THE AYURVEDIC PHARMACOPOEIA OF INDIA

THE AYURVEDIC PHARMACOPOEIA OF INDIA

PART - I
VOLUME - VIII

FIRST EDITION

GOVERNMENT OF INDIA
MINISTRY OF HEALTH AND FAMILY WELFARE
DEPARTMENT OF AYURVEDA, YOGA & NATUROPATHY, UNANI, SIDDHA AND HOMOEOPATHY,
NEW DELHI
2010

PDH.68. Pt. I, Vol. VIII
5000-2010 (DSK-II)

Effective from 1st December, 2011

Price	:	Inland	Rs. 500/-
		Foreign	US $ 100/-
ISBN	:	978-81-906489-4-3	
Published by	:	THE CONTROLLER OF PUBLICATIONS CIVIL LINES, DELHI - 110054	
On Behalf of	:	**GOVERNMENT OF INDIA** DEPARTMENT OF AYURVEDA, YOGA & NATUROPATHY, UNANI, SIDDHA AND HOMOEOPATHY (AYUSH), INDIAN RED CROSS SOCIETY BUILDING, SANSAD MARG, NEW DELHI	
WEBSITE	:	www.indianmedicine.nic.in	
Printed at	:	Cirrus Graphics Pvt. Ltd., New Delhi	

सचिव
भारत सरकार
स्वास्थ्य एवं परिवार कल्याण मंत्रालय
आयुर्वेद, योग व प्राकृतिक चिकित्सा,
यूनानी, सिद्ध एवं होम्योपैथी (आयुष) विभाग
रैड क्रॉस भवन, नई दिल्ली – 110001
SECRETARY
GOVERNMENT OF INDIA
MINISTRY OF HEALTH & FAMILY WELFARE
DEPTT. OF AYURVEDA, YOGA & NATUROPATHY,
UNANI, SIDDHA AND HOMOEOPATHY (AYUSH)
RED CROSS BUILDING, NEW DELHI-110001
Tel. : 011-23715564, Telefax : 011-23327660
E-mail : secy-ayush@nic.in
Mailing No. 110 108

सत्यमेव जयते

एस. जलजा.
S. JALAJA

FOREWORD

For the implementation of the Drugs and Cosmetics Act and Rules thereunder, it is necessary to work out standards for establishing the quality of the drugs. The Department of AYUSH with the help of Ayurvedic Pharmacopoeia Committee (APC), has already prescribed standards for 519 single plant drugs, 21 minerals and metals, which have been published till now in seven volumes of the Ayurvedic Pharmacopoeia of India, Part I (API-I). The Department has also published standards for 152 classical compound formulations and given in API, part II (Formulations) in 3 volumes.

Updating the existing monographs and adding new monographs with standards are the ongoing activities of the APC. The Drugs and Cosmetic Act in recent Rule 158 (B) clause IV has provided for the use of *Aushadh Ghanas* which include aqueous, hydro-alcoholic extracts and other than aqueous and hydro-alcoholic extracts both wet and dry, using plants mentioned in the books in the first Schedule to Act. In order to ensure the quality of such *Aushadh Ghanas*, APC selected 15 most widely used single plant drugs, their powders and their extracts for prescribing pharmacopoeial standards along with chromatographic finger print profile using Phyto-chemical Reference Standard (PRS) and also assays. Such standards allow the establishment of source plants used for the preparation of their extracts. These monographs also provide the SOPs for the preparation of their extracts, and form the Volume VIII of Part I of the API. This volume contains 60 monographs on 15 medicinal plants and their extracts. The Ayurvedic Pharmacopoeia Committee and the three industries involved in this new initiative of introducing quality standards of *Aushadh Ghanas* (medicinal plant extracts) in API Part-I, Vol. VIII deserve appreciation.

I want to place my appreciations on record for Ayurvedic Pharmacopoeia Committee, Prof. S.S. Handa, Dr. S.K. Sharma, Advisor (Ayurveda), Dr. Ramesh Babu, DG- CCRAS and APC entire team to bring this scientific work for the use of industry and other stakeholders of Ayurveda.

It is hoped that the addition of the volume VIII of API Part I will be of great utility to all the stakeholders.

(S. Jalaja)

CONTENTS

Appendices

LEGAL NOTICES

In India, there are several laws dealing with drugs for which monographs with quality standards and certain other requirements are prescribed. These monographs should be interpreted subject to the restrictions imposed by these laws wherever they are applicable.

In general, the Drugs and Cosmetic Act, 1940, the Dangerous Drugs Act, 1930, the Poisons Act, 1919, Drugs and Magic Remedies (Objectionable Advertisement) Act, 1954, the Narcotic Drugs and Psychotropic Substances Act 1985, and the Biodiversity Act, 2002, all as amended from time to time, along with the Rules framed thereunder, should be consulted to ensure that the provisions of such laws are being complied with.

Under the Drugs and Cosmetics Act, the Ayurvedic Pharmacopoeia of India, represented by its Parts and Volumes is the book of standards for substances included therein and such standards are Official. If considered necessary these standards can be amended and the Chairman of the Ayurvedic Pharmacopoeia Committee is authorized to issue such amendments. Whenever such amendments are issued, the specific Ayurvedic Pharmacopoeia of India intended thereby would be deemed to have been amended accordingly.

GENERAL NOTICES

Title: The title of the book is "Ayurvedic Pharmacopoeia of India, Part-I, Volume-VIII." Wherever the abbreviation "API, Pt.-I, Vol.-VIII" is used, it stands for the same and for the Supplements or Amendments thereto.

Name of the monograph: The name given on top of each monograph is in Saṁskṛta as mentioned in the Ayurvedic classics and/or Ayurvedic Formulary of India (AFI) and will be considered *Official*. These names have been arranged in alphabetical order in English.

If a preparation is intended to be stored over a period of time, deterioration due to microbial contamination may be inhibited by the addition of a permitted preservative. In such circumstances the label should state the name and the concentration of the preservative and the appropriate storage conditions. Mere presence of a monograph in the Pharmacopoeia shall not qualify the ingredient as a drug. The primary purpose of the monographs is to specify the quality parameters that can be employed to assess the "fitness for use" for the desired purpose which could be as a drug, dietary supplement, cosmetic or a functional food. The ingredients mentioned in this Pharmacopoeia may be prepared and their quality assessed as per the methods mentioned in the respective monographs. These ingredients may be regarded as Pharmacopoeial grade, even if they are to be used for non therapeutic purposes.

Introductory para: Each monograph begins with a Definition in an introductory paragraph. For drugs of plant origin, the part used has also been specified.

The requirements given in the monographs are not framed to provide against all impurities, contaminants or adulterants; they provide appropriate limits only for possible impurities that may be permitted to a certain extent. Material found to contain an impurity, contaminant or adulterant which is not detected by means of the prescribed tests in the Appendix 2 are also to be considered as impurity, should rational consideration require its absence.

Hydroalcohol: 50 per cent v/v of ethanol in purified water.

Yield of extract: The yield of extract mentioned in the monographs is meant to be indicative only. This is so, as newer techniques for extraction are being developed having higher efficiencies. Further, extractive values and thus yields are known to exhibit a high degree of inherent variability due to seasonal, geographical, edaphic and ontogenic factors.

Standards: For statutory purposes, the following shall be considered Official Standards: Definition, Identification, Quantitative parameters, Assay and Other requirements.

Added Substances: An article for which a monograph has been recommended contains no added substances/excipients, except when specifically permitted in the individual monograph. Unless otherwise specified in the individual monograph, or elsewhere in the General Notices, such added substances/excipients shall be from the approved list of Drugs and Cosmetics Rules, under Rule 169 to enhance its stability, usefulness, elegance, or to facilitate its preparation. Such added

substances shall comply with the quality indicated for it, shall be harmless in the amounts used, shall not exceed the minimum quantity required to provide their intended effect, shall not impair the therapeutic efficacy or the bioavailability and safety of the preparation and shall not interfere with the tests and assays prescribed for determining compliance with the official standards. Particular care should be taken to ensure that such substances are free from harmful organisms. Though the manufacturer of an extract is given the freedom to use an added substance, the manufacturer must guarantee the innocuousness of the added substance. The manufacturer shall also be responsible to explain to the appropriate authority, if needed, regarding the purpose of the added substance(s).

Meanings of Terms

Alcohol: The term 'alcohol' without qualification means ethanol (95 percent). Other dilution of ethanol are indicated by the term 'alcohol' followed by a statement of the percentage by volume of ethanol (C_2H_6O) required.

Desiccator: A tightly closed container of suitable size and design that maintains an atmosphere of low moisture content by means of silica gel or phosphorus pentoxide or other suitable desiccant.

Drying and ignition to constant weight: Two consecutive weighings after the drying or igniting operations do not differ by more than 0.5 mg, the second weighing following an additional period of drying or of ignition respectively appropriate to the nature and quantity of the residue.

Ethanol: The term 'ethanol' without qualification means anhydrous ethanol or absolute alcohol.

Filtration: Unless otherwise stated, filtration is the passing of a liquid through a suitable filter paper or equivalent device until the filtrate is clear.

Freshly prepared: Made not more than 24 hours before it is issued for use.

Label: Any printed packing material, including package inserts that provide information on the article.

Negligible: A quantity not exceeding 0.50 mg.

Solution: Where the name of the solvent is not stated 'solution' implies a solution in water. The water used complies with the requirements of the monographs on Purified Water. The term 'distilled water' indicates Purified Water prepared by distillation.

Temperature: The symbol '0' used without qualification indicates the use of the Celsius thermometric scale.

Water: If the term is used without qualification means Purified Water of the Pharmacopoeia. The term 'distilled water' indicates Purified Water prepared by distillation.

Water-bath: A bath of boiling water unless water at another temperature is indicated. Other methods of heating may be used provided the required temperature is approximately maintained but not exceeded.

Capital Letters in the Text: The names of the Pharmacopoeial substances, preparations and other materials in the text are printed in capital initial letters, and these infer that materials of Pharmacopoeial quality have been used.

Italics: Italic types are used for Scientific names of the plant drugs and microorganisms, and for some sub-headings and certain notations of the chemical names. Italic types have also been used for words which refer to solvent system in TLC procedure, reagents and substances, processes covered under Appendices. Chemicals and Reagents and Substances of Processes in Appendices have also been printed in Italics.

Odour and Taste: Wherever a specific odour has been observed, it has been mentioned as characteristic for that substance, but the description as 'odourless' or 'no odour' has generally been avoided in the Description where a substance has no odour. Where an 'odour' is said to be present, it is examined by smelling the drug directly after opening the container. If an odour is discernible, the contents are rapidly transferred to an open vessel and re-examined after 15 minutes. If odour persists to be discernible, the sample complies with the description for 'odour', as a characteristic for that substance.

The taste of a drug is examined by taking a small quantity of drug by the tip of a moist glass rod and allowing it to remain on the tongue. ***This does not apply in the case of poisonous substances.***

Powders: Drug substances are subjected to comminuting during preparation. It is desirable that such powders maintain certain average particle size for effective processing.

To provide for such situations, the fineness of a powder is given in terms of sieve sizes, using the BIS sieves as standard. The sieve sizes follow the latest revision of the BIS. For the convenience of users, the equivalents or nearest equivalent numbers according to the earlier BIS have also been given.

Weights and Measures: The metric system of weights and measures is employed. Weights are given in multiples or fractions of a gram (g) or of a milligram (mg). Fluid measures are given in multiples of fraction of milliliter (ml). The amount stated is approximate but the quantity actually used must be accurately weighed and must not deviate by more than 10 per cent from the one stated.

When the term "drop" is used measurement is to be made by means of a tube which delivers 20 drops per gram of distilled water at 15^0.

Identity, Purity and Strength: Under the heading "Identification", tests are provided as an aid to identification and are described in the respective monographs and included.

Vegetable drugs should be duly identified and authenticated and be free from insects, pests, fungi, microorganisms, pesticides, and other animal matter including animal excreta, be within the permitted and specified limits for lead, arsenic and heavy metals, and show no abnormal odour, colour, sliminess, mould or any sign of deterioration.

Quantitative tests like total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, water-soluble extractive, moisture content, volatile oil content and assays are the parameters upon which the standards of Pharmacopoeia depend. Except for Assays, which are covered under each monograph, the methods of determination for the others are given in Appendices, with a suitable reference in the monograph to the specific Appendix.

An analyst is not precluded from employing an alternate method in any instance if one is satisfied that the method, which one uses, will give the same result as the Pharmacopoeial method described under assay. However, in the event of doubt or dispute the methods of analysis of the Pharmacopoeia are alone authoritative. Unless otherwise prescribed, the assays and tests are carried out at a temperature between 20^0 and 30^0.

In the performance of an assay or any test procedure, *not less* than the specified number of dosage units or quantities should be taken for analysis. Proportionately larger or smaller quantities than the specified weights and volumes may be taken for substances under assay or test substances, Reference Standards or Standard Preparations, provided the measurement is made with at least equivalent accuracy and provided that any subsequent steps, such as dilutions, are adjusted accordingly to yield concentrations equivalent to those specified and are made in such a manner as to provide at least equivalent accuracy.

Expression of Results: Total ash, acid-insoluble ash, water-soluble matter, alcohol-soluble matter, water content, content of essential oil and content of active principle are calculated with reference to the drug that has not been specially dried, unless otherwise prescribed in the monograph. In other words, all limits are thus proposed on "as such basis" unless specified otherwise.

Limits for Heavy metals, Microbial load, Pesticide residues and Aflatoxins: Articles included in this volume are required to comply with the limits for heavy metals, microbial load, pesticide residues and aflatoxins prescribed in the individual monographs and wherever limit is not given in the monograph, they must comply with the limits given in the respective Appendices. The methods for determination of these parameters are given in the Appendices.

Thin Layer Chromatography (TLC): Under this title, wherever given, the R_f values given in the monographs are not absolute but only indicative. The analyst may use any other solvent system and detecting reagent to establish the identity of any particular chemical constituent reported to be present in the test substance. However, in case of dispute the pharmacopoeial method would prevail. Unless specified in the individual monograph all TLC have been carried out on pre-coated Silica gel $60F_{254}$ aluminium plates.

Reference Standards: Reference substance and standard preparation are authentic substances that have been verified for their suitability for use as standards for comparison in some assays, tests and TLC of the API. The reference standards, abbreviated as *RS* are issued by Pharmacopoeial Laboratory of Indian Medicine (PLIM).

Quantities to be weighed for Assays and Tests: In all description quantity of the substance to be taken for testing is indicated. The amount stated is approximate but the quantity actually used must be accurately weighed and must not deviate by more than 10 per cent from the one stated.

Constant Weight: The term "constant weight" when it refers to drying or ignition means that two consecutive weighing do not differ by more than 1.0 mg per gram of the substance taken for the determination, the second weighing following an additional hour of drying or further ignition.

Percentage of Solutions: In defining standards, the expression per cent (%), is used, according to circumstances, with one of the four meanings given below.

Per cent w/w (percentage weight in weight) expresses the number of grams of active substance in 100 grams of product.

Per cent w/v (percentage weight in volume) expresses the number of grams of active substance in 100 milliliters of product.

Per cent v/v (percentage volume in volume) expresses the number of milliliters of active substance in 100 milliliters of product.

Per cent v/w (percentage volume in weight) expresses the number of milliliters of active substance in 100 grams of product.

Percentage of Alcohol: All statements of percentage of alcohol (C_2H_5OH) refer to percentage by volumes at 15.56^0C.

Solubility: When stating the solubilities of Chemical substances the term "Soluble" is necessarily sometimes used in a general sense irrespective of concomitant chemical changes.

Statements of solubilities, which are expressed as a precise relation of weights of dissolved substance of volume of solvent, at a stated temperature, are intended to apply at that temperature. Statements of approximate solubilities for which no figures are given, are intended to apply at ordinary room temperature.

Pharmacopoeial chemicals when dissolved may show slight physical impurities, such as fragment of filter papers, fibres, and dust particles, unless excluded by definite tests in the individual monographs.

When the expression "parts" is used in defining the solubility of a substance, it is to be understood to mean that 1 gramme of a solid or 1 millilitre of a liquid is soluble in that number of millilitres of the solvent represented by the stated number of parts.

When the exact solubility of pharmacopoeial substance is not known, a descriptive term is used to indicate its solubility.

The following table indicates the meaning of such terms :-

Descriptive terms	***Relative quantities of solvent***
Very soluble	Less than 1 part.
Freely soluble	From 1 to 10 parts.
Soluble	From 10 to 30 parts.
Sparingly soluble	From 30 to 100 parts.
Slightly soluble	From 100 to 1000 parts.
Very slightly soluble	From 1000 to 10,000 parts.
Practically insoluble	More than 10,000 parts.

Reagents and Solutions: Reagents required for the assay and tests of the Pharmacopoeia are defined in the Appendix showing the nature, degree of the purity and strength of solutions to be made from them.

Therapeutic uses: Therapeutic uses wherever given are as mentioned in the API.

Doses: The doses mentioned in the monograph are in the metric system, which are approximate conversions from classical weights mentioned in Ayurvedic texts. A conversion table is appended giving classical weights with their metric equivalents (Appendix 5). Doses mentioned in the A.P.I. are intended merely for general guidance and represent, unless otherwise stated, the average range of quantities per dose which is generally regarded suitable by clinicians for adults only when administered orally. They are not to be regarded as binding upon the prescribers.

Storage: Statement under the heading 'Storage' constitutes non-mandatory advice. The substances and preparations of the Pharmacopoeia are to be stored under conditions that prevent contamination and, as far as possible, deterioration. Precautions that should be taken in relation to the effects of the atmosphere, moisture, heat and light are indicated, where appropriate, in the individual monographs.

Specific directions are given in some monographs with respect to the temperatures at which Pharmacopoeial articles should be stored, where it is considered that storage at a lower or higher temperature may produce undesirable results. The conditions are defined by the following terms.

Cold- Any temperature not exceeding 8^0 and usually between 2^0 and 8^0. A refrigerator provides a cold place in which the temperature is maintained thermostatically between 2^0 and 8^0.

Cool- Any temperature between 8^0 and 25^0. An article for which storage in a cool place is directed may, alternately, be stored in a refrigerator, unless otherwise specified in the individual monograph.

Room temperature - The temperature prevailing in a working area.

Warm - Any temperature between 30^0 and 40^0.

Excessive heat- Any temperature above 40^0.

Protection from freezing- Where, in addition to the risk of breaking of the container, freezing results in loss of strength or potency or in destructive alteration of the characteristics of an article, the label on the container bears an appropriate instruction to protect from freezing.

Storage under non-specific conditions- Where no specific storage directions or limitations are given in the individual monograph, it is to be understood that the storage conditions include protection from moisture, freezing and excessive heat.

Packaging and Containers: In general the ASU extracts should be packed in well closed container i.e. one that protects the contents from extraneous matter, moisture or loss of material under normal condition of handling.

The preferred packaging for extracts are a primary cover made up of 12 μ polyester, 100 μ polyethylene and a secondary cover made up of 9 μ aluminum sandwiched between 2 layers of 12 μ polyester and 100 μ polyethylene. The tertiary package can be HDPE drums.

The container is the device that holds the article. The immediate container is that which is in direct contact with the article at all times. The closure is a part of the container.

The container is designed so that the contents may be taken out for the intended purpose in a convenient manner.

It provides the required degree of protection to the contents from environmental hazards.

The container should not interact physically or chemically with the article placed in it so as to alter the strength, quality or purity of the article beyond the official requirements.

Prior to its being filled, the container should be clean. Special precautions and cleaning procedures may be necessary to ensure that each container is clean and that extraneous matter is not introduced into or onto the container.

Light-resistant Container- A light resistant container protects the contents from the effects of actinic light by virtue of the specific properties of the material of which it is made. Alternatively, a clear and colourless or a translucent container may be made light-resistant by means of an opaque (light-resistant) covering and/or stored in a dark place: in such cases, the label on the container should bear a statement that an opaque covering or storage in dark place is needed until the contents have been used up.

Well-closed Container- A well-closed container protects the contents from extraneous contamination and from loss of contents under normal conditions of handling, shipment, storage and distribution.

Tightly-closed Container- A tightly-closed container protects the contents from contamination by extraneous liquids solids or vapours, and from loss or deterioration of contents from effervescence, deliquescence or evaporation under normal conditions of handling, shipment, storage and distribution.

Single Unit Container- A single unit container is one that is designed to hold a quantity of the drug product intended for administration as a single finished device intended for use promptly

after the container is opened. The immediate container and/or outer container or protective packaging is so designed as to reveal evidence of tampering, if any.

Multiple Unit Container- A multiple unit container is a container that permits withdrawals of successive portions of the contents without changing the strength, quality or purity of the remaining portion.

Tamper-evident Container- A tamper-evident container is fitted with a device or mechanism that reveals irreversibly whether the container has been opened.

Labeling: In general, the labeling of drugs and pharmaceuticals is governed by the Drugs and Cosmetics Act, 1940 and Rules there under.

ABBREVIATIONS FOR TECHNICAL TERMS

^0C	–	–	^{0}
Cells containing pigments	–	–	ccp
centimeter(s)	–	–	cm
Cluster crystals of calcium oxalate	–	–	clr
concentrated	–	–	con.
diameter	–	–	dia.
dilute	–	–	dil.
gram per liter	–	–	g/l
gram(s)	–	–	g
hour(s)	–	–	h
kilogram(s)	–	–	kg
litre(s)	–	–	l
meta	–	–	*m*
meter(s)	–	–	m
micro	–	–	μ
microgram per milliliter	–	–	μg/ml
microliter	–	–	μl
micrometer	–	–	μm

milligram(s)	–	–	mg
milliliter per minute	–	–	ml/min
milliliter(s)	–	–	ml
millimeter	–	–	mm
minute(s)	–	–	min
Molarity	–	–	M
nanogram	–	–	ng
nanoliter	–	–	nl
nanometer	–	–	nm
Normality	–	–	N
ortho	–	–	*o*
para	–	–	*p*
parts per billion	–	–	ppb
parts per million	–	–	ppm
Phloem fibres	–	–	phf
rate per minute	–	–	rpm
Reference Standard	–	–	*RS*
Refractive index detector	–	–	RI detector
Rosette crystals of calcium oxalate	–	–	ro
specific gravity	–	–	sp.gr.
Starch grains	–	–	sg
ultraviolet	–	–	UV
volume	–	–	vol
volume in volume	–	–	v/v
volume in weight	–	–	v/w
weight	–	–	wt
weight in volume	–	–	w/v
weight in weight	–	–	w/w

INDO-ROMANIC EQUIVALENTS FOR DEVANĀGARĪ ALPHABETS

अ	A	a
आ	Ā	ā
इ	I	i
ई	Ī	ī
उ	U	u
ऊ	Ū	ū
ऋ	Ṛ	ṛ
ए	E	e
ऐ	AI	ai
ओ	O	o
औ	AU	au
ं	Ṁ	ṁ
ः	Ḥ	ḥ
क	KA	ka
ख	KHA	kha
ग	GA	ga
घ	GHA	gha
ङ	Ṅ A	ṅa
च	CA	ca
छ	CHA	cha
ज	JA	ja
झ	JHA	jha
ञ	Ñ A	ña
ट	Ṭ A	ṭa
ठ	Ṭ HA	ṭha
ड	Ḍ A	ḍa
ढ	Ḍ HA	ḍha
ण	Ṇ A	ṇa
त	TA	ta
थ	THA	tha
द	DA	da
ध	DHA	dha
न	NA	na
प	PA	pa
फ	PHA	pha
ब	BA	ba
भ	BHA	bha
म	MA	ma
य	YA	ya
र	RA	ra
ल	LA	la
व	VA	va
श	Ś A	śa
ष	Ṣ A	ṣa
स	SA	sa
ह	HA	ha
ळ	Ḷ A	ḷa
क्ष	KṢA	kṣa
त्र	TRA	tra
ज्ञ	JÑA	jña

ACKNOWLEDGMENTS

The Ayurvedic Pharmacopoeia Committee duly acknowledges the contributions made by the staff of the participating institutions viz., Natural Remedies Pvt. Ltd. Bangalore, Chemolids Pvt. Ltd. Vijayawada and Sami Labs Pvt. Ltd. associated with the APC project work for developing quality standards of plant drugs and their hydroalcoholic and water extracts.

The Committee expresses gratitude to the Secretary and Joint Secretary, Department of AYUSH, for providing financial and administrative support. The Committee also sincerely thanks Dr. M.M. Padhi, Deputy Director [Tech.]; Dr. Pramila Pant, Assistant Director [Chem.]; Dr. Bishnupriya Dhar, Assistant Director [P'cognosy]; Dr. Shruti Khanduri, Research Officer [Ay.]; Ms. Talat Anjum, Research Officer [Bot.]; Dr. Chhote Lal, Dr. A.K.S. Bhadoria and Dr. M.N. Rangne; Dr. Nikhil Jirankalgikar, S.R.F. [Ayu.]; Mr. Chinmay Rath, S.R.F. [Bot.]; Dr. Sunil Dutt, S.R.F.; Dr. Shelvi Agarwal, S.R.F. and other associated officers, for their constant efforts in bringing out this volume. Thanks are also due to Dr. Rajeev Kumar Sharma, Director I/C, Sh. Ravindra Singh, Assistant Director [Chem.]; Dr. Jayprakash, Research Officer (Chem), Sh. N.S. Mehra, Research Officer (Bot). The Committee also acknowledges Ms. Jyoti Bageja, Consultant, for arranging and giving shape to the technical data; Mr. Ashish and Ms. Meenakshi, D.E.O., for typing and providing assistance as required.

INTRODUCTION

The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization. Though Ayurveda has undergone many changes in the course of its long history, it still remains the mainstay of medical relief to a large section of population of the nation. Due to urbanization and dwindling of forests, the Vaidya by and large is no longer a self contained unit collecting and preparing his own medicines as before. He has now to depend on the newly developed agencies like one collecting and supplying the crude drugs and the other undertaking mass production of medicines in the Ayurvedic Pharmaceutical units run on commercial scale.

2. In view of the new trend in Ayurvedic Pharmaceutical field, Government of India considered it expedient to utilize the existing Drugs and Cosmetics Act 1940, to also control to a limited measure the Ayurvedic, Siddha and Unani drugs by amending the Act.

3. The act was accordingly amended in 1964, to ensure only a limited control over the production and sale of these medicines namely:-

i) The manufacture should be carried under prescribed hygienic conditions, under supervision of a person having a prescribed qualification;

ii) The raw materials used in the preparation of drugs should be genuine and properly identified and

iii) The formula or the true list of all the ingredients, contained in the drugs, should be displayed on the label of every container.

The present Ayurvedic Pharmacopoeial Committee (APC) was constituted under the Department of AYUSH (Vide letter No.5-5/CCRAS-2006/Tech/APC/Hqrs. dated 12th March, 2009) Ministry of Health and Family Welfare, Govt. of India:-

Prof. S. S. Handa **CHAIRMAN**
(Former Director,
Indian Institute of Integrative Medicine (IIIM), CSIR, Jammu)
Executive Villa, House No. 522-A,
Block –C, Sushant Lok Phase-I,
Gurgaon, Haryana-122 001.

Dr. S. K. Sharma **VICE- CHAIRMAN**
Adviser (Ayurveda)
Department of AYUSH,
Ministry of Health and Family Welfare,
Government of India, IRCS Building,
Red Cross Road, New Delhi-110 001.

Dr. G. S. Lavekar Director General, Central Council for Research in Ayurveda & Siddha, 61-65 Institutional Area, Opp-'D' Block, Janakpuri, New Delhi-110 058.	**MEMBER-SECRETARY** from March, 2009 to February, 2010
Dr.Ramesh Babu Devalla Director General, Central Council for Research in Ayurveda & Siddha, 61-65 Institutional Area, Opp-'D' Block, Janakpuri, New Delhi-110 058.	**MEMBER- SECRETARY** From 19th April, 2010 to onwards

OFFICIAL MEMBERS

Drug Controller General (I) or his representative, Ministry of Health & Family Welfare, Nirman Bhawan, New Delhi-110 011.	Member
Dr. Rajeev Kumar Sharma Director Pharmacopoeial Laboratory for Indian Medicine, Kamla Nehru Nagar, Ghaziabad.	Member
Managing Director I/c Indian Medicine Pharmaceutical Corporation Ltd. (IMPCL), Mohan, Uttranchal-244 715	Member

NON OFFICIAL MEMBERS

Sub-committee of Phyto-chemistry & Chemistry

Dr. P.D. Sethi Former Director, CIPL, B-140, Shivalik Enclave (Near Malviya Nagar), New Delhi-110 017.	Chairman
Dr. (Miss.) A. Saraswathy Scientist In charge, CSMDRIA & S, Arignar Anna Govt. Hospital Campus Arumbakkam, Chennai- 600 106.	Member
Dr. Jayaraman Arunachalam, Head, National center for Compositional Characterization of Materials – Bhabha Atomic Research Centre (BARC), DAE/Govt. of India, ECIL, Hyderabad-500 062.	Member

Prof. V. K. Kapoor — Member
Formerly Dean & Chairman,
Deptt. of Pharmaceutical Chemistry,
University Institute of Pharmaceutical Sciences,
Punjab University, Chandigarh -160 0014.

Dr. D. Vijaya Kumar, — Member
Scientist E-I,
Reaction Engineering Laboratory,
Indian Institute of Chemical Technology (CSIR),
Hyderabad - 500 007.

Sub-Committee on Pharmacognosy

Ms. S. Satakopan, — Chairperson
Ex-Chairperson APC,
7/4, Padmam Flats,
Seventh Street, Nagandllur
Chennai-600 061.

Dr. Indira Balachandran, — Member
Centre of Medicinal Plants Research,
Aryavaidyasala, Kottakal, Malappuram,
Kerala- 676 503.

Prof. M.A. Iyengar — Member
Retd. Prof. of Pharmacognosy,
14, H.I.G, HUDCO,
Maniphal – 576 104.

Dr. (Mrs.) Shanta Mehrotra, — Member
Emeritus Scientist,
Pharmacognosy & Ethnopharmacology Division,
National Botanical Research Institute,
Rana Pratap Marg,
Lucknow.

Formulary Sub-committee of APC
(Ras Shastra/Bhaishjya Kalpana – Ayurvedic Pharmacy)

Dr. S. K. Dixit, — Chairperson
Former Head of Department of Rasasastra, BHU,
B-3/402, Shivala, Varanasi-221 005.

Dr. P. Madhvankutty Varier, Member
Aryavaidyasala, Kottakal,
Malappuram, Kerala- 676 503
(Representative of Industry)

Dr. Banwarilal Gaur, Member
Vice Chancellor,
Rajasthan Ayurveda University,
Kadwad, Nagaur Road, Jodhpur.

Dr. S.S. Savrikar, Member
Formerly Vice Chancellor, GAU, Jamnagar,
Nand Jyot, Patel Colony, Road No.2,
Jamnagar – 361 008

Ayurveda Sub-committee of APC
(Single drugs of Plants, Minerals, Metals and related Pharmacology)

Dr. V.K. Joshi, Chairperson
Deptt. of Dravyaguna, Faculty of Ayurveda,
Institute of Medical Sciences,
Banaras Hindu University,
Varanasi -221 005.

Dr. M.C. Sharma, Member
Director,
NIA, Jaipur

Prof. (Dr.) Adhithan, Member
HOD Pharmacology,
JIPMER, Pondicherry

Dr. Maya Ram Uniyal, Member
Adviser
Medicinal and Aromatic Plants
E-141, Nehru Colony,
Dharampur, Dehradun (Uttarakhand)

Dr. K. Raghunathan, Member
Formerly Deputy Director, CCRAS
H.No. 664, Sector -28
Faridabad-121 008

1. The term of the Committee shall be for a period of 3 years from the date of its first meeting and the members shall hold office for that period.

2. The Chairman of the APC shall have the powers to form sub-committees whenever required and to co-opt experts from outsider for such sub-committees.

3. The committee shall have the power to frame procedure of functioning.

4. The functions of the Committee shall be as follows:

   i. To prepare a Ayurvedic Pharmacopoeia of India of single and compound drugs.

   ii. To prescribe the working standards for compound Ayurvedic formulations including tests for identity, purity, strength and quality so as to ensure uniformity of the finished formulations.

   iii. Keeping in view the time constraint, to identify such methods, procedures and plan of work as would enable to publish the formulary and standard of all commonly used drugs to be brought out in a phased manner.

   iv. To prepare remaining parts of the official formulary of compound preparations from the classical texts including standardized composition of reputed institution.

   v. To develop and standardize methods of preparations, dosage form, toxicity profile etc.

   vi. To develop quality standard, safety, efficacy profile of Intermediates like extracts of Ayurvedic raw drugs.

   vii. To develop the Quality standards, Safety, efficacy profile of different parts of the plants; as well as to inclusion of new plants as Ayruvedic drugs.

   viii. Any other matter relating to the Quality standards, Shelf Life, identification, new formulations etc.

5. The following are the targets focus of the Committee:

   i) To evolve standards of single drugs mentioned in the Ayurvedic formularies in India.

   ii) To evolve standards for compound formulations mentioned in the Ayurvedic formularies of India & other Ayurvedic formulations of National Priority.

   iii) To prepare drafts SOP of Ayurvedic formularies of India from the classical texts and other authentic sources.

iv) To achieve the targets of the committee/Sub-Committee of APC will invariable meet once in two months or so.

6. The non-Official and co-opted members on the Committee shall be entitled to TA & DA as admissible under the rules of the Govt. of India.

7. The expenditure involved will be met from the sanctioned grant of the Ministry of Health & Family Welfare, Deptt. of AYUSH-APC head.

SPECIAL INTRODUCTION TO VOLUME VIII

(EXTRACTS)

Background:-

Contrary to popular preparation, Ayurvedic therapeutic 'modes' or presentations in current usage have had a long history of development. Ayurvedic techniques of formulating compound mixtures (yogas) developed gradually from the pre-Vedic period through the Vedic, the Samhita, and the Sangraha periods and continue to develop. In the Samhita period, ancient indigenous science was at the peak of its glory and we find that almost all the pharmaceutical modes, now identified as 'classical', were known during this period.

From the scanty evidences and interpretations available about the pre-Vedic period, one naturally concluded that the pre-Vedic Indian employed only a few pharmaceutical modes and that methods in pharmacy were rather simple. Raw materials were used in their crude form, whole, or at best comminuted, to assist application. Extraction was limited to expression of fresh juice or extracting by decoction.

During the Vedic period preparation of medicines might have gained importance, as fire and pyre sacrifices were popular practices followed during the period, but complex formulations and combination of drugs did not appear during this period. Single drugs, their juices and pastes were the main forms in use. It is doubtful whether the formulation, in its contemporary sense, was practised in the Vedic period. In the Vedas, we find praises of single drugs. No complex mixtures of medicines are traceable in the Vedic literature.

We find that systematized information on Ayurvedic pharmacy appears in the compendia of the Samhita period. It is in these texts that complicated compounding procedures as well as multi-ingredient formulations are recorded. Tips on formulations, concepts of compatibility and incompatibility among ingredients, systematic classification of preparations, etc. are also available in these books. Though the oldest available Samhita contains references to almost all the classical pharmaceutical modes, it is not logical to conclude that all of them developed simultaneously. Hence, we may consider that primary preparations or basic modes were the most ancient. These work technically termed "kaṣaya kalpana". Five primary preparations, such as expressed juice (SVARASA), paste (KALKA), decoction (KVĀTHA), cold infusion (SEETA KASAYA) and hot infusion (PHANT'A KASHAYA) are mentioned in the ancient classics. All these modes have very short shelf life and hence were prepared as and when needed. More stable forms generated as secondary and tertiary preparations or derived modes such as medicated fatty preparations (oils, ghṛta, etc.), Jelly or semi solid preparations (AVALEHA), fermented products (ARIṢṬA, ASĀVA, etc.) Pills (GUṬIKĀ) were yet another means of presentation of drugs. Apart from many other factors, fats, sugar and alcohol were known to be natural preservatives.

During the Sangraha period, we find from recorded literature that formulations based on metals and minerals gained usage. This is on par with the development of Ayurvedic iatro-

chemistry known as Rasasastra. Some pharmaceutical modes such as syrups also appeared in Ayurvedic pharmacy. This was mainly due to the influence of Unani medicine that emanated from Middle East. In fact, it started even as early as the invasion of Alexander the Great, but attained great growth due to the active contribution by Moghuls in Medieval India.

A gradual shift in practice from extemporaneous and need based production, to a more organized and planned long term production ensured ready medicines round the year. This trend was evident as early as the 10^{th} century AD, but got established at the dawn of the 20^{th} century, resulting in a change in the pattern of drug production, and improvement in its technology. Ayurvedic pharmacy by this time faced challenges from Western medicines and modern methods of pharmacy during the British rule. There were efforts to replace aqueous decoctions of indigenous medicines with tinctures but such changes faced stiff opposition from the conservative section of Ayurvedic physicians.

Present trend

Since the last half a century, Ayurveda has had to compete with modern medicines, which are proven to be quick-acting, strong and effective. Convenience and acceptability of these medicines by patients was another factor that necessitated the Ayurvedic fraternity to modernize. Many manufacturers shifted their traditional preparation like Vatis and Gutikas to compressed tablets and capsules. There were attempts to achieve greater shelf life for traditional medicines, by adopting newer techniques of extraction, chemical preservation and application of modern principles in Ayurvedic pharmacy.

A recent trend in Ayurvedic manufacturing pharmacy aims at (1) enhancement of potency and reduction in bulk of dosage form (2) convenience in administering doses and (3) acceptability by improving palatability.

This trend is an outcome of significant gains in knowledge of phyto-chemical contents of the source plant, and improved methods of assessing the pharmacological and therapeutic actions of such phytochemical contents. A direct development of this awareness is the introduction of extracts of plants as a more effective means of obtaining desirable results.

EXTRACTS

Extraction, as the term is used in Pharmacy, involves the separation of medicinally active portion from plant or animal tissues using selective solvents through standard extraction procedures. The general techniques of extraction of medicinal plants include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (soxhlet), aqueous alcoholic extraction by fermentation (such as Asvas') counter current extraction (CCE), microwave assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction (SFE), etc. Such extraction techniques separate out soluble plant metabolites leaving behind insoluble cellular

marc. The product so obtained from plants are relatively complex mixtures of a number of groups of plant metabolites.

Extracts are prepared by using an appropriate menstruum, with a view to extraction of active principles or at least elimination of the inert bulk. Hence, modernization of Ayurvedic drug industry is experimenting with various extraction techniques. More and more capsules and tablets appearing in the market are based on products using extraction techniques. Even liquids like syrups, medicated oils and other oral suspensions depend on the extracts. Extraction is essential to reduce the bulk of the drug material and enhance its potency, acceptability and convenience of administration of the drug. The purpose of standardized extraction procedures for crude drugs is to acquire the therapeutically desired portion and eliminate inert material, by treatment with a selective solvent known as menstruum. The extract thus obtained is used as a medicinal agent directly, or further processed to be incorporated in any dosage form such as tablet, capsule or syrup etc. Standardized extract for use in a pharmacopoeia indicates an extract having an acceptable limit of the given content, specified by a biomarker or chemical/analytical marker. The extract should specify the defined range for the constituents (biomarker or chemical/analytical marker). Dry extracts usually have a loss on drying or water content not greater than 5 per cent w/w, unless specified otherwise in any monograph. In the cases of standardized extracts, the presence and content of the inert permissible excipients including preservatives, if any, should be declared on the label.

Extracts shall be free from solvents used for the extraction and shall comply with the respective limits (Appendix 3.8). Harmful and carcinogenic solvents shall not be used for extraction purposes.

Extracts may be exposed to ethylene oxide for fumigation or low dose gamma radiation for the purpose of avoiding microbial contamination. In cases where the extracts are fumigated, the final extracts exposed shall meet residual levels of ethylene dioxide limits as applicable. Herbs treated with low dose of gamma radiations shall meet national regulations related to such a treatment and shall be labeled as per law.

Phyto-chemical Reference Standards as Markers

Extracts are usually complex mixtures of several chemical constituents. For a large majority of botanical extracts it is not known with certainty which of the various components is responsible for the reported pharmacological effect. It is generally believed that several constituents act synergistically to provide the reported effect. For articles for which compendial monographs are provided, certain chemical constituents of the article are chosen and quantitative test procedures for determining their content are provided. The choice of such constituents, known generally as marker compounds, is based on certain considerations. Currently, the following types of marker compounds are specified in compendial monographs and may be identified in raw materials:

Active Principles - These are constituents that have proven clinical activity. A minimum content or range for the active principles is usually specified in the individual monograph. A quantitative determination of active principles carried out periodically during stability studies of dosage forms provides necessary information for arriving at suitable expiry dates.

Active Markers – These are constituents that have known pharmacological activity contributing in some extent to its efficacy. However, the clinical efficacy for these constituents may not be proven. A minimum content or range for active markers is usually specified in individual monographs. A quantitative determination of active markers during stability studies of dosage forms provides necessary information for arriving at suitable expiry dates.

Analytical Markers - Where neither defined active principles nor active markers are known, other constituents of the botanical extract amenable to quantitative determination are chosen. These markers aid in the positive identification of the article under text. In addition, maintaining a minimum content or a specified range of the analytical markers helps to achieve standardization of the plant extract and to arrive at a suitable expiry date during stability studies.

Negative markers – These are constituents that may have toxic or allergenic properties, rendering their presence in the botanical extract undesirable as for example B-asarone from Vacha (*Acorus calamus*). A stringent limit for such negative markers may be specified in individual monographs.

Monograph on Plant Extracts – Monograph of an extract in the pharmacopoeia is to provide qualitative and quantitative standards of quality for the extract for its use as a food item or a food supplement, dietary supplement/ nutraceutical, as a drug and/or as an ingredient in cosmetics. Each of such use would need to comply with applicable regulations. As per quality criteria, the plant extract in its entirety, is defined as the active substance. Consequently, all relevant aspects of quality of an extract must be considered and these include plant material, solvent used for extraction, extraction technology employed and manufacturing equipment used.

Methodology - Fifteen single plant drugs were selected for preparing aqueous and hydro-alcoholic dried extracts. Authenticity, purity and quality of each of the fifteen plant drugs and their powders was confirmed before preparing hydro-alcoholic extract and water extract. In order to make this volume self containing and comprehensive, monograph of the selected whole plant drug, its powder, hydro-alcoholic extract and water extract have been prepared.

The work of preparing extracts using identical standard operating procedures was allotted to three extract manufacturers viz., Natural Remedies, Bangalore, Sami Lab., Bangalore and Chemeloids, Vijayawada, and coordinated by Natural Remedies. Raw material was procured in one lot and distributed among the three collaborators. Data generated was shared on three batches of each. Comprehensive reproducible data has been incorporated in the monographs.

The monographs on fifteen plant drugs have been already included in earlier volumes of API Part I. They have now been upgraded by the addition of Thin Layer Chromatography finger print profiling using Phyto-Chemical Reference Standard (PRS). New addition of assay for the PRS has been also added to make the monograph comparable to international standards.

Keeping in view the use of extracts in Ayurvedic formulations, the Drugs and Cosmetics 5th Amendment Rule, 2010 under 158 (B) clause IV issues guidelines with respect to *Aushadh Ghana* (Medicinal Plant extracts- dry/wet) obtained from plants mentioned in books of First Schedule of the Act including Aqueous, hydro-alcoholic and other than Aqueous and hydro-alcoholic extracts.

In the light of this latest amendment in the Drugs and Cosmetics Rule 158 B clause IV, the Ayurvedic Pharmacopoeia Committee considers it appropriate to prepare monographs on plant extracts and in this Volume VIII of the API, Part I, standards for aqueous and hydro-alcoholic extracts are presented for the first time. To start with, 15 traditionally well known medicinal plants have been selected and this volume contains upgraded monographs on 15 source plants, their powders, their aqueous and hydro-alcoholic extracts, thus comprising a total of 60 monographs, for ensuring their quality for use as drug ingredients.

We offer this Volume for public use and welcome comments and criticisms to enhance its value in future revisions and additions.

MONOGRAPHS

ĀMALAKĪ

Āmalakī consists of dried pericarp of mature fruits of *Phyllanthus emblica* L. syn. *Emblica officinalis* Gaertn. (Fam. Euphorbiaceae); a small or medium sized monoecious tree, found both in mixed deciduous forests of the country ascending to 1300 m on hills; cultivated in plantations and home gardens; fruits are dried whole or without seeds. The seeds are removed by treating the fruits with boiling *water* followed by crushing. Āmalakī contains not less than 0.8 per cent of gallic acid when assayed.

Synonyms: Āmalaka, Amṛtaphala, Dhātrīphala

Regional Language Names:

Assamese	:	Amlaku, Amlakhi, Amlakhu
Bengali	:	Amla, Dhatri
English	:	Emblic Myrobalan
Gujarati	:	Ambala, Amala
Hindi	:	Amla, Aonla
Kannada	:	Nellikayi, Bela nelli, Pottadenollikayi
Kashmiri	:	Embali, Amli
Malayalam	:	Nellikka
Marathi	:	Avala, Avalkathi
Oriya	:	Anala, Ainla
Punjabi	:	Aula, Amla
Tamil	:	Nellikkai, Nelli
Telugu	:	Usirika
Urdu	:	Amla, Amlaj

Description:-

a) *Macroscopic:*

Drug consists of curled pieces of epicarp and mesocarp of dried fruit occurring either whole or as separated single segment 1 to 2 cm long or united as 3 or 4 segments; bulk colour grey to black, pieces showing, a broad, highly shrivelled and wrinkled; external surface convex to somewhat concave, transversely wrinkled showing a few whitish specks; occasionally some pieces may show a portion of stony endocarp; fracture, tough, cartilaginous, taste, sour and astringent; seeds and endocarp must be within the limits prescribed for foreign matter.

b) Microscopic:

TS of pericarp of fruit shows epicarp consisting of a single layer of epidermis, cell appearing tabular and polygonal in surface view; cuticle present; a few small rosette crystals of calcium oxalate present in epidermal cells; mesocarp cells tangentially elongated parenchymatous and cells with walls showing irregular thickenings; ramnified vascular elements occasionally present, lignified, having wide lumen; stone cells present, either isolated or in small groups toward

endocarp; pitted fibres with walls appearing serrated due to the pit canals leading into lumen, present.

c) Powder:

Fine powder shows epidermis with uniformly thickened straight walls; isodiametric parenchyma cells with irregular thickened walls; occasionally short fibres and tracheids; a few lignified stone cells having wide lumen (Fig. 1).

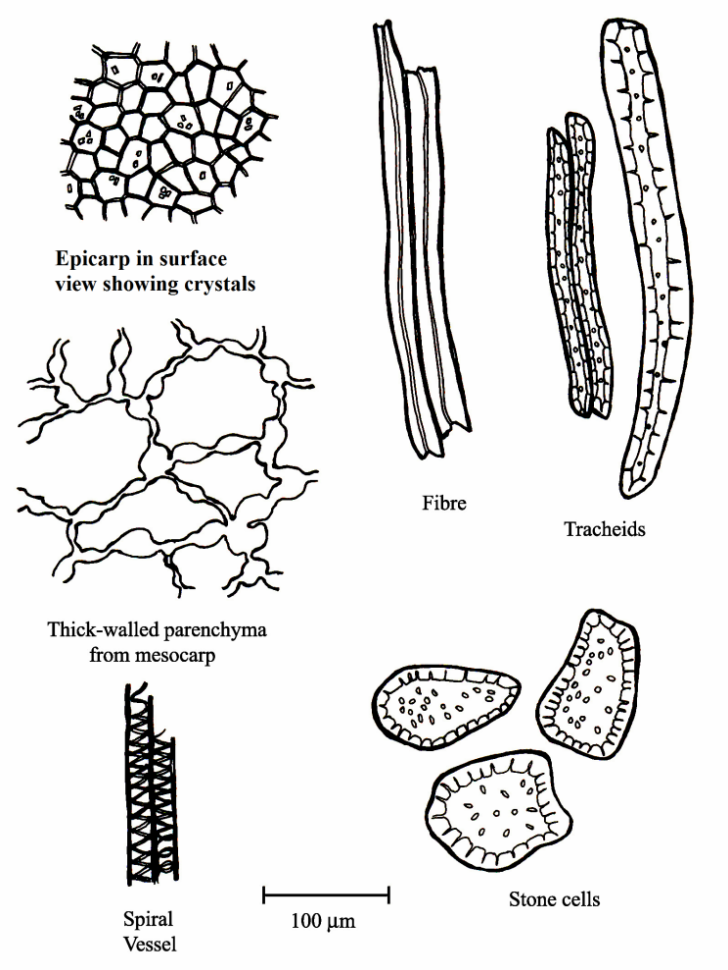


Fig.1. Powdered drug of Āmalakī (*Phyllanthus emblica* L.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 3.0 per cent, (Including seed and seed coat)	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total Ash:	Not more than 7.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 2.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 40.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 50.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig. 2).

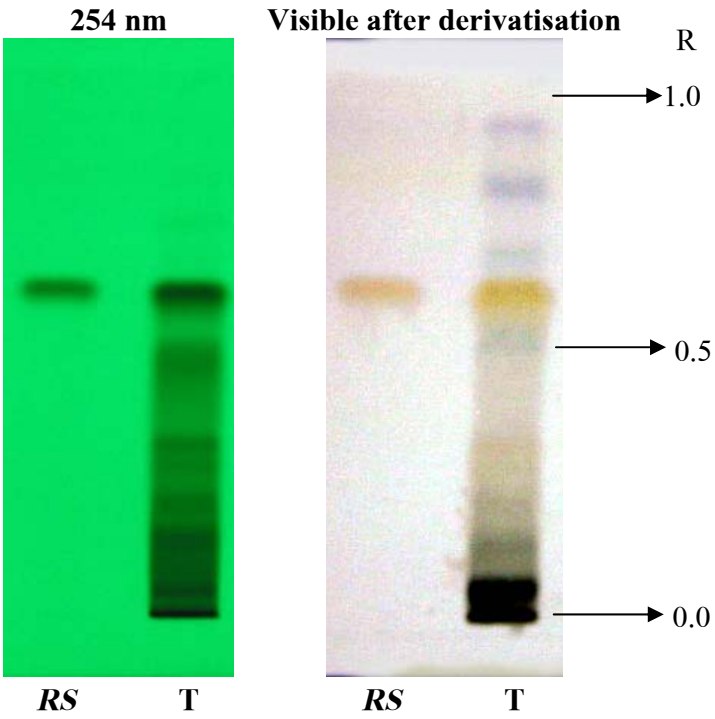


Fig.2. Thin-Layer Chromatogram of Āmalakī.
***RS*: *Gallic acid*, T: Test solution**

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water.* Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of *acetonitrile* and *water* containing 0.05 per cent of *glacial acetic acid* in the following proportions:-

Time (minutes)	*Water* containing 0.05 per cent acetic acid (per cent)	*Acetonitrile* (per cent)
0.01	100	0
10	80	20
25	78	22
30	60	40
35	100	0
38	100	0

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *gallic acid* in the substance being examined from the peak response of analyte and from the declared content of *gallic acid* in *gallic acid RS*.

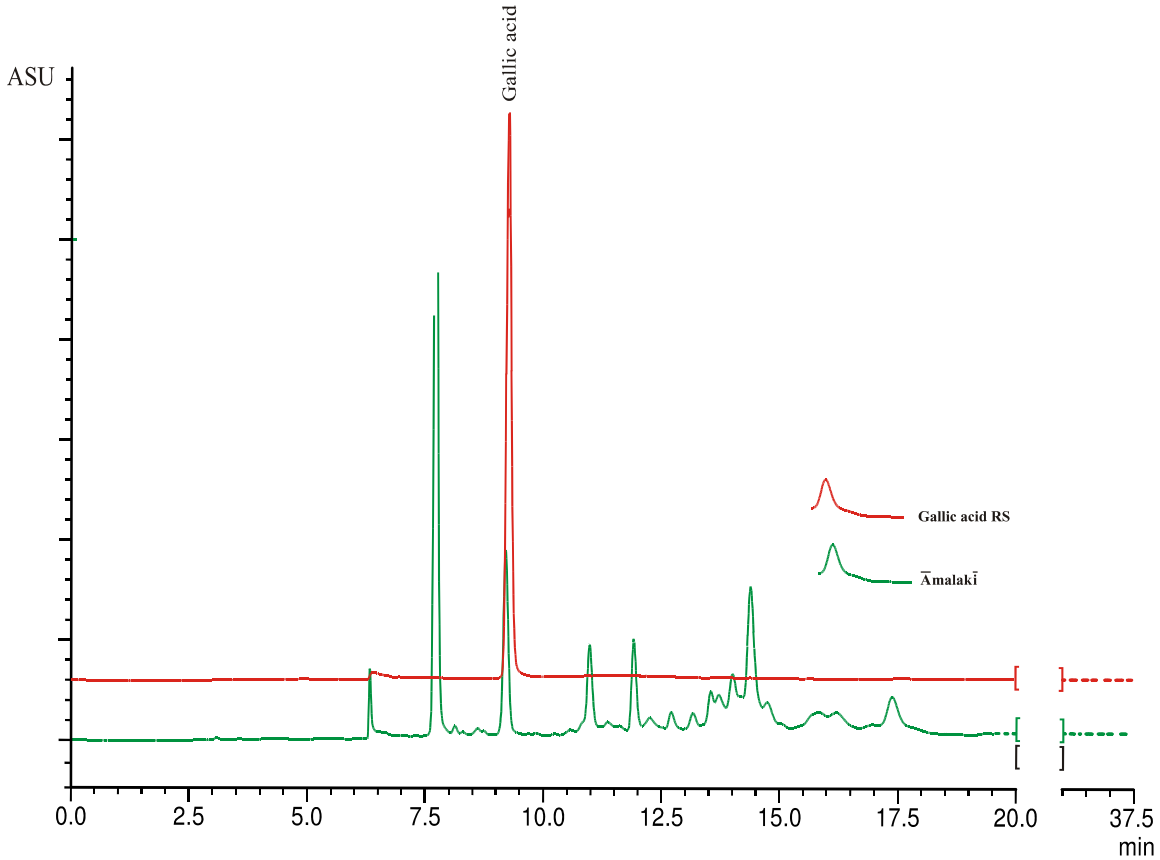


Fig.3. HPLC chromatogram of Āmalakī with *Gallic acid* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binominal and the part of the plant contained in the article.

API reference standards: API *Gallic acid RS.*

Constituents: Tannins, gallic acid, ellagic acid, phyllemblic acid, emblicol; alkaloids - phyllantidine and phyllantine; pectins; minerals.

Properties and Action:

Rasa	:	Amla, Kaṣāya, Madhura, Tikta, Kaṭu
Guṇa	:	Rūkṣa, Laghu
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Tridoṣajit, Vṛṣya, Rasāyana, Cakṣuṣya

Important formulations: Cyavanaprāśa; Dhātrī lauha; Dhātryādi ghṛta; Triphalā cūrṇa.

Therapeutic uses: Raktapitta (bleeding disorders); Amlapitta (hyperacidity); Prameha (increased frequency and turbidity of urine); Dāha (burning sensation).

Dose: 3 to 6 g of the drug in powder form.

ĀMALAKĪ (POWDER)

Āmalakī (Powder) consists of powder of Āmalakī complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, dark brown; odour characteristic; taste astringent and sour; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows thick-walled, rectangular or rounded stone cells and brachysclereids with pits; fragments of epicarp present, occasionally showing paracytic stomata; abundant thin-walled polygonal parenchymatous cells of the epicarp containing a few crystals of silica; fragments of simple pitted fibres; a few groups or isolated pitted vessels; fragments of thin irregular collenchymatous cells with corner thickening from mesocarp; cells containing simple rounded to oval starch grains measuring upto 8 μm.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Āmalakī.

Assay: Complies with the limits for Assay as per method stated under Āmalakī.

Additional requirements:

Packaging and Storage: As given under Āmalakī.

Labeling: As given under Āmalakī.

API reference standards: As given under Āmalakī.

ĀMALAKĪ HYDRO-ALCOHOLIC EXTRACT

Āmalakī Hydro-alcoholic Extract is a dried and powdered extract prepared from Āmalakī. The extract contains not less than 5.5 per cent of *gallic acid* when assayed.

Method of Preparation:

Take Āmalakī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 30.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig. 1).

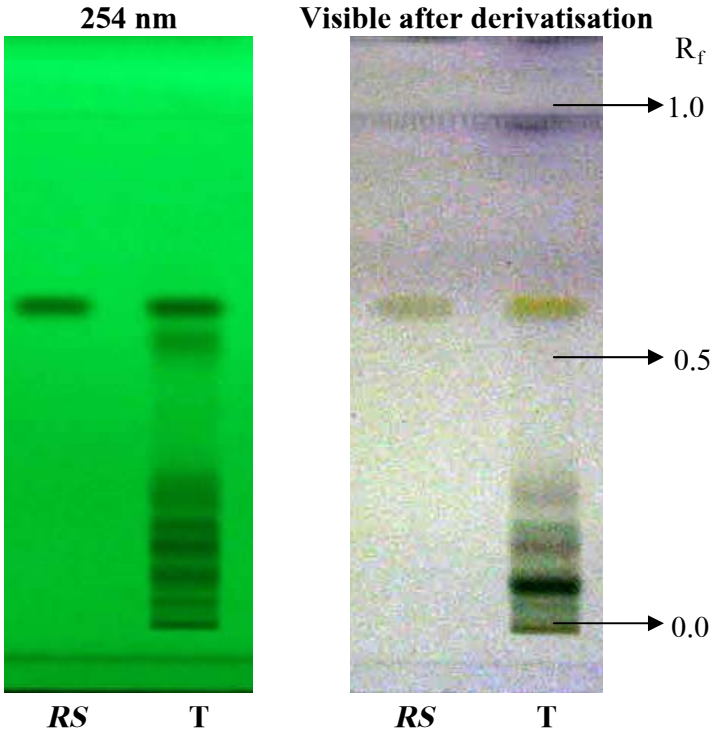


Fig.1. Thin-Layer Chromatogram of Āmalakī hydro-alcoholic extract. *RS* : *Gallic acid*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	3.0 - 4.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water.* Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of *acetonitrile* and *water* containing 0.05 per cent of *glacial acetic acid* in the following proportions:-

Time (minutes)	*Water* containing 0.05 percent acetic acid (per cent)	*Acetonitrile* (per cent)
0.01	100	0
10	80	20
25	78	22
30	60	40
35	100	0
38	100	0

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *gallic acid* in the substance being examined from the peak response of analyte and from the declared content of *gallic acid* in *gallic acid RS*.

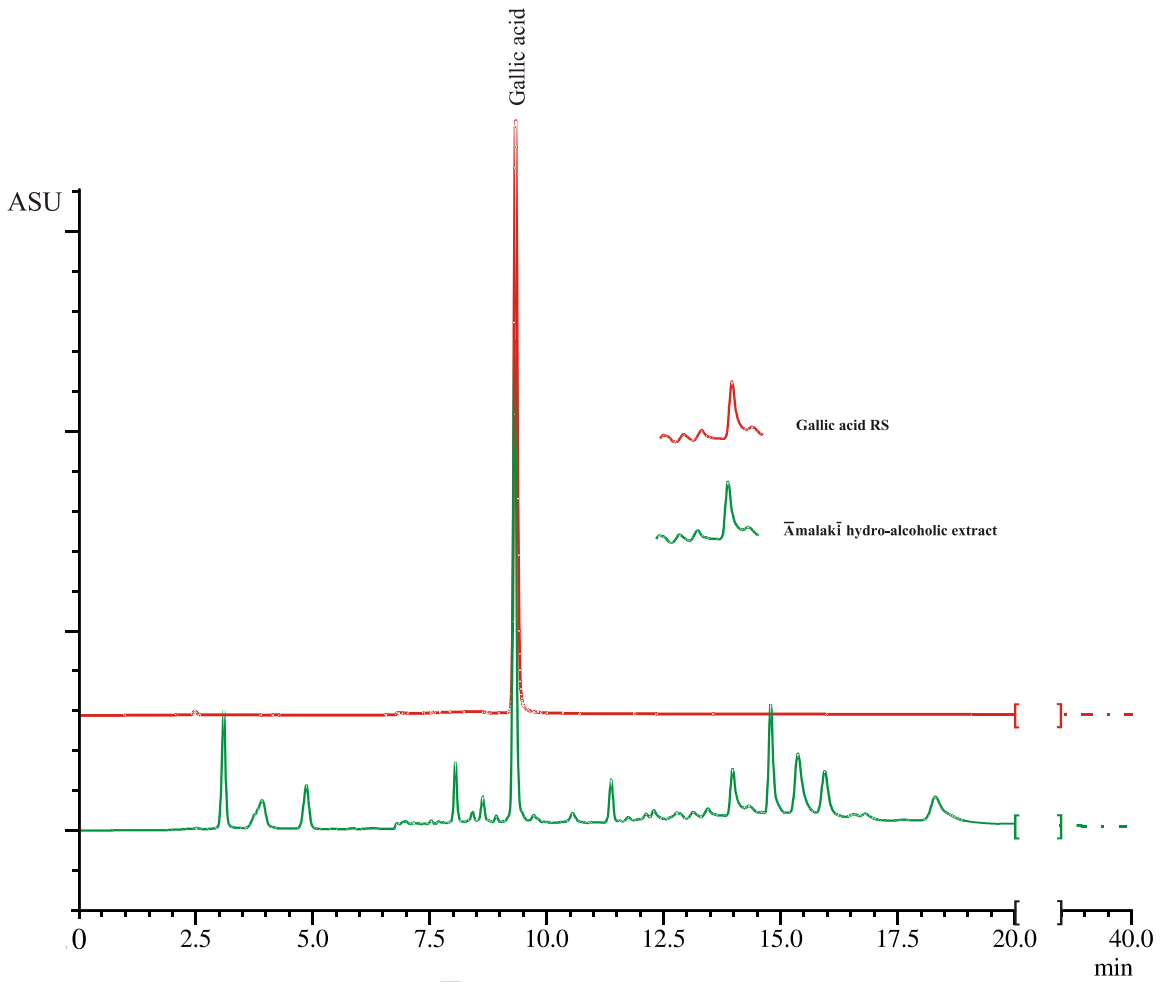


Fig.2. HPLC chromatogram of Āmalakī hydro-alcoholic extract with *Gallic acid* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS.*

ĀMALAKĪ WATER EXTRACT

Āmalakī Water Extract is a dried and powdered extract prepared from Āmalakī. The extract contains not less than 7.5 per cent of *gallic acid* when assayed.

Method of Preparation:

Take Āmalakī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 35.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig.1).

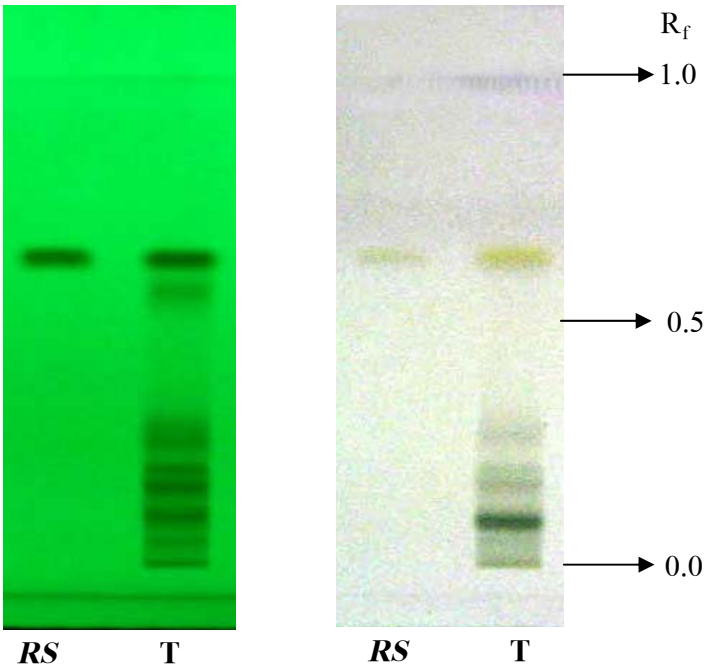


Fig.1. Thin-Layer Chromatogram of Āmalakī water extract. *RS*: *Gallic acid*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.25 per cent,	Appendix 2.1.7
pH:	Less than 3.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits,	Appendix 3.1
Microbial contamination:	Complies with the prescribed limits,	Appendix 3.2
Pesticide residues:	Complies with the prescribed limits,	Appendix 3.3
Aflatoxins:	Complies with the prescribed limits,	Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of *acetonitrile* and *water* containing 0.05 per cent of *glacial acetic acid* in the following proportions:-

Time (minutes)	*Water* containing 0.05 percent acetic acid (per cent)	*Acetonitrile* (per cent)
0.01	100	0
10	80	20
25	78	22
30	60	40
35	100	0
38	100	0

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *gallic acid* in the substance being examined from the peak response of analyte and from the declared content of *gallic acid* in *gallic acid RS*.

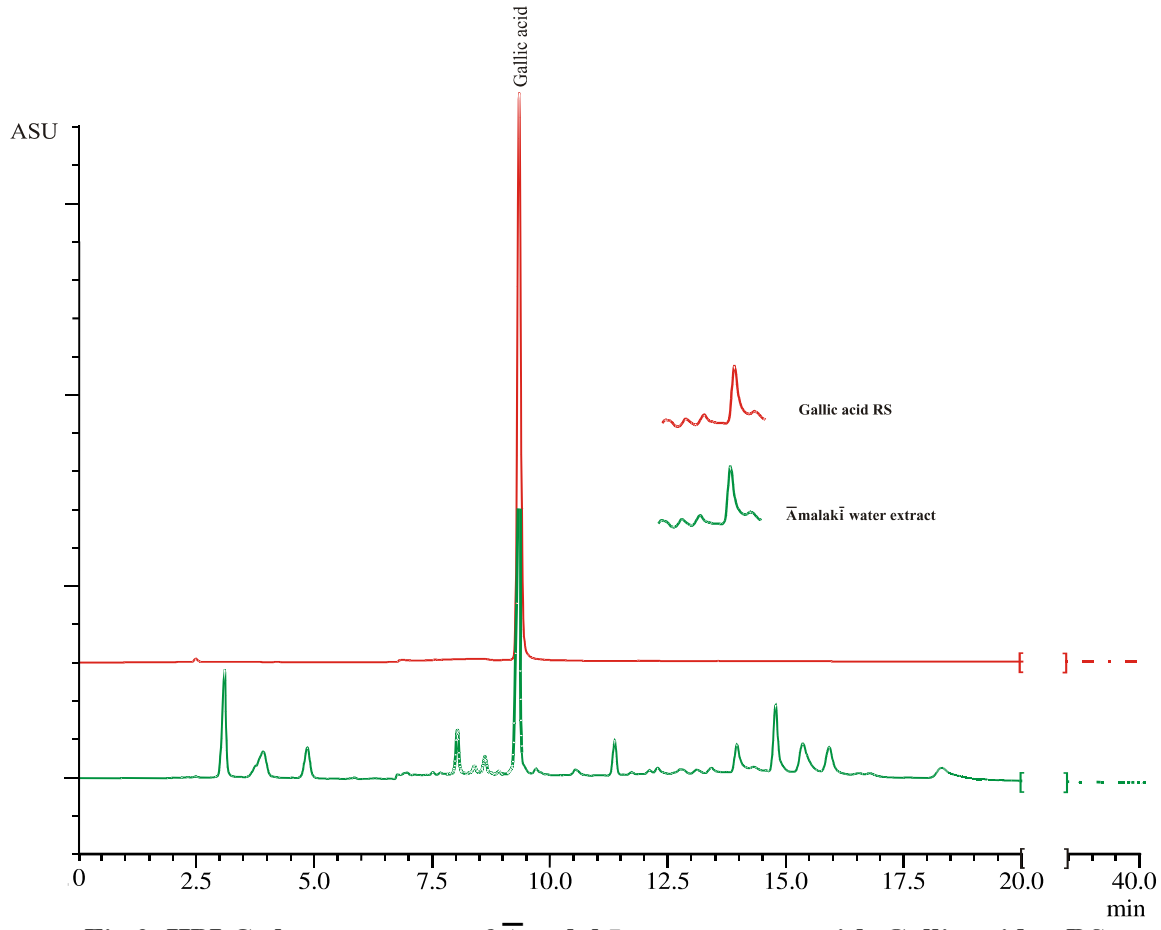


Fig.2. HPLC chromatogram of Āmalakī water extract with *Gallic acid* as RS

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light and moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS.*

ARJUNA

Arjuna consists of the stem bark of *Terminalia arjuna* W. & A. (Fam. Combretaceae); a large deciduous tree, commonly found throughout the greater parts of the country. Arjuna contains not less than 10.0 per cent of total polyphenols calculated as *pyrogallol* and not less than 0.1 per cent of total triterpenes (sum of *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* calculated as *arjungenin*) when assayed.

Synonyms: Kakubha, Pārtha, Śvetavāha

Regional Language Names:

Assamese	:	Arjun
Bengali	:	Arjuna
English	:	---
Gujarati	:	Sadad, Arjuna, Sajada
Hindi	:	Arjuna
Kannada	:	Matti, Bilimatti, Neermatti, Mathichakke, Kudare Kivimase
Kashmiri	:	---
Malayalam	:	Nirmasuthu, Vellamaruthi, Kellemasuthu, Mattimora, Torematti
Marathi	:	Arjuna, Sadada
Oriya	:	Arjuna
Punjabi	:	Arjon
Tamil	:	Marudam
Telugu	:	Maddi
Urdu	:	Arjun

Description:

a) *Macroscopic*

Bark available in pieces, flat, curved, recurved, channelled to half quilled, 0.2 to1.5 cm thick, market samples upto 10 cm in length and upto 7 cm in width, outer surface somewhat smooth and grey, inner surface somewhat fibrous and pinkish, transversely cut smoothened bark shows pinkish surface, fracture, short in inner and laminated in outer part; taste, bitter and astringent.

b) *Microscopic*

Stem Bark -Mature bark shows cork consisting of 9 to 10 layers of tangentially elongated cells, a few outer layers filled with brown colouring matter; cork cambium and secondary cortex not distinct and medullary rays observed traversing almost upto outer bark; secondary phloem occupies a wide zone, consisting of sieve tubes, companion cells, phloem parenchyma and phloem fibres, traversed by phloem rays, usually uniseriate but biseriate rays also occasionally seen; in the middle and outer phloem region, sieve tubes get collapsed and form ceratenchyma; phloem fibres distributed in rows and present in groups of 2 to10; rosette crystals of calcium oxalate measuring

80 to 180 μ in dia., present in most of the phloem parenchyma, alternating with fibres; idioblasts consisting of large cells having aggregates of prismatic and rhomboidal crystals of calcium oxalate in row throughout the zone, measuring 260 to 600 μ in dia., starch grains, mostly simple, compound of 2 to 3 components, sometimes upto 5 components, round to oval, elliptical, measuring 5 to 13 μ in dia., distributed throughout the tissue (absent in *T. alata*); in a tangential section the uniseriate phloem rays 2 to10 cells high and biseriate, 4 to 12 cells high; in longitudinal section rosette crystals of calcium oxalate found in the form of strands in phloem parenchyma.

c) *Powder*

Fine powder shows fragments of cork cells, uniseriate phloem rays, fibres, a number of rosette crystals of calcium oxalate, a few rhomboidal crystals, starch grains simple and compound, round to oval, elliptic, having 2 to 3 components with concentric striations and small narrow hilum, measuring 5 to 13 μ in diameter (Fig.1).

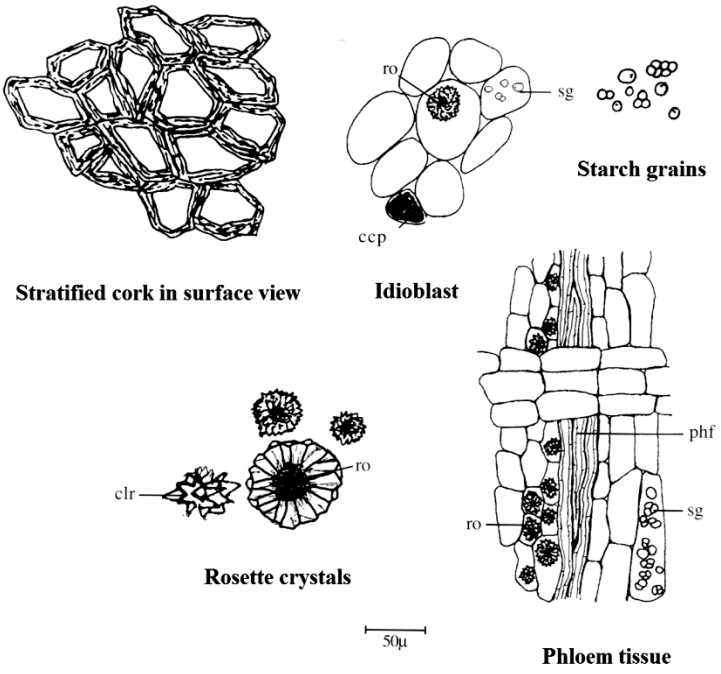


Fig.1. Powdered drug of Arjuna (*Terminalia arjuna* W. & A.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 25.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 20.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 20.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* as reference standards. Solvent system: *Ethyl acetate* : *toluene* : *formic acid* : *glacial acetic acid* (6.0 : 3.0 : 0.5 : 1.0). Test solution: To 3 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 1 mg each of *arjunic acid RS*, *arjungenin RS*, *arjunolic acid RS* and *arjunetin RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f~0.80 corresponding to that of *arjunic acid*, R_f ~0.65 corresponding to that of *arjunolic acid*, R_f ~0.60 corresponding to that of *arjungenin* and at R_f ~0.30 corresponding to *arjunetin* and the profile should be similar to the one given in the TLC (Fig. 2).

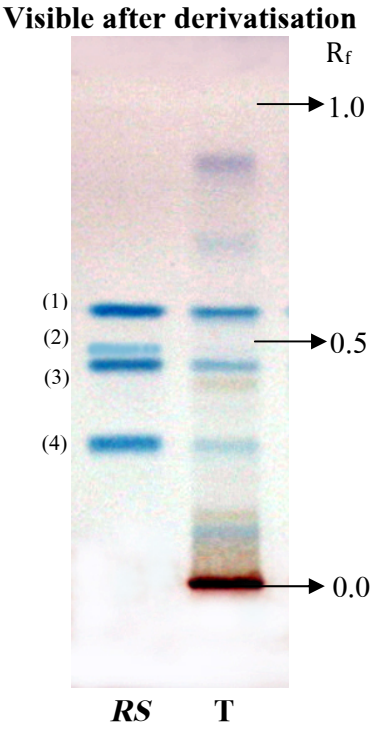


Fig.2. Thin-Layer Chromatogram of Arjuna. *RS*: (1) *Arjunic acid*, (2) *arjunolic acid*, (3) *arjungenin* and (4) *arjunetin,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay for total polyphenols:

Carry out the assay by *Spectrophotometry* (Appendix 3.7). Test solution: Always prepare fresh solution. Take about 100 mg, accurately weighed, of the substance being examined in a 250-ml conical flask, add 50 ml of 50.0 per cent *aqueous methanol* and reflux, cool and filter in a 100-ml volumetric flask. Reflux the residue further with 50 ml of 50.0 per cent *aqueous methanol* and filter to the same volumetric flask and make up the volume. Further dilute 5.0 ml aliquot to 50 ml with *water* in a volumetric flask. Standard solution: Take about 100 mg, accurately weighed, *pyrogallol RS* in a 100-ml volumetric flask and dissolve in 50.0 per cent *aqueous methanol* and

make up to 100 ml with 50.0 per cent *aqueous methanol*. Further dilute 5.0 ml to 50 ml with *water* in volumetric flask. Procedure: Pipette out 2, 5, 7 and 10 ml of standard solution and 5, 7 ml of test solution to different 100-ml volumetric flasks. Add 40 ml of *water*, 5 ml of *Folins reagent*, 10 ml of 30.0 per cent *sodium carbonate solution* in each of the volumetric flasks. Allow to stand for 30 minutes. Make up the volume with *water*, shake and allow to stand for 20 minutes more. Read in a suitable spectrophotometer at 750 nm using *water* as blank. Prepare a calibration curve from the values obtained. Calculate the content of polyphenols in the sample from the absorbance using the calibration curve.

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 5 g, accurately weighed, of the substance being examined and reflux with 100 ml *diethyl ether* on water bath for 15 minutes, cool and filter. Reflux the residue further with *diethyl ether* for two times more, cool and filter. Combine all the filtrates and evaporate to dryness under reduced pressure. Dissolve the residue in 10 ml of *methanol* in a volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *arjungenin RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions: -

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
18	40	60
20	15	85
22	15	85
25	70	30
30	70	30

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention time.

Analyte	**Relative retention time**
Arjunetin	0.67
Arjungenin	1.00
Arjunolic acid	1.50
Arjunic acid	1.70

Calculate the content of *arjunetin, arjungenin, arjunolic acid* and *arjunic acid* in the substance being examined as *arjungenin* from the peak response of analytes and from the declared content of *arjungenin RS*.

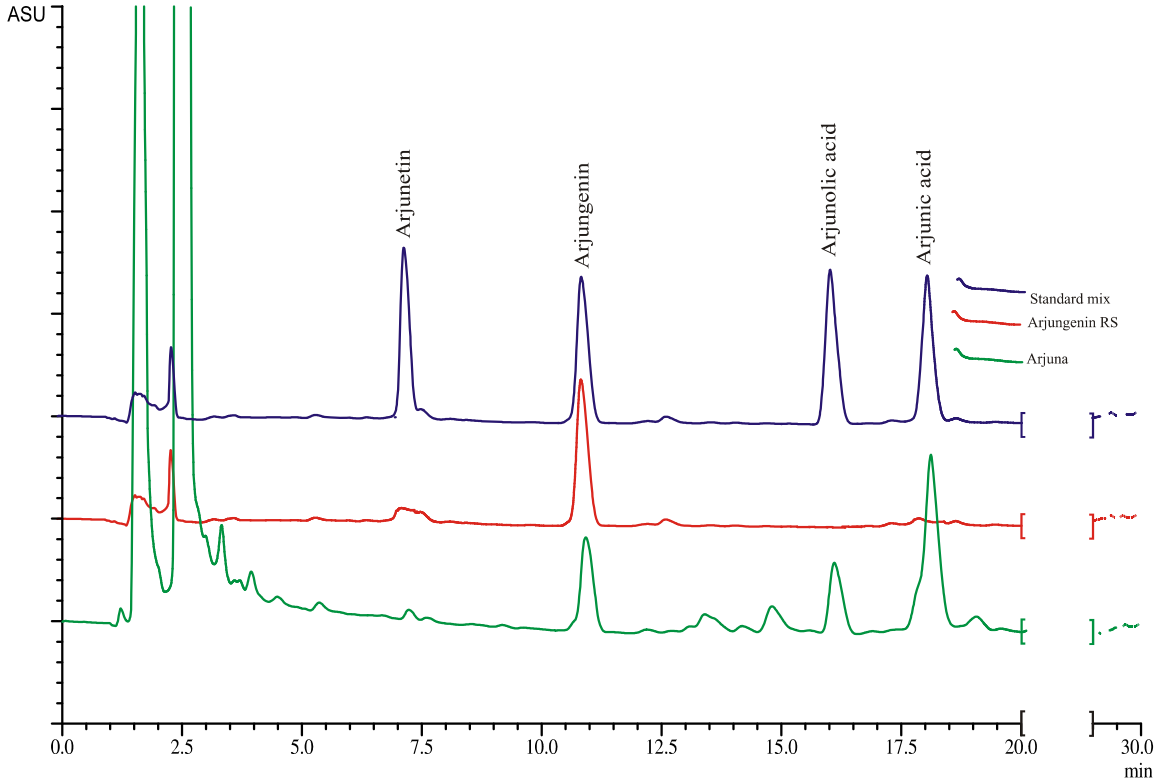


Fig.3. HPLC chromatograms of Arjuna with *Arjungenin* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binominal and the part of the plant contained in the article.

API reference standards: API *Arjungenin RS, Arjunolic acid RS, Arjunic acid RS* and *Arjunetin RS.*

Constituents: Tannins, arjunin, arjunic acid, arjunolic acid, arjunetin, arjunolitin, friedelin, terminoic acid, arjungenin and arjunglucosides.

Properties and Action:

Rasa : Kaṣāya
Guṇa : Rūkṣa
Vīrya : Śīta
Vipāka : Kaṭu
Karma : Kaphahara, Pittahara, Hṛdya, Vraṇanāśana, Bhagnasandhānakara, Vyaṅgahara

Important formulations: Pārthādyariṣṭa, Nāgārjunābhra rasa, Arjuna ghṛta.

Therapeutic uses: Hṛdroga (heart disease); Kṣatakṣaya (emaciation due to injury); Medoroga (obesity); Prameha (increased frequency and turbidity of urine); Vraṇa (ulcer); Tṛṣā (thirst); Vyaṅga (dark shade on face due to stress and excessive exercise / localized hyper pigmentation of skin).

Dose: 3 to 6 g of the drug in powder form.

ARJUNA (POWDER)

Arjuna (Powder) consists of powder of Arjuna complying with the following requirements:

Description:

(a) *Macroscopic:*

Coarse powder, brownish pink; odour characteristic; tasteless; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

(b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*; treat a few mg of powder with ferric chloride solution and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of stratified cork consisting of tangential thin walled cells in sectional view or polygonal cells in surface view; fragments of cortical parenchymatous cells and phloem tissue debris with idioblasts containing cluster and rosette crystals of calcium oxalate; starch grains measuring up to 12μ; fragment of cells containing tannins and dark reddish brown pigment.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Arjuna.

Assay: Complies with the limits for Assay as per method stated under Arjuna.

Additional requirements:

Packaging and Storage: As given under Arjuna.

Labeling: As given under Arjuna.

API reference standards: As given under Arjuna.

ARJUNA HYDRO-ALCOHOLIC EXTRACT

Arjuna Hydro-alcoholic Extract is a dried and powdered extract prepared from Arjuna. The extract contains not less than 30.0 per cent of total polyphenols calculated as *pyrogallol* and not less than 0.1 per cent of total triterpenes (sum of *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* calculated as *arjungenin*) when assayed.

Method of Preparation:

Take Arjuna suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* as reference standards. Solvent system: *Ethyl acetate* : *toluene* : *formic acid* : *glacial acetic acid* (6.0 : 3.0 : 0.5 : 1.0). Test solution: To 3 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 1 mg each of *arjunic acid RS*, *arjungenin RS*, *arjunolic acid RS* and *arjunetin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.80 corresponding to that of *arjunic acid*, at R_f ~0.60 corresponding to that of *arjungenin* and the profile should be similar to the one given in the TLC (Fig. 1).

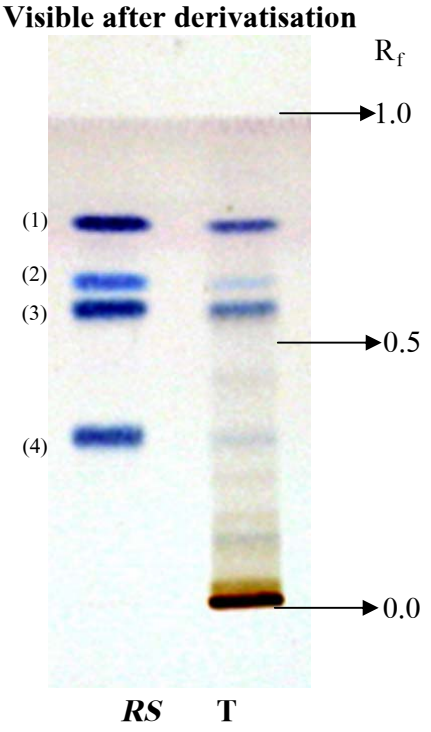


Fig.1. Thin-Layer Chromatogram of Arjuna hydro-alcoholic extract. *RS*: (1) *Arjunic acid*, (2) *arjunolic acid,* (3) *arjungenin* and (4) *arjunetin,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 5.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 0.5 per cent,	Appendix 2.1.7
pH:	4.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay for total polyphenols:

Carry out the assay by *Spectrophotometry* (Appendix 3.7). Test solution: Always prepare fresh solution. Take about 100 mg, accurately weighed, of the substance being examined in a 250-ml conical flask, add 50 ml of 50.0 per cent *aqueous methanol* and reflux, cool and filter in a 100-ml volumetric flask. Reflux the residue further with 50 ml of 50.0 per cent *aqueous methanol* and filter to the same volumetric flask and make up the volume. Further dilute 5.0 ml aliquot to 50 ml with *water* in a volumetric flask. Standard solution: Take about 100 mg, accurately weighed, *pyrogallol RS* in a 100-ml volumetric flask and dissolve in 50.0 per cent *aqueous methanol* and make up to 100-ml with 50.0 per cent *aqueous methanol*. Further dilute 5.0 ml to 50 ml with *water* in a volumetric flask. Procedure: Pipette out 2, 5, 7 and 10 ml of standard solution and 5, 7 ml of test solution to different 100-ml volumetric flasks. Add 40 ml of *water*, 5 ml of *Folins reagent*, 10 ml of 30.0 per cent *sodium carbonate solution* to each of the volumetric flasks. Allow to stand for 30 minutes. Make up the volume with *water*, shake and allow to stand for 20 minutes more. Read in a suitable spectrophotometer at 750 nm using *water* as blank. Prepare a calibration curve from the values obtained. Calculate the content of polyphenols in the sample from the absorbance using the calibration curve.

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 5 g, accurately weighed, of the substance being examined and reflux with 100 ml *diethyl ether* on water bath for 15 minutes, cool and filter. Reflux the residue further with *diethyl ether* for two times more, cool and filter. Combine all the filtrates and evaporate to dryness under reduced pressure. Dissolve the residue in 10 ml of *methanol* in a volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *arjungenin RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of

orthophosphoric acid and making up the volume to 1000 ml) and *acetonitrile* in the following proportions: -

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
18	40	60
20	15	85
22	15	85
25	70	30
30	70	30

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention time.

Analyte	**Relative retention time**
Arjunetin	0.67
Arjungenin	1.00
Arjunolic acid	1.50
Arjunic acid	1.70

Calculate the content of *arjunetin, arjungenin, arjunolic acid* and *arjunic acid* in the substance being examined as *arjungenin* from the peak response of analytes and from the declared content of *arjungenin RS*.

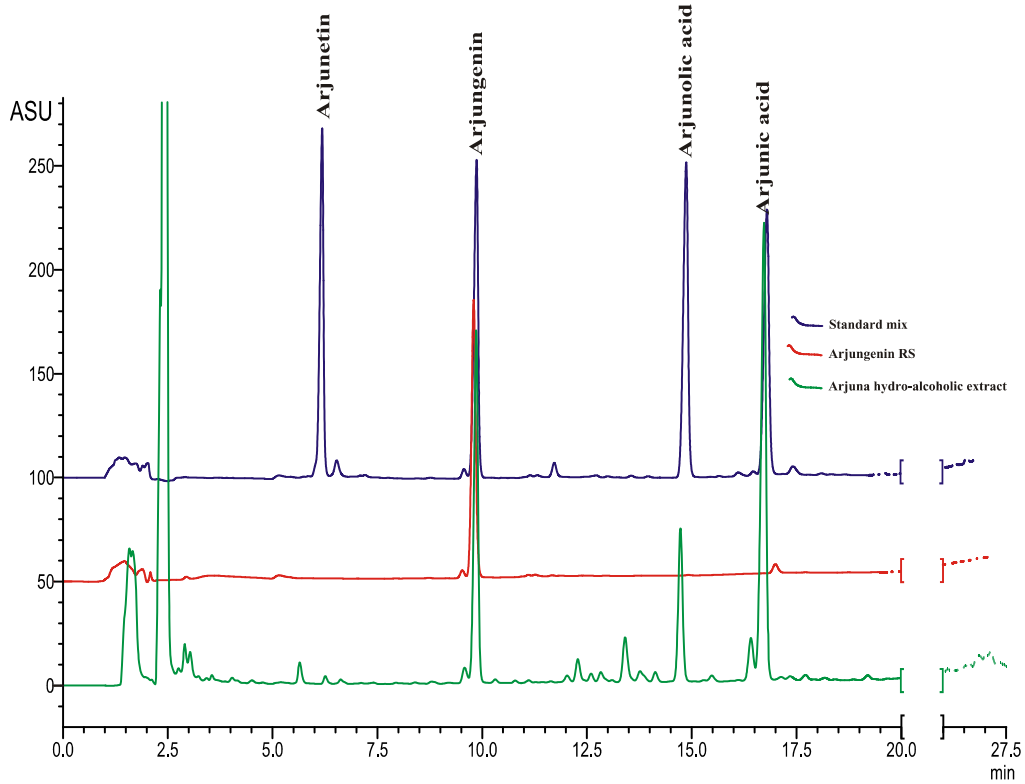


Fig.2. HPLC chromatograms of Arjuna hydro-alcoholic extract with *Arjungenin* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Arjungenin RS, Arjunolic acid RS, Arjunic acid RS* and *Arjunetin RS.*

ARJUNA WATER EXTRACT

Arjuna Water Extract is a dried and powdered extract prepared from Arjuna. The extract contains not less than 30.0 per cent of total polyphenols calculated as *pyrogallol and* not less than 0.05 per cent of total triterpenes (sum of *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin*) calculated as *arjungenin* when assayed.

Method of Preparation:

Take Arjuna suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 20.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *arjunic acid*, *arjungenin*, *arjunolic acid* and *arjunetin* as reference standards. Solvent system: *Ethyl acetate* : *toluene* : *formic acid* : *glacial acetic acid* (6.0 : 3.0 : 0.5 : 1.0). Test solution: To 3 g of the substance being examined, add 50 ml of *methanol* heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 1 mg each of *arjunic acid RS*, *arjungenin RS*, *arjunolic acid RS* and *arjunetin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f~0.80 corresponding to that of *arjunic acid*, at R_f ~0.60 corresponding to that of *arjungenin* and the profile should be similar to the one given in the TLC (Fig. 1).

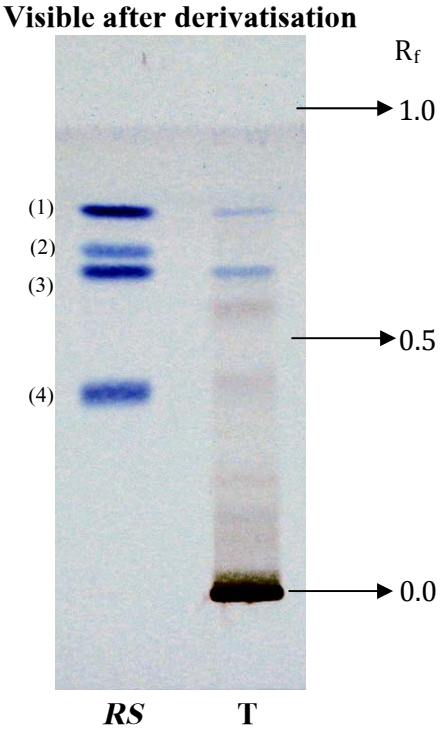


Fig.1. Thin-Layer Chromatogram of Arjuna water extract. *RS*: (1) *Arjunic acid*, (2) *arjunolic acid*, (3) *arjungenin* and (4) *arjunetin,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 7.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 0.5 per cent,	Appendix 2.1.7
pH:	4.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay for total polyphenols:

Carry out the assay by *Spectrophotometry* (Appendix 3.7). Test solution: Always prepare fresh solution. Take about 100 mg, accurately weighed, of the substance being examined in a 250 ml conical flask, add 50 ml of 50.0 per cent *aqueous methanol* and reflux, cool and filter in a 100-ml volumetric flask. Reflux the residue further with 50 ml of 50.0 per cent *aqueous methanol* and filter to the same volumetric flask and make up the volume. Further dilute 5.0 ml aliquot to 50 ml with *water* in a volumetric flask. Standard solution: Take about 100 mg, accurately weighed, *pyrogallol RS* in a 100-ml volumetric flask and dissolve in 50.0 per cent *aqueous methanol* and make up to 100-ml with 50.0 per cent *aqueous methanol*. Further dilute 5.0 ml to 50 ml with *water* in a volumetric flask. Procedure: Pipette out 2, 5, 7 and 10 ml of standard solution and 5, 7 ml of test solution to different 100-ml volumetric flasks. Add 40 ml of *water*, 5 ml of *Folins reagent*, 10 ml of 30.0 per cent *sodium carbonate solution* in each of the volumetric flasks. Allow to stand for 30 minutes. Make up the volume with *water*, shake and allow to stand for 20 minutes more. Read in a suitable spectrophotometer at 750 nm using *water* as blank. Prepare a calibration curve. Calculate the content of polyphenols in the sample from the absorbance using the calibration curve.

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 7 g, accurately weighed, of the substance being examined and reflux with 100 ml *diethyl ether* on water bath for 15 minutes, cool and filter. Reflux the residue further with *diethyl ether* for two times more, cool and filter. Combine all the filtrates and evaporate to dryness under reduced pressure. Dissolve the residue in 10 ml of *methanol* in a volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *arjungenin RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions: -

Time (minutes)	Phosphate Buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
18	40	60
20	15	85
22	15	85
25	70	30
30	70	30

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention time.

Analyte	**Relative retention time**
Arjunetin	0.67
Arjungenin	1.00
Arjunolic acid	1.50
Arjunic acid	1.70

Calculate the content of *arjunetin, arjungenin, arjunolic acid* and *arjunic acid* in the substance being examined as *arjungenin* from the peak response of analytes and from the declared content of *arjungenin RS*.

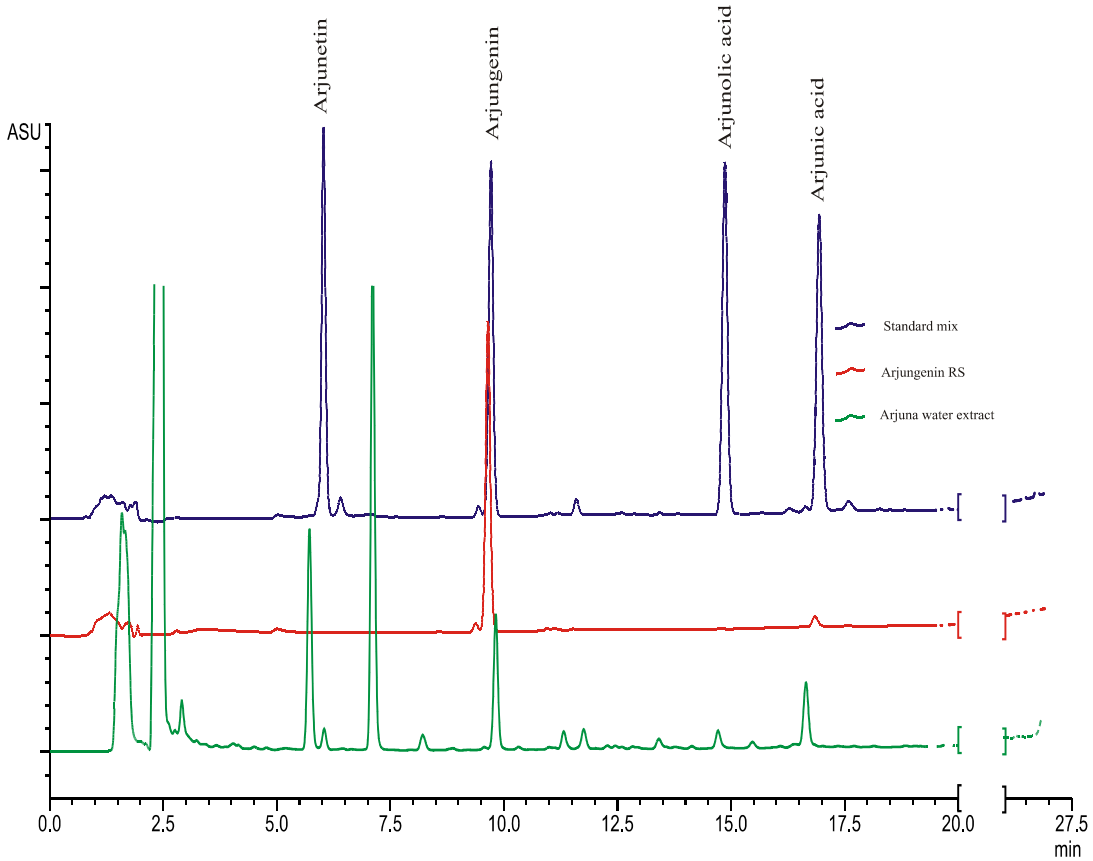


Fig.2. HPLC chromatograms of Arjuna water extract with *Arjungenin* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Arjungenin RS, Arjunolic acid RS, Arjunic acid RS* and *Arjunetin RS.*

AŚVAGANDHĀ

Aśvagandhā consists of dried mature roots of *Withania somnifera* (L.) Dunal. (Fam. Solanaceae), a perennial shrub, found in waste lands, cultivated fields and open grounds throughout India; widely cultivated in certain areas of Madhya Pradesh and Rajasthan; roots collected in winter, washed and cut into short pieces. Aśvagandhā contains not less than 0.15 per cent of total withanolides when assayed.

Synonyms: Hayagandhā, Vājigandhā

Regional Language Names:

Assamese	:	Ashvagandha
Bengali	:	Ashvagandha
Gujarati	:	Asgandha
Hindi	:	Asgandh
Kannada	:	Angarberu, Hiremaddina-gida
Kashmiri	:	Asagandh
Malayalam	:	Amukkuram
Marathi	:	Asagandha, Askagandha
Oriya	:	Aswagandha
Punjabi	:	Asgandh
Tamil	:	Amukkaramkizangu
Telugu	:	Pennerugadda
Urdu	:	Asgand

Description:

a) *Macroscopic:*

Roots straight, unbranched, thickness varying with age, roots bear fibre-like secondary roots, outer surface buff to greyish-yellow with longitudinal wrinkles; crown shows the remains of variously thickened stem bases; fracture, short and uneven; odour, characteristic; taste, bitter and acrid.

b) *Microscopic:*

TS of root shows exfoliated or crushed cork; cells, isodiametric and non-lignified; cork cambium of 2 to 4 diffused rows of cells; cortex about twenty layers of compact parenchymatous cells; phloem consists of sieve tubes, companion cells, phloem parenchyma; cambium 4 or 5 rows of tangentially elongated cells; secondary xylem forming a closed vascular ring separated by multiseriate medullary rays; xylem parenchyma few; pith absent; numerous simple, oval to rounded starch grains with hilum in centre, measuring up to 15 μ in diameter and several compound grains having 2 or 3 components found in secondary cortex and phloem; crystals absent.

c) *Powder:*

Fine powder shows fragments of parenchyma cells with starch grains; vessel scalariform, pitted: numerous simple, oval to rounded starch grains containing hilum in centre, measuring up to 15 μ in diameter and several compound grains having 2 or 3 components (Fig.1).

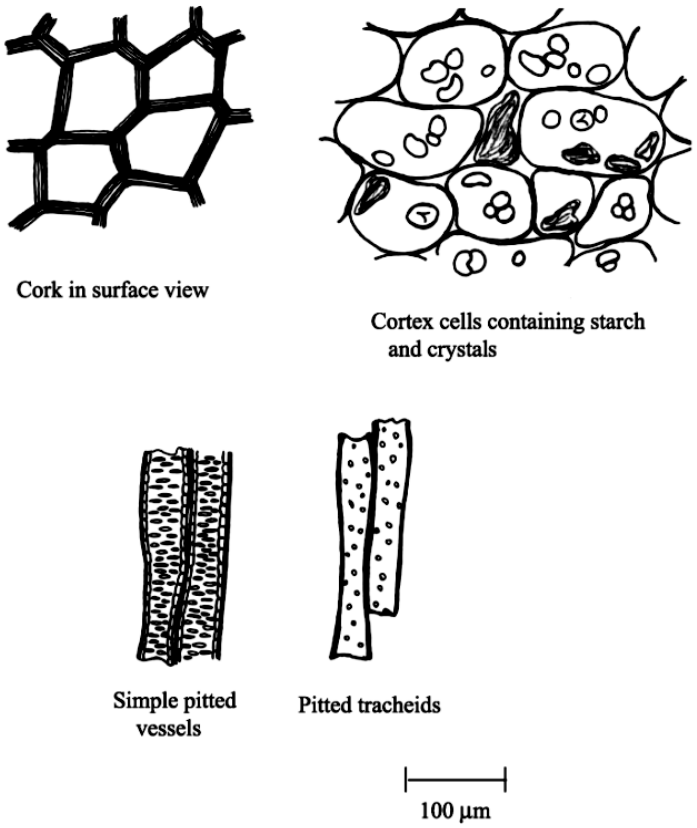


Fig.1. Powdered drug of Aśvagandhā (*Withania somnifera* (L.) Dunal**)**

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 7.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 15.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 7.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *withaferin A* as a reference standard. Solvent system: *Chloroform : methanol* (9.0 : 1.0). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for

10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *withaferin A* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *withaferin A* and the profile should be similar to the one given in the TLC (Fig. 2).

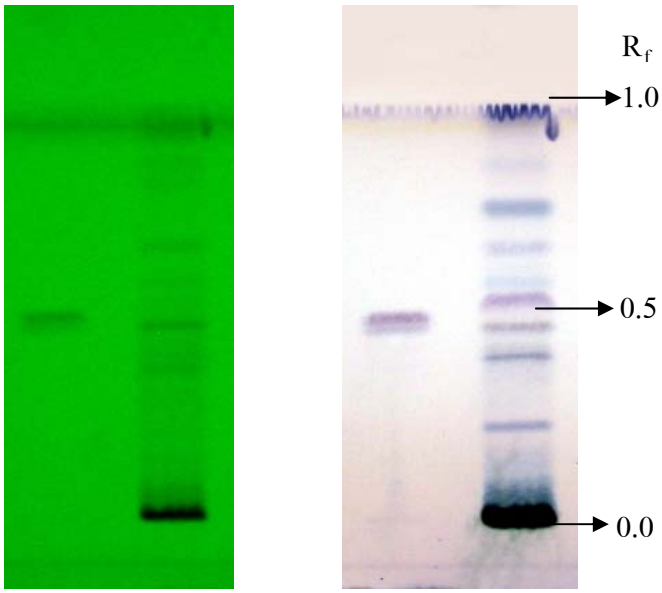


Fig.2. Thin-Layer Chromatogram of Aśvagandhā. *RS*: *Withaferin A*, T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *withanoside IV RS* and *withanolide A RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 227 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Withanolide glycosides	
Withanoside IV	0.70
Withanoside V & VI	0.89
Withanolide aglycones	
12- Deoxywithastramonolide	0.96
Withanolide A	1.00
Withanolide B	1.14

Calculate the content of *withanolide glycosides* in the sample as *withanoside IV* by summing the peak areas of *withanoside IV, V* and *VI* and from the declared content of *withanoside IV* in *withanoside IV RS.* Calculate the content of *withanolide aglycones* in the sample as *withanolide A* by summing the peak areas of *12-deoxywithastramonolide, withanolide A* and *withanolide B* and from the declared content of *withanolide A* in *withanolide A RS.* Sum the content of *withanolide glycosides* and *withanolide aglycones* to get total withanolides.

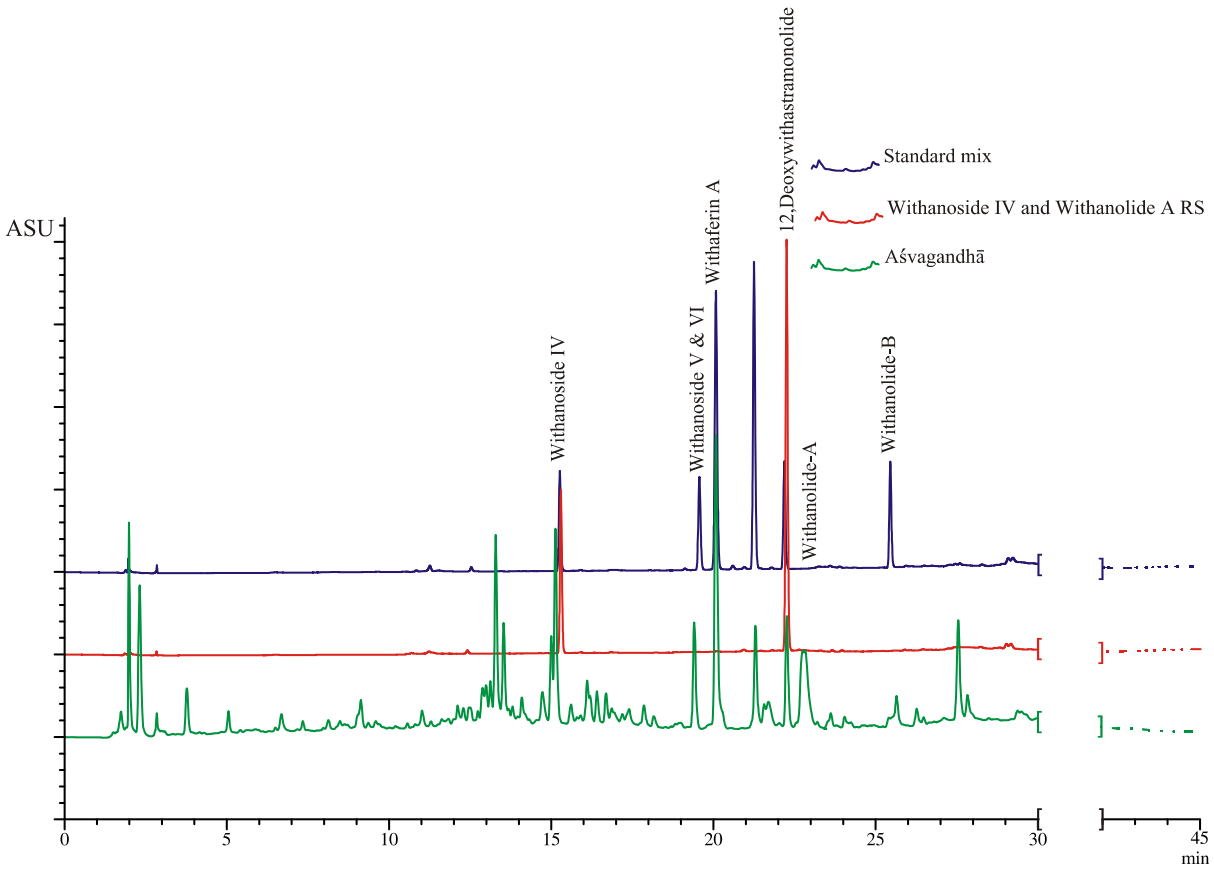


Fig.3. HPLC chromatograms of Aśvagandhā with *Withanoside IV*, *Withanolide A* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Withaferin A RS*, *Withanoside IV RS* and *Withanolide A RS*.

Constituents: Alkaloids, withanone, withaferin A, withanolides and withanosides.

Properties and Action:

Rasa	:	Tikta, Kaṣāya
Guṇa	:	Laghu
Vīrya	:	Uṣṇa
Vipāka	:	Madhura
Karma	:	Vātakaphapaha, Balya, Rasāyana, Vajikarana

Important formulations: Aśvangandhādyariṣṭa; Aśvangandhādi leha; Balāśvangandhā lākṣādi taila.

Therapeutic uses: Kṣaya (pthisis); Daurbalya (weakness); Vātaroga (diseases due to vāta doṣa); Śotha (inflammation); Klaibya (male impotence).

Dose: 3 to 6 g of the drug in powder form.

AŚVAGANDHĀ (POWDER)

Aśvagandhā (Powder) consists of powder of Aśvagandhā complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, creamish yellow, odour characteristic; taste bitter and acrid; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of cork with cubical to slightly tangentially elongated cells; starch grains generally simple, rounded, measuring up to 9μ, rarely compound starch grains with 2 or 3 components; detached components muller shaped; parenchymatous cells from cortex, xylem and medullary rays contain microsphenoidal crystals of calcium oxalate; debris from vascular elements present, showing short vessels with various kinds of thickenings on their walls.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Aśvagandhā.

Assay: Complies with the limits for Assay as per method stated under Aśvagandhā.

Additional requirements:

Packaging and Storage: As given under Aśvagandhā.

Labeling: As given under Aśvagandhā.

API reference standards: As given under Aśvagandhā.

AŚVAGANDHĀ HYDRO-ALCOHOLIC EXTRACT

Aśvagandhā Hydro-alcoholic Extract is a dried and powdered extract prepared from Aśvagandhā. The extract contains not less than 1.0 per cent of total withanolides when assayed.

Method of preparation:

Take Aśvagandhā suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 16.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *withaferin A* as a reference standard. Solvent system: *Chloroform* : *methanol* (9.0 : 1.0). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *withaferin A* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *withaferin A* and the profile should be similar to the one given in the TLC (Fig. 1).

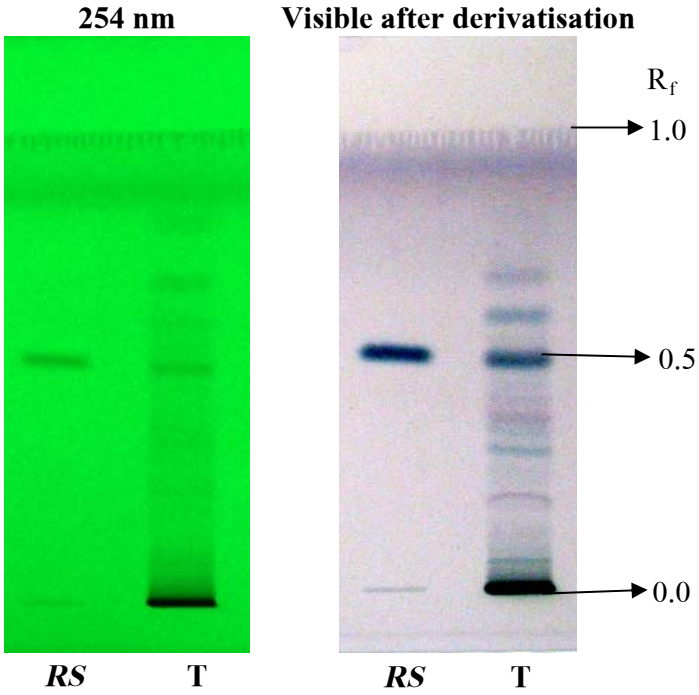


Fig.1. Thin-Layer Chromatogram of Aśvagandhā hydro-alcoholic extract
***RS*: *Withaferin A*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 16.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 2.0 per cent,	Appendix 2.1.7
pH:	4.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *withanoside IV RS* and *withanolide A RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 227 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Withanolide glycosides	
Withanoside IV	0.70
Withanoside V & VI	0.89
Withanolide aglycones	
12-Deoxywithastramonolide	0.96
Withanolide A	1.00
Withanolide B	1.14

Calculate the content of *withanolide glycosides* in the sample as *withanoside IV* by summing the peak areas of *withanoside IV, V* and *VI* and from the declared content of *withanoside IV* in *withanoside IV RS.* Calculate the content of *withanolide aglycones* in the sample as *withanolide A* by summing the peak areas of *12, deoxywithastramonolide, withanolide A* and *withanolide B* and from the declared content of *withanolide A* in *withanolide A RS.* Sum the content of *withanolide glycosides* and *withanolide aglycones* to get total withanolides.

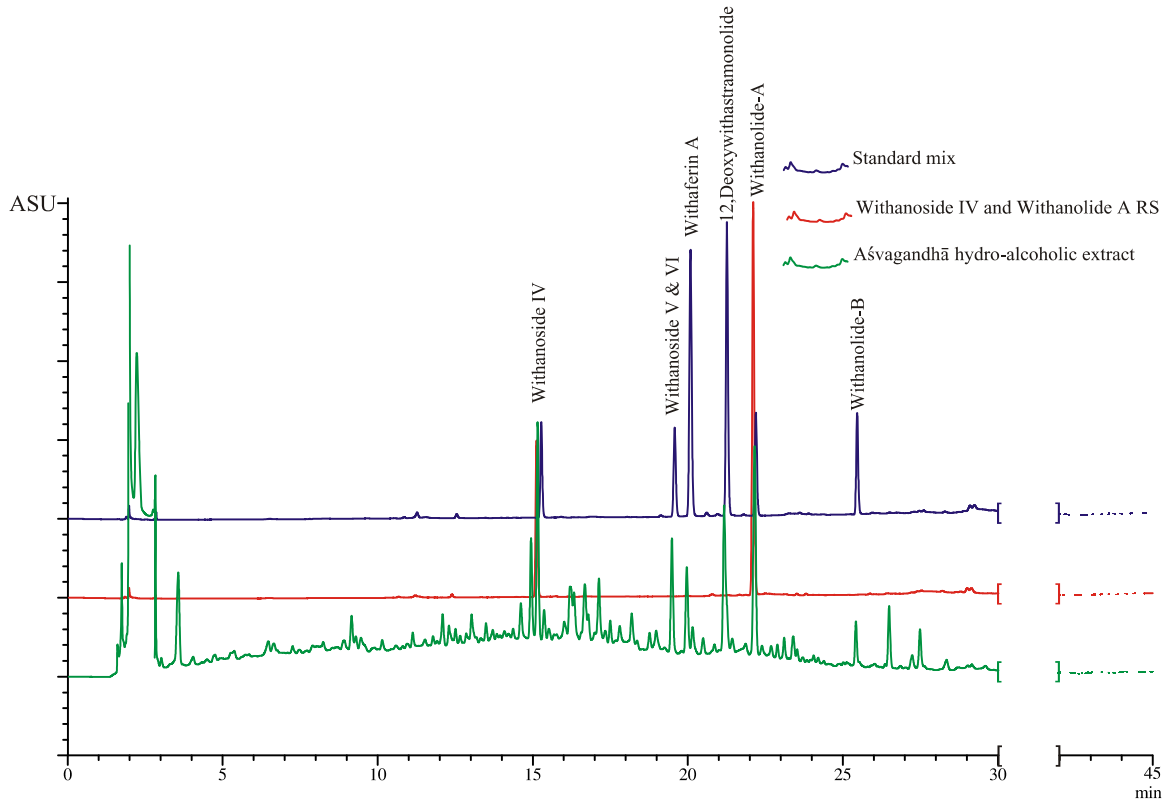


Fig.2. HPLC chromatograms of Aśvagandhā hydro-alcoholic extract with *Withanoside IV*, *Withanolide A* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Withaferin A RS*, *Withanoside IV RS* and *Withanolide A RS*.

AŚVAGANDHĀ WATER EXTRACT

Aśvagandhā Water Extract is a dried and powdered extract prepared from Aśvagandhā. The extract should contain not less than 0.3 per cent of total withanolides when assayed.

Method of preparation:

Take Aśvagandhā suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *withaferin A* as a reference standard. Solvent system: *Chloroform* : *methanol* (9.0 : 1.0). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *withaferin A* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *withaferin A* and the profile should be similar to the one given in the TLC (Fig. 1).

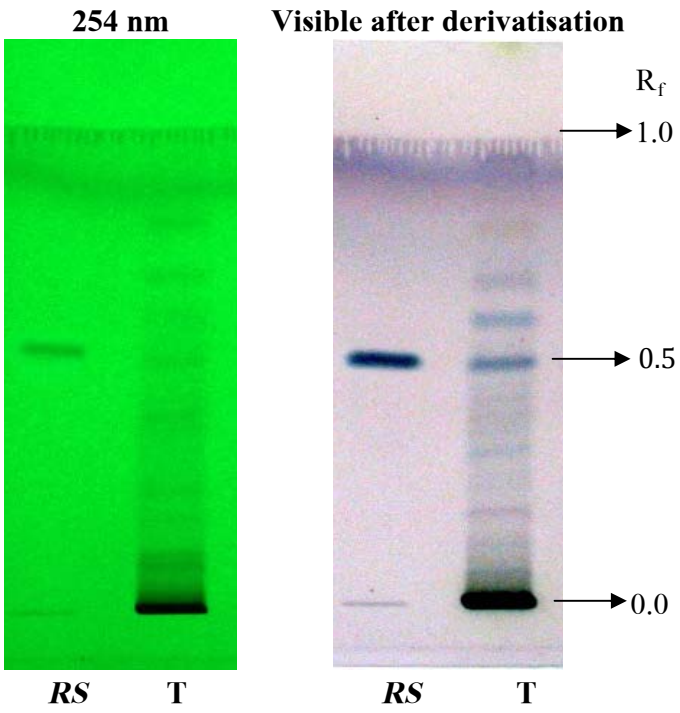


Fig.1. Thin-Layer Chromatogram of Aśvagandhā water extract. *RS*: *Withaferin A*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 12.5 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 2.0 per cent,	Appendix 2.1.7
pH:	4.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *withanoside IV RS* and *withanolide A RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 227 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Withanolide glycosides	
Withanoside IV	0.70
Withanoside V & VI	0.89

Withanolide aglycones	
12-Deoxywithastramonolide	0.96
Withanolide A	1.00
Withanolide B	1.14

Calculate the content of *withanolide glycosides* in the sample as *withanoside IV* by summing the peak areas of *withanoside IV, V* and *VI* and from the declared content of *withanoside IV* in *withanoside IV RS.* Calculate the content of *withanolide aglycones* in the sample as *withanolide A* by summing the peak areas of *12, deoxywithastramonolide, withanolide A* and *withanolide B* and from the declared content of *withanolide A* in *withanolide A RS.* Sum the content of *withanolide glycosides* and *withanolide aglycones* to get total withanolides.

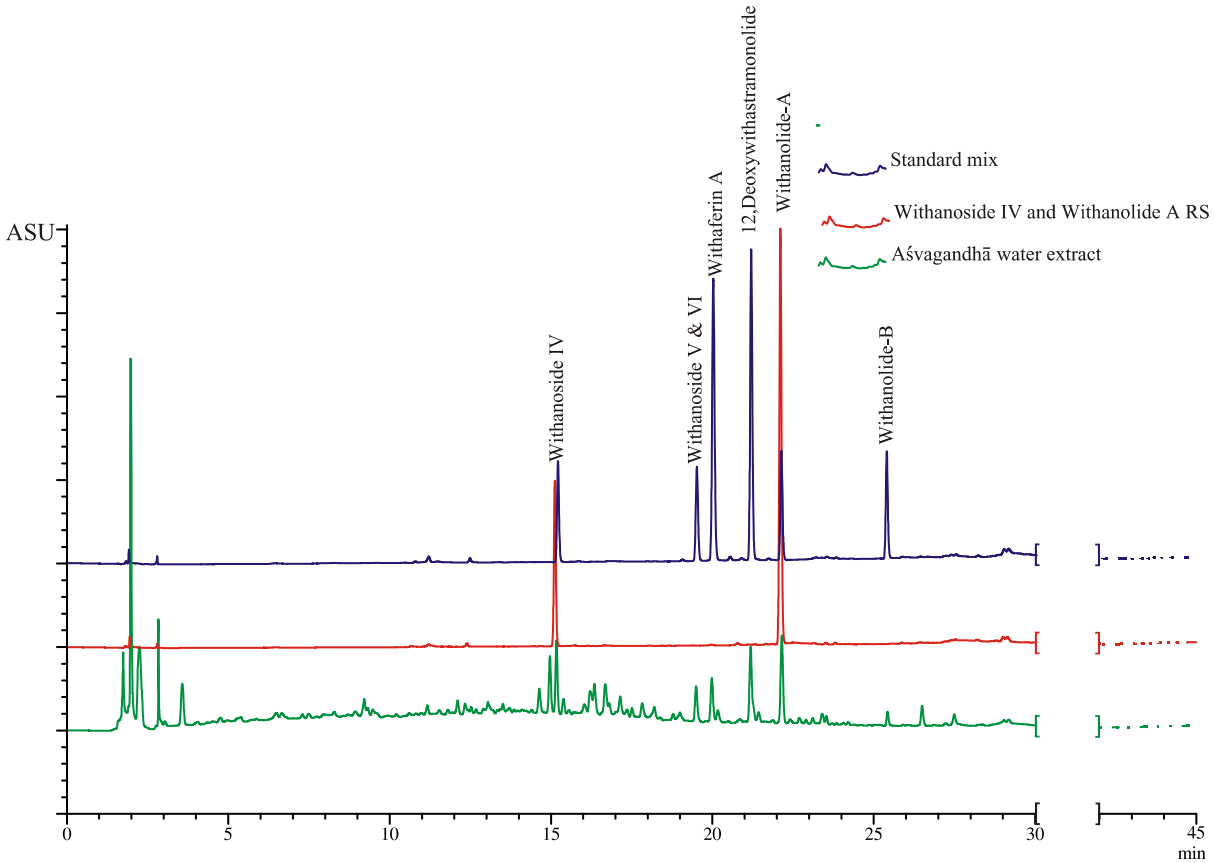


Fig.2. HPLC chromatograms of Aśvagandhā water extract with *Withanoside IV*, *Withanolide A* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Withaferin A RS*, *Withanoside IV RS* and *Withanolide A RS*.

BIBHĪTAKA

Bibhītaka consists of pericarp of ripe fruit of *Terminalia belerica* Roxb. (Fam. Combretaceae), a large deciduous tree, 10 to 20 m or more high, commonly found in plains and forests up to 900 m elevation; fruits ripen towards November, and form a minor non timber forest product, seeds are removed prior to use, and only pericarp is taken, although entire fruits are available in trade. Bibhītaka contains not less than 5.0 per cent of total polyphenols calculated as *gallic acid* [sum of *gallic acid*, *chebulic acid, corilagin*, *1, 3, 6-trigalloyl glucose* and *ellagic acid*] when assayed.

Synonyms: Vibhīta, Akṣa, Akṣaka

Regional Language Names:

Assamese	:	Bhomora, Bhomra, Bhaira
Bengali	:	Bayada, Baheda
English	:	Beleric Myrobalan
Gujarati	:	Bahedan
Hindi	:	Bahera
Kannada	:	Tare kai, Shanti Kayi
Kashmiri	:	Babelo, Balali
Malayalam	:	Tannikka
Marathi	:	Baheda
Oriya	:	Baheda
Punjabi	:	Bahera
Tamil	:	Thanrikkai
Telugu	:	Thanikkaya
Urdu	:	Bahera

Description :

a) *Macroscopic:*

Fruit nearly spherical to ovoid, 2.5 to 4.0 cm in diameter, fresh ripe fruits slightly silvery or with whitish shiny pubescent surface; mature fruits grey or greyish-brown with slightly wrinked appearance; taste, astringent; seeds upto 1.0 cm long and 0.5 cm wide embedded in hard endocarp, cream in colour where seeds are removed, the drug occurs as broken pieces of pericarp, yellow to brown, in varying sizes and shapes.

b) *Microscopic:*

TS of fruit shows a layer of epidermis, most cells elongating to form hair like protuberance with swollen base; mesocarp composed of a zone of parenchymatous cells, slightly tangentially elongated and irregularly arranged, interspersed with stone cells of varying shapes and sizes; elongated stone cells found towards periphery and spherical in the inner zone of mesocarp in groups of 3 to 10; mesocarp traversed in various directions by numerous collateral vascular

bundles; simple, round to oval starch grains measuring upto 11 μ in diameter and compound starch grains present in cells of mesocarp; a few peripheral layers devoid of starch grains; rosettes of calcium oxalate present in parenchymatous cells; endocarp composed of stone cells running longitudinally as well as transversely.

c) Powder:

Fine powder shows epidermal cells having hair-like projection, lignified stone cells having pitted walls and wide lumen, clusters of calcium oxalate, a few oil globules, numerous simple round to oval starch grains measuring up to 11 μ in diameter (Fig.1).

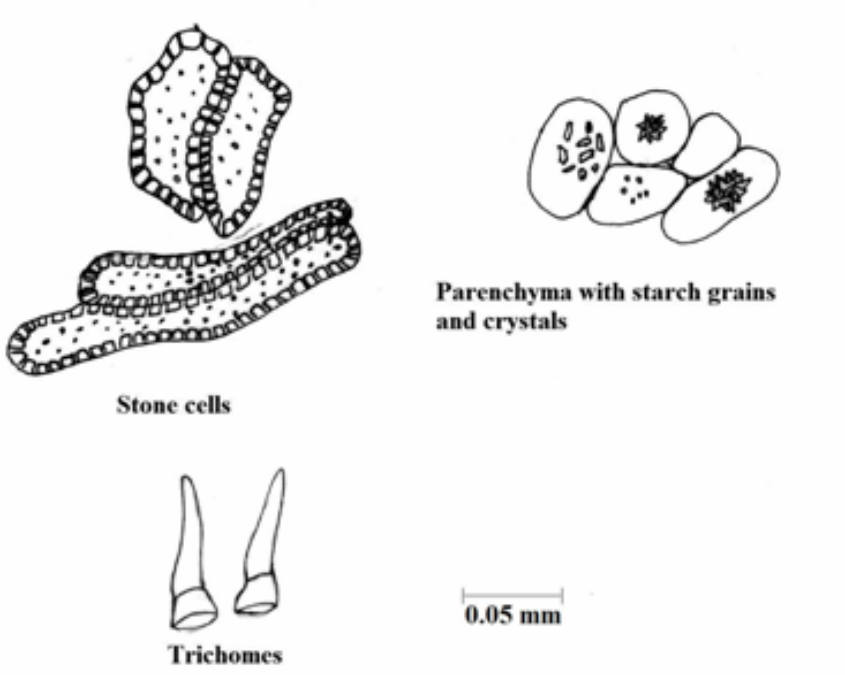


Fig.1. Powdered drug of Bibhītaka (*Terminalia belerica* Roxb.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 7.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 8.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 35.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a

water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 5 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig. 2).

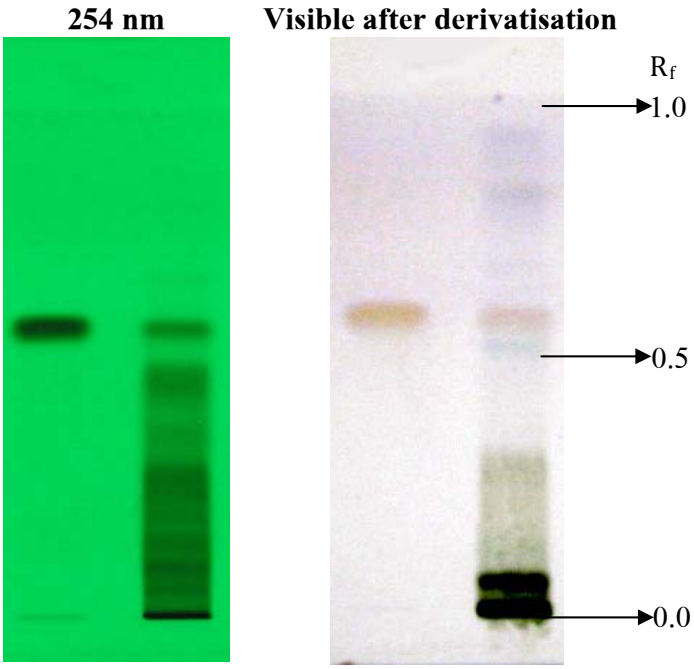


Fig.2. Thin-Layer Chromatogram of Bibhītaka. *RS*: *Gallic acid,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.3 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Gallic acid	1.00
Chebulic acid	0.73
Corilagin	2.23
1,3,6-Trigalloyl glucose	2.45
Ellagic acid	2.99

Calculate the content of total polyphenols (sum of *gallic acid, chebulic acid, corilagin*, *1, 3, 6-trigalloyl glucose* and *ellagic acid*) in the sample being examined from the peak response of analytes and from the declared content of *gallic acid* in *gallic acid RS*.

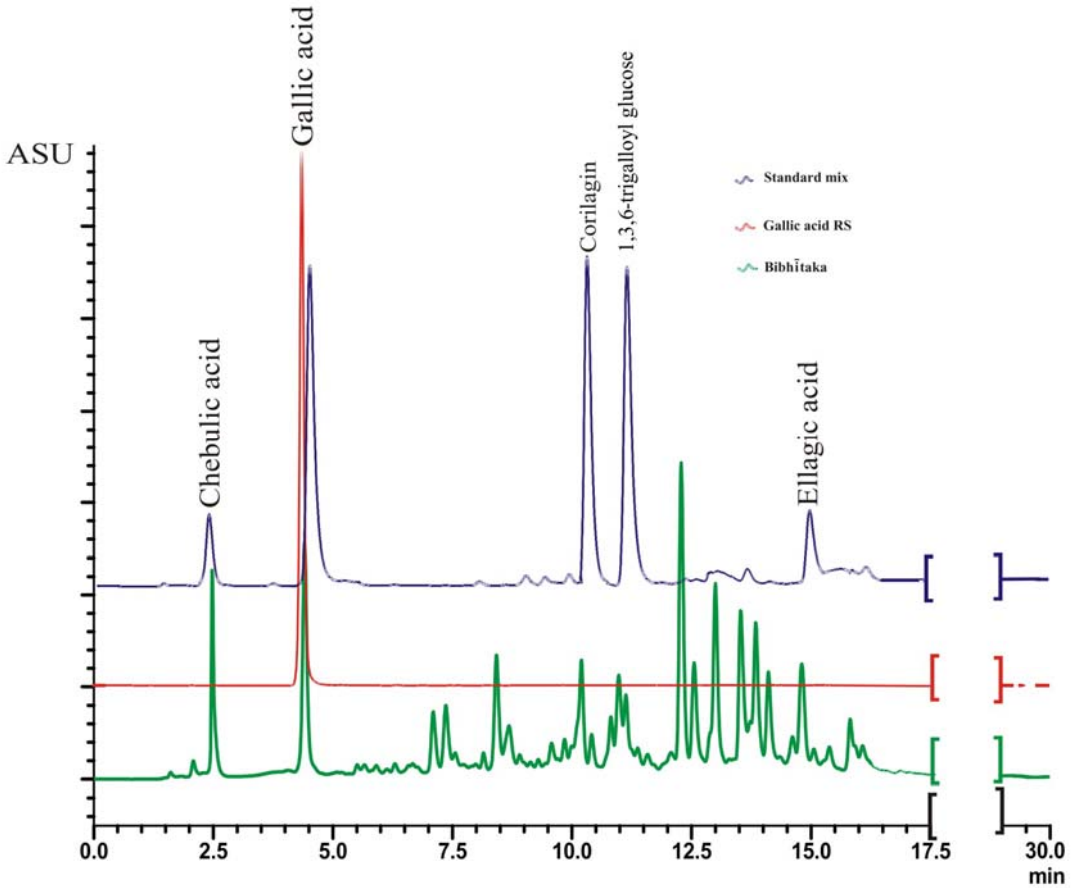


Fig.3. HPLC chromatograms of Bibhītaka with *Gallic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial, the part of the plant contained in the article.

API reference standards: API *Gallic acid RS.*

Constituents: Bellericagenin A & B, bellericaside A & B, termilignan, gallic acid, ellagic acid, ethyl gallate, chebulagic acid, corilagin, 1,3,6-trigalloyl glucose, bellericanin, phyllemblin and thannilignam.

Properties and Action:

Rasa : Kaṣāya
Guṇa : Rūkṣa, Laghu
Vīrya : Uṣṇa
Vipāka : Madhura
Karma : Kaphapittajit, Bhedaka, Kṛmināśana, Cakṣuṣya, Keśya, Kāsahara

Important formulations: Triphalā cūrṇa; Triphalādi taila; Lavaṅgādi vaṭī.

Therapeutic uses: Svarabheda (hoarseness of voice); Netraroga (diseases of eye); Kāsa (cough); Chardi (emesis); Kṛmiroga (worm infestation); Vibandha (constipation).

Dose: 3 to 6 g of the drug in powder form.

BIBHĪTAKA (POWDER)

Bibhītaka (Powder) consists of powder of Bibhītaka complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, light brown; odour characteristic; taste astringent; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows numerous groups or isolated stone cells elongated to nearly circular forms, thick walled, having wide lumen with pits; unicellular trichomes with sharp tips and bulbous base; epidermal fragments with cicatrices; a few thin walled polygonal parenchymatous cells; a few vessels with spiral thickenings; a few cells containing tannin; plenty of mostly rounded starch grains measuring up to 8 μ present in parenchymatous cells of mesocarp.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Bibhītaka.

Assay: Complies with the limits for Assay as per method stated under Bibhītaka.

Additional requirements:

Packaging and Storage: As given under Bibhītaka.

Labeling: As given under Bibhītaka.

API reference standards: As given under Bibhītaka.

BIBHĪTAKA HYDRO-ALCOHOLIC EXTRACT

Bibhītaka Hydro-alcoholic Extract is a dried and powdered extract prepared from Bibhītaka. The extract contains not less than 10.0 per cent of total polyphenols calculated as *gallic acid* [sum of *gallic acid*, *chebulic acid, corilagin*, *1, 3, 6-trigalloyl glucose* and *ellagic acid*] when assayed.

Method of Preparation:

Take Bibhītaka suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 40.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig. 1).

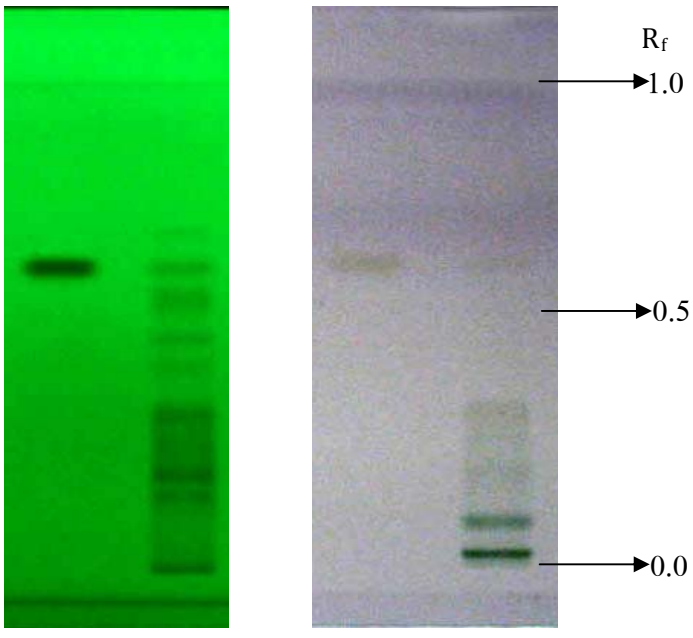


Fig.1. Thin-Layer Chromatogram of Bibhītaka hydro-alcoholic extract.
***RS*: *Gallic acid*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.25 per cent,	Appendix 2.1.7
pH:	3.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.1 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Gallic acid	1.00
Chebulic acid	0.73
Corilagin	2.23
1,3,6-Trigalloyl glucose	2.45
Ellagic acid	2.99

Calculate the content of total polyphenols (sum of *gallic acid, chebulic acid, corilagin*, *1,3,6-trigalloyl glucose* and *ellagic acid*) in the sample being examined from the peak response of analytes and from the declared content of *gallic acid* in *gallic acid RS*.

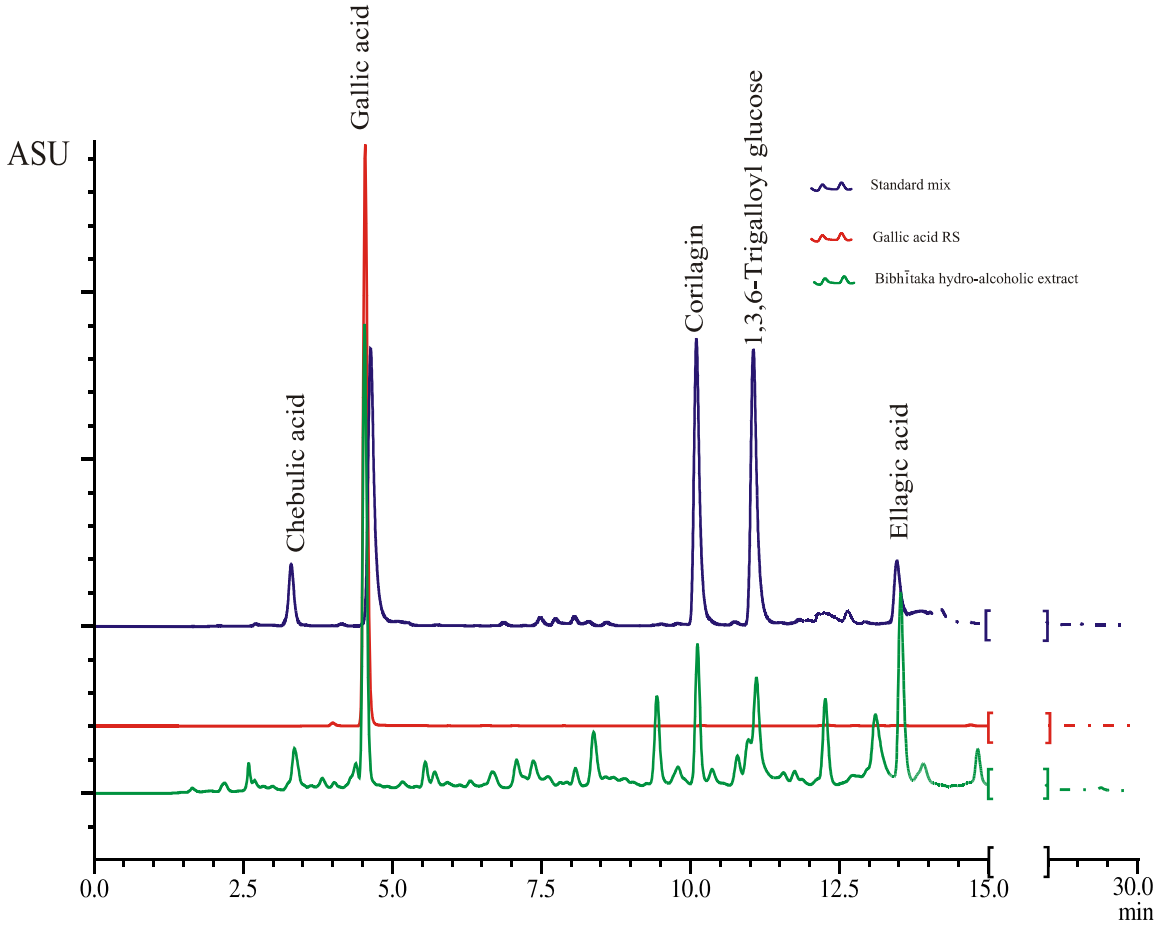


Fig.2. HPLC chromatograms of Bibhītaka hydro-alcoholic extract with *Gallic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS.*

BIBHĪTAKA WATER EXTRACT

Bibhītaka Water Extract is a dried and powdered extract prepared from Bibhītaka. The extract contains not less than 5.0 per cent of total polyphenols calculated as *gallic acid* [sum of *gallic acid*, *chebulic acid, corilagin*, *1,3,6-trigalloyl glucose* and *ellagic acid*] when assayed.

Method of Preparation:

Take Bibhītaka suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 45.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *gallic acid* as a reference standard. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 0.2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *gallic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R$_f$ ~0.60 corresponding to that of *gallic acid* and the profile should be similar to the one given in the TLC (Fig. 1).

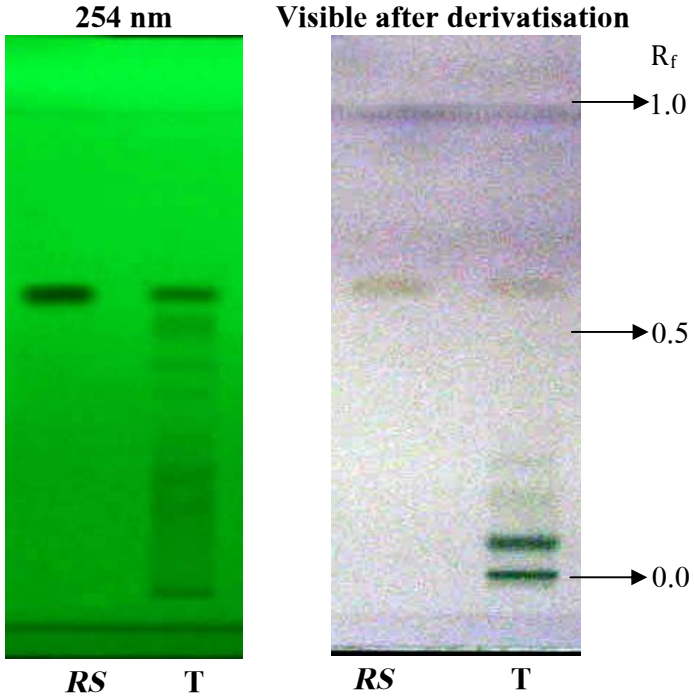


Fig.1. Thin-Layer Chromatogram of Bibhītaka water extract. *RS*: *Gallic acid,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	3.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.1 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 10 mg, accurately weighed, *gallic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *water* and make up the volume with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Gallic acid	1.00
Chebulic acid	0.73
Corilagin	2.23
1,3,6-Trigalloyl glucose	2.45
Ellagic acid	2.99

Calculate the content of total polyphenols (sum of *gallic acid, chebulic acid, corilagin*, *1, 3, 6-trigalloyl glucose* and *ellagic acid*) in the sample being examined from the peak response of analytes and from the declared content of *gallic acid* in *gallic acid RS*.

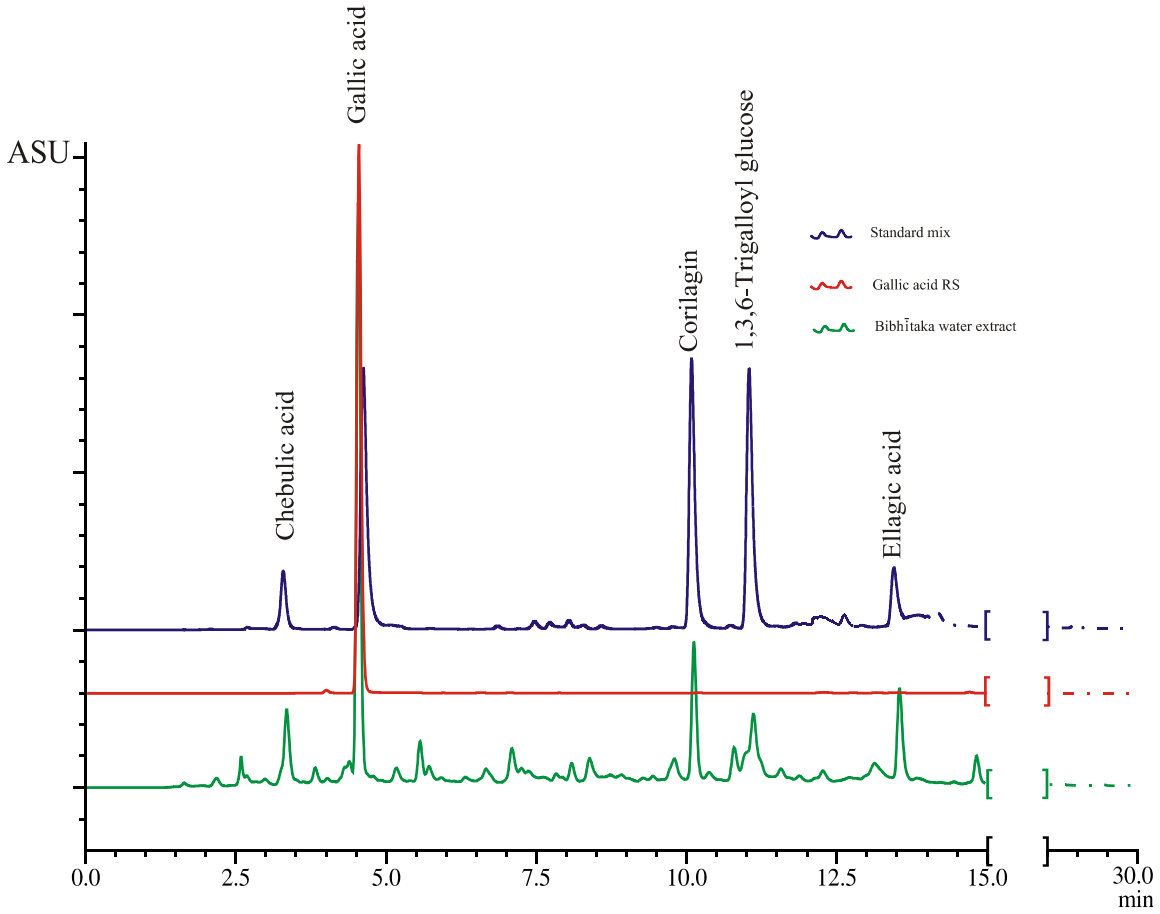


Fig.2. HPLC chromatograms of Bibhītaka water extract with *Gallic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS.*

BHṚṄGARĀJA

Bhṛṅgarāja consists of whole plant of *Eclipta alba* Hassk. (Fam. Asteraceae); a herbaceous annual, 30 - 50 cm high, erect or prostrate, much branched, strigosely hirsute, often rooting at nodes, a common weed of moist places found throughout India ascending upto 1700 m. Bhṛṅgarāja contains not less than 0.05 per cent of *wedelolactone* when assayed.

Synonyms: Tekaraja, Bhṛṅga, Mārkava, Bhṛṅgaja

Regional Language Names:

Assamese	:	Bhrngaraja
Bengali	:	Bheemraja, Kesuriya, Kesari
English	:	---
Gujarati	:	Bhangaro, Bhangro
Hindi	:	Bhangara, Bhangaraiya
Kannada	:	Garujalu, Gurugada Soppu, Keshavardhana, Kodigaraju
Kashmiri	:	--
Malayalam	:	Kayyonni, Knnunni
Marathi	:	Bhangra, Bhringiraja, Maka
Oriya	:	--
Punjabi	:	Bhangra
Tamil	:	Karisalankanni,Karisalanganni, Karisalai
Telugu	:	Guntakalagara, Guntagalagara
Urdu	:	Bhangra

Description:

a) *Macroscopic*

Root - Well developed, a number of secondary branches arise from main root, upto about 7 mm in dia., cylindrical, greyish.

Stem - Herbaceous, branched, occasionally rooting at nodes, cylindrical or flat, rough due to oppressed white hairs, node distinct, greenish, occasionally brownish.

Leaf - Opposite, sessile to subsessile, 2.2 - 8.5 cm long, 1.2 - 2.3 cm wide, usually oblong, lanceolate, sub-entire, sub-acute or acute, strigose with appressed hairs on both surfaces.

Flower - Solitary or 2, together on unequal axillary peduncles; involucral bracts about 8, ovate, obtuse or acute, herbaceous, strigose with oppressed hairs; ray flowers ligulate, ligule small, spreading, scarcely as long as bracts, not toothed, white; disc flowers tubular, corolla often 4 toothed; pappus absent, except occasionally very minute teeth on the top of achene; stamen 5, filaments epipetalous, free, anthers united into a tube with base obtuse; pistil bicarpellary; ovary inferior, unilocular with one basal ovule.

Fruit - Achenial cypsella, one seeded, cuneate, with a narrow wing, covered with warty excrescences, brown.

Seed - 0.2 - 0.25 cm long, 0.1 cm wide, dark brown, hairy and non endospermic.

b) *Microscopic*:

Root - Mature root shows poorly developed cork, consisting of 3-5 rows of thin-walled, tangentially elongated cells; secondary cortex consists of outer one or two rows of tangentially elongated or rounded cells with air cavities, inner secondary cortex of tangentially elongated to irregular shaped, parenchymatous cells with conspicuous air cavities; stone cells found scattered in secondary cortex and cork, in singles or in groups of various shape and size; pericyclic fibres in tangentially arranged bands of many cells or in singles; secondary phloem consists of sieve elements including phloem fibres traversed by multiseriate phloem rays; phloem rays broader towards periphery, consisting of rounded cells; xylem composed of vessels, fibre tracheids, fibres and xylem parenchyma, traversed by xylem rays; vessels numerous, found scattered throughout wood, in macerated preparation vessels small, drum-shaped, cylindrical elongated with pitted walls and perforations, simple, rarely slightly oblique; fibre tracheids, pitted, with very pointed tips, xylem fibres long with pointed tapering ends and short lumen, a few fibres show peg-like outgrowths towards the tapering ends; xylem parenchyma sparse usually squarish to rectangular having simple pits on their walls, xylem ray distinct, run straight in tangential section, generally 5-32 cells in height and 3-5 cells in width although very rarely uniseriate and biseriate rays also found, ray cells pitted.

Leaf- Petiole - shows single layered upper and lower epidermis consisting of tubular cells, covered with striated cuticle; trichomes of two types, non-glandular, uniseriate, 1-5 celled, warty, and with pointed apical cell; epidermis followed by wide cortex, consisting of 2-5 layered collenchyma on both, upper and lower side with distinct angular thickening; parenchyma 4-6 layered on upper side and 5-8 layered on lower side consisting of isodiametric, thin-walled cells with intercellular spaces; five vascular bundles central one largest while four others small flanking to either side of central bundle, consists of xylem on dorsal side and phloem on ventral side; xylem vessels arranged in radial rows traversed by xylem rays.

Midrib - cut at basal region shows both upper and lower single layered epidermis, externally covered with cuticle, a few epidermal cells elongate outwards to form uniseriate hairs; epidermis followed by cortex, consisting of 3-5 layered collenchymatous cells on both sides; section cut at middle region shows 3-4 layered collenchymatous cells on dorsal and 1-3 layered on ventral side, while the section cut at apical region, shows 2 layered collenchymatous cells on both sides, similarly transverse section cut at a basal, middle and apical regions shows 4-6 layered parenchymatous cells on dorsal side and 6-9 layered parenchyma on ventral side, in section cut at basal region 4-6 layered parenchyma on both the sides in the middle region with thin-walled cells and intercellular spaces, 2-3 layered parenchymatous cells on both side in the apical region; in the basal region section shows vascular bundle similar to that of petiole while in the section cut at middle and apical region section shows 4 smaller bundles shifting towards lamina.

Lamina - shows a dorsi ventral structure, epidermis single layered, externally covered with cuticle, followed by single layered palisade parenchyma containing chlorophyll contents; spongy parenchyma irregularly arranged with distinct intercellular spaces and filled with chlorophyll contents; mesophyll traversed by number of veins; anisocytic and anomocytic stomata present on both surface, more abundant on lower surfaces; stomatal index 20.0-22.5 on upper and 23.5 -26.0

on lower surface; palisade ratio 3.8 -4.5; hairs stiff, pointed, wide at the base, about 3 celled, uniseriate, middle cells longest, uppermost generally not exceeding the basal cell in length, septa thick-walled.

Stem - Mature stem shows single layered epidermis, externally covered with cuticle, a few epidermal cells elongate to form characteristic non-glandular trichomes, the cork where formed, poorly developed consisting of rectangular cells; secondary cortex composed of large, rounded or irregular shaped parenchymatous cells having wide air spaces; endodermis single layered consists of tangentially elongated cells; pericyclic fibres distinct, arranged in tangential strands; vascular bundles in a ring, collateral, endarch, of varying sizes traversed by medullary rays; phloem a narrow strip composed of sieve elements and phloem parenchyma; xylem consists of large number of vessels, xylem fibres and xylem parenchyma; xylem vessels appear evenly distributed throughout the xylem; in macerated preparation vessels barrel-shaped, some elongated with simple perforations, pitted with spiral thickening; xylem fibres with wide lumen, pointed tips and pitted walls, a few often bifurcate and a few other large, peg-like outgrowth; xylem parenchyma rectangular with pitted thickening; xylem rays triseriate to pentaseriate, normally biseriate and uniseriate, 8-15 cells in height and 3-5 cells in width; centre occupied by a wide pith consisting of isodiametric cells of parenchyma.

c) Powder:

Fine powder shows vessels in large groups or single broken pieces with pitted walls, numerous fibres entire or in pieces, trichomes entire or in pieces, warty, a few attached with epidermal and subsidiary cells, anomocytic and anisocytic stomata (Fig.1).

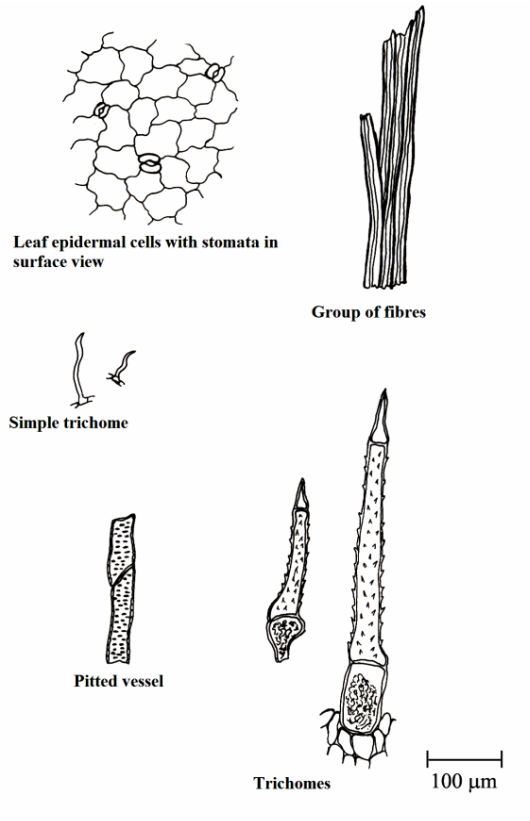


Fig.1. Powdered drug of Bhṛṅgarāja (*Eclipta alba* Hassk.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 22.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 11.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 5.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 15.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *wedelolactone* as a reference standard. Solvent system: *Toluene* : *acetone* : *formic acid* (11.0 : 6.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 5 mg of *wedelolactone RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 366 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *wedelolactone* and the profile should be similar to the one given in the TLC (Fig. 2).

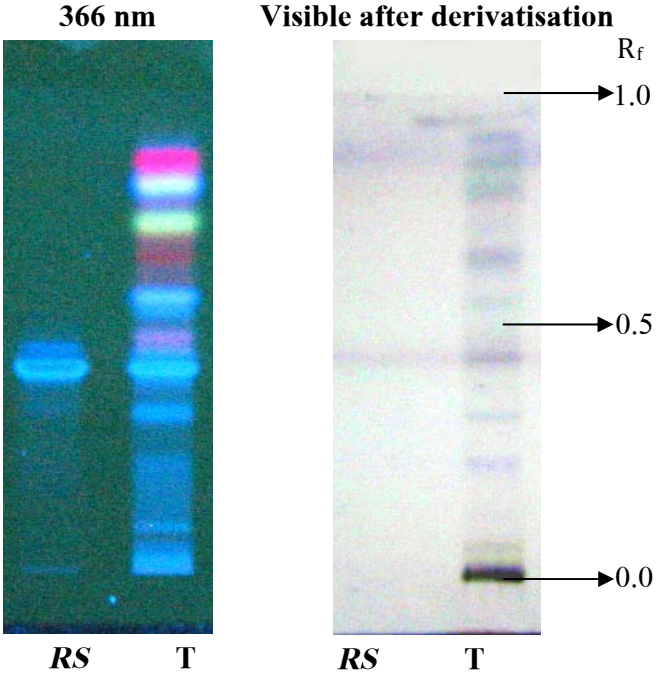


Fig.2. Thin-Layer Chromatogram of Bhṛṅgarāja. *RS*: *Wedelolactone,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2.5 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water

bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *wedelolactone RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	70	30
10	50	50
13	20	80
16	20	80
21	95	5
25	95	5

Injection volume: 20µl. Flow rate: 1.6 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *wedelolactone* in the substance being examined from the peak response of analyte and from the declared content of *wedelolactone* in *wedelolactone RS*.

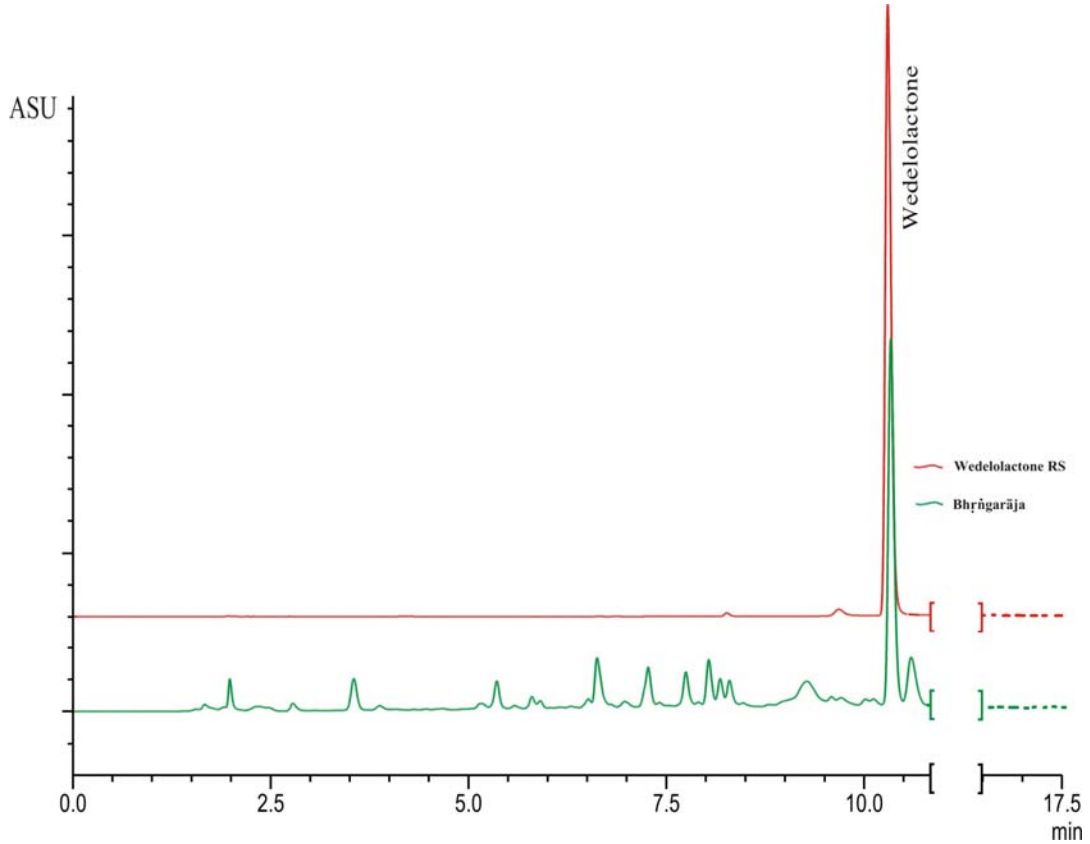


Fig.2. HPLC chromatogram of Bhṛṅgarāja with *Wedelolactone* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Wedelolactone RS.*

Constituents: Alkaloids, ecliptine, nicotine and wedelolactone.

Properties and Action:

Rasa	:	Kaṭu, Tikta
Guṇa	:	Rūkṣa, Tīkṣṇa
Vīrya	:	Uṣṇa
Vipāka	:	Kaṭu
Karma	:	Vātahara, Kaphahara, Āmahara, Balya, Rasāyana, Keśya, Tvacya, Dantya, Cakṣuṣya, Viṣahara

Important formulations: Bhṛṅgāmalakādi taila, Bhṛṅgarāja taila, Nīlībhṛṅgādi taila, Bhṛṅgarājāsava.

Therapeutic uses: Yakṛdroga (diseases of liver); Kṛmiroga (worm infestation); Śotha (inflammation); Pāṇḍu (anaemia); Śvāsa (asthma); Kāsa (cough); Śiraḥśūla (headache); Hṛdroga (heart disease).

Dose: 3 to 6 ml of the drug in the juice form.
12 to 36 g of the drug in the powder form for decoction.

BHṚṄGARĀJA (POWDER)

Bhṛṅgarāja (Powder) consists of powder of Bhṛṅgarāja complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, yellowish brown; odour aromatic; taste slightly bitter; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Leaf debris showing epidermis with sinuous walls and stomata that are anomocytic at some places and anisocytic at others; abundant trichomesuniseriate, multicellular, with large basal cell, and a pointed small apical cell, walls thick and warty, length upto 800 μm; abundant fibres from pericycle and xylem of both stem and root, several showing peg-like growth or nearly bifurcating at the tips; lumen broad; fibre-tracheids also present; xylem elements broad, barrel shaped, along with pitted walls, associated with xylem parenchyma also pitted; crystals and starch grains absent; pollen grains upto 30 μm in diameter with spiny exine.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Bhṛṅgarāja.

Assay: Complies with the limits for Assay as per method stated under Bhṛṅgarāja.

Additional requirements:

Packaging and Storage: As given under Bhṛṅgarāja.

Labeling: As given under Bhṛṅgarāja.

API reference standards: As given under Bhṛṅgarāja.

BHṚṄGARĀJA HYDRO-ALCOHOLIC EXTRACT

Bhṛṅgarāja Hydro-alcoholic Extract is a dried and powdered extract prepared from Bhṛṅgarāja. The extract contains not less than 0.15 per cent of *wedelolactone* when assayed.

Method of Preparation:

Take Bhṛṅgarāja suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 14.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *wedelolactone* as a reference standard. Solvent system: *Toluene* : *acetone* : *formic acid* (11.0 : 6.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 5 mg of *wedelolactone RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 366 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *wedelolactone* and the profile should be similar to the one given in the TLC (Fig. 1).

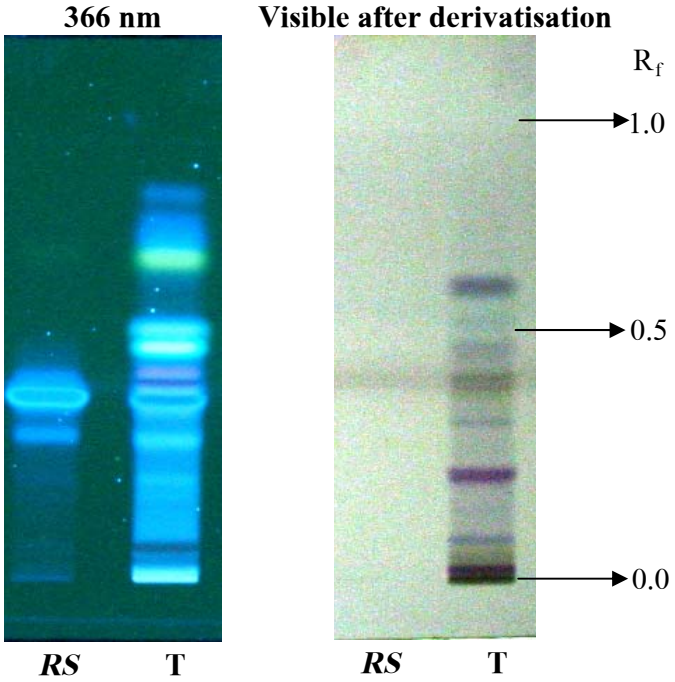


Fig.1. Thin-Layer Chromatogram of Bhṛṅgarāja hydro-alcoholic extract
***RS*: *Wedelolactone,* T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	5.0 - 7.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2.5 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *wedelolactone RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	70	30
10	50	50
13	20	80
16	20	80
21	95	5
25	95	5

Injection volume: 20 μl. Flow rate: 1.6 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *wedelolactone* in the substance being examined from the peak response of analyte and from the declared content of *wedelolactone* in *wedelolactone RS*.

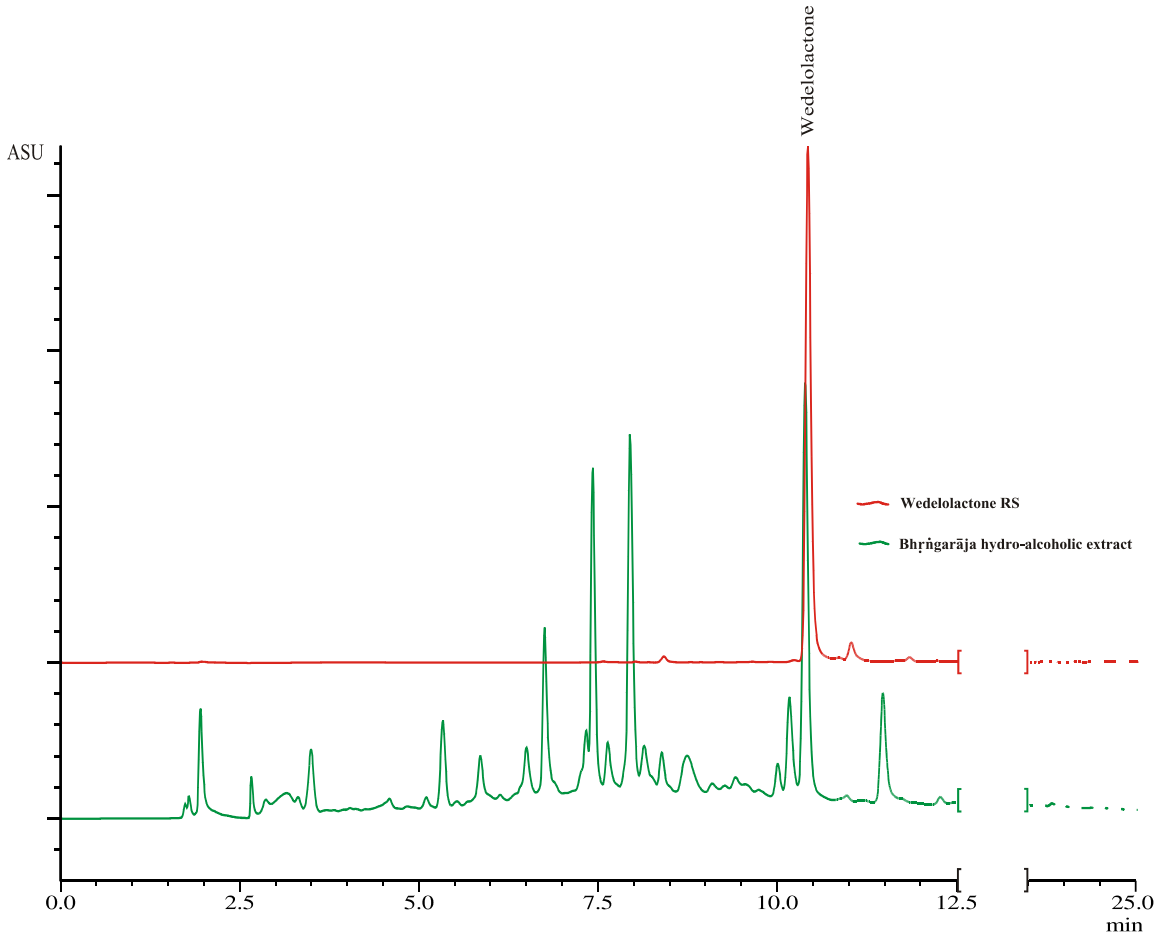


Fig.2. HPLC chromatogram of Bhṛṅgarāja hydro-alcoholic extract with *Wedelolactone* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Wedelolactone RS.*

BHṚṄGARĀJA WATER EXTRACT

Bhṛṅgarāja Water Extract is a dried and powdered extract prepared from Bhṛṅgarāja. The extract contains not less than 0.01 per cent of *wedelolactone* when assayed.

Method of Preparation:

Take Bhṛṅgarāja suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 12.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *wedelolactone* as a reference standard. Solvent system: *Toluene* : *acetone* : *formic acid* (11.0 : 6.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 5 mg of *wedelolactone RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm and 366 nm. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *wedelolactone* and the profile should be similar to the one given in the TLC (Fig. 1).

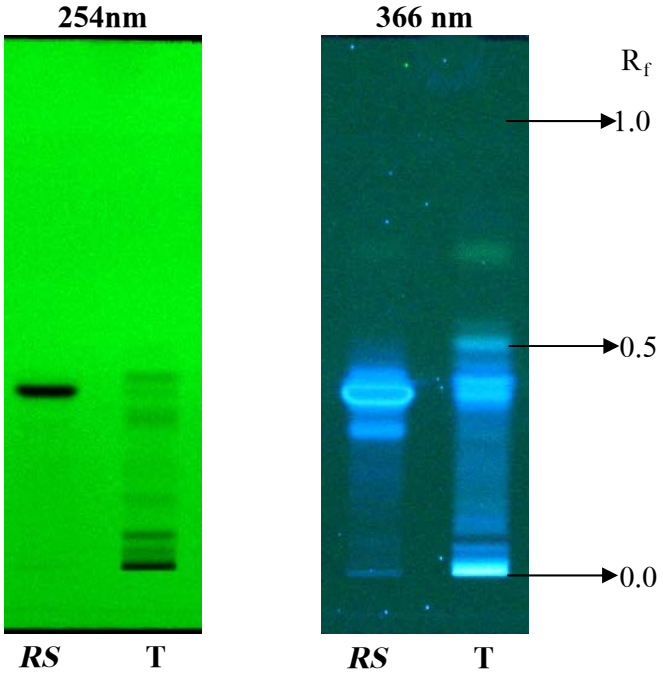


Fig.1. Thin-Layer Chromatogram of Bhṛṅgarāja water extract.
***RS*: *Wedelolactone,* T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	5.5 - 7.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 3.5 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *wedelolactone RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	70	30
10	50	50
13	20	80
16	20	80
21	95	5
25	95	5

Injection volume: 20μl. Flow rate: 1.6 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *wedelolactone* in the substance being examined from the peak response of analyte and from the declared content of *wedelolactone* in *wedelolactone RS*.

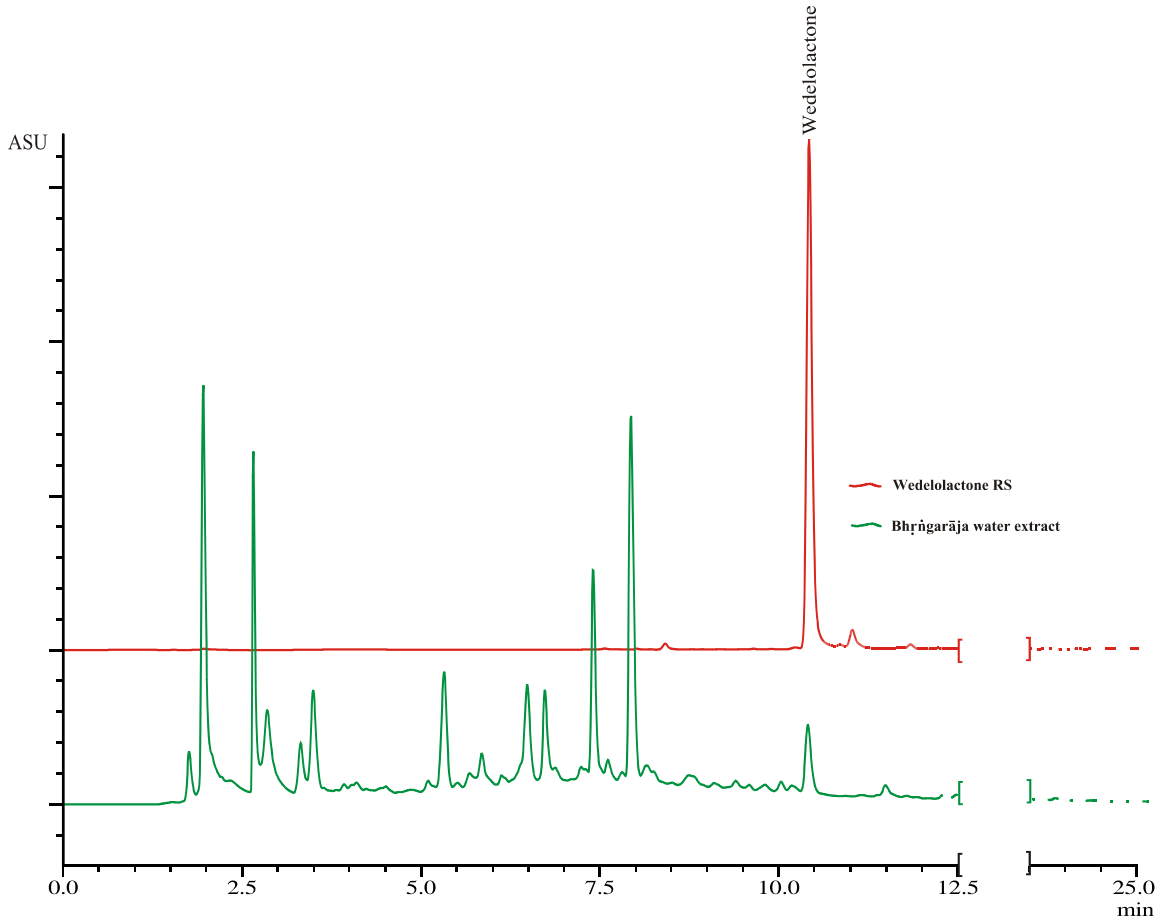


Fig.2. HPLC chromatogram of Bhṛṅgarāja water extract with *Wedelolactone* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Wedelolactone RS*.

BRĀHMĪ

Brāhmī consists of dried whole plant of *Bacopa monnieri* (L.) Wettst. syn. *Herpestis monnieria* (L.) H.B.& K. (Fam. Scrophulariaceae); a glabrous, succulent, small, prostrate or creeping annual herb, found throughout India in wet and damp places. Brāhmī contains not less than 2.0 per cent of *bacoside A* when assayed.

Synonyms: Sarasvatī, Kapotavaṅkā

Regional Language Names:

Assamese	:	Brahmi
Bengali	:	--
English	:	Thyme Leaved Gratiola
Gujarati	:	Neerbrahmi, Bamanevari
Hindi	:	Manduka Parni
Kannada	:	Nirubrahmi, Valabrahmi, Ondelaga, Mandukaparni
Kashmiri	:	--
Malayalam	:	Bhahmi
Marathi	:	Jalnam, Brahmi, Birami
Oriya	:	Brahmi
Punjabi	:	Brahmibuti
Tamil	:	Nirabrahmi, Brahmi vazhukkai
Telugu	:	Sambarenu, Sambarani
Urdu	:	Brahmi

Description:

a) *Macroscopic*:

Root - Thin, wiry, small, branched creamish-yellow.

Stem - Thin, green or purplish green, about 1-2 mm thick, soft, nodes and internodes prominent, glabrous; taste, slightly bitter.

Leaf - Simple, opposite, decussate, green, sessile, 1-2 cm long, obovate-oblong; taste, slightly bitter.

Flower - Small, axillary and solitary, pedicels 6-30 mm long, bracteoles shorter than pedicels.

Fruit - Capsules upto 5 mm long, ovoid and glabrous.

b) *Microscopic:*

Root - Shows a single layer of epidermis, cortex having large air cavities; endodermis single layered; pericycle not distinct; stele consists of a thin layer of phloem with a few sieve elements and isolated material from xylem shows vessels with reticulate thickenings.

Stem - Shows single layer of epidermis followed by a wide cortex of thin-walled cells with very large intercellular spaces; endodermis single layered; pericycle consisting of 1-2 layers; vascular

ring continuous, composed of a narrow zone of phloem towards periphery and a wide ring of xylem towards centre; centre occupied by a small pith with distinct intercellular spaces; starch grains simple, round to oval, present in a few cells of cortex and endodermis, measuring 4-14 μ in dia. and 8.0-14.0 x 2.5-9.0 μ in dia. respectively.

Leaf -Shows a single layer of upper and lower epidermis covered with thin cuticle; glandular hairs sessile, subsidiary cells present on both surfaces; a few prismatic crystals of calcium oxalate occasionally found distributed in mesophyll cells; mesophyll traversed by small veins surrounded by bundle sheath; no distinct midrib present.

c) *Powder*:

Fine powder shows xylem vessels with reticulate thickening, glandular hairs, simple, round and oval starch grains, measuring 4-14 μ in diameter (Fig.1).

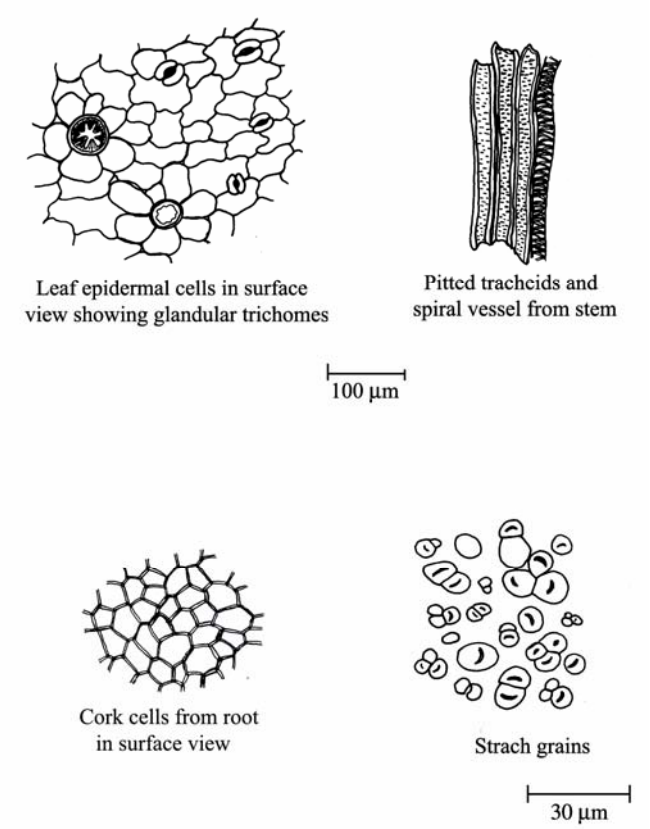


Fig.1. Powdered drug of Brāhmī (*Bacopa monnieri* (L.) Wettst.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 18.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 6.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 6.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 15.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *bacoside A* as a reference standard. Solvent system: *Ethyl acetate* : *methanol* : *water* (7.0 : 2.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *bacoside A RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *vanillin sulphuric acid reagent*. Heat the plate at 60^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *bacoside A* and the profile should be similar to the one given in the TLC (Fig. 2).

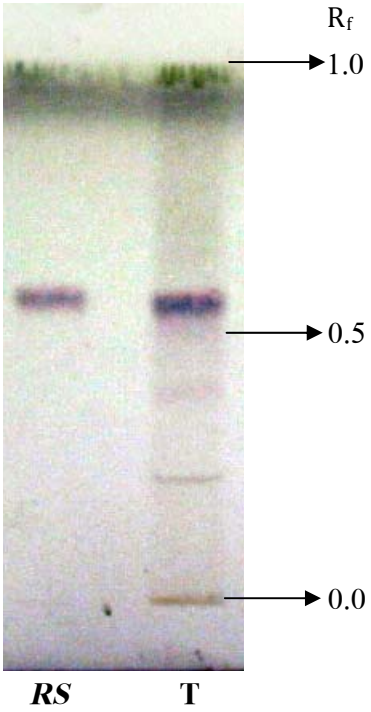


Fig.2. Thin-Layer Chromatogram of Brāhmī. *RS*: *Bacoside A,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 75 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. *Standard solution*: Take about 5 mg, accurately weighed, *bacoside A RS* (mixture of *bacoside* A_3, *bacopaside-II*, *jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in a 25-ml volumetric flask, dissolve in 10 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
25	40	60
30	70	30
35	70	30

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Bacoside A_3	1.00
Bacopaside-II	1.04
Jujubogenin isomer of bacopasaponin C	1.13
Bacopasaponin C	1.19

Calculate the content of *bacoside A* (mixture of *bacoside A_3, bacopaside-II, jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in the substance being examined from the peak response of analytes and from the declared content of *bacoside A* in *bacoside A RS*.

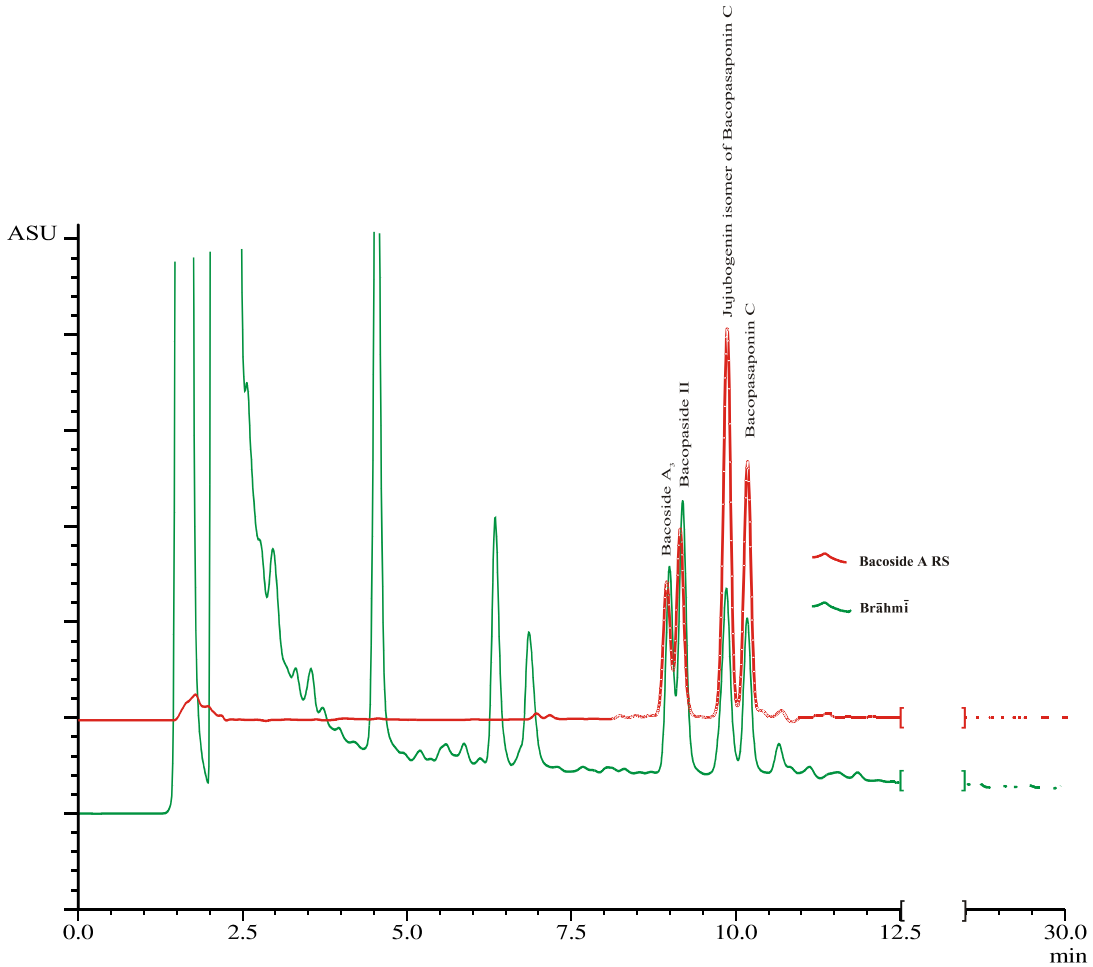


Fig.3. HPLC chromatogram of Brāhmī with *Bacoside A* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Bacoside A RS*.

Constituents: Brahmine, herpestine, α-mannitol, hersaponin, monnierin, luteolin, apigenin, bacopasaponins and bacosides.

Properies and Action:

Rasa	:	Tikta, Kaṣāya, Madhura
Guṇa	:	Laghu, Sara
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Vātahara, Kaphahara, Rasāyana, Āyuṣya, Medhya, Matiprada, Svarya, Prajāsthāpana, Viṣahara, Mohahara

Important formulations: Sārasvatāriṣṭa, Brāhmī ghṛta, Ratnagiri rasa, Brāhmī vaṭī, Sārasvata cūrṇa, Smṛtisāgara rasa.

Therapeutic uses: Kuṣṭha (diseases of skin); Jvara (fever); Śopha (oedema); Pāṇḍu (anaemia); Prameha (increased frequency and turbidity of urine); Mānasavikāra (mental disorders).

Dose: 1-3 g in powder form.

BRĀHMĪ (POWDER)

Brāhmī (Powder) consists of powder of Brāhmī complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, light brown; odour characteristic; taste slightly bitter; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows a few sessile glandular trichomes from leaf with 4 to 8 celled head; numerous anomocytic, occasionally diacytic, stomata in surface view; numerous simple, round to oval starch grains measuring up to 14 μ; fragments of thin walled, polygonal, parenchymatous cells of mesophyll with rosette crystals, prismatic and rosette crystals found isolated also; a few oil globules scattered as such and within the parenchymatous cells; fragments of thin walled cotyledon; thick walled, rectangular sclerified cells of testa; a few fragments of vessels with annular and spiral thickenings.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Brāhmī.

Assay: Complies with the limits for Assay as per method stated under Brāhmī.

Additional requirements:

Packaging and Storage: As given under Brāhmī.

Labeling: As given under Brāhmī.

API reference standards: As given under Brāhmī.

BRĀHMĪ HYDRO-ALCOHOLIC EXTRACT

Brāhmī Hydro-alcoholic Extract is a dried and powdered extract prepared from Brāhmī. The extract contains not less than 5.0 per cent of *bacoside A* when assayed.

Method of preparation:

Take Brāhmī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 14.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *bacoside A* as a reference standard. Solvent system: *Ethyl acetate* : *methanol* : *water* (7.0 : 2.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *bacoside A RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *vanillin sulphuric acid reagent*. Heat the plate at 60^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *bacoside A* and the profile should be similar to the one given in the TLC (Fig. 1).

Visible after derivatisation

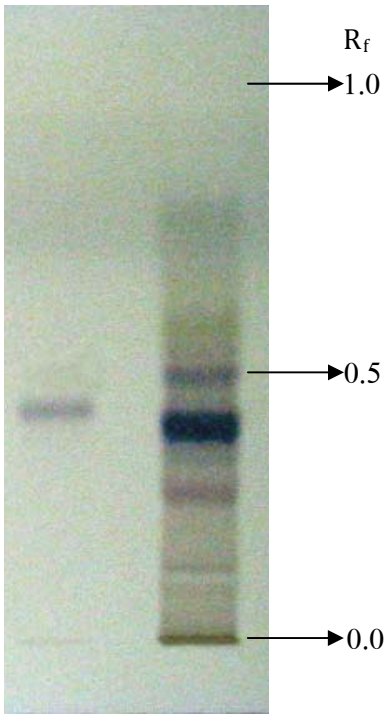


Fig.1. Thin-Layer Chromatogram of Brāhmī hydro-alcoholic extract
***RS*: *Bacoside A*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 35.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7

pH:	5.0 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. *Standard solution*: Take about 5 mg, accurately weighed, *bacoside A RS* (mixture of *bacoside* A_3, *bacopaside-II*, *jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in a 25-ml volumetric flask and dissolve in 10 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
25	40	60
30	70	30
35	70	30

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Bacoside A_3	1.00
Bacopaside-II	1.04
Jujubogenin isomer of bacopasaponin C	1.13
Bacopasaponin C	1.19

Calculate the content of *bacoside A* (mixture of *bacoside* A_3, *bacopaside-II, jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in the substance being examined from the peak response of analytes and from the declared content of *bacoside A* in *bacoside A RS*.

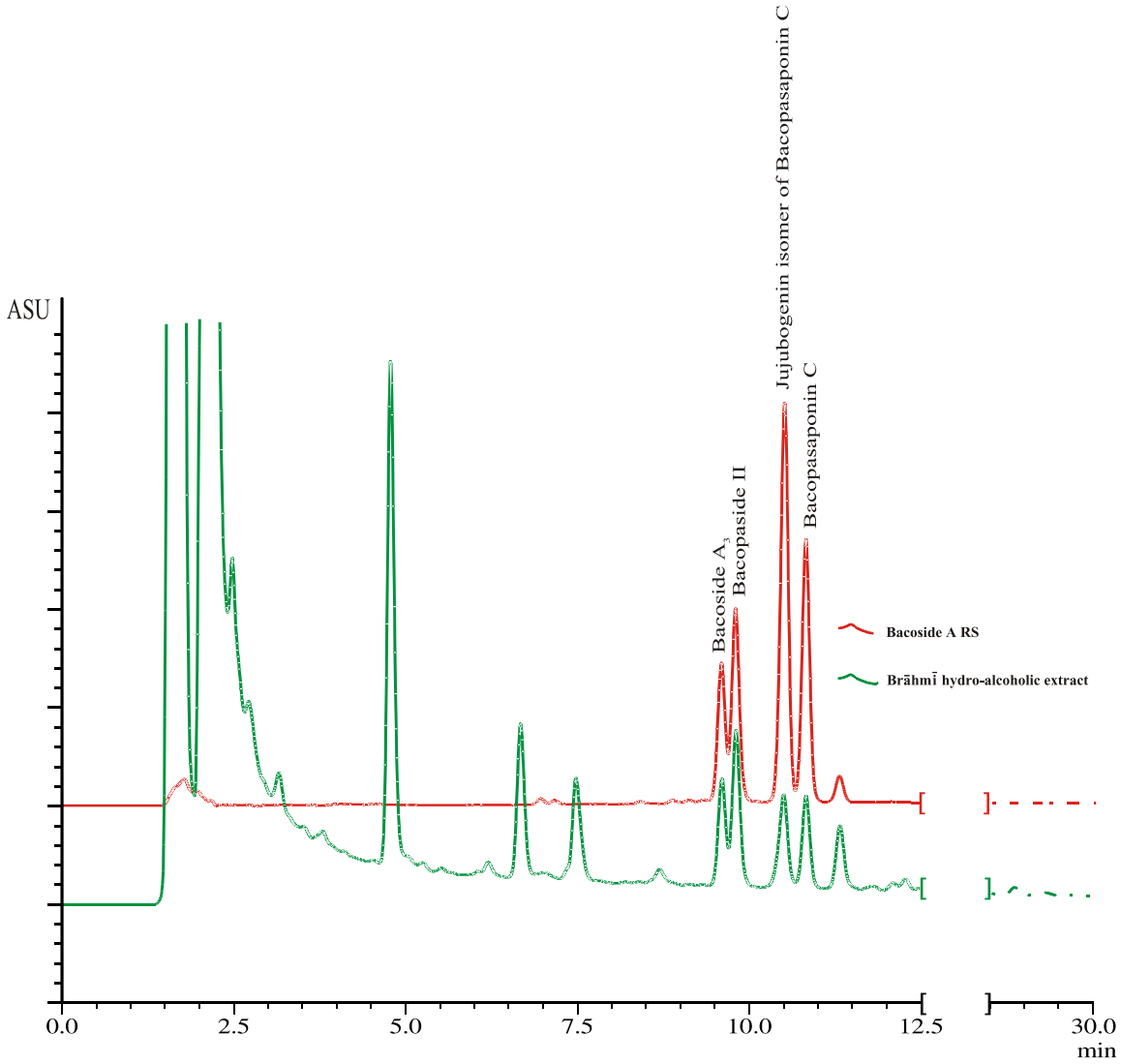


Fig.2. HPLC chromatogram of Brāhmī hydro-alcoholic extract with *Bacoside A* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Bacoside A RS.*

BRĀHMĪ WATER EXTRACT

Brāhmī Water Extract is a dried and powdered extract prepared from Brāhmī. The extract contains not less than 0.75 per cent of *bacoside A* when assayed.

Method of preparation:

Take Brāhmī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 18.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *bacoside A* as a reference standard. Solvent system: *Ethyl acetate* : *methanol* : *water* (7.0 : 2.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *bacoside A RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *vanillin sulphuric acid reagent*. Heat the plate at 60^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *bacoside A* and the profile should be similar to the one given in the TLC (Fig. 1).

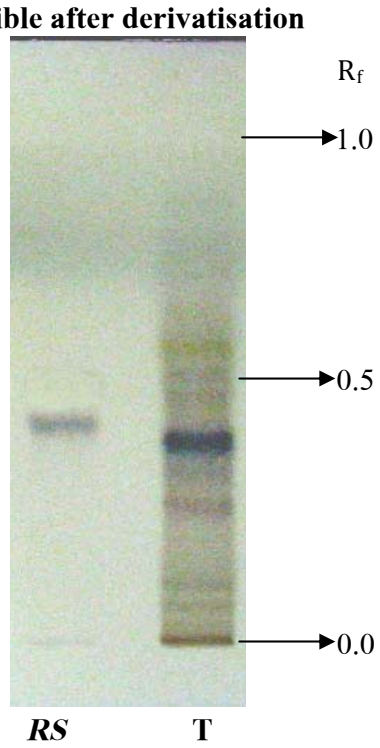


Fig.1. Thin-Layer Chromatogram of Brāhmī water extract. *RS*: *Bacoside A,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 35.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7
pH:	4.5 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 4 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. *Standard solution*: Take about 5 mg, accurately weighed, *bacoside A RS* (mixture of *bacoside* A_3, *bacopaside-II*, *jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in a 25-ml volumetric flask and dissolve in 10 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	70	30
25	40	60
30	70	30
35	70	30

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Bacoside A_3	1.00
Bacopaside-II	1.04
Jujubogenin isomer of bacopasaponin C	1.13
Bacopasaponin C	1.19

Calculate the content of *bacoside A* (mixture of *bacoside A*$_3$, *bacopaside-II, jujubogenin isomer of bacopasaponin C* and *bacopasaponin C*) in the substance being examined from the peak response of analytes and from the declared content of *bacoside A* in *bacoside A RS*.

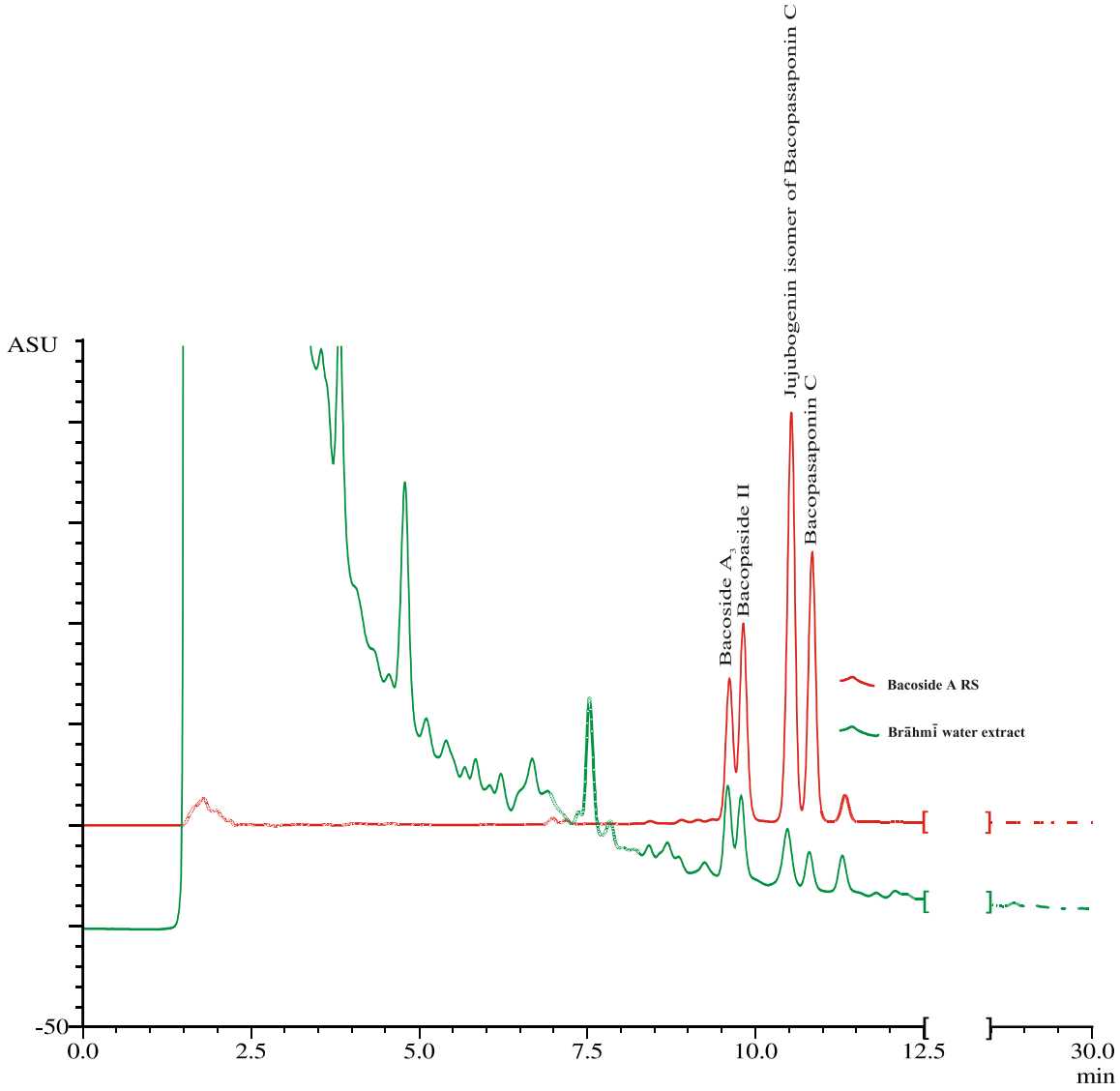


Fig.2. HPLC chromatogram of Brāhmī water extract with *Bacoside A* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Bacoside A RS.*

HARIDRĀ

Haridrā consists of the dried and cured rhizomes of *Curcuma longa* L. (Fam. Zingiberaceae); a perennial herb extensively cultivated in many parts of the country; crop is harvested after 9 to 10 months when lower leaves turn yellow; rhizomes carefully dug up with picks between October-April and cured by boiling in a thin aqueous solution of turmeric and drying. Haridrā contains not less than 2.0 per cent of total curcuminoids when assayed.

Synonyms: Rajanī, Niśā, Niśi, Ratri, Kṣaṇadā, Doṣā

Regional Language Names:

Assamese	:	Haldhi, Haladhi
Bengali	:	Halud, Haldi
English	:	Turmeric
Gujarati	:	Haldar
Hindi	:	Haldi, Hardi
Kannada	:	Arishina
Kashmiri	:	Ledar, Ladhir
Malayalam	:	Manjal
Marathi	:	Halad
Oriya	:	Haladi
Punjabi	:	Haldi, Haldar
Tamil	:	Manjal
Telugu	:	Pasupu
Urdu	:	Haldi

Description:

a) *Macroscopic:*

Rhizomes ovate, oblong or pyriform (round turmeric) or cylindrical, often short branched (long turmeric), former about half as broad as long, latter 2 to 5 cm long and about 1 to 2 cm thick, externally yellowish to yellowish-brown with root scars and annulations of leaf bases; fracture horny, fractured surface orange to reddish brown; central cylinder twice as broad as cortex; odour and taste characteristic.

b) *Microscopic:*

TS of rhizome shows epidermis with thick-walled, cubical cells of various dimensions; cortex characterized by the presence of mostly thin-walled rounded parenchyma cells scattered collateral vascular bundles; a few layers of cork developed under epidermis and scattered oleo-resin cells with brownish contents; cork generally composed of 4 to 6 layers of thin-walled, brick-shaped parenchyma; cells of ground tissue contain starch grains of 4 to 15 μ in diameter; these starch grains get gelatinized to form a compact mass in each of the cells; oil cell with suberised walls containing either orange-yellow oil globules or amorphous resinous matter, vessels mainly spirally thickened along with a few reticulate and annular.

c) *Powder:*

Fine powder shows fragments of parenchymatous cells containing brownish content vessel with spiral thickenings; a few oil globules; starch grains partially gelatinized, simple, some times groups or single, rounded, measuring 4 to 15 μ in diameter; gelatinized starch grains form a compact mass in each of the cells (Fig.1).

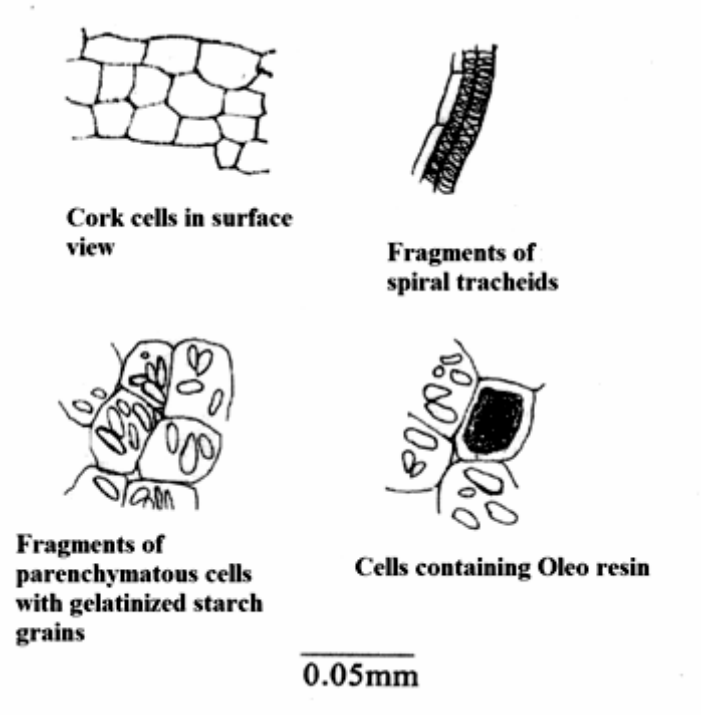


Fig.1. Powdered drug of Haridrā (*Curcuma longa* L.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 9.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than1.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 8.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 12.0 per cent,	Appendix 2.1.9
Volatile oil:	Not less than 4.0 per cent,	Appendix 2.1.12

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *curcuminoids* as a reference standard. Solvent system: *Chloroform* : *ethanol* : *glacial acetic acid* (9.5 : 0.5 : 0.1). Test solution: To 2 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard

solution: Dissolve 1 mg of *curcuminoids RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 366 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with the test solution shows a band at R_f~0.50 corresponding to that of *curcumin*, R_f~0.45 corresponding to that of *demethoxycurcumin* and at R_f~0.30 corresponding to that of *bisdemethoxy-curcumin* and the profile should be similar to the one given in the TLC (Fig. 2).

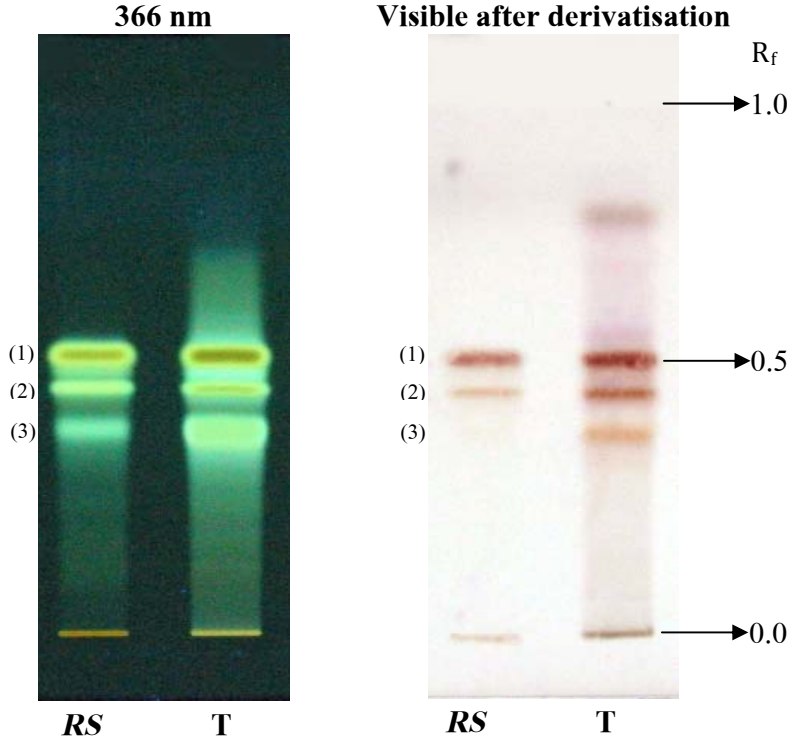


Fig. 2. Thin-Layer Chromatogram of Haridrā. RS: Curcuminoids [(1) Curcumin, (2) demethoxycurcumin, (3) bisdemethoxycurcumin], T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.75 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*. Standard solution: Take about 10 mg, accurately weighed, *curcuminoids RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 65 volumes of buffer (prepared by dissolving 10 g of *citric acid* in 1000 ml of *water* and adjusting the *p*H to 3.0 using *ammonia solution*), 35 volumes of *tetrahydrofuran*. Injection volume: 20 µl. Flow rate: 1.2 ml per minute.

Detection: UV, 430 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *curcuminoids* in the substance being examined from the peak response of analyte and from the declared content of *curcuminoids in curcuminoids RS*.

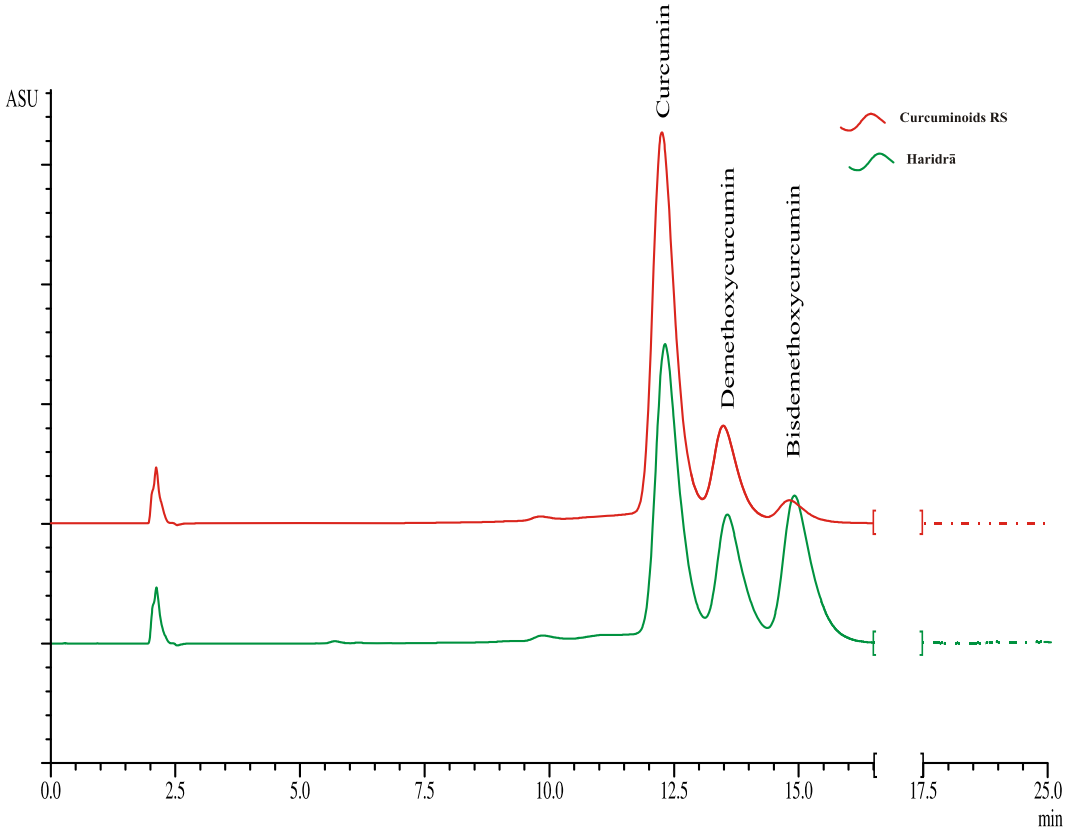


Fig.3. HPLC chromatogram of Haridrā with *Curcuminoids* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Curcuminoids RS.*

Constituents: Curcuminoids, curcumin; essential oil; *ar*-termerone, α and β termerone, curcumol.

Properties and action:

Rasa : Tikta, Kaṭu
Guṇa : Rūkṣa
Vīrya : Uṣṇa
Vipāka : Kaṭu
Karma : Kaphapittanut, Viṣaghna, Varṇya, Kuṣṭhaghna, Kṛmighna, Pramehanāśaka

Important formulations: Haridrā khaṇḍa.

Therapeutic uses: Viṣavikāra (disorders due to poison); Kuṣṭha (diseases of skin); Vraṇa (ulcer); Tvagroga (skin disease); Prameha (increased frequency and turbidity of urine); Pāṇḍu (anaemia); Śītapitta (urticaria); Pīnasa (chronic rhinitis/sinusitis).

Dose: 1 to 3 g of the drug in powder form.

HARIDRĀ (POWDER)

Haridrā (Powder) consists of powder of Haridrā complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, bright yellow; odour aromatic; taste characteristics; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*; treat a few mg of powder with Sudan III solution and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of cork; fragments of cortical and stelar parenchymatous cells containing simple round starch grains measuring up to 15μ; starch grains are in general gelatinized; a few cells show the presence of intact oleo-resin; a few vascular bundles and fragments of pitted fibres present; longitudinal reticulate, spiral and annular vessels present.

A small quantity of powder on a microslide flooded with dil. sulphuric acid covered with a cover slip observed through microscope shows many particles turning a deep red.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Haridrā.

Assay: Complies with the limits for Assay as per method stated under Haridrā.

Additional requirements:

Packaging and Storage: As given under Haridrā.

Labeling: As given under Haridrā.

API reference standards: As given under Haridrā.

HARIDRĀ HYDRO-ALCOHOLIC EXTRACT

Haridrā Hydro-alcoholic Extract is a dried and powdered extract prepared from Haridrā. The extract contains not less than 4.0 per cent of total curcuminoids when assayed.

Method of Preparation:

Take Haridrā suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 8.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *curcuminoids* as a reference standard. Solvent system: *Chloroform* : *ethanol* : *glacial acetic acid* (9.5 : 0.5 : 0.1). Test solution: To 2 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard solution: Dissolve 1 mg of *curcuminoids RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 366 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with the test solution shows a band at R_f ~0.50 corresponding to that of *curcumin*, R_f ~0.45 corresponding to that of *demethoxycurcumin* and at R_f ~0.30 corresponding to that of *bisdemethoxycurcumin* and the profile should be similar to the one given in the TLC (Fig. 1).

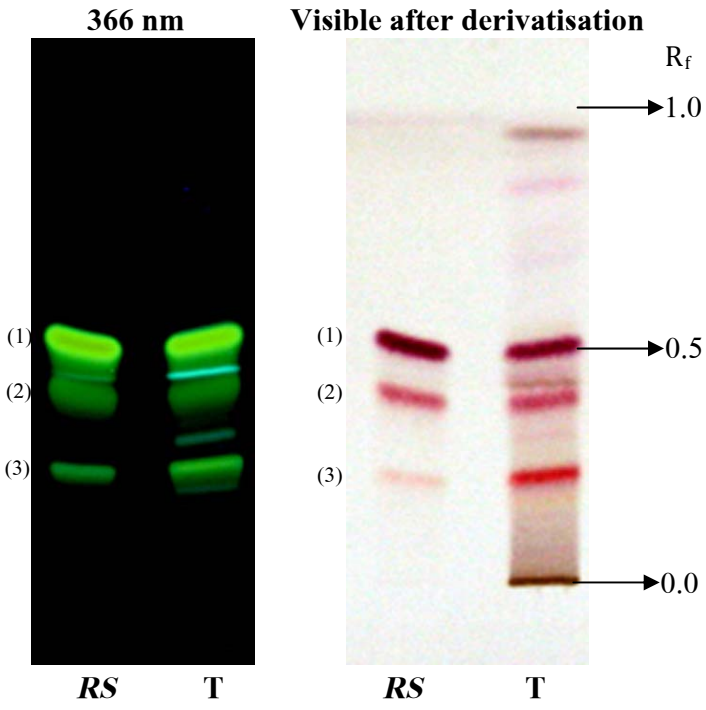


Fig.1. Thin-Layer Chromatogram of Haridrā hydro-alcoholic extract. *RS*: *Curcuminoids* [(1) *Curcumin*, (2) *demethoxycurcumin*, (3) *bisdemethoxycurcumin*], T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 30.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 percent,	Appendix 2.1.7
pH:	5.5 - 7.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.75 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*. Standard solution: Take about 10 mg, accurately weighed, *curcuminoids RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 65 volumes of buffer (prepared by dissolving 10 g of *citric acid* in 1000 ml of *water* and adjusting the *p*H to 3.0 using *ammonia solution*), 35 volumes of *tetrahydrofuran*. Injection volume: 20 µl. Flow rate: 1.2 ml per minute. Detection: UV, 430 nm. Procedure: Inject 20 µl of the filtered standard solution and record the

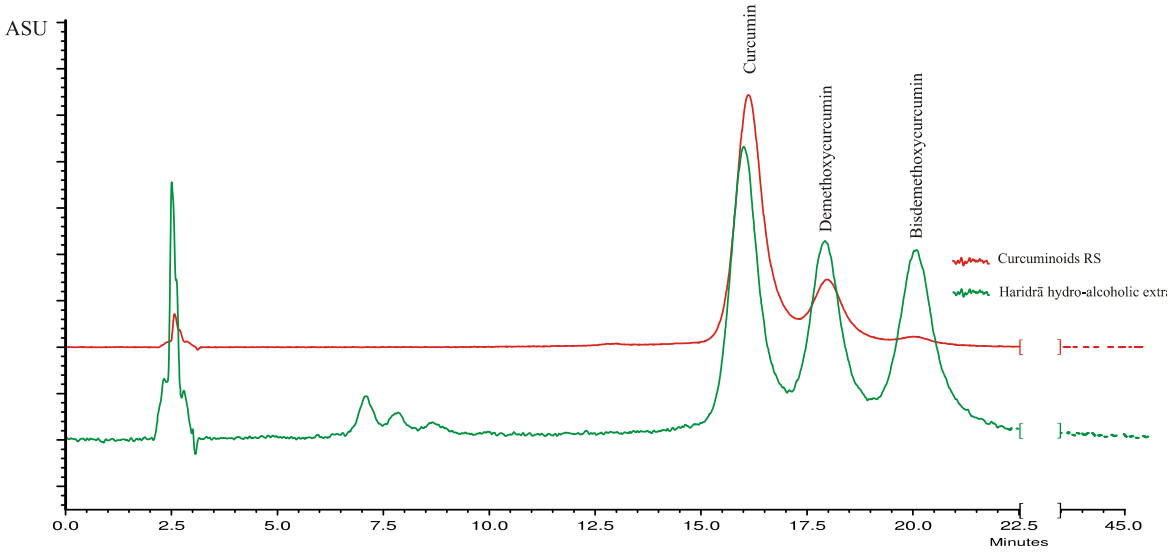


Fig.2. HPLC chromatogram of Haridrā hydro-alcoholic extract with *curcuminoids* as *RS*

chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *curcuminoids* in the substance being examined from the peak response of analytes and from the declared content of *curcuminoids in curcuminoids RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Curcuminoids RS*

HARIDRĀ WATER EXTRACT

Haridrā Water Extract is a dried and powdered extract prepared from Haridrā. The extract contains not less than 0.3 per cent of total curcuminoids when assayed.

Method of Preparation:

Take Haridrā suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 20.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *curcuminoids* as a reference standard. Solvent system: *Chloroform* : *ethanol* : *glacial acetic acid* (9.5 : 0.5 : 0.1). Test solution: To 4 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard solution: Dissolve 1 mg of *curcuminoids RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solution on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 366 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5-10 minutes or till the bands are clearly visible. The chromatogram obtained with the test solution shows a band at R_f ~0.50 corresponding to that of *curcumin*, R_f ~0.45 corresponding to that of *demethoxycurcumin* and at R_f ~0.30 corresponding to that of *bisdemethoxycurcumin* and the profile should be similar to the one given in the TLC (Fig. 1).

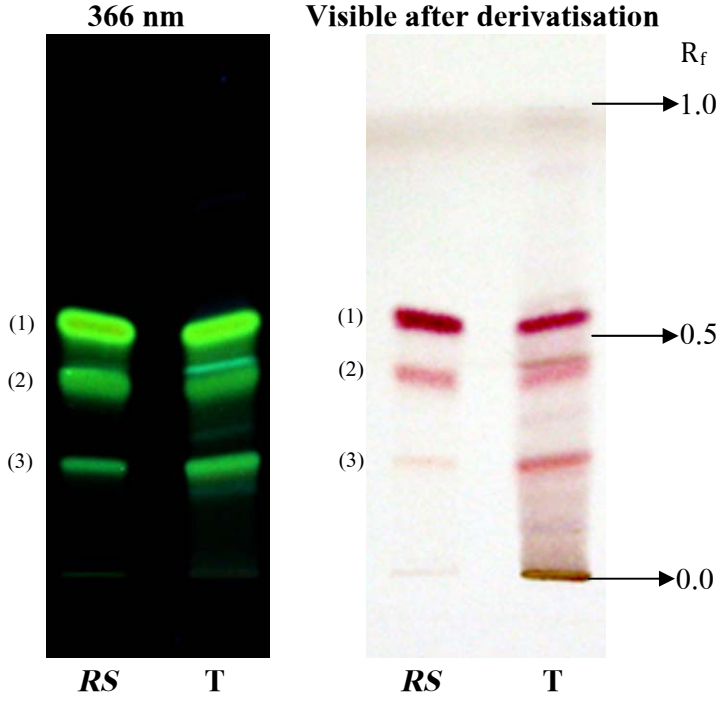


Fig.1. Thin-Layer Chromatogram of Haridrā water extract. *RS*: *Curcuminoids* [(1) *Curcumin*, (2) *demethoxycurcumin*, (3)*bisdemethoxycurcumin*] T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 30.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
pH:	5.5 to 7.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 3 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*. Standard solution: Take about 10 mg, accurately weighed, *curcuminoids RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 65 volumes of buffer (prepared by dissolving 10 g of *citric acid* in 1000 ml of *water* and adjusting the *p*H to 3.0 using *ammonia solution*), 35 volumes of *tetrahydrofuran*. Injection volume: 20 µl. Flow rate: 1.2 ml per minute.

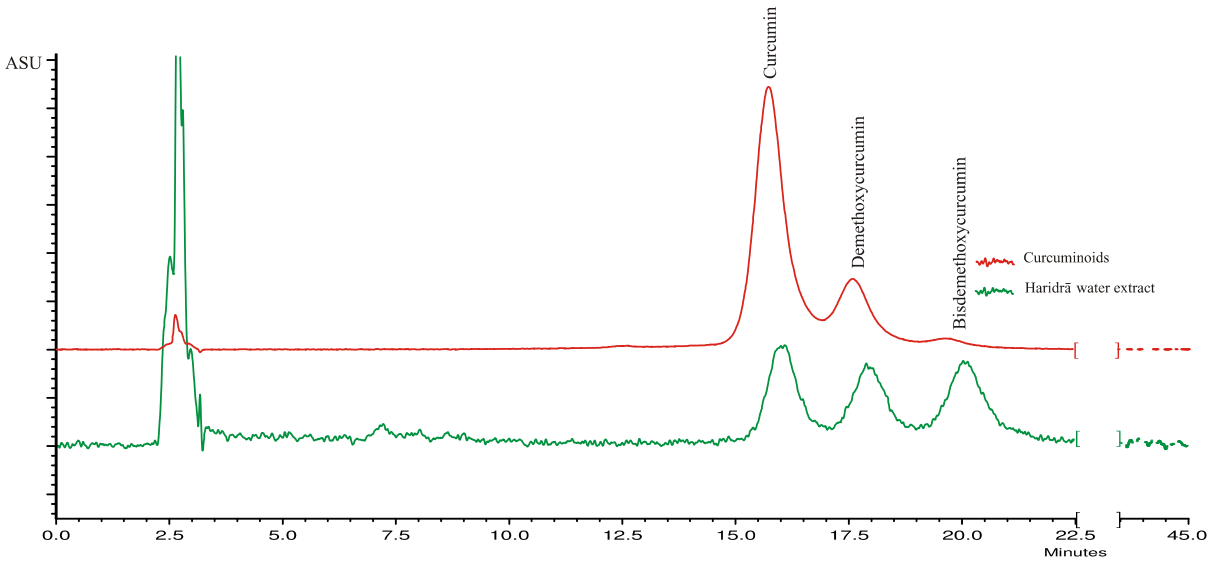


Fig.2. HPLC chromatogram of Haridrā water extract with *Curcuminoids* as *RS*

Detection: UV, 430 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *curcuminoids* in the substance being examined from the peak response of analytes and from the declared content of *curcuminoids* in *curcuminoids RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name and, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Curcuminoids RS*

HARĪTAKĪ

Harītakī consists of pericarp of the mature fruit of *Terminalia chebula* Retz. (Fam. Combretaceae), a moderate sized or large tree found throughout India, chiefly in deciduous forests, but occasionally also in slightly moist forests, upto about 1500 m elevation; flowers appear from April-August and fruits ripen from October-January; seed are removed, and only the pericarp is used, although in trade entire fruits are sold. Harītakī contains not less than 15.0 per cent of total polyphenols calculated as *chebulinic acid* [sum of *corilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid*] when assayed.

Synonyms: Abhayā, Kāyasthā, Śivā, Pathyā, Vijayā (Not Bhaṅgā)

Regional Language Names:

Assamese	:	Shilikha
Bengali	:	Haritaki
English	:	Myrobalan
Gujarati	:	Hirdo, Himaja, Pulo-harda
Hindi	:	Harre, Harad, Harar
Kannada	:	Alalekai
Kashmiri	:	Halela
Malayalam	:	Katukka
Marathi	:	Hirda, Haritaki, Harda, Hireda
Oriya	:	Harida
Punjabi	:	Halela, Harar
Tamil	:	Kadukkai
Telugu	:	Karaka, Karakkaya
Urdu	:	Halela

Description:

a) *Macroscopic:*

Fruits are broken by crushing and seeds removed; pericarp occurs in pieces of various sizes; taste, astringent and bitter.

b) *Microscopic:*

TS of pericarp shows epicarp consisting of one layer of epidermal cells, inner tangential and upper portions of radial wall thick; mesocarp, 2 to 3 layers of collenchyma, followed by a broad zone of parenchyma in which are scattered groups of fibres and sclereids and vascular bundles; fibres present with peg like outgrowth and simple pitted walls; sclereids of various shapes and sizes but mostly elongated; tannins, raphides and rosette crystals of calcium oxalate occur in parenchyma; endocarp consists of thick-walled sclereids of various shapes and sizes, mostly elongated; surface view of epidermis reveal polygonal, beaded cells; several of them divided into two by a thin septa;

starch grains simple and compound with 2 to 8 components rounded or oval in shape, measuring 2 to 7 μ in diameter, found in plenty in almost all cells of mesocarp.

c) Powder:

Fine powder shows a few fibres, with peg-like out growth, vessels with simple pits and groups of sclereids; starch grains simple, and compound consisting of 2 to 8 components, rounded to oval, simple component measuring 2 to 7 μ in diameter (Fig.1).

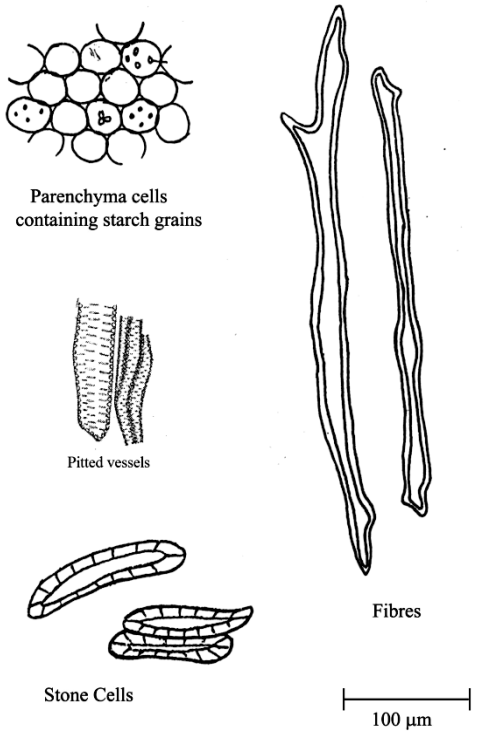


Fig.1. Powdered drug of Harītakī (*Terminalia chebula* Retz.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 1.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 6.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 3.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 40.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 60.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *gallic acid* and *chebulinic acid* as reference standards. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 125 mg of the substance being examined, add 25 ml

of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *gallic acid RS* and *chebulinic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and at R_f ~0.20 corresponding to that of *chebulinic acid* and the profile should be similar to the one given in the TLC (Fig.2).

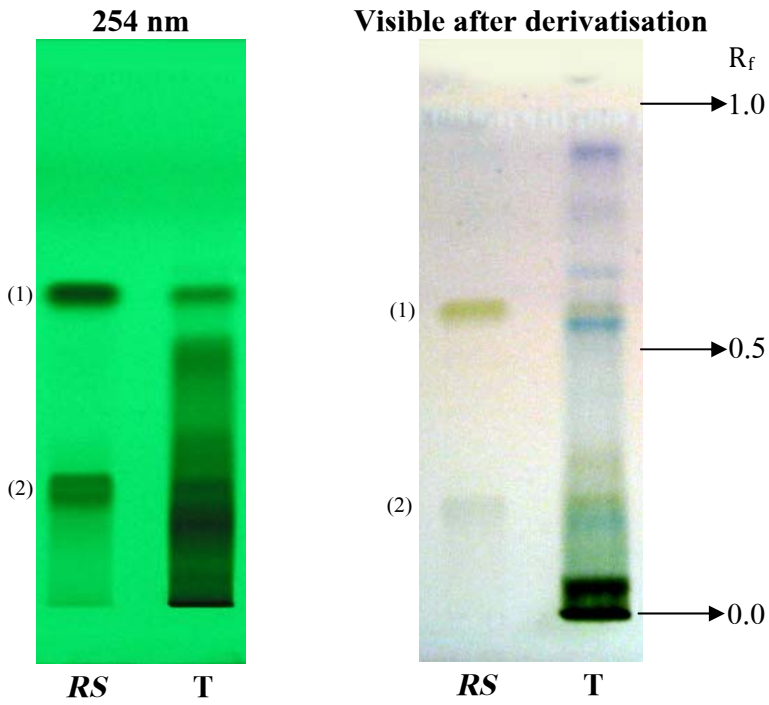


Fig.2. Thin-Layer Chromatogram of Harītakī
RS: (1) Gallic acid and (2) Chebulinic acid,
T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6).Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 5 mg, accurately weighed, *chebulinic acid RS* in a 10-ml volumetric flask and dissolve in 5 ml of *water* and dilute to 10 ml with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Chebulinic acid	1.00
Corilagin	0.70
1,3,6-Trigalloyl glucose	0.79
Chebulagic acid	0.88

Calculate the content of total polyphenols in the substance being examined from the sum of the peak areas of *corilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid* and from the declared content of *chebulinic acid* in *chebulinic acid RS*.

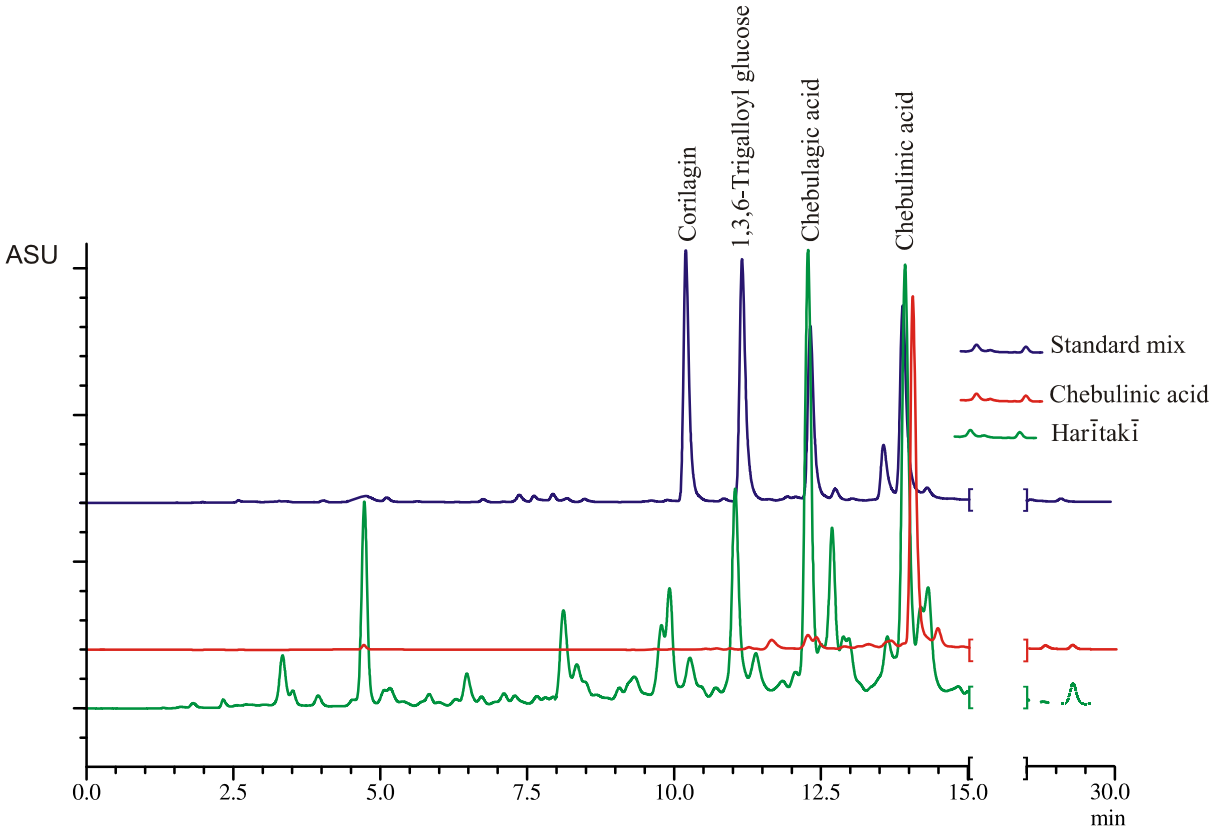


Fig.3. HPLC chromatograms of Harītakī with *Chebulinic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Gallic acid RS* and *Chebulinic acid RS.*

Constituents: Tannins; chebulagic acid, chebulic acid, chebulinic acid, ellagic acid, gallic acid; terchebin, ellagi tannin, terchebulin and syryngic acid.

Properties and Action:

Rasa	:	Kaṣāya, Kaṭu, Tikta, Amla, Madhura
Guṇa	:	Laghu, Rūkṣa
Vīrya	:	Uṣṇa
Vipāka	:	Madhura
Karma	:	Sarvadoṣapraśamana, Rasāyana, Cakṣuṣya, Dīpana, Anulomana, Hṛdya, Medhya

Important formulations: Abhayāriṣṭa; Agastya harītakī rasāyana; Citraka harītakī; Dantī harītakī; Daśamūla harītakī; Brāhma rasāyana; Triphalā cūrṇa; Triphalādi taila; Abhayā lavaṇa; Pathyādi lepa.

Therapeutic uses: Vibandha (constipation); Aruci (tastelessness); Udāvarta (upward movement of gases); Gulma (abdominal lump); Udararoga (diseases of abdomen); Arśa (piles); Pāṇḍu (anaemia)); Śotha (inflammation); Jīrṇajvara (chronic fever); Viṣamajvara (intermittent fever); Prameha (increased frequency and turbidity of urine); Śiroroga (diseases of head); Kāsa (cough); Tamakaśvāsa (bronchial asthma); Hṛdroga (heart disease).

Dose: 3 to 6 g of the drug in powder form.

HARĪTAKĪ (POWDER)

Harītakī (Powder) consists of powder of Harītakī complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, yellowish-brown; odour characteristic; taste astringent; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Groups of elongated thick walled sclereids with pits and broad lumen, starch grains simple rounded or oval in shape measuring up to 7 μ in diameter, found in plenty in almost all cells of mesocarp, rosette crystals of calcium oxalate up to 25 μ in size; criss-cross thin walled fibres with broad lumen and pegged tips, thin walled parenchyma cells, polygonal epidermal cells with slightly beaded wall and transverse septa; stone cells with wide lumen and pits.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Harītakī.

Assay: Complies with the limits for Assay as per method stated under Harītakī.

Additional requirements:

Packaging and Storage: As given under Harītakī.

Labeling: As given under Harītakī.

API reference standards: As given under Harītakī.

HARĪTAKĪ HYDRO-ALCOHOLIC EXTRACT

Harītakī Hydro-alcoholic Extract is a dried and powdered extract prepared from Harītakī. The extract contains not less than 25.0 per cent of total polyphenols calculated as *chebulinic acid* [sum of *corilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid*] when assayed.

Method of Preparation:

Take Harītakī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 50.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *gallic acid* and *chebulinic acid* as reference standards. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 125 mg of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *gallic acid RS* and *chebulinic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and at R_f ~0.20 corresponding to that of *chebulinic acid* and the profile should be similar to the one given in the TLC (Fig.1).

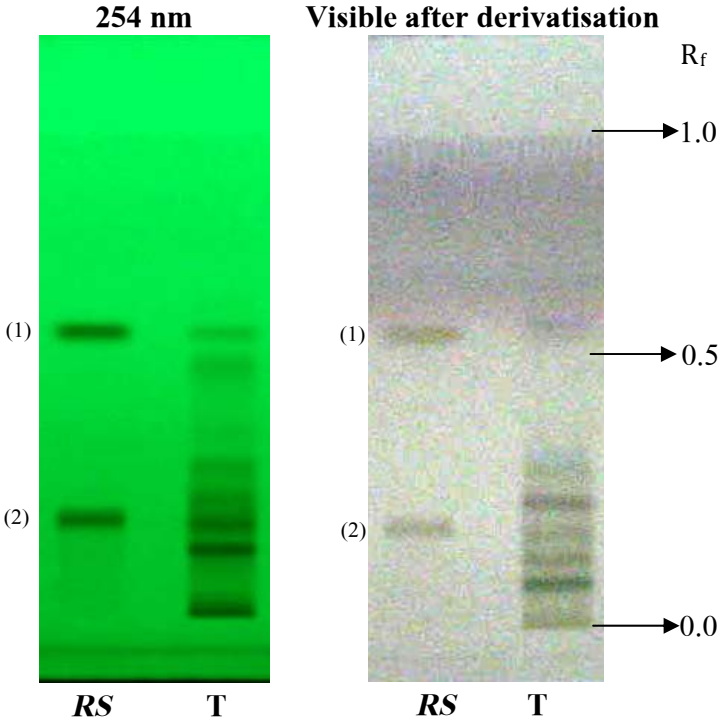


Fig.1. Thin-Layer Chromatogram of Harītakī hydro-alcoholic extract. *RS*: (1) *Gallic acid* and (2) *Chebulinic acid,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	3.0 to 4.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml with *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 5 mg, accurately weighed, *chebulinic acid RS* in a 10-ml volumetric flask and dissolve in 5 ml of *water* and dilute to 10 ml with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Chebulinic acid	1.00
Corilagin	0.70
1,3,6-Trigalloyl glucose	0.79
Chebulagic acid	0.88

Calculate the content of total polyphenols in the substance being examined from the sum of the peak areas of *corilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid,* and from the declared content of *chebulinic acid* in *chebulinic acid RS*.

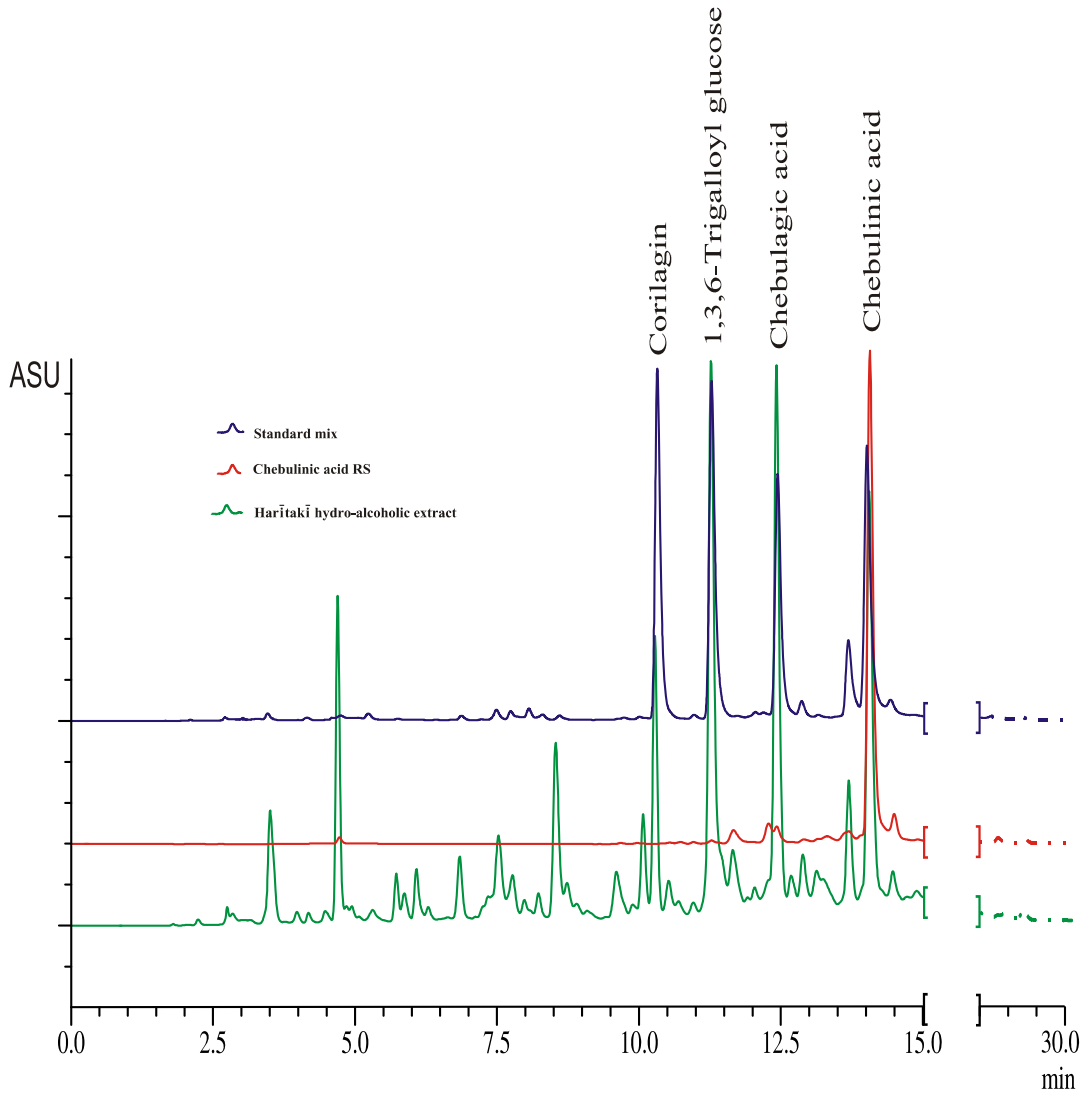


Fig.2 HPLC chromatograms of Harītakī hydro-alcoholic extract with *Chebulinic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS* and *Chebulinic acid RS.*

HARĪTAKĪ WATER EXTRACT

Harītakī Water Extract is a dried and powdered extract prepared from Harītakī. The extract contains not less than 20.0 per cent of total polyphenols calculated as *chebulinic acid* [sum of c*orilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid*] when assayed.

Method of Preparation:

Take Harītakī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 45.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *gallic acid* and *chebulinic acid* as reference standards. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (2.0 : 5.0 : 1.5). Test solution: To 125 mg of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *gallic acid RS* and *chebulinic acid RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *gallic acid* and at R_f ~0.20 corresponding to that of *chebulinic acid* and the profile should be similar to the one given in the TLC (Fig.1).

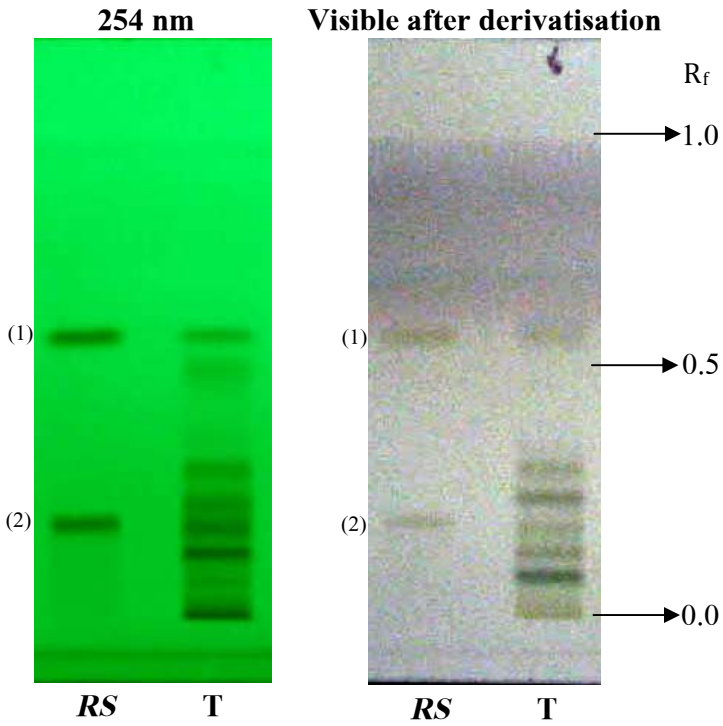


Fig.1. Thin-Layer Chromatogram of Harītakī water extract. *RS*: (1) *Gallic acid* and (2) *Chebulinic acid,* T: Test soultion

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	3.0 - 4.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 0.4 g, accurately weighed, of the substance being examined and reflux with 25 ml *water* on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *water* for two times more, cool and filter. Combine all the filtrates and transfer to 100-ml volumetric flask, and make up the volume. Standard solution: Take about 5 mg, accurately weighed, of *chebulinic acid RS* in a 10-ml volumetric flask and dissolve in 5 ml of *water* and dilute to 10 ml with *water*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:-

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	95	5
18	65	35
25	45	55
30	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 270 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Chebulinic acid	1.00
Corilagin	0.70
1,3,6-Trigalloyl glucose	0.79
Chebulagic acid	0.88

Calculate the content of total polyphenols in the substance being examined from the sum of the peak areas of *corilagin*, *1,3,6-trigalloyl glucose*, *chebulagic acid* and *chebulinic acid,* and from the declared content of *chebulinic acid* in *chebulinic acid RS*.

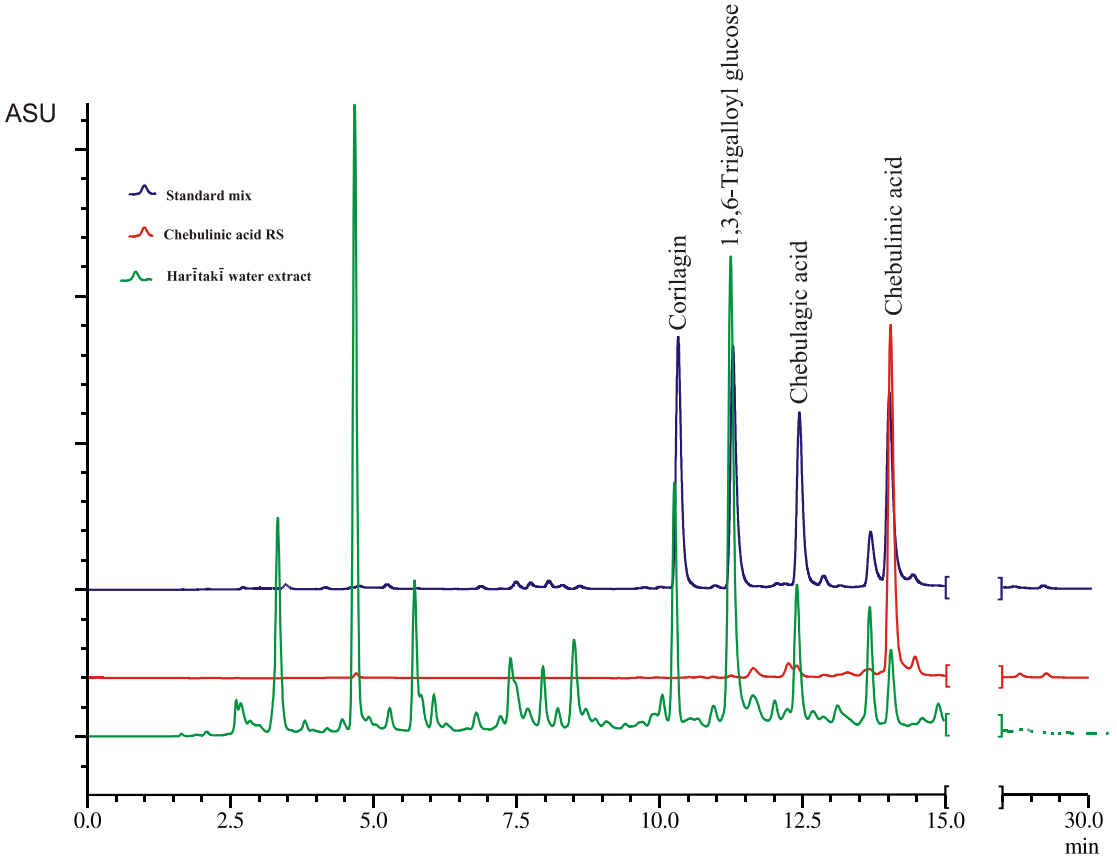


Fig.2. HPLC chromatograms of Harītakī water extract with *Chebulinic acid* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Gallic acid RS* and *Chebulinic acid RS.*

KĀLAMEGHA

Kālamegha consists of aerial parts of *Andrographis paniculata* (Burm.f.) Wall. ex Nees (Fam. Acanthaceae), is a dark green annual herb with quadrangular stem and white flowers found wild in barren lands and cultivated fields throughout India upto an altitude of 600 m. Kālamegha contains not less than 1.0 per cent of *Andrographolide* when assayed.

Synonyms: Bhūnimba

Regional Language Names:

Assamese	:	Kalmegh
Bengali	:	Kalmegh
English	:	Green chiretta, Kalmegh
Gujarati	:	Kariyatu, Kirayata, Kiriyati
Hindi	:	Kalmegh, Kalpanatha, Kiriyat
Kannada	:	Nelaberu
Kashmiri	:	--
Malayalam	:	Nilaveppu, Kiriyatta, Nelavepu
Marathi	:	Aalen kiraiyit, Oli-kiryat
Oriya	:	Bhuinimo
Punjabi	:	--
Tamil	:	Nilavempu, Nilavembu
Telugu	:	Nelavemu
Urdu	:	--

Description:

a) *Macroscopic*:

Commercial sample consists of chopped pieces of the whole plant with stem, roots, leaves, inflorescence stalk, flowers and fruits along with a few seeds.

Stem - Dark green, hard, quadrangular and narrowly winged in upper parts; glabrous about 6 mm thick; scars of detached leaves opposite and decussate; node swollen; fracture short; taste bitter.

Leaf - Present in broken pieces, fragile, deep green in adaxial surface, paler on the abaxial surface; winged, short petiole of about 8 mm long present; tip acute to acuminate.

Flower - Small, zygomorphic, pedicellate with a stalk of about a centimeter; solitary detached or intact on lax, spreading axillary or terminal racemes or panicles; bracts present, lanceolate, about 5 mm long; sepal linear – lanceolate, glandular, pubescent; corolla two – lipped with a slightly enlarged tube; generally white with purplish tinge; outer surface of petal hairy, upper lip two lobed, lower three lobed.

Fruit - A capsule; linear, pubescent with yellowish brown numerous seeds supported on retinacula; seeds subquadrate rugose.

b) *Microscopic:*

Stem- TS square in outline with four arrow winged; epidermis with a thin cuticle and few glandular trichomes; cortex narrow; collenchymatous tissue present in the wings and below the epidermis; rest of cortex is parenchymatous containing chlorophyll; epidermal cells contain cystoliths; endodermis present; phloem narrow with a few isolated fibres and small accicular calcium oxalate crystals present occasionally; xylem wide, vessels scattered; accicular fibres and tracheids present; pith present small, with occasional cystoliths and acicular crystals of calcium oxalate.

Leaf -

Petiole -TS shows lateral wings; epidermis followed by collenchyma and chlorenchymatous tissue; two vascular bundle present in each of the wings on the adaxial side; meristele central in the form of an arc surrounded by parenchyma; small acicular crystals of calcium oxalate present in the parenchymatous tissue.

Midrib - TS shows convex outline on the adaxial surface and a laterally flattened outline on the abaxial side, several cells of epidermis contains cystoliths, followed by collenchyma adjacent to both upper and lower epidermis, meristele similar to that of petiole.

Lamina - Dorsiventral; epidermal cells contains cystoliths; surface view of upper epidermis shows straight walled cells; with abundant diacytic stomata lower epidermal cells sinuous trichomes present, ordinary with one to three cells more on the margins; glandular with unicellular stalk and multicellular head present. Pallisade of one layer continues below the midrib also.

c) *Powder*:

Shows abundant simple, uni-and 2 to 3 celled multicellular short and long trichomes; glandular trichomes with short and long multicellular stalk and multicellular head from leaf, stem and all parts of the flower; long acicular fibres from xylem region of stem, epidermal cells of the leaf and petiole in surface view embedded with diacytic stomata and cystolith; parenchymatous cells of the pith of stem embedded with acicular crystals of calcium oxalate and simple starch grains; fragments of spiral, pitted and scalariform vessels, polygonal thick-walled cells of the corolla tube, pollen grains and sclereidal layers from the stamen; fragments of cotyledon and endosperm, fibres and sclereids from the fruit wall (Fig.1).

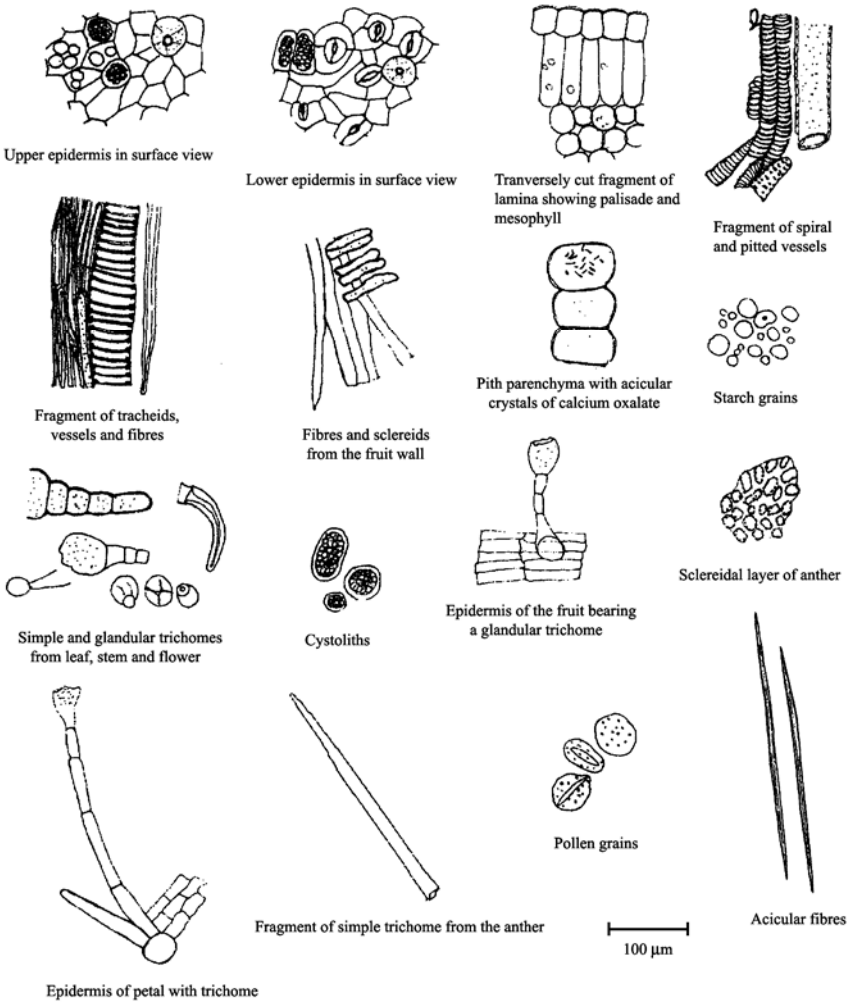


Fig.1. Powdered drug of Kālamegha (*Andrographis paniculata* (Burm.f.) Wall. ex Nees)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 15.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 3.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 12.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 19.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *andrographolide* and *andrograpanin* as reference standards. Solvent system: *Toluene* : *acetone* : *formic acid* (6.0 : 4.0 : 0.4). Test solution: To 2 g of the substance being examined, add 25 ml of

methanol, heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *andrographolide RS* and 3 mg of *andrograpanin RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *andrograpanin* and at R_f ~0.40 corresponding to that of *andrographolide* and the profile should be similar to the one given in the TLC (Fig. 2).

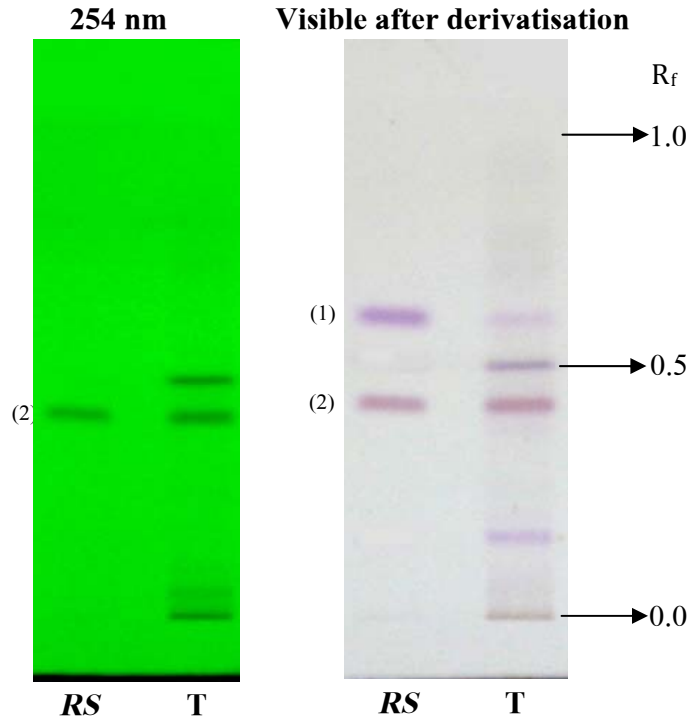


Fig. 2. Thin-Layer Chromatogram of Kālamegha *RS*: (1) *Andrograpanin* and (2) *Andrographolide,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *andrographolide RS* in a 50-ml volumetric flask, dissolve in 25 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions.

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 223 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *andrographolide* in the substance being examined from the peak response of analyte and from the declared content of *andrographolide* in *andrographolide RS*.

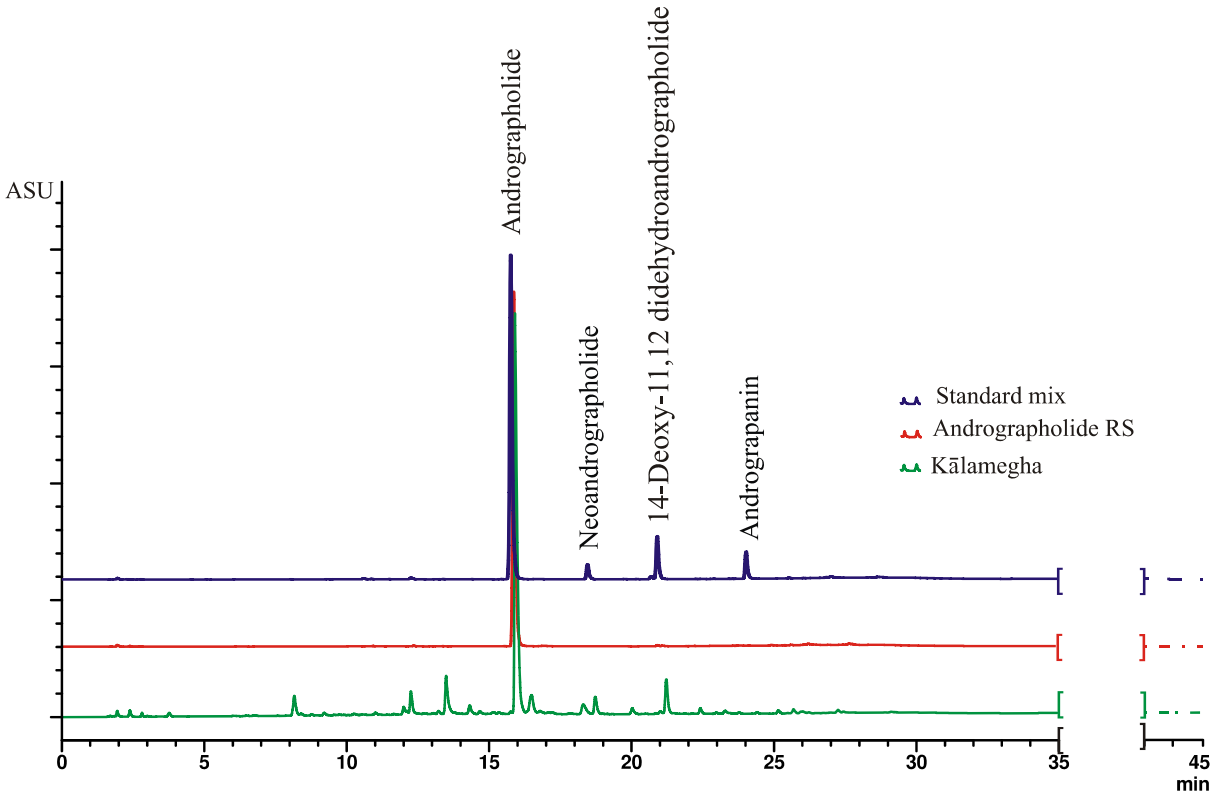


Fig.3. HPLC chromatograms of Kālamegha with Andrographolide as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Andrographolide RS* and *Andrographanin RS.*

Constituents: Andrographolide, neoandrographolide, andrographanin. 14-deoxy-11,12-didehydroandrographolide

Properties and Action:

Rasa	:	Tikta
Guṇa	:	Laghu, Rūkṣa
Vīrya	:	Śīta
Vipāka	:	Kaṭu
Karma	:	Dīpana, Jvaraghna, Kṛmighna, Kāsaghna, Pācana, Raktamokṣaṇa

Important formulations: Kālameghāsava.

Therapeutic uses: Ajīrṇa (dyspepsia); Arśa (piles); Atisāra (diarrhoea); Jvara (fever); Kaṇḍū (itching); Kāmalā (jaundice); Kuṣṭha (diseases of skin); Prameha (increased frequency and turbidity of urine); Pravāhikā (dysentery); Tvakvikāra (skin disorders); Vraṇa (wound); Yakṛtvikāra (disorders of liver).

Dose: 1 to 3 g of the drug in powder form.

KĀLAMEGHA (POWDER)

Kālamegha (Powder) consists of powder of Kālamegha complying with the following requirements:

Description:

a) *Macroscopic*:

Coarse powder, greenish brown; odour characteristic; taste bitter; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic*:

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of acicular fibres from xylem region of stem, fragment of spiral, pitted and scalariform vessels; a few thin walled striated cork in sectional view; parenchymatous pith cells containing minute acicular crystals of calcium oxalate and starch grains measuring up to 8μ; fragments of epidermis with numerous diacytic stomata; abundant cystoliths; uni, bi, and tri cellular short and long trichomes; glandular trichomes short and long with multicellular stalk and head; fragments of fibres and sclereids; a few oval to spherical shaped pollen grains.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Kālamegha.

Assay: Complies with the limits for Assay as per method stated under Kālamegha.

Additional requirements:

Packaging and Storage: As given under Kālamegha.

Labeling: As given under Kālamegha.

API reference standards: As given under Kālamegha.

KĀLAMEGHA HYDRO-ALCOHOLIC EXTRACT

Kālamegha Hydro-alcoholic Extract is a dried and powdered extract prepared from Kālamegha. The extract contains not less than 5.0 per cent of *andrographolides* when assayed.

Method of preparation:

Take Kālamegha suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 13.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *andrographolide and andrograpanin* as reference standards. Solvent system: *Toluene* : *acetone* : *formic acid* (6.0 : 4.0 : 0.4). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *andrographolide RS* and 3 mg of *andrograpanin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *andrograpanin* and at R_f ~0.40 corresponding to that of *andrographolide* and the profile should be similar to the one given in the TLC (Fig. 1).

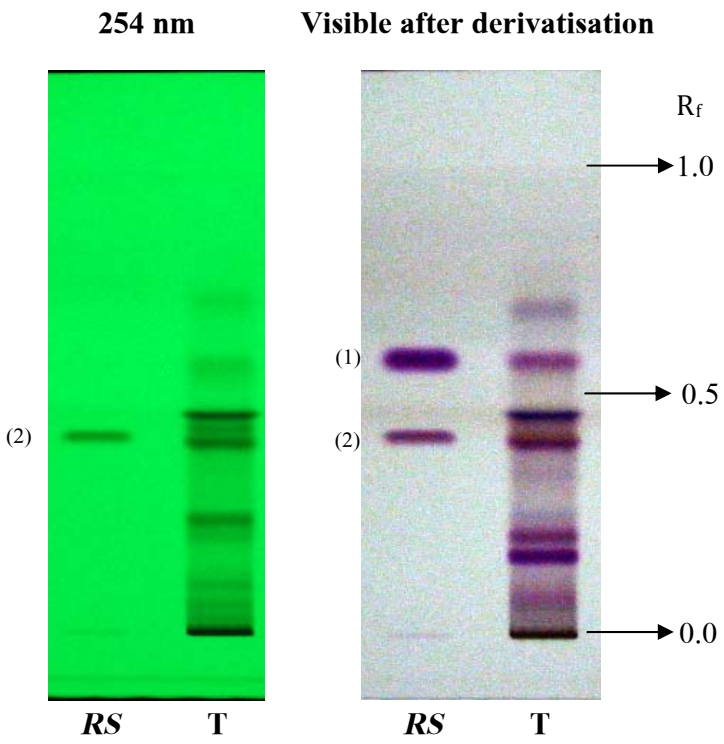


Fig. 1.Thin-Layer Chromatogram of Kālamegha hydro-alcoholic extract. *RS*: (1) *Andrograpanin* and (2) *Andrographolide*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 28.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7
pH:	6.0 - 8.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 4 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *andrographolide RS* in a 50-ml volumetric flask, dissolve in 25 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions.

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 223 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention time, as below.

Analyte	Relative retention time
Andrographolide	1.0
Neoandrographolide	1.2
14-Deoxy-11,12-didehydroandrographolide	1.4
Andrograpanin	1.6

Calculate the content of *andrographolides* (sum the peak areas of all the four peaks) in the substance being examined from the peak response of analytes and from the declared content of *andrographolide* in *andrographolide RS*.

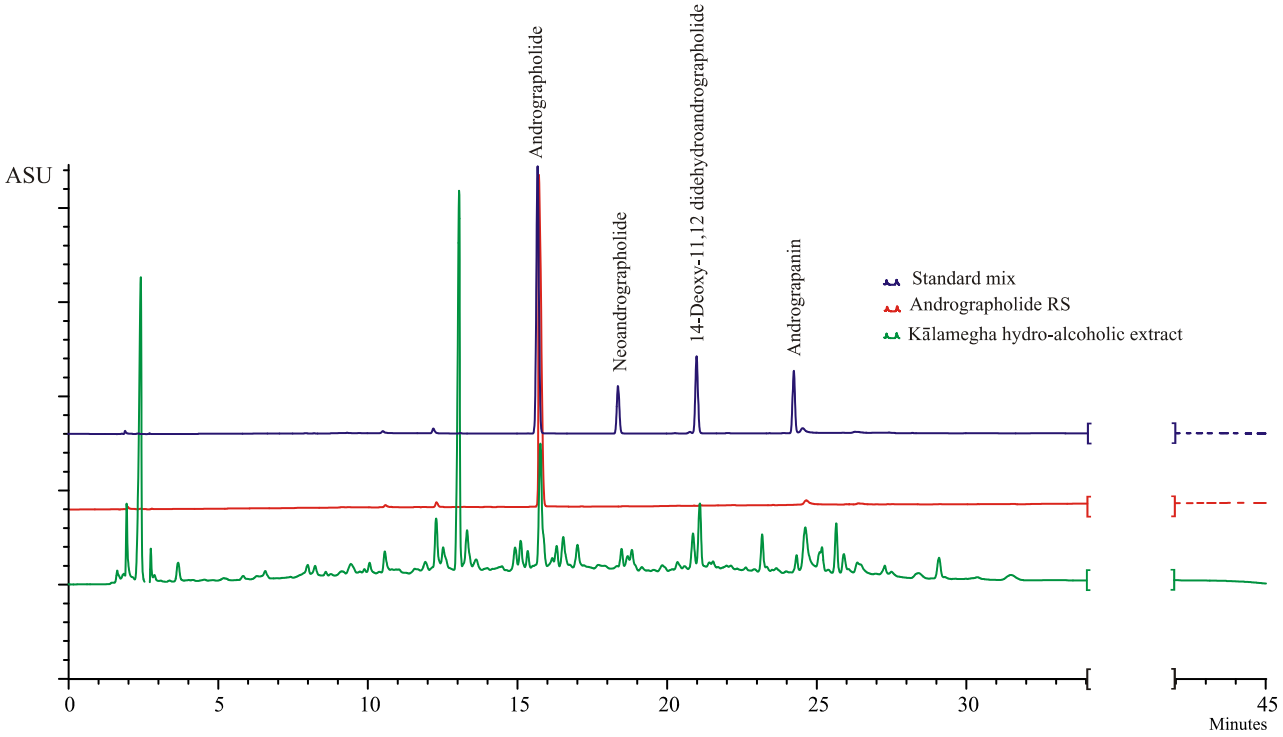


Fig.2. HPLC chromatograms of Kālamegha hydro-alcoholic extract with Andrographolide as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Andrographolide RS* and *Andrograpanin RS.*

KĀLAMEGHA WATER EXTRACT

Kālamegha Water Extract is a dried and powdered extract prepared from Kālamegha. The extract contains not less than 0.5 per cent of *andrographolides* when assayed.

Method of preparation:

Take Kālamegha suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80–85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400–600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh and pack. The yield obtained is about 16.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *andrographolide and andrograpanin* as reference standards. Solvent system: *Toluene* : *acetone* : *formic acid* (6.0 : 4.0 : 0.4). Test solution: To 4 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 10-15 minutes, cool and filter. Standard solution: Dissolve 10 mg of *andrographolide RS* and 3 mg of *andrograpanin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of *anisaldehyde sulphuric acid reagent.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *andrograpanin* and at R_f ~0.40 corresponding to that of *andrographolide* and the profile should be similar to the one given in the TLC (Fig. 1).

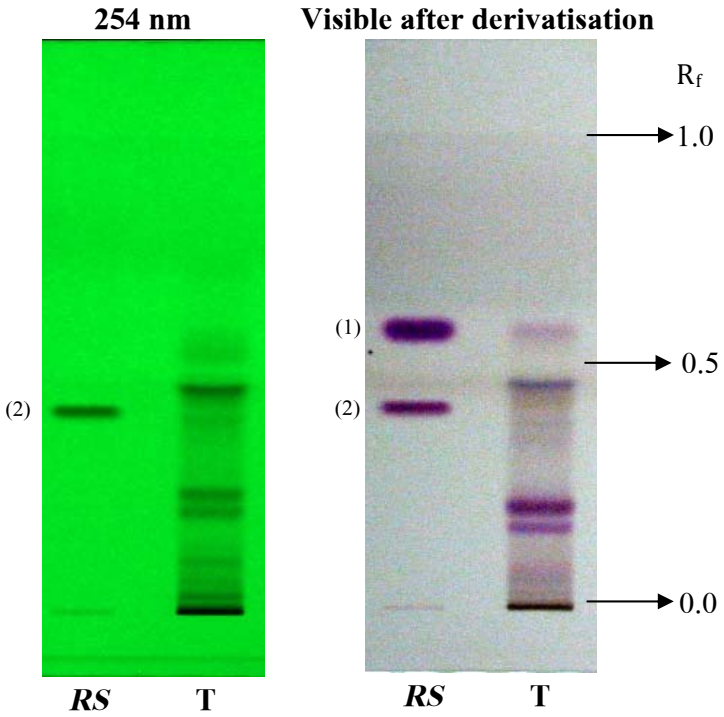


Fig.1. Thin-Layer Chromatogram of Kālamegha water extract. *RS*: (1) *Andrograpanin* and (2) *Andrographolide*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 32.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 7.0 per cent,	Appendix 2.1.7
pH:	5.5 - 7.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 4 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *andrographolide RS* in a 50-ml volumetric flask and dissolve in 25 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml and *acetonitrile* in the following proportions.

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
18	55	45
25	20	80
28	20	80
35	55	45
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 223 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention time, as below.

Analyte	Relative retention time
Andrographolide	1.0
Neoandrographolide	1.2
14-Deoxy-11,12-didehydroandrographolide	1.4
Andrograpanin	1.6

Calculate the content of *andrographolides* (sum the peak areas of all the four peaks) in the substance being examined from the peak response of analytes and from the declared content of *andrographolide* in *andrographolide RS.*

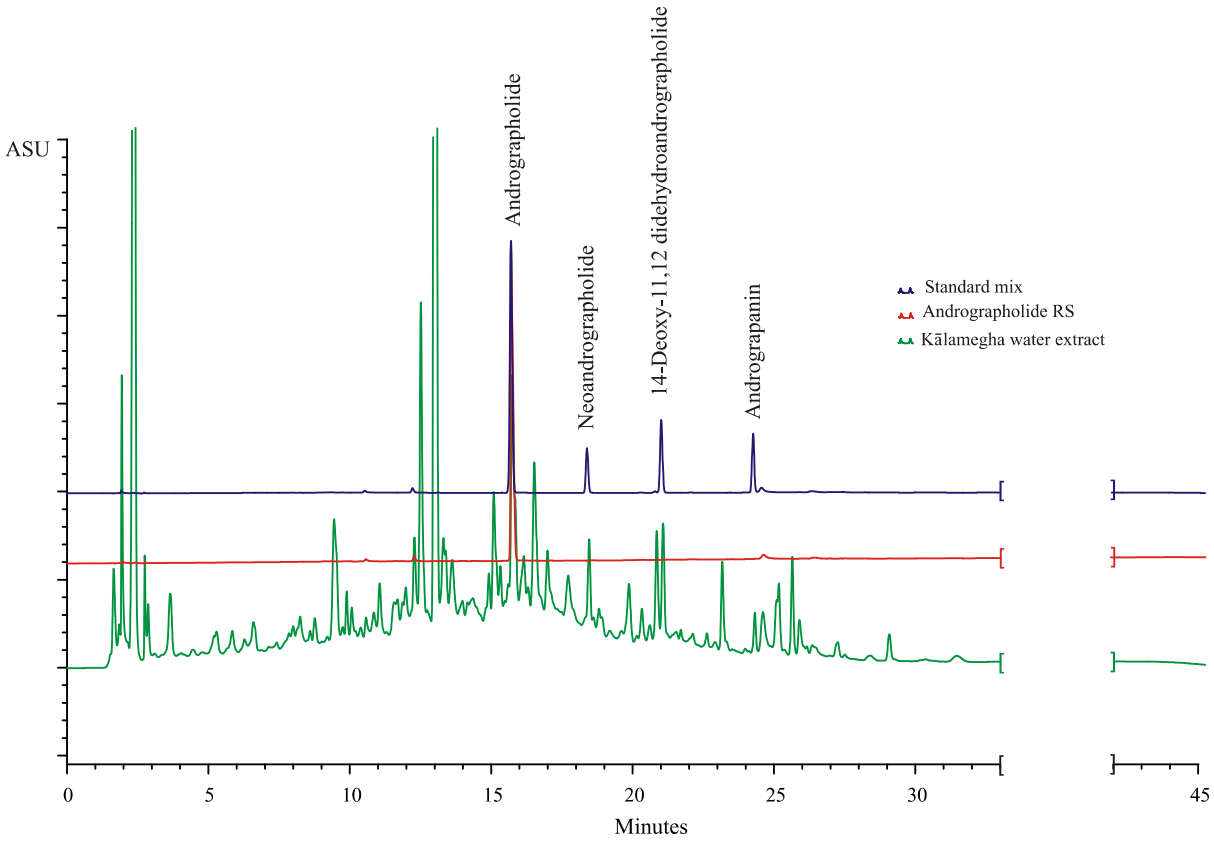


Fig.2. HPLC chromatograms of Kālamegha water extract with *Andrographolide* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the the part of the plant used and type of extract.

API reference standards: API *Andrographolide RS* and *Andrograpanin RS.*

KAṆṬAKĀRĪ

Kaṇṭakārī consists of mature, dried whole plant of *Solanum surattense* Burm. f. syn. *Solanum xanthocarpum* Schrad. & Wendl. (Fam. Solanaceae), a perennial, very prickly diffused herb of wasteland, found throughout India. Kaṇṭakārī contains not less than 0.02 per cent of *solasodine* when assayed.

Synonyms: Vyāghrī, Nidigdhikā, Kṣudrā, Kaṇṭakārikā, Dhāvanī, Nidigdha, Duḥsparśā

Regional Language Names:

Assamese	:	Katvaedana, Kantakar
Bengali	:	Kantakari
English	:	Febrifuge plant
Gujarati	:	Bharingani
Hindi	:	Katai, Katali, Ringani, Bhatakataiya, Chhotikateri
Kannada	:	Nelagulla, Kiragulla
Kashmiri	:	---
Malayalam	:	Kantakari chunda
Marathi	:	Bhauringani, Kataringani
Oriya	:	Bhejibaugana, Ankarati, Chakada Bhoji
Punjabi	:	Kandiari
Tamil	:	Kandangatri, Kandankatri, Kandanghathiri
Telugu	:	Nelamulaka, Pinnamulaka, Mulaka, Chinnamulaka, Vakudu
Urdu	:	---

Description:

a) *Macroscopic:*

Root – 10 to 45 cm long, upto 2 cm thick, almost cylindrical and tapering, bearing a number of fine longitudinal and few transverse wrinkles with occasional scars of a few lenticels and small rootlets; transversely smoothened surface shows a thin bark and a wide compact cylinder of wood; fracture, short; taste, bitter.

Stem – Herbaceous, glabrous, prickly with prominent nodes and internodes; stem pieces 8 to 10 mm thick of variable lengths, external surface yellowish green and smooth; transversely smoothened surface shows a very thin bark and prominent wood, centre shows a large and distinct pith, often hollow; fracture, short to slightly fibrous.

Leaf – Petiolate, exstipulate, ovate-oblong or elliptic, sinuate or sub-pinnatifid, sub-acute hairy; 4 to 12.5 cm long and 2 to 7.5 cm wide; green; veins and midrib covered with sharp prickles.

Flower – Ebracteate, pedicellate, bisexual, pentamerous, regular, complete, bright blue or bluish purple; calyx-persistent, gamosepalous, tube short, globose, linear-lanceolate, acute, hairy, 0.5 to 1.3 cm long and densely prickly; corolla-gamopetalous, lobes deltoid, acute, hairy; 1 to 2 cm long and purple in colour; stamens 5, epipetalous, basifixed, filament short, 1 to 1.5 mm long; anther,

oblong lanceolate, 0.7 to 0.8 cm long; ovary superior, ovoid, glabrous, bilocular with axile placentation having numerous ovules.

Fruit – Berry yellow, globular, measuring upto 1 cm in diameter, surrounded by persistent calyx at base; unripe fruits when present, variegated with green and white strips.

Seed – Circular, flat, numerous, embedded in a fleshy mesocarp, about 0.2 cm in diameter, glabrous, taste, bitter and acrid.

b) *Microscopic:*

Root – TS of mature root shows cork composed of 3 to 6 layers of thin-walled, rectangular and tangentially elongated cells; cork cambium single layered followed by 6 to 15 layers of thin-walled, tangentially elongated to oval or circular parenchymatous cells of cortex; stone cells either single or in groups of 2 to 20 or even more present in this region and also in the secondary phloem composed of sieve elements and phloem parenchyma traversed by medullary rays; phloem rays 1 to 4 cells wide and 2 to 22 cells high; cambium 3 to 5 layered of thin-walled rectangular cells; xylem composed of vessels, tracheids, fibre tracheids, parenchyma and traversed by medullary rays, all elements being lignified; vessels and tracheids with bordered pits; fibres with a few simple pits; xylem parenchyma rectangular or lightly elongated with simple pits and rarely with reticulate thickenings; xylem rays 1 to 3 cells wide and 1 to 20 cells high; microsphenoidal crystals of calcium oxalate as sandy masses and simple starch grains present in secondary cortex, phloem and medullary rays.

Stem – TS of mature stem shows a bicollateral structure; 1.5 to 2 cm thick consists of 6 to 12 layers of cork of thin-walled somewhat rectangular cells; secondary cortex consists of 7 to 11 layers of parenchymatous cells, some cells thickened and lignified forming stone cells; primary cortex remains intact even at quite mature stage but later gets crushed; pericyclic fibres, occur singly or in small groups of 2 or 3; secondary phloem consists of sieve elements, parenchyma, a few fibres, stone cells and traversed by phloem rays; fibres found scattered in singles or in small groups in outer and middle phloem region; inner phloem devoid of fibres; stone cells present in singles or in small groups of 2 to 4; phloem rays, 1 or 2 or rarely 3 cells wide; cambium composed of 2 or 3 layers; xylem consists of vessels, tracheids, parenchyma, fibres and traversed by xylem rays; vessels vary greatly in shape and size and show bordered pits; tracheids elongated with irregular walls and bordered pits; fibres much elongated, thick-walled and lignified with tapering and pointed ends, some having truncated ends or bifurcated at one or both ends with a few simple pits; fibre tracheids with both ends tapering and have reticulate thickenings; xylem parenchyma cubical to rectangular with simple or bordered pits or reticulate thickenings; xylem rays conspicuous by their pitted thickenings, longer size and radial elongation of cells, 1 or 2 or rarely 3 cells wide and 2 or 25 cells high; internal phloem composed of sieve elements and parenchyma, forming more or less a continuous band in the perimedullary zone; a few phloem fibres similar to those of outer phloem region also present; central region occupied by a large pith; microsphenoidal crystals of calcium oxalate as sandy masses and simple starch grains present in cortex, secondary cortex, phloem, medullary rays and pith cells.

Leaf:

Petiole – TS of petiole shows circular to wavy outline; epidermis single layered, covered externally by a thick cuticle; hypodermis consists of 3 or 4 layers of collenchymatous cells; one large-crescent-shaped, bicollateral, central vascular bundle and two small lateral bundles present; rest of tissue of petiole composed of polygonal, angular, thin-walled, parenchymatous cells; epidermis shows mostly stellate and rarely uni to tricellular hairs.

Midrib – TS of midrib shows a biconvex structure; epidermis on either side covered externally by a thick cuticle; below epidermis 3 or 4 layers of collenchyma present; stele composed of crescent-shaped, bicollateral, central vascular bundle and two small lateral vascular bundles; rest of tissue composed of thin-walled, parenchyma, some stellate hair present on epidermis.

Lamina-TS shows a dorsiventral structure; epidermis on either side, wavy in outline, covered externally by a thick cuticle; mesophyll composed of a single layered palisade on upper side and 4 to 6 layers of loosely arranged spongy parenchyma on lower side; 4 to 8 armed stellate hairs present on both surfaces of epidermis; anisocytic stomata present on both surfaces; vein-islet number 46 to 80 on lower epidermis, 61 to 80 on upper epidermis; stomatal index 20 to 25 on lower epidermis, 14 to 24 on upper epidermis, palisade ratio 2 to 4.

Fruit –TS of mature fruit shows single layered epicarp, covered externally by a thin cuticle; 1 or 2 layers of collenchyma present below epicarp; mesocarp composed of thin-walled, oval to polygonal cells; a few vascular bundles scattered in this region seed consists of thick-walled, radially elongated testa, narrow endosperm with embryo; cells of endosperm contain oil globules.

c) Powder:

Fine powder shows single or groups of stone cells, groups of aseptate fibres with tapering ends, pitted vessels, groups of spongy parenchyma, fragments of palisade tissue, anisocytic stomata, stellate hairs; testa irregular in shape in surface view; cell debris containg microsphenoidal crystals of calcium oxalate; simple, rounded to oval starch grains measuring 3 to 11 μ in diameter (Fig.1).

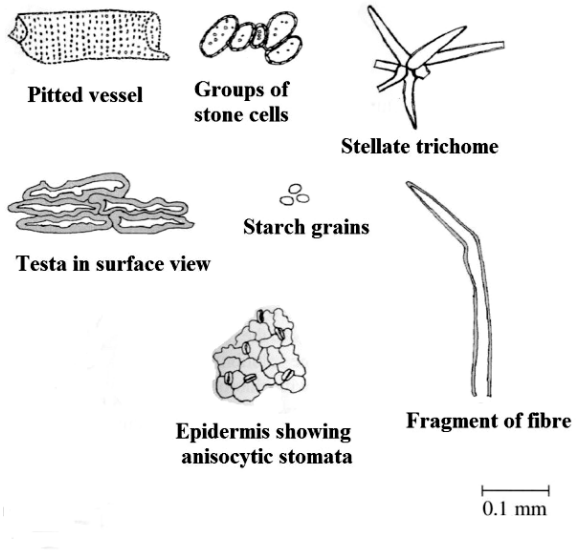


Fig.1. Powdered drug of Kaṇṭakārī (*Solanum surattense* Burm. f.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 9.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 3.0 per cent,	Appendix 2.1.7
Alcohol- soluble extractive:	Not less than 6.0 per cent,	Appendix 2.1.8
Water- soluble extractive:	Not less than 16.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *solasodine* as a reference standard. Solvent system: *Chloroform* : *methanol* (5.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *water*. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask, extract thrice with 25 ml of *chloroform* and combine the *chloroform* layers. Wash the *chloroform* layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10-ml volumetric flask, make up the volume and filter. Standard solution: Dissolve 1 mg of *solasodine RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *solasodine* and the profile should be similar to the one given in the TLC (Fig. 2).

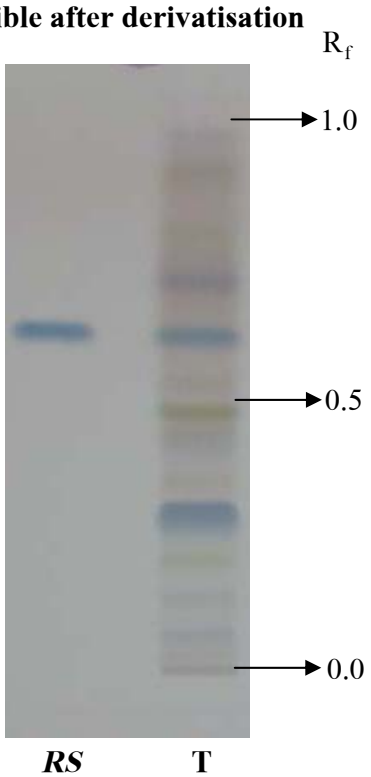


Fig.2. Thin-Layer Chromatogram of Kaṇṭakārī. *RS*: *Solasodine*, T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 5 g, accurately weighed, of the substance being examined, add 25 ml of *water* and sonicate for 5 minutes. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask and extract thrice with 25 ml of *chloroform* and combine the chloroform layers. Wash the chloroform layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10 ml volumetric flask, make up the volume and filter. Standard solution: Take about 1 mg, accurately weighed, *solasodine RS* in a 10-ml volumetric flask, dissolve in 5 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	75	25
15	45	55
20	20	80
25	75	25
30	75	25

Injection volume: 20 μl. Flow rate: 1.6 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *solasodine* in the substance being examined from the peak response of analyte and from the declared content of *solasodine* in *solasodine RS*.

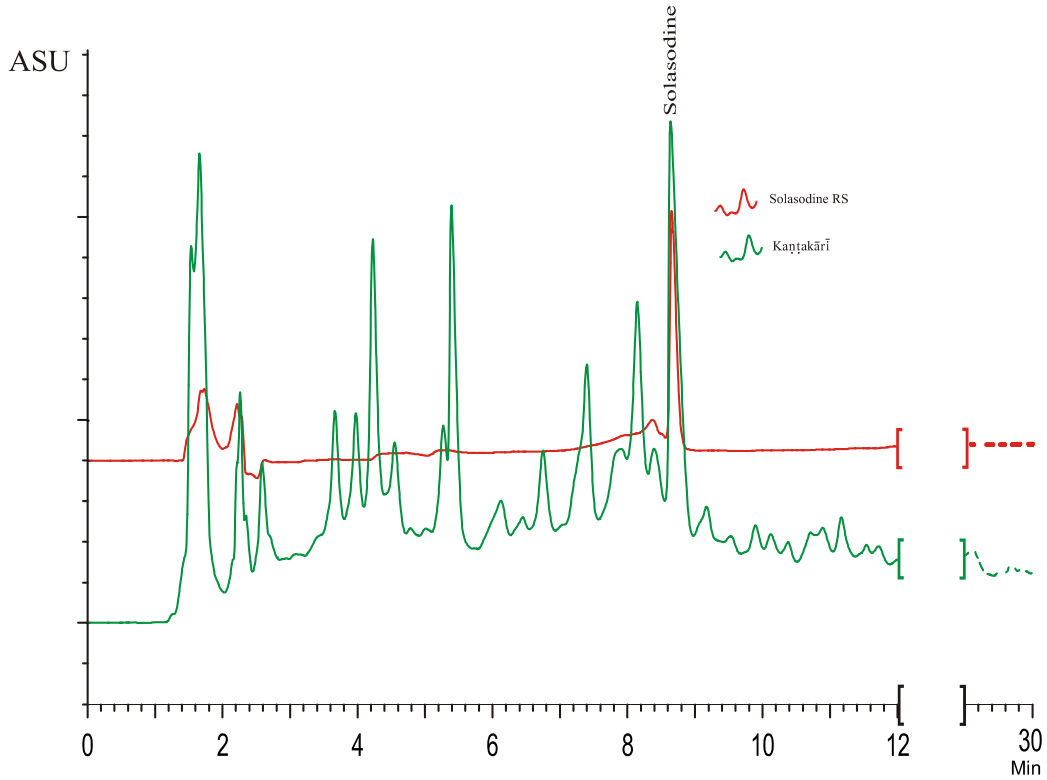


Fig.3. HPLC chromatogram of Kaṇṭakārī with *Solasodine* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Solasodine RS.*

Constituents: Solamargine, α-solamargine, solasonine, solasodine; sterols, cycloartenol, morcarpesterol.

Properties and Action:

Rasa : Kaṭu, Tikta
Guṇa : Laghu, Rūkṣa
Vīrya : Uṣṇa
Vipāka : Kaṭu
Karma : Dīpana, Pācana, Āmadoṣanāśaka, Kaṇṭhya, Śothahara

Important formulations: Kaṇṭakāryavaleha; Pañcatiktaka ghṛta; Vyāghrīharātakī.

Therapeutic uses: Śvāsa (asthma); Kāsa (cough); Jvara (fever); Aruci (tastelessness); Pīnasa (chronic rhinitis/sinusitis); Pārśvaśūla (intercostal neuralgia and pleurodynia); Svarabheda (hoarseness of voice).

Dose: 20 to 30 g of the drug for decoction.

KAṆṬAKĀRĪ (POWDER)

Kaṇṭakārī (Powder) consists of powder of Kaṇṭakārī complying with the following requirements:

Description:

a) *Macroscopic*:

Coarse powder, brown; odour characteristic; taste slightly bitter; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic*:

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*; treat a few mg of powder with Sudan III solution and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows thick walled, lignified, isolated or groups of pitted stone cells; thin walled polygonal parenchyma containing sandy microsphenoidal calcium oxalate crystals; polygonal cells of cork; fragments of lignified, pitted vessels and spiral thickenings; fragment of straight walled epidermis of stem with anomocytic stomata; several fragments and entire, pointed, stellate trichomes; fragments of sinuously walled epidermal cells of leaf with anomocytic and anisocytic stomata; a few oil globules and simple, rounded, starch grains with hilum measuring up to 9 μ; fragments of several layers of thick walled, horse shoe shaped stone cells of seed in transverse and surface view; a few endosperm cells and fibres; very few pollen grains seen occasionally; fragments of closely arranged sclerefiedfibres from spines.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Kaṇṭakārī.

Assay: Complies with the limits for Assay as per method stated under Kaṇṭakārī.

Additional requirements:

Packaging and Storage: As stated under Kaṇṭakārī.

Labeling: As stated under Kaṇṭakārī.

API reference standards: As stated under Kaṇṭakārī.

KAṆṬAKĀRĪ HYDRO-ALCOHOLIC EXTRACT

Kaṇṭakārī Hydro-alcoholic Extract is a dried and powdered extract prepared from Kaṇṭakārī. The extract contains not less than 0.05 per cent of *solasodine* when assayed.

Method of Preparation:

Take Kaṇṭakārī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 10.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *solasodine* as a reference standard. Solvent system: *Chloroform* : *methanol* (5.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *water*. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask, extract thrice with 25 ml of *chloroform* and combine the *chloroform* layers. Wash the *chloroform* layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10- ml volumetric flask, make up the volume and filter. Standard solution: Dissolve 1 mg of *solasodine RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *solasodine* and the profile should be similar to the one given in the TLC (Fig. 1).

Visible after derivatisation

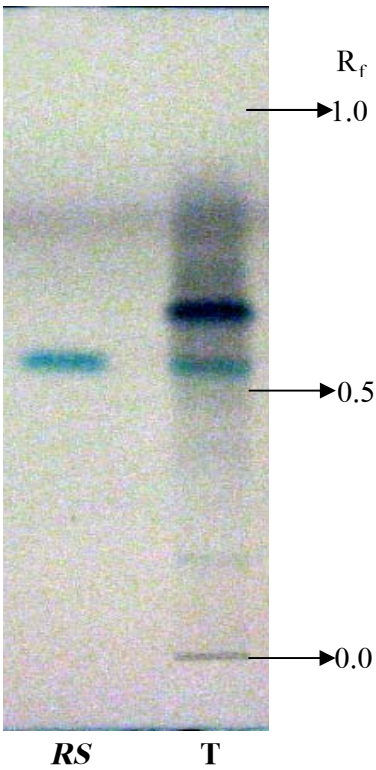


Fig.1. Thin-Layer Chromatogram of Kaṇṭakārī hydro-alcoholic extract
***RS*: *Solasodine*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	5.0 - 7.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined, add 25 ml of *water* and sonicate for 5 minutes. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask and extract thrice with 25 ml of *chloroform* and combine the chloroform layers. Wash the chloroform layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10-ml volumetric flask, make up the volume and filter. Standard solution: Take about 1 mg, accurately weighed, *solasodine RS* in a 10-ml volumetric flask, dissolve in 5 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	75	25
15	45	55
20	20	80
25	75	25
30	75	25

Injection volume: 20 μl. Flow rate: 1.6 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *solasodine* in the substance being examined from the analyte peak response and from the declared content of *solasodine* in *solasodine RS*.

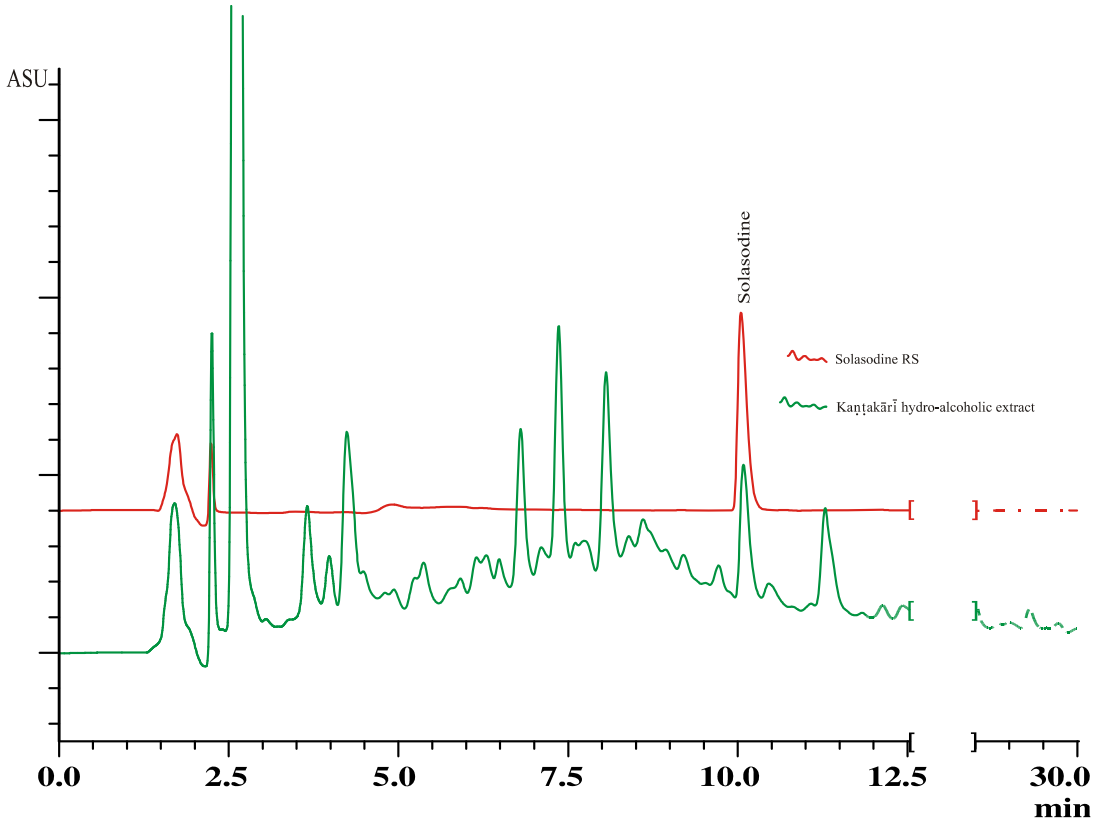


Fig.2. HPLC chromatogram of Kaṇṭakārī hydro-alcoholic extract with *Solasodine* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Solasodine RS.*

KAṆṬAKĀRĪ WATER EXTRACT

Kaṇṭakārī Water Extract is a dried and powdered extract prepared from Kaṇṭakārī. The extract contains not less than 0.05 per cent of *solasodine* when assayed.

Method of Preparation:

Take Kaṇṭakārī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 12.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *solasodine* as a reference standard. Solvent system: *Chloroform* : *methanol* (5.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *water*. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask, extract thrice with 25 ml of *chloroform* and combine the *chloroform* layers. Wash the *chloroform* layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10-ml volumetric flask, make up the volume and filter. Standard solution: Dissolve 1 mg of *solasodine RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solution on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.60 corresponding to that of *solasodine* and the profile should be similar to the one given in the TLC (Fig. 1).

Visible after derivatisation

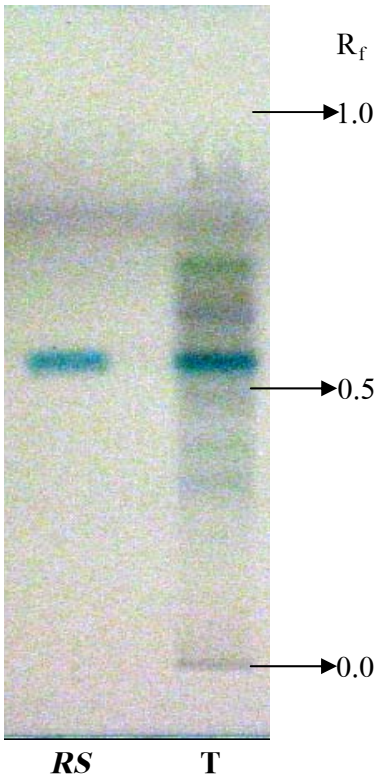


Fig.1. Thin-Layer Chromatogram of Kaṇṭakārī water extract.
***RS*: *Solasodine*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.5 per cent,	Appendix 2.1.7
pH:	5.0 - 7.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined, add 25 ml of *water* and sonicate for 5 minutes. Add 5 ml of 5N *hydrochloric acid*, heat under reflux on a water bath for 15 minutes, cool and add *ammonia solution* to adjust the *p*H to 8-9. Transfer the solution to separating flask and extract thrice with 25 ml of *chloroform* and combine the chloroform layers. Wash the chloroform layer with *water* and pass through *anhydrous sodium sulphate*. Evaporate the *chloroform* from the filtrate under vacuum to dryness. Dissolve the residue in 5 ml of *methanol*, transfer to a 10-ml volumetric flask, make up the volume and filter. Standard solution: Take about 1 mg, accurately weighed, *solasodine RS* in a 10-ml volumetric flask, dissolve in 5 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0	75	25
15	45	55
20	20	80
25	75	25
30	75	25

Injection volume: 20 μl. Flow rate: 1.6 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the standard solution and record the chromatogram. Inject 20 μl of the test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *solasodine* in the substance being examined from the analyte peak response and from the declared content of *solasodine* in *solasodine RS*.

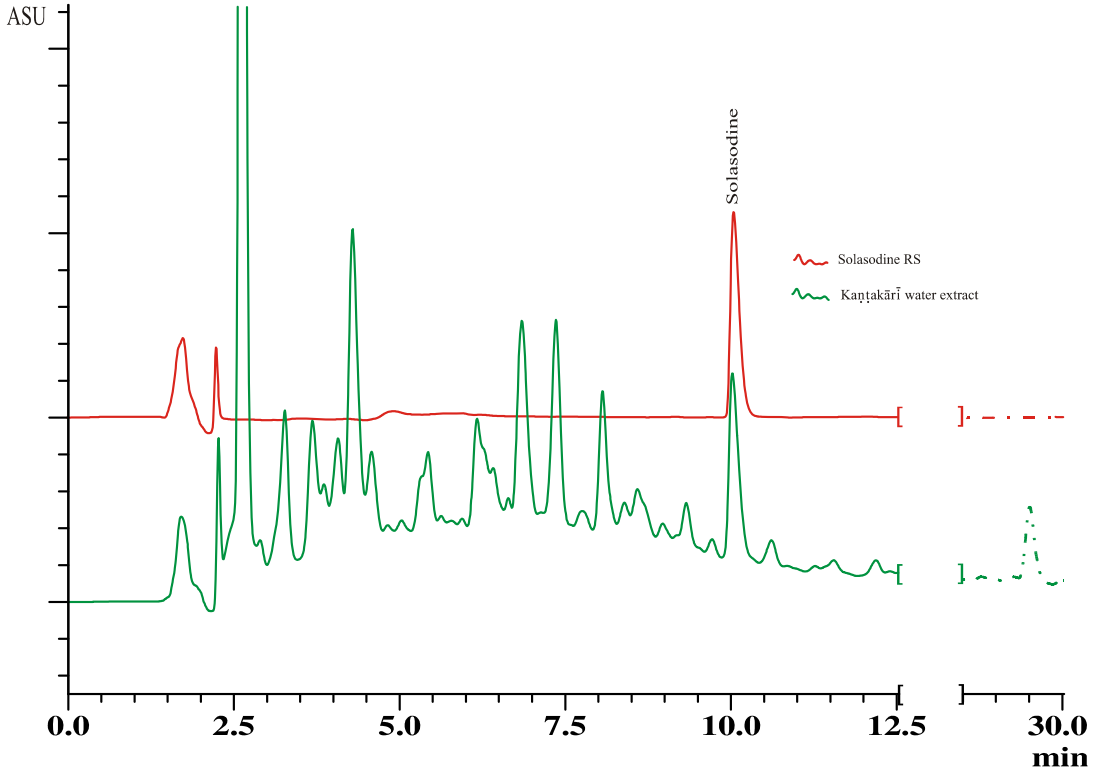


Fig.2. HPLC chromatogram of Kaṇṭakārī water extract with *Solasodine* as *RS*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Solasodine RS.*

MAṆḌŪKAPARṆĪ

Mandūkaparṇī consists of dried whole plant of *Centella asiatica* (L.) Urban. syn. *Hydrocotyle asiatica* L. (Fam. Apiaceae), a prostrate, faintly aromatic, stoloniferous perennial herb, commonly found as a weed in crop fields and other waste places throughout India up to an altitude of 600 m. Mandūkaparṇī contains not less than 1.0 per cent of total triterpenes (sum of *madecassoside, asiaticoside B*, *asiaticoside*, *madecassic acid, terminolic acid* and *asiatic acid*) when assayed.

Synonyms: Maṇḍūkī, Darduracchadā

Regional Language Names:

Assamese	:	Manimuni
Bengali	:	Jholkhuri, Thalkuri, Thankuni
English	:	Indian Pennywort
Gujarati	:	Khodabrahmi, Khadbhrammi
Hindi	:	Brahma Manduki, Brahmi
Kannada	:	Ondelaga, Brahmi soppu
Kashmiri	:	---
Malayalam	:	Kodangal
Marathi	:	Karivana
Oriya	:	---
Punjabi	:	Brahmi
Tamil	:	Vallarai
Telugu	:	Saraswati Aku, Vauari
Urdu	:	Brahmi

Description:

a) *Macroscopic*:

Small creeping herb with slender stem, rooting at nodes giving rise to thin, brownish-grey, roots of about 2.5 to 6.0 cm in length; leaves 1 to 3 from each node, orbicular-reniform, crenate, base cordate, petioles channelled with adnate stipules; flowers fascicled umbels each carrying 3 or 4 flowers, short stalked; fruits cremocarp, ovoid, with laterally compressed seeds.

b) *Microscopic*:

Root - Shows wavy outline, consisting of 3 to 5 layered, rectangular, cork cells having exfoliated cells, followed by 3 or 4 layers of parenchyma cells containing oval to round, simple, starch grains measuring 8 to 16 μ in dia., having centric hilum and microsphenoidal crystals of calcium oxalate; secondary cortex composed of thin-walled, oval to polygonal, parenchymatous cells; secretory cells present, scattered towards periphery region; secondary phloem and secondary xylem consisting of usual elements; vessels lignified with reticulate and spiral thickening; pith nearly obliterated.

Stem - More or less concave-convex outline, shows single layered epidermis composed of round to cubical cells covered by striated cuticle; below this 2 or 3 layers of collenchymatous cells, followed by 6 to 8 layers of thin-walled, isodiametric, parenchymatous cells with intercellular spaces present; vascular bundles collateral, open, arranged in a ring, capped by patches of sclerenchyma and traversed by wide medullary rays; vessels with spiral thickening present, resin duct present in parenchymatous cells of cortex and generally one in between vascular bundles; pith of isodiametric, parenchyma with intercellular spaces.

Leaf- Petiole - shows a characteristic outline due to two projections adjacent to ventral groove; epidermis single layered, cells cubical covered by a thick cuticle; inner walls of epidermal cells adjoining the cortex much thickened; hairs absent; collenchyma 2 or 3 layered, absent on the projections, a broad zone of more or less rounded parenchyma cells present with intercellular spaces, and a few containing rosette crystals of calcium oxalate; resin canal present on dorsal side of each vascular bundle except in the vascular bundles occurring projecting arms; vascular bundles seven in number, two of which less developed and present in projections.

Midrib - shows a single layered epidermis, 2 or 3 layered collenchyma on both surfaces, 4 or 5 layered parenchyma, mostly devoid of chloroplasts; central zone occupied by vascular bundles differentiated into xylem towards ventral side and phloem towards dorsal side; phloem consisting of sieve tubes, companion cells and phloem parenchyma; xylem consisting of radial rows of vessels with xylem parenchyma in between.

Lamina -shows an epidermis of tangentially elongated cells on both surfaces, larger on the upper surface, covered by striated cuticle; mesophyll differentiated into 2 or 3 layers of palisade cells, 5 to 7 layers of loosely arranged, somewhat isodiametric spongy parenchyma; rosette crystals of calcium oxalate present in a few cells; stomata more on the lower surface, anisocytic in general, but anomocytic type also occurs on both surfaces, palisade ratio 3 to 5, stomatal index on upper surface, 9 to 12, and lower surface 11 to 17.

Fruit - shows several ridges in outline; epicarp consists of single layered epidermis covered externally with thick cuticle; mesocarp consists of polygonal, thin walled parenchymatous cells having patches of sclerenchymatous cells on both lateral side; each ridge having a vittae and patch of sclerenchyma; endocarp consists of columnar shaped sclereids arranged in wavy layers; endosperm and embryo composed of oval to polygonal, thin-walled parenchymatous cells.

c) *Powder*:

Fine powder shows fragments of epidermal cells polygonal in surface view with stomata, palisade cells, vessels with spiral, reticulate and annular thickening; microsphenoidal and rosette crystals of calcium oxalate; simple, oval to round starch grains measuring 8 to 16 μ in dia. (Fig.1).

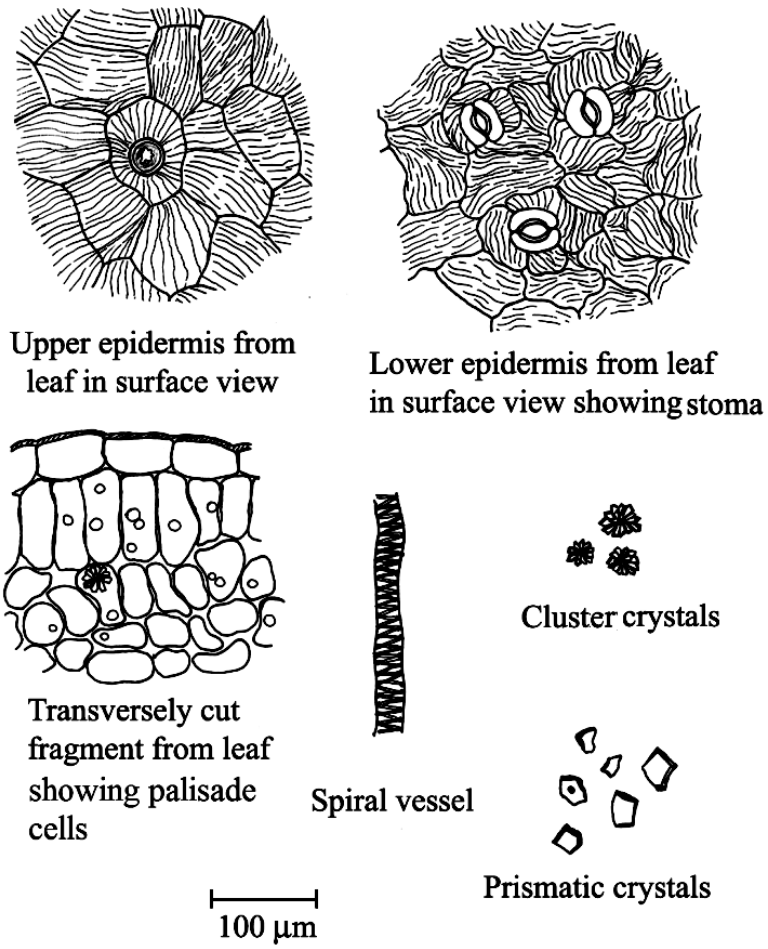


Fig.1. Powdered drug of Maṇḍūkaparṇī (*Centella asiatica* (L.) Urban.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 10.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 17.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 9.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 20.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *asiaticoside* and *madecassoside* as reference standards. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water* (6.0 : 3.2 : 1.2 : 0.8). Test solution: To 3 g of the substance being examined, add

25 ml of *methanol*, heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *asiaticoside RS* and *madecassoside RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows band at R_f~0.45 corresponding to that of *asiaticoside* and at R_f~0.30 corresponding to that of *madecassoside* and the profile should be similar to the one given in the TLC (Fig. 2).

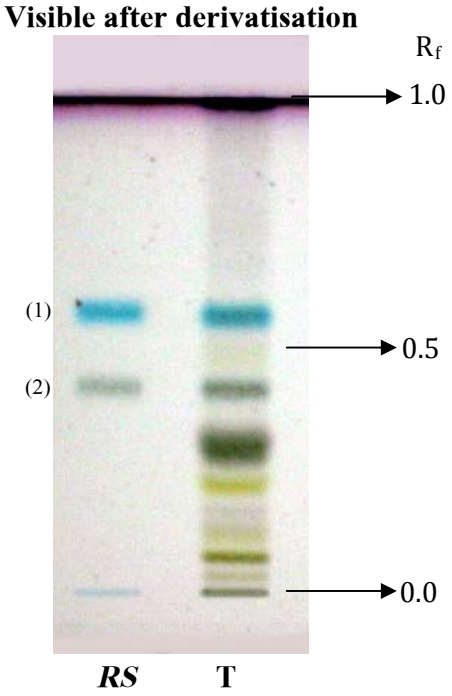


Fig. 2 Thin-Layer Chromatogram of Maṇḍūkaparṇī. RS: (1) *Asiaticoside* and (2) *Madecassoside*, T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 3 g, accurately weighed, of the substance being examined, add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *asiaticoside RS* and *madecassoside RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	80	20
15	50	50
20	20	80

23	20	80
30	50	50
35	80	20
40	95	5
45	95	5

Injection volume: 20 µl. Flow rate: 1.8 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	**Relative retention time**
Madecassoside + Asiaticoside B	0.92
Asiaticoside	1.00
Madecassic acid + Terminolic acid	1.46
Asiatic acid	1.63

Calculate the content of madecassoside (mixture of madecassoside and asiaticoside B), madecassic acid (mixture of madecassic acid and terminolic acid) as madecassoside. Calculate the content of asiaticoside and asiatic acid as asiaticoside in the substance being examined from the analyte peak response and from the declared content of madecassoside in madecassoside RS and asiaticoside in asiaticoside RS. Sum both to obtain total triterpenes.

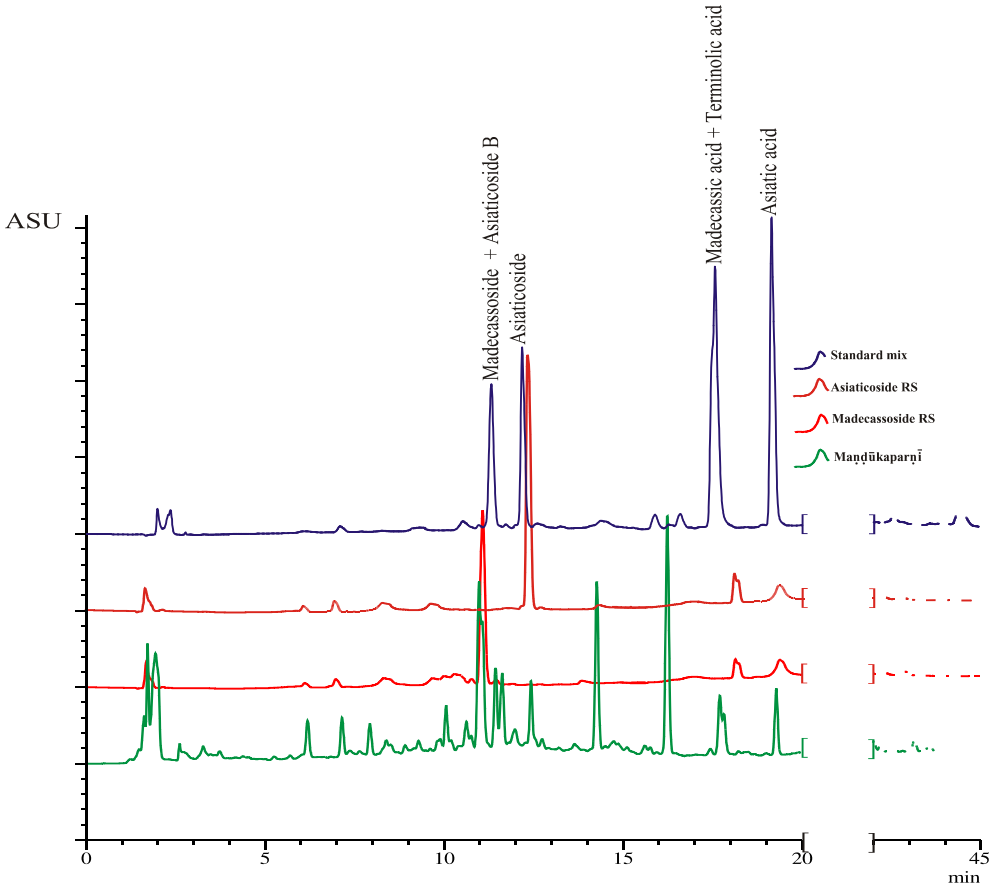


Fig. 3.HPLC chromatograms of Maṇḍūkaparṇī with *Asiaticoside, Madecassoside* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name and, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Asiaticoside RS* and *Madecassoside RS.*

Constituents: Madecassoside, asiaticoside, madecassic acid, terminolic acid and asiatic acid.

Properties and Action:

Rasa	:	Tikta, Kaṣāya, Madhura,Kaṭu
Guṇa	:	Laghu, Sara
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Kaphapittahara, Hṛdya, Medhya, Svarya, Rasāyana, Dīpana, Varṇya, Viṣaghna, Āyuṣya, Balya, Smṛtiprada.

Important formulations: Brāhma rasāyana.

Therapeutic uses: Raktapitta (bleeding disorders); Kuṣṭha (diseases of skin); Meha (excessive flow of urine); Jvara (fever); Śvāsa (asthma); Kāsa (cough); Aruci (tastelessness); Pāṇḍu (anaemia); Śotha (inflammation); Kaṇḍū (itching); Raktadoṣa (disorders of blood).

Dose: 3 to 6 g.

MAṆḌŪKAPARṆĪ (POWDER)

Maṇḍūkaparṇī (Powder) consists of Maṇḍūkaparṇī complying with the following requirements:

Description:

a) *Macroscopic*:

Coarse powder, yellowish brown; odour characteristic; taste slightly bitter; all particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic*:

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of epidermal cells polygonal in surface view with straight anticlinal walls and striated cuticle, with anisocytic and anomocytic stomata; palisade cells present isolated or intact beneath the epidermis; microsphenoidal and rosette crystals of calcium oxalate scattered as such throughout or within parenchymatous cells; very rarely a few unicellular and multicellular covering trichomes; longitudinal view of spiral, annular, reticulate and pitted vessels and thin walled fibres; secretory ducts; fragments of cork from root in surface view; parquetry layer and sclereids from the mesocarp of fruits, endosperm cells from seeds filled with aleurone grains and fixed oil globules; simple, oval to round starch grains measuring up to 16μ.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Maṇḍūkaparṇī.

Assay: Complies with the limits for Assay as per method stated under Maṇḍūkaparṇī

Additional requirements:

Packaging and Storage: As given under Maṇḍūkaparṇī.

Labeling: As given under Maṇḍūkaparṇī.

API reference standards: As given under Maṇḍūkaparṇī.

MAṆḌŪKAPARṆĪ HYDRO-ALCOHOLIC EXTRACT

Maṇḍūkaparṇī Hydro-alcoholic Extract is a dried and powdered extract prepared from Maṇḍūkaparṇī. The extract contains not less than 5.0 per cent of total triterpenes (sum of *madecassoside, asiaticoside B*, *asiaticoside*, *madecassic acid, terminolic acid* and *asiatic acid*) when assayed.

Method of preparation:

Take Maṇḍūkaparṇī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *asiaticoside* and *madecassoside* as reference standards. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water* (6.0 : 3.2 : 1.2 : 0.8). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *asiaticoside RS* and *madecassoside RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows band at R_f~0.45 corresponding to that of *asiaticoside* and at R_f~0.30 corresponding to that of *madecassoside* and the profile should be similar to the one given in the TLC (Fig. 1).

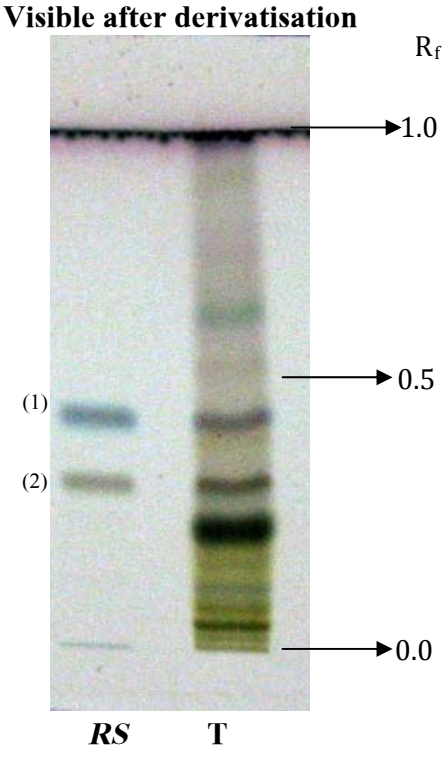


Fig. 1. Thin-Layer Chromatogram of Maṇḍūkaparṇī hydro-alcoholic extract *RS*: (1) *Asiaticoside* and (2) *Madecassoside*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 22.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7
pH:	4.5 - 6.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined, add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *asiaticoside RS* and *madecassoside RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	80	20
15	50	50
20	20	80
23	20	80
30	50	50
35	80	20
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.8 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Madecassoside + Asiaticoside B	0.92
Asiaticoside	1.00
Madecassic acid + Terminolic acid	1.46
Asiatic acid	1.63

Calculate the content of *madecassoside* (mixture of *madecassoside* and *asiaticoside B*), *madecassic acid* (mixture of *madecassic acid* and *terminolic acid*) as *madecassoside*. Calculate the content of *asiaticoside* and *asiatic acid* as *asiaticoside* in the substance being examined from the analyte peak response and from the declared content of *madecassoside* in *madecassoside RS* and *asiaticoside* in *asiaticoside RS*. Sum both to obtain total triterpenes.

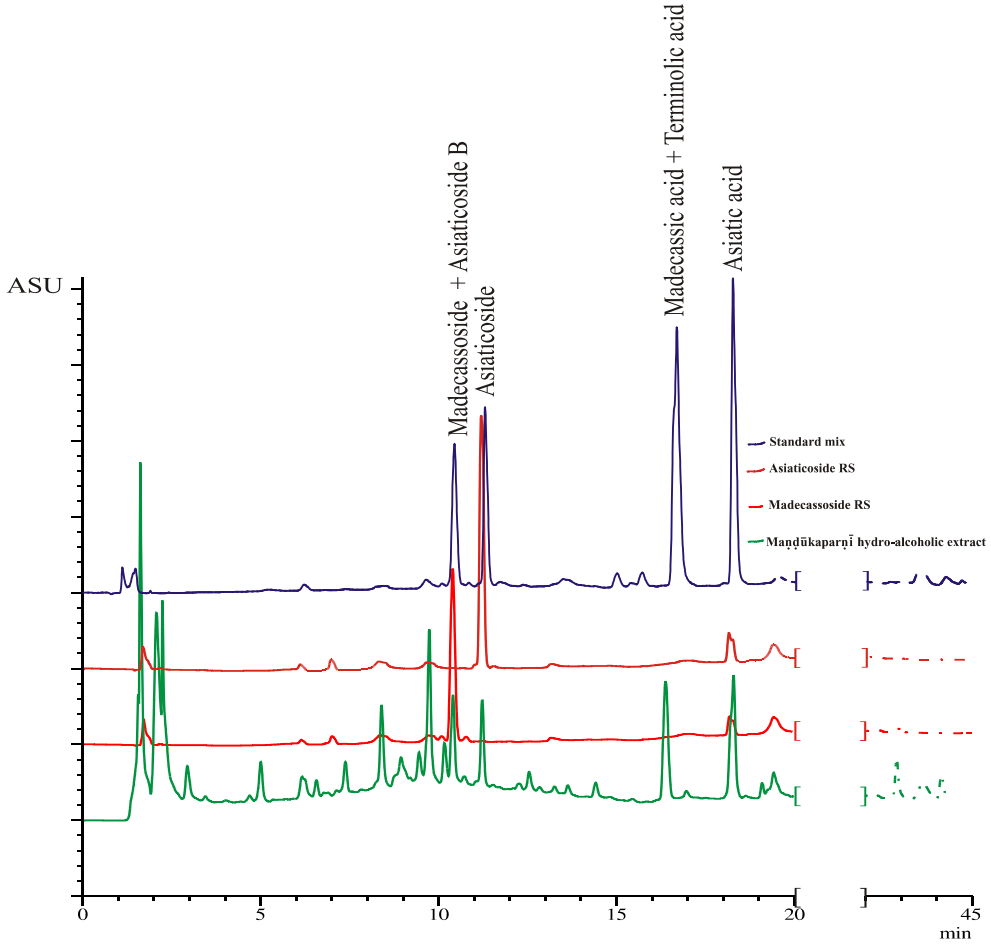


Fig.2.HPLC chromatograms of Maṇḍūkaparṇī hydro-alcoholic extract with *Asiaticoside, Madecassoside* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Asiaticoside RS* and *Madecassoside RS.*

MAṆḌŪKAPARṆĪ WATER EXTRACT

Maṇḍūkaparṇī Water Extract is a dried and powdered extract prepared from Maṇḍūkaparṇī. The extract contains not less than 1.5 per cent of total triterpenes (sum of *madecassoside, asiaticoside B*, *asiaticoside*, *madecassic acid, terminolic acid* and *asiatic acid*) when assayed.

Method of preparation:

Take Maṇḍūkaparṇī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 20.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *asiaticoside* and *madecassoside* as reference standards. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water* (6.0 : 3.2 : 1.2 : 0.8). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes, cool and filter. Standard solution: Dissolve 10 mg each of *asiaticoside RS* and *madecassoside RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows bands at R_f ~0.45 corresponding to that of *asiaticoside* and at R_f ~0.30 corresponding to that of *madecassoside* and the profile should be similar to the one shown in (Fig. 1).

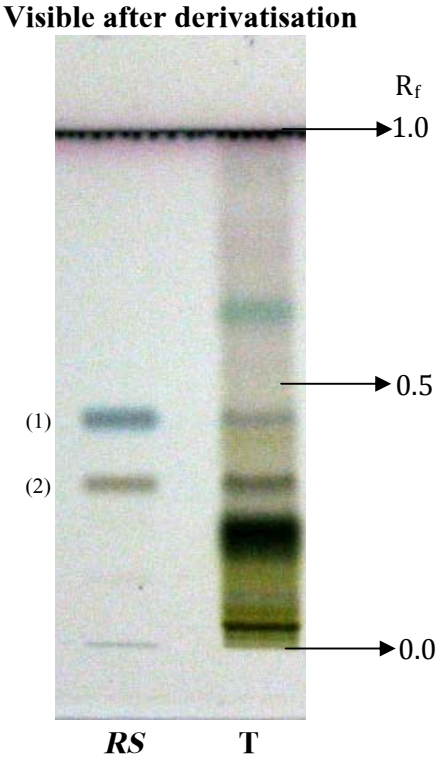


Fig. 1. Thin-Layer Chromatogram of Maṇḍūkaparṇī water extract. RS: (1) *Asiaticoside* and (2) *Madecassoside*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 22.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 5.0 per cent,	Appendix 2.1.7
pH:	5.0 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 4 g, accurately weighed, of the substance being examined, add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *asiaticoside RS* and *madecassoside RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water*, adding 0.5 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and *acetonitrile* in the following proportions:

Time (minutes)	Phosphate buffer (per cent)	*Acetonitrile* (per cent)
0.01	95	5
5	80	20
15	50	50
20	20	80
23	20	80
30	50	50
35	80	20
40	95	5
45	95	5

Injection volume: 20 μl. Flow rate: 1.8 ml per minute. Detection: UV, 205 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Madecassoside + Asiaticoside B	0.92
Asiaticoside	1.00
Madecassic acid + Terminolic acid	1.46
Asiatic acid	1.63

Calculate the content of *madecassoside* (mixture of *madecassoside* and *asiaticoside B*), *madecassic acid* (mixture of *madecassic acid* and *terminolic acid*) as *madecassoside*. Calculate the content of *asiaticoside* and *asiatic acid* as *asiaticoside* in the substance being examined from the analyte peak response and from the declared content of *madecassoside* in *madecassoside RS* and *asiaticoside* in *asiaticoside RS*. Sum both to obtain total triterpenes.

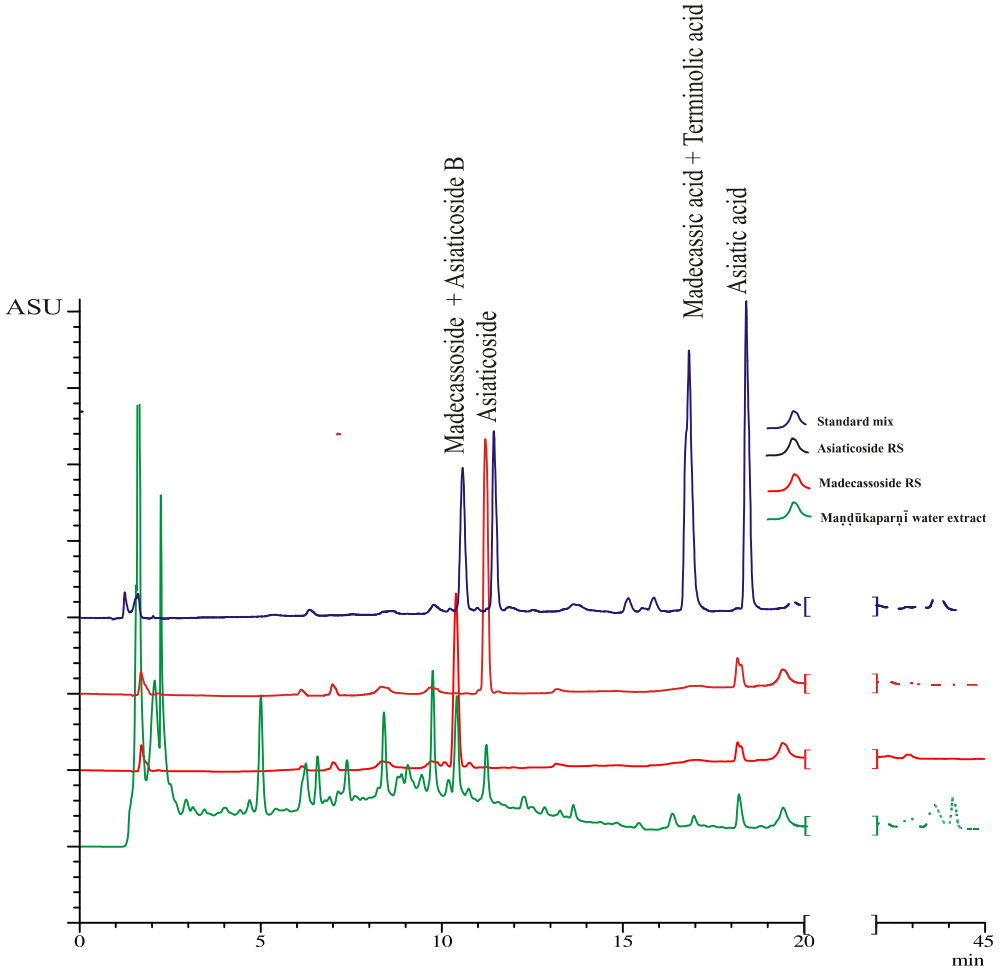


Fig. 2.HPLC chromatograms of Maṇḍūkaparṇī water extract with *Asiaticoside, Madecassoside* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Asiaticoside RS* and *Madecassoside RS.*

ŚATĀVARĪ

Śatāvarī consists of tuberous roots of *Asparagus recemosus* Willd. (Fam. Liliaceae), an ascending, spinous much branched, perennial climber found throughout the country. Śatāvarī contains not less than 0.5 per cent of total shatavarins (sum of *shatavarin-I* and *shatavarin-IV*) calculated as *shatavarin-IV* when assayed.

Synonyms: Nārāyaṇī, Varī, Abhīru, Atirasā

Regional Language Names:

Assamese	:	Satmull
Bengali	:	Satamuli, Satmuli, Shatamuli
English	:	Asparagus
Gujarati	:	Satavari
Hindi	:	Satavar, Satamul
Kannada	:	Ashadi poeru, Halavu Bau, Narayani, Makkala
Kashmiri	:	---
Malayalam	:	Satavari Kizhangu
Marathi	:	Satavari
Oriya	:	---
Punjabi	:	Satavar
Tamil	:	Shimai-Shadvari, Nilichedi Kishangu
Telugu	:	Sima-Shatawari (Dry Root), Pippipichara, Pilliteegalu (Fresh Root)
Urdu	:	Satavari

Description:

a) *Macroscopic:*

Root tuberous, 10 to 30 cm in length and 0.1 to 0.5 cm thick, tapering at both ends with longitudinal wrinkles; colour cream; taste sweetish.

b) *Microscopic:*

Shows an outer layer of piliferous cells, ruptured at places, composed of small, thin-walled, rectangular asymetrical cells, a number of cells elongated to form unicellular root hairs; cortex comprises of 25 to 29 layers, distinct in two zones, outer and inner cortex; outer cortex consists of 6 or 7 layers, compactly arranged, irregular to polygonal, thick walled, lignified cells; inner cortex comprise of 21 to 23 layers, oval to polygonal, thin-walled, tangentially elongated cells with intercellular spaces; stone cells, either singly or in groups, form a discontinuous to continuous ring in the upper part of this region; raphides of calcium oxalate also present in this region; 2 or 3 layers of stone cells encirle the endodermis; endodermis composed of thin-walled parenchymatous cells; pericycle present below endodermis; stele ex arch and radial in position; xylem consist of vessels, tracheids and parenchyma; xylem vessels have pitted thickening; phloem patches consists of usual element; pith composed of circular to oval parenchymatous cells, a few cells slightly lignified.

c) Powder:

Fine powder shows fragments of lignified, thick-walled cells; vessels with simple pits, pieces of raphides, numerous, lignified, rectangular elongated' stone cells having clear striations with wide as well as narrow lumen and groups of parenchyma (Fig.1).

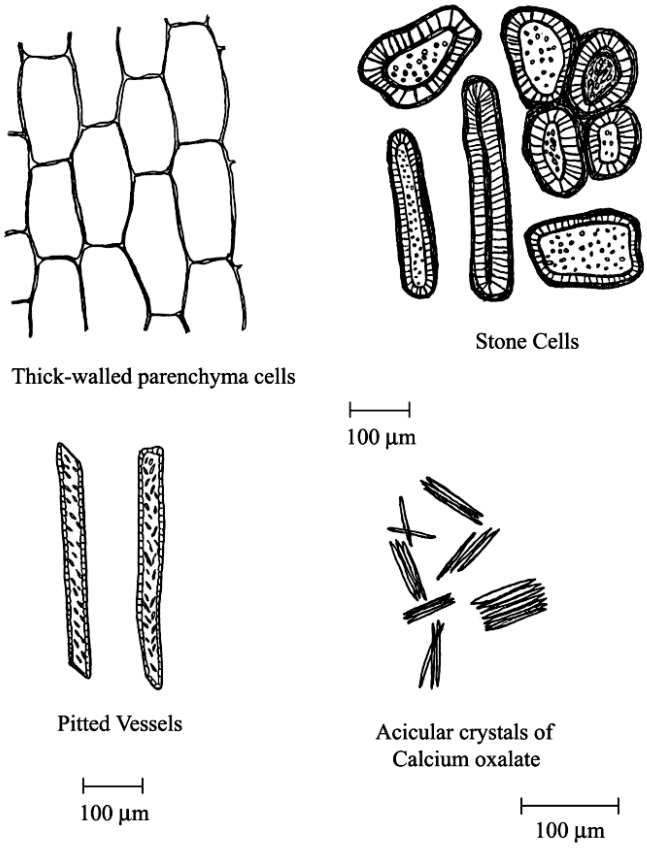


Fig.1: Powdered drug of Śatāvarī (*Asparagus racemosus* Willd.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 1.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 10.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 5.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 0.5 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 10.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 45.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *shatavarin-IV* as a reference standard. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water*

(5.0 : 3.5 : 1.5 : 1.0). Test solution: To 4 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *shatavarin-IV RS* in 25 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.80 corresponding to that of *shatavarin-IV* and the profile should be similar to the one given in the TLC (Fig. 2).

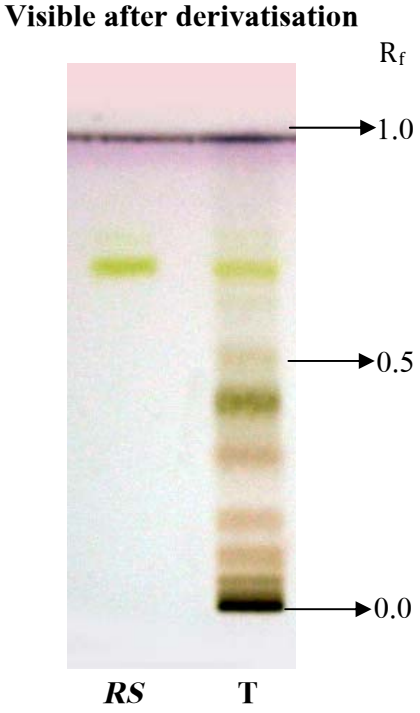


Fig. 2 Thin-Layer Chromatogram of Śatāvarī. *RS*: *Shatavarin-IV,* T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *high performance thin-layer chromatography* (Appendix 3.5.1). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol,* reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *shatavarin-IV RS* in a 100-ml volumetric flask. Dissolve in 50 ml *methanol* and make up the volume with *methanol*. Chromatographic system: High performance thin-layer chromatography. Stationary phase: Glass or aluminium plates coated with silica gel $60F_{254}$ plate. Mobile phase: *Ethyl acetate* : *methanol* : *water* (7.5 : 1.5 : 1.0). Detection: UV-Vis. Procedure: Set the applicator as per the condition prescribed by the manufacturer. By means of suitable syringe apply 3, 6, 9, 12 µl of standard solution in 4 different tracks at 1 cm height, with 4 mm band-width and 7 mm distance between the tracks. Apply 6 & 9 µl of test solution in another two tracks. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *vanillin sulphuric acid reagent*. Heat the plate at $50\text{-}60^0$ till yellow colour spots appear. Scan the TLC plate in the densitometer at 429 nm. Record the chromatograms and measure the responses for the analyte peaks. Calculate the content of

shatavarin-I and *shatavarin-IV* as *shatavarin-IV* in the substance being examined from the peak response of analyte and from the declared content of *shatavarin-IVin shatavarin-IV RS*.

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Shatavarin-IV RS.*

Constituents: Sugar, glycosides, saponin, sitosterol and shatavarins.

Properties and Action:

Rasa	:	Madhura, Tikta
Guṇa	:	Snigdha, Guru
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Vṛṣya, Balya, Medhya, Rasāyana, Kaphavātaghna, Pittahara, Vātahara, Stanyakara, Hṛdya, Netrya, Śukrala, Agnipuṣṭikara

Important formulations: Śatāvarī guḍa, Brāhma rasāyana, Pūga khaṇḍa, Saubhāgyaśuṇṭhī, Mahānārāyaṇa taila, Bṛhacchāgalādya ghṛta, Śatāvarī ghṛta, Śatāvarī kalpa, Aśvangandhāriṣṭa, Nārasiṁha cūrṇa.

Therapeutic uses: Śotha (inflammation); Kṣaya (pthisis); Pariṇāmaśūla (duodenal ulcer); Gulma (abdominal lump); Atisāra (diarrhoea); Raktātisara (diarrhoea with blood); Raktavikāra (disorders of blood); Mūtrarakta (haematuria); Amlapitta (hyperacidity); Arśa (piles); Vātajvara (fever due to vāta doṣa); Svarabheda (hoarseness of voice); Naktāndhya (night blindness); Vātarakta (gout); Raktapitta (bleeding disorders); Visarpa (erysepales); Sūtikā Roga (puerperal diseases); Stanya Doṣa (disorders of breast milk); Stanya Kṣaya (decrease in breast milk).

Dose: 3 to 6 g of the drug.

ŚATĀVARĪ (POWDER)

Śatāvarī (Powder) consists of powder of Śatāvarī complying with the following requirements:

Description:

a) *Macroscopic*:

Coarse powder, creamish white; odour characteristic; tasteless; all particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Debris from piliferous layer occasionally seen; cork debris also present; stone cells with narrow as well as wide lumen present; vessel elements showing oblique end wall with perforations that are elongated and irregularly distributed; abundant fragments of thin walled, polygonal parenchymatous cells containing raphides; isolated raphides also found; numerous fragments of vessels with scalariform and pitted walls; a few rectangular sclereids; number of simple, rounded starch grains measuring up to 7 μ.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Śatāvarī.

Assay: Complies with the limits for Assay as per method stated under Śatāvarī.

Additional requirements:

Packaging and Storage: As given under Śatāvarī.

Labeling: As given under Śatāvarī.

API reference standards: As given under Śatāvarī.

ŚATĀVARĪ HYDRO-ALCOHOLIC EXTRACT

Śatāvarī Hydro-Alcoholic Extract is a dried and powdered extract prepared from Śatāvarī. The extract contains not less than 1.0 per cent of total shatavarins (sum of *shatavarin I* and *shatavarin IV*) calculated as *shatavarin IV* when assayed.

Method of Preparation:

Take Śatāvarī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 60-65^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using s*hatavarin-IV* as a reference standard. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water* (5.0 : 3.5 : 1.5 : 1.0). Test solution: To 4 g of the substance being examined, add 50 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *shatavarin-IV RS* in 25 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.80 corresponding to that of *shatavarin-IV* and the profile should be similar to the one given in the TLC (Fig. 1).

Visible after derivatisation

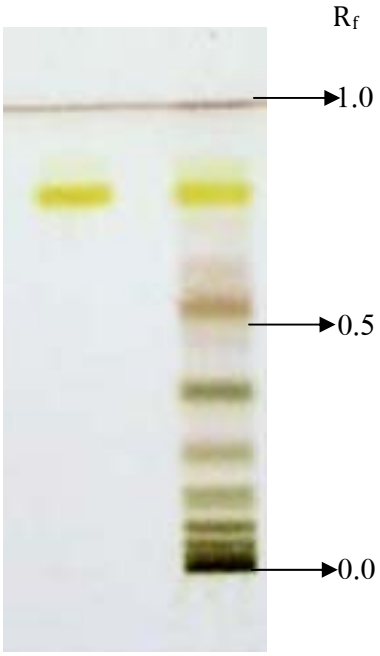


Fig. 1 Thin-Layer Chromatogram of Śatāvarī hydro-alcoholic extract
***RS*: *Shatavarin-IV*, T: Test solution**

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 5.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
pH:	4.5 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *high performance thin-layer chromatography* (Appendix 3.5.1). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol,* reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *shatavarin-IV RS* in a 100-ml volumetric flask. Dissolve in 50 ml *methanol* and make up the volume with *methanol*. Chromatographic system: High performance thin-layer chromatography. Stationary phase: Glass or aluminium plates coated with silica gel $60F_{254}$ plate. Mobile phase: *Ethyl acetate* : *methanol* : *water* (7.5 : 1.5 : 1.0). Detection: UV-Vis. Procedure: Set the applicator as per the condition prescribed by the manufacturer. By means of suitable syringe apply 3, 6, 9, 12 µl of standard solution in 4 different tracks at 1 cm height, with 4 mm band-width and 7 mm distance between the tracks. Apply 6 & 9 µl of test solution in another two tracks. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *vanillin sulphuric acid reagent*. Heat the plate at $50\text{-}60^0$ till yellow colour spots appear. Scan the TLC plate in the densitometer at 429 nm. Record the chromatograms and measure the responses for the analyte peaks. Calculate the content of *shatavarin-I* and *shatavarin-IV* as *shatavarin-IV* in the substance being examined from the peak response of analyte and from the declared content of *shatavarin-IV* in *shatavarin-IV RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Shatavarin-IV RS.*

ŚATĀVARĪ WATER EXTRACT

Śatāvarī Water Extract is a dried and powdered extract prepared from Śatāvarī. The extract contains not less than 0.75 per cent of total shatavarins (sum of s*hatavarin-I* and *shatavarin-IV*) calculated as *shatavarin-IV* when assayed.

Method of Preparation:

Take Śatāvarī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 60-65^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using s*hatavarin-IV* as a reference standard. Solvent system: *Chloroform* : *acetic acid* : *methanol* : *water* (5.0 : 3.5 : 1.5 : 1.0). Test solution: To 4 g of the substance being examined, add 50 ml of *methanol,* heat on a water bath for 15 minutes and cool. Dilute to 100 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *shatavarin-IV RS* in 25 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.80 corresponding to that of *shatavarin-IV* and the profile should be similar to the one given in the TLC (Fig. 1).

Visible after derivatisation

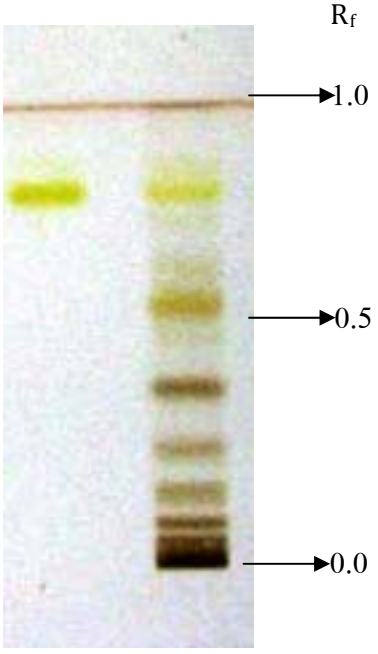


Fig. 1. Thin-Layer Chromatogram of Śatāvarī water extract.
RS: *Shatavarin-IV*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 5.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7

pH:	4.5 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *high performance thin-layer chromatography* (Appendix 3.5.1). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol,* reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, *shatavarin-IV RS* in a 100-ml volumetric flask. Dissolve in 50 ml *methanol* and make up the volume with *methanol*. Chromatographic system: High performance thin-layer chromatography. Stationary phase: Glass or aluminium plates coated with silica gel $60F_{254}$ plate. Mobile phase: *Ethyl acetate* : *methanol* : *water* (7.5 : 1.5 : 1.0). Detection: UV-Vis. Procedure: Set the applicator as per the condition prescribed by the manufacturer. By means of suitable syringe apply 3, 6, 9, 12 µl of standard solution in 4 different tracks at 1 cm height, with 4 mm band-width and 7 mm distance between the tracks. Apply 6 & 9 µl of test solution in another two tracks. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and spray the plate with a solution of *vanillin sulphuric acid reagent*. Heat the plate at $50\text{-}60^0$ till yellow colour spots appear. Scan the TLC plate in the densitometer at 429 nm. Record the chromatograms and measure the responses for the analyte peaks. Calculate the content of *shatavarin-I* and *shatavarin-IV* as *shatavarin-IV* in the substance being examined from the peak response of analyte and from the declared content of *shatavarin-IV in shatavarin-IV RS.*

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Shatavarin-IV RS.*

TĀMALAKĪ

Tāmalakī consists of roots, stems and leaves of *Phyllanthus fraternus* Webst. syn. *Phyllanthus niruri* Hook. f. non L. (Fam. Euphorbiaceae), a monoecious annual herb, 20 to 60 cm high, occurring all over the plains of India as a weed. Tāmalakī contains not less than 0.08 per cent of *phyllanthin* and 0.02 per cent of *hypophyllanthin* when assayed.

Synonyms: Mahīdhātrikā, Bhūmyāmalakī, Bahuphalā

Regional Language Names:

Assamese	:	Bhuin Amla
Bengali	:	Bhumamla, Bhumi Amalaki
English:	:	---
Gujarati	:	Bhoi Amali, Bhony amari, Bhonyamali
Hindi	:	Bhui Amala
Kannada	:	Nelanelli
Kashmiri	:	---
Malayalam	:	Kizanelli, Keezhanelli, Ajjhada
Marathi	:	Bhuiawali
Oriya	:	Bhuin Amla
Punjabi	:	---
Tamil	:	Kizhukai nelli, Kizanelli
Telugu	:	Nela usirika
Urdu	:	---

Description:

a) *Macroscopic:*

Root – Small, 2.5 to 11.0 cm long, nearly straight, gradually tapering, with a number of fibrous secondary and tertiary roots, light brown; fracture, short.

Stem – Slender, glabrous; light brown, 20 to 75 cm long, with a crown of numerous branches with small leaves at the upper region; they may be intact or detached from the stem.

Leaf – Each leaf is simple, arranged in two rows in the branches; alternate, dishticous almost sessile, stipulate, oblong, entire; up to 1.5 cm long and 0.5 cm wide, greenish-brown; taste, slightly bitter; male and female flowers and fruits are borne on the axils of the leaves.

b) *Microscopic:*

Root – TS of root shows 4 or 6 layers of cork consisting of thin-walled, rectangular, tangentially elongated and radially arranged cells, filled with reddish-brown content; cortex consists of 8 to 10 layers of thin-walled, tangentially elongated, parenchymatous cells; phloem narrow, consisting of sieve elements, phloem parenchyma and traversed by narrow phloem rays; xylem represented by a broad zone of tissue, composed of vessels, tracheids, fibres and parenchyma, all elements being thick-walled and lignified having simple pits; xylem rays uniseriate.

Stem – TS of stem shows, a single layered epidermis composed of thick-walled, flattened, tangentially elongated cells; older stem shows 4 to 5 layers of cork, composed of thin-walled, tabular, tangentially elongated and radially arranged cells, filled with reddish-brown content; cortex composed of 4 to 6 layers of oval, tangentially elongated, thin-walled, parenchymatous cells, some cortical cells filled with yellowish-brown content; endodermis quite distinct; pericycle represented by a discontinuous ring, composed of several tangentially elongated strands of lignified fibres with thick walls and narrow lumen; secondary phloem narrow, composed of sieve elements and parenchyma; xylem composed of vessels, fibres, parenchyma and traversed by numerous uniseriate rays; vessels mostly simple pitted, a few show spiral thickenings; fibres narrow elongated, with narrow or sometimes blunt ends with simple pits; centre, occupied by a pith composed of thin-walled, circular to oval parenchymatous cells, a few cluster crystals of calcium oxalate present in parenchymatous cells of cortex and phloem parenchyma.

Leaf- Lamina shows a dorsiventral structure, epidermis on either surface composed of thin-walled, tangentially elongated cells, covered externally by a thick cuticle; anisocytic stomata present on both epidermis; palisade single layered; spongy parenchyma composed of 3 to 5 layers of loosely arranged cells having a number of veins traversing, a few cluster crystals of calcium oxalate present in spongy parenchyma.

c) Powder:

Fine powder shows polygonal epidermal cells in surface view, fragments of rectangular cork cells, spiral vessels and fibres; a few cluster crystals of calcium oxalate (Fig. 1).

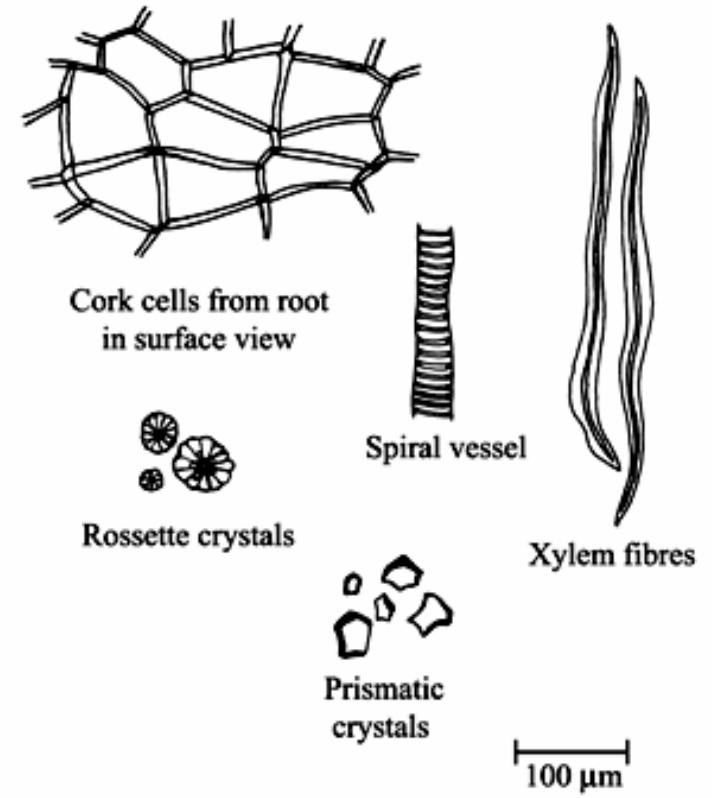


Fig.1. Powdered drug of Tāmalakī (*Phyllanthus fraternus* Webst.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 16.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 7.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 3.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 13.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *hypophyllanthin* and *phyllanthin* as reference standards. Solvent system : *Toluene* : *ethyl acetate* : *formic acid* (5.0 : 1.5 : 0.5). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 5 mg of each of *hypophyllanthin RS* and *phyllanthin RS* in 10 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of 10.0 per cent *methanolic sulphuric acid.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *hypophyllanthin* and at R_f ~0.35 corresponding to that of *phyllanthin* and the profile should be similar to the one given in the TLC (Fig. 2).

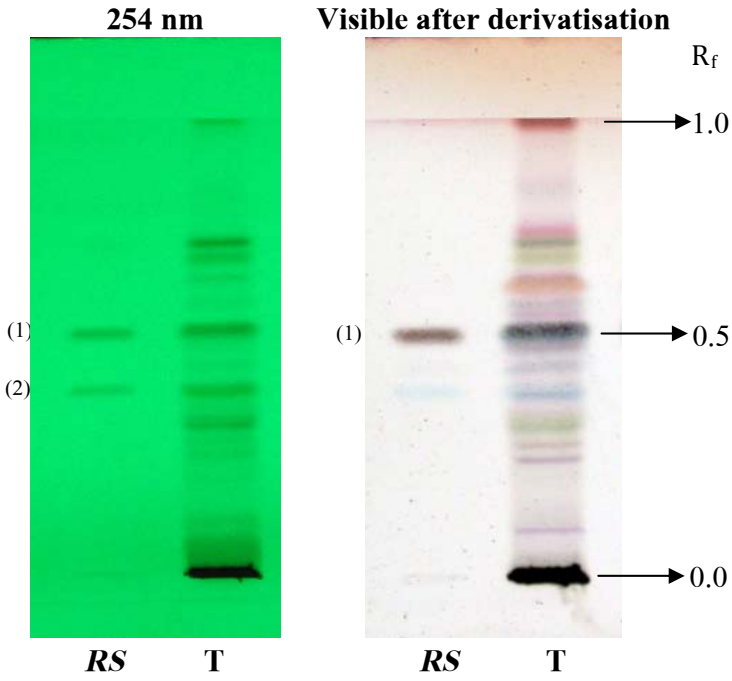


Fig. 2. Thin-Layer Chromatogram of Tāmalakī
RS*: (1) *Hypophyllanthin* and (2) *Phyllanthin,
T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2.5 g, accurately weighed, of the substance being examined. Add 25 ml of 65.0 per cent *aqueous methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *methanol* for three times more, cool and filter. Combine the filtrates, make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *hypophyllanthin RS* and *phyllanthin RS* in a 100-ml volumetric flask and dissolve in 50 ml of 65.0 per cent *methanol* and make up to 100-ml with 65.0 per cent *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 3.5 volumes of *water* and 6.5 volumes of *methanol*. Injection volume: 20 µl. Flow rate: 1 ml per minute. Detection: UV, 230 nm. Procedure: Inject 20 µl of the filtered standard solutions and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the responses for the analyte peaks. Calculate the content of the *hypophyllanthin, phyllanthin* in the substance being examined from the peak response of analytes and from the declared content of *hypophyllanthin* and *phyllanthin* in *hypophyllanthin RS* and *phyllanthin RS*.

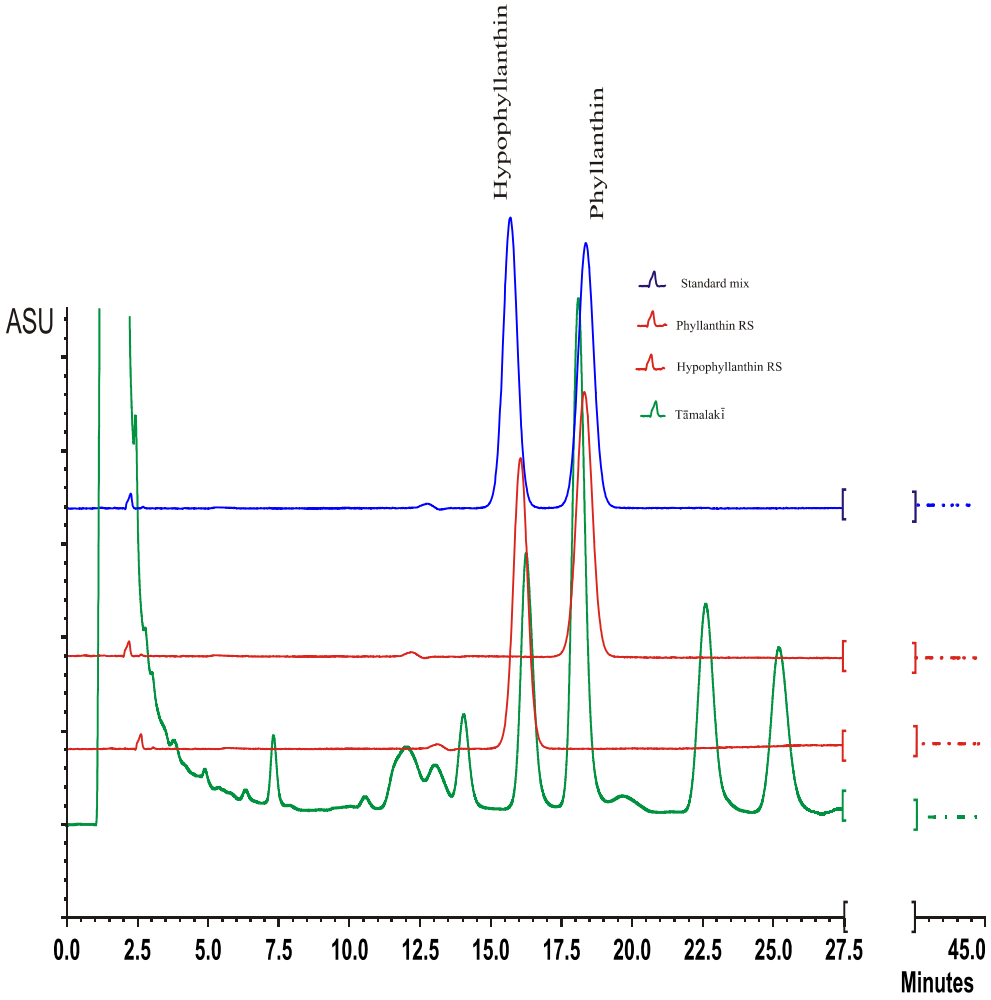


Fig.3. HPLC chromatograms of Tāmalakī with *Phyllanthin*, *Hypophyllanthin* as *RS* and Standard mix

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Hypophyllanthin RS* and *Phyllanthin RS.*

Constituents: Phyllanthin, hypophyllanthin.

Properties and Action:

Rasa	:	Kaṣāya, Tikta, Madhura
Guṇa	:	Laghu**,** Rūkṣa
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Rocana, Dāhanāśanī, Pittaśāmaka, Mūtrala

Important formulations: Citraka harītakī; Madhuyaṣṭyādi taila; Pippalyādi ghṛta; Cyavanaprāśa; Śatāvarīguḍa.

Therapeutic uses: Tṛṣā (thirst); Kāsa (cough); Amlapitta (hyperacidity); Pāṇḍu (anaemia); Kṣaya (pthisis); Kṣata (wound); Kuṣṭha (diseases of skin); Prameha (increased frequency and turbidity of urine); Mūtraroga (urinary diseases).

Dose: 10 to 20 ml of the drug in juice form.
3 to 6 g of the drug in powder form.

TĀMALAKĪ (POWDER)

Tāmalakī (Powder) consists of powder of Tāmalakī complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, light brownish yellow; odour characteristic; taste slightly bitter; all particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows fragments of fibres, pitted and vessels with spiral and pitted thickenings; fragments of sinuous walled epidermis with paracytic and anomocytic stomata; transversely cut fragments of lamina with single layered palisade, epidermis often papillate, parenchymatous cells with prismatic crystals of calcium oxalate; fragments of cork in surface view from the root, scleroidalfibres and simple starch grains measuring up to 11μ; fragments of pericarp with compactly placed thick walled column like cells; fragment of straight walled epicarp with anisocytic stomata; fragments of pitted and thick walled sclerified cells of testa; rectangular parenchyma of cotyledon containing oil drops, aleurone grains and rosette crystals of calcium oxalate.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Tāmalakī.

Assay: Complies with the limits for Assay as per method stated under Tāmalakī.

Additional requirements:

Packaging and Storage: As given under Tāmalakī.

Labeling: As given under Tāmalakī.

API reference standards: As given under Tāmalakī.

TĀMALAKĪ HYDRO-ALCOHOLIC EXTRACT

Tāmalakī Hydro-alcoholic Extract is a dried and powdered extract prepared from Tāmalakī. The extract contains not less than 0.4 per cent of *phyllanthin* and 0.1 per cent of *hypophyllanthin* when assayed.

Method of preparation:

Take Tāmalakī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *hypophyllanthin* and *phyllanthin* as reference standards. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (5.0 : 1.5 : 0.5). Test solution: To 3 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 5 mg of each of *hypophyllanthin RS* and *phyllanthin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of 10.0 per cent *methanolic sulphuric acid.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *hypophyllanthin* and at R_f ~0.35 corresponding to that of *phyllanthin* and the profile should be similar to the one given in the TLC (Fig. 1).

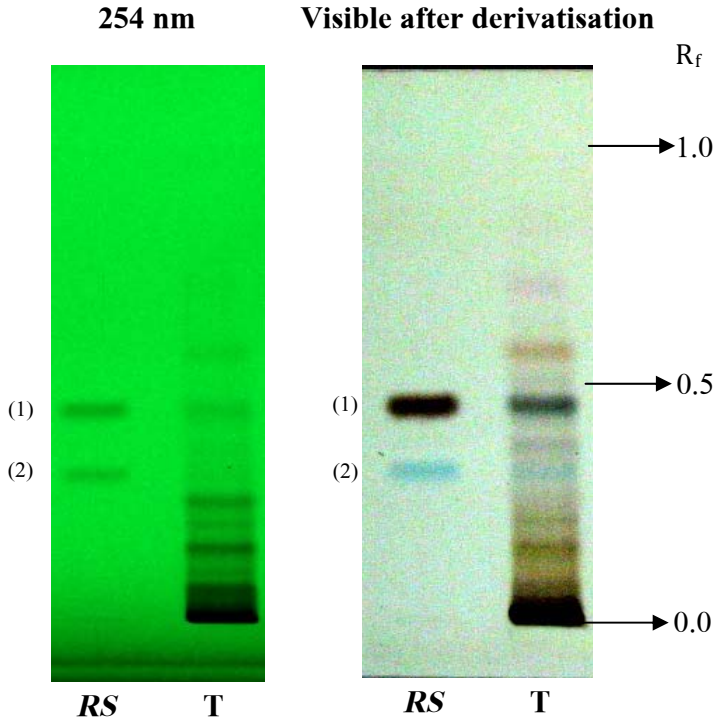


Fig. 1. Thin-Layer Chromatogram of Tāmalakī hydro-alcoholic extract. *RS*: (1) *Hypophyllanthin* and (2) *Phyllanthin*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 16.5 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 3.5 per cent,	Appendix 2.1.7
pH:	4.5 to 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2.5 g, accurately weighed, of the substance being examined. Add 25 ml of 65.0 per cent *aqueous methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *methanol* for three times more, cool and filter. Combine the filtrates, make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of

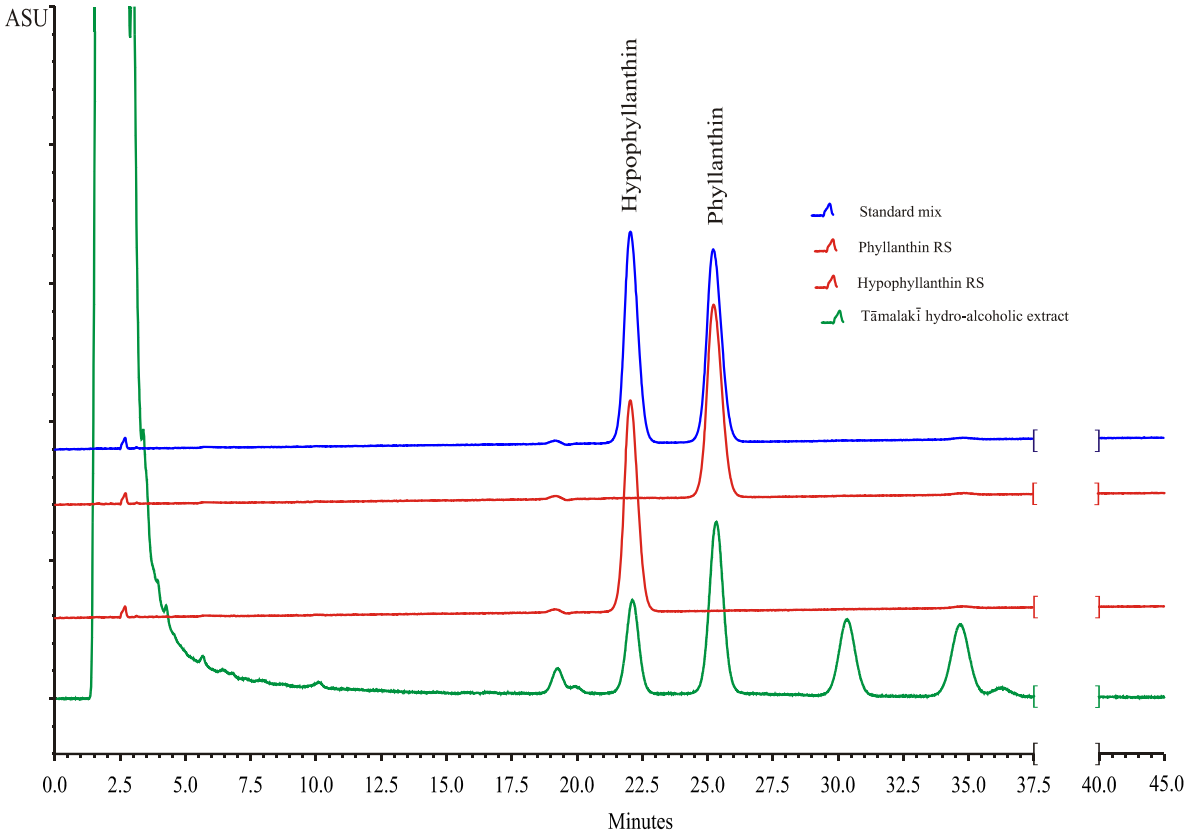


Fig.2. HPLC chromatograms of Tāmalakī hydro-alcoholic extract with *Phyllanthin*, *Hypophyllanthin* as *RS* and Standard mix

hypophyllanthin RS and *phyllanthin RS* in a 100-ml volumetric flask and dissolve in 50 ml of 65.0 per cent *methanol* and make up to 100-ml with 65.0 per cent *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed mixture of 3.5 volumes of *water* and 6.5 volumes of *methanol*. Injection volume: 20 μl. Flow rate: 1 ml per minute. Detection: UV, 230 nm. Procedure: Inject 20 μl of the filtered standard solutions and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the responses for the analyte peaks. Calculate the content of the *hypophyllanthin, phyllanthin* in the substance being examined from the peak response of analytes and from the declared content of *hypophyllanthin* and *phyllanthin* in *hypophyllanthin RS* and *phyllanthin RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Hypophyllanthin RS* and *Phyllanthin RS.*

TĀMALAKĪ WATER EXTRACT

Tāmalakī Water Extract is a dried and powdered extract prepared from Tāmalakī. The extract contains not less than 0.15 per cent of *phyllanthin* and 0.03 per cent of *hypophyllanthin* when assayed.

Method of preparation:

Take Tāmalakī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 16.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *hypophyllanthin* and *phyllanthin* as reference standards. Solvent system: *Toluene* : *ethyl acetate* : *formic acid* (5.0 : 1.5 : 0.5). Test solution: To 4 g of the substance being examined, add 25 ml of *methanol,* heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 5 mg of each of *hypophyllanthin RS* and *phyllanthin RS* in 10 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with a solution of 10.0 per cent *methanolic sulphuric acid.* Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.50 corresponding to that of *hypophyllanthin* and at R_f ~0.35 corresponding to that of *phyllanthin* and the profile should be similar to the one shown in (Fig. 1).

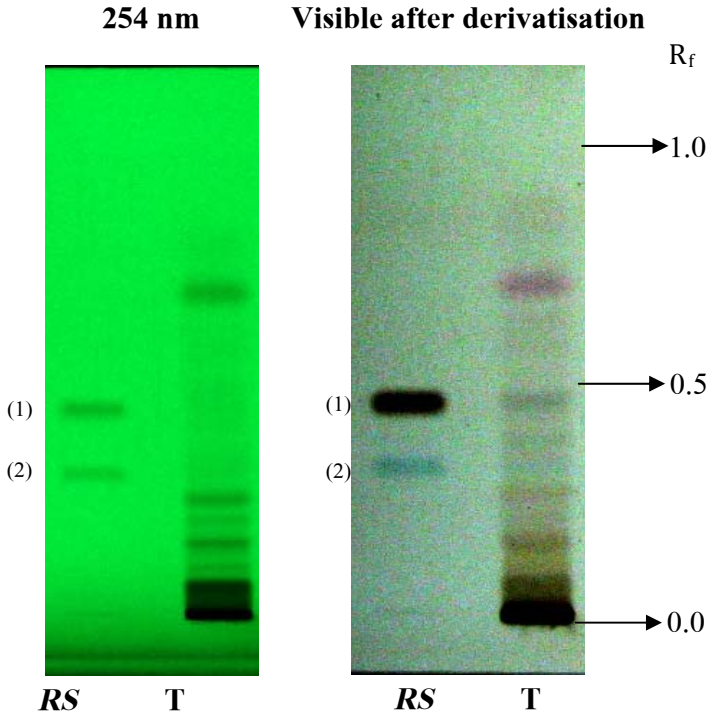


Fig.1. Thin-Layer Chromatogram of Tāmalakī water extract. RS: (1) *Hypophyllanthin* and (2) *Phyllanthin*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 7.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 25.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 7.5 per cent,	Appendix 2.1.7
pH:	4.5 - 5.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2.5 g, accurately weighed, of the substance being examined. Add 25 ml of 65.0 per cent *aqueous methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 25 ml of *methanol* for three times more, cool and filter. Combine the filtrates, make up the volume in a 100-ml volumetric flask. Standard solution: Take about 10 mg, accurately weighed, each of *hypophyllanthin RS* and *phyllanthin RS* in a 100-ml volumetric flask and dissolve in 50 ml of 65.0 per cent *methanol* and make up to 100-ml with 65.0 per cent *methanol*.

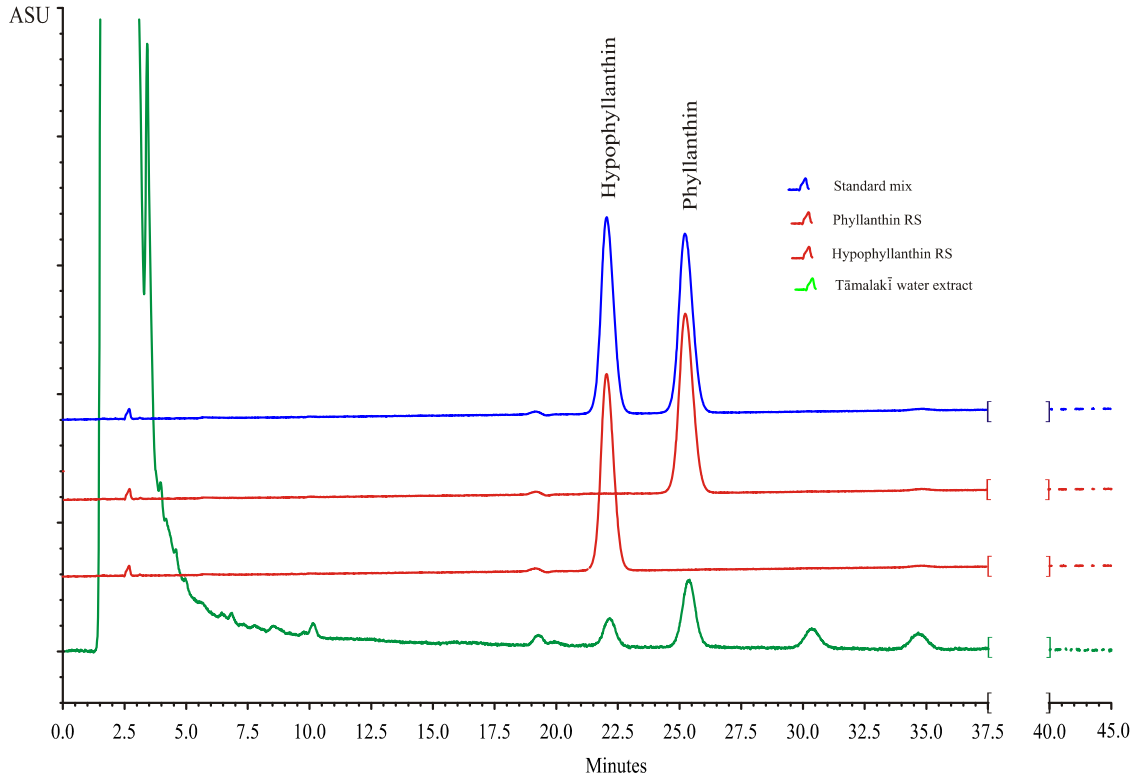


Fig.2. HPLC chromatograms of Tāmalakī water extract with *Phyllanthin*, *Hypophyllanthin* as *RS* and Standard mix

Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 3.5 volumes of *water* and 6.5 volumes of *methanol*. Injection volume: 20 µl. Flow rate: 1 ml per minute. Detection: UV, 230 nm. Procedure: Inject 20 µl of the filtered standard solutions and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the responses for the analyte peaks. Calculate the content of the *hypophyllanthin, phyllanthin* in the substance being examined from the peak response of analytes and from the declared content of *hypophyllanthin* and *phyllanthin* in *hypophyllanthin RS* and *phyllanthin RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Hypophyllanthin RS* and *Phyllanthin RS.*

VĀSĀ

Vāsā consists of fresh, dried, mature leaves of *Justicia adhatoda* L. syn. *Adhatoda zeylanica* Medicus (Fam. Acanthaceae), a sub-herbaceous bush, found throughout the year in plains and sub-Himalayan tracts in India, ascending up to 1200 m; flowers during February-March and also at the end of rainy season; leaves stripped off from older stems and dried in drying sheds. Vāsā contains not less than 0.3 per cent of *vasicine* when assayed.

Synonyms: Vṛṣa, Āṭarūṣa, Vāsaka

Regional Language Names:

Assamese	:	Titabahak, Bahak, Vachaka
Bengali	:	Baksa, Vasaka
English	:	Vasaka
Gujarati	:	Aduso, Ardusi, Adulso
Hindi	:	Aduss, Arusa
Kannada	:	Adsale, Adusoge, Atarusha, Adsole, Adasale
Kashmiri	:	Vasa
Malayalam	:	Attalatakam, Atalotakam
Marathi	:	Adulsa, Vasa
Oriya	:	Basanga
Punjabi	:	Bhekar, Vansa, Arusa
Tamil	:	Vasambu, Adathodai
Telugu	:	Addasaramu
Urdu	:	Adusa, Basa

Description:

a) *Macroscopic:*

Leaves, 10 to 30 cm long and 3 to 10 cm broad, lanceolate to ovate-lanceolate slightly acuminate, base tapering, petiolate; petioles 2 to 8 cm long, exstipulate, glabrescent, 8 to 10 pairs of lateral veins bearing a few hairs; dried leaves dull brown above, light greyish brown below; odour, characteristic; taste, bitter.

b) *Microscopic:*

Leaf -

Petiole – TS of petiole shows a single layered epidermis of parenchyma cells interrupted at places by multicellular trichomes; upto 10 layer hypodermis of parenchymatous cells; ground tissue of round parenchyma cells encircling a large, collateral arch-vascular bundle in the centre and two small vascular bundles in each wings bellow hypodermis.

Lamina - TS of lamina shows, dorsiventral structure with 2 layers of palisade cells; in surface view, epidermal cells sinuous with diacytic stomata on both surfaces, more numerous on the lower size; ordinary trichomes a few; 1 to 3, rarely upto 5 cells, uniseriate, upto 500 μ glandular

trichomes with unicellular stalk and 4 celled head measuring, 25 to 36 μ in diameter in surface view; spongy parenchyma 3 or 5 cells deep consisting of oval to circular cells, elongated cigar shaped cystoliths in midrib, spongy parenchyma and palisade cells, elongated and cigar shaped; acicular and prismatic forms of calcium oxalate crystals present in spongy parenchyma cells; palisade ratio 5 to 8.5; stomatal index 10 to 18 for lower surface; vein islets number 6.5 to 7.5.

c) Powder:

Powder shows fragments of thin-walled, cystolith bearing parenchyma cells; sinuous epidermal cells, acicular and prismatic crystals of calcium oxalate and debris from lamina showing sinuous epidermal cells with dicytic stomata, glandular and warty trichomes (Fig.1).

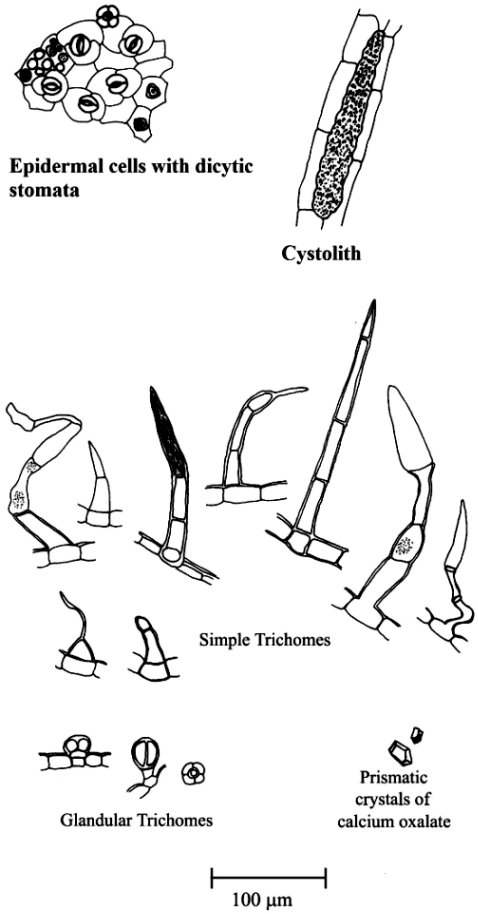


Fig.1. Powdered drug of Vāsā (*Justicia adhatoda* L.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 21.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 3.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 22.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5), using *vasicine hydrochloride* as a reference standard. Solvent system: *1,4-Dioxane* : *ammonia* (9.0 : 1.0). Test solution: To 5 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Reflux the residue futher with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume to 50-ml in a volumetric flask. Standard solution: Dissolve 10 mg of *vasicine hydrochloride RS* in 25 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *Dragendorff's reagent* and examine the plate in day light. The chromatogram obtained with test solution shows a band at R_f ~0.65 corresponding to that of *vasicine* and the profile should be similar to the one given in the TLC (Fig. 2).

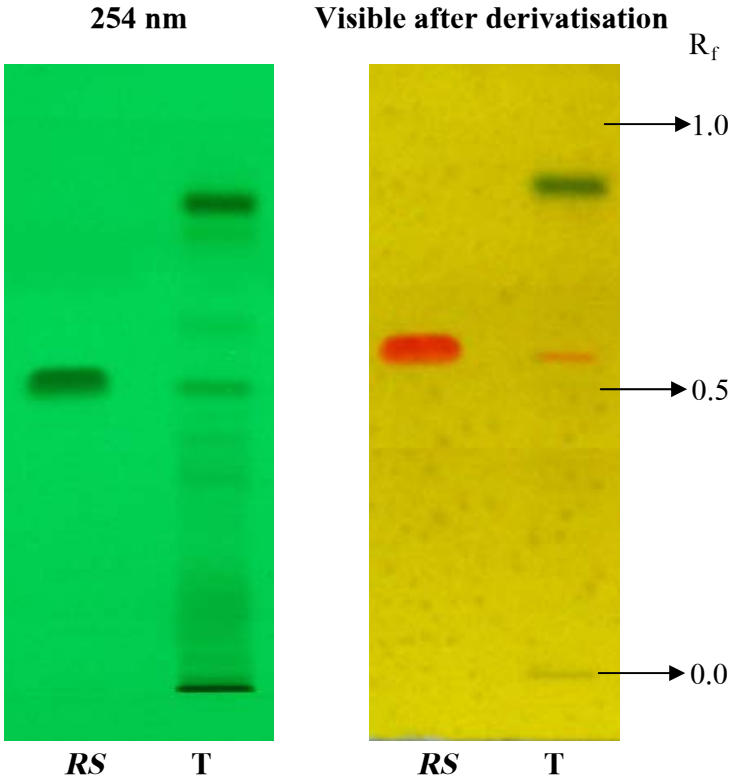


Fig.2. Thin-Layer Chromatogram of Vāsā
***RS*: *Vasicine hydrochloride*, T: Test solution**

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume to 100-ml in a volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*. Standard solution:

Take about 10 mg, accurately weighed, *vasicine hydrochloride RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5µm. Mobile phase: Filtered and degassed mixture of 92 volumes of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water* and adjusting the *p*H to 2.8 using *orthophosphoric acid*), 5 volumes of *acetonitrile* and 3 volumes of *tetrahydrofuran*. Injection volume: 20 µl. Flow rate: 1.0 ml per minute. Detection: UV, 280 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *vasicine* in the substance being examined from the peak response of analyte and from the declared content of *vasicine* in *vasicine hydrochloride RS*.

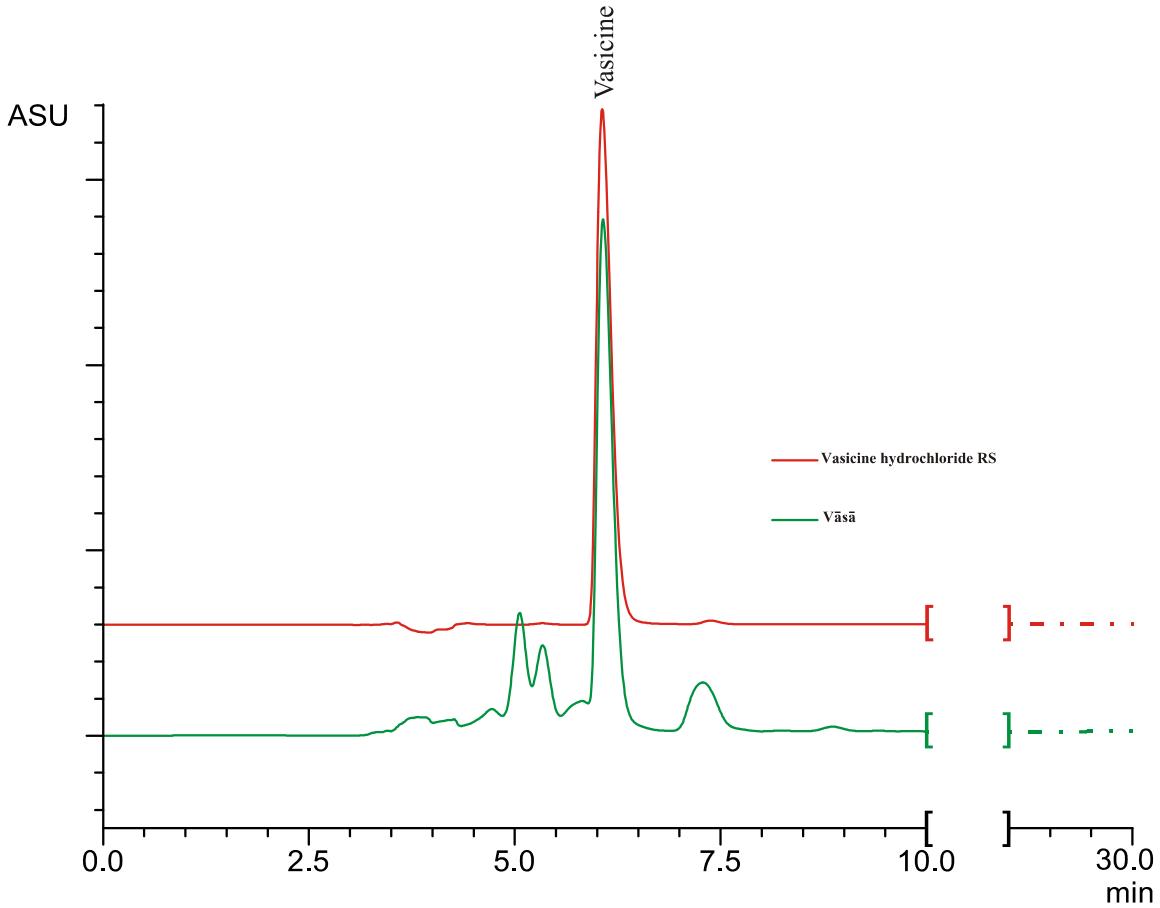


Fig.3. HPLC chromatogram of Vāsā with *Vasicine Hydrochloride* as *RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Vasicine hydrochloride RS*.

Constituents: Vasicine, vasicinone, deoxyvasicine, adhatonine, vasicinol.

Properties and Action:

Rasa	:	Tikta, Kaṣāya
Guṇa	:	Laghu
Vīrya	:	Śīta
Vipāka	:	Kaṭu
Karma	:	Kaphapittahara, Raktasaṅgrāhika, Kāsaghna, Hṛdya

Important formulations: Vāsakāsava; Vāsāvaleha.

Therapeutic uses: Kāsa (cough); Śvāsa (asthma); Kṣaya (pthisis); Raktapitta (bleeding disorders); Prameha (increased frequency and turbidity of urine); Kāmalā (jaundice); Kuṣṭha (diseases of skin).

Dose: 10 to 20 ml of juice of fresh leaves.
10 to 20 g of the dried drug for decoction.

VĀSĀ (POWDER)

Vāsā (Powder) consists of powder of Vāsā complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, greenish; odour not characteristic; taste slightly bitter; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash and mount a small portion in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Leaf debris showing diacytic stomata, abundant simple, multicellular, uniseriate ordinary trichomes and sessile glandular trichomes of various sizes and shapes; fragments of palisade cells embedded with cigar shaped cystolith and fatty oil globules; warty cysloliths embedded in parenchymatous cells of midrib and petiole; fragments of xylem vessels with reticulate, pitted, spiral and annular thickenings; a few acicular and prismatic crystals of calcium oxalate present in the mesophyll region.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Vāsā.

Assay: Complies with the limits for Assay as per method stated under Vāsā.

Additional requirements:

Packaging and Storage: As given under Vāsā.

Labeling: As given under Vāsā.

API reference standards: As given under Vāsā.

VĀSĀ HYDRO-ALCOHOLIC EXTRACT

Vāsā Hydro-alcoholic Extract is a dried and powdered extract prepared from Vāsā. The extract contains not less than 2.0 per cent of *vasicine* when assayed.

Method of Preparation:

Take Vāsā suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 18.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5), using *vasicine hydrochloride* as a reference standard. Solvent system: *1,4-Dioxane* : *ammonia* (9.0 : 1.0). Test solution: To 1 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *vasicine hydrochloride RS* in 25 ml of *methanol*. Procedure: Apply 10 μl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *Dragendorff's reagent* and examine the plate in day light. The chromatogram obtained with test solution shows a band at R_f ~0.65 corresponding to that of *vasicine* and the profile should be similar to the one given in the TLC (Fig. 1).

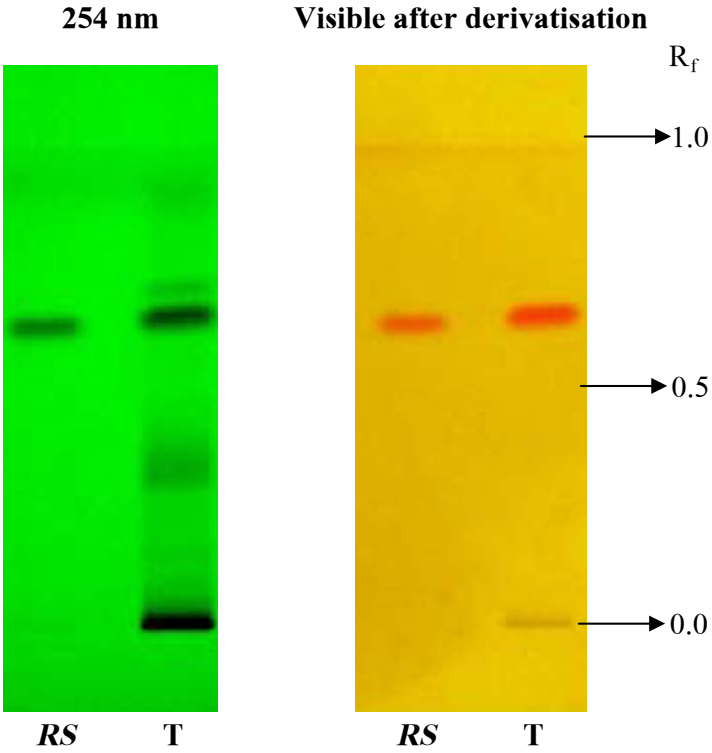


Fig.1. Thin-Layer Chromatogram of Vāsā hydro-alcoholic extract. *RS*: *Vasicine hydrocholoride,* T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 25.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
pH:	6.0 - 8.0,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 1 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume to 100-ml in a volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*.

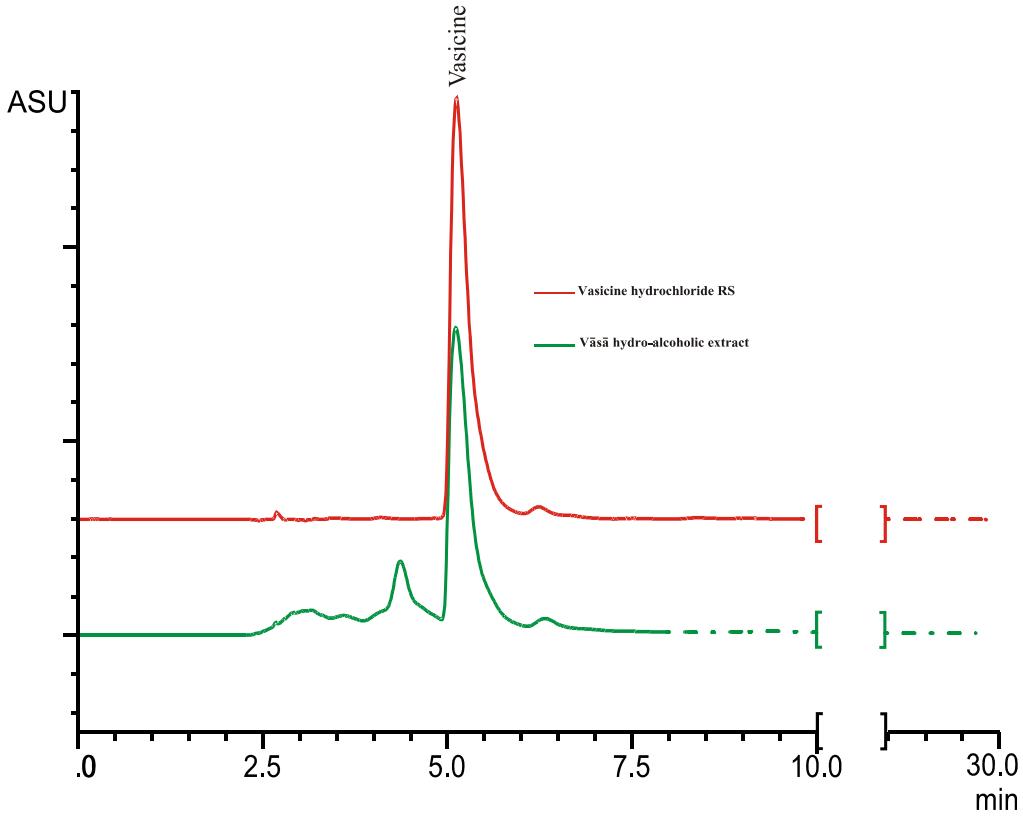


Fig.2. HPLC chromatogram of Vāsā hydro-alcoholic extract with *Vasicine hydrochloride* as *RS*

Standard solution: Take about 10 mg, accurately weighed, *vasicine hydrochloride RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5μm. Mobile phase: Filtered and degassed mixture of 92 volumes of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water* and adjusting the *p*H to 2.8 using *orthophosphoric acid*), 5 volumes of acetonitrile and 3 volumes of *tetrahydrofuran*. Injection volume: 20 μl. Flow rate: 1.0 ml per minute. Detection: UV, 280 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *vasicine* in the substance being examined from the peak response of analyte and from the declared content of *vasicine* in *vasicine hydrochloride RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Vasicine hydrochloride RS.*

VĀSĀ WATER EXTRACT

Vāsā Water Extract is a dried and powdered extract prepared from Vāsā. The extract contains not less than 1.0 per cent of *vasicine* when assayed.

Method of Preparation:

Take Vāsā suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 µm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5), using *vasicine hydrochloride* as a reference standard. Solvent system: *1,4-Dioxane* : *ammonia* (9.0 : 1.0). Test solution: To 2 g of the substance being examined, add 25 ml of *methanol*, heat on a water bath for 15 minutes and cool. Dilute to 50 ml with *methanol* and filter. Standard solution: Dissolve 10 mg of *vasicine hydrochloride RS* in 25 ml of *methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *Dragendorff's reagent* and examine the plate in day light. The chromatogram obtained with test solution shows a band at R_f ~0.65 corresponding to that of *vasicine* and the profile should be similar to the one given in the TLC (Fig. 1).

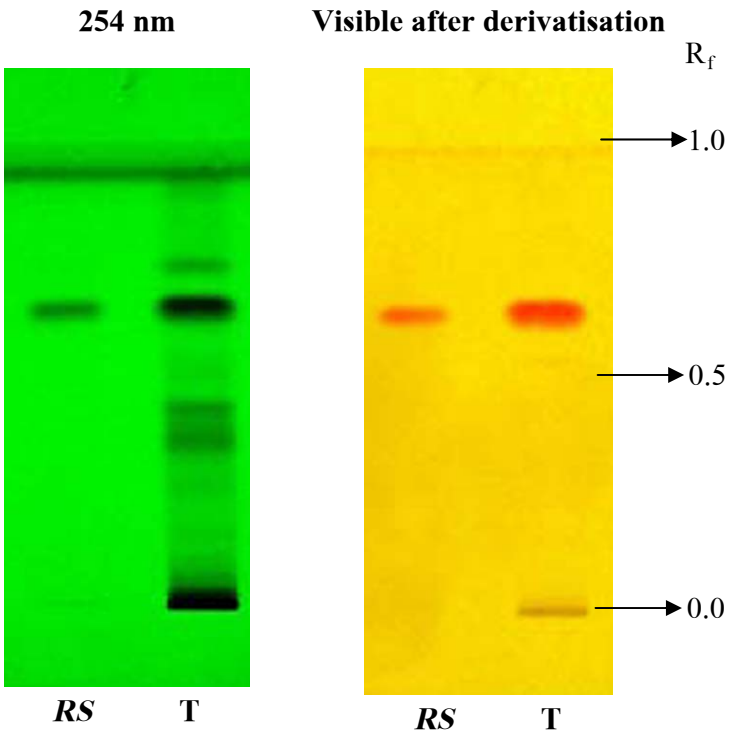


Fig.1. Thin-Layer Chromatogram of Vāsā water extract *RS*: *Vasicine hydrochloride*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 6.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 32.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 1.0 per cent,	Appendix 2.1.7
pH:	6.0 to 8.0,	Appendix 2.1.10
Total soluble matter:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 2 g, accurately weighed, of the substance being examined. Add 50 ml of *methanol*, reflux on a water bath for 15 minutes, cool and filter. Reflux the residue further with 50 ml of *methanol* for two times more, cool and filter. Combine all the filtrates, concentrate and make up the volume to 100-ml in a volumetric flask. Dilute 10 ml of this solution to 25 ml with *methanol*. Standard solution: Take about 10 mg, accurately weighed, *vasicine hydrochloride RS* in a 100-ml volumetric flask

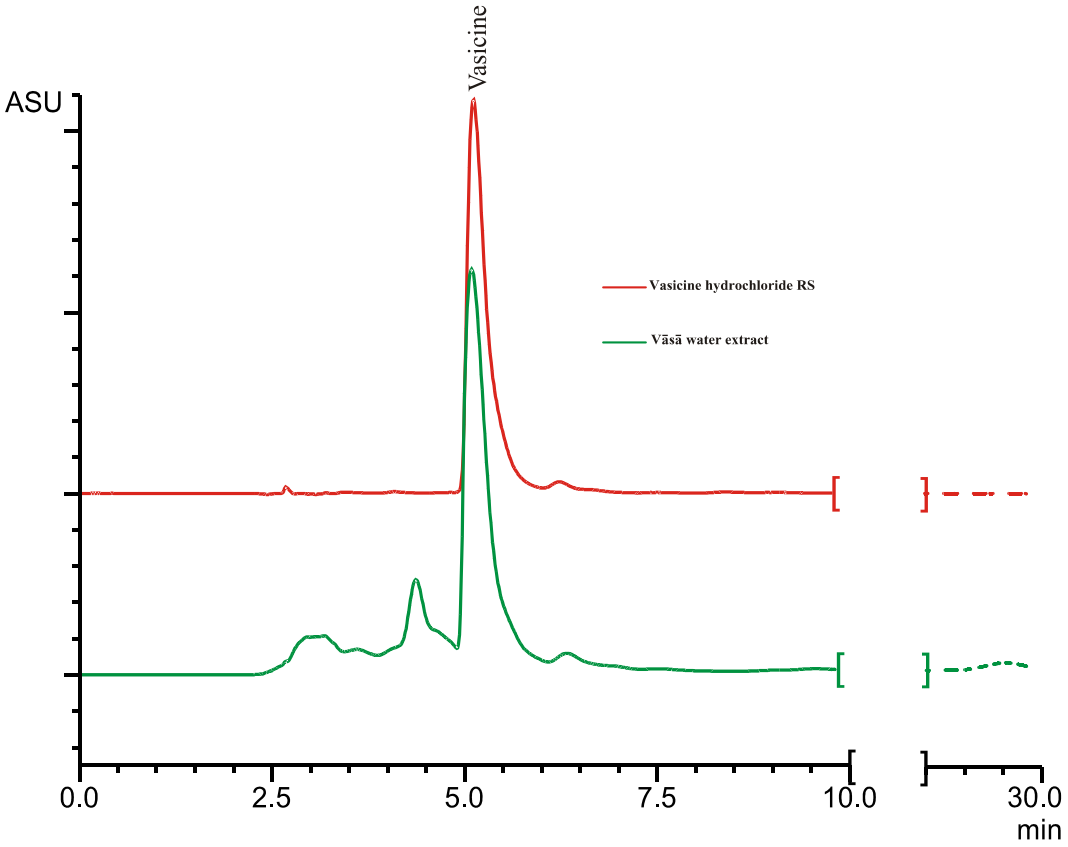


Fig.2. HPLC chromatogram of Vāsā water extract with *Vasicine hydrochloride* as *RS*

and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: Silica CN (250 mm x 4.6 mm), 5µm. Mobile phase: Filtered and degassed mixture of 92 volumes of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 500 ml of *water* and adjusting the *p*H to 2.8 using *orthophosphoric acid*), 5 volumes of acetonitrile and 3 volumes of *tetrahydrofuran*. Injection volume: 20 µl. Flow rate: 1.0 ml per minute. Detection: UV, 280 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *vasicine* in the substance being examined from the peak response of analyte and from the declared content of *vasicine* in *vasicine hydrochloride RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Vasicine hydrochloride RS.*

YAṢṬĪ

Yaṣṭī consists of dried, unpeeled, stolons and roots of *Glycyrrhiza glabra* L. (Fam. Fabaceae), a tall perennial herb, up to 2 m high found cultivated to a little extent in some parts of India, most of the yasti in trade are from import (Russia is a major producer). Yaṣṭī contains not less than 2.5 per cent of *glycyrrhizin* when assayed.

Synonyms: Yaṣṭīmadhuka, Yaṣṭika, Madhuka, Madhuyaṣṭī, Yaṣṭyāhva

Regional Language Names:

Assamese	:	Jesthimadhu, Yeshtmadhu
Bengali	:	Yashtimadhu
English	:	Liquorice root
Gujarati	:	Jethimadha, Jethimard, Jethimadh
Hindi	:	Mulethi, Mulathi, Muleti, Jethimadhu, Jethimadh
Kannada	:	Jestamadu, Madhuka, Jyeshtamadhu, Atimadhura
Kashmiri	:	Multhi
Malayalam	:	Irattimadhuram
Marathi	:	Jesthmadh
Oriya	:	Jatimadhu, Jastimadhu
Punjabi	:	Jethimadh, Mulathi
Tamil	:	Athimadhuram
Telugu	:	Atimadhuramu
Urdu	:	Mulethi, Asl-us-sus

Description:

a) *Macroscopic:*

Stolon consists of yellowish brown or dark brown, cylindrical, longitudinally wrinkled pieces, with occasional small buds and encircling scale leaves, transversely, cut and smoothened surface shows a cambium ring about one-third of radius from outer surface and a small central pith; root similar without a pith; fracture, coarsely fibrous in bark and splintery in wood; odour, faint and characteristic; taste, sweetish.

b) *Microscopic:*

Stolon – TS of stolon shows cork of 10 or 20 to more layers of tabular cells, outer layers with reddish-brown amorphous contents, inner 3 or 4 rows having thicker, colourless walls; secondary cortex usually of 1 or 3 layers of radially arranged parenchymatous cells containing isolated prisms of calcium oxalate; phloem a broad band, parenchymatous cells of inner part cellulosic and outer lignified; radially arranged groups of about 10 or 50 fibres, surrounded by a sheath of parenchyma cells, each usually containing a prism of calcium oxalate; cambium form a tissue of 3 or more layers of cells; secondary xylem distinctly radiate with medullary rays, 3 or 5 cells wide, vessels about 80 or 200 μ in diameter with thick, yellow, pitted, reticulately thickened walls; groups of lignified fibres with crystal sheaths similar to those in phloem; xylem parenchyma of

two kinds, those between the vessels having thick pitted walls, the remaining with thin walls; pith of parenchymatous cells, with intercellular spaces.

Root – TS of root shows structure closely resembling to that of stolon except that no pith is present; xylem tetrarch; usually four principal medullary rays at right angles to each other; all parenchymatous tissues containing abundant, simple, and compound consisting of 2 to 4 components, rounded to oval starch grains, measuring 6 to 13 μ in diameter.

c) Powder:

Fine powder shows fragments of fibres, vessels with simple and bordered pits; starch grains simple, oval to rounded, 2 to 4 or more components, measuring 6 to 13 μ in diameter (Fig.1).

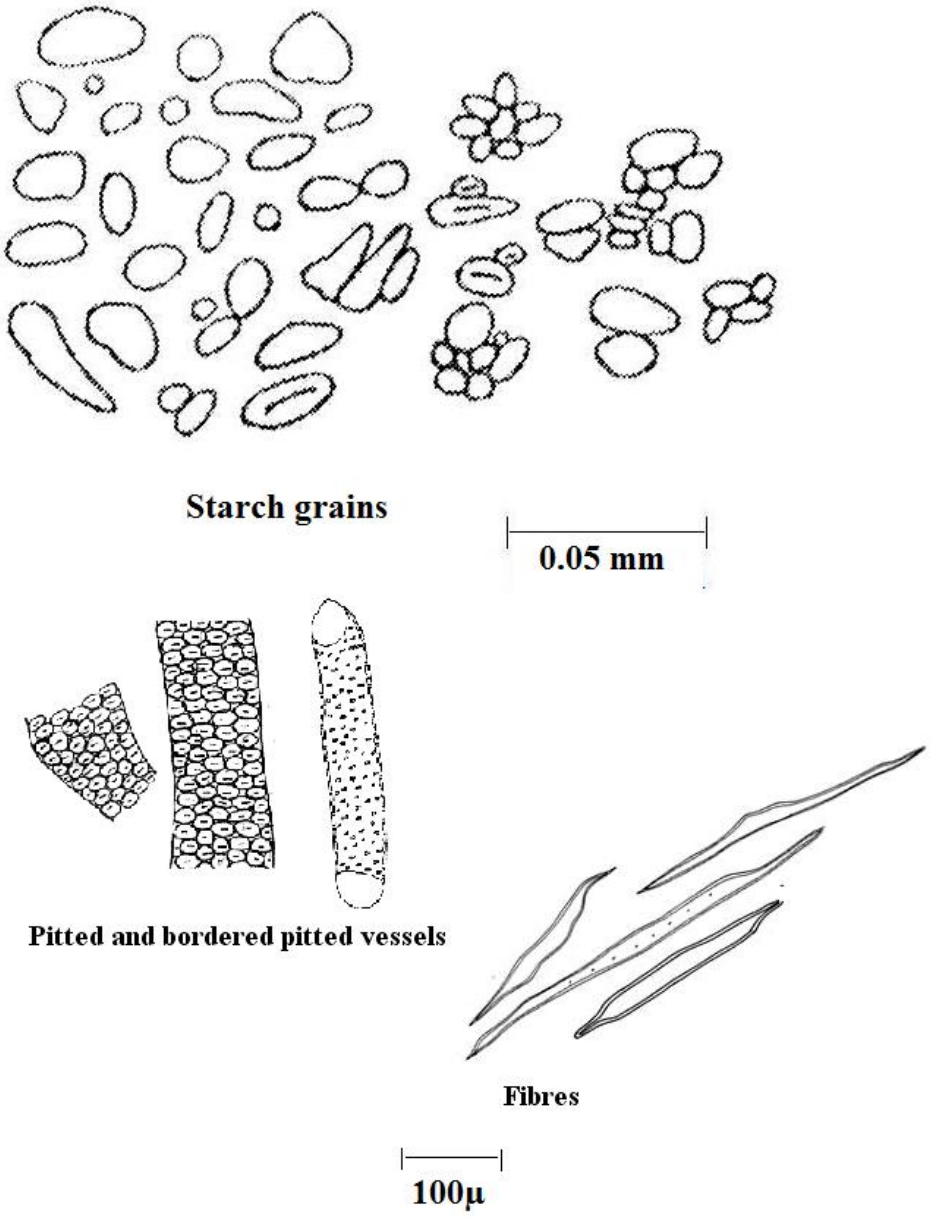


Fig.1. Powdered drug of Yaṣṭī (*Glycyrrhiza glabra* L.)

Identity, Purity and Strength:

Quantitative parameters:

Foreign matter:	Not more than 2.0 per cent,	Appendix 2.1.3
Loss on drying:	Not more than 12.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 2.5 per cent,	Appendix 2.1.7
Alcohol-soluble extractive:	Not less than 10.0 per cent,	Appendix 2.1.8
Water-soluble extractive:	Not less than 20.0 per cent,	Appendix 2.1.9

Identification:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel $60F_{254}$ plate (Appendix 3.5) using *glycyrrhizin ammonical hydrate* as a reference standard. Solvent system: *n-Butanol* : *water* : *glacial acetic acid* (7.0 : 2.0 : 1.0). Test solution: To 1 g of the substance being examined, add 50 ml of 70.0 per cent *aqueous methanol* heat on a water bath for 15 minutes and cool. Dilute to 100 ml with 70.0 per cent *aqueous methanol* and filter. Standard solution: Dissolve 10 mg of *glycyrrhizin ammonical hydrate RS* in 10 ml of 70.0 per cent *aqueous methanol*. Procedure: Apply 10 µl each of the test and standard solutions on a TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.30 corresponding to that of *glycyrrhizin* and the profile should be similar to the one given in the TLC (Fig. 2).

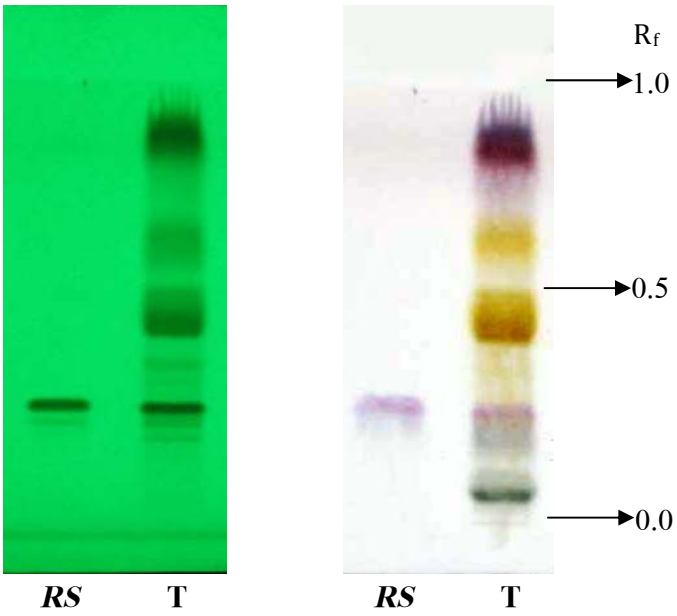


Fig.2. Thin-Layer Chromatogram of Yaṣṭī
***RS*: *Glycyrrhizin ammonical hydrate*,**
T: Test solution

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 1 g, accurately weighed, of the substance being examined, shake with 100 ml of 0.1M *ammonia solution* and sonicate for 30 minutes. Filter the suspension into a 100-ml volumetric flask and make up the volume with 0.1M *ammonia solution*. Dilute the stock solution appropriately to get the solution of the desired strength. Standard solution: Take about 5 mg, accurately weighed, *glycyrrhizin ammonical hydrate RS* in a 100-ml volumetric flask and dissolve in 50 ml of 0.1 M *ammonia solution* and make up the volume with 0.1 M *ammonia solution*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water*. Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *glycyrrhizin* in the substance being examined from the peak response of analyte and from the declared content of *glycyrrhizin* in *glycyrrhizin ammonical hydrate RS*.

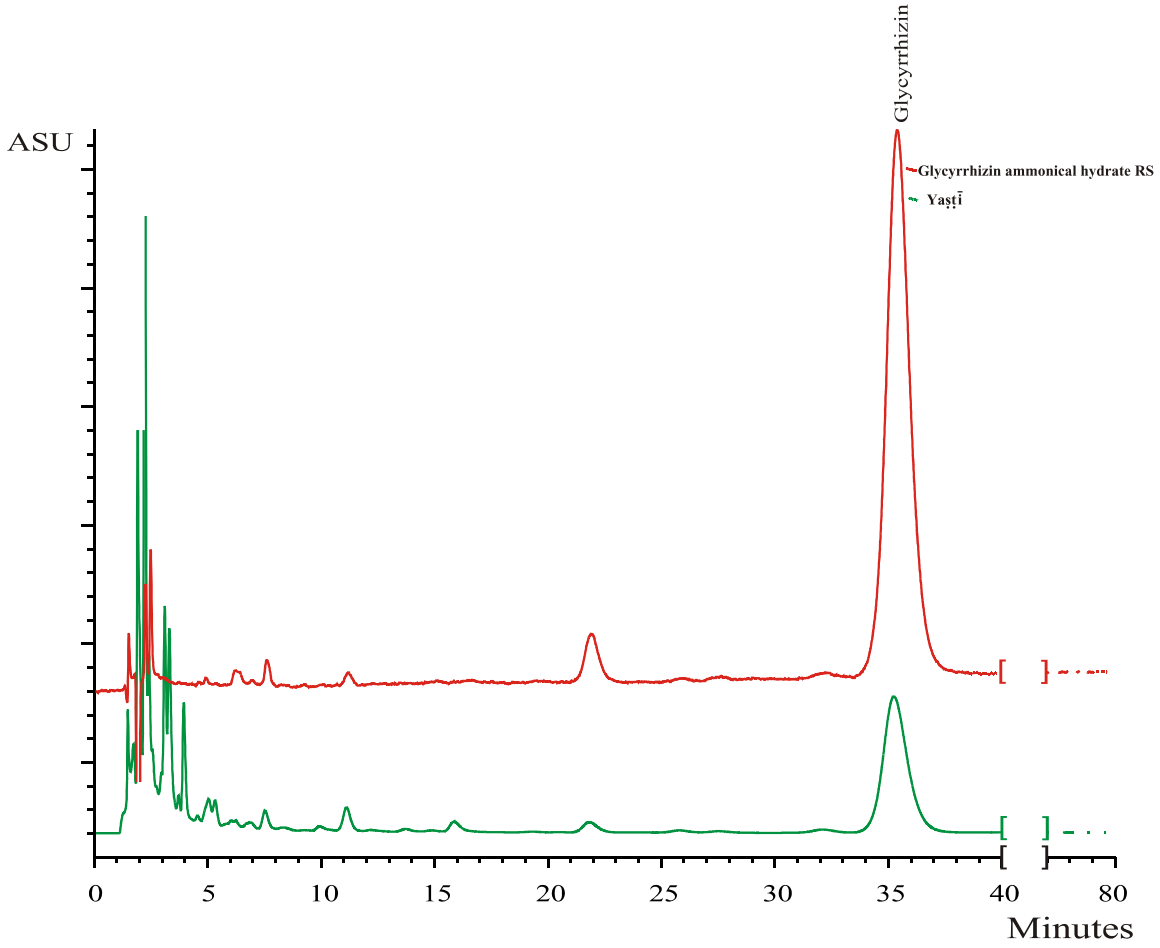


Fig.3. HPLC chromatogram of Yaṣṭī with *Glycyrrhizin ammonical hydrate as RS*

Additional requirements:

Packaging and Storage: Store in clean, well ventilated area protected from light, moisture and against attack by insects and rodents.

Labeling: The label states the official name, following the Latin binomial and the part of the plant contained in the article.

API reference standards: API *Glycyrrhizin ammonical hydrate RS.*

Constituents: Glycyrrhizin, glycyrrhetinic acid, glycryrrhetol, glabrolide, isoglabrolide, asparagine, sugars, resin and starch.

Properties and Action:

Rasa	:	Madhura
Guṇa	:	Guru, Snigdha
Vīrya	:	Śīta
Vipāka	:	Madhura
Karma	:	Vātapittajit, Raktaprasādana, Balya, Varṇya, Vṛṣya, Cakṣuṣya

Important formulations: Elādi guṭikā; Yaṣṭīmadhuka taila; Madhuyaṣṭyādi taila.

Therapeutic uses: Kāsa (cough); Svarabheda (hoarseness of voice); Kṣaya (pthisis); Vraṇa (ulcer); Vātarakta (gout).

Dose: 2 to 4 g of the drug in powder form.

YAṢṬĪ (POWDER)

Yaṣṭī (Powder) consists of powder of Yaṣṭī complying with the following requirements:

Description:

a) *Macroscopic:*

Coarse powder, brownish yellow; odour aromatic; taste sweet; all the particles to pass through a sieve with a nominal mesh aperture of 1.70 mm and not more than 40.0 per cent through a sieve with a nominal mesh aperture of 355 μm.

b) *Microscopic:*

Take a few mg of powder and warm with *chloral hydrate* over water bath, wash, and mount a small portion in *glycerin*; treat a few mg with *iodine* in *potassium iodide* solution and mount in *glycerin*; treat a few mg of powder with solution of *phloroglucinol*, allow to dry, add a few drops of *hydrochloric acid* and mount in *glycerin*.

Observe the following characteristics in the different mounts.

Shows abundant rectangular parenchyma cells with simple, spherical to ovoid and slightly flattened, starch grains with slit like hilum and a few compound starch grains with 2 to 4 components, measuring up to 15 μ; a few collenchyma cells also present; lignified vessel found singly or in small groups, border pitted; smaller, narrower vessels show a simple perforation in some what oblique end wall, larger vessels usually accompanied by lignified, moderately thin walled, xylem parenchyma cells; a few crystals of calcium oxalate occurs isolated, and in parenchyma, medullary rays and pith; abundant crystal fibres present.

Identity, Purity and Strength:

Complies with the tests for Identity, Purity, Strength and Thin-layer chromatography as stated under Yaṣṭī.

Assay: Complies with the limits for Assay as per method stated under Yaṣṭī.

Additional requirements:

Packaging and Storage: As given under Yaṣṭī.

Labeling: As given under Yaṣṭī.

API reference standards: As given under Yaṣṭī.

YAṢṬĪ HYDRO-ALCOHOLIC EXTRACT

Yaṣṭī Hydro-alcoholic Extract is a dried and powdered extract prepared from Yaṣṭī. The extract contains not less than 10.0 per cent of *glycyrrhizin* when assayed.

Method of Preparation:

Take Yaṣṭī suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 20.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F_{254} plate (Appendix 3.5) using *glycyrrhizin ammonical hydrate* as a reference standard. Solvent system: *n-Butanol* : *water*: *glacial acetic acid* (7.0 : 2.0 : 1.0). Test solution: To 1 g of the substance being examined, add 50 ml of 70.0 per cent *aqueous methanol* heat on a water bath for 15 minutes and cool. Dilute to 100 ml with 70.0 per cent *aqueous methanol* and filter. Standard solution: Dissolve 10 mg of *glycyrrhizin ammonical hydrate RS* in 10 ml of 70.0 per cent *aqueous methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.30 corresponding to that of *glycyrrhizin* and the profile should be similar to the one given in the TLC (Fig. 1).

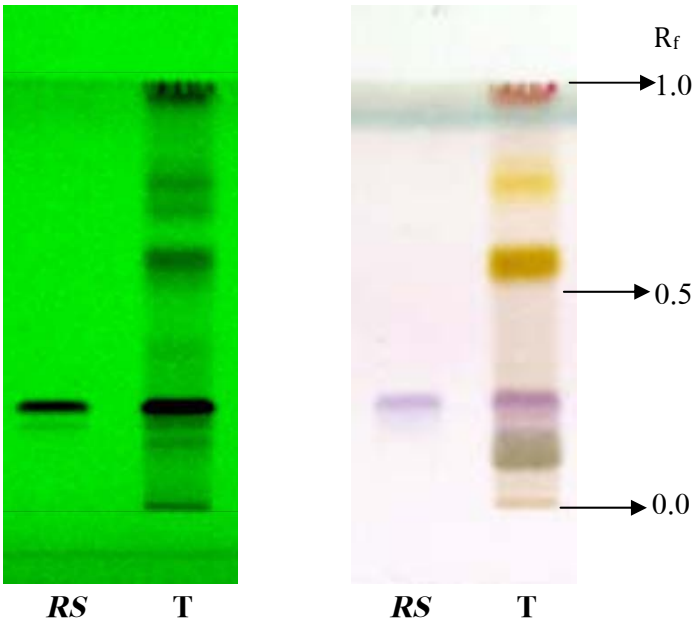


Fig.1. Thin-Layer Chromatogram of Yaṣṭī hydro-alcoholic extract. *RS*: *Glycyrrhizin ammonical hydrate*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 7.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 0.5 per cent,	Appendix 2.1.7
pH :	5.0 - 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-I)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Residual solvent:	Complies with the prescribed limits, Appendix 3.8
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 1 g, accurately weighed, of the substance being examined, shake with 100-ml of 0.1M *ammonia solution* and sonicate for 3 minutes. Filter the suspension into a 100-ml volumetric flask and make up the volume with 0.1M *ammonia solution*. Dilute the stock solution appropriately to get the solution of the desired strength.

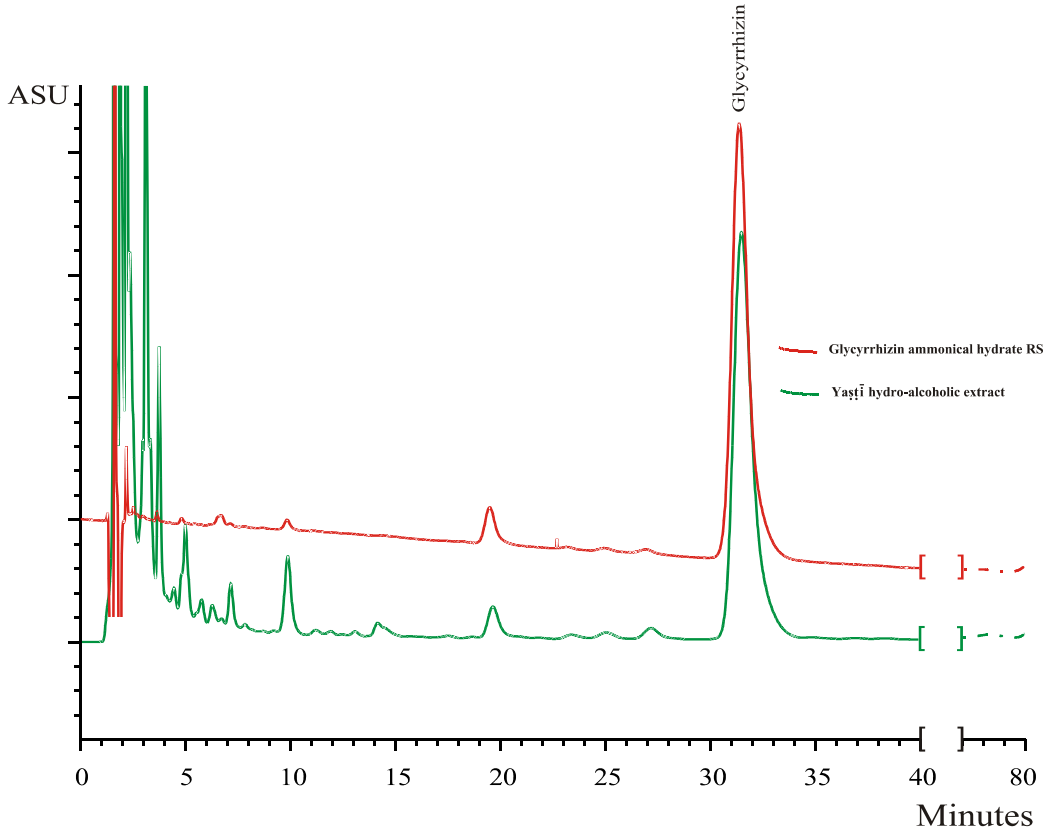


Fig.2. HPLC chromatogram of Yaṣṭī hydro-alcoholic extract with *Glycyrrhizin ammonical hydrate* as RS

Standard solution: Take about 5 mg, accurately weighed, *glycyrrhizin ammonical hydrate RS* in a 100-ml volumetric flask and dissolve in 50 ml of 0.1 M *ammonia solution* and make up the volume with 0.1 M *ammonia solution*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 μm. Mobile phase: Filtered and degassed mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water*. Injection volume: 20 μl. Flow rate: 1.5 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 μl of the filtered standard solution and record the chromatogram. Inject 20 μl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *glycyrrhizin* in the substance being examined from the peak response of analyte and from the declared content of *glycyrrhizin* in *glycyrrhizin ammonical hydrate RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light, moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Glycyrrhizin ammonical hydrate RS*.

YAṢṬĪ WATER EXTRACT

Yaṣṭī Water Extract is a dried and powdered extract prepared from Yaṣṭī. The extract contains not less than 8.0 per cent of *glycyrrhizin* when assayed.

Method of Preparation:

Take Yaṣṭī suitably sized (powder or pieces) in an extractor. Add *water*, about 3 times the quantity of raw material and heat at a temperature between 80-85^0 for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted three times more, filtering the extract each time into the same vessel. Concentrate the combined filtrate to a syrupy consistency and dry under vacuum (between 400-600 mm of Hg) at a temperature not exceeding 80^0 till the moisture is below 5.0 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 25.0 per cent.

Identity, Purity and Strength:

Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F$_{254}$ plate (Appendix 3.5) using *glycyrrhizin ammonical hydrate* as a reference standard. Solvent system: *n-butanol* : *water* : *glacial acetic acid* (7.0 : 2.0 : 1.0). Test solution: To 1 g of the substance being examined, add 50 ml of 70.0 per cent *aqueous methanol* heat on a water bath for 15 minutes and cool. Dilute to 100 ml with 70.0 per cent *aqueous methanol* and filter. Standard solution: Dissolve 10 mg of *glycyrrhizin ammonical hydrate RS* in 10 ml of 70.0 per cent *aqueous methanol*. Procedure: Apply 10 μl each of the test and standard solutions on TLC plate as bands of 10 mm. Develop the plate to a distance of 8 cm from the line of application. Dry the plate in air and examine under 254 nm. Spray the plate with solution of *anisaldehyde sulphuric acid reagent*. Heat the plate at 110^0 for about 5 minutes or till the bands are clearly visible. The chromatogram obtained with test solution shows a band at R_f ~0.30 corresponding to that of *glycyrrhizin* and the profile should be similar to the one given in the TLC (Fig. 1).

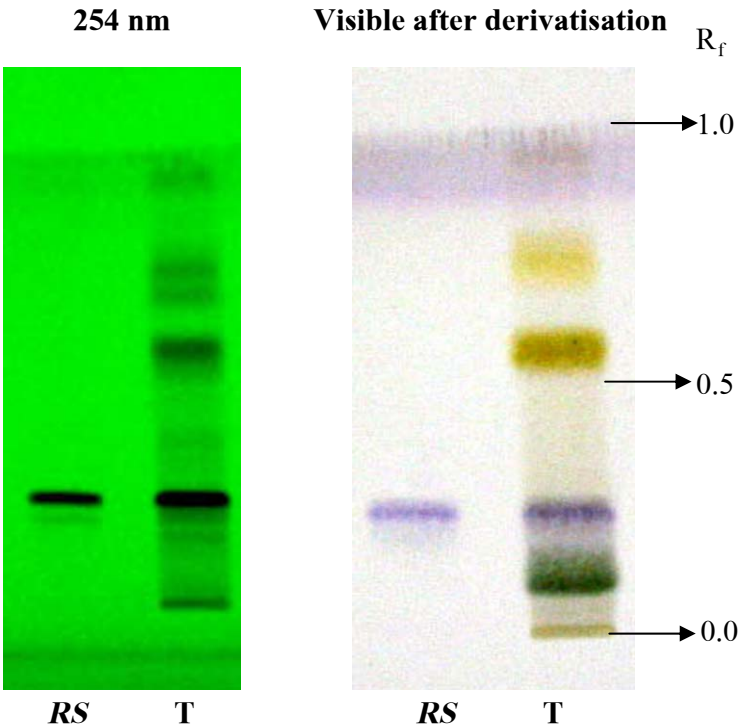


Fig.1. Thin-Layer Chromatogram of Yaṣṭī water extract. *RS*: *Glycyrrhizin ammonical hydrate*, T: Test solution

Quantitative parameters:

Loss on drying:	Not more than 5.0 per cent,	Appendix 2.1.4
Total ash:	Not more than 10.0 per cent,	Appendix 2.1.5
Acid-insoluble ash:	Not more than 0.5 per cent,	Appendix 2.1.7
pH:	5.5 to 6.5,	Appendix 2.1.10
Total soluble solids:	Not less than 90.0 per cent,	Appendix 2.1.11 (Method-II)

Other requirements:

Heavy metals:	Complies with the prescribed limits, Appendix 3.1
Microbial contamination:	Complies with the prescribed limits, Appendix 3.2
Pesticide residues:	Complies with the prescribed limits, Appendix 3.3
Aflatoxins:	Complies with the prescribed limits, Appendix 3.4

Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). Test solution: Take about 1 g, accurately weighed, of the substance being examined, shake with 100-ml of 0.1M *ammonia solution* and sonicate for 3 minutes. Filter the suspension into a 100-ml volumetric flask and make up the volume with 0.1M *ammonia solution*. Dilute the stock solution appropriately to get the solution of the desired strength. Standard solution: Take about 5 mg, accurately weighed, *glycyrrhizin ammonical hydrate RS* in a 100-ml volumetric flask and dissolve in 50 ml of 0.1 M

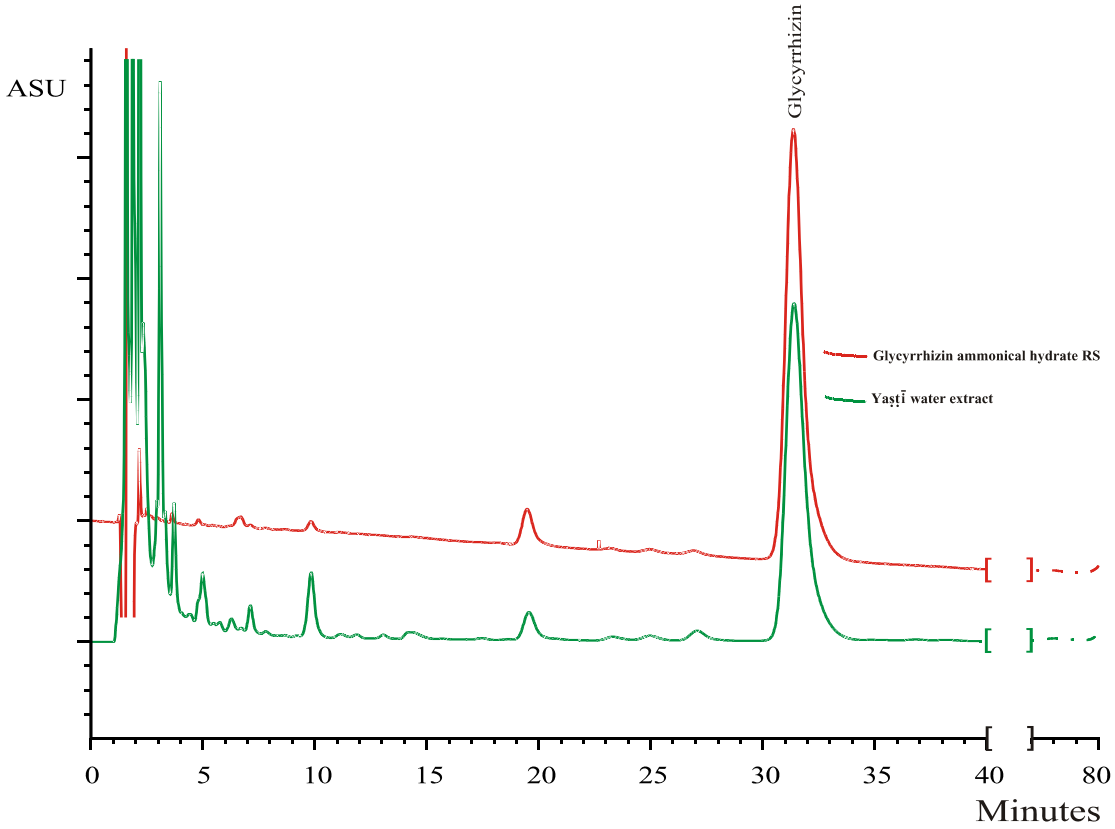


Fig.2. HPLC chromatogram of Yaṣṭī water extract with *Glycyrrhizin ammonical hydrate* as *RS*

ammonia solution and make up the volume with 0.1 M *ammonia solution*. Chromatographic system: High performance liquid chromatography. Column and stationary phase: C18 (250 mm x 4.6 mm), 5 µm. Mobile phase: Filtered and degassed mixture of 6 volumes of *glacial acetic acid*, 30 volumes of *acetonitrile* and 64 volumes of *water*. Injection volume: 20 µl. Flow rate: 1.5 ml per minute. Detection: UV, 254 nm. Procedure: Inject 20 µl of the filtered standard solution and record the chromatogram. Inject 20 µl of the filtered test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *glycyrrhizin* in the substance being examined from the peak response of analyte and from the declared content of *glycyrrhizin* in *glycyrrhizin ammonical hydrate RS*.

Additional requirements:

Packaging and Storage: Preserve in well closed containers, protected from light and moisture and store at room temperature.

Labeling: The label states the official name, following the Latin binomial, the part of the plant used and type of extract.

API reference standards: API *Glycyrrhizin ammonical hydrate RS.*

APPENDICES

APPENDIX – 1

1.1 Apparatus for Tests and Assays

1.1.1. - Nessler Cylinders

Nessler cylinders which are used for comparative tests are matched tubes of clear colourless glass with a uniform internal diameter and flat, transparent base. These comply with Indian Standard 4161-1967 and are of transparent glass with a nominal capacity of 50 ml. The overall height is about 150 mm, the external height to the 50 ml mark 110 to 124 mm, the thickness of the wall 1.0 to 1.5 mm and the thickness of the base 1.5 to 3.0 mm. The external height to the 50 ml mark of the cylinder used for a test must not vary by more than 1 mm.

1.1.2. - Sieves

Sieves for pharmacopoeial testing are constructed from wire cloth with square meshes, woven from wire of brass, bronze, stainless steel or any other suitable material. The wires should be of uniform circular cross-section and should not be coated or plated. There must be no reaction between the material of the sieve and the substance being sifted.

Sieves conform to the following specifications –

Table 1

IS 460 (Pt I) 1985 (Reaffirmed 1998)	IS 460-1978
mm	--
4.0	4
2.8	6
2.0	8
1.7	10
1.4	12
1.0	16
μm	--
710	22
600	25
500	30
425	36
355	44
250	60
180	85

150	100
125	120
106	150
90	170
75	200
63	240
53	300
45	350

Designation

Test sieves of metal wire cloth are designated by the nominal size of aperture of the wire cloth, followed by the inscription 'IS Sieve'.

Examples:

a. 5.60-mm IS Sieve

b. 425 μm IS Sieve

Nominal aperture sizes of 1 mm and above, as well as their associated tolerances and wire diameters, are expressed in millimetres (mm) and for aperture sizes smaller than 1 mm, these are expressed in micrometres (μm).

1.1.3. - Thermometers

Unless otherwise specified, thermometers suitable for pharmacopoeial tests conform to Indian Standard 4825-1968 and are standardised in accordance with the 'Indian Standard Method of Calibrating Liquid-in-Glass Thermometers', 6274-1971.

The thermometers are of the mercury-in-glass type and are filled with a dried inert gas, preferably nitrogen. They may be standardised for total immersion or for partial immersion. Each thermometer should be employed according to the condition of immersion under which it was standardised. In the selection of the thermometer it is essential to consider the conditions under which it is to be used.

1.1.4. - Ultraviolet Lamp (For general purposes and for chromatography work)

An instrument consisting of mercury vapour lamp and a filter which gives an emission band with maximum intensity at about 254 nm (near UV rays) and 366 nm (far UV rays) is used. To ensure that the required emission is being given by the lamp, carry out the following test periodically. Apply to a plate coated with *silica gel* G, 5 μl of a 0.04 per cent w/v solution of *sodium salicylate* in *ethanol* (95 per cent) for lamps of maximum output at 254 nm and 5 μl of a 0.2 per cent w/v solution in *ethanol* (95 per cent) for lamps of maximum output at 365 nm. Examine the spot in a position normal to the radiation. The distance between the lamp and the plate under examination used in a pharmacopoeial test should not exceed the distance used to carry out the above test.

1.1.5. - Volumetric Glassware

Volumetric apparatus is normally calibrated at 27^0. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25^0. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27^0.

Pharmacopoeial assays involving volumetric measurements require the use of accurately calibrated glassware. Volumetric apparatus must be suitably designed to assure accuracy. The design, construction and capacity of volumetric glassware should be in accordance with those laid down by the Bureau of Indian Standards. The tolerances on capacity for volumetric flasks, pipettes and burettes, as laid down in the relevant Indian Standards, are permissible.

1.1.6. - Weights and Balances

Pharmacopoeial tests and assays require the use of analytical balances that vary in capacity, sensitivity and reproducibility. The accuracy needed for a weighing should dictate the type of balance. Where substances are to be "accurately weighed", the weighing is to be performed so as to limit the error to not more than 0.1 per cent. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and quantity of 10 g is to be weighed to the nearest 10 mg. A balance should be chosen such that the value of three times the standard deviation of the reproducibility of the balance, divided by the amount to be weighed, does not exceed 0.001.

APPENDIX - 2

2.1 Tests and Determinations

2.1.1 Microscopic Identification of Botanical Substances:

Microscopic identification of the botanical ingredients is a standard for statutory purposes in several monographs. Appropriate processing for separation and isolation with suitable clearing reagents and stains, and finally mounting a little part on a slide that helps to show the unit structures is to be followed. Identification of the discrete, but disoriented units will not be possible without proper isolation and should not be attempted.

Monographs where the test is prescribed give both a relevant method of isolation and diagnostic features specific to the expected ingredient. Only a brief method and a few of the characteristics for each ingredient are given, but an analyst may use other methods of isolation and choose more characteristics to draw a correct conclusion.

A. Stains and Reagents for Microchemical Reactions:

For the purpose of identification and characterization of materials expected to be included in the prescribed standards, the following stains and reagents are recommended for use wherever relevant, in addition to those mentioned in the monograph.

Acetic Acid: Dilute 6 ml of *glacial acetic acid* with 100 ml of distilled *water*; *used for identification of cystoliths, which dissolve with effervescence.*

Aniline Chloride Solution: Dissolve 2 g in a mixture of 65 ml of 30 per cent *ethyl alcohol* and 15 ml distilled *water* and add 2 ml of conc. *hydrochloric acid. Lignified tissues are stained bright yellow.*

Bismarck Brown: Dissolve 1 g in 100 ml of 95 per cent of *ethyl alcohol*; *used as a general stain for macerated material (with Schultze's).*

Chlorinated Soda Solution (Bleaching Solution): Dissolve 75 g of *sodium carbonate* in 125 ml of distilled *water*; triturate 50 g of chlorinated lime (bleaching powder) in a mortar with 75 ml of distilled *water*, adding it little by little. Mix the two liquids and shake occasionally for three or four hours. Filter and store, protected from light. *Used for lightening highly coloured material, by warming in it and washing the tissues thoroughly.*

Breamer's Reagent: Dissolve 1 g of *sodium tungstate* and 2 g of *sodium acetate* in sufficient quantity of *water* to make 10 ml. Yellowish to brown precipitates; *indicate the presence of tannins.*

Canada Balsam (as a Mountant): Heat Canada balsam on a *water* bath until volatile matter is removed and the residue sets to a hard mass on cooling. Dissolve residue in xylene to form a thin syrupy liquid. *Used for making permanent mounts of reference slides of selected debris.*

Chloral Hydrate Solution: Dissolve 50 g of *chloral hydrate* in 20 ml of distilled *water*. *A valuable clarifying agent for rendering tissues transparent and clear, by freeing them from most of the ergastic substances, but leaving calcium oxalate crystals unaffected.*

Chloral Iodine: Saturate *chloral hydrate* solution with *iodine*, leaving a few crystals undissolved; *useful for detecting minute grains of starch otherwise undetectable.*

Chlorziniciodine (Iodinated Zinc Chloride Solution): Dissolve 20 g of *zinc chloride* and 6.5 g of *potassium iodide* in 10 ml of distilled *water*. Add 0.5 g of *iodine* and shake for about fifteen minutes before filtering. Dilute, if needed, prior to use. *Renders cellulosic walls bluish violet and lignified walls yellowish brown to brown.*

Chromic Acid Solution: 10 g of *potassium chromate* dissolved in 90 ml of dilute *sulphuric acid*: *A macerating agent similar to Schultze's.*

Corallin Soda: Dissolve 5 g of corallin in 100 ml of 90 per cent ethyl alcohol. Dissolve 25 g of *sodium carbonate* in 100 ml distilled *water*; keep the solutions separate and mix when required, by adding 1 ml of the corallin solution to 20 ml of the aqueous *sodium carbonate* solution. Prepare fresh each time, as the mixture will not keep for long. *Used for staining sieve plates and callus bright pink and imparts a reddish tinge to starch grains and lignified tissues.*

Ammoniacal Solution of Copper Oxide (Cuoxam): Triturate 0.5 g of *copper carbonate* in a mortar with 10 ml of distilled *water* and gradually add 10 ml of strong solution of *ammonia* (sp. gr. 0.880) with continued stirring; *used for dissolving cellulosic materials.*

Eosin: 1 per cent solution in 90 per cent *ethyl alcohol*; *Stains cellulose and aleurone grains red.*

Ferric Chloride Solution: A 5 per cent solution of *ferric chloride* in distilled *water*. *Taninns containing tissues coloured bluish or greenish black.*

Glycerin: Pure or diluted as required with one or two volumes of distilled *water*. *Used as a general mountant.*

Haematoxylin, Delafield's: Prepare a saturated solution of *ammonia* alum. To 100 ml of this add a solution of one g of *Haematoxylin* in 6 ml of ethyl alcohol (97 per cent). Leave the mixed solution exposed to air and light in an unstopped bottle for three or four days. Filter and add to the filtrate 25 ml of *glycerin* and 25 ml of *methyl alcohol*. Allow the solution to stand exposed to light, till it acquires a dark colour (about two months). Refilter and store as a stock solution. Dilute it 3 or 4 times volumes with distilled *water*. *Stains cellulosic fibers blue; used only on water washed material.*

Iodine Water: Mix one volume of decinormal iodine with 4 volumes of distilled *water*. *Stains starch blue, and reveals crystalloids and globoids when present in aleurone grains.*

Iodine in Potassium Iodide Solution: Dissolve one g of *potassium iodide* in 200 ml of distilled *water*, add 2 g of iodine to the solution and dissolved it; *stains lignified walls yellow and cellulosic walls blue.*

Lactophenol (Amman's Fluid): *Phenol* 20 g, *Lactic acid* 20 g, *Glycerin* 40 g, dissolved in *distilled water* 20 ml; *reveals starch grains in polarised light with a well marked cross at hilum, and also minute crystals of calcium oxalate as brightly polarising points of light.*

Methylene Blue: A solution of 0.1 g of *Methylene blue* in 25 ml of *ethyl alcohol* (95 per cent). *A general stain for nucleus and bacteria.*

Millon's Reagent: Dissolve one volume of mercury in 9 volumes of fuming *nitric acid* (Sp. gr. 1.52), keeping the mixture well cooled during reaction. Add equal volume distilled *water* when cool. *Stains proteins red.*

Naphthol Solution: Dissolve 10 g of Naphthol in 100 ml of *ethyl alcohol*; *a specific stain for detection of inulin; cells containing inulin turn deep reddish violet.*

Phloroglucinol: 1 g of *phloroglucinol* dissolved in 100 ml of 90 per cent *ethyl alcohol*; mount debris in a few drops, allow to react for a minute, draw off excess of reagent with a filter paper strip and add a drop of conc. *hydrochloric acid* to the slide; *lignified tissues acquire a deep purplish red colour; very effective on water washed material but not in chloral hydrate washed debris,* for which alcoholic solution of safranin is more effective (See Safranin).

Picric Acid Solution (Trinitrophenol Solution): A saturated aqueous solution made by dissolving 1 g of picric acid in 95 ml of distilled *water*; *stains animal and insect tissues, a light to deep yellow; in a solution with ethyl alcohol, aleurone grains and fungal hyphae are stained yellow.*

Potash, Caustic: A 5 per cent aqueous solution*; used to separate tenacious tissues of epidermis and also laticiferous elements and vittae, both of which are stained brown.*

Ruthenium Red: Dissolve 0.008 g of ruthenium red in 10 ml of a 10 per cent solution of lead acetate; (to be freshly prepared) *used for identification of most kinds of mucilage containing tissues, which turn pink. A 0.0008 g ruthenium red dissolved in 10 ml of distilled water and used immediately stains cuticular tissues in debris to a light pink*.

Safranin: A one per cent solution in 50 per cent *ethyl alcohol*; *used to stain lignified cell walls deep red, even after clearing with choral hydrate.*

Schultze's Maceration Fluid: Add isolated debris to 50 per cent conc. *nitric acid* in a test tube and warm over *water* bath: add a few crystals of *potassium chlorate* while warming, till tissues soften; cool, wash with *water* thoroughly and tease out for mounting hard tissues; *isolated cell structures are clearly revealed, but the structures are not useful for measurement of dimensions*.

Sehweitzer's Reagent: Same as Ammoniacal Copper Oxide Solution (Cuoxam).

Sudan Red III: Dissolve 0.01 g of sudan red III in 5 ml of *ethyl alcohol* (90 per cent) and 5 ml of pure *glycerin*; *suberised walls of cork cells, and fatty material in cells are stained bright red.*

Sulphovanadic Acid (Mandelin's Reagent)**:** Triturate one g of ammonium vandate with 100 ml conc. s*ulphuric acid*. Allow the deposit to subside and use the clear liquid. *This is to be prepared fresh; useful for identification of alkaloids, particularly strychnine which turns violet in the cells containing it.*

2.1.2. Net Content:

The content of the final or retail pack shall be not less than 98 percent of the declared net content.

2.1.3. Determination of Foreign Matter

A. FOREIGN MATTER

The sample shall be free from visible signs of mould growth, sliminess, contamination by insects and other animal and animal products including animal excreta or any other noxious foreign matter. Foreign matter consists of any organism, part or product of an organism, other than that named in the definition of the product and mineral admixtures, such as soils, stones, sand and dust. It shall also include other than official parts of organism beyond their specified limits.

Take 100 g of sample (unless otherwise specified) and spread in a thin layer on a suitable platform. Examine in daylight with unawed eye or using 6x or 10x magnifying glass and separate the foreign matter. Appropriate sieve can also be used to separate the foreign matter. Dust regarded as mineral admixture is separated by sifting the sample through a 250 μm sieve. Weigh the sorted foreign matter and calculate the foreign matter content in per cent with reference to drug sample.

2.1.4. Determination of Moisture Content (Loss on Drying):

Dry the evaporating dish for 30 minutes under the same conditions to be employed in the determination. Place about 5 to 10 g of powder/drug accurately weighed in a tared evaporating dish. For unpowdereded drug, prepare about 10 g of the sample by cutting, shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. By gentle, sidewise shaking, distribute the test specimen as evenly as practicable to a depth of about 5 mm generally, and not more than 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber. Dry the test specimen at 105^0 for 3 hours and weigh. Continue the drying and weighing at half an hour interval until difference between two successive weighing corresponds to, not more than 0.25 per cent.

2.1.5. Determination of Total Ash:

Incinerate about 2 to 3 g, accurately weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 600^0 until free from carbon, cool in a desiccator for 30 minutes and weigh without delay. If carbon free ash cannot be obtained in this way, exhaust the charred mass with hot *water*, collect the residue on an ashless filter paper, incinerate the residue and filter paper,

add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 600^0. Calculate the percentage of ash with reference to the air-dried drug.

2.1.6. Determination of Water-Soluble Ash:

Boil the ash obtained in (2.1.5) for 5 minutes with 25 ml of *water*; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot *water*, and ignite for 15 minutes at a temperature not exceeding 450^0. Substract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

2.1.7. Determination of Acid-insoluble Ash:

To the crucible containing total ash, add dropwise 25 ml of *dilute hydrochloric acid*. Collect the insoluble matter on an ashless filter paper (Whatman 41) and wash with hot *water* until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccator for 30 minutes and weigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried drug.

2.1.8. Determination of Alcohol-soluble Extractive:

Macerate 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol of specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish and dry at 105^0, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

2.1.9. Determination of Water-soluble Extractive:

Proceed as directed for the determination of Alcohol-soluble extractive, using *chloroform water* (2.5 ml chloroform in purified *water* to produce 1000 ml) instead of ethanol.

2.1.10. Determination of *p*H Values:

The *p*H value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in g per litre. For the purpose of pharmacopoeia *p*H is defined as the value given by a suitable, properly standardized, *p*H meter capable of reproducing *p*H values to 0.05 *p*H unit using an indicator electrode sensitive to hydrogen-ion activity, the glass electrode and a suitable reference electrode. The instrument should be capable of sensing the potential across the electrode pair and for *p*H standardization purposes, applying an adjustable potential to the circuit by manipulation of "standardization," "zero," "asymmetry," or "calibration" control, and should be able to control the change in millivolts per unit change in *p*H

reading through a "temperature" and/or "slope" control. Measurements are made at 25 ± 2^0, unless otherwise specified.

To standardize the *p*H meter, select two Buffer Solutions whose difference in *p*H does not exceed 4 units such that the expected *p*H of the material under test falls between them. Commercially available buffer solutions for *p*H meter standardization, having traceability to the National Standards can be used.

Fill the cell with one of the Buffer Solutions for Standardization at the temperature at which the test material is to be measured. Set the "temperature" control at the temperature of the solution, and adjust the calibration control to make the observed *p*H value identical with that of the declared *p*H. Rinse the electrodes and cell with several portions of the second Buffer Solution for Standardization, then fill the cell with it, at the same temperature as the material to be measured. The *p*H of the second buffer solution is within ±0.07 *p*H unit of the declared value. If a larger deviation is noted, examine the electrodes and if they are faulty, replace them. Repeat the standardization until both Buffer Solutions for Standardization give observed *p*H values within 0.05 *p*H unit of the declared value without further adjustment of the controls.

When the system is functioning satisfactorily, rinse the electrodes and cell several times with a few portions of the test material, fill the cell with the test material, and read the *p*H value. Use carbon dioxide-free *water* for solution or dilution of test material in *p*H determinations. In all *p*H measurements, allow a sufficient time for stabilization.

Unless otherwise specified in the monograph prepare 5 per cent w/v of the sample. Filter if it is not soluble completely and use the filtrate to measure the *p*H.

2.1.11. Determination of Total Soluble Solids: Method-I

Take about one g, accurately weighed, of the substance being examined in a 100-ml volumetric flask, dissolve in 50 ml of 50 per cent v/v aqueous ethanol, sonicate for 10 minutes, heat on *water* bath (avoiding evaporation), cool and dilute to 100 ml with 50 per cent v/v aqueous ethanol. Mix and quickly pipette out 25 ml solution to a tared glass dish and evaporate. Centrifuge the remaining liquid for 10 minutes at 3000 rpm. Pipette out 25 ml of the supernatant obtained after centrifugation to a tared glass dish and evaporate. After evaporation of solvent, place the glass dishes in oven at 105^0 to dry to a constant weight. The weight of residue obtained after centrifugation is not less than 90 per cent of the weight of the residue obtained before centrifugation.

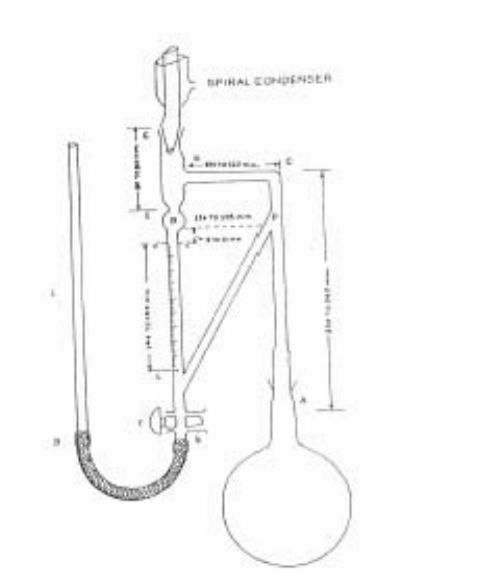
Determination of Total Soluble Solids: Method-II

Replace the aqueous ethanol with *water* and follow the procedure as given in **Method- I**.

2.1.12. Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of *water* and *glycerin,* collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w.

The apparatus consists of the following parts (See Fig.). The apparatus described below is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.



(a)Distilling Flask –A spherical flask, 1,000 ml capacity with ground neck, taper of ground socket 1 in 10, internal dia. of larger end 34.35 to 34.65 mm.

(b)Still Head – Graduated measuring tube and return flow tube made in one piece, in accordance with the following specifications. External diameter of the smaller end 31.0 to 31.2 mm. Minimum length of the ground zone –34 mm.

Tube AC, length –220 to 240 mm.

Internal diameter –13 to 15 mm.

Bulb CD, length –100 to 110 mm.

Internal diameter –13 to 15 mm.

Spiral Condenser – Ground joint accurately fitting in the ground neck of the tube EG, taper 1 in 10.

Tube EG, length –80 to 90 mm.

Internal Diameter –30 to 40 mm.

Bulb B –length 20 to 22 mm.

Internal diameter –15 to 20 mm.

The distance between B and P is 120 to 125 mm.

Junction P and the centre of the bulb B must be in the same horizontal plane.

Measuring tube JL – Length of the graduated portion 144 to 155 mm capacity 2 millilitres graduated into fifths and fiftieths of a millilitre.

Tube PL – Return flow tube –Internal diameter –7 to 8 mm.

Levelling tube I, length –450 to 500 mm. Internal diameter 10 to 12 mm tapering at the lower end with a wide top (20 to 25 mm diameter).

Rubber tubing a—b length 450 to 500 mm. Internal diameter 5 to 8 mm.

(c)Burner – A luminous Argand burner with chimney and sensitive regulative tap.

(d)Stand –A retort stand with asbestos covered ring and clamp carrying a piece of metal tubing connected by a short length of rubber tubing with the *water* inlet tube of the condenser jacket.

The whole of the apparatus is effectively screened from draught.

The apparatus is cleaned before each distillation by washing successively with *acetone* and *water,* then inverting it, filling it with *chromic sulphuric acid* mixture, after closing the open end at G, and allowing to stand and finally rinsing with *water*.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml of *glycerin* and 175 ml of *water* in the one litre distilling flask and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, *water* is run into the graduated receiver, keeping the tap T open until the *water* overflows, at P. Any air bubbles in the rubber tubing a-b are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap T is opened and the tube L_1 lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

The tube L_1 is then raised till the level of *water* in it is above the level of B, when the tap T is slowly opened to return the oil to the bulb. The distillation is again continued for another hour and

the volume of oil is again read, after cooling the apparatus as before. If necessary, the distillation is again continued until successive readings of the volatile oil do not differ.

The measured yield of volatile oil is taken to be the content of volatile oil in the drug.

The dimensions of the apparatus may be suitably modified in case of necessity.

APPENDIX – 3

3.1. Test for Heavy Metals

3.1.1. Limits for Heavy Metals:

Table 2- Permissible Limits of Heavy Metals

S.No.	Heavy Metal contents	Permissible limits
1	Lead	10 ppm
2	Arsenic	3 ppm
3	Cadmium	0.3 ppm
4	Mercury	1 ppm

3.1.2. Determination of lead, cadmium, arsenic and mercury by Atomic absorption Spectrophotometry or by inductively coupled plasma:

Procedure: Prepare a test solution of the substance being examined as follows:
Transfer 3g of the test substance to a clean, dry, 300-ml Kjeldahl flask. [Note -A 800-ml flask may be used if the reaction foams excessively]. Clamp the flask at an angle of 45^0 and add a sufficient quantity of a concentrated nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside and add portions of the same acid mixture, heating after each addition, until a total of 18 ml of the acid has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 ml of nitric acid and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool cautiously add 5 ml of *water*, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few ml. Cool, cautiously add 5 ml of *water*, and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of 30 per cent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 ml. If the solution is still yellow, repeat the addition of 5 ml of *water* and the peroxide treatment. Cool, dilute cautiously with a few ml of *water*, and rinse into a 50-ml colour-comparison tube, taking care that the combined volume does not exceed 25 ml. Prepare a blank solution following the same procedure omitting the sample.

Prepare not less than 3 standard solutions of the element being examined of different concentrations, covering the 25 to 200 percentage of the range that may be present in the sample solution. Add separately the corresponding reagents as that for the test solution and prepare the blank reference solution with the corresponding reagents.

Calibrate, operate the instruments as per manufacturer's recommendations and set the analytical condition suitable for the analysis of lead, cadmium, arsenic, and mercury.

Measure the absorbances of the blank reference solution and each reference solution of different concentrations separately, record the readings and prepare a calibration curve with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa.

Interpolate the mean value of the readings obtained with the test solution on the calibration curve to determine the concentration of each heavy metal.

For more information on Apparatus refer API, Part I, Volume-VI, Appendix, 2.3.7 & 2.3.8

3.2. Microbial Limit Tests:

Table 3- Microbial Contamination Limits

Sl. No.	Parameters	Permissible limits for herbal extracts and powders	Permissible limits for plant materials which will be treated before use
1	*Staphylococcus aureus*/g	Absent	-
2	*Salmonella* sp./g	Absent	Absent
3	*Pseudomonas aeruginosa*/g	Absent	-
4	*Escherichia coli*	Absent	10
5	Total microbial plate count (TPC)	10^5/g*	10^7
6	Total Yeast & Mould	10^3/g	10^5

*For topical use, the limits shall be 10^7/g

The following tests are designed for the estimation of the number of viable aerobic microorganisms present and for detecting the presence of designated microbial species in the extract. The term 'growth' is used to designate the presence and presumed proliferation of viable microorganisms.

Preliminary Testing: The methods given here in are invalid unless it is demonstrated that the test specimens (extracts) to which they are applied do not, of themselves, inhibit the multiplication under the test conditions of microorganisms that can be present. Therefore, prior to doing the tests, inoculate diluted extracts being examined with separate viable cultures of *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This is done by adding 1 ml of 24 hours broth culture containing not less than 1000 microorganisms to the first dilution (in buffer solution *p*H 7.2, fluid soyabean-casein digest medium or fluid lactose medium) of the test material and following the test procedure. If the organisms fail to grow in the relevant medium the procedure should be modified by (a) increasing the volume of diluent with the quantity of test material remaining the same, or (b) incorporating a sufficient quantity of a suitable

inactivating agent in the diluents, or (c) combining the afore mentioned modifications so as to permit growth of the organisms in the media. If inhibitory substances are present in the extracts, 0.5 per cent of soya lecithin and 4 per cent of polysorbate 20 may be added to the culture medium. Alternatively, repeat the test as described in the previous paragraph, using fluid casein digest-soya lecithin-polysorbate 20 medium, to demonstrate neutralization of preservatives or other antimicrobial agents in the test material. Where inhibitory substances are contained in the extracts and the latter is soluble, the membrane filtration method described under Total Aerobic Microbial Count may be used.

If in spite of incorporation of suitable inactivating agents and a substantial increase in the volume of diluent it is still not possible to recover the viable cultures described above and where the article is not suitable for applying the membrane filtration method it can be assumed that the failure to isolate the inoculated organism may be due to the bactericidal activity of the product. This may indicate that the article is not likely to be contaminated with the given species of microorganisms. However, monitoring should be continued to establish the spectrum of inhibition and bactericidal activity of the article.

Media

Culture media may be prepared as given below or dehydrated culture media may be used provided that, when reconstituted as directed by the manufacturer, they have similar ingredients and / or yield media comparable to those obtained from the formulae given below.

Where agar is specified in a formula, use agar that has a moisture content of not more than 15 per cent. Where *water* is called for in a formula, use purified *water*. Unless otherwise indicated, the media should be sterilized by heating in an autoclave (15 psi) at 121^0 for 15 minutes. In preparing media by the formulas given below, dissolve the soluble solids in the *water*, using heat if necessary, to effect complete solution, add solutions of 0.1N hydrochloric acid or 0.1N sodium hydroxide in quantities sufficient to yield the required *p*H in the medium when it is ready for use. Determine the *p*H at $25^0 \pm 2^0$.

Baird-Parker Agar Medium

Pancreatic digest of casein	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Lithium chloride	5.0 g
Agar	20.0 g
Glycine	12.0 g
Sodium pyruvate	10.0 g
Water to	1000 ml

Heat with frequent agitation and boil for 1 minute. Sterilize, cool in between 45^0-50^0, add 10 ml of a one per cent w/v solution of sterile potassium tellurite and 50 ml of egg yolk emulsion. Mix

thoroughly, but gently and pour into plates. (Prepare the egg-yolk emulsion by disinfecting the surface of whole shell eggs, aseptically cracking the eggs, and separating out intact yolks into a sterile graduated cylinder. Add sterile saline solution, get a 3 to 7 ratio of egg-yolk to saline. Add to a sterile blender cup and mix at high speed for 5 seconds). Adjust the *p*H after sterilization to 6.8 ± 0.2.

Bismuth Sulphite Agar Medium

Solution (1)

Beef extract	6 g
Peptone	10 g
Agar	24 g
Ferric citrate	0.4 g
Brilliant green	10 mg
Water to	1000 ml

Dissolve with the aid of heat and sterilize by maintaining at 115^0 for 30 minutes.

Solution (2)

Ammonium bismuth citrate	3 g
Sodium sulphite	10 g
Anhydrous disodium hydrogen phosphate	5 g
Dextrose monohydrate	5 g
Water to	100 ml

Mix, heat to boiling, cool to room temperature, add 1 volume of solution (2) to 10 volumes of solution (1) previously melted and cooled to a temperature of 55^0 and pour.

Bismuth Sulphite Agar Medium should be stored at 2^0 to 8^0 for 5 days before use.

Brilliant Green Agar Medium

Peptone	10.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium chloride	5.0 g
Phenol red	80.0 g
Brilliant green	12.5 mg
Agar	12.0 g
Water to	1000 ml

Mix, allow to stand for 15 minutes, sterilize by maintaining at 115^0 for 30 minutes and mix before pouring.

Buffered Sodium Chloride-Peptone Solution *p*H 7.0

Potassium dihydrogen phosphate	3.56 g
Disodium hydrogen phosphate	7.23 g
Sodium chloride	4.30 g
Peptone (meat or casein)	1.0 g
Water to	1000 ml

0.1 to 1.0 per cent w/v Polysorbate 20 or polysorbate 80 may be added. Sterilize by heating in an autoclave at 121^0 for 15 minutes.

Casein Soyabean Digest Agar Medium

Pancreatic digest of casein	15.0 g
Papaic digest of soyabean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water to 1000 ml	

Adjust the *p*H after sterilization to $7.3 \pm 0.2^*$.

Cetrimide Agar Medium

Pancreatic digest of gelatin	20.0 g
Magnesium chloride	1.4 g
Potassium sulphate	10.0 g
Cetrimide	0.3 g
Agar	13.6 g
Glycerin	10.0 g
Water to	1000 ml

Heat to boiling for 1 minute with shaking. Adjust the *p*H so that after sterilization it is 7.0 to 7.4.*

Desoxycholate-Citrate Agar Medium

Beef extract	5.0 g
Peptone	5.0 g
Lactose	10.0 g
Trisodium citrate	8.5 g

* Sterilize at 121^0 for 15 minutes in an autoclave

Sodium thiosulphate	5.4 g
Ferric citrate	1.0 g
Sodium desoxycholate	5.0 g
Neutral red	0.02 g
Agar	12.0 g
Water to	1000 ml

Mix and allow to stand for 15 minutes. Gently boil with continuous stirring and continue boiling until solution is complete. Cool to 80^0, mix, pour and cool rapidly.

Care should be taken not to overheat Desoxycholate Citrate Agar during preparation. It should not be remelted and the surface of the plates should be dried before use.

Fluid Casein Digest-Soya Lecithin-Polysorbate 20 Medium

Pancreatic digest of casein	20 g
Soya lecithin	5 g
Polysorbate 20	40 ml
Water to	1000 ml

Dissolve the pancreatic digest of casein and soya lecithin in *water*, heating in a water-bath at 48^0 to 50^0 for about 30 minutes to effect solution. Add polysorbate 20, mix and dispense as desired*.

Fluid Lactose Medium

Beef extract	3.0 g
Pancreatic digest of gelatin	5.0 g
Lactose	5.0 g
Water to	1000 ml

Cool as quickly as possible after sterilization. Adjust the *p*H after sterilization to 6.9 ± 0.2.

Lactose Broth Medium

Beef extract	3.0 g
Pancreatic digest of gelatin	5.0 g
Lactose	5.0 g
Water to 1000 ml	

Adjust the *p*H after sterilization to 6.9 ± 0.2.*

---

* Sterilize at 121^0 for 15 minutes in an autoclave

Levine Eosin-Methylene Blue Agar Medium

Pancreatic digest of gelatin	10.0 g
Dibasic potassium phosphate	2.0 g
Agar	15.0 g
Lactose	10.0 g
Eosin Y	400 mg
Methylene blue	65 mg
Water to	1000 ml

Dissolve the pancreatic digest of gelatin, *dibasic potassium phosphate* and agar in *water* with warming and allow to cool. Just prior to use, liquify the gelled agar solution and the remaining ingredients, as solutions, in the following amounts and mix. For each 100 ml of the liquified agar solution use 5 ml of a 20 per cent w/v solution of lactose, 2 ml of a 2 per cent w/v solution of eosin Y and 2 ml of a 0.33 per cent w/v solution of *methylene blue*. The finished medium may not be clear. Adjust the *p*H after sterilization to 7.1±0.2.

MacConkey Agar Medium

Pancreatic digest of gelatin	17.0 g
Peptone (meat and casein, equal parts)	3.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Bile salts	1.5 g
Agar	13.5 g
Neutral red	30 mg
Crystal violet	1 mg
Water to	1000 ml

Boil the mixture of solids and *water* for 1 minute to effect solution. Adjust the *p*H after sterilization to 7.1 ± 0.2.*

MacConkey Broth Medium

Pancreatic digest of gelatin	20.0 g
Lactose	10.0 g
Dehydrated ox bile	5.0 g
Bromocresol purple	10 mg
Water to	1000 ml

Adjust the *p*H after sterilization to 7.3 ± 0.2*

* Sterilize at 121^0 for 15 minutes in an autoclave

Mannitol-Salt Agar Medium

Pancreatic digest of gelatin	5.0 g
Peptic digest of animal tissue	5.0 g
Beef extract	1.0 g
D-Mannitol	10.0 g
Sodium chloride	75.0 g
Agar	15.0 g
Phenol red	25 mg
Water to	1000 ml

Mix, heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilization to 7.4 ± 0.2*.

Nutrient Agar Medium: Nutrient broth gelled by the addition of 1 to 2 per cent w/v of agar.

Nutrient Broth Medium

Beef extract	10.0 g
Peptone	10.0 g
Sodium chloride	5 mg
Water to	1000 ml

Dissolve with the aid of heat. Adjust the *p*H to 8.0 to 8.4 with 5M *sodium hydroxide* and boil for 10 minutes. Filter and sterilize by maintaining at 115^0 for 30 minutes and adjust the *p*H to 7.3 ± 0.1.

Pseudomonas Agar Medium for Detection of Flourescein

Pancreatic digest of casein	10.0 g
Peptic digest of animal tissue	10.0 g
Anhydrous dibasic potassium phosphate	1.5 g
Magnesium sulphate hepta hydrate	1.5 g
Glycerin	10.0 ml
Agar	15.0 g
Water to	1000 ml

Dissolve the solid components in *water* before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilization to 7.2 ± 0.2*.

* Sterilize at 121^0 for 15 minutes in an autoclave

Pseudomonas Agar Medium for Detection of Pyocyanin

Pancreatic digest of gelatin	20.0 g
Anhydrous magnesium chloride	1.4 g
Anhydrous potassium sulphate	10.0 g
Agar	15.0 g
Glycerin	10.0 ml
Water to	1000 ml

Dissolve the solid components in water before adding glycerin. Heat with frequent agitation and boil for 1 minute to effect solution. Adjust the *p*H after sterilization to 7.2 ± 0.2*.

Sabouraud Dextrose Agar Medium

Dextrose	40 g
Peptic digest of animal tissue and Pancreatic digest of casein (1:1)	10 g
Agar	15 g
Water to	1000 ml

Mix, and boil to effect solution. Adjust the *p*H after sterilization to 5.6 ± 0.2*.

Sabouraud Dextrose Agar Medium with Antibiotics

To 1 liter of Sabouraud Dextrose Agar Medium, add 0.1 g of *benzylpenicillin* sodium and 0.1 g of *tetracycline HCL* or alternatively add 50 mg of *chloramphenicol* immediately before use.

Selenite F Broth

Peptone	5 g
Lactose	4 g
Disodium hydrogen phosphate	10 g
Sodium hydrogen selenite	4 g
Water to	1000 ml

Dissolve, distribute in sterile containers and sterilize by maintaining at 100^0 for 30 minutes.

Fluid Selenite-Cystine Medium

Pancreatic digest of casein	5.0 g
Lactose	4.0 g
Sodium phosphate	10.0 g

* Sterilize at 121^0 for 15 minutes in an autoclave

Sodium hydrogen selenite	4.0 g
l-Cystine	10.0 mg
Water to	1000 ml

Mix and heat in flowing steam for 15 minutes. Adjust the final *p*H to 7.0 ± 0.2. Do not sterilize.

Tetrathionate Broth Medium

Beef extract	0.9 g
Peptone	4.5 g
Yeast extract	1.8 g
Sodium chloride	4.5 g
Calcium carbonate	25.0 g
Sodium thiosulphate	40.7 g
Water to	1000 ml

Dissolve the solids in *water* and heat the solution to boil. On the day of use, add a solution prepared by dissolving 5 g of potassium iodide and 6 g of iodine in 20 ml of *water*.

Tetrathionate-Bile-Brilliant Green Broth Medium

Peptone	8.6 g
Dehydrated ox bile	8.0 g
Sodium chloride	6.4 g
Calcium carbonate	20.0 g
Potassium tetrathionate	20.0 g
Brilliant green	70 mg
Water to	1000 ml

Heat just to boiling; do not reheat. Adjust the *p*H so that after heating it is 7.0 ± 0.2.

Triple Sugar-Iron Agar Medium

Beef extract	3.0 g
Yeast extract	3.0 g
Peptone	20.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose monohydrate	1.0 g
Ferrous sulphate	0.2 g
Sodium chloride	5.0 g
Sodium thiosulphate	0.3 g
Phenol red	24 mg
Water to	1000 ml

Mix, allow standing for 15 minutes, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 121^0 for 15 minutes. Allow to stand in a sloped form with a butt about 2.5 cm long.

Urea Broth Medium

Potassium dihydrogen orthophosphate	9.1 g
Anhydrous disodium hydrogen phosphate	9.5 g
Urea	20.0 g
Yeast extract	0.1 g
Phenol red	10 mg
Water to	1000 ml

Mix, sterilize by filtration and distribute aseptically in sterile containers.

Vogel-Johnson Agar Medium

Pancreatic digest of casein	10.0 g
Yeast extract	5.0 g
Mannitol	10.0 g
Dibasic potassium phosphate	5.0 g
Lithium chloride	5.0 g
Glycerin	10.0 g
Agar	16.0 g
Phenol red	25.0 mg
Water to	1000 ml

Boil the solution of solids for 1 minute. Sterilize, cool to between 45^0-50^0 and add 20 ml of 1 per cent w/v sterile solution of potassium tellurite. Adjust the *p*H after sterilization to 7.0 ± 0.2*.

Xylose-Lysine-Desoxycholate Agar Medium

Xylose	3.5 g
l-Lysine	5.0 g
Lactose	7.5 g
Sucrose	7.5 g
Sodium chloride	5.0 g
Yeast extract	3.0 g
Phenol red	80 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g

* Sterilize at 121^0 for 15 minutes in an autoclave

Sodium thiosulphate	6.8 g
Ferric ammonium citrate	800 mg
Water to	1000 ml

Heat the mixture of solids and *water*, with swirling, just to the boiling point. Do not overheat or sterilize. Transfer at once to a water-bath maintained at about 50^0 and pour into plates as soon as the medium has cooled. Adjust the final *p*H to 7.4 ± 0.2.

Sampling: Use 10 ml or 10 g specimens for each of the tests specified in the individual monograph.

Precautions: The microbial limit tests should be carried out under conditions designed to avoid accidental contamination during the test. The precautions taken to avoid contamination must be such that they do not adversely affect any microorganisms that should be revealed in the test.

3.2.1. Total Aerobic Microbial Count:

Pre treat the extracts and raw materials being examined as described below.

Note: The raw materials needs to be ground as a coarse powder before analysis.

Water-soluble products: Dissolve 10 g or dilute 10 ml of the extract preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution *p*H 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the *p*H to about 7.

Products insoluble in water (non-fatty): Suspend 10 g or 10 ml of the extract preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution *p*H 7.0 or any other suitable medium shown not to have antimicrobial activity under the conditions of the test and dilute to 100 ml with the same medium. If necessary, divide the preparation being examined and homogenize the suspension mechanically. A suitable surface-active agent such as 0.1 per cent w/v of polysorbate 80 may be added to assist the suspension of poorly wettable substances. If necessary, adjust the *p*H of the suspension to about 7.

Fatty products: Homogenise 10 g or 10 ml of the extract preparation being examined, unless otherwise specified, with 5 g of polysorbate 20 or polysorbate 80. If necessary, heat to not more than 40^0. Mix carefully while maintaining the temperature in the water-bath or in an oven. Add 85 ml of buffered *sodium chloride-peptone solution p*H 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of the test, heated to not more than 40^0 if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 minutes. If necessary, adjust the *p*H to about 7.

Examination of the sample: Determine the total aerobic microbial count in the extract being examined by any of the following methods.

Membrane filtration: Use membrane filters 50 mm in diameter and having a nominal pore size not greater than 0.45 μm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined. Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated extract preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as *buffered sodium chloride-peptone solution pH 7.0.* For fatty substances add to the liquid *polysorbate 20* or *polysorbate 80.* Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of *casein soyabean digest agar* and the other, intended for the enumeration of fungi, to the surface of a plate of *Sabouraud dextrose agar* with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30^0 to 35^0 in the test for bacteria and 20^0 to 25^0 in the test for fungi. Count the number of colonies that are formed. Calculate the number of microorganisms per g or per ml of the extract preparation being examined, if necessary count bacteria and fungi separately.

Plate count for bacteria: Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated extract preparation and about 15 ml of liquified *casein soyabean digest agar* at not more than 45^0. Alternatively, spread the pretreated extract preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated extract preparation as described above so that a colony count of not more than 300 may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30^0 to 35^0 for 5 days, unless a more reliable count is obtained in a shorter time.

Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

Plate count for fungi: Proceed as described in the test for bacteria but use *Sabouraud dextrose agar with antibiotics* in place of *casein soyabean digest agar* and incubate the plates at 20^0 to 25^0 for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

Multiple-tube or serial dilution method : In each of fourteen test-tubes of similar size place 9.0 ml of sterile *fluid soyabean casein digest medium.* Arrange twelve of the tubes in four sets of three tubes each. Put aside one set of three tubes to serve as controls. Into each of three tubes of one set ("100") and into fourth tube (A) pipette 1 ml of the solution of suspension of the test specimen (extract) and mix. From tube A pipette 1 ml of its contents into the one remaining tube (B) not included in the set and mix. These two tubes contain 100 mg (or 100 μl) and 10 mg (or 10 μl) of the specimen respectively. Into each of the second set ("10") of three tubes pipette 1 ml from tube A, and into each tube of the third set ("1") pipette 1 ml from tube B. Discard the unused contents of tube A and B. Close well and incubate all of the tubes. Following the incubation

period, examine the tubes for growth. The three control tubes remain clear. Observations in the tubes containing the test specimen, when interpreted by reference to Table 4, indicate the most probable number of microorganisms per g or per ml of the test specimen.

Table 4 – Most Probable Total Count by Multiple-Tube Or Serial Dilution Method

Observed combination of numbers of tubes showing growth in each set			**Most probable number of microorganisms per g or per ml**
Number of mg (or ml) of specimen per tube			
100 (100 µL)	**10 (10 µL)**	**1 (1 µL)**	
3	3	3	>1100
3	3	2	1100
3	3	1	500
3	3	0	200
3	2	3	290
3	2	2	210
3	2	1	150
3	2	0	90
3	1	3	160
3	1	2	120
3	1	1	70
3	1	0	40
3	0	3	95
3	0	2	60
3	0	1	40
3	0	0	23

3.2.2. Tests for Specified Microorganisms:

Pretreatment of the extract sample being examined: Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution *p*H 7.0.

Escherichia coli: Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37^0 for 18-24 hours.

Primary test: Add 1.0 ml of the enrichment culture to a tube containing 5 ml of MacConkey broth. Incubate in a water-bath at $36\text{-}38^0$ for 48 hours. If the contents of the tube show acid and gas, carry out the secondary test.

Secondary test: Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth and (b) 5 ml of peptone *water*. Incubate in a water-bath at 43.5 - 44.5^0 for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole, add 0.5 ml of Kovac′s reagent, shake well and allow to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of *Escherichia coli*.

Carry out a control test by repeating the primary and secondary tests, adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Escherichia coli* (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient broth, to 5 ml of MacConkey broth. The test is not valid unless the results indicate that the control contains *Escherichia coli*.

Alternative test: By means of an inoculating loop, streak a portion from the enrichment culture (obtained in the previous test) on the surface of MacConkey agar medium. Cover and invert the dishes and incubate. Upon examination, if none of the colonies are brick-red in colour and have a surrounding zone of precipitated bile the sample meets the requirements of the test for the absence of *Escherichia coli*.

If the colonies described above are found, transfer the suspect colonies individually to the surface of Levine eosin-methylene blue agar medium, plated on Petri dishes. Cover and invert the plates and incubate. Upon examination, if none of the colonies exhibits both a characteristic metallic sheen under reflected light and a blue-black appearance under transmitted light, the sample meets the requirements of the test for the absence of *Escherichia coli*. The presence of *Escherichia coli* may be confirmed by further suitable cultural and biochemical tests.

Salmonella : Transfer a quantity of the pretreated extract preparation being examined containing 1 g or 1 ml of the product to 100 ml of nutrient broth in a sterile screw-capped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35-37^0 for 24 hours.

Table 5 – Test for *Salmonella*

Medium	***Description*** **of colony**
Bismuth sulphite agar	Black or green
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate-citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxycholate agar	Red with or without black centres

Primary test: Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36-38^0 for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, deoxycholate citrate agar and xylose-lysine-

deoxycholate agar. Incubate the plates at 36-38^0 for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 5, the sample meets the requirements of the test for the absence of the genus *Salmonella*. If any colonies conforming to the description in Table 5 are produced, carry out the secondary test.

Secondary test: Subculture any colonies showing the characteristics given in Table 5 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36^0 to 38^0 for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in urea broth indicates the presence of Salmonella . If acid but no gas is produced in the cultures, the identity of the organisms should be confirmed by agglutination tests.

Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 *Salmonella abony* (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains *Salmonella.*

***Pseudomonas aeruginosa*:** Pretreat the extract preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35^0 to 37^0 for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium on the surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35^0 to 37^0 for 18 to 24 hours. If, upon examination, none of the plates contains colonies having the characteristics listed in Table 6 for the media used, the sample meets the requirement for freedom from *Pseudomonas aeruginosa*. If any colonies conforming to the description in Table 6 are produced, carry out the oxidase and pigment tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of *Pseudomonas* agar medium for detection of fluorescein and *Pseudomonas* agar medium for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media and incubate at 33^0 to 37^0 for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 6 are present. If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1 per cent w/v solution of N,N,N',N'-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the sample meets the requirements of the test for the absence of *Pseudomonas aeruginosa.*

Table 6 – Tests for *Pseudomonas aeruginosa*

Medium	Characteristic colonial morphology	Fluorescence in UV light	Oxidase test	Gram stain
Cetrimide agar	Generally Greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for detection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

Staphylococcus aureus: Proceed as described under *Pseudomonas aeruginosa,* if upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 7 for the media used, the sample meets the requirements for the absence of *Staphylococcus aureus*. If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 7 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives.

Incubate in water-bath at 37^0 examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of *Staphylococcus aureus*.

Table 7 – Tests for *Staphylococcus aureus*

Selective medium	Characteristic colonial morphology	Gram stain
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Baird-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

Validity of the tests for total aerobic microbial count:

Grow the following test strains separately in tubes containing fluid soyabean-casein digest medium at 30^0 to 35^0 for 18 to 24 hours or, for *Candida albicans*, at 20^0 for 48 hours.

Staphylococcus aureus	(ATCC 6538; NCTC 10788)
Bacillus subtilis	(ATCC 6633; NCIB 8054)
Escherichia coli	(ATCC 8739; NCIB 8545)
Candida albicans	(ATCC 2091; ATCC 10231)

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution *p*H 7.0 to make test suspensions containing about 100 viable microorganisms per ml. Use the suspension of each of the microorganisms separately as a control of the counting methods, in the presence and absence of the preparation being examined, if necessary.

A count for any of the test organisms differing by not more than a factor of 10 from the calculated value for the inoculum should be obtained. To test the sterility of the medium and of the diluent and the aseptic performance of the test, carry out the total aerobic microbial count method using sterile buffered sodium chloride-peptone solution *p*H 7.0 as the test preparation. There should be no growth of microorganisms.

Validity of the tests for specified microorganisms: Grow separately the test strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in fluid soyabean-casein digest medium and *Escherichia coli* and *Salmonella typhimurium* at 30^0 to 35^0 for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution *p*H 7.0 to make test suspensions containing about 10^3 viable microorganisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 10^2 micro-organisms of each strain) as an inoculum in the test for *E. coli, S. typhimurium*, *P. aeruginosa* and *S. aureus*, in the presence and absence of the extract preparation being examined, if necessary. A positive result for the respective strain of microorganism should be obtained.

3.3 Pesticide Residue:

Definition: For the purposes of the Pharmacopoeia, a pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of vegetable drugs.

Table -8. Permissible Limits for Pesticide Residue:

Substance	Limit (mg/kg)
Alachlor	0.02
Aldrin and Dieldrin (sum of)	0.05
Azinphos-methyl	1.0
Bromopropylate	3.0
Chlordane (sum of *cis-, trans* - and Oxythlordane)	0.05
Chlorfenvinphos	0.5
Chlorpyrifos	0.2
Chlorpyrifos-methyl	0.1
Cypermethrin (and isomers)	1.0
DDT (sum of p,p'-DDT, o,p'-DDT, p,p-DDE and p,p'-TDE)	1.0
Deltamethrin	0.5
Diazinon	0.5

Substance	Limit (mg/kg)
Dichlorvos	1.0
Dithiocarbamates (as CS2)	2.0
Endosulfan (sum of isomers and endosulfan sulphate)	3.0
Endrin	0.05
Ethion	2.0
Fenitrothion	0.5
Fenvalerate	1.5
Fonofos	0.05
Heptachlor (sum of heptachlor and heptachlor epoxide)	0.05
Hexachlorobenzene	0.1
Hexachlorocyclohexane isomers (other than γ)	0.3
Lindane (γ-hexachlorocyclohexane)	0.6
Malathion	1.0
Methidathion	0.2
Parathion	0.5
Parathion-methyl	0.2
Permethrin	1.0
Phosalone	0.1
Piperonyl butoxide	3.0
Pirimiphos-methyl	4.0
Pyrethrins (sum of)	3.0
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide)	1.0

Note: Apart from the above, if any pesticides applied to the herb before or after harvesting should also be tested. The limit should be calculated using the following formula.

$$\frac{\text{ADI x M}}{\text{MDD x 100}}$$

ADI= Acceptable daily intake as published by FAO-WHO, in milligrams per kilogram of body mass,

M= body mass in kilograms (60 kg),

MDD= daily dose of the drug, in kilograms.

If the drug is intended for the preparation of extracts, tinctures or other pharmaceutical forms whose preparation method modifies the content of pesticides in the finished product, the limits are calculated using the following expression:

$$\frac{\text{ADI x M x E}}{\text{MDD x 100}}$$

E= Extraction factor for of the method of preparation, determined experimentally.

Higher limits can also be authorised, in exceptional cases, especially when a plant requires a particular cultivation method or has a metabolism or a structure that gives rise to a higher than normal content of pesticides.

Reagents: All reagents and solvents are free from any contaminants, especially pesticides, that might interfere with the analysis. It is often necessary to use special quality solvents or, if this is not possible, solvents that have recently been re-distilled in an apparatus made entirely of glass. In any case, suitable blank tests must be carried out.

Apparatus: Clean the apparatus and especially glassware to ensure that they are free from pesticides, for example, soak for at least 16 hours in a solution of phosphate-free detergent, rinse with large quantities of *distilled water* and wash with *acetone* and *hexane* or *heptane*.

3.3.1. Test for Pesticides:

The following methods may be used depending on the substance being examined, it may be necessary to modify, sometimes extensively, the procedure described hereafter. In any case, it may be necessary to use, in addition, another column with a different polarity or another detection method (mass spectrometry) or a different method (immunochemical methods) to confirm the results obtained. This procedure is valid only for the analysis of samples of vegetable drugs containing less than 15 per cent of *water*. Samples with a higher content of *water* may be dried, provided it has been shown that the drying procedure does not affect significantly the pesticide content.

Extraction (Method-I): To 10 g of the substance being examined, add 100 ml of *acetone* and allow to stand for 20 minutes. Add 1 ml of a solution containing 1.8 μg/ml of *carbophenothion* in *toluene.* Homogenise using a high-speed blender for 3 minutes. Filter and wash the filter cake with two quantities, each of 25 ml of *acetone.* Combine the filtrate and the washings and heat using a rotary evaporator at a temperature not exceeding 40^0 until the solvent has almost completely evaporated. To the residue add a few milliliters of *toluene* and heat again until the *acetone* is completely removed. Dissolve the residue in 8 ml of *toluene.* Filter through a membrane filter (45 μm), rinse the flask and the filter with *toluene* and dilute to 10.0 ml with the same solvent (solution A).

Purification: Examine by size-exclusion chromatography. The chromatographic procedure may be carried out using:

- a stainless steel column 0.30 m long and 7.8 mm in internal diameter packed with styrene-divinylbenzene copolymer (5 μm).
- as mobile phase *toluene* at a flow rate of 1 ml/min.

Performance of the column: Inject 100 µl of a solution containing 0.5 g/l of *methyl red* and 0.5 g/l of *oracet blue* in *toluene* and proceed with the chromatography. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10.3 ml. If necessary calibrate the column, using a solution containing *toluene,* at a suitable concentration, the insecticide to be analysed with the lowest molecular mass (for example, dichlorvos) and that with the highest molecular mass (for example, deltamethrin). Determine which fraction of the eluate contains both insecticides.

Purification of the test solution: Inject a suitable volume of solution A (100 µl to 500 µl) and proceed with the chromatography. Collect the fraction as determined above (solution B). Organophosphorus insecticides are usually eluted between 8.8 ml and 10.9 ml. Organochlorine and pyrethroid insecticides are usually eluted between 8.5 ml and 10.3 ml.

In a chromatography column, 0.10 m long and 5 mm in internal diameter, introduce a piece of defatted cotton and 0.5 g of silica gel treated as follows: heat silica gel for chromatography in an oven at 1500 for at least 4 hours. Allow to cool and add dropwise a quantity of *water* corresponding to 1.5 per cent of the mass of silica gel used; shake vigorously until agglomerates have disappeared and continue shaking for 2 hours using a mechanical shaker. Condition the column using 1.5 ml of *hexane.* Prepacked columns containing about 0.50 g of a suitable silica gel may also be used provided they are previously validated.

Concentrate solution B in a current of helium for chromatography or oxygen-free nitrogen almost to dryness and dilute to a suitable volume with *toluene* (200 µl to 1 ml according to the volume injected in the preparation of solution B). Transfer quantitatively onto the column and proceed with the chromatography using 1.8 ml of *toluene* as the mobile phase. Collect the eluate (solution C).

Extraction (Method-II): To 25 g of the substance being examined, add 300ml of acetonitrile : *water* (3 : 1) and homogenise using a high-speed blender for 5 minutes. Filter and wash the filter cake with two quantities, each of 25 ml of *acetonitrile water mixture.* Transfer filtrate and rinse to a separating funnel.

Add 50 ml of saturated sodium chloride and mix vigorously for 30 seconds. Add 50 ml hexane to the separating funnel and extract. Repeat extraction with hexane for another two times. Collect the hexane layer and pass the combined hexane layer through sodium sulphate. Collect the hexane and evaporate to dryness. Dissolve the residue in 25 ml hexane.

Florisil column clean up: Use florisil solid phase extraction cartridges. Using bulb pipet transfer 2 ml of the hexane solution containing the pesticide residue in to the florisil cartridge. Elute with 12 ml of 15 per cent diethyl ether in hexane. Further elute with 12 ml of 50 per cent diethyl ether in hexane. Collect the elutes separately and evaporate and dry using rotary evaporator. Dissolve in 0.2 ml of *n*-hexane containing 10 ng/ml of *carbophenothion* and sonicate.

3.3.2. Quantitative Analysis: Refer API, Part I, Volume VI, Section 2.5.1. page 282 to 286

3.4. Test for Aflatoxins:

Table 9 - Permissible Limit of Aflatoxins

Sl. No	Aflatoxin	Permissible Limit
1	B1	< 2 ppb
2	B1+B2+G1+G2	< 5 ppb

Caution: Aflatoxins are highly dangerous and extreme care should be exercised in handling aflatoxin materials. This test is provided to detect the possible presence of aflatoxins B1, B2, G1 and G2 in any material of plant origin. Unless otherwise specified in the individual monograph, use the following method.

Zinc Acetate – Aluminum Chloride Reagent: Dissolve 20 g of *zinc acetate* and 5 g of *aluminum chloride* in sufficient *water* to make 100 ml.

Sodium Chloride Solution: Dissolve 5 g of *sodium chloride* in 50 ml of purified *water*.

Test Solution 1: Transfer about 5 g of the powdered material, accurately weighed, to a glass-stoppered flask. Add 200 ml of a mixture of *methanol* and *water* (17 : 3). Shake vigorously by mechanical means for not less than 30 minutes and filter. [Note – If the solution has interfering plant pigments, proceed as directed for *Test Solution 2*]. Discard the first 50 ml of the filtrate and collect the next 40 ml portion. Transfer the filtrate to a separating funnel. Add 40 ml of sodium chloride solution and 25 ml of *hexane* and shake for 1 minute. Allow the layers to separate and transfer the lower aqueous layer to a second separating funnel. Extract the aqueous layer in the separating funnel twice, each time with 25 ml of *methylene chloride*, by shaking for 1 minute. Allow the layers to separate each time, separate the lower organic layer and remove the solvent from the combined and evaporate layers on a water bath. Cool the residue. If interferences exist in the residue, proceed as directed for *Cleanup Procedure;* otherwise, dissolve the residue obtained above in 0.2 ml of a mixture of *chloroform* and *acetonitrile* (9.8 : 0.2) and shake by mechanical means, if necessary.

Test Solution 2: Collect 100 ml of the filtrate from the start of the flow and transfer to a 250 ml beaker. Add 20 ml of *zinc acetate-aluminum chloride reagent* and 80 ml of *water*. Stir and allow to stand for 5 minutes. Add 5 g of a suitable filtering aid, such as diatomaceous earth, mix and filter. Discard the first 50 ml of the filtrate, and collect the next 80 ml portion. Proceed as directed for *Test Solution 1,* beginning with "Transfer the filtrate to a separating funnel."

Cleanup Procedure: Place a medium-porosity sintered-glass disk or a glass wool plug at the bottom of a 10 mm x 300 mm chromatographic tube. Prepare slurry of 2 g of silica gel with a mixture of *diethyl ether* and *hexane* (3 : 1), pour the slurry into the column and wash with 5 ml of the same solvent mixture. Allow the absorbent to settle and add to the top of the column a layer of

1.5 g of *anhydrous sodium sulphate*. Dissolve the residue obtained above in 3 ml of *methylene chloride* and transfer it to the column. Rinse the flask twice with 1 ml portions of *methylene chloride*, transfer the rinses to the column and elute at a rate not greater than 1 ml per minute. Add successively to the column 3 ml of *hexane*, 3 ml of *diethyl ether* and 3 ml of *methylene chloride*; elute at a rate not greater than 3 ml per minute; and discard the eluates. Add to the column 6 ml of a mixture of *methylene chloride* and *acetone* (9 : 1) and elute at a rate not greater than 1 ml per minute, preferably without the aid of vacuum. Collect this eluate in a small vial, add a boiling chip if necessary and evaporate to dryness on a water bath. Dissolve the residue in 2 ml of a mixture of *chloroform* and *acetonitrile* (9.8 : 0.2) and shake by mechanical means if necessary.

Aflatoxin Solution: Dissolve accurately weighed quantities of aflatoxins B1, B2, G1 and G2 in a mixture of *chloroform* and *acetonitrile* (9.8 : 0.2) to obtain a solution having concentration of 1.0 μg/ml each for aflatoxins B1 and G1, 0.2 μg/ml each for aflatoxins B_2 and G2.

Procedure: Separately apply 2.5, 5, 7.5 and 10 μl of the Aflatoxin Solution and three 10 μl applications of either *Test Solution 1* or *Test Solution 2* to a suitable thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel. Superimpose 5 μl of the *Aflatoxin Solution* on one of the three 10 μl applications of the *Test Solution.* Allow the spots to dry and develop the chromatogram in an unsaturated chamber containing a solvent system consisting of a mixture of *chloroform*, *acetone* and *isopropyl alcohol* (85 :10 :5) until the solvent front has moved not less than 8 cm from the origin. Remove the plate from the developing chamber, mark the solvent front and allow the plate to air-dry. Locate the spots on the plate by examination under UV light at 366 nm: the four applications of the *Aflatoxin Solution* appear as four clearly separated blue fluorescent spots; the spot obtained from the *Test Solution* that was superimposed on the *Aflatoxin Solution* is no more intense than that of the corresponding *Aflatoxin Solution;* and no spot from any of the other *Test Solutions* corresponds to any of the spots obtained from the applications of the *Aflatoxin Solution.* If any spot of aflatoxins is obtained in the *Test Solution,* the colour match the position of each fluorescent spot of the *Test Solution* with those of the *Aflatoxin Solution* to identify the type of aflatoxin present. The intensity of the aflatoxins spot, if present in the *Test Solution,* when compared with that of the corresponding aflatoxin in the *Aflatoxin Solution* will give an approximate concentration of aflatoxin in the *Test Solution.*

3.5. Thin-Layer Chromatography (TLC):

Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, stationary phase and a mobile phase. The stationary phase acts as an adsorbent in a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet. Precoated plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of stationary phase, its preparation and its use with different solvents.

Identification can be effected by comparison of spots of identical R_f value and colour in unknown sample to a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Apparatus:

(a) Flat uniformly thick glass plates of appropriate dimensions coated with a layer of adsorbent that allow the application of the necessary number of the solutions being examined along with reference solutions. The plates are prepared as described below; alternatively, commercially prepared plates may be used.

(b) An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

(c) The coating substance consists of finely divided adsorbent materials, normally between 5 to 40 μm in diameter is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of plaster of paris (hydrated calcium sulphate) or with any other suitable binder. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.

(d) A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

(e) A storage rack to support the plates during drying and transportation.

(f) A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.

(g) Graduated micro-pipettes capable of delivering microlitre quantities say 10 μl and less.

(h) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.

(i) An ultra-violet light, suitable for observation at short (254 nm) and long (366 nm) ultra-violet wavelengths.

Preparation of plates: Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.20 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100^0 to 105^0 for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs. Now a days pre coated plates of silica gel on glass/aluminium/ plastic sheets are also available.

Method:

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of the mobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate. Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specifies 'protected from light' or 'in subdued light' it is intended that the entire procedure is carried out under these conditions.

Visualisation:

The phrases *ultra-violet light (254 nm)* and *ultra-violet light (366 nm)* indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be. The term *secondary spot* means any spot other than the principal spot. Similarly, a *secondary band* is any band other than the principal band.

R_f Value:

Measure and record the distance of each spot from the point of its application and calculate the R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

3.5.1. Quantitative measurement (HPTLC)

The substances that have been separated after development of the plate and that respond to UV-Vis irradiation can be estimated directly on the plate with suitable instrumentation. Measurement is of the reflectance of the incident light from the spots by moving the plate or the measuring device. Likewise, fluorescence may be measured using an appropriate optical system.

Apparatus: The apparatus for direct measurement consist of:

- a device for extract positioning and reproducible application of the amount of solutions onto the plate,
- a mechanical device for moving the plate or the measuring device along the x-axis or the y-axis, (Applicator)
- a recorder and a suitable integrator or a computer, and
- a photometer with a source of light, an optical device for generating monochromatic light and a photocell of adequate sensitivity; for measurement of fluorescence, a suitable filter to prevent light used for excitation from reaching the detector while permitting emitted light or a specific portion thereof to pass (Densitometer)

Method: Prepare the test solution and reference solution as prescribed in the individual monograph. Use the same solvent for all the solutions and apply the same volume of each and develop the plate. Prepare and apply new fewer than 3 reference solutions of the substance under examination, the concentrations of which span the expected value in the test solution (about 80 per cent, 100 per cent and 120 per cent). Treat with the prescribed reagent, if necessary, and record the reflectance, the transmittance or fluorescence in the chromatograms obtained with all the solutions. Use the measured results to calculate the amount of substance in the test solution.

The requirement for resolution and separation are prescribed in the individual monograph.

3.6. Liquid chromatography:

Liquid chromatography is one of the widely used methods for separation and quantitative estimation of marker compounds present in herbal drugs. It is a liquid chromatographic system that uses narrow columns (~ 5 mm in diameter), pumping system operating at pressures up to 200 atm and suitable detectors. Reversed phase silica columns are widely used. A guard column is recommended to be fitted before the column to prevent the entry of unwanted compounds of the sample solution into the column. Sample introduction is done by syringe and a loop injector may be fitted with a fixed volume loop between 1-200 μl to facilitate accurate sample injection. Detection of the compound of interest is by retention time, UV absorbance fluorescence and electrical conductions. For majority of analyses, variable wavelength UV or photodiode array UV and RI detectors are used. Details of chromatographic conditions, *e.g.,* column type, mobile phases, flow rats, detectors, *etc.* are given in detail in individual monographs. For accurate analysis, high purity reagents and HPLC grade solvents must be used.

Columns

1. Silica C 18 - Octadecyl silane chemically bonded to porous silica or ceramic particles.

2. Silica Nitrile- Nitrile groups chemically bonded to porous silica microparticles

3.7. Spectrophotometry

Ultraviolet and visible absorption spectrophotometry is the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185 nm to 380 nm, and 380 nm to 780 nm of the spectrum, respectively.

The magnitude of the absorption of a solution is expressed in terms of the absorbance, A, defined as the logarithm to base 10 of the reciprocal of transmittance (T) for monochromatic radiation:

$A=\log 10\ (I_0/I)$

Where I_0 is the intensity of the incident radiation. I is the intensity of the transmitted radiation. The absorbance depends on the concentration of the absorbing substance in the solution and the thickness of the absorbing layer taken for measurement.

For convenience of reference and for ease in calculations, the specific absorbance of a 1 per cent w/v solutions is adopted in this Pharmacopoeia for several substances unless otherwise indicated, and it refers to the absorbance of a 1 per cent w/v solution in a 1 cm cell and measured at a defined wavelength. It is evaluated by the expression.

$A(1\ \text{per cent},1\ \text{cm}) = A/cl$

Where c is the concentration of the absorbing substance expressed as percentage w/v and l is the thickness of the absorbing layers in cm. The value of A (1 per cent, 1 cm) at a particular wavelength in a given solvent is a property of the absorbing substance.

Unless otherwise stated, measure the absorbance at the prescribed wavelength using a path length of 1 cm and at 24^0 to 26^0. Unless otherwise stated, the measurements are carried out with reference to the same solvent or the same mixture of solvents.

Determination of absorbance: Unless otherwise directed, measure the absorbance at the prescribed wavelength using a path length of 1 cm at 24^0 and 26^0. If necessary, the path length may be varied provided that compliance with Beer's Law has been shown over the range in question.

A statement in assay or test of the wavelength at which maximum absorption occurs implies that the maximum occurs either precisely at or within ± 2 nm of the given wavelength.

Likewise, a statement in a test of the absorbance, A, at a given wavelength or at the maximum at about a specified wavelength implies that the measured absorbance is within ± 3 per cent of the stated value.

When an assay or test prescribes the use of a reference substance, make the spectrophotometric measurements with the solution prepared from the reference substance by the official directions and then with the corresponding solution prepared from the substance under examination. Carry out the second measurement as quickly as possible after the first, using the same cell and same experimental conditions.

Unless otherwise specified, the requirements in the monographs for light absorption in the tests and assay apply to the dried or anhydrous material, where a standard is given for solvent content. In calculating the result, the loss on drying or contents of *water* solvent, determined by the method specified in the monograph, are taken in to account.

3.8. Test for Residual Solvent:

Residual ethanol limits : Not more than 5000 ppm

Quantitative analysis of residual solvents:

Gas chromatography (GC) is a chromatographic separation technique based on the difference in the distribution of species between two non-miscible phases in which the mobile phase is a carrier gas moving through or passing the stationary phase contained in a column. It is applicable to substances or their derivatives which are volatilised under the temperatures employed.

GC is based on mechanisms of adsorption, mass distribution or size exclusion.

Apparatus: The apparatus consists of an injector, a chromatographic column contained in an oven, a detector and a data acquisition system (or an integrator or a chart recorder). The carrier gas flows through the column at a controlled rate or pressure and then through the detector.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

Injectors: *Direct injections* of solutions are the usual mode of injection, unless otherwise prescribed in the monograph. Injection may be carried out either directly at the head of the column using a syringe or an injection valve, or into a vaporisation chamber which may be equipped with a stream splitter.

Stationary phases

Stationary phases are contained in columns which may be:

- a capillary column of fused-silica whose wall is coated with the stationary phase,

- a column packed with inert particles impregnated with the stationary phase,

- a column packed with solid stationary phase.

Capillary columns are 0.1 mm to 0.53 mm in internal diameter (Ñ) and 5 m to 60 m in length. The liquid or stationary phase, which may be chemically bonded to the inner surface, is a film 0.1 ñm to 5.0 ñm thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter (Ñ) of 2 mm to 4 mm. Stationary phases usually consist of porous polymers or solid supports impregnated with liquid phase.

Supports for analysis of polar compounds on columns packed with low-capacity, low-polarity stationary phase must be inert to avoid peak tailing. The reactivity of support materials can be reduced by silanising prior to coating with liquid phase. Acid-washed, flux-calcinated diatomaceous earth is often used. Materials are available in various particle sizes, the most commonly used particles are in the ranges of 150 ñm to 180 ñm and 125 ñm to 150 ñm.

Mobile phases: Retention time and peak efficiency depend on the carrier gas flow rate; retention time is directly proportional to column length and resolution is proportional to the square root of the column length. For packed columns, the carrier gas flow rate is usually expressed in millilitres per minute at atmospheric pressure and room temperature. Flow rate is measured at the detector outlet, either with a calibrated mechanical device or with a bubble tube, while the column is at operating temperature. The linear velocity of the carrier gas through a packed column is inversely proportional to the square root of the internal diameter of the column for a given flow volume. Flow rates of 60 ml/min in a 4 mm internal diameter column and 15 ml/min in a 2 mm internal diameter column, give identical linear velocities and thus similar retention times.

Helium or nitrogen is usually employed as the carrier gas for packed columns, whereas commonly used carrier gases for capillary columns are nitrogen, helium and hydrogen.

Detectors: Flame-ionisation detectors are usually employed but additional detectors which may be used include: electron-capture, nitrogen-phosphorus, mass spectrometric, thermal conductivity, Fourier transform infrared spectrophotometric, and others, depending on the purpose of the analysis.

Method: Equilibrate the column, the injector and the detector at the temperatures and the gas flow rates specified in the monograph until a stable baseline is achieved. Prepare the test solution(s) and the reference solution(s) as prescribed. The solutions must be free from solid particles.

Performance: Criteria for assessing the suitability of the system are described in the chapter on Chromatographic separation techniques. The extent to which adjustments of parameters of the chromatographic system can be made to satisfy the criteria of system suitability are also given in this chapter.

Reagents: Solvents and reagents used in the preparation of solutions for examination should be of a quality suitable for use in gas chromatography. A wide range of chemical substances is used as stationary phases, including polyethylene glycols, high-molecular weight esters and amides, hydrocarbons, silicone gums and fluids (polysiloxanes often substituted with methyl, phenyl, nitrilo, vinyl or fluoroalkyl groups or mixtures of these) and microporous cross-linked

polyaromatic beads. A suitable stationary phase, its concentration and the nature and grade of a suitable solid support are stated in the monograph. The column should be conditioned in accordance with the manufacturer's instructions. In most cases reference is made to a particular commercial brand that has been found to be suitable for the purpose, but such statements do not imply that a different but equivalent commercial brand may not be used.

The chromatography is carried out either at a constant temperature or according to a given temperature programme.

Analytical procedure:

Test solution: Place in the round bottom flask, accurately weigh about 1g, of the substance being examined, dissolve in 15 ml of *dimethylformamide*. Heat the flask and collect the exactly 10ml of distillate in a graduated cylinder. Cooling by circulating *water* is essential. Measure and record the volume.

Standard preparation ethanol: Prepare 500ppm of ethanol in *dimethylformamide separately.*

Chromatographic condition:

Detector: Flame ionization detector

Column with stationary phase: A fused-silica capillary column 30 m long and 0.25 or 0.32 or 53 mm in internal diameter coated with cross-linked 6 per cent polycyanopropylphenylsiloxane and 94 per cent polydimethylsiloxane having film thickness: 1.4 ñm, 1.8ñm or 3ñm.

Temperature: Coloumn 34^0 to 100^0 at 15^0/min., then increase to 180^0 @ 25^0/min then increase to 225^0 at 40^0/min. Injection port temperature 250^0; detector temperature 275^0.

Carrier Gas: Nitrogen for chromatography at an appropriate flow

Procedure: Inject 1ñl standard solution and record the chromatogram. In the chromatogram obtained with test solution. If there is any peak corresponding to ethanol the peak area is not greater than the peak area in the chromatogram obtained with standard solution for ethanol.

3.9. Stability Testing and Shelf Life Determination for New and Existing Ayurvedic Drugs

(This guideline is not limited only to ASU extracts covered under this volume of API. It shall be applicable to all the licensed ASU medicines.)

3.9.1 Scope and Objective

The objective of this guideline is to specify the method of arriving at shelf life by stability testing. The shelf life determined by the process mentioned in this guideline can be used to decide the

expiry date, in case a manufacturer wishes to assign a shelf life longer than one specified by the notification GSR 764(E) dated October 15, 2009.

The guideline can be used for all patented and proprietary Ayurvedic medicines, both new and existing products.

3.9.2 General Information on Stability

Information of shelf life (expiry date) is mandatory requirement for all licensed Ayurvedic medicines. The stability depends on various factors like the nature of the product, the ingredients of the products, the packaging material etc. Stability studies are carried out to demonstrate that the medicine will remain suitable for consumption during shelf period when stored under the condition(s) mentioned on the packaging. If there is no mention about any specific storage condition, then it is assumed that the product can be stored at room temperature (below 30^{o}). For a suitable drug substance, retest period is more appropriate than expiry date.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of variety of environmental factors such as temperature, humidity and light to establish a retest period for drug substance or a shelf life for drug products.

Two approaches can be followed to monitor the stability of the product. The first approach is to store the samples of same batch material at standard storage and accelerated storage conditions and test them periodically. Based on the evaluation of the results, the expiry date or shelf life may be determined.

The second approach is to select samples from batches manufactured over a period of last five years spanning six months and evaluate them simultaneously. Based on the result obtained the expiry date or shelf life may be determined. This approach is applicable for existing products which do not have yet a declared shelf life. This approach has been referred in scientific literature as the "cross sectional approach".

3.9.3 Selection of batches

Formal stability studies should be conducted on at least three primary batches. The primary batches should be of the same formulation as proposed for marketing. For new products, the batches should be manufactured to a minimum of pilot scale by the same route and using a method of manufacture and procedure that simulates the final process to be used for production batches. Pilot batches which are at least 1/10 of the commercial batch size can be used. The overall quality of the batches of drug placed in formal stability studies should be representative of the quality of the material made on production scale. Where possible, batches of drug product should be manufactured by using different batches of drug substance. Stability to be performed on each individual strength and container size of the product unless bracketing and matrixing is applied.

For cross sectional approach at least two batches per year to be selected. For example if stability to be evaluated for four years eight batches should be selected.

3.9.4 Container and closure system

The stability studies should be conducted on the dosage form packaged in the container and closure proposed for marketing (including as appropriate, any secondary packaging and container label). If the container is too large for drug substances the stability studies should be conducted in a container and closure system that is the same as or simulates the packaging proposed for storage and distribution.

3.9.5 Specification

Specification is a list of tests, reference to analytical procedures and proposed acceptance criteria.

Stability study should include testing of those attributes of the drug that are susceptible to change during storage and are likely to influence quality, safety, and/or efficacy. The testing should cover as appropriate, the physical, chemical, biological, and microbiological attributes. Validated stability-indicating analytical procedures should be applied. Whether and to what extent replication should be performed will depend on the results from validation studies.

The physical parameters included in the specification need not be limited to colour, odour, appearance, shape and taste only. The chemical parameters should include colour reaction, *p*H value, weight variation, disintegration, bulk density, extractive values, estimation of active or marker or category compound by suitable methods and chromatographic profiling. A suitable bioassay may be employed wherever possible.

The limits of acceptance for the products should be those specified in pharmacopoeia. If limits are not available these should be derived from release specification. Shelf life acceptance criteria should be derived from consideration of all available stability information. It may be appropriate to have justifiable differences between the shelf life and release acceptance criteria based on the stability evaluation and the changes observed on storage. Any differences between the release and shelf life acceptance criteria for anti microbial preservative content should be supported by a validated correlation of chemical content and preservative effectiveness demonstrated during development of the product in its final formulation (except for preservative concentration) intended for marketing.

3.9.6 Testing frequency

For long term studies frequency of testing should be sufficient to establish the stability profile of the drug. For drug with proposed shelf life of at least 12 months the frequency of testing at long term storage condition should normally be every six months over first year, and the second year and annually thereafter through the proposed re-test period or shelf life.

At the accelerated storage condition, a minimum of three time points including the initial and final time points (*e.g.* 0, 3 and 6 months) from a 6 study is recommended

Reduced designs *i.e.*, matrixing or bracketing, where the testing frequency is reduced or certain factor combinations are not tested at all, can be applied if justified.

3.9.7 Storage condition

The world can be divided in to four climatic zones I – IV. This guideline address zone IV. The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the zone. Recommended storage conditions are

S.No	Study	Storage condition	Minimum time
1	Accelerated	$40^{o} \pm 2^{o}$/ 75 per cent RH ± 5 per cent	6 months
2	Long term	$30^{o} \pm 2^{o}$/ 60 per cent RH ± 5 per cent	12 months

Other storage conditions are allowable if suitably justified. For products which are temperature sensitive, to be stored in lower temperature which will then become the condition designated long term storage temperature. The accelerated testing should be then carried out at least 10^{o}C more than the long term storage condition along with appropriate relative humidity condition for that temperature.

The reference samples for the above study should be stored in a temperature less than 10^{o}

3.9.8 Evaluation

The purpose of stability is to establish, based on testing a minimum of atleast three batches of the drug, a retest period applicable to all future batches for the drug substance, or a shelf life and label storage instructions applicable for all future batches of the drug product manufactured and packed under similar circumstances.

An Ayurvedic drug can be considered to be stable if "no significant change" occurs during at any time of testing at accelerated storage condition or at real time storage condition.

"Significant change" for a drug is defined as

1. A + or – 20 per cent change from the initial assay value (If the drug is analysed for its marker). A + or – 15 per cent change from the initial assay value (If the drug is analysed for its active compound).

2. Appearance of new spots in Identification by TLC (when compared with the sample stored in less than 10^{o}) or completely disappearance of existing spot.

3. The physico-chemical parameters (Moisture, Ash, Particle size) shall not vary beyond 25 per cent of the initial value.

4. Failure to meet the acceptance criteria as per individual monographs or specification

5. Failure to meet acceptance criteria for appearance (Physical attributes, and functionality tests *e.g*., Colour, phase separation, caking, hardness).

APPENDIX - 4

REAGENTS AND CHEMICALS

Acetic Acid – Contains approximately 33 per cent w/v of $C_2H_4O_2$. Dilute 315 ml of glacial acetic acid to 1000 ml with *water*.

Acetic Acid, Glacial – CH_3COOH =60.05.

Contains not less than 99.0 per cent w/w of $C_2H_4O_2$. About 17.5 N in strength.

Description – At temperature above its freezing point a clear colourless liquid, odour, pungent and characteristic; crystallises when cooled to about 10^0 and does not completely re-melt until warmed to about 15^0.

Solubility – Miscible with *water,* with *glycerin* and most fixed and volatile oils.

Boiling range –Between 117^0 and 119^0.

Congealing temperature –Not lower than 14.8^0.

Wt. per ml –At 25^0about 1.047 g.

Heavy metals –Evaporate 5 ml to dryness in a porcelain dish on water-bath, warm the residue with 2 ml of 0.1 *N hydrochloric acid* and *water* to make 25 ml; the limit of heavy metals is 10 parts per million, Appendix 2.3.3.

Chloride –5 ml complies with the limit test for chlorides, Appendix 2.3.2.

Sulphate –5 ml complies with the limit test for sulphates,

Certain aldehydic substances – To 5 ml add 10 ml of *mercuric chloride solution* and make alkaline with *sodium hydroxide solution,* allow to stand for five minutes and acidify with dilute *sulphuric acid*; the solution does not show more than a faint turbidity.

Formic acid and oxidisable impurities – Dilute 5 ml with 10 ml of *water*, to 5 ml of this solution add 2.0 ml of 0.1 *N potassium dichromate* and 6 ml of *sulphuric acid*, and allow to stand for one minute, add 25 ml of *water*, cool to 15^0, and add 1 ml of freshly prepared *potassium iodide* solution and titrate the liberated iodine with 0.1 *N sodium thiosulphate*, using starch solution as indicator. Not less than 1 ml of 0.*N sodium thiosulphate* is required.

Odorous impurities –Neutralise 1.5 ml with *sodium hydroxide solution*; the solution has no odour other than a faint acetous odour.

Readily oxidisable impurities – To 5 ml of the solution prepared for the test for Formic Acid and Oxidisable Impurities, add 20 ml of *water* and 0.5 ml of 0.1 *N potassium permanganate;* the pink colour does not entirely disappear within half a minute.

Non-volatile matter – Leaves not more than 0.01 per cent w/w of residue when evaporated to dryness and dried to constant weight at 105^0.

Assay –Weigh accurately about 1 g into a stoppered flask containing 50 ml of *water* and titrate with *N sodium hydroxide,* using *phenolphthalein solution* as indicator. Each ml of *sodium hydroxide* is equivalent to 0.06005 g of $C_2H_4O_2$.

Acetic Acid, Lead-Free –Acetic acid which complies with following additional test, boil 25 ml until the volume is reduced to about 15 ml, cool make alkaline with lead-free ammonia solution, add 1 ml of lead free *potassium cyanide solution, dilute* to 50 ml with *water*, add 2 drops of *sodium sulphide solution;* no darkening is produced.

Acetone – Propan-2-one; C_3H_6O = 58.08 (67-64-1)

Analytical reagent grade of commerce. A volatile, flammable liquid; boiling point, about 56^0; weight per ml, about 0.79 g. Complies with the following test. *Water* Not more than 0.3 per cent w/w, Appendix IX C, using anhydrous pyridine as the solvent.

Acetonitrile – Methyl Cyanide; CH_3CN = 41.05

General laboratory reagent grade of commerce.
Colourless liquid; bp, about 81^0; wt. per ml, about 0.78 g.
Acetonitrile intended for use in spectrophotometry complies with the following test.
Transmittance : Not less than 98 per cent in the range 255 to 420 nm using *water* as the blank.

Alcohol –

Description – Clear, colourless, mobile, volatile liquid, odour, characteristic and spirituous; taste, burning, readily volatilised even at low temperature, and boils at about 78^0, flammable. Alcohol containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of C_2H_5OH at 15.56^0.

Solubility –Miscible in all proportions with *water,* with *chloroform* and with *solvent ether.*

Acidity or alkalinity – To 20 ml add five drops of *phenolphthalein solution*; the solution remains colourless and requires not more than 2.0 ml of 0.1*N sodium hydroxide* to produce a pink colour.

Specific gravity –Between 0.8084 and 0.8104 at 25^0.

Clarity of solution –Dilute 5 ml to 100 ml with *water* in glass cylinder; the solution remains clear when examined against a black background. Cool to 10^0 for thirty minutes; the solution remains clear.

Methanol – To one drop, add one of *water*, one drop of *dilute phosphoric acid,* and one drop of *potassium permanganate solution.* Mix, allow to stand for one minute and add sodium bisulphite solution dropwise, until the permanganate colour is discharged. If a brown colour remains, add one drop of *dilute phosphoric* acid. To the colourless solution add 5 ml of freshly prepared *chromotropic acid* solution and heat on a water-bath at 60^0 for ten minutes; no violet colour is produced.

Foreign organic substances – Clean a glass-stoppered cylinder thoroughly with *hydrochloric acid,* rinse with *water* and finally rinse with the alcohol under examination. Put 20 ml in the cylinder, cool to about 15^0 and then add from a carefully cleaned pipette 0.1 ml of 0.1 *N potassium permanganate.* Mix at once by inverting the stoppered cylinder and allow to stand at 15^0 for five minutes; the pink colour does not entirely disappear.

Isopropyl alcohol and t-butyl alcohol – To 1 ml add 2 ml of *water* and 10 ml of *mercuric sulphate solution* and heat in a boiling water-bath; no precipitate is formed within three minutes.

Aldehydes and ketones – Heat 100 ml of *hydroxylamine hydrochloride solution* in a loosely stoppered flask on a water-bath for thirty minutes, cool, and if necessary, add sufficient 0.05 *N sodium hydroxide* to restore the green colour. To 50 ml of this solution add 25 ml of the alcohol and heat on a water bath for ten minutes in a loosely stoppered flask. Cool, transfer to a *Nesseler cylinder*, and titrate with 0.05 *N sodium hydroxide* until the colour matches that of the remainder of the *hydroxylamine hydrochloride solution* contained in a similar cylinder, both solutions being viewed down the axis of the cylinder. Not more than 0.9 ml of 0.05 *N sodium hydroxide* is required.

Fusel oil constituents – Mix 10 ml with 5 ml of *water* and 1 ml of *glycerin* and allow the mixture to evaporate spontaneously from clean, odourless absorbent paper; no foreign odour is perceptible at any stage of the evaporation.

Non-volatile matter – Evaporate 40 ml in a tared dish on a water-bath and dry the residue at 105n for one hour; the weight of the residue does not exceed 1 mg.

Storage – Store in tightly-closed containers, away from fire.

Labelling – The label on the container states "Flammable".

Alcohol, Aldehyde-free. –Alcohol which complies with the following additional test :

Aldehyde – To 25 ml, contained in 300 ml flask, add 75 ml of *dinitrophenyl hydrazine solution,* heat on a water bath under a reflux condenser for twenty four hours, remove the alcohol by distillation, dilute to 200 ml with a 2 per cent v/v solution of sulphuric acid, and set aside for twenty four hours; no crystals are produced.

Alcohol, Sulphate-free. –Shake alcohol with an excess of anion exchange resin for thirty minutes and filter.

Ammonia solution Sp. – *Strong ammonia solution* which complies with the following additional test.

Evaporate 10 ml to dryness on a water-bath. To the residue add 1 ml of *dilute hydrochloric acid Sp.* and evaporate to dryness. Dissolve the residue in 2 ml of *dilute acetic acid Sp.*, add sufficient *water* to produce 25 ml and add 10 ml of *hydrogen sulphide solution*. Any darkening produced is not greater than that of a blank solution containing 2 ml of dilute *acetic acid* Sp., 1.0 ml of *standard lead solution* and sufficient *water* to produce 25 ml.

0.1M ammonia – Solution of any molarity x M may be prepared by diluting 75 x ml of strong ammonia solution to 1000 ml with *water*

Anisaldehyde Sulphuric Acid Reagents – Mix in the following order 0.5 ml of anisaldehyde, 10 ml of glacial acetic acid, 85 ml of methanol and 5 ml of sulphuric acid.

Bismuth nitrate – Analytical reagent grade

Bromine – Br_2 = 159.8 (7726-95-6).

Analytical reagent grade of commerce.

A heavy, brownish-red, fuming liquid, highly corrosive to the skin; d_{20}^{20}, about 3.1.

To prepare 0.05M bromine dissolve 3 g of potassium bromate and 15 g of potassium bromide in sufficient *water* to produce 1000 ml. Weaker solutions should be prepared using proportionately lesser amounts of reagents or by appropriate dilution.

Butan-1-ol – n-Butyl alcohol; butanol; $C_4H_{10}O$ = 74.12 (71-36-3).

Analytical reagent grade of commerce.

A colourless liquid; boiling point, 116^0 to 119^0; d_{20}^{20}, about 0.81.

Chloroform – Trichloromethane; $CHCl_3$ = 119.4 (67-66-3)

Analytical reagent grade of commerce containing 0.4 to 1.0 per cent w/w of ethanol.

A colourless liquid with a sweet, penetrating odour; boiling point, about 60^0; d_{20}^{20}, 1.475 to 1.481.

Citric Acid – $C_6H_8O_7,H_2O$ = 210.1 (5949-29-1). Analytical reagent grade of commerce. When used in the limit test for iron, complies with the following requirement.

Dissolve 0.5 g in 10 ml of *water*, add 0.1 ml of mercaptoacetic acid, mix, make alkaline with 10M ammonia and add sufficient *water* to produce 20 ml. No pink colour is produced.

Ether – $C_4H_{10}O$ = 74.12 (60-29-7)

Analytical reagent grade of commerce.

A volatile, highly flammable, colourless liquid; boiling point, 34^0 to 35^0; d_{20}^{20}, 0.713 to 0.715.

Do not distil unless the ether complies with the following test for peroxides.

Peroxides Place 8 ml of potassium iodide and starch solution in a 12 ml ground-glass-stoppered cylinder about 1.5 cm in diameter. Fill completely with the reagent being examined, shake vigorously and allow to stand in the dark for 30 minutes. No colour is produced.

Store protected from light at a temperature not exceeding 15^0. The name and concentration of any added stabiliser are stated on the label.

1,4-Dioxane — 1,4-Dioxan; Diethylene Dioxide: $C_4H_8O_2$ = 88.11

Analytical reagent grade of commerce.

Colourless liquid with an ethereal odour; bp, about 101^0; wt. per ml, about 1.03 g.

Do not distil unless the dioxan complies with the test for peroxides.

Peroxides : Place 8 ml of starch iodide solution in a 12 ml glass-stopperred cylinder about 1.5 cm in diameter. Fill completely with the reagent under examination, shake vigourously and allow to stand protected from light for 30 minutes; no colour is produced.

Dragendorff's Reagent

Solution A — 0.85g Basic *Bismuth nitrate* is dissolved in a mixture of 10ml *glacial acetic acid* and 40 ml *water*.

Solution B — A solution is made of 8 g potassium iodide in 20 ml *water*.

Stock solution – Equal volumes of solution A and B are mixed (Can be stored for a long time in dark glasses vessels)

Spray reagent – 1ml Stock solution is mixed with 2 ml *glacial acetic acid* and 10 ml *water* before use.

Ethanol (96 per cent) — Alcohol - C_2H_6O.

Analytical reagent grade ethanol of commerce containing not less than 95.1 per cent v/v and not more than 96.9 per cent v/v of C_2H_6O.

A colourless liquid; weight per ml, about 0.81 g.

Diluted ethanols may be prepared by diluting the volumes of ethanol (96 per cent) indicated in the following table to 1000 ml with *water*.

Strength per cent v/v	Volume of Ethanol (96 per cent) (Apprx) ml	Weight per ml (g)
90	934	0.83
85	885	0.85
80	831	0.86
70	727	0.89
65	676	0.90
60	623	0.91
50	519	0.93
45	468	0.94
25	259	0.97
20	207	0.975
10	104	0.986

Ethyl Acetate – $C_4H_8O_2$ = 88.1 (141-78-6)

Analytical reagent grade of commerce.

A colourless liquid with a fruity odour; boiling point, about 76^0 to 78^0; d_{20}^{20}, 0.901 to 0.904.

Folin Ciocalteu Reagent- Dilute commercially available Folin-Ciocalteu reagent (2N) with an equal volume of distilled *water*. Transfer it in a brown bottle and store in a refrigerator (4^0). It should be goldern in colour. Do not use it if it turns olive green.

Phosphomolybdotungstic Reagent (Folins reagent) Folin Ciocalteau phenol reagent of commerce.

Dissolve 100 g of sodium tungstate and 25 g of *sodium molybdate* in 700 ml of *water* , add 100 ml of *hydrochloric acid* and 50 ml of *orthophosphoric acid* and heat the mixture under a reflux condenser for 10 hours. Add 150 g of *lithium sulphate*, 50 ml of *water* and 0.2 ml of bromine and boil to remove excess bromine (about 15 minutes), cool, dilute to 1000 ml with *water* and filter. The reagent should be yellow in colour. If it acquires a greenish tint, it is unsatisfactory for use but may be regenerated by boiling with 0.2 ml of bromine. Care must be taken to remove excess bromine by boiling.

Store at 2^0 to 8^0.

Formic Acid, Anhydrous – CH_2O_2 = 46.03

Analytical reagent grade formic acid of commerce containing not less than 98.0 per cent w/w of CH_2O_2.

A colourless, corrosive liquid with a pungent odour; d_{20}^{20}, about 1.22.

Assay : Weigh accurately a conical flask containing 10 ml of *water* , quickly add about 1 ml of the reagent and weigh again. Add 50 ml of *water* and titrate with 1M sodium hydroxide VS using 0.5 ml of *phenolphthalein* solution as indicator. Each ml of 1M *sodium hydroxide* VS is equivalent to 46.03 mg of CH_2O_2.

Hydrochloric Acid – HCl = 36.46 (7647-01-0)

Where no molarity is indicated use analytical reagent grade of commerce with a relative density of about 1.18, containing not less than 35 per cent w/w and not more than 38 per cent w/w of HCl and about 11.5M in strength.

A colourless, fuming liquid.

Solutions of molarity xM should be prepared by diluting 85x ml of *hydrochloric acid* to 1000 ml with *water*.

Store in a container of *polyethylene* or other non-reacting material at a temperature not exceeding 30^0.

Lithium Sulphate – Li_2SO_4,H_2O = 128.0 (10102-25-7)

Analytical reagent grade of commerce.

Methanol – Methyl alcohol; CH_4O = 32.04 (67-56-1)

Analytical reagent grade of commerce.

A colourless liquid; boiling point, 64^0 to 65^0; d_{20}^{20}, 0.791 to 0.793.

When 'methanol ' is followed by a percentage figure, an instruction to use *methanol* diluted with *water* to produce the specified percentage v/v of *methanol* is implied.

Orthophosphoric Acid – Phosphoric acid; H_3PO_4 = 98.00 (7664-38-2)

Analytical reagent grade of commerce containing not less than 84 per cent w/w of H_3PO_4 and about 15.7M in strength.

A corrosive liquid; weight per ml, about 1.75 g.

Potassium Dihydrogen Orthophosphate – Potassium dihydrogen phosphate; KH_2PO_4 = 136.1 (7778-77-0)

Analytical reagent grade of commerce.

Colourless crystals.

Potassium Iodide – KI = 166.0 (7681-11-0)

Analytical reagent grade of commerce.

A white, crystalline powder.

Sodium Carbonate – $Na_2CO_3,10H_2O$ = 286.2 (6132-02-1)

Analytical reagent grade of commerce.

Melting point, greater than 300^0.

Sodium Molybdate – $Na_2MoO_4,2H_2O$ = 242.0 (10102-40-6)

Analytical reagent grade of commerce.

Sodium Tungstate – $Na_2WO_4,2H_2O$ = 329.9 (10213-10-2)

Analytical reagent grade of commerce.

Sulphuric Acid – H_2SO_4 = 98.08 (7664-93-9)

When no molarity is indicated use analytical reagent grade of commerce containing about 96 per cent w/w of *sulphuric acid* and about 18M in strength; an oily, corrosive liquid; weight per ml, about 1.84 g.

When solutions of molarity xM are required, they should be prepared by carefully adding 54x ml of *sulphuric acid* to an equal volume of *water* and diluting to 1000 ml with *water*.

When 'sulphuric acid' is followed by a percentage figure, an instruction to add, carefully, sulphuric acid to *water* to produce the specified percentage v/v (or, if required, w/w) proportion of *sulphuric acid* is implied.

Methanolic Sulphuric Reagent

Add dropwise 10ml of sulphuric acid to 90ml of ice- cold methanol.

Sodium Sulphate, Anhydrous – Na_2SO_4 = 142.0 (7757-82- 6)

Analytical reagent grade of commerce complying with the following test.

Loss on drying : When dried at 130^0, loses not more than 0.5 per cent of its weight.

Sodium Carbonate Solution

30g of sodium carbonate is dissolved in *water* and make up to 100 ml.

Tetrahydrofuran – Tetramethylene oxide; C_4H_8O = 72.11 (109-99-9)

Analytical reagent grade of commerce.

A clear, colourless, flammable liquid; boiling point, about 66^0; d_{20}^{20}, about 0.89.

Do not distil unless it complies with the following test.

Peroxides : Place 8 ml of potassium iodide and starch solution in a ground-glass-stoppered cylinder with a capacity of 12 ml and about 1.5 cm in diameter and add sufficient of the substance being examined to fill the cylinder completely, shake vigorously and allow to stand for 30 minutes protected from light. No colour is produced.

Tetrahydrofuran used in spectrophotometry complies with the following additional requirement.

Transmittance Not less than 20 per cent at 255 nm, 80 per cent at 270 nm and 98 per cent at 310 nm determined using *water* in the reference cell.

Toluene – Methylbenzene; C_7H_8 = 92.14 (108-88-3)

Analytical reagent grade of commerce.

A colourless liquid with a characteristic odour; weight per ml, 0.865 to 0.870 g; boiling point, about 110^0.

Vanillin – 4-Hydroxy-3-methoxybenzaldehyde; $C_8H_8O_3$ = 152.2 (121-33-5)

Analytical reagent grade of commerce.

White to yellowish white, needles or crystalline powder, with an odour of vanilla.

Melting point, about 81^0, determined without previous drying.

Vanillin Sulphuric Acid Reagent

Dissolve 1g of vanillin in 90ml of ethanol; add dropwise 10ml of sulphuric acid to ice- cold ethanol.

Water HPLC Grade

Ultra pure *water*.

Water, Carbon Dioxide-free

Water, Carbon Dioxide-free : *Water* that has been boiled vigorously for a few minutes and protected from the atmosphere during cooling and storage

APPENDIX - 5

WEIGHTS AND MEASURES

5.1. Metric Equivalents of Classical Weights and Measures

The following table of metric equivalents of weights and measures, linear measures and measurement of time used in the Ayurvedic classics have been approved by the Ayurvedic Pharmacopoeia committee in consultation with Indian Standards Institution.

I. WEIGHTS AND MEASURES

Classical Unit		Metric Equivalent
1 Rattī or Guñjā		= 125 mg
8 Rattī or Guñjā	= 1 Māṣa	= 1 g
12 Maṣas	= 1 Karṣa (Tolā)	= 12 g
2 Karṣas	= 1 Śukti	= 24 g
2 Śukti	= 1 Palam	= 48 g
2 Palas	= 1 Prasṛti	= 96 g
2 Prasṛtis	= 1 Kuḍava	= 192 g
2 Kuḍavas	= 1 Mānikā	= 384 g
2 Mānikās	= 1 Prastha	= 768 g
4 Prasthas	= 1 Āḍhaka	= 3 kg 72 g
4 Āḍhakas	= 1 Droṇa	= 12 kg 288 g
2 Droṇas	= 1 Śūrpa	= 24 kg 576 g
2 Śūrpas	=1 Droṇī (Vāhī)	= 49 kg 152 g
4 Droṇīs	= 1 Khārī	= 196 kg 608 g
100 Palas	= 1 Tulā	= 4 kg 800 g
20 Tulās	= 1 Bhāra	= 96 kg

In case of liquids, the metric equivalents would be the corresponding litre and milliliter.

II. LINEAR MEASURES

Classical Unit	Inches	Metric Equivalent
Yavodara	1/8 of 3/4"	0.24 cm
Aṅgula	3/4"	1.95 cm
Vitasti	9"	22.86 cm
Aratni	10 1/2"	41.91 cm
Hasta	18"	45.72 cm
Nṛpahasta (Rājahasta)	22"	55.88 cm
Vyāma	72"	182.88 cm

III. MEASUREMENT OF TIME

Unit		Equivalent (in hours, minutes & seconds)
2 Kṣaṇas	= 1 Lava	
2 Lavas	= 1 Nimeṣa	
3 Nimeṣas	= 1 Kāṣṭhā	= 4.66 seconds
1 Ghaṭīs		= 24 minutes
30 Kāṣṭhās	= 1 Kalā	= 2 minutes 20 seconds
20 Kalās + 3 Kāṣṭhās	= 1 Muhūrta	= 48 minutes
30 Muhūrtas	= 1 Ahorātra	= 24 hours
15 Ahorātras	= 1 Pakṣa	= 15 days
2 Pakṣas	= 1 Māsa	= 30 days/1 month
2 Māsas	= 1 Ṛtu	= 60 days/ Two Months
3 Ṛtus	= 1 Ayana	= 6 Months
2 Ayanas	= 1 Saṁvatsara	= 12 months/1 Year
5 Saṁvatsara	= 1Yuga	= 5 Years
1 Ahorātra of Devas		= 1 Year
1 Ahorātra of Pitaras		= 1 Month

5.2. Metric System

Measure of Mass (Weights)

1 Kilogram (Kg)	–	is the mass of the International Prototype Kilogram.
1 Gramme (g)	–	the 1000^{th} part of 1 Kilogram
1Milligram (mg)	–	the 1000^{th} part of 1 gramme
1 Microgram (μg)	–	the 1000^{th} part of 1 milligram

Measures of capacity (Volumes)

1 Litre (1) is the volume occupied at its temperature of maximum density by a quantity of *water* having a mass of 1 Kilogram.

1 Millilitre (ml) is the 1000^{th} part of 1 litre.

The accepted relation between the litre and the cubic centimetre is 1 litre – 1000.027 cubic centimeters.

Relation of capacity of Weight (Metric)

One litre of *water* at 20^0 weighs 997.18 grams when weighed in air of density 0.0012 gram per millilitre against brass weights of density 84 grams per millilitre.

Measures of Length

1 Metre (m) is the length of the International Prototype Metre at 0.

1 Centimetre (cm)	–	the 100^{th} part of 1 metre
1 Millimetre (mm)	–	the 1000^{th} part of 1 metre
1 Micron (μ)	–	the 1000^{th} part of 1 millimetre
1 Milliimicron (mμ)	–	the 1000^{th} part of micron

INDEX