

# **THE AYURVEDIC PHARMACOPOEIA OF INDIA**

**PART - I  
VOLUME - IX**

**First Edition**



**सत्यमेव जयते**

**Government of India  
Ministry of AYUSH  
2016**

**Published by  
PHARMACOPOEIA COMMISSION FOR INDIAN MEDICINE &  
HOMOEOPATHY  
GHAZIABAD**

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सत्यमेव जयते



अजीत मोहन शरण  
**AJIT M. SHARAN**



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भारत सरकार  
आयुर्वेद, योग व प्राकृतिक चिकित्सा  
यूनानी, सिद्ध एवं होम्योपैथी (आयुष) मंत्रालय  
आयुष भवन, 'बी' ब्लाक, जी.पी.ओ. कॉम्प्लेक्स,  
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GOVERNMENT OF INDIA  
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## FOREWORD

India is one of the few countries which have taken major strides towards establishing the pharmacopoeial standards of its traditional medicines of ancient origin. For ensuring the manufacturing of the quality drugs the Drugs and Cosmetics Act, 1940, was amended in 1964 to bring within the range of its operation and Rules there under. This has led necessary to work out standards of Ayurvedic drugs under the Ayurvedic Pharmacopoeia of India.

2. The Ayurvedic Pharmacopoeia is a book of standards narrating the quality of preferred drugs that are manufactured, distributed and sold by licensed drug manufacturers. The Ayurvedic Pharmacopoeia Committee (APC) under the Ministry of AYUSH, Government of India has already published standards for more than 550 single drugs and 152 classical compound formulations. This Pharmacopoeia activity is continuing with the addition of new monographs as ongoing processes of the APC.

3. The present Ayurvedic Pharmacopoeia of India, Part-I, Vol. IX comprises of 45 monographs on the most frequently used plant drugs with standards of source plant, hydro-alcoholic extracts and aqueous extracts with their chromatographic finger print. The work carried out for this volume is of high scientific quality and will provide guidelines for the different groups of stakeholders in the use of plants including preparation and standardization of medicinal plant extracts, drugs etc. The work comprised in this volume is accomplished by different participating institutions under APC project.

4. I deeply appreciate the efforts of the participating institutions, Prof. S. S. Handa, Chairman, Scientific Body, PCIM&H, Prof. V. K. Joshi, Chairman and the members of the Ayurvedic Pharmacopoeia Committee, Prof. K. S. Dhiman, Director General, CCRAS, Dr. M. M. Padhi, Deputy Director General, CCRAS and former Director I/c, PCIM, and Dr. Rajeev Kr. Sharma, Director, PCIM&H and the entire team to bring out this volume of Ayurvedic Pharmacopoeia of India.

5. I am confident that this Ayurvedic Pharmacopoeia of India, Part-I, Volume-IX will be of great utility to all the stakeholders involved in the quality control of Ayurvedic Drugs.

(Ajit M. Sharan)

NEW DELHI  
1<sup>st</sup> March, 2016





सत्यमेव जयते

भारत सरकार  
आयुर्वेद, योग व प्राकृतिक चिकित्सा  
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GOVERNMENT OF INDIA  
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**Dated: 01<sup>st</sup> March, 2016**

## PREFACE

India has a living tradition of use of plants as food, fodder and medicine since ancient times. The rational use of plants as medicine is found in Charaka Samhita and Sushruta Samhita dated back to 1000 BCE. In India as well other parts of the world, the plant based medicine has gained increased acceptance, which has drawn the attention of the Government organizations and World Health Organization to provide quality standard drugs to the consumers.

The Ayurvedic Pharmacopoeia committee under the Ministry of AYUSH is working for quality standard in this field and the present volume in the series of already published 8 volumes contains quality standards of 15 plants with their monographs on the single drug, the hydro-alcoholic extract and water extract. Thus, total 45 monographs are contained in this present volume.

Each monograph is titled with the classical name of the plant with its synonyms in Sanskrit and the regional language names, following this, description of the useful part(s) of the plant with illustrated powder microscopy, thin layer chromatography, heavy metal limits, microbial contamination, pesticide residue, aflatoxins, liquid chromatography etc. has been scientifically presented.

It is believed that the current volume similar to the previous Volume-VIII would be of immense interest to the Ayurvedic drug industry, academicians and research organizations and others.

**(JITENDRA SHARMA)**



## 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 Pharmacopoeia Commission for Indian Medicine & Homoeopathy 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-IX.” Wherever the abbreviation “API, Pt.-I, Vol.-IX” 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 Saṃskṛta 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.

**Hydro-alcohol:** 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 per cent). Other dilutions of ethanol are indicated by the term ‘alcohol’ followed by a statement of the percentage by volume of ethanol (C<sub>2</sub>H<sub>6</sub>O/C<sub>2</sub>H<sub>5</sub>OH) 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, per g of the drug taken 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 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*.

**Temperature:** The symbol ‘°’ 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 containing 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.***

**Powder:** Drug substances are subjected to comminution 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 or 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<sup>0</sup>.

**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.

Herbal/Plant drugs should be duly identified and authenticated and should be free from insects, pests, fungi, microorganisms, pesticides, and other animal matter including animal excreta, should 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.

Herbal/plant drugs should be duly identified and authenticated and should be free from insects, pests and other animal matter including animal excreta, should have, within the permitted and specified limits, for fungi, microorganisms, pesticides, 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 and 30<sup>0</sup>.

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 extractive, alcohol-soluble extractive, 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 contamination, 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, 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<sub>254</sub> 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. 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 descriptions 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.

**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<sub>2</sub>H<sub>5</sub>OH) refer to percentage by volumes at 15.56°.

**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 gram 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:-

<b>Descriptive terms</b>	<b>Relative quantities of solvent</b>
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 API 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<sup>0</sup> and usually between 2<sup>0</sup> and 8<sup>0</sup>. A refrigerator provides a cold place in which the temperature is maintained thermostatically between 2<sup>0</sup> and 8<sup>0</sup>.

*Cool-* Any temperature between 8<sup>0</sup> and 25<sup>0</sup>. 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<sup>0</sup> and 40<sup>0</sup>.

*Excessive heat-* Any temperature above 40<sup>0</sup>.

*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  $\mu$  polyester, 100  $\mu$  polyethylene and a secondary cover made up of 9 $\mu$  aluminium sandwiched between 2 layers of 12  $\mu$  polyester and 100  $\mu$  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.



## PHARMACOPOEIA COMMISSION FOR INDIAN MEDICINE & HOMOEOPATHY

Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H) is an autonomous organization under Ministry of AYUSH, Govt. of India with a primary mandate to develop pharmacopoeial standards for drugs/formulations used under Ayurveda, Siddha, Unani and Homoeopathic systems of medicine. It serves as an umbrella organization for Ayurvedic Pharmacopoeia Committee (APC), Siddha Pharmacopoeia Committee (SPC), Unani Pharmacopoeia Committee (UPC) and Homoeopathic Pharmacopoeia Committee (HPC). Pharmacopoeial Laboratory for Indian Medicine (PLIM) and Homoeopathic Pharmacopoeia Laboratory (HPL) are its permanent supporting structures.

The Commission was initially established as Pharmacopoeia Commission for Indian Medicine (PCIM) in the year 2010. In pursuance to the decision of Central Government, Homoeopathy was incorporated and the Commission was renamed as Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H) on 25<sup>th</sup> June 2014. Commission has a three-tier structure of Governance comprising of the General Body, Standing Finance Committee and Scientific Body. The Secretary, Ministry of AYUSH, Govt. of India is ex-officio Chairman of the Commission.

### Objectives

1. Publication and revision of the Ayurvedic, Siddha, Unani and Homoeopathic Pharmacopoeia of India at suitable intervals and of such addenda or supplementary compendia during the intervening periods as may be deemed necessary; releasing the publications for public use from a date when they are to become official.
2. Publication and revision of the Ayurvedic, Siddha and Unani Formularies of India, Homoeopathic pharmacopoeia as well as Homoeopathic Pharmaceutical Codex at regular intervals with a view to make it an authentic source of information on rational combination and use of medicines including their methods of preparation, therapeutic indications, adverse reactions, contra-indications, drug-drug interactions and similar issues concerning Indian medicines for safe use in humans and animals. Identification of Ayurvedic, Siddha and Unani formulations and Homoeopathic pharmacopoeia as well as Homoeopathic Pharmaceutical Codex with a view to develop their quality standards and to ensure quality and safety of ASU & H medicine.
3. To nurture and promote awareness of quality in Ayurvedic, Siddha and Unani drugs/formulations, Homoeopathic pharmacopoeia as well as Homoeopathic Pharmaceutical Codex and drug research on ASU products and publish regularly or at suitable intervals other related scientific information as authorized under the rules and procedures of the Commission.
4. Exchange information and interact with expert committees of the World Health Organization and other international bodies with a view to harmonize and develop the Ayurvedic, Siddha, Unani and Homoeopathic Pharmacopoeial standards to make those internationally acceptable.
5. Arranging studies either under its own auspices or through collaboration with other institutions to develop standards and quality specifications for identity, purity and strength of raw materials and compound formulations and to develop Standard Operating Procedures for the process of manufacture included or to be included in the Ayurvedic, Siddha, Unani and Homeopathic Pharmacopoeia/formulary and its addenda or supplementary compendia or other authorized publications.
6. Maintain National repository of authentic reference raw materials used in the manufacture of Ayurveda, Siddha, Unani and Homeopathic medicines for the purpose of reference and supply of reference standards to the stake holders at a price.

7. To assign responsibilities described for Pharmacopoeial Laboratory for Indian Medicine and Homoeopathic Pharmacopoeia Laboratory under the Drugs & Cosmetics Act.
8. Generate and maintain repository of chemical reference marker compounds of the plants or other ingredients used in standardizing Ayurveda, Siddha, Unani and Homeopathy medicines and supply them as reference standards to the stake holders on price.
9. Furtherance of the provision of Chapter IVA of Drugs and Cosmetic Act, 1940 in case ASU drugs & 4A of Schedule II of Drugs & Cosmetics Act in case of Homoeopathy medicine and rules there under related to Ayurvedic, Siddha and Unani drugs and Homoeopathy medicine respectively.
10. Acting as a coordinating centre for analytical laboratories, industry and academia by encouraging exchange of scientific and technical information and staff and by undertaking sponsored funded research as well as consultancy projects.
11. Organizing national/international symposia, seminars, meetings and conferences in selected areas from time to time and to provide updated regular training to the regulatory authorities and stake holders.

### **The General Body**

The General Body is the apex body and is responsible for overall governance of the Commission.

#### **Composition:**

- |       |   |                   |
|-------|---|-------------------|
| i)    | Secretary, Ministry of AYUSH<br>Sh. Nilanjan Sanyal until 31 <sup>st</sup> August, 2015;<br>Sh. Ajit M. Sharan from 1 <sup>st</sup> Sept., 2015   | Chairman          |
| ii)   | Joint Secretary, Ministry of AYUSH<br>Sh. Raj Pratap Singh until 1 <sup>st</sup> Dec., 2014<br>Sh. Anurag Srivastav until 1 <sup>st</sup> Nov., 2015<br>Sh. Jitendra Sharma from 2 <sup>nd</sup> Nov., 2015 | Vice-Chairman - 1 |
| iii)  | Chairman, Scientific Body, PCIM&H<br>Prof. S. S. Handa  | Vice-Chairman - 2 |
| iv)   | Secretary and Director General, ICMR<br>Dr. Soumya Swaminathan  | Member            |
| v)    | Chairman, CII or his nominee<br>Sh. Sumit Mazumder  | Member            |
| vi)   | Chairman, FICCI or his nominee<br>Mr. Harshavardhan Neotia  | Member            |
| vii)  | Drugs Controller General (India)<br>Dr. G. N. Singh   | Member            |
| viii) | Central Drug Controller (AYUSH)   | Member            |
| ix)   | Adviser (Ayurveda), Ministry of AYUSH<br>Dr. Manoj Nesari   | Member            |
| x)    | Adviser (Unani), Ministry of AYUSH<br>Prof. Rais-Ur-Rahman  | Member            |
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- xiv) Director, PCIM&H Member Secretary  
Dr. Rajeev Kr. Sharma

### **The Standing Finance Committee**

All matters with respect to financial approvals are dealt by Standing Finance Committee. Standing Finance Committee is responsible for screening/appraising/evaluating the projects/works etc. of the Commission and recommend for the approval of these projects /works by the General Body.

### **Composition:**

- i) Joint Secretary (AYUSH) Chairman  
Sh. Raj Pratap Singh until 1<sup>st</sup> Dec., 2014  
Sh. Anurag Srivastava until 1<sup>st</sup> Nov., 2015  
Sh. Jitendra Sharma from 2<sup>nd</sup> Nov., 2015
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Prof. S.S. Handa
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- iv) Central Drug Controller (AYUSH) Member
- v) Director, PCIM&H Member Secretary  
Dr. Rajeev Kr. Sharma

## The Scientific Body

The Scientific Body is responsible for designing/preparing and according technical approval for all the scientific & technical works/projects and execution of these works/projects through different Pharmacopoeia Committees / other agencies, publication of validated Pharmacopoeia after obtaining the approval of the General Body.

### Composition:

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- xx) Director, PCIM&H Member Secretary  
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## **Execution of Pharmacopoeial Work**

### **Pharmacopoeia Committees**

The Scientific and technical work of the Commission is being executed through Ayurvedic, Siddha, Unani & Homoeopathic Pharmacopoeia committees under the supervision of the Scientific Body. The function of Pharmacopoeia committees is to prepare official formularies, Pharmacopoeias of single drugs and compound formulations, Pharmacopoeial codex and other technical documents related to standards for drugs. The present composition of ASU&H Pharmacopoeia committees is as below:

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| Chemistry:              | Principal Scientific Officer- Dr. S. C. Verma; Scientific Officer- Dr. Anupam Maurya  |
| Pharmacognosy:          | Principal Scientific Officer- Dr. Jayanthi A.; Scientific Officer- Dr. (Ms.) Nitin Rai; Research Assistant- Sh. Jeetendra Kumar Vaishya, Sh. Suman Halder |
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| Secretarial Assistance: | Sh. Pankaj Nagdeve, Sh. Afzal Hashmi, Sh. Sanjay Singh Rawat, Sh. Ashish Kumar, Sh. Sunil Dahiya  |

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In last, thanks are due to all those who have directly or indirectly contributed in the preparation of this volume.

## 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, *vaidya* by and large is no longer 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.

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.

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 Ayurvedic Pharmacopoeia Committee, (APC) constituted under the erstwhile Department of AYUSH (vide letter No. 5-5/CCRAS-2006/Tech/APC/Hqrs. dated 12<sup>th</sup> March, 2009) Ministry of Health and Family Welfare, Govt. of India initiated the exercise on present volume. This Pharmacopoeia Committee included Prof. S. S. Handa (Chairman), Dr. S. K. Sharma (Vice Chairman), Dr. G. S. Lavekar (Member Secretary until February 2010) and Dr. Ramesh Babu Devalla (Member Secretary) and other eminent experts in respective fields. The work was further carried out under auspices of PCIM&H and duly approved by its Governing Body.

## SPECIAL INTRODUCTION TO VOLUME IX

### (EXTRACTS)

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 *Saṅgraha* 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 practiced 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 "*Kaṣāya Kalpanā*". Five primary preparations, such as expressed juice (*Svarasa*), paste (*Kalka*), decoction (*Kvātha*), cold infusion (*Śīta Kaṣāya*) and hot infusion (*Phāṇṭa Kaṣāya*) 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*, *Āsava* etc.) & Pills (*Guṭīkā*) 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 *Saṅgraha* 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 *Rasaśāstra*. 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<sup>th</sup> century AD, but got established at the dawn of the 20<sup>th</sup> 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 *Vatī* and *Gutīkā* 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 *Āsava*) 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  $\beta$ -asarone from *Vacā* (*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, hydro-alcoholic extract and water extract have been prepared.

The work of preparing extracts using identical standard operating procedures was allotted to different extract manufacturers in two groups viz., Natural Remedies Pvt. Ltd. Bangalore, Chemolids Pvt. Ltd. Vijayawada and Green Chem Pvt. Ltd. Bangalore in one group and Sanat Product Pvt. Ltd. Bulendashar, Arjuna Products Pvt. Ltd, Kochi and Amsar Pvt. Ltd. Indore in other group. Both the groups are co-ordinated by Natural Remedies Pvt. Ltd. Bangalore. 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 5<sup>th</sup> Amendment Rule, 2010 under 158 (B) clause IV issues guidelines with respect to *Auṣadha 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 IX of the API, Part I, standards for aqueous and hydro-alcoholic extracts are presented. To start with, 15 traditionally well-known medicinal plants have been selected and this volume contains upgraded monographs on 15 source plants, their aqueous and hydro-alcoholic extracts, thus comprising a total of 45 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.

## ABBREVIATIONS FOR TECHNICAL TERMS

$^{\circ}\text{C}$	-	-	$^{\circ}$
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	-	-	<i>m</i>
meter(s)	-	-	m
micron (0.001mm)	-	-	$\mu$
microgram per milliliter	-	-	$\mu\text{g/ml}$
microliter	-	-	$\mu\text{l}$
micrometer	-	-	$\mu\text{m}$
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	-	-	<i>o</i>
para	-	-	<i>p</i>
parts per billion	-	-	ppb
parts per million	-	-	ppm
phloem fibres	-	-	phf
rate per minute	-	-	rpm

Reference Standard	-	-	<i>RS</i>
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	ड	ḌA	ḍa
आ	Ā	ā	ढ	ḌHA	ḍha
इ	I	i	ण	ṆA	ṇa
ई	Ī	ī	त	TA	ta
उ	U	u	थ	THA	tha
ऊ	Ū	ū	द	DA	da
ऋ	Ṛ	ṛ	ध	DHA	dha
ए	E	e	न	NA	na
ऐ	AI	ai	प	PA	pa
ओ	O	o	फ	PHA	pha
औ	AU	au	ब	BA	ba
·	Ṃ	ṃ	भ	BHA	bha
:	Ḥ	ḥ	म	MA	ma
क	KA	ka	य	YA	ya
ख	KHA	kha	र	RA	ra
ग	GA	ga	ल	LA	la
घ	GHA	gha	व	VA	va
ङ	ṆA	ṇa	श	ŚA	śa
च	CA	ca	ष	ṢA	ṣa
छ	CHA	cha	स	SA	sa
ज	JA	ja	ह	HA	ha
झ	JHA	jha	क्ष	KṢA	kṣa
ञ	ÑA	ña	त्र	TRA	tra
ट	ṬA	ṭa	ज्ञ	JÑA	jña
ठ	ṬHA	ṭha			

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## APĀMĀRGA

Apāmārga consists of dried whole plant of *Achyranthes aspera* L. (Fam. Amaranthaceae); a stiff, erect, 0.3-0.9 m high herb, found commonly as a weed throughout India up to 900 m. Apāmārga contains not less than 0.002 per cent of *oleanolic acid* when assayed.

**Synonyms:** Mayūraka, Pratyakpuṣpa, Kharamañjarī, Śikharī

### Other/Regional Language Names:

*Assamese:* Chirchita; *Bengali:* Apamg; *English:* Prickly Chaff Flower; *Gujarati:* Aghedo; *Hindi:* Chirchita, Latjira; *Kannada:* Uttarani; *Malayalam:* Katalati; *Marathi:* Aghada; *Punjabi:* Puthakanda; *Tamil:* Nayuruvi; *Telugu:* Uttarenu; *Urdu:* Chirchita

### Description:

#### a) Macroscopic:

**Root** - Cylindrical tap root, slightly ribbed, 0.1-1.0 cm in thickness, gradually tapering, rough due to presence of some root scars, secondary and tertiary roots present, yellowish-brown; odour, not distinct

**Stem** - 0.3-0.5 cm in cut pieces, yellowish-brown, erect, branched, cylindrical, hairy, solid, hollow when dry

**Leaf** - Simple, sessile, exstipulate, opposite, decussate, wavy margin, obovate, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs

**Flower** - Arranged in inflorescence of long spikes, greenish-white, numerous, sessile, bracteate with two bracteoles, one spine lipped, bisexual, actinomorphic, hypogynous; perianth segments 5, free, membranous, contorted or quincuncial, stamens 5, opposite the perianth lobes, connate forming a membranous tube-like structure, alternating with truncate and fimbriate staminodes, filament short; anther, two celled, dorsifixed; gynoecium bicarpellary, syncarpous; ovary superior, unilocular with single ovule; style single; stigma capitate

**Fruit** - Indehiscent, dry utricle enclosed within persistent perianth and bracteoles

**Seed** - Sub-cylindric, truncate at the apex, round at the base, endospermic, brown

#### b) Microscopic:

**Root** - Mature root shows 3-8 layered, rectangular, tangentially elongated, thin-walled cork cells; secondary cortex consisting of 6-9 layers, oval to rectangular, thin-walled, parenchymatous cells having a few scattered single or groups of stone cells; followed by 4-6 discontinuous rings of anomalous secondary thickening composed of vascular tissues; small patches of sieve tubes distinct in phloem parenchyma, demarcating the xylem rings; xylem composed of usual elements; vessels simple pitted; medullary rays 1-3 cells wide; small prismatic crystals of calcium oxalate present in cortical region and numerous in medullary rays

**Stem** - Young stem shows 6-10 prominent ridges, which diminish downwards upto the base where it becomes almost cylindrical; epidermis single layered, covered by thick cuticle having uniseriate, 2-5 celled, covering trichomes and glandular with globular head, 3-4 celled stalk; cortex 6-10 layered, composed of parenchymatous cells, most of them containing rosette crystals of calcium oxalate; in the ridges cortex collenchymatous; vascular bundles lie facing each ridge capped by pericyclic fibres; transverse section of mature stem shows lignified, thin-walled cork cells; pericycle a discontinuous ring of lignified fibres; vascular tissues show anomalous secondary growth having 4-6 incomplete rings of xylem and phloem; secondary phloem consisting of usual elements form incomplete rings; cambial strips present between secondary xylem and phloem; secondary xylem consists of vessels annular, spiral, scalariform and elongately pitted, fibres elongated, lignified; pith wide consisting of oval to polygonal, parenchymatous cells

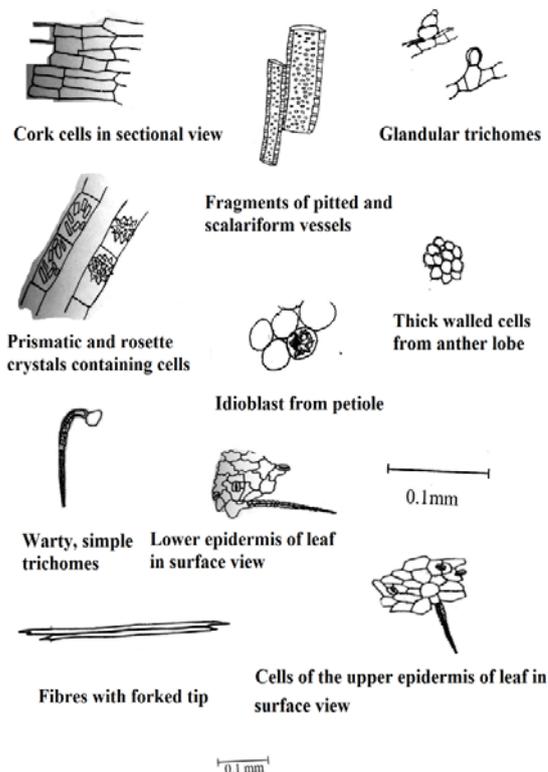
**Leaf** - Petiole - Shows crescent-shaped outline, having single-layered epidermis with thick cuticle; ground tissues consisting of thin-walled, parenchymatous cells containing rosette crystals of calcium oxalate; 4-5 vascular bundles situated in mid region

**Midrib** - Shows a single layered epidermis, on both surfaces; epidermis followed by 4-5 layered collenchyma on upper side and 2-3 layered on lower side; ground tissue consisting of thin-walled, parenchymatous cells having a number of vascular bundles; each vascular bundle shows below the xylem vessels, thin layers of cambium, followed by phloem and a pericycle represented by 2-3 layers of thick-walled, non-lignified cells; rosette crystals of calcium oxalate found scattered in ground tissues

**Lamina** - Shows single layered, tangentially elongated epidermal cells covered with thick cuticle having covering trichomes, similar to those of stem, on both surfaces; mesophyll differentiated into palisade and spongy parenchyma, palisade 2-4 layered of thick parenchyma; larger, slightly elongated in upper, while smaller and rectangular in lower surface; spongy parenchyma 3-5 layers thick, consisting of more or less isodiametric parenchymatous cells; idioblast containing large rosette crystals of calcium oxalate distributed in palisade and spongy parenchyma cells; stomata anisocytic and anomocytic, on both surfaces; stomatal index 4.5-9.0 on upper surface, 9.0-20.0 on lower surface; palisade ratio 7.0-11; vein islet number 7-13

c) *Powder*:

Powder shows simple, multicellular, sharp or blunt ended, warty or smooth trichomes; lower epidermal cells of leaf showing sinuous walls, and upper with fairly straight walls, glandular trichomes with globular head of 3 or 4 cells, anisocytic and anomocytic stomata, thick-walled and thin-walled fibres with sharp or forked ends; cork tissue, fragments of pitted vessels; prismatic and rosette and sandy crystals of calcium oxalate scattered as such throughout or in idioblasts (Fig. 1)



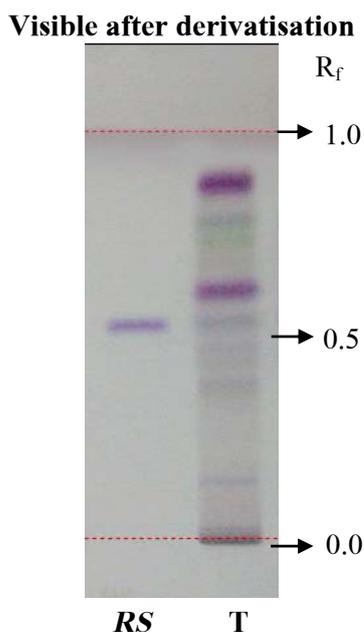
**Fig. 1: Powdered drug of APĀMĀRGA (*Achyranthes aspera* L.)**

**Identity, Purity and Strength:**

**Identification:**

*Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *oleanolic acid* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 per cent *methanol* (50 ml x 3) for a period of 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with *water*. Pass the combined *chloroform* extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 10 ml of *methanol*. *Standard solution*: Dissolve 10 mg of *oleanolic acid RS* in about 10 ml of *methanol*.



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

**Procedure:** Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate : toluene : formic acid* (45.0 : 0.5 : 0.1). Dry the plate in air. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

#### 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 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 2.0 per cent (Appendix 2.1.8); **Water-soluble extractive:** not less than 12.0 per cent (Appendix 2.1.9)

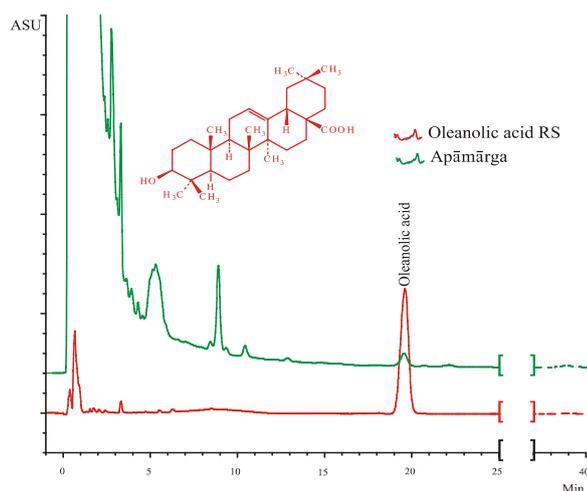
#### Other requirements:

**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 and reflux with 50 per cent methanol (50 ml x 3) on a water bath for 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100 ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with *water*, pass the combined *chloroform* extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 5 ml of *methanol*, transfer to a 10-ml volumetric flask and make up the volume. Filter through 0.42 µm membrane. **Standard solution:** Take about 10 mg, accurately weighed, *oleanolic acid RS* in a 100 ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (100 mm x 3.0 mm, 2.5 µm). **Mobile phase:** Filtered and degassed mixture of 33 volumes of *phosphate* buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, adding 1 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and 67 volumes of *acetonitrile*. **Injection volume:** 20 µl. **Flow rate:** 0.3 ml per min. **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 peak. Calculate the content of *oleanolic acid* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Apāmārga with Oleanolic acid as RS**

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standard:

API Oleanolic acid RS

**Constituents:** Oleanolic acid glycosides; ecdysterone, ecdysone, betaine, tritriacontanone, pentatriacontan-17-ol, 27-cyclohexylheptacosan-7-ol, 16-hydroxy-26-methylheptacosan-2-one, 4-methylheptatriacont-1-en-10-ol, estracontanol,  $\beta$ -sitosterol, pentatriacontane, pentatriacontan-6-one, hexatriacontane, triacontane, hentriacontane, octacosan-10-one, triacosan-4-one; lauric, myristic, palmitic, stearic, oleic, linoleic, arachidic and behenic acids

**Properties and Action:** Rasa: Kaṭu, Tikta; Guṇa: Sara, Tīkṣṇa; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Chedana, Kaphahara, Medohara, Pācana, Vātahara

**Important formulations:** Abhyā lavaṇa, Apāmārgakṣāra taila, Apāmārgakṣāra, Guḍapippalī, Jyotiṣmatī taila

**Therapeutic uses:** Apacī (chronic lymphadenopathy/scrofula), Arśa (piles), Kaṇḍū (pruritis), Medoroga (obesity), Śūla (pain), Udararoga (disease of abdomen)

**Dose:** Cūrṇa (powder): 3-6 g

## APĀMĀRGA HYDRO-ALCOHOLIC EXTRACT

Apāmārga Hydro-alcoholic Extract is a dried and powdered extract prepared from Apāmārga (appropriately powdered). The extract contains not less than 0.08 per cent of *oleanolic acid* when assayed.

### Method of Preparation:

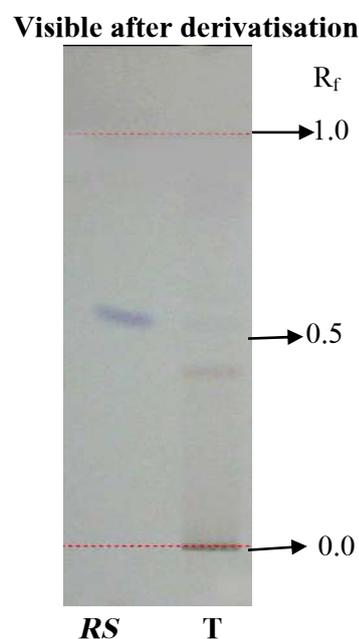
Take Apāmārga suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 7 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *oleanolic acid* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 per cent *methanol* (50 ml x 3) for a period of 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with *water*. Pass the combined *chloroform* extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 10 ml of *methanol*. *Standard solution*: Dissolve 10 mg of *oleanolic acid* RS in about 10 ml of

*methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *toluene* : *formic acid* (45.0 : 0.5 : 0.1). Dry the plate in air. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Apāmārga hydro-alcoholic extract**  
**RS: Oleanolic 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-6.5 (Appendix 2.1.10); *Total soluble solids*: not less than 90.0 per cent (Appendix 2.1.11) (Method-I)

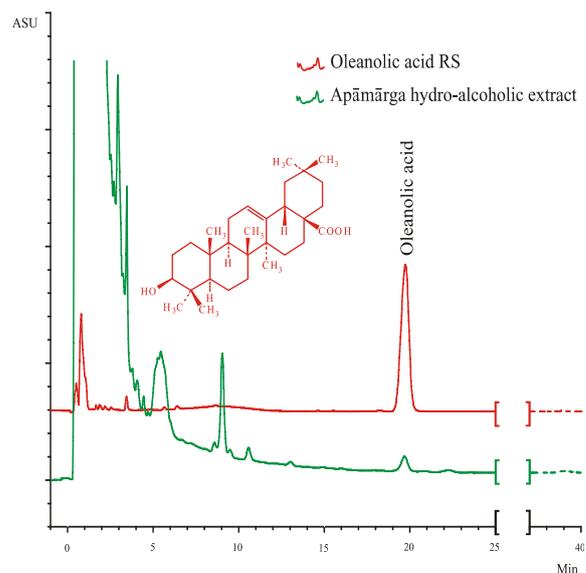
**Other requirements:**

**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 and reflux with 50 per cent *methanol* (50 ml x 3) on a water bath for 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with *water*, pass the combined *chloroform* extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 5 ml of *methanol*, transfer to a 10-ml volumetric flask and make up the volume. Filter through 0.42 µm membrane. **Standard solution:** Take about 10 mg, accurately weighed, *oleanolic acid RS* in a 100 ml flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (100 mm x 3.0 mm, 2.5 µm). **Mobile phase:** Filtered and degassed mixture of 33 volumes of *phosphate* buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, adding 1 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and 67 volumes of *acetonitrile*. **Injection volume:** 20 µl. **Flow rate:** 0.3 ml per min. **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 peak. Calculate the content of *oleanolic acid* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Apāmārga hydro-alcoholic extract with *Oleanolic acid* as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Oleanolic acid RS*

## APĀMĀRGA WATER EXTRACT

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

### Method of Preparation:

Take Apāmārga 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<sup>0</sup> 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 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 9 per cent.

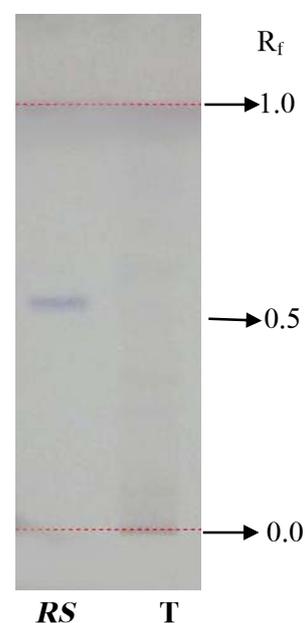
### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *oleanolic acid* as a reference standard. **Test solution:** Extract 2 g of substance by refluxing with 50 per cent *methanol* (50 ml x 3) for a period of 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with water. Pass the combined chloroform extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 10 ml of *methanol*. **Standard solution:** Dissolve 10 mg of *oleanolic acid* RS in about 10 ml of *methanol*. **Procedure:** Apply 10 μl each of the test

and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *ethyl acetate* : *toluene* : *formic acid* (45.0 : 0.5 : 0.1). Dry the plate in air. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).

#### Visible after derivatisation



**Fig. 1: Thin-Layer Chromatogram of Apāmārga water extract**  
**RS: Oleanolic 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 25.0 per cent (Appendix 2.1.5); **Acid-insoluble ash:** not more than 2.0 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-II)

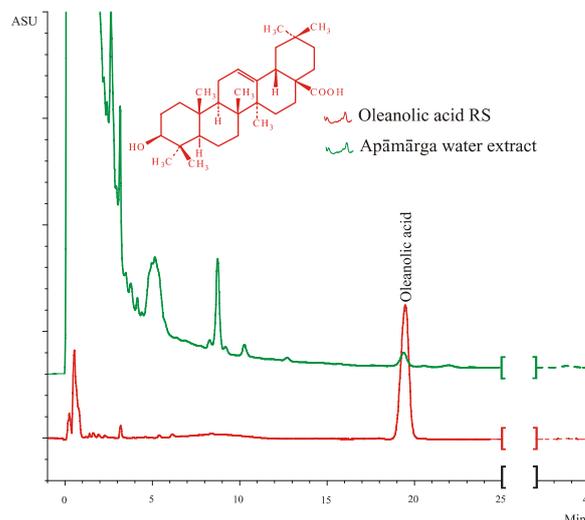
**Other requirements:**

*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 and reflux with 50 per cent *methanol* (50 ml x 3) on a water bath for 30 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100 ml round bottomed flask. Add 20 ml of 2 M *methanolic hydrochloric acid* and reflux at 60-70<sup>0</sup> on a water bath for 3 hours, cool and transfer the solution to a separating flask, extract with *chloroform* (25 ml x 3). Combine all the organic extracts and wash gently with *water*, pass the combined *chloroform* extract through *anhydrous sodium sulphate* and evaporate the *chloroform* under vacuum. Dissolve the residue obtained in 5 ml of *methanol*, transfer to a 10 ml volumetric flask and make up the volume. Filter through 0.42 µm membrane. *Standard solution:* Take about 10 mg, accurately weighed, *oleanolic acid RS* in a 100-ml volumetric flask and dissolve in 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *Chromatographic system:* High performance liquid chromatography. *Column and stationary phase:* C18 (100 mm x 3.0 mm, 2.5 µm). *Mobile phase:* Filtered and degassed mixture of 33 volumes of *phosphate buffer* (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 900 ml of *water*, adding 1 ml of *orthophosphoric acid* and making up the volume to 1000 ml) and 67 volumes of *acetonitrile*. *Injection volume:* 20 µl. *Flow rate:* 0.3 ml per min. *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 peak. Calculate the content of *oleanolic acid* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Apāmārga water extract with *Oleanolic acid* as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Oleanolic acid RS*

## ASANA

Asana consists of heart-wood of *Pterocarpus marsupium* Roxb. (Fam. Fabaceae); a moderate to large sized, deciduous tree, upto 30 m high and 2.5 m in girth, with straight clear bole, found mostly throughout Gujarat, Madhya Pradesh, Bihar and Orissa. Asana contains not less than 0.25 per cent of *pterostilbene* when assayed.

**Synonyms:** Bījaka, Pītasāra, Asanaka, Bijasāra

### Other/Regional Language Names:

*Assamese:* Aajar; *Bengali:* Piyasala, Pitasala; *English:* Indian Kino Tree; *Gujarati:* Biyo; *Hindi:* Vijayasara, Bija; *Kannada:* Bijasara, Asana; *Kashmiri:* Lal chandeur; *Malayalam:* Venga; *Marathi:* Bibala; *Oriya:* Piashala; *Punjabi:* Chandan Lal, Channanlala; *Tamil:* Vengai; *Telugu:* Yegi, Vegisa; *Urdu:* Bijasar

### Description:

#### a) Macroscopic:

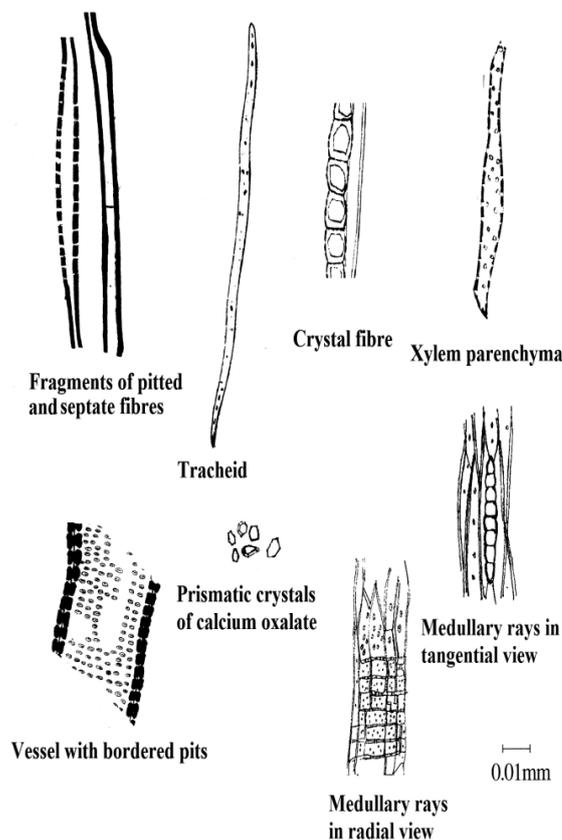
Drug occurs as irregular pieces of variable size and thickness, golden yellowish brown with darker streaks, on soaking in water gives yellow colour solution with blue fluorescence; fracture strong, tough, very hard, moderately heavy difficult to break but brittle; taste astringent.

#### b) Microscopic:

Transverse section shows alternating bands of larger and smaller polygonal cells consisting of tracheids, fibre tracheids, xylem parenchyma and traversed by xylem rays, numerous xylem vessels distributed throughout, in singles or in groups of 2-3, showing tyloses in isolated preparations, vessels drum or barrel shaped with well-marked perforation rims and bordered pits, tracheids numerous, long, thick-walled with tapering ends and simple pits, fibre tracheids elongated, thick-walled with narrow lumen and simple pits, xylem parenchyma rectangular with simple pits, paratracheal surrounding vessels, xylem rays uni to biseriate, 3-5-7 cells high, prismatic crystals of calcium oxalate present in crystal fibres, starch absent

#### c) Powder:

Powder shows vessels with bordered pits, fibre tracheids, tracheids, fragments of xylem rays and few crystal fibres, starch absent (Fig. 1).



**Fig. 1: Powdered drug of ASANA** { $\times e$  "ASANA"} (*Pterocarpus marsupium* Roxb.)

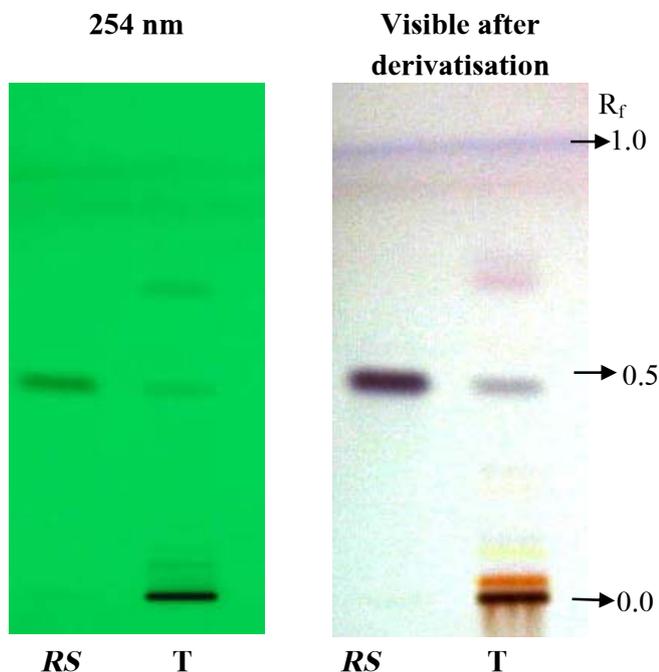
### Identity, Purity and Strength:

#### Identification:

##### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *pterostilbene* as a reference standard. *Test solution:* Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 10 mg of *pterostilbene RS* in about 10 ml of *methanol*. *Procedure:* Apply 10  $\mu$ l

each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *ethyl acetate* : *n-hexane* (5.5 : 4.5).



**Fig. 2: Thin-Layer Chromatogram of Asana**  
**RS: *Pterostilbene*, T: Test solution**

Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

#### 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 2.0 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 7.0 per cent (Appendix 2.1.8); *Water-soluble extractive*: not less than 5.0 per cent (Appendix 2.1.9)

#### 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)

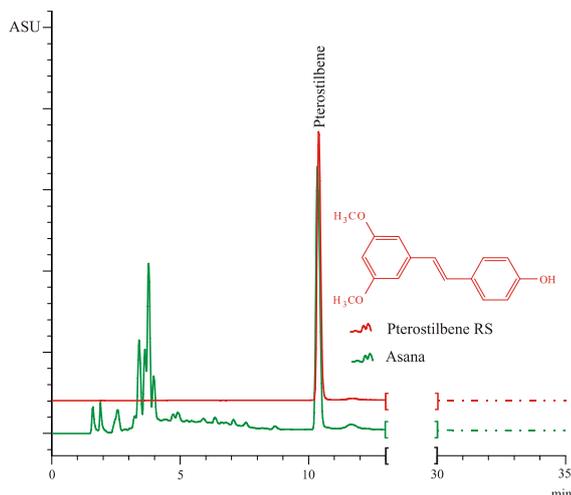
#### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume with *methanol*. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *pterostilbene RS* in a 100 ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
5	45	55
10	35	65
20	35	65
25	45	55
30	85	15
35	85	15

*Injection volume*: 20 µl. *Flow rate*: 1.5 ml per min. *Detection*: UV 320 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 peak. Calculate the content of *pterostilbene* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Asana with *Pterostilbene* as RS**

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standard:**

API *Pterostilbene* RS

**Constituents:** Pterostilbene, marsupsin, liquiritigenin, isoliquiritigenin, pterosupin, *p*-hydroxybenzaldehyde, 7,4'-dihydroxyflavone, (2R)-3-(*p*-hydroxyphenyl) lactic acid, propterol, marsupol, carpusin

**Properties and Action:** Rasa: Kaṭu, Tikta, Kaṣāya; Guṇa: Laghu, Rūkṣa; Vīrya: Śīta; Vipāka: Kaṭu; Karma: Kaphamedoviśoṣaṇa, Keśya, Kuṣṭhaghna, Raktaśodhana, Rasāyana, Stambhana, Tvacya

**Important formulations:** Asanabilvādi taila, Nyagrodhādi cūrṇa

**Therapeutic uses:** Pāṇḍu (anaemia), Prameha (increased frequency and turbidity of urine)

**Dose:** Cūrṇa (powder): 3-6 g

## ASANA HYDRO-ALCOHOLIC EXTRACT

Asana Hydro-alcoholic Extract is a dried and powdered extract prepared from Asana (appropriately powdered). The extract contains not less than 0.5 per cent of *pterostilbene* when assayed.

### Method of preparation:

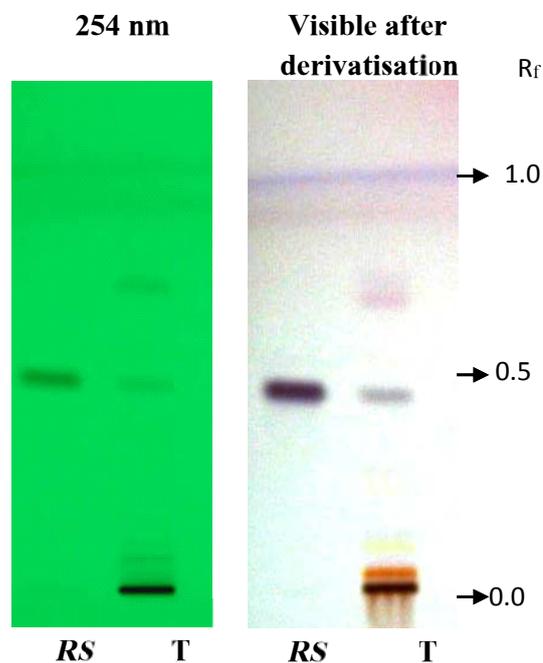
Take Asana suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 µm pore size) to a suitable sized 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 6 per cent.

### Identity, Purity and Strength:

#### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *pterostilbene* as a reference standard. *Test solution:* Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 10 mg of *pterostilbene RS* in about 10 ml of *methanol*. *Procedure:* Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *ethyl acetate : n-hexane* (5.5 : 4.5). Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution

shows a band corresponding to that of the standard solution (Fig. 1).



**RS: Pterostilbene, T: Test solution**

**Fig. 1: Thin-Layer Chromatogram of Asana hydro-alcoholic extract**

### Quantitative parameters:

*Loss on drying:* not more than 7.0 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 2.0 per cent (Appendix 2.1.7); *pH:* 4.0-7.0 (Appendix 2.1.10); *Total soluble solids:* not less than 85.0 per cent (Appendix 2.1.11) (Method-I)

### Other requirements:

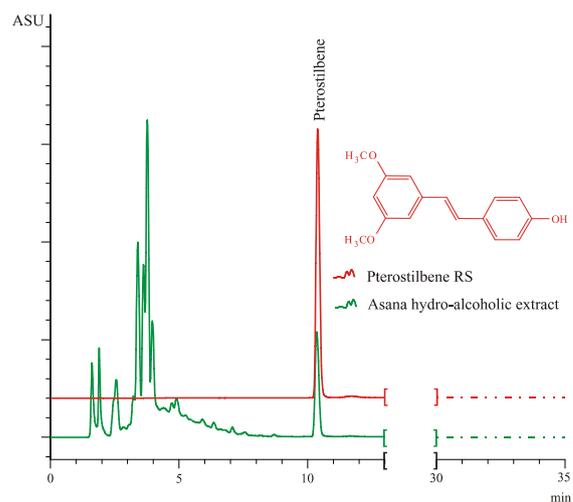
*Heavy metals:* Complies with the prescribed limits, (Appendix 3.1); *Microbial contamination:* Complies with the prescribed limits, (Appendix 3.2); *Pesticide residues:* Complies with the prescribed limits, (Appendix 3.3); *Residual solvent:* Complies with the prescribed limits, (Appendix 3.8); *Aflatoxins:* 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 and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume with *methanol*. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *pterostilbene RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
5	45	55
10	35	65
20	35	65
25	45	55
30	85	15
35	85	15

*Injection volume*: 20 µl. *Flow rate*: 1.5 ml per min. *Detection*: UV 320 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 peak. Calculate the content of *pterostilbene* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Asana hydro-alcoholic extract with *Pterostilbene* as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Pterostilbene RS*

## ASANA WATER EXTRACT

Asana Water Extract is a dried and powdered extract prepared from Asana. The extract contains not less than 0.1 per cent of *pterostilbene* when assayed.

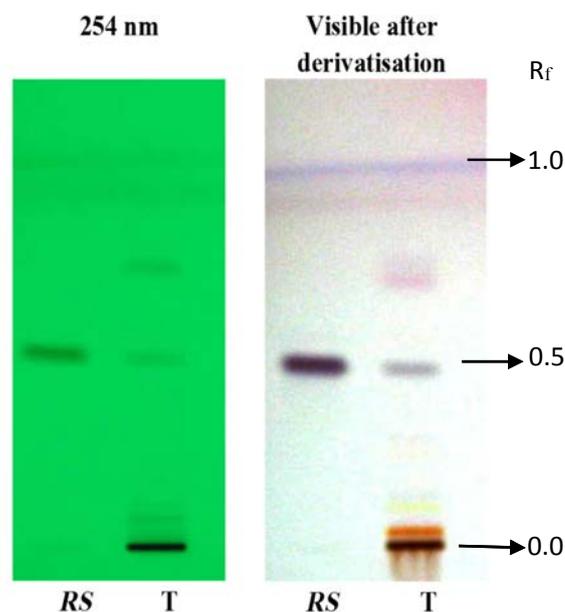
### Method of preparation:

Take Asana 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 µm mesh and pack. The yield obtained is about 8 per cent.

### Identity, Purity and Strength:

#### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *pterostilbene* as a reference standard. *Test solution:* Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10–15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 10 mg of *pterostilbene* RS in about 10 ml of *methanol*. *Procedure:* Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *ethyl acetate* : *n-hexane* (5.5 : 4.5). Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Asana water extract**

**RS: *Pterostilbene*, T: Test solution**

### Quantitative parameters:

*Loss on drying:* not more than 7.0 per cent (Appendix 2.1.4); *Total ash:* not more than 8.0 per cent (Appendix 2.1.5); *Acid-insoluble ash:* not more than 2.0 per cent (Appendix 2.1.7); *pH:* 4.0–7.0 (Appendix 2.1.10); *Total soluble solids:* not less than 65.0 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)

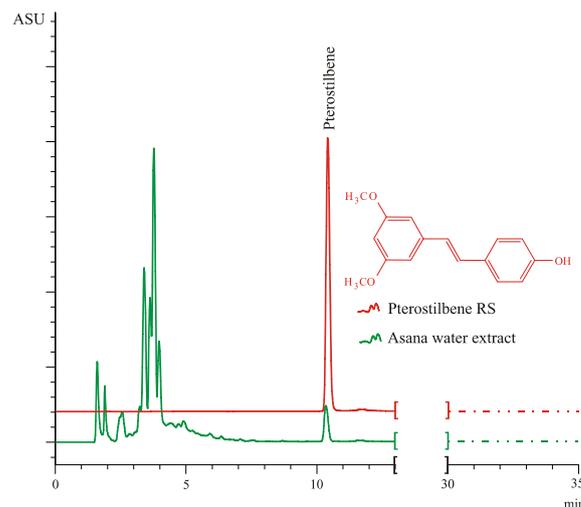
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution:* Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter.

Combine all the filtrates and transfer to a 100 ml volumetric flask and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 10 mg, accurately weighed, *pterostilbene RS* in a 100 ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
5	45	55
10	35	65
20	35	65
25	45	55
30	85	15
35	85	15

**Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 320 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *pterostilbene* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Asana water extract with *Pterostilbene* as RS**

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standard:**

API *Pterostilbene RS*

## DĀRUHARIDRĀ

Dāruharidrā consists of dried stem of *Berberis aristata* DC. (Fam. Berberidaceae); an erect, spinous, deciduous shrub, usually 1.8-3.6 m in height found in the Himalayan ranges at an elevation of 1000-3000 m, and in the Nilgiri hills in south India. Dāruharidrā contains not less than 0.4 per cent of *berberine* when assayed.

**Synonyms:** Kaṭaṅkaṭerī, Dārvī

### Other/Regional Language Names:

*Bengali:* Daruharidra; *English:* Indian Berberry; *Gujarati:* Daruharidra, Daruhuladur; *Hindi:* Daruhaldi, Darhald; *Kannada:* Maradarishana, Maradrishina, Daruhaldi; *Kashmiri:* Ras ashud, Rasvat; *Malayalam:* Maramannal, Maramanjali; *Marathi:* Daruhalad; *Oriya:* Daruharidra, Daruhalidi; *Punjabi:* Sumalu; *Tamil:* Gangeti, Varatiu manjal; *Telugu:* Manupasupu; *Urdu:* Darhald

### Description:

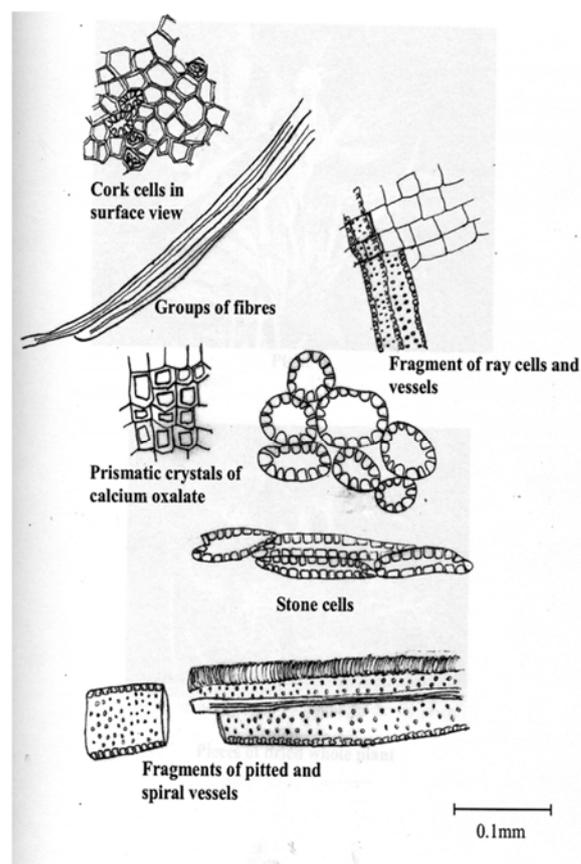
#### a) Macroscopic:

Drug available in pieces of variable length and thickness, bark about 0.4-0.8 cm thick, pale yellowish-brown, soft, closely and rather deeply furrowed, rough, brittle, xylem portion yellow, more or less hard, radiate with xylem rays, pith mostly absent, when present small, yellowish-brown when dried, fracture short in bark region, splintery in wood; taste bitter

#### b) Microscopic:

**Stem** - Shows rhytidoma with cork consisting of 3-45 rectangular and squarish, yellow coloured, thin-walled cells, arranged radially; sieve elements irregular in shape, thin walled, a few cells containing yellowish-brown contents; phloem fibres arranged in tangential rows, consisting of 1-4 cells, each fibre short thick-walled, spindle-shaped, lignified having wide lumen; half inner portion of rhytidoma traversed by secondary phloem rays; phloem rays run obliquely consisting of radially elongated parenchymatous cells, almost all phloem ray cells having single prismatic crystals of calcium oxalate, a few cells of rhytidoma also contain

prismatic crystals of calcium oxalate; stone cells also found scattered in phloem ray cells in groups, rarely single, mostly elongated, a few rounded; secondary phloem, a broad zone, consisting of sieve elements and phloem fibres, traversed by multiseriate phloem rays; sieve elements arranged in tangential bands and tangentially compressed cells alternating with single to five rows of phloem fibres, secondary xylem broad consisting of xylem vessels, tracheids, xylem fibres and traversed by multi seriate xylem rays; xylem vessels numerous, small to medium sized, distributed throughout xylem region in groups or in singles, groups of vessels usually arranged radially; isolated vessels cylindrical with rounded or projected at one or both ends with spiral thickenings; fibres numerous, lignified, large, thick-walled with wide lumen, and pointed tips; xylem rays quite distinct, straight, multiseriate, consisting of radially arranged rectangular cells, each ray 30-53 cells high, 8-12 cells wide, a few ray cells containing brown contents



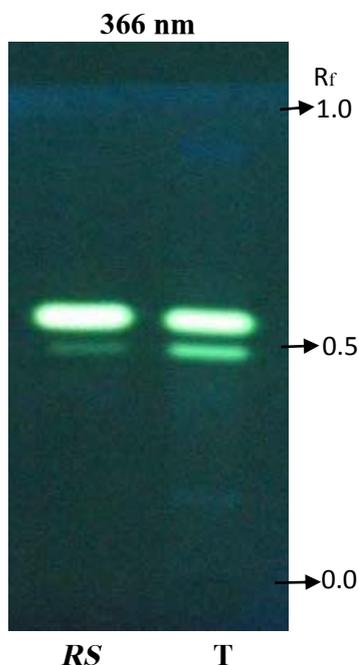
**Fig. 1: Powdered drug of DĀRUHARIDRĀ  
(*Berberis aristata* DC.)**

c) *Powder*:

Fine powder shows mostly fragments of cork cells, yellow coloured phloem fibres entire or in pieces, stone cells in singles or in groups, numerous prismatic crystals of calcium oxalate, xylem vessels having spiral thickenings, thick-walled, lignified xylem fibres and ray cells (Fig. 1)

**Identity, Purity and Strength:****Identification:***Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *berberine chloride* as a reference standard.



**Fig. 2: Thin-Layer Chromatogram of Dāruharidrā**

**RS: Berberine chloride, T: Test solution**

*Test solution:* Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 10 mg of *berberine chloride RS* in about 100 ml of *methanol*. *Procedure:* Apply 10  $\mu$ l each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *n-butanol* : *glacial acetic*

*acid* : *Water* (6.5 : 1.5 : 2.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

**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 14.0 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 6.0 per cent (Appendix 2.1.8); *Water-soluble extractive:* not less than 8.0 per cent (Appendix 2.1.9)

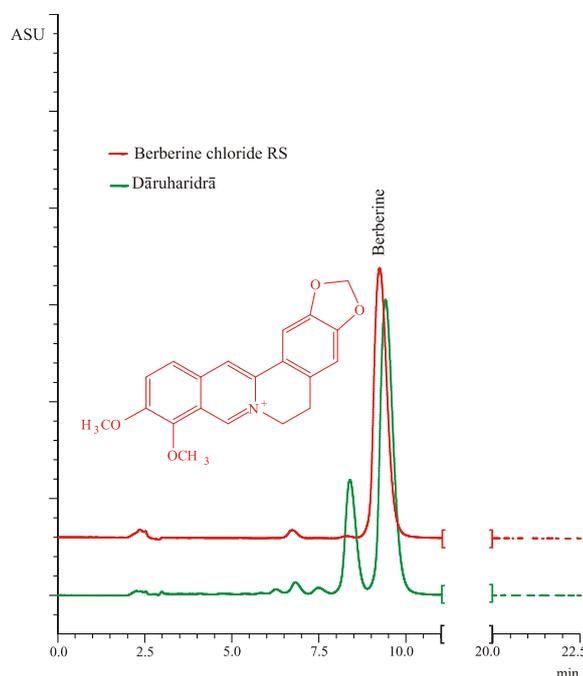
**Other requirements:**

*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 and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume with *methanol*. Dilute the solution to match the standard concentration. Filter through 0.42  $\mu$ m membrane. *Standard solution:* Take about 10 mg, accurately weighed, *berberine chloride RS* in a 100 ml volumetric flask and dissolve in about 25 ml of *methanol* and make up the volume with *methanol*. Dilute 5 ml of this solution to 25 ml. Filter through 0.42  $\mu$ m membrane. *Chromatographic system:* High performance liquid chromatography. *Column and stationary phase:* Silica CN (250 mm x 4.6 mm, 5  $\mu$ m). *Mobile phase:* Filtered and degassed mixture of

35 volumes of *acetonitrile* and 65 volumes of *water* containing 0.3 per cent w/v of *orthophosphoric acid*. *Injection volume*: 20  $\mu$ l. *Flow rate*: 1.5 ml per min. *Detection*: UV 346 nm. *Procedure*: Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *berberine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Dāruharidrā with *Berberine chloride* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Berberine chloride* RS

**Constituents:** Berberine, palmatine, oxyberberine, oxyacanthine, karachine.

**Properties and Action:** Rasa: Tikta; Guṇa: Rūkṣa, Laghu; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Doṣapācana, Stanyaśodhana, Stanyadoṣahara

### Important formulations:

Aśvagandhādyariṣṭa, Bhṛṅgarāja taila, Jātyādi taila, Khadirādi guṭika, Khadirāriṣṭa, Triphalā ghr̥ta

### Therapeutic uses:

Āmātisāra (diarrhoea due to indigestion), Kaṇḍū (pruritis), Kapharoga (disease due to kapha doṣa), Karṇaroga (disease of ear), Medoroga (obesity), Mukharoga (disease of mouth), Netraroga (disease of eye), Prameha (increased frequency and turbidity of urine), Ūrustambha (stiffness in thigh muscles), Vraṇa (wound)

**Dose:** Cūrṇa (powder): 3-6 g

## DĀRUHARIDRĀ HYDRO-ALCOHOLIC EXTRACT

Dāruharidrā Hydro-alcoholic Extract is a dried and powdered extract prepared from Dāruharidrā (appropriately powdered). The extract contains not less than 6 per cent of *berberine* when assayed.

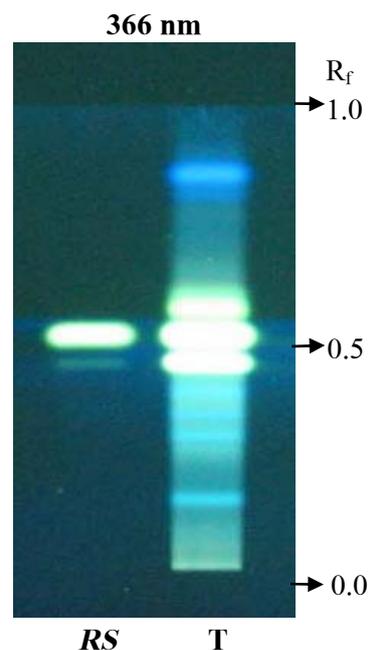
### Method of preparation:

Take Dāruharidrā 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<sup>0</sup> 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<sup>0</sup> 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *berberine chloride* as a reference standard. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *berberine chloride* RS in about 100 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *n-butanol* : *glacial acetic acid* : *Water* (6.5 : 1.5 : 2.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Dāruharidrā hydro-alcoholic extract**  
**RS: Berberine chloride T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 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 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter.

Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume with *methanol*. Dilute the solution to match the standard concentration. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 10 mg, accurately weighed, *berberine chloride RS* in a 100-ml volumetric flask and dissolve in about 25 ml of *methanol* and make up the volume with *methanol*. Dilute 5 ml of this solution to 25 ml. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** Silica CN (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed mixture of 35 volumes of *acetonitrile* and 65 volumes of *water* containing 0.3 per cent w/v of *orthophosphoric acid*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 346 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak.

Calculate the content of *berberine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

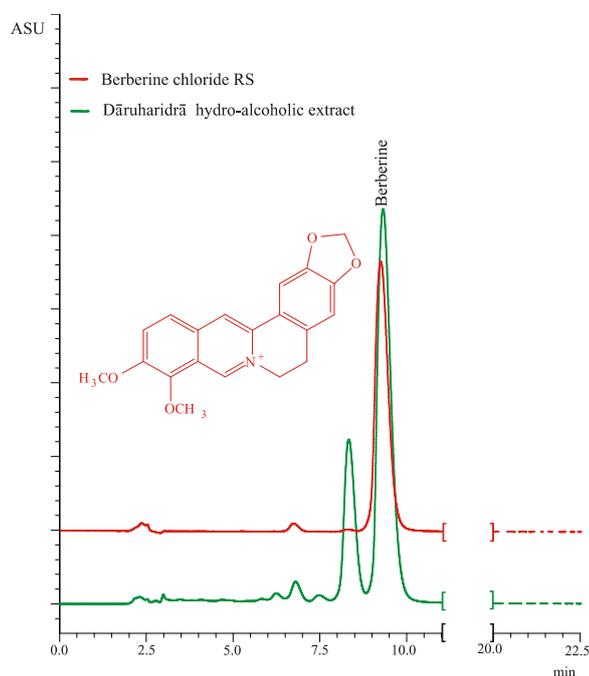
**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Berberine chloride RS*



**Fig. 2: HPLC chromatogram of Dāruharidrā hydro-alcoholic extract with *Berberine chloride* as *RS***

## DĀRUHARIDRĀ WATER EXTRACT

Dāruharidrā Water Extract is a dried and powdered extract prepared from Dāruharidrā. The extract contains not less than 4 per cent of *berberine* when assayed.

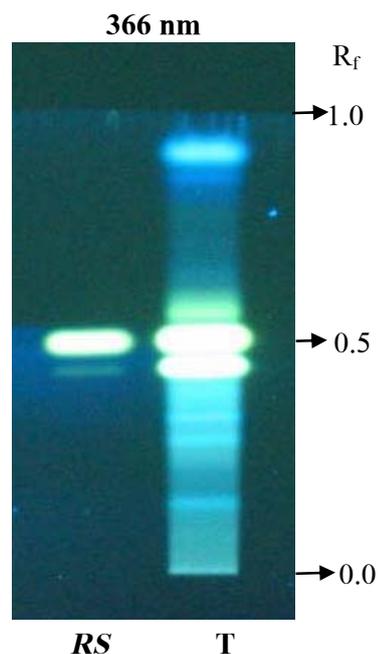
### Method of preparation:

Take Dāruharidrā 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<sup>0</sup> 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<sup>0</sup> 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *berberine chloride* as a reference standard. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10–15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *berberine chloride* RS in about 100 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *n-butanol* : *glacial acetic acid* : *Water* (6.5 : 1.5 : 2.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Dāruharidrā water extract**  
**RS: Berberine chloride, T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 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-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 and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100 ml

volumetric flask and make up the volume with *methanol*. Dilute the solution to match the standard concentration. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 10 mg, accurately weighed, *berberine chloride RS* in a 100 ml volumetric flask and dissolve in about 25 ml of *methanol* and make up the volume with *methanol*. Dilute 5 ml of this solution to 25 ml. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** Silica CN (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed mixture of 35 volumes of *acetonitrile* and 65 volumes of *water* containing 0.3 per cent w/v of *orthophosphoric acid*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 346 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak.

Calculate the content of *berberine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

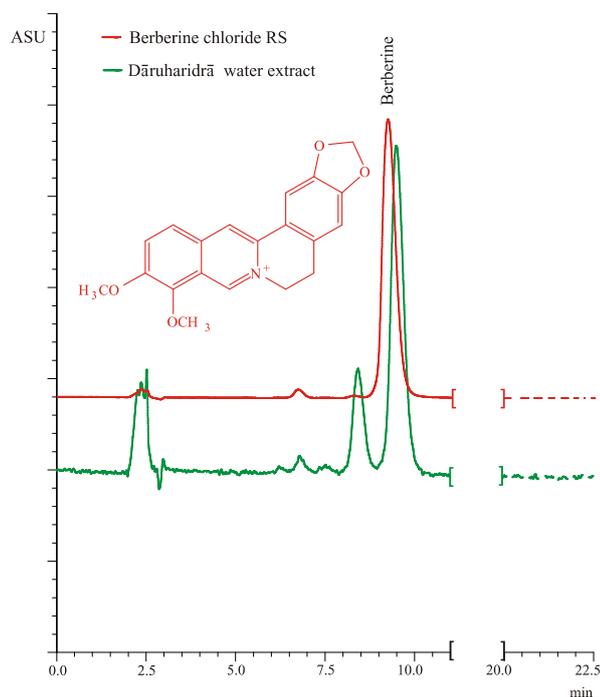
#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standard:

API *Berberine chloride RS*



**Fig. 2:** HPLC chromatogram of *Dāruharidrā* water extract with *Berberine chloride* as *RS*

## DHĀRĀ VṚKṢĀMLA

Dhārā Vṛkṣāmla consists of dried fruit of *Garcinia gummi-gutta* (L.) Rob. syn. *Garcinia cambogia* (Gaertn.) Desr. (Fam. Clusiaceae); a small tree, common in evergreen forests of Western ghats, from Konkan southwards to Travancore, and in the Shola forests of the Nilgiris up to an altitude of 1800 m. Dhārā Vṛkṣāmla contains not less than 5 per cent of *hydroxycitric acid* and not less than 5 per cent of *lactone* when assayed.

**Synonym:** Kṣīrī Vṛkṣāmla

**Regional Language Names:** English: Malabar Tamarind, Kokum, Butter Tree; Gujarati: Kokam, Kokan; *Hindi*: Kokam; *Kannada*: Murgin huli, Murgala; *Malayalam*: Panampuli; *Marathi*: Kokam, Ratamba, Amsol, Amsul, Ratambi; Oriya: Raktasrava; *Tamil*: Kodukkappuli; *Telugu*: Vrksamta, Simachinta

### Description:

#### a) Macroscopic:

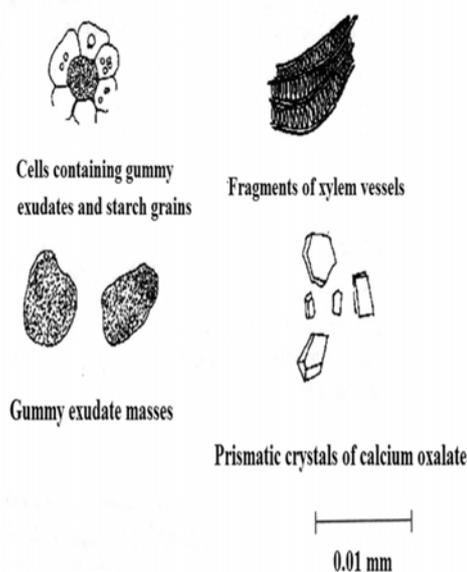
Fruits are ovoid, yellow or red when ripe and become black when dried. 6-8 grooves are seen up to the middle. Dried pieces of drug consists of longitudinal fragments of pericarp of various size and shapes strongly inwardly curved, boat or half moon shaped, dark brownish black, wrinkled irregularly and internally smooth. Odour characteristic, taste sour, astringent and slightly bitter

#### b) Microscopic:

TS of pericarp shows a layer of epicarp, composed of rectangular to tangentially elongated cells covered externally with thin cuticle; mesocarp very wide composed of 100 to 150 rows of parenchymatous cells of various size and shape which possess simple and compound starch grains and prismatic crystals of calcium oxalate; vascular bundles consists of phloem and xylem with spiral vessels, rectangular to irregular shaped parenchyma cells, traversing throughout the mesocarp but more prominently in inner zone of pericarp

#### c) Powder:

Shows isolated cells of mesocarp, containing dark reddish brown gummy exudates, prismatic crystals of calcium oxalate and starch grains; fragments of longitudinally cut spiral and annular vessels (Fig. 1)



**Fig. 1: Powdered drug of Dhārā Vṛkṣāmla (*Garcinia gummi-gutta* (L.) Rob.)**

### Identity, Purity and Strength:

#### Identification:

##### High performance liquid chromatography:

Carry out *liquid chromatography* (Appendix 3.6) using (-)-*hydroxycitric acid lactone* and *calcium (-)-hydroxycitrate* as a reference standards. Test solution, Standard solution, Chromatographic system, Mobile phase, Injection volume, Detection and Procedure follow as mentioned under Assay. The chromatogram obtained with test solution shows peaks corresponding to the retention time of (-)-*hydroxycitric acid lactone* and (-)-*hydroxycitric acid* (Fig. 2).

#### Quantitative parameters:

*Foreign matter*: not more than 2.0 per cent (Appendix 2.1.3); *Loss on drying*: not more than 20.0 per cent (Appendix 2.1.4); *Total ash*: not more

than 8.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 1.5 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 35 per cent (Appendix 2.1.9)

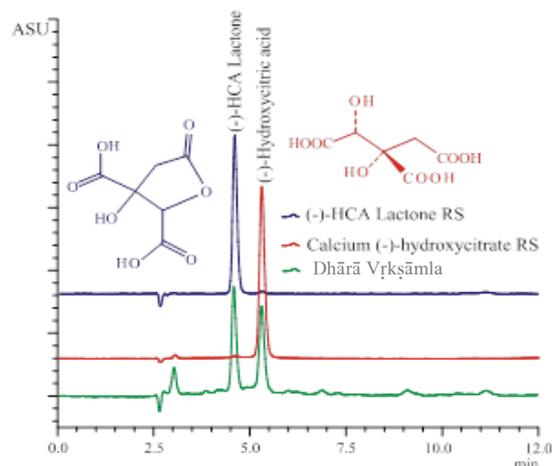
### Other requirements:

*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 0.5 g, accurately weighed, of the substance being examined and reflux with 25 ml of *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution) on a water bath for 10 min and transfer to a 50-ml volumetric flask. Cool, filter and make up the volume with *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution). Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, (-)-hydroxycitric acid lactone RS and 50 mg of calcium (-)-hydroxycitrate RS in a 25-ml volumetric flask and dissolve in about 10 ml of *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution) and make up the volume with *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution). Filter through 0.42 µm membrane. *Chromatographic system*: High performance liquid chromatography. *Column and stationary phase*: C18 (250 mm x 4.6 mm, 5 µm). *Mobile phase*: Filtered and degassed 0.05 M sodium sulphate in *water* (adjusted to *pH* 2.3 with *sulphuric acid* solution). *Injection volume*: 20 µl. *Flow rate*: 1.0 ml per min. *Detection*: UV 210 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, identify the analyte peak using relative retention time. The relative retention time for (-)-hydroxycitric acid lactone is 1 and for (-)-hydroxycitric acid is about 1.1. Calculate the contents of (-)-hydroxycitric acid lactone and (-)-hydroxycitric

*acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Dhārā Vṛkṣāmla with (-)-Hydroxycitric acid lactone and Calcium (-)-hydroxycitrate as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API (-)-Hydroxycitric acid lactone RS and Calcium (-)-hydroxycitrate RS

**Constituents:** (-)-Hydroxycitric acid, (-)-hydroxycitric acid lactone, citric acid, tartaric acid

**Properties and Action:** Rasa: Amla; Guṇa: Laghu, Rūkṣa; Vīrya: Uṣṇa; Vipāka: Amla; Karma: Arśoghna, Dīpana, Kapha-vātahara, Rucya, Sandhānīya, Śūlaghna, Tṛṣṇānigrahaṇa

**Therapeutic uses:** Agnimāndya (digestive impairment), Arśa (piles), Gulma (abdominal lump), Śūla (pain), Vibandha (constipation)

**Dose:** Cūrṇa (powder): 3-6 g

## DHĀRĀ VR̥KṢĀMLA HYDRO-ALCOHOLIC EXTRACT

Dhārā Vr̥kṣāmla Hydro-alcoholic Extract is a dried and powdered extract prepared from Dhārā Vr̥kṣāmla (appropriately powdered). The extract contains not less than 3 per cent of (-)-hydroxycitric acid and not less than 14 per cent of (-)-hydroxycitric acid lactone when assayed.

### Method of Preparation:

Take Dhārā Vr̥kṣāmla (Powder) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85° for 3-4 hours. Filter the extract through an appropriate 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 80° till the moisture is less than 7 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 38 per cent.

### Identity, Purity and Strength:

#### High performance liquid chromatography:

Carry out *liquid chromatography* (Appendix 3.6) using (-)-hydroxycitric acid lactone and calcium (-)-hydroxycitrate as a reference standards. Test solution, Standard solution, Chromatographic system, Mobile phase, Injection volume, Detection and Procedure follow as mentioned under Assay. The chromatogram obtained with test solution shows peaks corresponding to the retention time of (-)-hydroxycitric acid lactone and (-)-hydroxycitric acid (Fig. 1).

### 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 2.0 per cent (Appendix 2.1.7); *pH*: 2.0-3.5 (Appendix 2.1.10); *Total soluble solids*: not less than 90.0 per cent (Appendix 2.1.11) (Method-I)

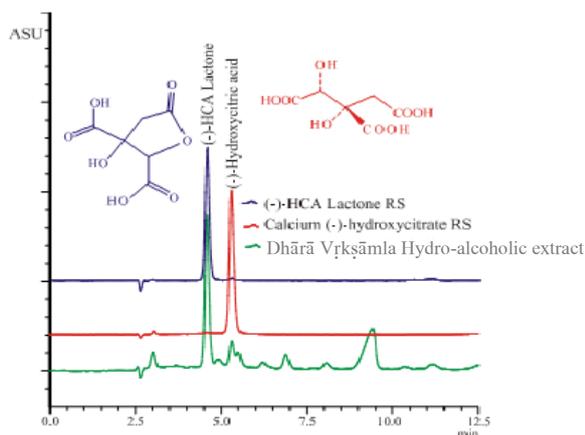
### Other requirements:

*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 0.5 g, accurately weighed, of the substance being examined and reflux with 25 ml of *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution) on a water bath for 10 min and transfer to a 50-ml volumetric flask. Cool, filter and make up the volume with *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution). Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, (-)-hydroxycitric acid lactone RS and 50 mg of calcium (-)-hydroxycitrate RS in a 25-ml volumetric flask and dissolve in about 10 ml of *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution) and make up the volume with *water* (adjusted to *pH* 2.1 with *sulphuric acid* solution). Filter through 0.42 µm membrane. *Chromatographic system*: High performance liquid chromatography. *Column and stationary phase*: C18 (250 mm x 4.6 mm, 5 µm). *Mobile phase*: Filtered and degassed 0.05 M *sodium sulphate* in *water* (adjusted to *pH* 2.3 with *sulphuric acid* solution). *Injection volume*: 20 µl. *Flow rate*: 1.0 ml per min. *Detection*: UV 210 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, identify the analyte peak using relative retention time. The relative retention time for (-)-hydroxycitric acid lactone is 1 and for (-)-

*hydroxycitric acid* is about 1.1. Calculate the contents of (-)-*hydroxycitric acid lactone* and (-)-*hydroxycitric acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 1: HPLC chromatograms of Dhārā Vṛkṣāmla hydro-alcoholic extract with (-)-*Hydroxycitric acid lactone* and Calcium (-)-*hydroxycitrate as RS***

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API (-)-*Hydroxycitric acid lactone RS* and Calcium (-)-*hydroxycitrate RS*

## DHĀRĀ VṚKṢĀMLĀ WATER EXTRACT

Dhārā Vṛkṣāmla Water Extract is a dried and powdered extract prepared from Dhārā Vṛkṣāmla. The extract contains not less than 6 per cent of (-)-hydroxycitric acid and not less than 20 per cent of (-)-hydroxycitric acid lactone when assayed.

### Method of Preparation:

Take Dhārā Vṛkṣāmla (Powder) in an extractor. Add water, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85° for 3-4 hours. Filter the extract through an appropriate 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 80° till the moisture is less than 7 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 38 per cent.

### Identity, Purity and Strength:

*High performance liquid chromatography:*

Carry out *liquid chromatography* (Appendix 3.6) using (-)-hydroxycitric acid lactone and calcium (-)-hydroxycitrate as a reference standards. Test solution, Standard solution, Chromatographic system, Mobile phase, Injection volume, Detection and Procedure follow as mentioned under Assay. The chromatogram obtained with test solution shows peaks corresponding to the retention time of (-)-hydroxycitric acid lactone and (-)-hydroxycitric acid (Fig. 1).

### 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 2.0 per cent (Appendix 2.1.7); *pH:* 2.0-3.5 (Appendix 2.1.10); *Total soluble solids:* not less than 90.0 per cent (Appendix 2.1.11) (Method-II)

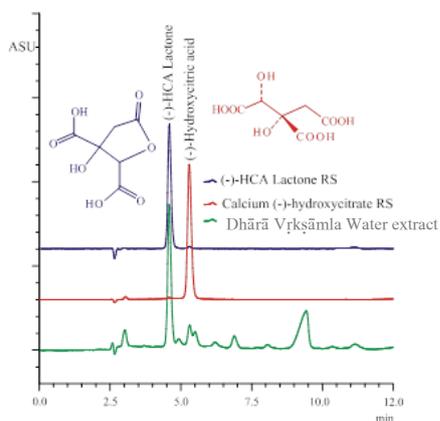
### Other requirements:

*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 0.5 g, accurately weighed, of the substance being examined and reflux with 25 ml of water (adjusted to pH 2.1 with sulphuric acid solution) on a water bath for 10 min and transfer to a 50-ml volumetric flask. Cool, filter and make up the volume with water (adjusted to pH 2.1 with sulphuric acid solution). Filter through 0.42 µm membrane. *Standard solution:* Take about 10 mg, accurately weighed, (-)-hydroxycitric acid lactone RS and 50 mg of calcium (-)-hydroxycitrate RS in a 25-ml volumetric flask and dissolve in about 10 ml of water (adjusted to pH 2.1 with sulphuric acid solution) and make up the volume with water (adjusted to pH 2.1 with sulphuric acid solution). Filter through 0.42 µm membrane. *Chromatographic system:* High performance liquid chromatography. *Column and stationary phase:* C18 (250 mm x 4.6 mm, 5 µm). *Mobile phase:* Filtered and degassed 0.05 M sodium sulphate in water (adjusted to pH 2.3 with sulphuric acid solution). *Injection volume:* 20 µl. *Flow rate:* 1.0 ml per min. *Detection:* UV 210 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, identify the analyte peak using relative retention time. The relative retention time for (-)-hydroxycitric acid lactone is 1 and for (-)-hydroxycitric acid is about 1.1. Calculate the contents of (-)-hydroxycitric acid lactone and (-)-

*hydroxycitric acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 1: HPLC chromatograms of Dhārā Vr̥kṣāmla water extract with (-)-Hydroxycitric acid lactone and Calcium (-)-hydroxycitrate as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API (-)-Hydroxycitric acid lactone RS and Calcium (-)-hydroxycitrate RS

## KATUKĀ

Katukā consists of the dried rhizome with root of *Picrorrhiza kurroa* Royle ex Benth. (Fam. Scrophulariaceae); a perennial, more or less hairy herb common on the north-western Himalayas from Kashmir to Sikkim. Rhizome is cut into small pieces. It contains not less than 6.0 per cent of bitters and not less than 4.0 per cent of sum of *picroside I* and *picroside II* when assayed.

**Synonyms:** Tiktā, Tiktaroḥiṇī, Kaṭuroḥiṇī, Kaṭvī, Matsyaśakalā

### Other/Regional Language Names:

*Assamese:* Katki, Kutki; *English:* Hellebore; *Gujarati:* Kadu, Katu; *Hindi:* Kutki; *Kannada:* Katukarohini; *Malayalam:* Katukurohini, Katuruhini; *Marathi:* Kutki, Kali kutki; *Oriya:* Katuki; *Punjabi:* Karru, Kaur; *Tamil:* Kadugurohini; *Telugu:* Katukarohini, Katki

### Description:

#### a) Macroscopic:

**Rhizome** - 2.5 cm long and 4-8 mm thick, subcylindrical, straight or slightly curved, externally greyish-brown, surface rough due to longitudinal wrinkles, circular scars of roots and bud scales and sometimes roots attached; tip ends in a growing bud surrounded by tufted crown of leaves; at places cork exfoliates exposing dark cortex; fracture short; odour pleasant; taste bitter

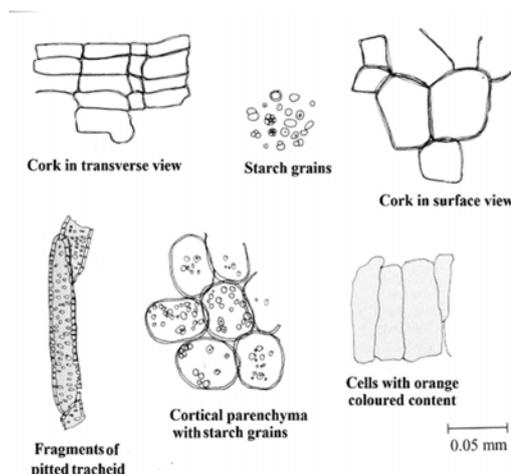
**Root** - Thin, cylindrical, 5-10 cm long, 0.05-0.1 cm in diameter, straight or slightly curved with a few longitudinal wrinkles and dotted scars, mostly attached with rhizomes, dusty grey, fracture short, inner surface black with whitish xylem; odour pleasant; taste bitter

#### b) Microscopic:

**Rhizome**- Shows 20-25 layers of cork consisting of tangentially elongated, suberised cells; cork cambium 1-2 layered; cortex single layered or absent, primary cortex persists in some cases, one or two small vascular bundles present in cortex;

vascular bundles surrounded by single layered endodermis of thick walled cells; secondary phloem composed of phloem parenchyma and a few scattered fibres; cambium 2-4 layered; secondary xylem consists of vessels, tracheids, xylem fibres and xylem parenchyma, vessels vary in shape and size having transverse oblique articulation; tracheids long, parenchyma thin walled and polygonal in shape; centre occupied by a small pith consisting of thin-walled cells; simple round to oval, starch grains abundantly found in all cells

**Root** - Young root shows single layered epidermis, some epidermal cells elongate forming unicellular hairs; hypodermis single layered; cortex 8-14 layered; consisting of oval to polygonal, thick-walled, parenchymatous cells; primary stele tetrach to heptarch, enclosed by single layered pericycle and single layered, thick-walled cells of endodermis; mature root shows 4-15 layers of cork, 1-2 layers of cork cambium; secondary phloem poorly developed; secondary xylem consisting of vessels, tracheids, parenchyma and fibres; vessels have varying shape and size, some cylindrical with tail-like, tapering ends, some drum shaped with perforation on end walls or lateral walls; tracheids cylindrical with tapering pointed ends; fibres aseptate, thick walled, lignified with tapering, blunt, chisel-like pointed ends.



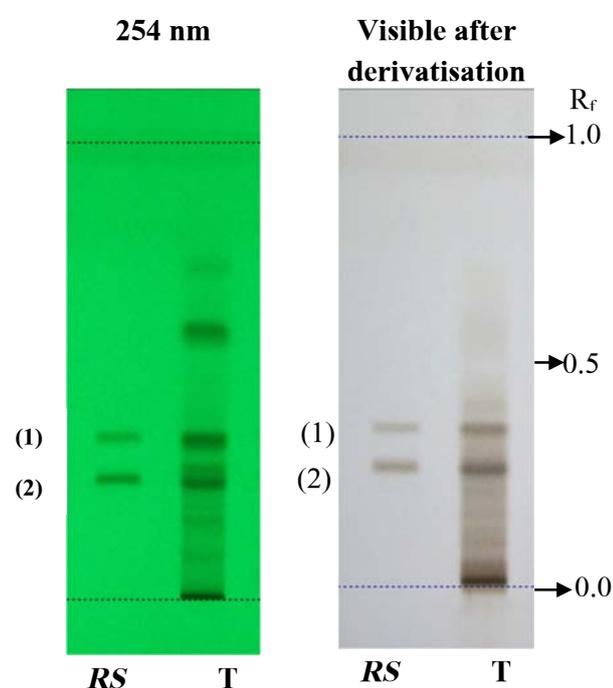
**Fig. 1: Powdered drug of KATUKĀ (*Picrorrhiza kurroa* Royle ex Benth.)**

c) *Powder*:

Powder dusty grey, shows group of fragments of cork cells, thick walled parenchyma, pitted vessels and single round to oval starch grains (Fig. 1)

**Identity, Purity and Strength:****Identification:***Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate Appendix 3.5) using *picroside-I* and *picroside-II* as reference standards.



**Fig. 2: Thin-Layer Chromatogram of Kaṭukā**  
**RS: (1) *Picroside-I* and (2) *Picroside-II*,**  
**T: Test solution**

*Test solution:* Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 5 mg each of *picroside-I* RS and *picroside-II* RS in 25 ml of *methanol*. *Procedure:* Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *water* (82.0 : 10.0 : 8.0). Dry the plate in air and examine under UV 254 nm. Spray the

plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 2).

**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 10.0 per cent (Appendix 2.1.8); *Water-soluble extractive:* not less than 20.0 per cent (Appendix 2.1.9)

**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)

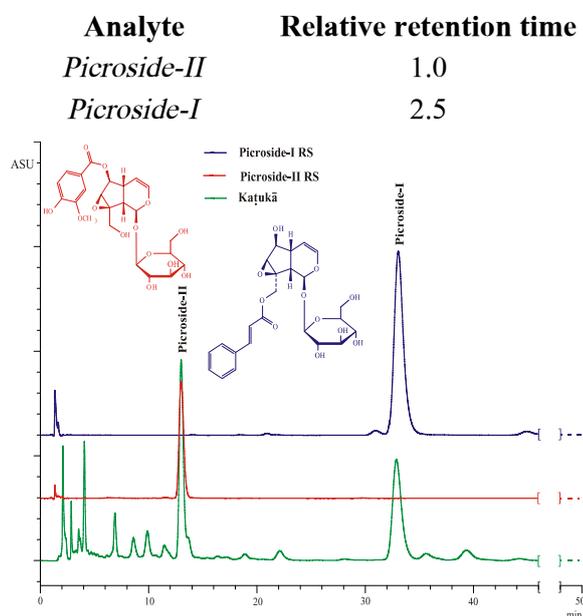
**Assay of bitters:**

Carry out the assay by *Gravimetry*. Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate and evaporate to dryness under reduced pressure. Dissolve the residue in hot *water* and filter. Extract the filtrate repeatedly with 50, 50, 50, 25, 25 ml of *ethyl acetate*. Combine all the *ethyl acetate* extracts and filter and evaporate to dryness under reduced pressure. Dry the residue at 100<sup>0</sup> for one hour and weigh the residue. Calculate the content of bitters from the weight of the residue and from the weight of substance taken for the test.

**Assay:**

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution:* Take about 0.2 g,

accurately weighed, of the substance being examined and reflux with water (25 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml volumetric flask and make up the volume with water. Filter through 0.42 µm membrane. *Standard solution*: Take about 5 mg, accurately weighed, each of *picroside-I RS* and *picroside-II RS* in 25 ml volumetric flask and dissolve in about 20 ml of water and make up the volume with water. Filter through 0.42 µm membrane. *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 17 volumes of acetonitrile and 83 volumes of water containing 0.1 per cent phosphoric acid. *Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detector*: UV 262 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 responses for the analyte peaks.



**Fig. 3: HPLC chromatogram of Kaṭukā with *Picroside-I* and *Picroside-II* as RS**

Calculate the content of *picroside-I* and *picroside-II* in the substance being examined from peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standards:

API *Picroside-I RS* and *Picroside-II RS*

**Constituents:** Picrosides I, II and III, pikuroside, kutkoside, cucurbitacins, cucurbitacin glycosides, apocynin, androsin, picein, vanillic acid, veronicoside, minecoside, 6-feruloylcatalpol

**Properties and Action:** Rasa: Tikta; Guṇa: Laghu; Vīrya: Śīta; Vipāka: Kaṭu; Karma: Bhedinī, Dīpana, Āmapacanī, Gulmaghnī, Jvarahara, Kaṇḍūghna, Lekhanīya, Pittahara, Stanyaśodhana, Śūlahara, Śleṣmahara, Viṣaghna

**Important formulations:** Ārogyavardhinī guṭikā, Mahātikataka ghṛta, Sarvajvarahara lauha, Tiktaka ghṛta

**Therapeutic uses:** Dāha (burning sensation), Āmajavara (fever due to indigestion), Āmavāta (rheumatism), Jvara (fever), Kāmalā (Jaundice), Kuṣṭha (diseases of skin), Plīhodara (splenomegaly), Sthaulya (obesity), Śvāsa (dyspnoea), Viṣamajvara (intermittent fever), Vraṇa (wound), Yakṛdśoṭha (hepatitis)

**Dose:** Cūrṇa (powder): 1-3 g

## KAṬUKĀ HYDRO-ALCOHOLIC EXTRACT

Kaṭukā Hydro-alcoholic Extract is a dried and powdered extract prepared from Kaṭukā (appropriately powdered). The extract contains not less than 6 per cent of bitters and not less than 1 per cent of sum of *picroside-I* and *picroside-II* when assayed.

### Method of preparation:

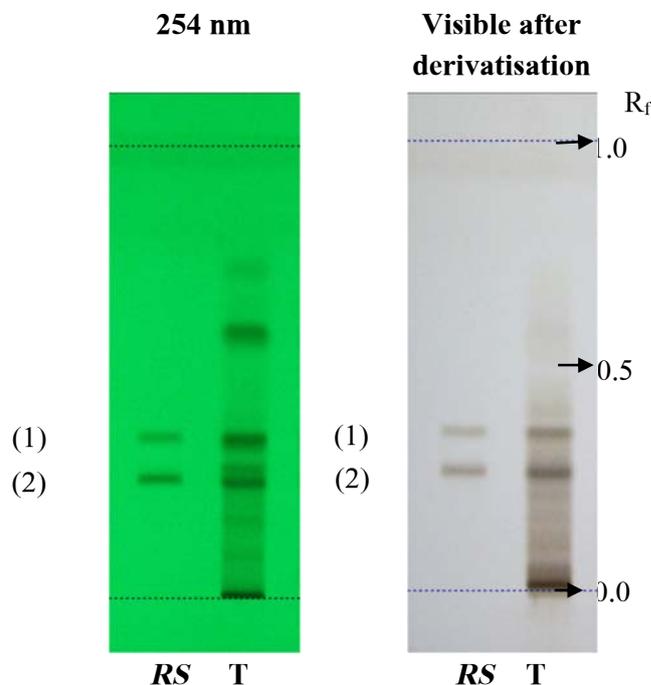
Take Kaṭukā suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85° for 3-4 hours. Filter the extract through a filter (preferably 10 µm pore size) to a suitable sized vessel. 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° till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *picroside-I* and *picroside-II* as reference standards. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of *picroside-I* RS and *picroside-II* RS in 25 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *water* (82.0 : 10.0 : 8.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105° till the colour of the spots/bands appear without charring. The

chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Kaṭukā hydro-alcoholic extract**  
**RS: (1) Picroside-I and (2) Picroside-II,**  
**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 1.0 per cent (Appendix 2.1.7); *pH*: 4.0-6.0 (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 of bitters:**

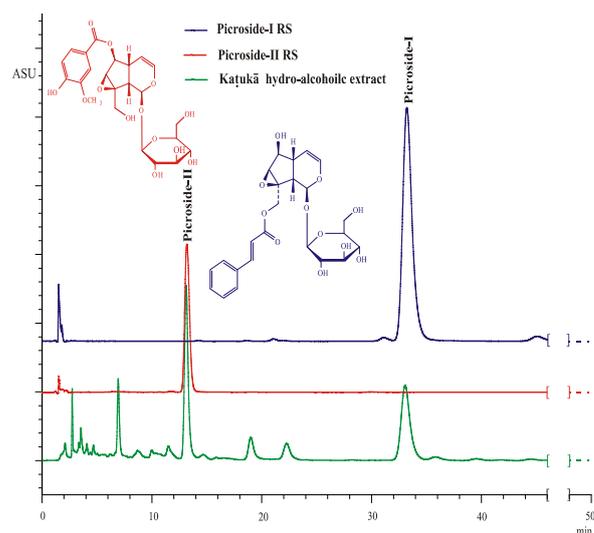
Carry out the assay by *Gravimetry*. Take about 2 g, accurately weighed, of the substance being examined, and reflux with *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate and evaporate to dryness under reduced pressure. Dissolve the residue in hot *water* and filter. Extract the filtrate repeatedly with 50, 50, 50, 25, 25 ml of *ethyl acetate*. Combine all the *ethyl acetate* extracts and filter and evaporate to dryness under reduced pressure. Dry the residue at 100<sup>0</sup> for one hour and weigh the residue. Calculate the content of bitters from the weight of the residue and from the weight of substance taken for the test.

**Assay:**

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.2 g, accurately weighed, of the substance being examined and reflux with *water* (25 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml volumetric flask and make up the volume with *water*. Filter through 0.42 µm membrane. *Standard solution*: Take about 5 mg, accurately weighed, each of *picroside-I RS* and *picroside-II RS* in 25-ml volumetric flask and dissolve in about 20 ml of *water* and make up the volume with *water*. Filter through 0.42 µm membrane. *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 17 volumes of *acetonitrile* and 83 volumes of *water* containing 0.1 per cent *phosphoric acid*. *Injection volume*: 20 µl. *Flow rate*: 1.0 ml per min. *Detector*: UV 262 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 responses for the analyte peaks.

Analyte	Relative retention time
<i>Picroside-II</i>	1.0
<i>Picroside-I</i>	2.5

Calculate the content of *picroside-I* and *picroside-II* in the substance being examined from peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Kaṭukā hydro-alcoholic extract with *Picroside-I* and *Picroside-II* as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standards:**

API *Picroside-I RS* and *Picroside-II RS*

## KAṬUKĀ WATER EXTRACT

Kaṭukā Water Extract is dried and powdered extract prepared from Kaṭukā. The extract contains not less than 8 per cent of bitters and not less than 5 per cent of sum of *picroside-I* and *picroside-II* when assayed.

### Method of preparation:

Take Kaṭukā 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh and pack. The yield obtained is about 20 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *picroside-I* and *picroside-II* as reference standards. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of *picroside-I RS* and *picroside-II RS* in 25 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate: methanol: water* (82.0 : 10.0 : 8.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without

charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).

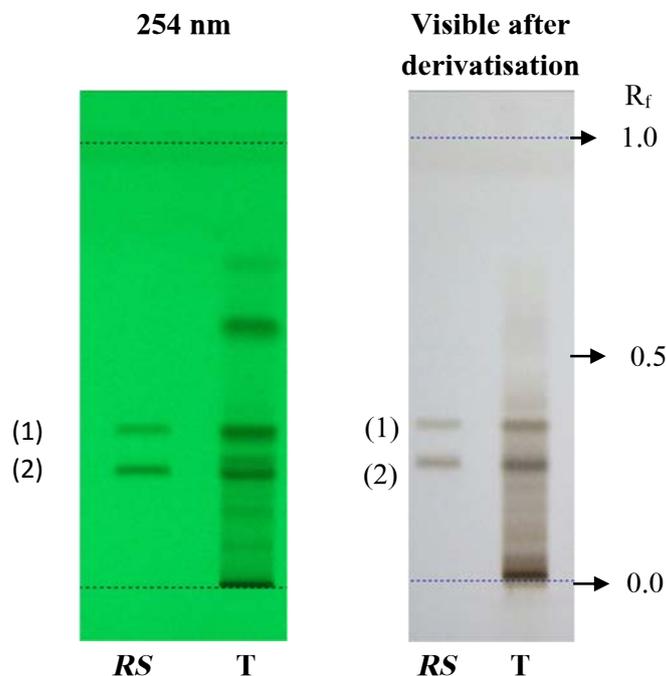


Fig. 2: Thin-Layer Chromatogram of Kaṭukā water extract  
RS: (1) *Picroside-I* and (2) *Picroside-II*,  
T: Test solution

### 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 1.0 per cent (Appendix 2.1.7); *pH*: 4.0-7.0 (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 of bitters:

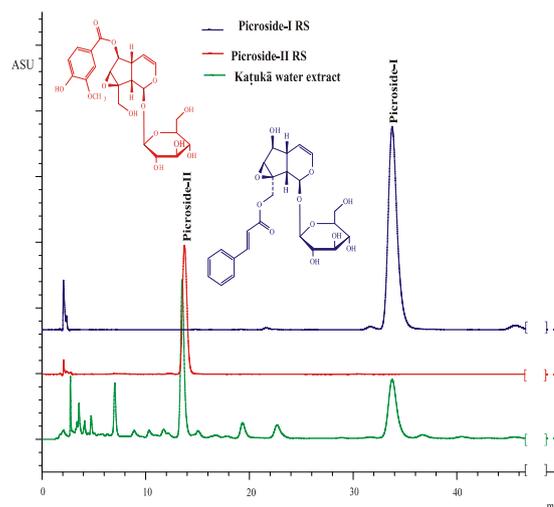
Carry out the assay by *Gravimetry*. Take about 2 g, accurately weighed, of the substance being

examined, and reflux with *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate and evaporate to dryness under reduced pressure. Dissolve the residue in hot *water* and filter. Extract the filtrate repeatedly with 50, 50, 50, 25, 25 ml of *ethyl acetate*. Combine all the *ethyl acetate* extracts and filter and evaporate to dryness under reduced pressure. Dry the residue at 100<sup>0</sup> for one hour and weigh the residue. Calculate the content of bitters from the weight of the residue and from the weight of substance taken for the test.

### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). **Test solution:** Take about 0.2 g, accurately weighed, of the substance being examined and reflux with *water* (25 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 100-ml volumetric flask and make up the volume with *water*. Filter through 0.42 µm membrane. **Standard solution:** Take about 5 mg, accurately weighed, each of *picroside-I RS* and *picroside-II RS* in 25-ml volumetric flask and dissolve in about 20 ml of *water* and make up the volume with *water*. Filter through 0.42 µm membrane. **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 17 volumes of *acetonitrile* and 83 volumes of *water* containing 0.1 per cent *phosphoric acid*. **Injection volume:** 20 µl. **Flow rate:** 1 ml per min. **Detector:** UV 262 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 responses for the analyte peaks.

Analyte	Relative retention time
<i>Picroside-II</i>	1.0
<i>Picroside-I</i>	2.5



**Fig. 2: HPLC chromatograms of Kaṭukā water extract with *Picroside-I* and *Picroside-II* as RS**

Calculate the content of *picroside-I* and *picroside-II* in the substance being examined from peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *Picroside-I RS* and *Picroside-II RS*

## MAÑJIṢṬHĀ

Mañjiṣṭhā consists of the dried root of *Rubia cordifolia* L. (Fam. Rubiaceae), a perennial herbaceous creeper or climber, with hooked prickles and whorls of four leaves, but without interpetiolar stipules, found throughout the country ascending to 3750 m. It contains not less than 0.04 per cent of *rubiadin* when assayed.

**Synonyms:** Yojanavallī, Tāmrvallī, Vastrarañjinī, Raktā

**Other/Regional Language Names:** *Assamese:* Phuvva; *Bengali:* Manjishtha, Manjith; *English:* Indian Madder; *Gujarati:* Manjitha; *Hindi:* Manjitha, Manjit; *Kannada:* Manjustha; *Malayalam:* Manjatti, Manchatti; *Marathi:* Manjishtha; *Punjabi:* Manjishtha, Manjit; *Tamil:* Manatte, Manjitti; *Telugu:* Manjishtha

### Description:

#### a) Macroscopic:

**Root** - Cylindrical, often surmounted by a knotty crown of root stock; about 2 to 9 cm in length and 0.2 to 0.6 cm in width; surface smooth finely striated longitudinally and occasionally grooved, often exhibiting lateral root scars; dark reddish brown both externally and internally. Fracture short, taste sweetish, acrid and disagreeable, odour pleasant

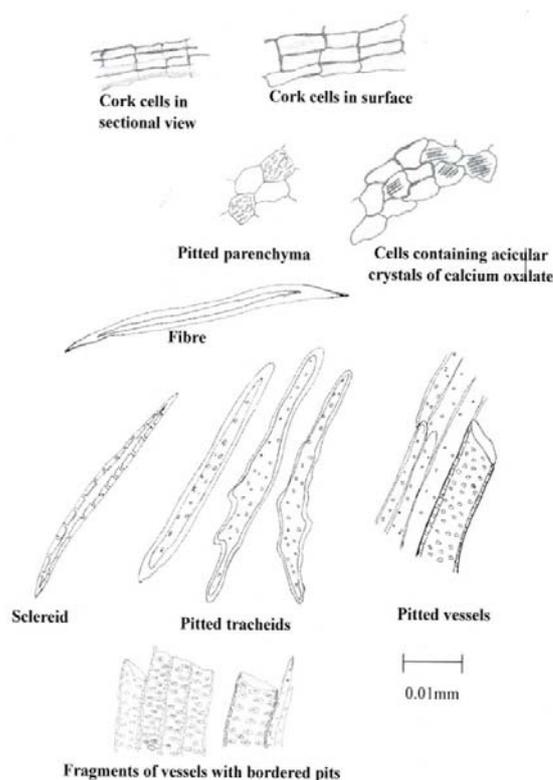
#### b) Microscopic:

TS of root shows a well developed cork, consisting of 3 to 8 layered suberized radially arranged cells, occasionally filled with reddish brown content, followed by a cortex of 3 to 10 cell layers; some cortical cells filled with acicular and sandy crystals of calcium oxalate more towards periphery. Phloem 8 to 12 layers wide, consists of sieve tubes, companion cells and phloem parenchyma. Xylem consists of vessels, fibres, tracheids and xylem parenchyma. Vessels are broader towards the peripheral region of the xylem. The size of vessels vary from 30 to 270  $\mu\text{m}$  in length and 18 to 90  $\mu\text{m}$  in breadth.

Medullary rays are uni- to multiseriate and oval to circular starch grains present in cortical and phloem parenchyma cells.

#### c) Powder:

Shows numerous fragments of cork, lignified xylem vessels, tracheids and fibres, raphides, clusters and sandy oxalate crystals, parenchyma with red content and starch grains (Fig. 1)



**Fig. 1: Powdered drug of MAÑJIṢṬHĀ**  
(*Rubia cordifolia* L.)

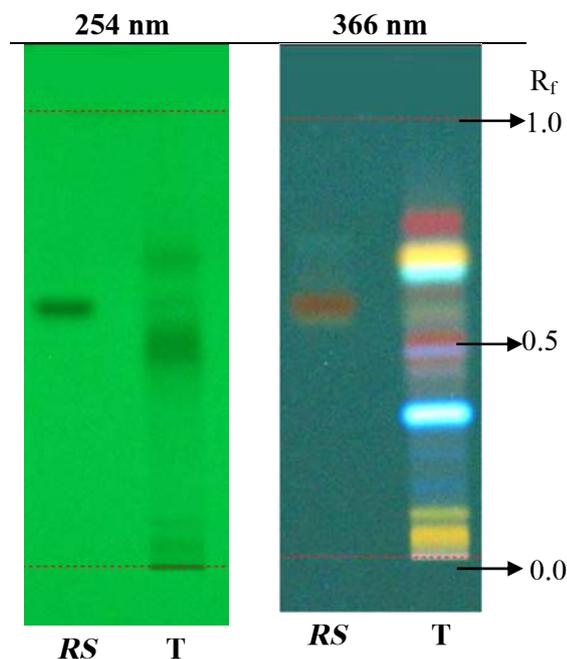
### Identity, Purity and Strength:

#### Identification:

##### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *rubiadin* as a reference standard. *Test solution:* Extract 2 g of substance by refluxing with *chloroform* (25 ml x 3) for a period of 10-15 min each. Filter and concentrate the combined extract to dryness. Dissolve the residue in 2 ml of

*methanol*. **Standard solution:** Dissolve 1 mg of *rubiadin RS* in about 10 ml of *methanol*.



**Fig. 2: Thin-Layer Chromatogram of Mañjisthā**

**RS: Rubiadin, T: Test solution**

**Procedure:** Apply 10  $\mu$ l each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop upto 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *chloroform* : *glacial acetic acid* (10.0 : 5.0 : 1.0 : 2.5). Dry the plate in air and examine under UV 254 nm and under UV 366 nm. The chromatographic profile of the test solution shows a band corresponding to that of standard solution (Fig.2).

#### 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 12.0 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 3.0 per cent (Appendix 2.1.8); **Water-soluble extractive:** not less than 10.0 per cent (Appendix 2.1.9)

#### 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)

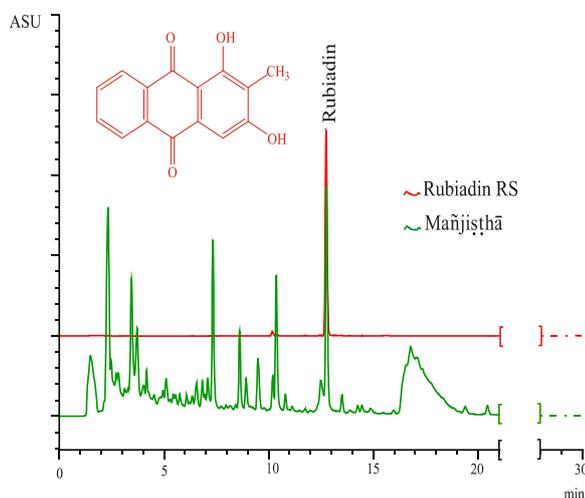
#### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). **Test solution:** Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (50 ml x 3) on water bath for 5-10 min each, cool and Filter. Combine all the filtrates, concentrate to 50 ml and transfer in a 100-ml volumetric flask and make up the volume with *methanol*. Filter through 0.42  $\mu$ m membrane. **Standard solution:** Take about 5 mg, accurately weighed, *rubiadin RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu$ m membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu$ m). **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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	65	35
5	50	50
15	20	80
20	15	85
25	65	35
30	65	35

**Injection volume:** 20  $\mu$ l. **Flow rate:** 1.5 ml per min. **Detection:** UV 278 nm. **Procedure:** Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and measure the

response for the analyte peak. Calculate the content of *rubiadin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Mañjiṣṭhā with Rubiadin as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API references standard:

API *Rubiadin RS*

**Constituents:** Rubiadin, anthraquinones, alizarin, purpurin, purpuroxanthin, ruberythric acid, 1,3-dihydroxy-2-ethoxymethyl-9,10-nthraquinone, lucidin primeveroside, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-*O*- (6'-*O*-acetyl)- $\alpha$ -rhamnosyl(1 $\rightarrow$ 2)- $\beta$ -glucoside, furomollugin, rubilactone, 2-carboxymethyl-3-prenyl-2,3-epoxy-1, 4-naphthoquinone, 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone, rubioncolin B, 1-hydroxy-2-methyl anthraquinone, nordamnacanthal, physcion, 1,4-dihydroxy-6-methylantraquinone, 1,4-

dihydroxy-2-methylantraquinone, 1,5-dihydroxy-2-methylantraquinone, 3-prenyl-5-methoxy-1,4-naphthoquinone, 1-hydroxy-2-methoxyanthraquinone, 1,4-dihydroxy-2-methyl-5-(or 8)-methoxyanthraquinone, 1,3-dimethoxy-2-carboxyanthraquinone

**Properties and Action:** Rasa: Kaṣāya, Tikta, Madhura; Guṇa: Laghu, Rūkṣa; Vīrya: Śīta; Vipāka: Kaṭu; Karma: Pittasaṁśamana, Sandhānīya, Varṇya, Vraṇaropaṇī

**Important formulations:** Methikādi cūrṇa, Palāśapuṣpāsava, Yogarājāsava

**Therapeutic uses:** Bhagna (fracture), Garbhapāta (abortion), Pakvātisāra (chronic diarrhoea), Tvakroga (skin disease), Vraṇa (wound), Vyaṅga (dark shade on face due to stress and excessive exercise)

**Dose:** 3-6 g

## MAÑJIṢṬHĀ HYDRO-ALCOHOLIC EXTRACT

Mañjiṣṭhā Hydro-alcoholic Extract is a dried and powdered extract prepared from Mañjiṣṭhā (appropriately powdered). The extract contains not less than 0.05 per cent of *rubiadin* when assayed.

### Method of Preparation:

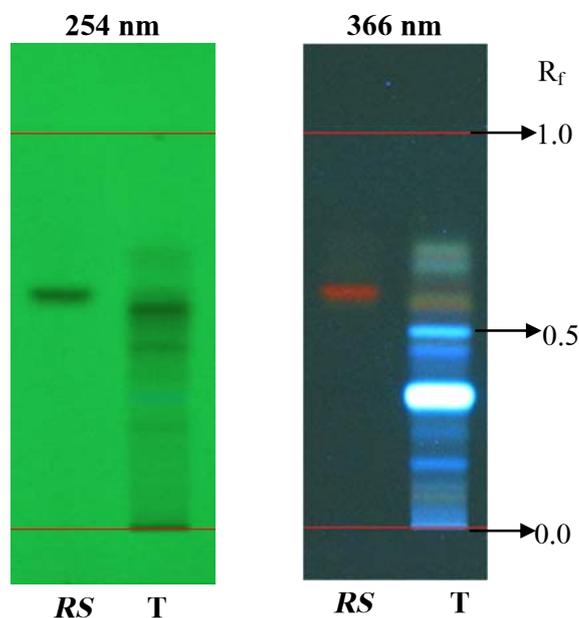
Take Mañjiṣṭhā suitably sized (powder or pieces) in an extractor. Add 50.0 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 µm pore size) to a suitable 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 4 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *rubiadin* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with *chloroform* (25 ml x 3) for a period of 10-15 min each. Filter and concentrate the combined extract to dryness. Dissolve the residue in 2 ml of *methanol*. *Standard solution*: Dissolve 1 mg of *rubiadin* RS in about 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop upto 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *chloroform* : *glacial acetic acid* (10.0 : 5.0 : 1.0 : 2.5). Dry the plate in air and examine under UV 254 nm and under UV 366 nm. The chromatographic profile of the test solution

shows a band corresponding to that of standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Mañjiṣṭhā hydro-alcoholic extract**  
RS: *Rubiadin*, 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.5-7.0 (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)

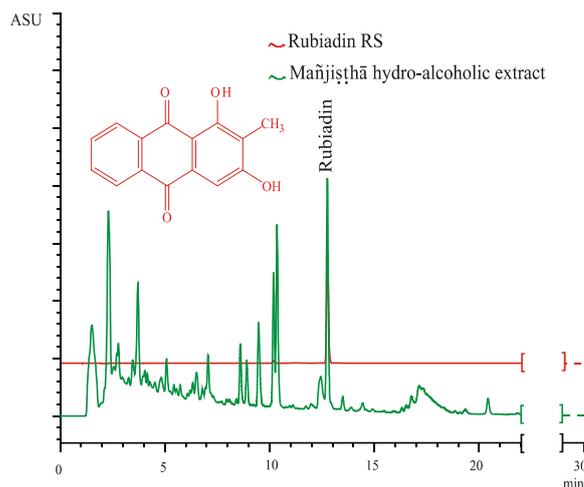
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 2 g,

accurately weighed, of the substance being examined and reflux with *methanol* (50 ml x 3) on water bath for 5-10 min each, cool and filter. Combine all the filtrates, concentrate to 50 ml and transfer in a 100 ml volumetric flask and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 5 mg, accurately weighed, *rubiadin RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	65	35
5	50	50
15	20	80
20	15	85
25	65	35
30	65	35

**Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 278 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *rubiadin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Mañjiṣṭhā hydro-alcoholic extract with *Rubiadin* as RS**

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standard:

API *Rubiadin RS*

## MAÑJIṢṬHĀ WATER EXTRACT

Mañjiṣṭhā Water Extract is a dried and powdered extract prepared from Mañjiṣṭhā. The extract contains not less than 0.02 per cent of *rubiadin* when assayed.

### Method of Preparation:

Take Mañjiṣṭhā 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *rubiadin* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with *chloroform* (25 ml x 3) for a period of 10-15 min each. Filter and concentrate the combined extract to dryness. Dissolve the residue in 2 ml of *methanol*. *Standard solution*: Dissolve 1 mg of *rubiadin* RS in about 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop upto 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *chloroform* : *glacial acetic acid* (10.0 : 5.0 : 1.0 : 2.5). Dry the plate in air and examine under UV 254 nm and under UV 366 nm. The chromatographic profile of the test solution shows a band corresponding to that of standard solution (Fig. 1).

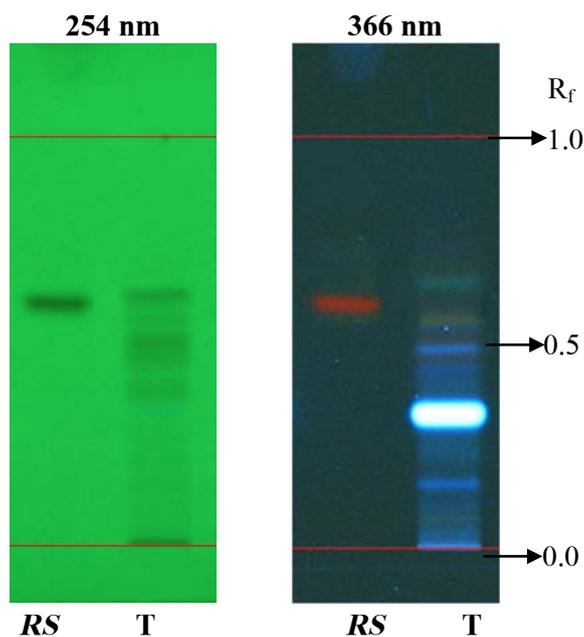


Fig. 1: Thin-Layer Chromatogram of Mañjiṣṭhā water extract  
RS: *Rubiadin*, T: Test solution

### Quantitative parameters:

*Loss on drying*: not more than 7.0 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-6.5 (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)

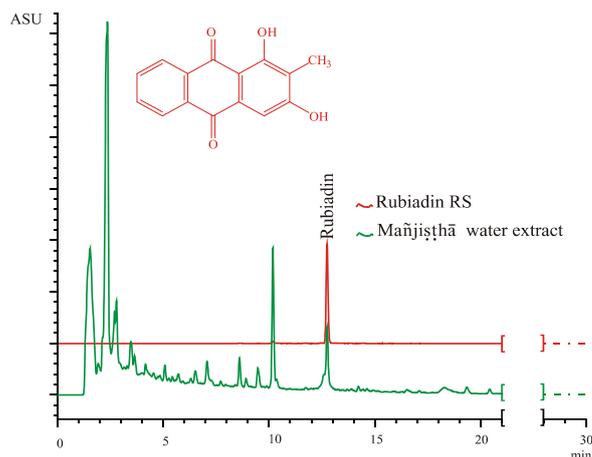
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (50 ml x 3) on water bath for 5-10 min each, cool and filter. Combine all the filtrates, concentrate to 50 ml and

transfer in a 100-ml volumetric flask and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 5 mg, accurately weighed, *rubiadin RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	65	35
5	50	50
15	20	80
20	15	85
25	65	35
30	65	35

**Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 278 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *rubiadin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Mañjiṣṭhā water extract with *Rubiadin* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Rubiadin RS*

## MEṢAŚRŅĠĪ

Meṣaśrᅇᅇᅇ consists of dried leaf of *Gymnema sylvestre* R.Br. (Fam. Asclepiadaceae), a large woody, much branched, climber, with pubescent young parts, found throughout India in dry forests upto 600 m. It contains not less than 8.0 per cent of *gymnemic acids* and not less than 2.0 per cent of *gymnemagenin* when assayed.

**Synonyms:** Madhunāśinī

**Other/Regional Language Names:** *Bengali:* Medhasingi; *English:* Periploca of the Woods; *Gujarati:* Kaavalee, Medhasinge; *Hindi:* Gudmaar, Medhasingi; *Kannada:* Kadhasige; *Malayalam:* Cakkarakkolli, Madhunaashini; *Marathi:* Kaavalee, Medhashingi; *Tamil:* Sirukurunjan, Shakkaraikkolli; *Telugu:* Padapatri

### Description:

#### a) Macroscopic:

Leaf simple, opposite, elliptical or ovate, petiolate, petiole 6 to 12 mm long and pubescent; lamina 3 to 6 cm long and 1 to 3 cm broad; acute or shortly acuminate; more or less pubescent on both sides, base rounded or cordate, venation reticulate; odour unpleasant; taste bitter and acrid, and leaves a numbing sensation

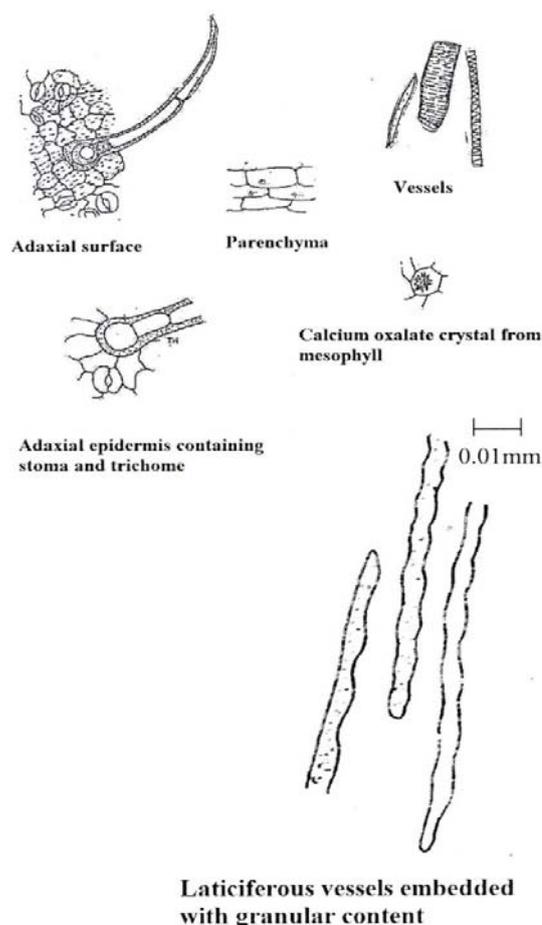
#### b) Microscopic:

**Leaf - Petiole -** Nearly semi-circular in outline having a deep furrow, shows a single layered epidermis covered with thick cuticle; multicellular uniseriate trichomes present; cortex composed of 3 or 4 layers of collenchyma and 3 or 4 layers of thin walled parenchymatous cells with intercellular spaces; vascular bundle bicollateral, conjoint and 3 in number, one central larger and crescent shaped and 2 lateral and smaller in size; a few rosette crystals of calcium oxalate present in cortical region

**Midrib -** Epidermis and trichome as in petiole; epidermis followed by 2 or 3 layers of collenchyma adjacent to the lower surface; vascular bundle crescent shaped, bicollateral,

conjoint and situated in centre; rest of the tissue between collenchyma and vascular bundles consisting of polygonal thin-walled parenchymatous cells with intercellular spaces, a few having rosette crystals of calcium oxalate

**Lamina -** Cuticle striated shows dorsiventral structure; epidermis with cells having beaded walls and trichome as in petiole and midrib; trichome consists of 3 to 6 cells nearly similar in width and variable in length, terminal cells blunt, most of them curved inwards from the leaf surface; palisade 1 or 2 layers; spongy parenchyma irregular, arranged with distinct intercellular spaces, rosette crystals of calcium oxalate present in this region; stomata paracytic, present only on lower surface; palisade ratio 7 or 8; stomatal index 20 to 25, and vein islet number 7 to 10



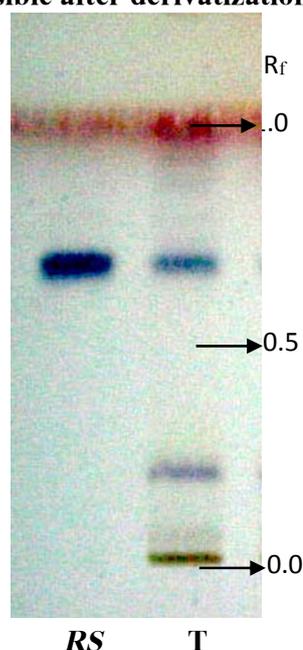
**Fig. 1: Powdered drug of MEṢAŚRŅĠĪ (*Gymnema sylvestre* R.Br.)**

c) *Powder*:

Light yellow; shows polygonal, thin walled parenchymatous cells, simple pitted fibres and vessels; laticiferous vessels embedded with granular contents, large and a few small rosette crystals of calcium oxalate, simple and compound starch grains, measuring 5 to 11  $\mu$  in dia (Fig. 1)

**Identity, Purity and Strength:****Identification:***Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *gymnemenin* as a reference standard. *Test solution*: Extract 0.5 g of substance by refluxing with a mixture of 50 per cent *methanol* and 11 per cent *potassium hydroxide* solution (10 : 2), for a period of 1 hour. Add 1.8 ml of concentrated *hydrochloric acid* and reflux again for one hour on water bath. Cool and adjust the pH between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution. Dilute to 50 ml with 50 per cent of *methanol* and filter. *Standard solution*: Dissolve 10 mg of *gymnemenin RS* in 10 ml of *methanol*.

**Visible after derivatization**

**Fig. 2: Thin-Layer Chromatogram of Meṣāṣṅgī**  
RS: *Gymnemenin*, T: Test solution

*Procedure*: Apply 10  $\mu$ l each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *formic acid* (82.0 : 10.0 : 8.0). Dry the plate in air. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

**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 12.0 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 7.0 per cent (Appendix 2.1.8); *Water-soluble extractive*: not less than 28.0 per cent (Appendix 2.1.9)

**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 of gymnemic acids:**

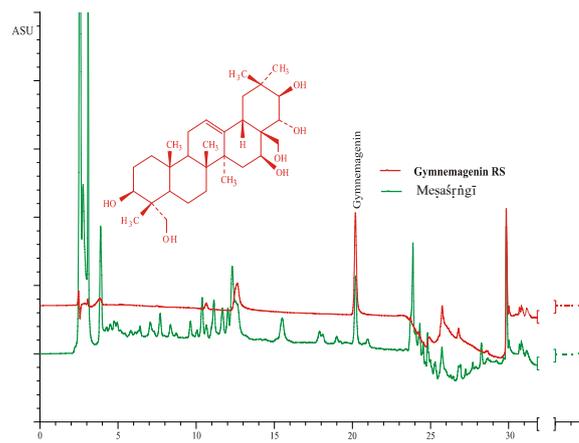
Carry out the assay by *Gravimetry*. Take about 5 g, accurately weighed, of the substance being examined, in a 100-ml round bottomed flask; reflux with 0.1 N *sodium hydroxide* solution (25 ml x 3) on a water bath for 10 min each. Cool and centrifuge, collect all the supernatants, concentrate to 25 ml and add dilute *sulphuric acid* drop wise till precipitate is completed. Centrifuge and wash the residue with cold water till the residue is acid free. Dissolve the residue in 50 ml of *methanol* and evaporate to dryness under reduced pressure. Dry the residue at 105<sup>0</sup> for one hour and weigh the residue. Calculate the content of gymnemic acids from the weight of the residue.

**Assay:**

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.5 g, accurately weighed, of the substance being examined, add 10 ml of 50 per cent *methanol* and 2 ml of 11 per cent *potassium hydroxide* solution, reflux for one hour on a water bath, cool and add 1.8 ml of concentrated *hydrochloric acid* and again reflux for one hour on a water bath. Cool and adjust *pH* between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution and make up the volume to 100 ml with 50 per cent *methanol*. Take 20 ml of this solution add 400 mg of *polyamide* (C-200), stir for one hour and filter the supernatant. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *gymnemagenin RS* in a 50 ml volumetric flask and dissolve in about 25 ml of 50 per cent *methanol* and make up the volume with 50 per cent *methanol*. Filter through 0.42 µm membrane. *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 0.1 per cent of *orthophosphoric acid* in the following proportions:-

Time (min)	Buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
20	58	42
22	0	100
26	0	100
28	85	15
35	85	15

*Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detection*: UV 210 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 peak. Calculate the content of *gymnemagenin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Meşaṅgī with Gymnemagenin as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Gymnemagenin RS*

**Constituents:** Triterpenoid saponins of gymnemic acid A, B, C and D with sugar residues such as glucuronic acid, galacturonic acid, ferulic and angelic acids attached as carboxylic acids. Several isopropylene derivatives of gymnemagenin, a hexahydroterpene, gymnemagenin, gymnemic acid. The leaves also contain betaine, choline, gymnamine alkaloids, inositol, *d*-quercitol. Hydrocarbons such as nonacosane, hentriacontane, tritriacontane, pentatriacontane, phytin, resin, tartaric acid, formic acid, butyric acid,  $\gamma$ -butyric acid, amino acids such as leucine, isoleucine, valine, alanine

**Properties and Action:** Rasa: Tikta, Kaṣāya; Guṇa: Laghu, Rūkṣa; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Cakṣuṣya, Dīpana, Gulmaghna, Kaphahara, Vātahara, Śūlaghna, Viṣaghna

**Important formulations:** Ayaskṛti, Mṛtasañjīvanī surā, Mahāviṣagarbha taila, Nyagrodhādi cūrṇa

**Therapeutic uses:** Gulma (abdominal lump), Kuṣṭha (disease of skin), Prameha (increased frequency and turbidity of urine), Sthaulya (obesity), Śiraḥśūla (headache), Vidradhi (abscess)

**Dose:** Cūrṇa (powder): 3-6 g

## MEṢAŚṚṄĪ HYDRO-ALCOHOLIC EXTRACT

Meṣaśṛṅġī Hydro-alcoholic Extract is a dried and powdered extract prepared from Meṣaśṛṅġī (appropriately powdered). The extract contains not less than 20 per cent of *gymnemic acids* and not less than 4 per cent of *gymnemagenin* when assayed.

### Method of preparation:

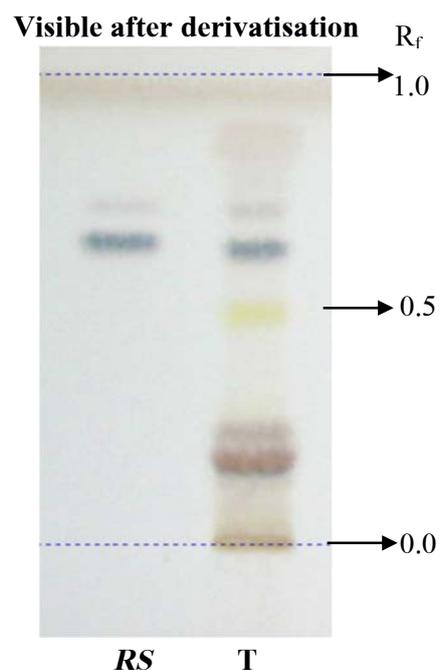
Take Meṣaśṛṅġī suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 15 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *gymnemagenin* as a reference standard. *Test solution*: Extract 0.5 g of substance by refluxing with a mixture of 50 per cent *methanol* and 11 per cent *potassium hydroxide* solution (10 : 2), for a period of 1 hour. Add 1.8 ml of concentrated *hydrochloric acid* and reflux again for one hour on water bath. Cool and adjust the *pH* between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution. Dilute to 50 ml with 50 per cent of *methanol* and filter. *Standard solution*: Dissolve 10 mg of *gymnemagenin RS* in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *formic acid* (82.0 :

10.0 : 8.0). Dry the plate in air. Spray the plate with 10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



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

### Quantitative parameters:

*Loss on drying*: not more than 7.0 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 1.0 per cent (Appendix 2.1.7); *pH*: 4.5-7.0 (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 of gymnemic acids:

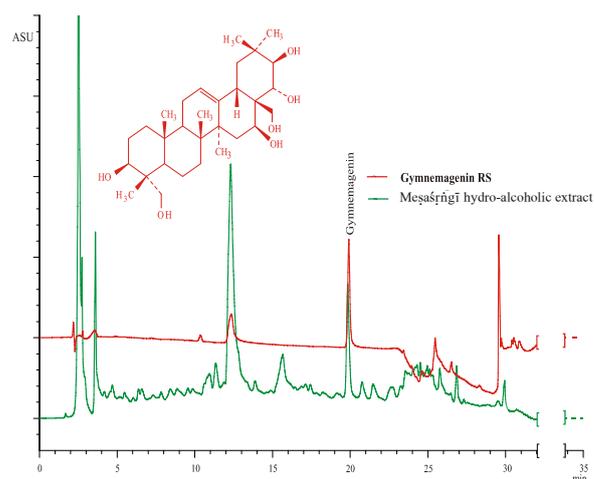
Carry out the assay by *Gravimetry*. Take about 5 g, accurately weighed, of the substance being examined, in a 100 ml round bottomed flask; reflux with 0.1 N *sodium hydroxide* solution (25 ml x 3) on a water bath for 10 min each. Cool and centrifuge, collect all the supernatants, concentrate to 25 ml and add dilute *sulphuric acid* drop wise till precipitate is completed. Centrifuge and wash the residue with cold water till the residue is acid free. Dissolve the residue in 50 ml of *methanol* and evaporate to dryness under reduced pressure. Dry the residue at 105<sup>0</sup> for one hour and weigh the residue. Calculate the content of gymnemic acids from the weight of the residue.

### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.5 g, accurately weighed, of the substance being examined, add 10 ml of 50 per cent *methanol* and 2 ml of 11 per cent *potassium hydroxide* solution, reflux for one hour on a water bath, cool and add 1.8 ml of concentrated *hydrochloric acid* and again reflux for one hour on a water bath. Cool and adjust *pH* between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution and make up the volume to 100 ml with 50 per cent *methanol*. Take 20 ml of this solution add 400 mg of *polyamide* (C-200), stir for one hour and filter the supernatant through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *gymnemagenin RS* in a 50-ml volumetric flask and dissolve in about 25 ml of 50 per cent *methanol* and make up the volume with 50 per cent *methanol*. Filter through 0.42 µm membrane. *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 0.1 per cent of *orthophosphoric acid* in the following proportions:-

Time (min)	Buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
20	58	42
22	0	100
26	0	100
28	85	15
35	85	15

*Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detection*: UV 210 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 peak. Calculate the content of *gymnemagenin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2:** HPLC chromatogram of Meşaşṛṅgī hydro-alcoholic extract with *Gymnemagenin* as RS

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Gymnemagenin RS*

## MEṢAŚRĀṄĪ WATER EXTRACT

Meṣaśrāṅī Water Extract is a dried and powdered extract prepared from Meṣaśrāṅī. The extract contains not less than 15 per cent of *gymnemic acids* and not less than 2.5 per cent of *gymnemagenin* when assayed.

### Method of preparation:

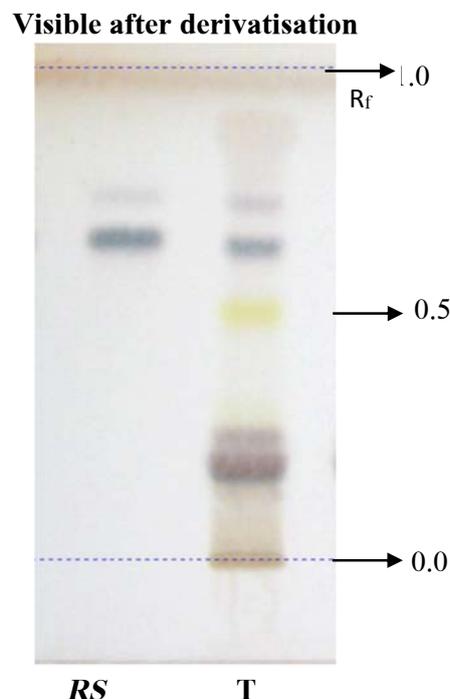
Take Meṣaśrāṅī suitably sized (powder or pieces) in an extractor. Add water, about 3 times the quantity of raw material and heat at a temperature between 80–85<sup>0</sup> for 3–4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized vessel. The marc is extracted 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh and pack. The yield obtained is about 15 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *gymnemagenin* as a reference standard. *Test solution*: Extract 0.5 g of substance by refluxing with a mixture of 50 per cent *methanol* and 11 per cent *potassium hydroxide* solution (10 : 2), for a period of 1 hour. Add 1.8 ml of concentrated *hydrochloric acid* and reflux again for one hour on water bath. Cool and adjust the *pH* between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution. Dilute to 50 ml with 50 per cent of *methanol* and filter. *Standard solution*: Dissolve 10 mg of *gymnemagenin RS* in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *formic acid* (82.0 : 10.0 : 8.0). Dry the plate in air. Spray the plate with

10 per cent *methanolic sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



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

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total Ash*: not more than 20.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 1.0 per cent (Appendix 2.1.7); *pH*: 4.5–8.0 (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 of gymnemic acids:

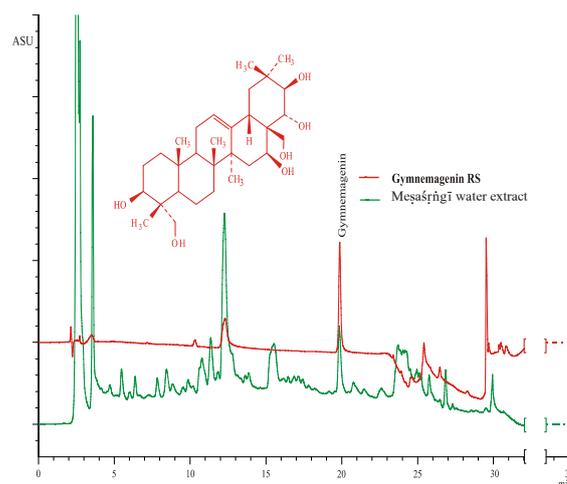
Carry out the assay by *Gravimetry*. Take about 5 g, accurately weighed, of the substance being examined, in a 100 ml round bottomed flask; reflux with 0.1 N *sodium hydroxide* solution (25 ml x 3) on a water bath for 10 min each. Cool and centrifuge, collect all the supernatants, concentrate to 25 ml and add dilute *sulphuric acid* drop wise till precipitate is completed. Centrifuge and wash the residue with cold water till the residue is acid free. Dissolve the residue in 50 ml of *methanol* and evaporate to dryness under reduced pressure. Dry the residue at 105<sup>0</sup> for one hour and weigh the residue. Calculate the content of gymnemic acids from the weight of the residue.

### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.5 g, accurately weighed, of the substance being examined, add 10 ml of 50 per cent *methanol* and 2 ml of 11 per cent *potassium hydroxide* solution, reflux for one hour on a water bath, cool and add 1.8 ml of concentrated *hydrochloric acid* and again reflux for one hour on a water bath. Cool and adjust *pH* between 7.5 to 8.5 with 11 per cent *potassium hydroxide* solution and make up the volume to 100 ml with 50 per cent *methanol*. Take 20 ml of this solution add 400 mg of *polyamide* (C-200), stir for one hour and filter the supernatant. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *gymnemenin RS* in a 50-ml volumetric flask and dissolve in about 25 ml of 50 per cent *methanol* and make up the volume with 50 per cent *methanol*. Filter through 0.42 µm membrane. *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 0.1 per cent of *orthophosphoric acid* in the following proportions:-

Time (min)	Buffer (per cent)	Acetonitrile (per cent)
0.01	85	15
20	58	42
22	0	100
26	0	100
28	85	15
35	85	15

*Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detection*: UV 210 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 peak. Calculate the content of *gymnemenin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Meşasringi water extract with *Gymnemenin* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Gymnemenin RS*

## METHĪ

Methī consists of seeds of *Trigonella foenum-graecum* L. (Fam. Fabaceae); an aromatic, 30 to 60 cm tall, annual herb, cultivated throughout the country. Methī contains not less than 5 per cent of *saponins* and not less than 0.2 per cent of 4-*hydroxyisoleucine* when assayed.

### Synonym:

**Other/Regional Language Names:** *Assamese:* Methi; *Bengali:* Methi; *English:* Fenugreek; *Gujarati:* Methi; *Hindi:* Methi; *Kannada:* Menthe, Mente; *Kashmiri:* Methi; *Malayalam:* Uluva; *Marathi:* Methi; *Punjabi:* Methi; *Tamil:* Ventayam; *Telugu:* Mentulu; *Urdu:* Methi

### Description:

#### a) Macroscopic:

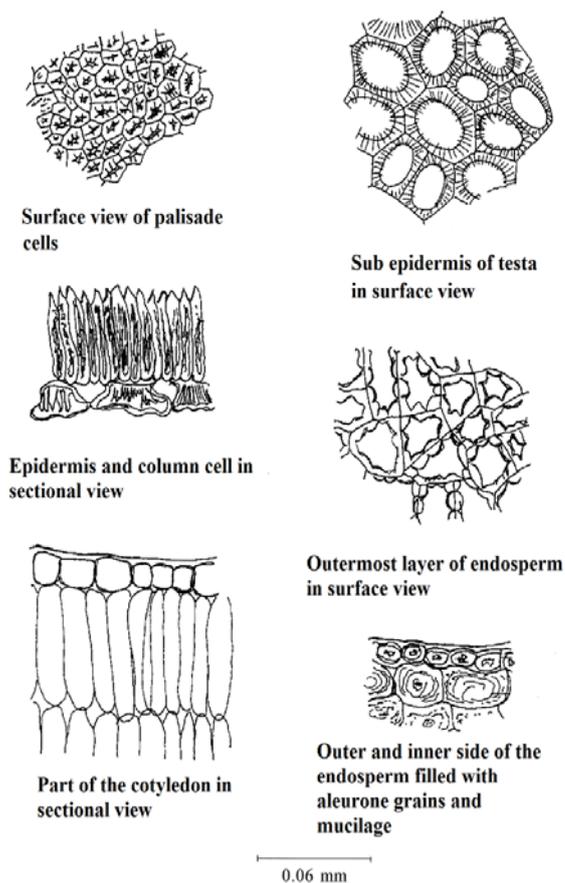
Seed oblong, rhomboidal with deep furrow running obliquely from one side, dividing seed into a larger and smaller part, 0.2 to 0.5 cm long, 0.15 to 0.35 cm broad, smooth, very hard; dull yellow; seed becomes mucilaginous when soaked in water; odour pleasant; taste bitter.

#### b) Microscopic:

**Seed** - Seed shows a layer of thick-walled, columnar palisade, covered externally with thick cuticle; cells flat at base, mostly pointed but a few flattened at apex, supported internally by a tangentially wide bearer cells having radial rib-like thickenings; followed by 4 to 5 layers of tangentially elongated, thin-walled, parenchymatous cells; endosperm consists of a layer of thick-walled cells containing aleurone grains, several layers of thin walled, mucilaginous cells, varying in size, long axis radially elongated in outer region and tangentially elongated in inner region; cotyledons consists of 3 to 4 layers of palisade cells varying in size with long axis and a few layers of rudimentary spongy tissue; rudimentary vascular tissue situated in spongy mesophyll; cells of cotyledon contain aleurone grains and oil globules.

#### c) Powder:

Powder shows groups of palisade parenchymatous and bearer cells in top and side views, aleurone grains, oil globules, endosperm and epidermal cells of testa (Fig. 1).



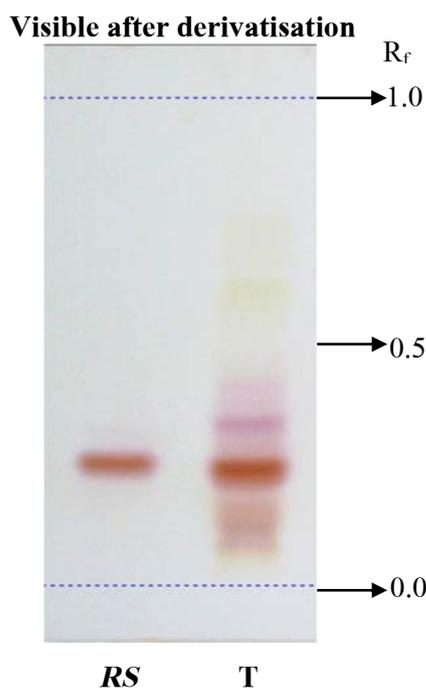
**Fig. 1: Powdered drug of Methī  
(*Trigonella foenum-graecum* L.)**

### Identity, Purity and Strength:

#### Identification:

##### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using 4-*hydroxyisoleucine* as a reference standard. *Test solution:* Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 2.5 mg of 4-*hydroxyisoleucine* RS in 10 ml of *methanol*.



**Fig. 2: Thin-Layer Chromatogram of Methi**  
**RS: 4-Hydroxyisoleucine, T: Test solution**

**Procedure:** Apply 10  $\mu\text{l}$  each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *n*-butanol : acetic acid : water (40 : 10 : 10). Dry the plate in air. Spray the plate with 1 per cent *ninhydrin* in *methanol* reagent and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

#### 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 4.0 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 5.0 per cent (Appendix 2.1.8); **Water-soluble extractive:** not less than 10.0 per cent (Appendix 2.1.9)

#### 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 of Saponins:

Carry out the assay by *gravimetry*. Take about 5 g, accurately weighed, of the substance being examined and reflux with 50 per cent of *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate and evaporate to dryness under reduced pressure. Add 25 ml of *petroleum ether* (40<sup>0</sup>-60<sup>0</sup>) to the residue and reflux for 10 min cool and decant the *petroleum ether* layer. Add 10 ml of *methanol* to the residue and dissolve, add 100 ml of *acetone*; filter the precipitate in a tared filter paper. Dry the residue at 80<sup>0</sup> for one hour and weigh the residue. Calculate the content of saponins from the weight of the residue.

#### Assay:

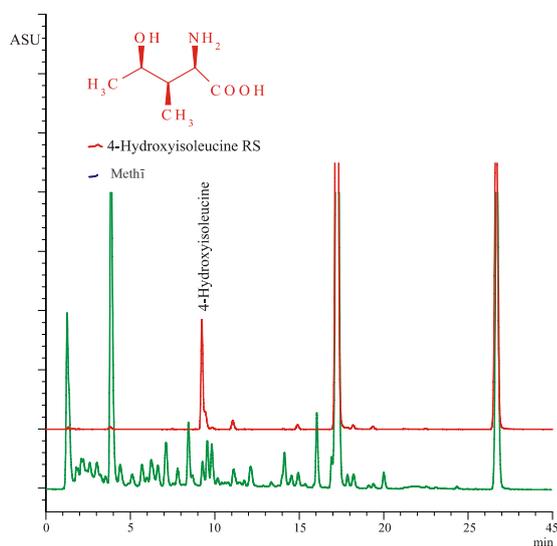
Carry out the assay by *liquid chromatography* (Appendix 3.6). **Test solution:** Take about 0.5 g, accurately weighed, of the substance being examined in a 25-ml of volumetric flask and add 2.5 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *Water*), sonicate until all the sample is dissolved. Add 125  $\mu\text{l}$  of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water*. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 2.5 mg, accurately weighed, 4-hydroxyisoleucine RS in a 10 ml volumetric flask and add 1 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *Water*), sonicate until the entire sample is dissolved. Add 50  $\mu\text{l}$  of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (100 mm x 3.0 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and

degassed gradient mixture of *acetonitrile* and *water* containing 0.1 per cent of *orthophosphoric acid* in the following proportions:

Time (min)	Water containing 0.1 per cent orthophosphoric acid (per cent)	Acetonitrile (per cent)
0.01	80	20
30	30	70
32	0	100
36	0	100
38	80	20
45	80	20
0.01	80	20

*Injection volume:* 5 µl. *Flow rate:* 0.4 ml per min.

*Detection:* UV 254 nm. *Procedure:* Inject 5 µl of the standard solution and record the chromatogram. Inject 5 µl of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *4-hydroxyisoleucine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Methi with 4-Hydroxyisoleucine as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *4-Hydroxyisoleucine RS*

**Constituents:** Graecunins H, I, J, K, L, M, N; trigofenosides A, D, F, G; trigoneosides IIa, Ib, diosgenin. Trigoneosides Ia, IIb, IIIa, IIIb, Xa, Xb, XIb, XIIa, XIIb and XIIIa, yamogenin tetroside B and C, smilagenin, sarsa-sapogenin, yamogenin, tigogenin and neotigogenin, yuccagenin, gitogenin and neogitogenin, vitexin, saponaretin, homoorientin, vicenin-1 and vicenin-2. Seed oil contains octadecatrienoic acid

**Properties and Action:** Rasa: Tikta; Guṇa: Laghu, Snigdha; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Dīpana, Kaphahara, Rucya, Vātahara

**Important formulations:** Mustakāriṣṭa, Mṛtasañjīvanī surā

**Therapeutic uses:** Aruci (tastelessness), Grahaṇī (malabsorption syndrome), Jvara (fever), Prameha (increased frequency turbidity of urine)

**Dose:** Cūrṇa (powder): 3-6 g

## METHĪ HYDRO-ALCOHOLIC EXTRACT

Methī Hydro-alcoholic Extract is a dried and powdered extract prepared from Methī (appropriately powdered). The extract contains not less than 50 per cent of *saponins* and not less than 0.5 per cent of *4-hydroxyisoleucine* when assayed.

### Method of preparation:

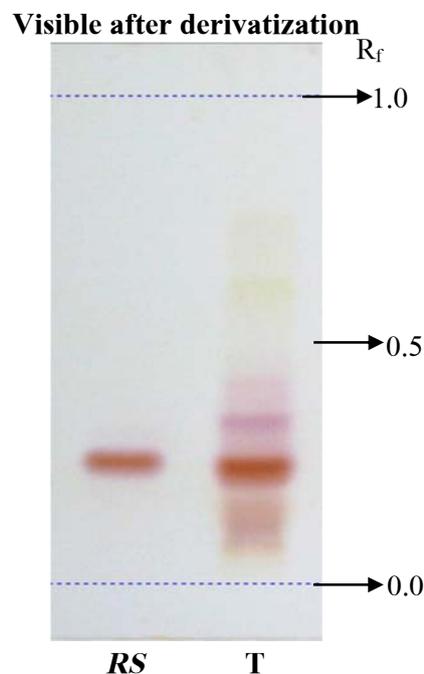
Take Methī suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 6 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *4-hydroxyisoleucine* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 2.5 mg of *4-hydroxyisoleucine* RS in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *n-butanol* : *acetic acid* : *water* (40 : 10 : 10). Dry the plate in air. Spray the plate with 1 per cent *ninhydrin* in *methanol* reagent and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The

chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Methī hydro-alcoholic extract**

**RS: 4-Hydroxyisoleucine, T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 7.0 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-7.0 (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 of Saponins:

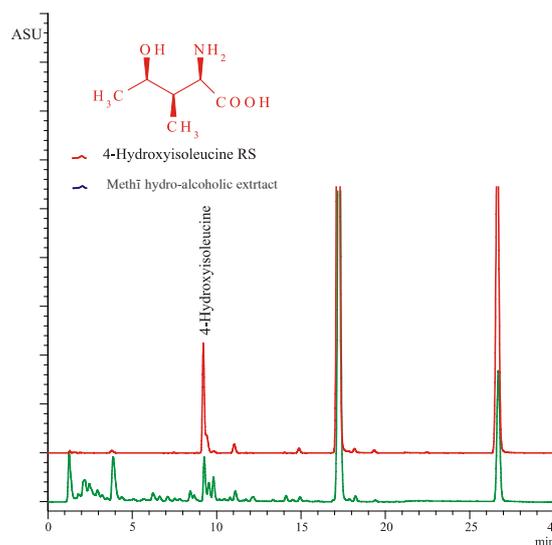
Carry out the assay by *gravimetry*. Take about 5 g, accurately weighed, of the substance being examined and reflux with 50 per cent of *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate and evaporate to dryness under reduced pressure. Add 25 ml of *petroleum ether* (40<sup>0</sup>-60<sup>0</sup>) to the residue and reflux for 10 min cool and decant the *petroleum ether* layer. Add 10 ml of *methanol* to the residue and dissolve, add 100 ml of *acetone*; filter the precipitate in a tared filter paper. Dry the residue at 80<sup>0</sup> for one hour and weigh the residue. Calculate the content of saponins from the weight of the residue.

### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). **Test solution:** Take about 0.5 g, accurately weighed, of the substance being examined in a 25-ml of volumetric flask and add 2.5 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *Water*), sonicate until all the sample is dissolved. Add 125 µl of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water* and filter through 0.42 µm membrane. **Standard solution:** Take about 2.5 mg, accurately weighed, 4-*hydroxyisoleucine RS* in a 10-ml volumetric flask and add 1 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *Water*), sonicate until the entire sample is dissolved. Add 50 µl of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water* and filter through 0.42 µm membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (100 mm x 3.0 mm, 5 µm). **Mobile phase:** Filtered and degassed gradient mixture of *acetonitrile* and *water* containing 0.1 per cent of *orthophosphoric acid* in the following proportions:-

Time (min)	Water containing 0.1 percent orthophosphoric acid (per cent)	Acetonitrile (per cent)
0.01	80	20
30	30	70
32	0	100
36	0	100
38	80	20
45	80	20
0.01	80	20

**Injection volume:** 5 µl. **Flow rate:** 0.4 ml per min. **Detection:** UV 254 nm. **Procedure:** Inject 5 µl of the standard solution and record the chromatogram. Inject 5 µl of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of 4-*hydroxyisoleucine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Methi hydro-alcoholic extract with 4- Hydroxyisoleucine as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API 4-*Hydroxyisoleucine RS*

## METHĪ WATER EXTRACT

Methī Water Extract is dried and powdered extract prepared from Methī. The extract contains not less than 20 per cent of *saponins* and not less than 0.2 per cent of *4-hydroxyisoleucine* when assayed.

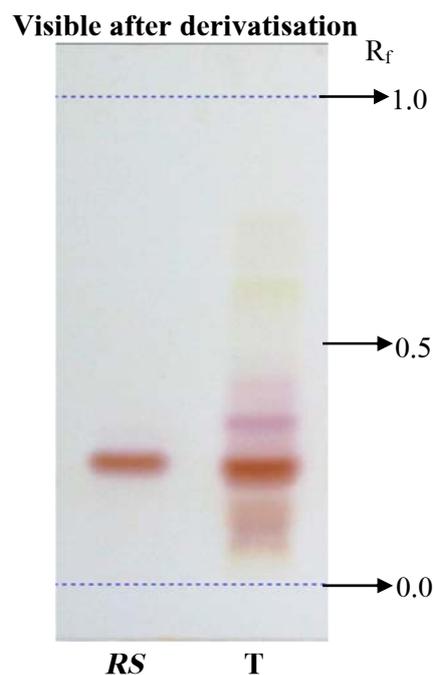
### Method of preparation:

Take Methī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh and pack. The yield obtained is about 6 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *4-hydroxyisoleucine* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 2.5 mg of *4-hydroxyisoleucine* RS in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *n-butanol* : *acetic acid* : *Water* (40 : 10 : 10). Dry the plate in air. Spray the plate with 1 per cent *ninhydrin* in *methanol* reagent and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Methī water extract**

**RS: 4-Hydroxyisoleucine, T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 8.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-7.0 (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 of Saponins:

Carry out the assay by *gravimetry*. Take about 5 g, accurately weighed, of the substance being examined and reflux with 50 per cent of *methanol* (50 ml x 3) on water bath for one hour each, cool and filter. Combine all the filtrates, concentrate

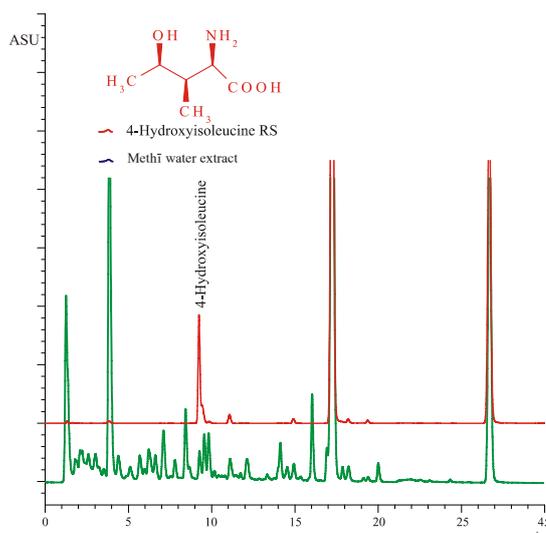
and evaporate to dryness under reduced pressure. Add 25 ml of *petroleum ether* (40<sup>0</sup>-60<sup>0</sup>) to the residue and reflux for 10 min cool and decant the *petroleum ether* layer. Add 10 ml of *methanol* to the residue and dissolve, add 100 ml of *acetone*; filter the precipitate in a tared filter paper. Dry the residue at 80<sup>0</sup> for one hour and weigh the residue. Calculate the content of saponins from the weight of the residue.

### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). **Test solution:** Take about 0.5 g, accurately weighed, of the substance being examined in a 25-ml of volumetric flask and add 2.5 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *Water*), sonicate until all the sample is dissolved. Add 125 µl of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water* and filter through 0.42 µm membrane. **Standard solution:** Take about 2.5 mg, accurately weighed, *4-hydroxyisoleucine RS* in a 10-ml volumetric flask and add 1 ml of coupling solution (40 ml of *acetonitrile* : 8 ml of *triethylamine* : 12 ml of *water*), sonicate until the entire sample is dissolved. Add 50 µl of *phenyl isothiocyanate* to the solution and sonicate for 5 min and make up the volume with *methanol*. Dilute 5 ml of this solution to 50 ml with mixture of 35 volumes of *methanol* and 65 volumes of *water* and filter through 0.42 µm membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (100 mm x 3.0 mm, 5 µm). **Mobile phase:** Filtered and degassed gradient mixture of *acetonitrile* and *water* containing 0.1 per cent of *orthophosphoric acid* in the following proportions:-

Time (min)	Water containing 0.1 per cent orthophosphoric acid (per cent)	Acetonitrile (per cent)
0.01	80	20
30	30	70
32	0	100
36	0	100
38	80	20
45	80	20
0.01	80	20

**Injection volume:** 5 µl. **Flow rate:** 0.4 ml per min. **Detection:** UV 254 nm. **Procedure:** Inject 5 µl of the standard solution and record the chromatogram. Inject 5 µl of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *4-hydroxyisoleucine* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Methi water extract with 4-Hydroxyisoleucine as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API 4-Hydroxyisoleucine RS

## NIRGUṄḌĪ

Nirguṅḍī consists of dried leaves of *Vitex negundo* (L.) Dunal. (Fam. Verbenaceae), a large aromatic shrub or a small tree, upto 4.5 m in height, common throughout the country ascending to an altitude of 1500 m in the outer Himalayas. It is common in waste places around villages, river banks, moist localities and in the deciduous forests. Nirguṅḍī contains not less than 0.25 per cent of *negundoside* and 1.0 per cent *agnuside* when assayed.

**Synonyms:** Sinduvāra, Śephālikā, Nīla

**Other/Regional Language Names:** *Assamese:* Aslak; *Bengali:* Nirgundi, Nishinda; *Gujarati:* Nagod; *Hindi:* Nirgundi, Sindur, Sambhalu; *Kannada:* Lakkigida, Nekkigida; *Malayalam:* Indranee, Nirgundi; *Marathi:* Nirgudi; *Punjabi:* Sambhalu, Banna; *Tamil:* Karunochchi, Nocchi; *Telugu:* Nallavavilli, Vavili; *Urdu:* Sambhalu, Panjangusht

### Description:

#### a) Macroscopic:

Leaves born on a rachis 2.5-3.8 cm long; palmately compound, mostly trifoliolate, occasionally pentafoliolate; in trifoliolate leaf, leaflet lanceolate or narrowly lanceolate, middle leaflet 5-10 cm long and 1.6-3.2 cm broad, with 1-1.3 cm long petiole, remaining two sub-sessile; in pentafoliolate leaf inner three leaflets have petiole and remaining two sub-sessile; surface glabrous above and tomentose beneath; texture leathery.

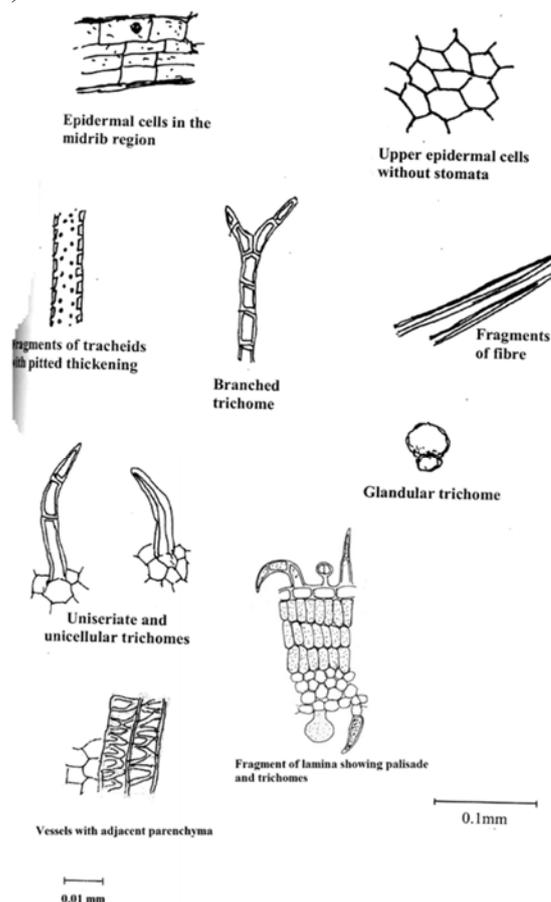
#### b) Microscopic:

**Rachis** - T.S. shows single layered epidermis having a number of unicellular, bicellular and uniseriate multicellular covering trichomes and also glandular trichomes with uni to tricellular stalk and uni to bicellular head; cortex composed of outer collenchymatous tissue and inner 6-8 layers of parenchymatous tissue; collenchyma well developed in basal region and gradually decreases towards middle and distal regions; pericyclic fibres absent in the basal region of petiole but present in the form of

a discontinuous ring in the distal region surrounding a central crescent shaped stele; a few smaller vascular bundles present below the crescent and two, or rarely three, bundles situated above the crescent.

**Lamina** - shows single layered epidermis having mostly unicellular hairs, bi and multicellular and glandular trichomes being rare; hypodermis 1-3 layered, interrupted at places by 4-8 palisade layers containing chlorophyll; mesophyll almost entirely of palisade cell layers, with isodiametric parenchymatous cells sparsely distributed in the spongy region, and present more at the edges of the lamina; a large number of veins enclosed by bundle sheath traverse mesophyll; stomata present only on the ventral surface, covered densely with trichomes; vein-islet and vein termination number of leaf are 23-25 and 5-7 respectively.

#### c) Powder:



**Fig. 1: Powdered drug of NIRGUṄḌĪ (*Vitex negundo* (L.) Dunal.)**

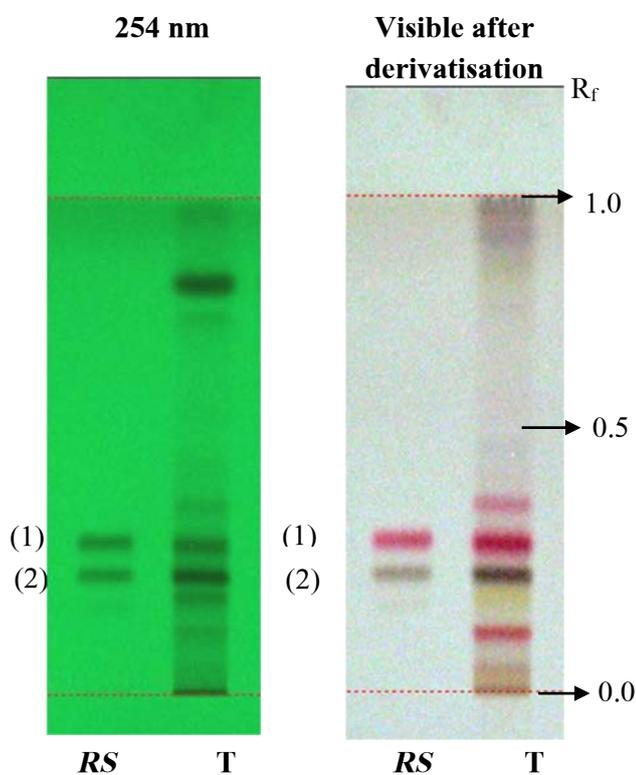
The powder shows bicellular trichomes and groups of vessels with scalariform thickenings besides tissue fragments comprising both thin and thick walled cells (Fig. 1).

### Identity, Purity and Strength:

#### Identification:

##### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *negundoside* and *agnuside* as reference standards.



**Fig. 2: Thin-Layer Chromatogram of Nirgundī**  
**RS: (1) Negundoside and (2) Agnuside,**  
**T: Test solution**

**Procedure:** Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *glacial acetic acid* : *water* (8.0 : 1.0 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with of *anisaldehyde-sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic

profile of the test solution shows bands corresponding to that of the standard solution (Fig. 2).

#### 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 8.0 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 10.0 per cent (Appendix 2.1.8); *Water-soluble extractive*: not less than 20.0 per cent (Appendix 2.1.9)

#### 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:

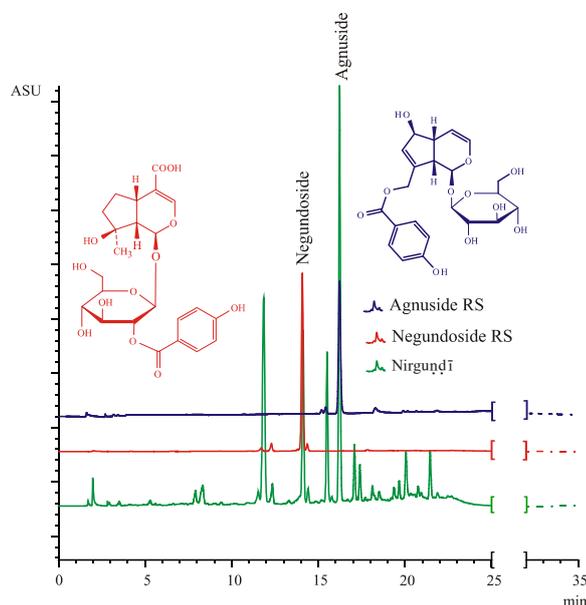
Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 1 g, accurately weighed, of the substance being examined to a 100-ml round bottomed flask, reflux with *methanol* (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, each of *negundoside RS* and *agnuside RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *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 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
10	85	15
20	70	30
23	85	15
25	95	5
35	95	5

**Injection volume:** 20  $\mu$ l. **Flow rate:** 1.5 ml per min. **Detection:** UV 254 nm. **Procedure:** Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
Negundoside	1.00
Agnuside	1.15

Calculate the content of *negundoside* and *agnuside* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatograms of Nirgunḍī with Negundoside and Agnuside as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *Negundoside RS* and *Agnuside RS*

**Constituents:** Negundoside, nishindaside, agnuside, mussaenoside tetraacetate, mussaenoside pentaacetate, 6'-*p*-hydroxybenzoyl mussaenosidic acid, 2'-*p*-hydroxybenzoyl mussaenosidic acid, sabinene, *p*-cymene,  $\beta$ -phellandrene,  $\gamma$ -terpinene, terpinen-4-ol,  $\beta$ -caryophyllene, viridiflorol,  $\beta$ -eudesmol,  $\alpha$ -pinene,  $\Delta$ -carene, limonene, camphene, citral, caryophyllene, methyl heptanone, linalool, camphor, 1,8-cineole,  $\alpha$ -terpineol, geraniol, caryophyllene oxide, terpenyl acetate, geranyl acetate, benzaldehyde, cinnamaldehyde, 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone, 5,3'-dihydroxy-7,8,4'-trimethoxy flavanone, 5,3'-dihydroxy-6,7,4'-trimethoxy flavanone, 4,4'-dimethoxy-trans-stilbene, 5,6,7,8,3'4'5'-heptamethoxy flavone, 5-*O*-desmethylnobiletin, gardenin A, B, corymbosin

**Properties and Action:** Rasa: Tikta, Kaṭu, Kaṣāya; Guṇa: Laghu; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Kāsaghna, Kṛmighna, Kaphahara, Vātahara, Vraṇaśodhana

**Important formulations:** Daśmūla taila, Mahāvātavidhvamśana rasa, Nirgunḍī taila, Tribhuvanakīrti rasa, Trivikrama rasa, Vātagajāṅkuśa rasa, Viṣatinduka taila, Yakṛtplīhāri lauha

**Therapeutic uses:** Aruci (anorexia), Śvāsa (dyspnoea), Kāsa (cough), Kṛmi (helminthiasis), Pratiśyāya (coryza), Sandhiśoṭha (arthritis), Śoṭha (inflammation), Vātavyādhi (disease due to vāta doṣa/neurological disease), Vraṇa (wound)

**Dose:** Cūrṇa (powder): 3-6 g

## NIRGUṄḌĪ HYDRO-ALCOHOLIC EXTRACT

Nirguṅḍī Hydro-alcoholic Extract is a dried and powdered extract prepared from Nirguṅḍī (appropriately powdered). The extract contains not less than 1 per cent of *negundoside* and 0.05 per cent *agnuside* when assayed.

### Method of preparation:

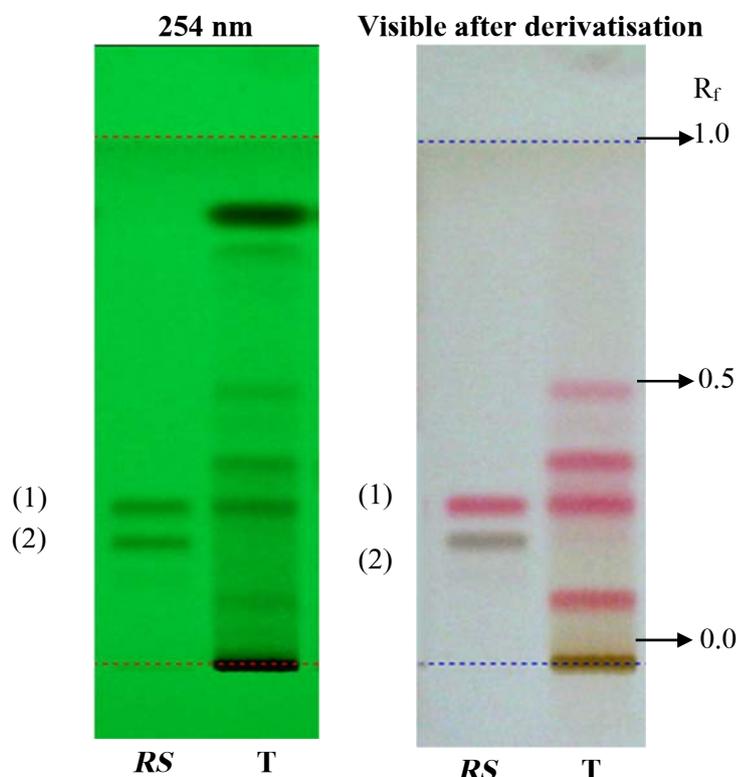
Take Nirguṅḍī suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 µm pore size) to a suitable sized 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<sup>0</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *negundoside* and *agnuside* as reference standards. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg each of *negundoside RS* and *agnuside RS* in 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate : glacial acetic acid : water* (8.0 : 1.0 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with of *anisaldehyde-sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear

without charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Nirguṅḍī hydro-alcoholic extract**  
**RS: (1) Negundoside and (2) Agnuside,**  
**T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 5.0 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-6.0 (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:

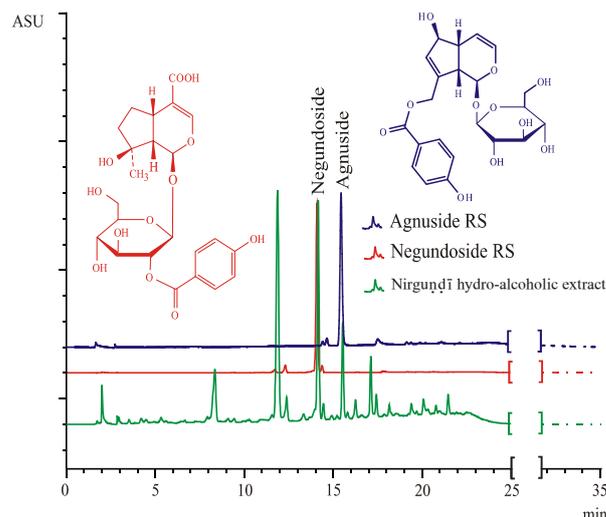
Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 1 g, accurately weighed, of the substance being examined to a 100-ml round bottomed flask, reflux with *methanol* (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, each of *negundoside RS* and *agnuside RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *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 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
10	85	15
20	70	30
23	85	15
25	95	5
35	95	5

*Injection volume*: 20 µl. *Flow rate*: 1.5 ml per min. *Detection*: UV 254 nm. *Procedure*: Inject 20 µl of the standard solution and record the chromatogram. Inject 20 µl of the test solution, record the chromatogram and identify the analyte peaks using the relative retention times.

Analyte	Relative retention time
<i>Negundoside</i>	1.00
<i>Agnuside</i>	1.15

Calculate the content of *negundoside* and *agnuside* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Nirgundī hydro-alcoholic extract with *Negundoside* and *Agnuside* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *Negundoside RS* and *Agnuside RS*

## NIRGUṆḌĪ WATER EXTRACT

Nirguṇḍī Water Extract is a dried and powdered extract prepared from Nirguṇḍī. The extract should contain not less than 1 per cent of *negundoside* and 0.05 per cent *agnuside* when assayed.

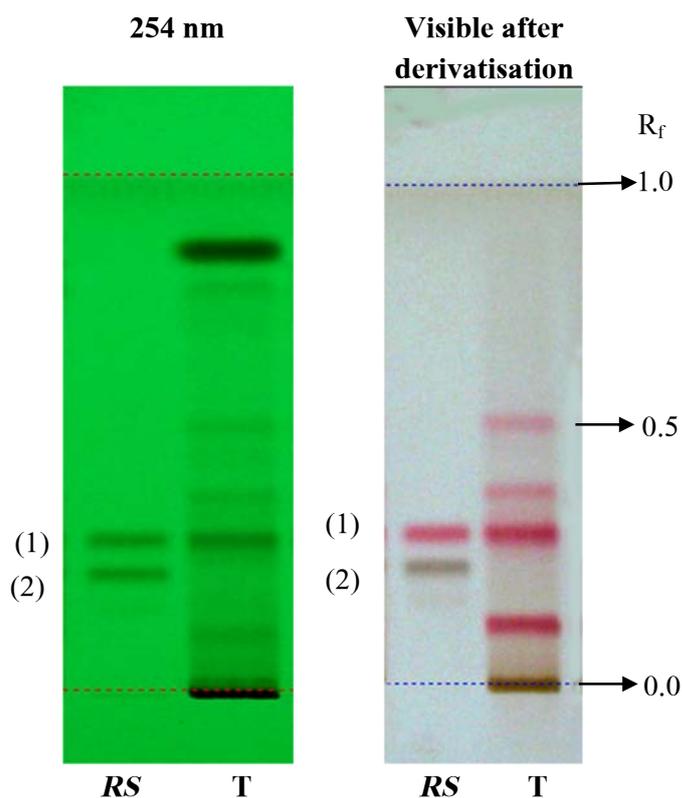
### Method of preparation:

Take Nirguṇḍī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 µm mesh to obtain the extract and pack. The yield obtained is about 23 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *negundoside* and *agnuside* as reference standards. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg each of *negundoside RS* and *agnuside RS* in 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate: glacial acetic acid : water* (8.0 : 1.0 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Nirguṇḍī water extract**  
**RS: (1) *Negundoside* and (2) *Agnuside*,**  
**T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 6.0 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); *pH*: 4.0-6.0 (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:**

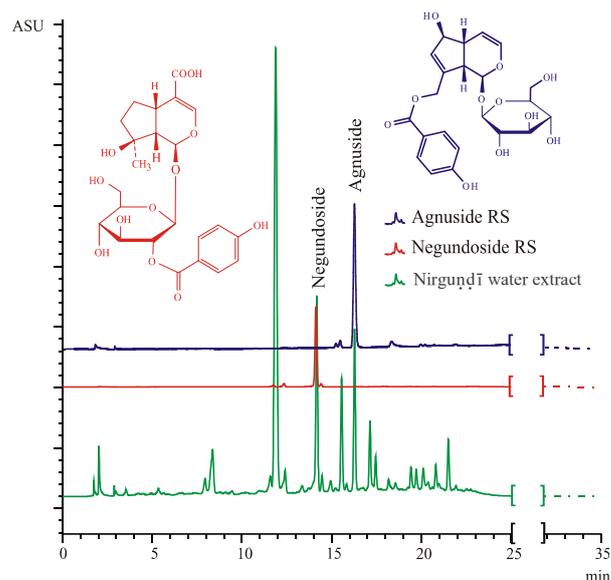
Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 1 g, accurately weighed, of the substance being examined to a 100 ml round bottomed flask, reflux with *methanol* (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, each of *negundoside RS* and *agnuside RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *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 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
10	85	15
20	70	30
23	85	15
25	95	5
35	95	5

*Injection volume*: 20 µl. *Flow rate*: 1.5 ml per min. *Detection*: UV 254 nm. *Procedure*: Inject 20 µl of the standard solution and record the chromatogram. Inject 20 µl of the test solution, record the chromatogram and identify the analyte peaks using the relative retention times, as below.

Analyte	Relative retention time
<i>Negundoside</i>	1.00
<i>Agnuside</i>	1.15

Calculate the content of *negundoside* and *agnuside* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Nirgundī water extract with *Negundoside* and *Agnuside* as RS**

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standards:**

API *Negundoside RS* and *Agnuside RS*

## PUNARNAVĀ

Punarnavā consists of dried root of *Boerhaavia diffusa* L. (Fam. Nyctaginaceae); a trailing herb with stout root stock and many diffused, slender, prostrate or ascending branches. The extract contains not less than 0.005 per cent of boeravinone B when assayed.

**Synonyms:** Kaṭhillā, Śophaghnī, Śothaghnī

Other/Regional Language Names: *Assamese:* Ranga Punanabha; *Bengali:* Rakta punarnava; *English:* Horse Purslane, Hog Weed; *Gujarati:* Dholisaturdi, Motostodo; *Hindi:* Gadapura, Lalpunarnava; *Kannada:* Snadika, Kommeberu, Komma; *Kashmiri:* Vanjula punarnava; *Malayalam:* Chuvanna Tazhutama, Tavilama, Tlutama; *Marathi:* Ghetuli, Vasuchi muli, Satodimula, Punarnava, Khaparkhuti; *Oriya:* Lalapuiruni, Malipuruni; *Punjabi:* ltcit (Ial), Khattan; *Tamil:* Mukurattai (Shihappu); *Telugu:* Atikammamidi, Erragalijeru; *Urdu:* Surkh punarnava

### Description:

#### a) Macroscopic:

Root well developed, fairly long, somewhat tortuous, cylindrical, 0.2 to 1.5 cm in diameter; yellowish to brown; surface rough due to minute longitudinal striations and root scars; fracture short; odour not distinct; taste slightly bitter.

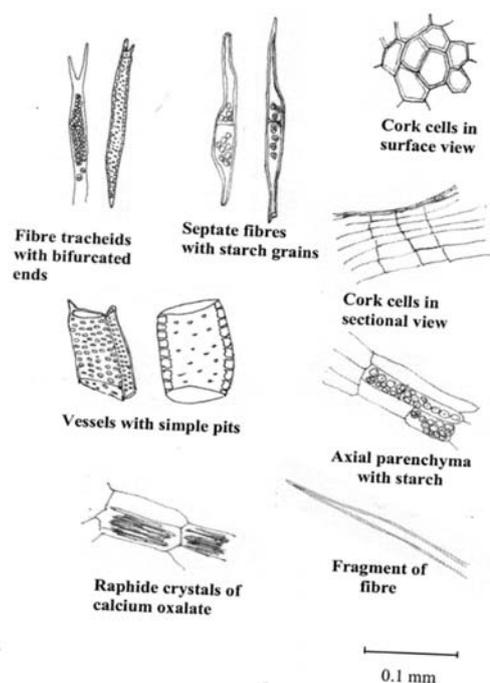
#### b) Microscopic:

Mature root shows anomalous growth; cork composed of thin-walled, tangentially elongated cells in the outer few layers; cork cambium 1 to 2 layers of thin-walled cells; secondary cortex consists of 2 to 3 layers of parenchymatous cells, followed by cortex composed of 5 to 12 layers of thin-walled, oval to polygonal cells; several concentric bands of xylem tissues, alternating with zone of parenchymatous tissue present below cortical region; number of bands vary according to thickness of root and consists of vessels, tracheids and fibres; vessels mostly found in groups of 2 to 8 in radial rows, having simple pits and reticulate

thickenings; tracheids, thick-walled with simple pits; fibres aseptate, elongate, thick-walled with pointed ends; phloem occurs as hemispherical or crescent patches outside each group of xylem vessels and composed of sieve elements and parenchyma; a broad zone of parenchymatous tissue, in between two successive rings of xylem elements, composed of thin-walled, more or less rectangular cells arranged in radial rows; central region of root occupied by primary vascular bundles; numerous raphides in single or in groups present in cortical region and in parenchymatous and xylem tissue; starch grains simple measuring up to 11  $\mu$  in diameter and compound, having 2 to 4 components, found in abundance in most of the cells of cortex and xylem elements.

#### c) Powder:

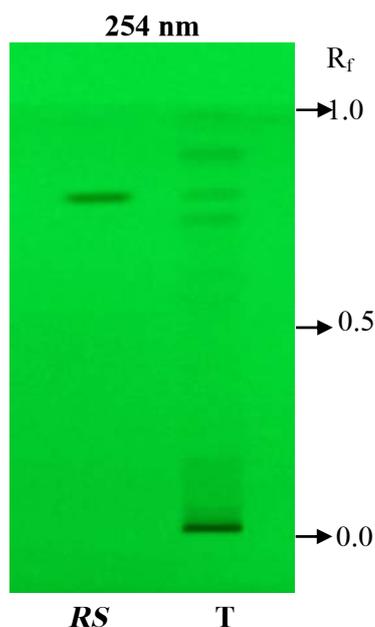
Light yellow; shows vessels with reticulate thickenings or simple pits, fibres, fragments of cork cells, cells containing raphides of calcium oxalate and simple, rounded, starch grains, measuring 2.75 to 11  $\mu$  in diameter and compound starch grains having 2 to 4 components (Fig. 1.)



**Fig. 1: Powdered drug of PUNARNAVĀ**  
(*Boerhaavia diffusa* L.)

**Identity, Purity and Strength:****Identification:***Thin-layer chromatography:*

Carry out thin-layer chromatography on a pre-coated silica gel 60F254 plate (Appendix 3.5) using boeravinone B as a reference standard. Test solution: Extract 2 g of substance by refluxing with 50 ml of methanol for a period of 15 min. Filter and concentrate the extract to 25 ml. Standard solution: Dissolve 2 mg of boeravinone B RS in 10 ml of methanol. Procedure: Apply 10  $\mu$ l each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: toluene : ethyl acetate : formic acid (4.0 : 5.0 : 1.0).



**Fig. 2: Thin-Layer Chromatogram of Punarnava**  
RS: Boeravinone B, T: Test solution

Dry the plate in air and examine under UV 254 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

**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 5.0 per cent (Appendix 2.1.8); *Water-soluble extractive:* not less than 8.0 per cent (Appendix 2.1.9)

**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)

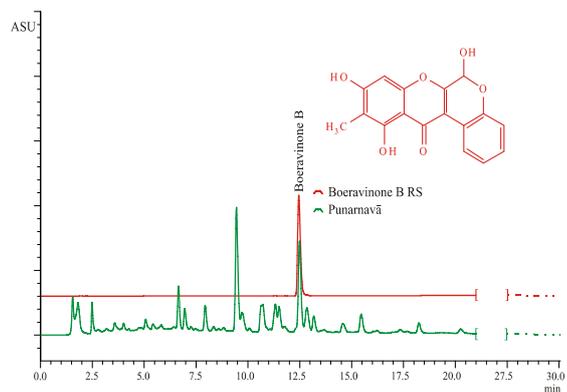
**Assay:**

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution:* Take about 5 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume. Filter through 0.42  $\mu$ m membrane. *Standard solution:* Take about 2.5 mg, accurately weighed, *boeravinone B RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu$ m membrane. *Chromatographic system:* High performance liquid chromatography. *Column and stationary phase:* C18 (250 mm x 4.6 mm, 5  $\mu$ m). *Mobile phase:* Filtered and degassed gradient mixture of *water* and *acetonitrile* in the following proportions:-

Time (min)	Water (per cent)	Acetonitrile (per cent)
0.01	80	20
5	55	45
15	20	80
20	20	80
23	55	45
25	80	20
30	80	20

*Injection volume:* 20  $\mu$ l. *Flow rate:* 1.5 ml per min. *Detection:* UV 280 nm. *Procedure:* Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution,

record the chromatogram and measure the response for the analyte peak. Calculate the content of *boeravinone B* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Punarnavā with *Boeravinone B* as RS**

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standard:

API *Boeravinone B* RS

**Constituents:** Punarnavoside, boeravinone C, liriiodendrin; hypoxanthine-9-L-arabinofuranoside, eupalitin-3-*O*- $\beta$ -D-galactopyranoside. Eupalitin, eupalitin-3-*O*- $\beta$ -D-galactopyranosyl-(1'''' $\rightarrow$ 2''')-*O*- $\beta$ -D-galactopyranoside, 3,3',5-trihydroxy-7-methoxyflavone, 4',7-dihydroxy-3'-methylflavone, 3,4-dimethoxy phenyl-1-*O*- $\beta$ -D-apiofuranosyl-(1'' $\rightarrow$ 3')-*O*- $\beta$ -D-glucopyranoside.

**Properties and Action:** Rasa: Madhura, Tikta; Guṇa: Rūkṣa; Vīrya: Uṣṇa; Vipāka: Madhura; Karma: Anulomana, Mūtravirecanīya, Rasāyana, Śothahara, Vātaśleṣmahara

**Important formulations:** Punarnavāṣṭaka kvātha cūrṇa, Punarnavādi maṇḍūra, Punarnavāsava, Sukumāra ghṛta

**Therapeutic uses:** Dhātu kṣaya (tissue wasting), Pāṇḍu (anaemia), Śoṭha (inflammation)

**Dose:** Cūrṇa (powder): 3-6 g

## PUNARNAVĀ HYDRO-ALCOHOLIC EXTRACT

Punarnavā Hydro-alcoholic Extract is a dried and powdered extract prepared from Punarnavā (appropriately powdered). The extract contains not less than 0.025 per cent of *boeravinone B* when assayed.

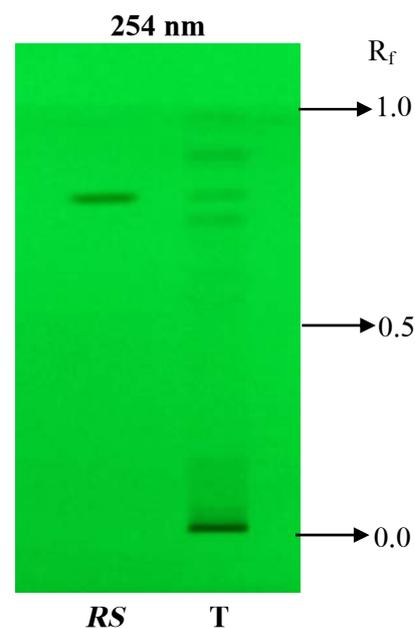
### Method of preparation:

Take Punarnavā 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *boeravinone B* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 2 mg of *boeravinone B* RS in 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *formic acid* (4.0 : 5.0 : 1.0). Dry the plate in air and examine under UV 254 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Punarnavā water extract**  
RS: *Boeravinone B*, T: Test solution

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 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)

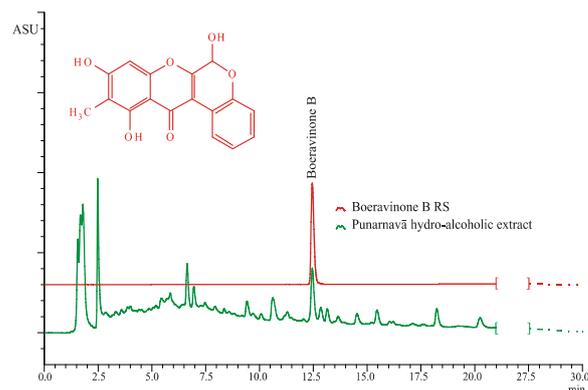
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 5 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3)

on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 2.5 mg, accurately weighed, *boeravinone B RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed gradient mixture of *water* and *acetonitrile* in the following proportions:-

Time (min)	Water (per cent)	Acetonitrile (per cent)
0.01	80	20
5	55	45
15	20	80
20	20	80
23	55	45
25	80	20
30	80	20

**Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.5 ml per min. **Detection:** UV 280 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *boeravinone B* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Punarnavā hydro-alcoholic extract with Boeravinone B as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Boeravinone B RS*

## PUNARNAVĀ WATER EXTRACT

Punarnavā Water Extract is a dried and powdered extract prepared from Punarnavā. The extract contains not less than 0.0025 per cent of *boeravinone B* when assayed.

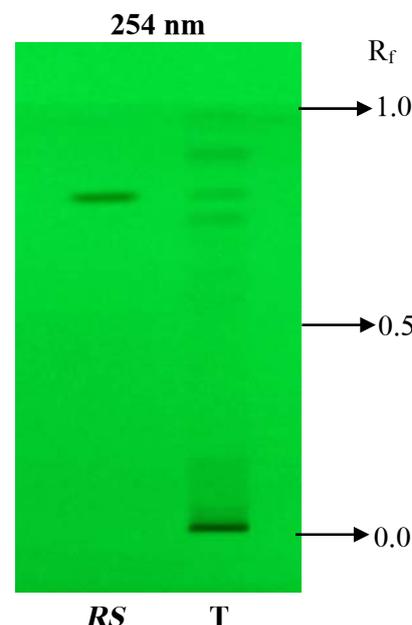
### Method of preparation:

Take Punarnavā 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° 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° till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 µm mesh and pack. The yield obtained is about 8 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *boeravinone B* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 2 mg of *boeravinone B RS* in 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase : *toluene* : *ethyl acetate* : *formic acid* (4.0 : 5.0 : 1.0). Dry the plate in air and examine under UV 254 nm. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Punarnavā water extract**  
**RS: Boeravinone B, T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 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-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)

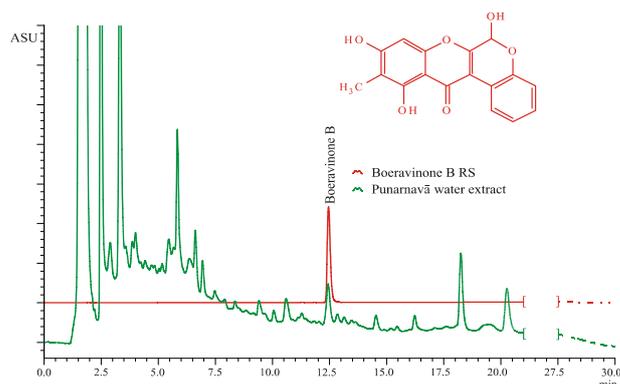
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 5 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml

volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. *Standard solution:* Take about 2.5 mg, accurately weighed, *boeravinone B RS* in a 100 ml volumetric flask and dissolve in about 50 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. *Chromatographic system:* High performance liquid chromatography. *Column and stationary phase:* C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). *Mobile phase:* Filtered and degassed gradient mixture of *water* and *acetonitrile* in the following proportions:

Time (min)	Water (per cent)	Acetonitrile (per cent)
0.01	80	20
5	55	45
15	20	80
20	20	80
23	55	45
25	80	20
30	80	20

*Injection volume:* 20  $\mu\text{l}$ . *Flow rate:* 1.5 ml per min. *Detection:* UV, 280 nm. *Procedure:* Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *boeravinone B* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Punarnavā water extract with *Boeravinone B* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Boeravinone B RS*

## ŚALLAKĪ (Exudate)

Śallakī consists of exudate of *Boswellia serrata* Roxb. (Fam. Burseraceae), a moderate sized, deciduous tree, upto 18 m in height and upto 2.4 m in girth, commonly found in the dry forests from Punjab to West Bengal and in peninsular India. It contains not less than 4.0 per cent of  $\beta$ -boswellic acid when assayed.

**Synonym:** Kunduru

### Other/Regional Language Names:

*Assamese:* Sallaki; *Bengali:* Salai, Salgai; *English:* Indian Olibanum Tree; *Gujarati:* Shaledum, Saleda, Saladi, Gugal, Saledhi; *Hindi:* Salai, Labana; *Kannada:* Madimar, Chilakdupa, Tallaki, Maddi; *Kashmiri:* Kunturukkam, Samprani; *Marathi:* Salai cha dink; *Punjabi:* Salai Gonda; *Tamil:* Kundurukam; *Telugu:* Anduga, Kondagugi tamu; *Urdu:* Kundur

### Description:

a) *Macroscopic:*

Drug occurs in stalactitic, transparent, tears forming agglomerates of various shapes and sizes, brownish-yellow, upto 5 cm long, 2 cm thick, fragrant, fracture brittle; fractured surface waxy and translucent; burns readily and emanates an agreeable characteristic, balsamic resinous odour; taste, aromatic and agreeable

Identification - Trituration with water forms an emulsion; when immersed in alcohol (90 per cent) a tear of Śallakī is not altered much in form but becomes almost opaque and white; when a drop of con.  $H_2SO_4$  is added to a freshly fractured surface, it becomes cherry red which, when washed with water changes to a white emulsion, turning later to a buff colour

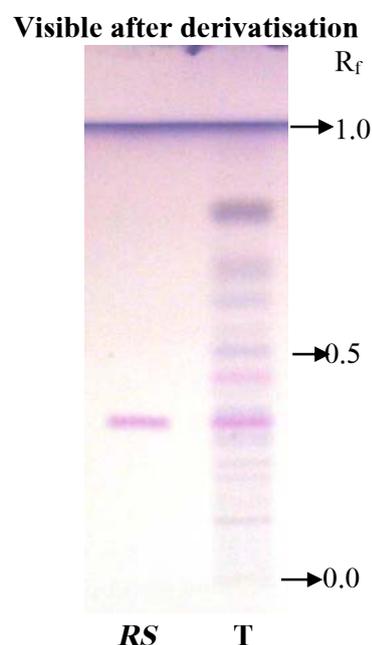
Fluorescence Test - Brownish-yellow colour in day light; aqueous extract under UV light (366 nm) light green and in (254 nm) shows dark blue colour; alcoholic extract under UV light (366 nm) is colourless and in (254 nm) shows light green colour

### Identity, Purity and Strength:

#### Identification:

*Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using  $\beta$ -boswellic acid as a reference standard. *Test solution:* Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml.



**Fig. 2: Thin-Layer Chromatogram of Śallakī**  
**RS:  $\beta$ -boswellic acid, T: Test solution**

*Standard solution:* Dissolve 10 mg of  $\beta$ -boswellic acid RS in 25 ml of *methanol*. *Procedure:* Apply 10  $\mu$ l each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *methanol* (8.0 : 1.5 : 0.5). Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/ bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

### Quantitative parameters:

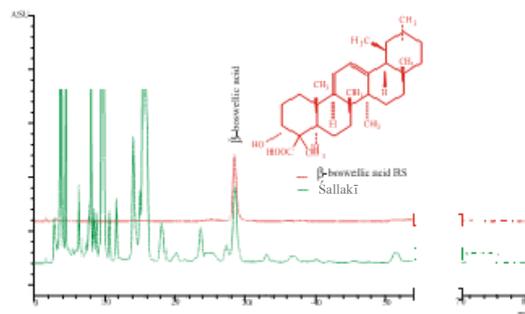
**Foreign matter:** not more than 5.0 per cent (Appendix 2.1.3); **Loss on drying:** not more than 12.0 per cent (Appendix 2.1.4); **Total ash:** not more than 10.0 per cent (Appendix 2.1.5); **Acid-insoluble ash:** not more than 8.0 per cent (Appendix 2.1.7); **Alcohol-soluble extractive:** not less than 45.0 per cent (Appendix 2.1.8); **Water-soluble extractive:** not less than 28.0 per cent (Appendix 2.1.9)

### 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 *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. **Standard solution:** Take about 10 mg, accurately weighed,  $\beta$ -boswellic acid RS in a 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume with *methanol*. **Chromatographic system:** High performance liquid Chromatography. **Column and stationary phase:** Reverse phase C18 (250 mm x 4.6 mm, 5  $\mu$ m). **Mobile phase:** Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *acetonitrile*. **Injection volume:** 20  $\mu$ l. **Flow rate:** 1.0 ml per min. **Detection:** UV 205 nm. **Procedure:** Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of,  $\beta$ -boswellic acid in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatogram of Śallakī with  $\beta$ -boswellic acid as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API  $\beta$ -boswellic acid RS

**Constituents:** Volatile oil (9 per cent), gum and resin; the volatile oil contains  $\alpha$ -thujene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene,  $\alpha$ -terpineol, limonene, camphene, myrcene,  $\alpha$ -terpene, *p*-cymene; active principles boswellic acids from resin viz.,  $\alpha$ -boswellic acid,  $\beta$ -boswellic acid, 3-*O*-acetyl- $\beta$ -boswellic acid, 11-keto- $\beta$ -boswellic acid, 3-*O*-acetyl-11-keto- $\beta$ -boswellic acid. A diterpene alcohol serratol and four tetracyclic triterpene acids 3- $\alpha$ -acetoxytirucall-8,24-dien-21-oic acid, 3-ketotirucall-8, 24-dien-21-oic acid, 3- $\alpha$ -hydroxytirucall-8, 24-dien-oic acid, 3- $\beta$ -hydroxytirucall-8, 24-dien-21-oic acid

**Properties and Action:** Rasa: Tikta, Madhura; Guṇa: Guru, Snigdha, Tikṣṇa; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Balya, Kaphahara, Kaphapittahara, Raktastambhana, Śothahara, Vātahara

**Important formulations:** Balāguḍūcyādi taila, Balā taila, Jīrkādi modaka, Karpūrādyarka

**Therapeutic uses:** Jvara (fever), Mukharoga (disease of mouth), Pittābhiṣyanda (conjunctivitis due to pitta doṣa), Pradara (excessive vaginal discharge), Śarkarāmeha (glycosuria), Sandhiśūla (joint pain), Śūla (pain), Śvāsa (dyspnoea)

**Dose:** Cūrṇa (powder): 1-3 g

## ŚALLAKĪ HYDRO-ALCOHOLIC EXTRACT

Śallakī Hydro-alcoholic Extract is a dried and powdered extract prepared from Śallakī (appropriately powdered). The extract contains not less than 0.4 per cent of *β-boswellic acid* when assayed.

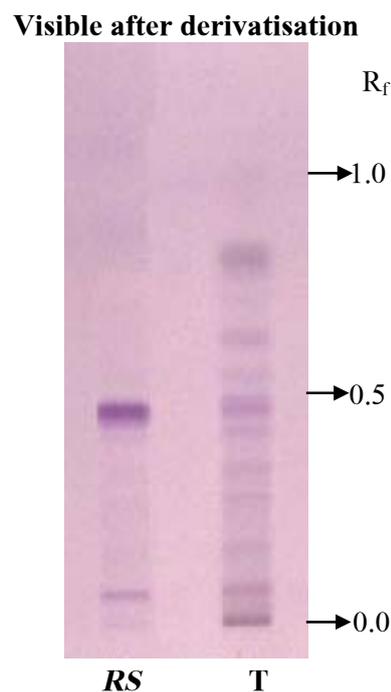
### Method of preparation:

Take Śallakī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *β-boswellic acid* as a reference standard. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *β-boswellic acid* RS in 25 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *Toluene* : *Ethyl acetate* : *Methanol* (8.0 : 1.5 : 0.5). Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots / bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Śallakī hydro-alcoholic extract**

**RS: *β-boswellic 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 4.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 1.0 per cent (Appendix 2.1.7); *pH*: 3.5-5.5 (Appendix 2.1.10); *Total soluble solids*: not less than 70.0 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:

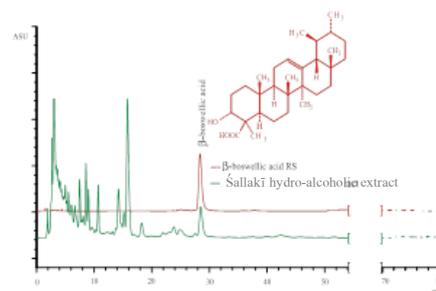
Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.4 g,

accurately weighed, of the substance being examined and reflux with *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane.

**Standard solution:** Take about 10 mg, accurately weighed,  *$\beta$ -boswellic acid RS* in a 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane.

**Chromatographic system:** High performance liquid Chromatography. **Column and stationary phase:** Reverse phase C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *acetonitrile*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.0 ml per min. **Detection:** UV 205 nm.

**Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of  *$\beta$ -boswellic acid* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Śallakī hydro-alcoholic extract with  *$\beta$ -boswellic acid as RS***

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standard:**

API  *$\beta$ -boswellic acid RS*

## ŚALLAKĪ WATER EXTRACT

Śallakī Water Extract is a dried and powdered extract prepared from Śallakī. The extract contains not less than 0.1 per cent of *β-boswellic acid* when assayed.

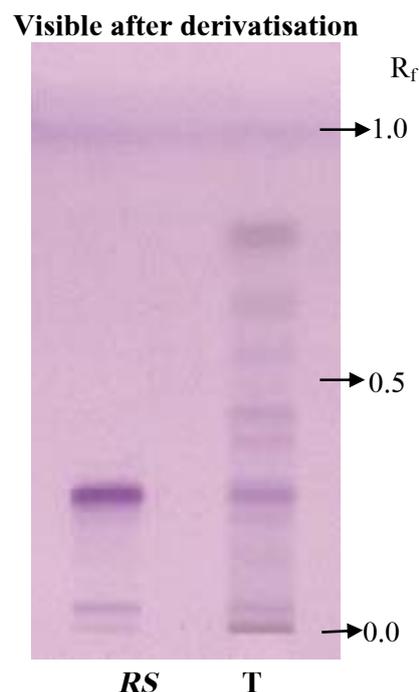
### Method of preparation:

Take Śallakī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh and pack. The yield obtained is about 12 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *β-boswellic acid* as a reference standard. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *β-boswellic acid RS* in 25 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *methanol* (8.0 : 1.5 : 0.5). Spray the plate with *anisaldehyde -sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/ bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Śallakī water extract**

**RS: *β-boswellic 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 5.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 1.0 per cent (Appendix 2.1.7); *pH*: 3.5-5.5 (Appendix 2.1.10); *Total soluble solids*: not less than 65.0 per cent (Appendix 2.1.11) (Method-II)

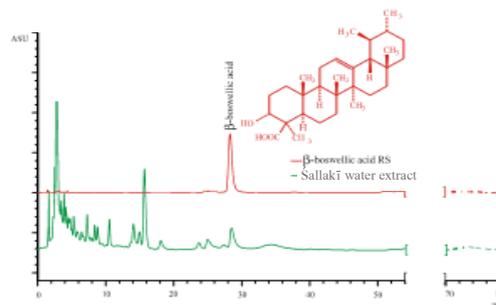
### Other requirements:

*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 *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter.

Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 10 mg, accurately weighed,  *$\beta$ -boswellic acid RS* in a 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid Chromatography. **Column and stationary phase:** Reverse phase C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *acetonitrile*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1.0 ml per min. **Detection:** UV 205 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of  *$\beta$ -boswellic acid* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Sallakī water extract with  *$\beta$ -boswellic acid* as RS**

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standard:**

API  *$\beta$ -boswellic acid RS*

## ŚIRĪṢA

Śirīṣa consists of stem bark of *Albizia lebbek* (L.) Benth. (Fam. Mimosaceae), a large tree, common throughout the country, ascending to 1200 m on the Himalayas. It contains not less than 5.0 per cent of total polyphenols calculated as *pyrogallol* and not less than 0.1 per cent of *catechin* when assayed.

**Synonyms:** Śukapriya, Mṛdupuṣpa

**Other/Regional Language Names:** *Bengali:*

Sirish, Siris; *English:* Siris Tree, Lebbeck Tree; *Gujarati:* Shrish; *Hindi:* Siris, Shris; *Kannada:* Bagey, Bage Mara, Hombage; *Malayalam:* Vaka, Nenmenivaka; *Marathi:* Shirish; *Oriya:* Sersuan, Sirisha; *Punjabi:* Sirish, Sareehn; *Tamil:* Vakai; *Telugu:* Dirisena; *Urdu:* Siris

### Description:

#### a) Macroscopic:

Bark 1.5–2.5 cm thick, external surface dark brown, rough due to longitudinal fissures and transverse cracks, rhytidoma forming major part of bark and peeling off in flakes exposing buff coloured surface, middle bark brown, inner bark much fibrous, light yellow to grey; fracture, laminated in outer region and fibrous in inner region; taste astringent

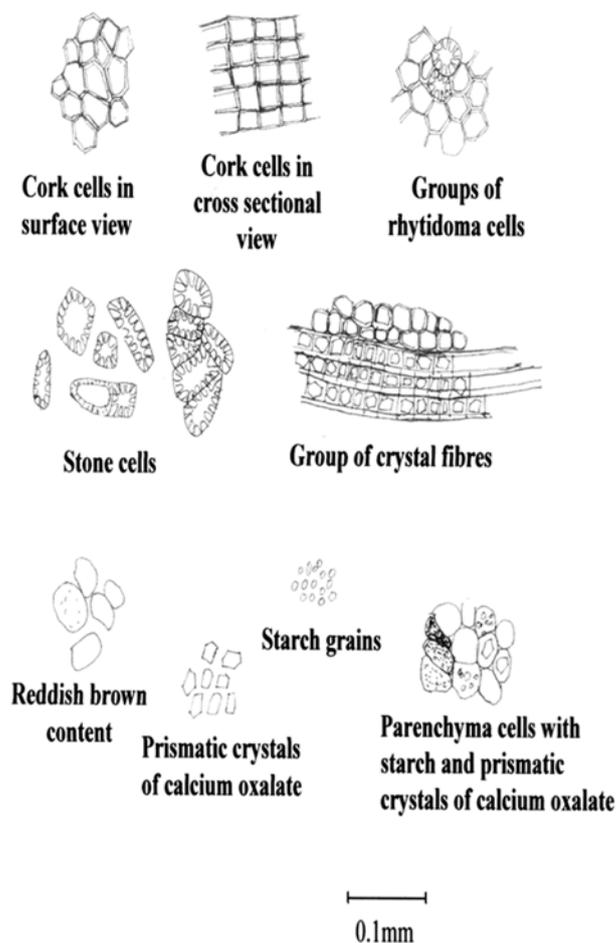
#### b) Microscopic:

Mature bark about 2 cm thick, shows dead tissue of rhytidoma; cork consists of a few layers of thin-walled, transversely elongated and radially arranged cells; secondary cortex wide, composed of radially elongated to squarish, moderately thick-walled cells containing orange to reddish-brown contents; a few of the cells contain prismatic crystals of calcium oxalate; stone cells variable in shape and size, present in singles or in groups throughout the region; secondary phloem consists of sieve elements, phloem parenchyma, phloemfibres and crystal fibres, traversed by phloem rays; prismatic crystals of calcium oxalate present in most of the phloem parenchyma cells; tangential bands of ceratenchyma

present in middle and outer phloem region; phloem fibres, elongated, thick-walled, lignified, present in many concentric strips, mostly enclosed by crystal sheath throughout the middle and inner regions of phloem; crystal fibres having a number of septa, each chamber containing a single prismatic crystal of calcium oxalate; phloem rays numerous, radially elongated, somewhat wavy in outer phloem region and bi to multiseriate in the inner phloem region, being 2-5 cells wide and 7-25 cells high

#### c) Powder:

Greyish-brown; shows large number of stone cells of different sizes and shapes from rhytidoma or cortex, prismatic crystals of calcium oxalate, crystal fibres, phloem fibres, cork cells and parenchymatous cells with starch grains (Fig.1).



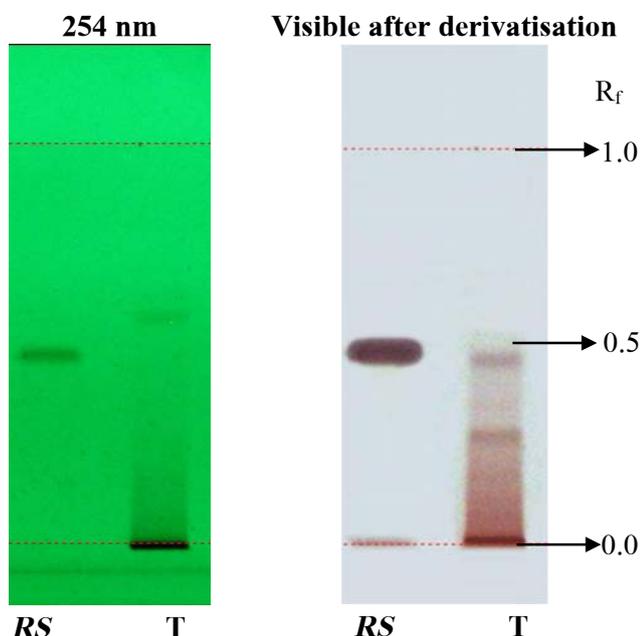
**Fig. 1: Powdered drug of ŚIRĪṢA  
(*Albizia lebbek* (L.) Benth.)**

## Identity, Purity and Strength:

### Identification:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *catechin* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *water* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *catechin RS* in 10 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *formic acid* (1.2 : 1.8 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *fast blue salt B reagent*, and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).



**Fig. 2: Thin-Layer Chromatogram of Śirīṣā**  
**RS: Catechin, T: Test solution**

### 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 8.0 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 12.0 per cent (Appendix 2.1.8); *Water-soluble extractive*: not less than 6.0 per cent (Appendix 2.1.9)

### 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).

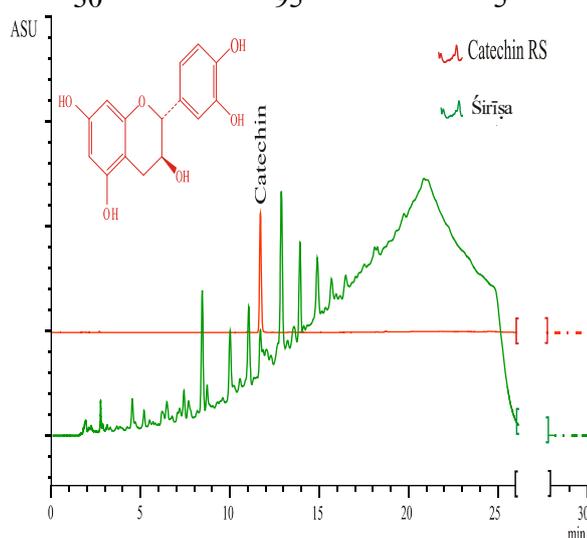
### Assay for total polyphenols:

Carry out the assay by *Spectrophotometry* (Appendix 3.7). *Test solution*: Always prepare fresh solution. Take about 500 mg, accurately weighed, of the substance being examined in a 100-ml round bottomed flask, reflux with 50 per cent *aqueous methanol* (25 ml x 3) on water bath for 15 min each, cool, filter and make up the volume to 100 ml in a volumetric flask. Further dilute 5 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 about 50 ml of 50 per cent *aqueous methanol* and make up to 100 ml with 50 per cent *aqueous methanol*. Further dilute 5 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* and 10 ml of 30.0 per cent *sodium carbonate* solution to each of the volumetric flasks. Allow to stand for 30 min. Make up the volume with *water*, shake and allow to stand for 20 min 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 4 g, accurately weighed, of the substance being examined and reflux with *water* (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100-ml volumetric flask. Filter through 0.42  $\mu\text{m}$  membrane. *Standard solution*: Take about 10 mg, accurately weighed, *catechin RS* in a 100-ml volumetric flask and dissolve in about 50 ml of *water* and make up the volume with *water*. Filter through 0.42  $\mu\text{m}$  membrane. *Chromatographic system*: High performance liquid chromatography. *Column and stationary phase*: C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). *Mobile phase*: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
18	75	25
22	75	25
25	95	5
30	95	5



**Fig. 3: HPLC chromatogram of Śirīṣa with Catechin as RS**

*Injection volume*: 20  $\mu\text{l}$ . *Flow rate*: 1.5 ml per min. *Detection*: UV 280 nm. *Procedure*: Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *catechin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standard:**

API *Catechin RS*

**Constituents:** (+)-Catechin, saponins, (+)-leucocyanidin, Albizia saponin A, Albizia saponin B, Albizia saponin C, acacic acid, albigenin

**Properties and Action:** Rasa: Tikta, Kaṣāya, Madhura; Guṇa: Laghu, Rūkṣa; Vīrya: Anuṣṇa; Vipāka: Kaṭu; Karma: Tridoṣahara, Tvagdoṣa, Varṇya, Viṣaghna

**Important formulations:** Ayaskṛti, Bṛhanmaricādyā taila, Daśāṅga lepa, Devadārvāriṣṭa, Vajraka taila

**Therapeutic uses:** Kāsa (cough), Kṛmi roga (worm infestation), Kaṇḍū (pruritis), Kuṣṭha (disease of skin), Mūṣaka viṣa (rat poisoning), Netṛābāhiṣyanda (conjunctivitis), Pāmā (eczema), Pīnasa (chronic rhinitis), Pratiśyāya (coryza), Sarpadaṁśa (snake bite), Sūryāvarta (chronic sinusitis), Śītapīta (urticaria), Śoṭha (inflammation), Ardhāvabhedaka (migraine), Śvāsa (dyspnoea), Viṣamajvara (intermittent fever), Visarpa (erysipales), Vraṇa (wound)

**Dose:** Cūrṇa (powder): 3-6 g

## ŚIRĪṢA HYDRO-ALCOHOLIC EXTRACT

Śirīṣa Hydro-alcoholic Extract is a dried and powdered extract prepared from Śirīṣa (appropriately powdered). The extract contains not less than 10 per cent of *total polyphenols* calculated as *pyrogallol* and not less than 0.1 per cent of *catechin* when assayed.

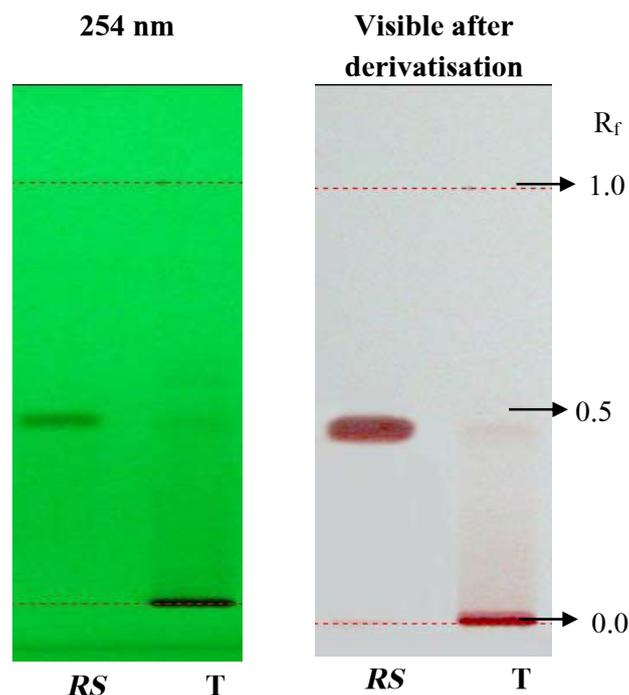
### Method of Preparation:

Take Śirīṣa 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 reflux at a temperature between 80-85<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 21 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60<sub>F<sub>254</sub></sub> plate (Appendix 3.5) using *catechin* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *water* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *catechin* RS in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *formic acid* (1.2 : 1.8 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *fast blue salt B reagent*, and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Śirīṣa hydro-alcoholic extract**  
**RS: Catechin, 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 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-6.5 (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 for total polyphenols:

Carry out the assay by *Spectrophotometry* (Appendix 3.7). *Test solution*: Always prepare fresh solution. Take about 500 mg, accurately weighed, of the substance being examined in a 100-ml round

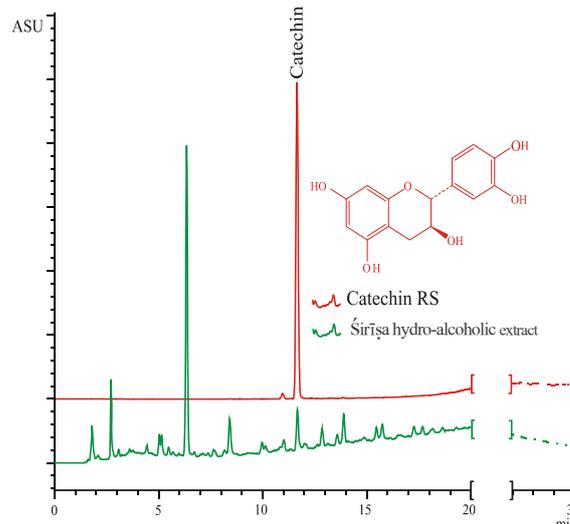
bottomed flask, reflux with 50 per cent *aqueous methanol* (25 ml x 3) on water bath for 15 min each, cool, filter and make up the volume to 100 ml in a volumetric flask. Further dilute 5 ml aliquot to 50 ml with *water* in a volumetric flask. *Standard solution*: Take about 100 mg, accurately weighed, *pyrogallol RS* in a 100-ml volumetric flask and dissolve in about 50 ml of 50 per cent *aqueous methanol* and make up to 100-ml with 50 per cent *aqueous methanol*. Further dilute 5 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* and 10 ml of 30.0 per cent *sodium carbonate* solution to each of the volumetric flasks. Allow to stand for 30 min. Make up the volume with *water*, shake and allow to stand for 20 min 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 4 g, accurately weighed, of the substance being examined and reflux with *water* (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100 ml volumetric flask. Filter through 0.42 µm membrane. *Standard solution*: Take about 10 mg, accurately weighed, *catechin RS* in a 100 ml volumetric flask and dissolve in about 50 ml of *water* and make up the volume with *water*. Filter through 0.42 µm membrane. *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 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
18	75	25
22	75	25
25	95	5
30	95	5

*Injection volume*: 20 µl. *Flow rate*: 1.5 ml per min. *Detection*: UV 280 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 peak. Calculate the content of *catechin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Śirīṣa hydro-alcoholic extract with *Catechin* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Catechin RS*

## ŚIRĪṢA WATER EXTRACT

Śirīṣa Water Extract is a dried and powdered extract prepared from Śirīṣa. The extract contains not less than 10 per cent of *total polyphenols* calculated as *pyrogallol* and not less than 0.1 per cent of *catechin* when assayed.

### Method of Preparation:

Take Śirīṣa 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 17 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60<sub>F254</sub> plate (Appendix 3.5) using *catechin* as a reference standard. *Test solution*: Extract 2 g of substance by refluxing with 50 ml of *water* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 10 mg of *catechin* RS in 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *formic acid* (1.2 : 1.8 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *fast blue salt B reagent*, and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 1).

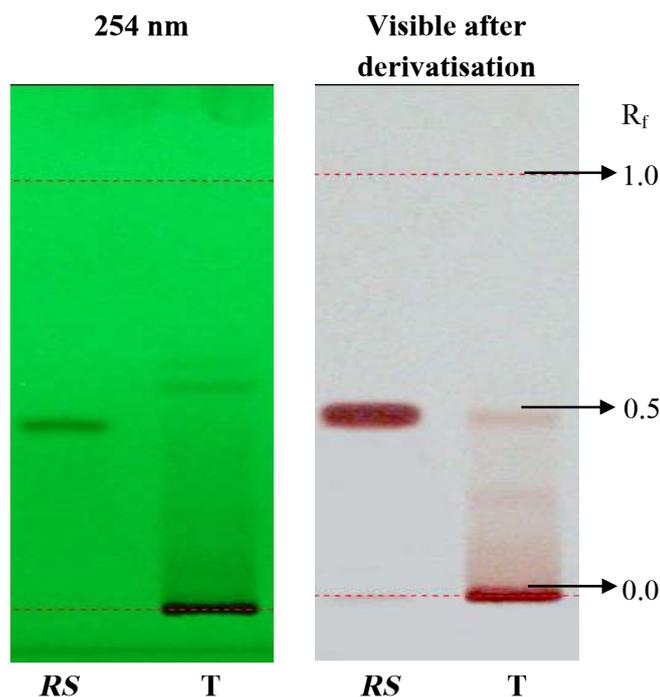


Fig. 1: Thin-Layer Chromatogram of Śirīṣa water extract

RS: *Catechin*, T: Test solution

### Quantitative parameters:

*Loss on drying*: not more than 5.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 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-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 500 mg, accurately weighed, of the substance being examined in a 100-ml round bottomed flask, reflux with 50 per cent *aqueous methanol* (25 ml x 3) on water bath

for 15 min each, cool, filter and make up the volume to 100 ml in a volumetric flask. Further dilute 5 ml aliquot to 50 ml with water in a volumetric flask. Filter through 0.42  $\mu\text{m}$  membrane. *Standard solution*: Take about 100 mg, accurately weighed, *pyrogallol RS* in a 100-ml volumetric flask and dissolve in about 50 ml of 50 per cent *aqueous methanol* and make up to 100-ml with 50 per cent *aqueous methanol*. Further dilute 5 ml to 50 ml with water in a volumetric flask. Filter through 0.42  $\mu\text{m}$  membrane. *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* and 10 ml of 30.0 per cent *sodium carbonate* solution to each of the volumetric flasks. Allow to stand for 30 min. Make up the volume with water, shake and allow to stand for 20 min. 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.

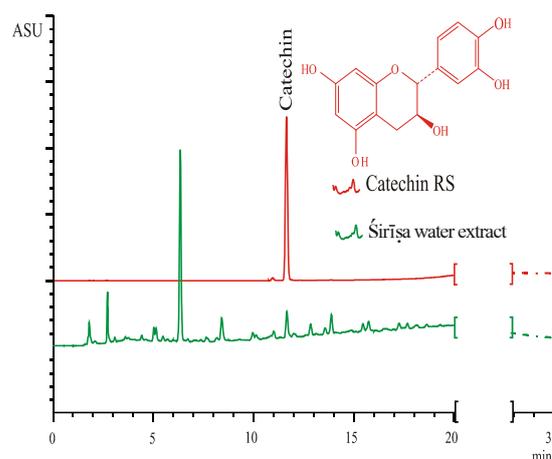
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 4 g, accurately weighed, of the substance being examined and reflux with water (50 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and make up the volume in a 100 ml volumetric flask. Filter through 0.42  $\mu\text{m}$  membrane. *Standard solution*: Take about 10 mg, accurately weighed, *catechin RS* and in a 100-ml volumetric flask and dissolve in about 50 ml of water and make up the volume with water. Filter through 0.42  $\mu\text{m}$  membrane. *Chromatographic system*: High performance liquid chromatography. *Column and stationary phase*: C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). *Mobile phase*: Filtered and degassed gradient mixture of phosphate buffer (prepared by dissolving 0.14 g of *potassium dihydrogen orthophosphate* in 900 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 (min)	Phosphate buffer (per cent)	Acetonitrile (per cent)
0.01	95	5
18	75	25
22	75	25
25	95	5
30	95	5

*Injection volume*: 20  $\mu\text{l}$ . *Flow rate*: 1.5 ml per min. *Detection*: UV 280 nm. *Procedure*: Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peak. Calculate the content of *catechin* in the substance being examined from the peak response of analyte. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Śirīṣa water extract with Catechin as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standard:

API *Catechin RS*

## ŚUNṬHĪ

Śunṭhī consists of dried rhizome of *Zingiber officinale* Roxb. (Fam. Zingiberaceae), widely cultivated in India, rhizomes dug in January-February, buds and roots removed, soaked overnight in water, decorticated, and some times treated with lime and dried. Śunṭhī contains not less than 0.75 per cent of total gingerols (sum of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol) when assayed.

**Synonyms:** Mahauṣadha, Nāgara, Viśvā, Viśvabheṣaja, Śrīagavera

**Other/Regional Language Names:** *Assamese:* Adasuth, Aadar Shuth; *Bengali:* Suntha, Sunthi; *English:* Ginger Root, Ginger; *Gujarati:* Sunth, Sundh, Suntha; *Hindi:* Sonth; *Kannada:* Shunthi; *Kashmiri:* Shonth; *Malayalam:* Chukku; *Marathi:* Sunth; *Oriya:* Sunthi; *Punjabi:* Sund; *Tamil:* Sukku, Chukku; *Telugu:* Sonthi, Sunthi; *Urdu:* Sonth, Zanjabeel

### Description:

#### a) Macroscopic:

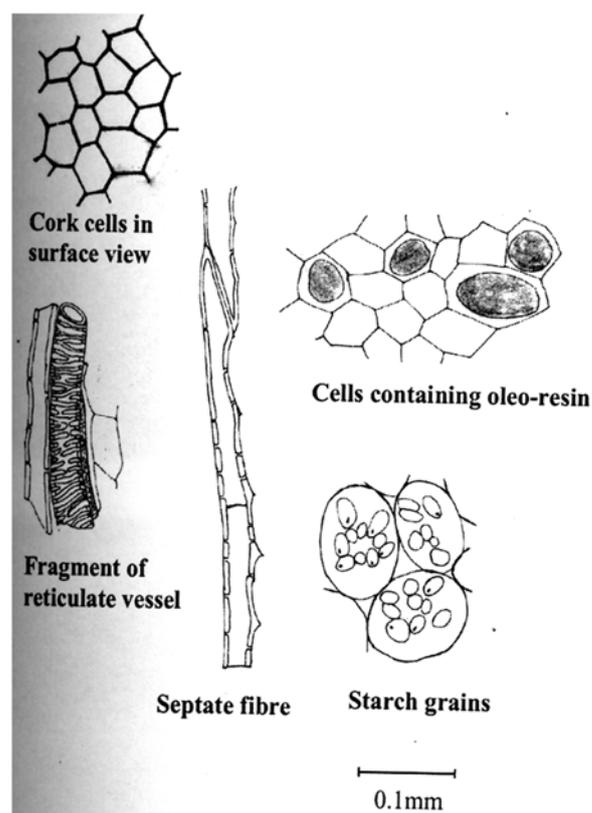
Rhizome, laterally compressed bearing short, flattish, ovate, oblique, branches on upper side each having at its apex a depressed scar, pieces about 5-15 cm long, 1.5-6.5 cm wide (usually 3-4 cm) and 1-1.5 cm thick, externally buff coloured showing longitudinal striations and occasional loose fibres, fracture short, smooth, transverse surface exhibiting narrow cortex (about one-third of radius), a well-marked endodermis and a wide stele showing numerous scattered fibro-vascular bundles and yellow secreting cells, odour agreeable and aromatic; taste agreeable and pungent

#### b) Microscopic:

Transverse section of rhizome shows cortex of isodiametric thin-walled parenchyma with scattered vascular strands and numerous isodiametric idioblasts, about 40-80  $\mu$  in diameter containing a yellowish to reddish-brown oleo-resin, endodermis slightly thick walled, free from starch, immediately inside endodermis a row of nearly cells, isodiametric

arranged radially around numerous scattered, collateral vascular bundles, each consisting of a few unligified, reticulate or spiral vessels upto about 70  $\mu$  in diameter, a group of phloem cells, unligified, thin-walled, septate fibres upto about 30  $\mu$  wide and 600  $\mu$  long with small oblique slit like pits, numerous scattered idioblasts, similar to those of cortex, and associated with vascular bundles, also present, idioblasts about 8-20  $\mu$  wide and up to 130  $\mu$  long with dark reddish-brown contents: in single or in axial rows, adjacent to vessels, parenchyma of cortex and stele packed with flattened, rectangular, ovate, starch grains, mostly 5-15  $\mu$  - 30-60  $\mu$  long about 25  $\mu$  wide and 7  $\mu$  thick, marked by five transverse striations

#### c) Powder:



**Fig. 1: Powdered drug of ŚUNṬHĪ**  
(*Zingiber officinale* Roxb.)

Powder shows parenchyma cell groups containing yellow brown oleo-resinous bodies; occur also as fragments or in droplets. Starch grains abundant, simple, ovoid or sac shaped upto 30-50  $\mu$  in length and have a distinct hilum at the narrower end with

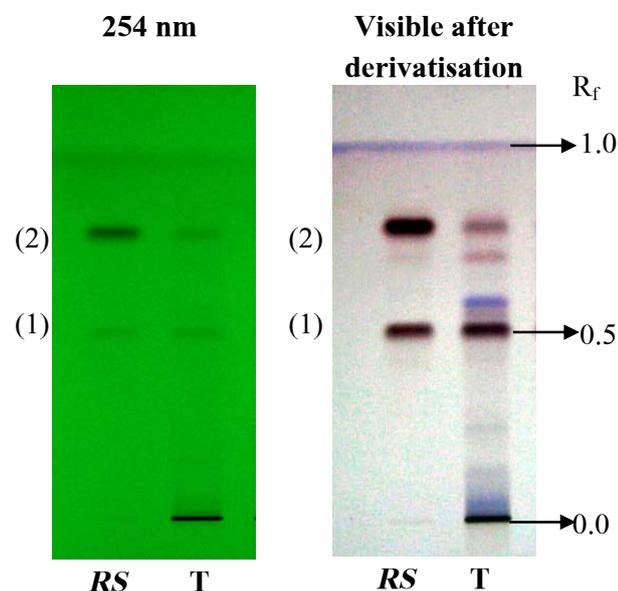
concentric striations; septate sclerenchymatous lignified fibres in groups associated with vessels. Vessels mostly non-lignified (Fig. 1)

### Identity, Purity and Strength:

#### Identification:

##### *Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *6-gingerol* and *6-shogaol* as reference standards. *Test solution:* Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 5 mg each of *6-gingerol RS* and *6-shogaol RS* in about 25 ml of *methanol*. *Procedure:* Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate : toluene : diethyl ether* (3.0 : 6.0 : 1.0).



**Fig. 2: Thin-Layer Chromatogram of Śunṭhī**  
**RS: (1) 6-Gingerol and (2) 6-Shogaol,**  
**T: Test solution**

Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde-sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The

chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 2).

#### Quantitative parameters:

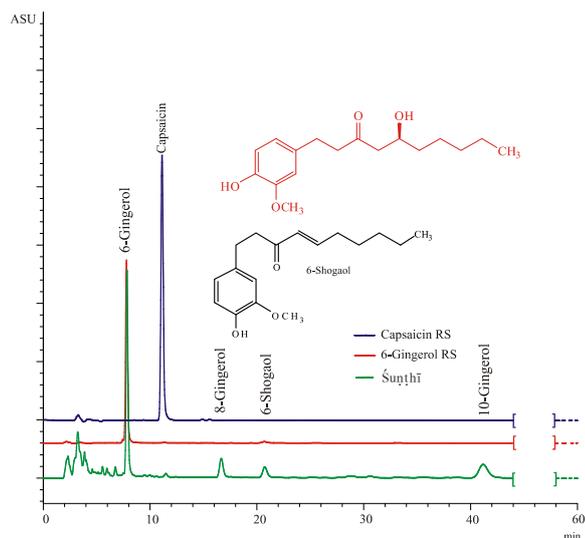
*Foreign matter:* not more than 1.0 per cent (Appendix 2.1.3); *Loss on drying:* not more than 12.0 per cent (Appendix 2.1.4); *Total ash:* not more than 6.0 per cent (Appendix 2.1.5); *Acid-insoluble ash:* not more than 1.5 per cent (Appendix 2.1.7); *Alcohol-soluble extractive:* not less than 3.0 per cent (Appendix 2.1.8); *Water-soluble extractive:* not less than 10.0 per cent (Appendix 2.1.9).

#### 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 and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume. Filter through 0.42 µm membrane. *Standard solution:* Take about 5 mg, accurately weighed, *capsaicin RS* in 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume. Filter through 0.42 µm membrane. *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 55 volumes of *acetonitrile*, 44 volumes of 0.1 per cent *phosphoric acid* and 1 volume of *methanol*. *Injection volume:* 20 µl. *Flow rate:* 1 ml per min. *Detection:* UV 282 nm.



**Fig. 3: HPLC chromatogram of Śuṅṭhī with Capsaicin as RS**

**Procedure:** Inject 20  $\mu$ l of the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	Relative retention time
6-Gingerol	0.8
Capsaicin	1.0
8-Gingerol	1.6
10-Gingerol	4.0
6-Shogaol	2.04

Sum the peak areas of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in the substance being examined. Calculate the content of total gingerols from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standards:** API 6-Gingerol RS, 6-Shogaol RS and Capsaicin RS

**Constituents:** 6-Shogaol, 6-gingerol, zingiberol,  $\beta$ -phellandrene,  $\alpha$ -zingiberene, ar-curcumen,  $\beta$ -bisabolene. Gingerenones A, B & C, isogingerenone B, hexahydrocurcumin, diaryl heptanoids, gingerdiols, 6-gingesulfonic acid, gingerglycolipids A, B & C, gingerenones, (+)-angelicoidenol-2-O- $\beta$ -D-glucopyranoside, geraniol glycosides,  $\alpha$ -santalol,  $\beta$ -eudesmol, nerolidol, farnesol, elemol, neral, geranial,  $\alpha$ - and  $\beta$ -pinene, camphene, sabinene, myrcene, limonene, 1,8-cineole, aliphatic alkanes, alcohols, aldehydes, ketones, sulphides

**Properties and Action:** Rasa: Kaṭu, Tikta; Guṇa: Laghu, Snigdha; Vīrya: Uṣṇa; Vipāka: Madhura; Karma: Anulomana, Dīpana, Āmaḍaḡhna, Hṛḍya, Kaphaḡhna, Pācana, Rucya, Vātakaphahara, Vṛṣya

**Important formulations:** Saubhāḡya vaṭī, Saubhāḡyaśuṅṭhī, Trikaṭu, Vaiśvānara Cūrṇa

**Therapeutic uses:** Agnimāndya (digestive impairment), Ādhmāna (flatulence with gurgling sound), Āmavāta (rheumatism), Gulma (abdominal lump), Pāṇḍu (anaemia), Pīnasa (Chronic rhinitis), Ślipada (filariasis), Śvāsa (dyspnoea), Udararoga (disease of abdomen)

**Dose:** Cūrṇa (powder): 1-3 g

## ŚUNṬHĪ HYDRO-ALCOHOLIC EXTRACT

Śunṭhī Hydro-alcoholic Extract is a dried and powdered extract prepared from Śunṭhī (appropriately powdered). The extract contains not less than 1.5 per cent of total gingerols (Sum of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol) when assayed.

### Method of preparation:

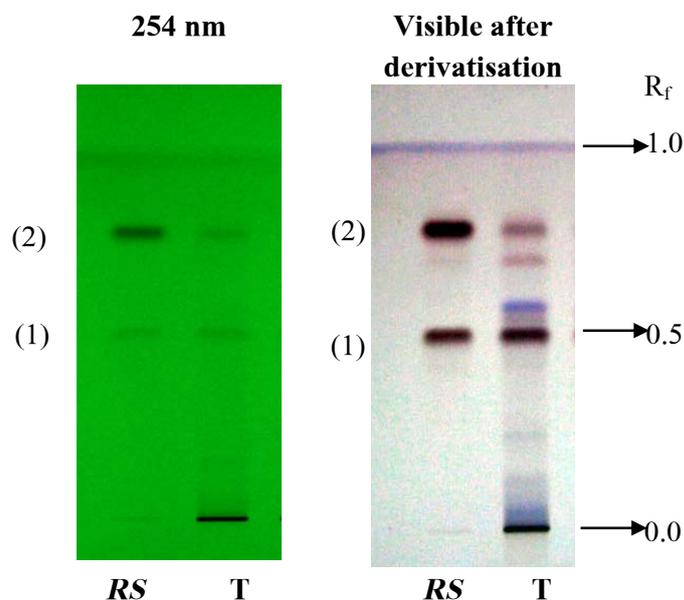
Take Śunṭhī suitably sized (powder or pieces) in an extractor. Add 50 per cent aqueous alcohol, about 3 times the quantity of raw material and heat under a reflux at a temperature between 80-85<sup>0</sup> for 3-4 hours. Filter the extract through a filter (preferably 10 μm pore size) to a suitable sized 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<sup>0</sup> till the moisture is below 7 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 11 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using 6-gingerol and 6-shogaol as reference standards. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of 6-gingerol RS and 6-shogaol RS in about 25 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *toluene* : *diethyl ether* (3.0 : 6.0 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde-sulphuric acid reagent* and

heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Śunṭhī hydro-alcoholic extract**

**RS: (1) 6-Gingerol and (2) 6-Shogaol,  
T: Test solution**

### Quantitative parameters:

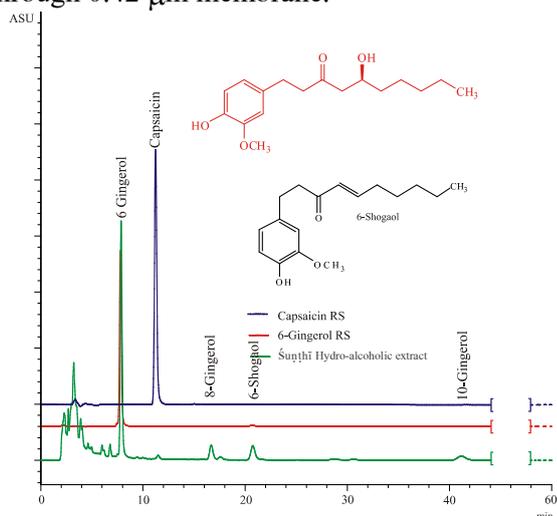
*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-7.0 (Appendix 2.1.10); *Total soluble solids*: not less than 90.0 per cent (Appendix 2.1.11) (Method-I)

### 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 and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 100-ml volumetric flask and make up the volume. Filter through 0.42 µm membrane.



**Fig. 2: HPLC chromatogram of Śunṭhī Hydro-alcoholic extract with Capsaicin as RS**

*Standard solution*: Take about 5 mg, accurately weighed, *capsaicin RS* in 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume. Filter through 0.42 µm membrane. *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 55 volumes of *acetonitrile*, 44 volumes of 0.1 per cent *orthophosphoric acid* and 1 volume of *methanol*. *Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detection*: UV 282 nm. *Procedure*: Inject 20 µl of the standard solution and record the chromatogram. Inject 20 µl of the test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	Relative retention time
6-Gingerol	0.8
Capsaicin	1.0
8-Gingerol	1.6
10-Gingerol	4.0
6-Shogaol	2.04

Sum the peak areas of *6-gingerol*, *8-gingerol*, *10-gingerol* and *6-shogaol* in the substance being examined. Calculate the content of *total gingerols* from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

**Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

**API reference standards:**

API 6-Gingerol RS, 6-Shogaol RS and Capsaicin RS

## ŚUNṬHĪ WATER EXTRACT

Śunṭhī Water Extract is a dried and powdered extract prepared from Śunṭhī. The extract contains not less than 0.2 per cent of total gingerols (Sum of *6-gingerol*, *8-gingerol*, *10-gingerol* and *6-shogaol*) when assayed.

### Method of preparation:

Take Śunṭhī 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° 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° till the moisture is below 7 per cent. Mill the mass and sieve the powder through 500 µm mesh and pack. The yield obtained is about 16 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *6-gingerol* and *6-shogaol* as reference standards. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of *6-gingerol RS* and *6-shogaol RS* in about 25 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *toluene* : *diethyl ether* (3.0 : 6.0 : 1.0). Dry the plate in air and examine under UV 254 nm. Spray the plate with *anisaldehyde-sulphuric acid reagent* and heat at 105° till the colour of the spots/bands appear without charring. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).

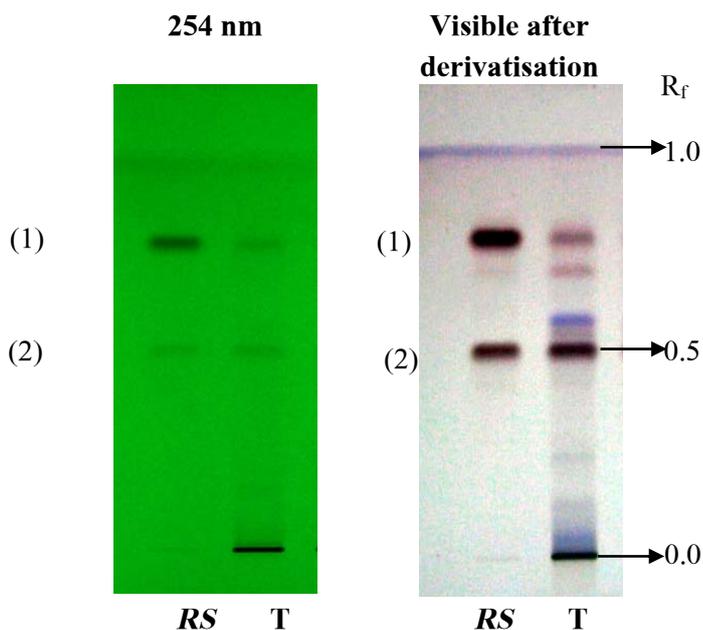


Fig. 1: Thin-Layer Chromatogram of Śunṭhī water extract  
RS: (1) *6-Gingerol* and (2) *6-Shogaol*, T: Test solution

### Quantitative parameters:

*Loss on drying*: not more than 7.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 2.0 per cent (Appendix 2.1.7); *pH*: 4.0-7.0 (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)

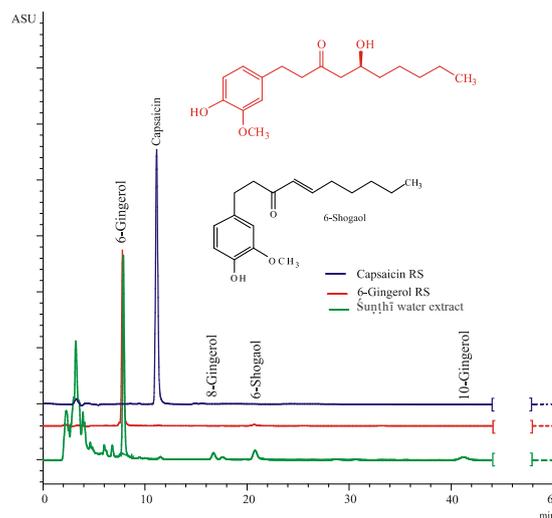
### Assay:

Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 2 g, accurately weighed, of the substance being examined and reflux with *methanol* (25 ml x 3) on water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer

to a 100-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 5 mg, accurately weighed, *capsaicin RS* in 25-ml volumetric flask and dissolve in about 10 ml of *methanol* and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm, 5  $\mu\text{m}$ ). **Mobile phase:** Filtered and degassed mixture of 55 volumes of *acetonitrile*, 44 volumes of 0.1 per cent *orthophosphoric acid* and 1 volume of *methanol*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1 ml per minu. **Detection:** UV 282 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and identify the peaks using the relative retention times, as below.

Analyte	Relative retention time
6-Gingerol	0.8
Capsaicin	1.0
8- Gingerol	1.6
10-Gingerol	4.0
6-Shogaol	2.04

Sum the peak areas of *6-gingerol*, *8-gingerol*, *10-gingerol* and *6-shogaol* in the substance being examined. Calculate the content of *total gingerols* from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatogram of Šunṭhī water extract with *Capsaicin* as *RS***

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *6-Gingerol RS*, *6-Shogaol RS* and *Capsaicin RS*

## SVARṆAPATRĪ

Svarṇapatrī consists of dried leaflets of *Cassia senna* L. syn. *C. angustifolia* L., (Fam. Caesalpiaceae), a small shrub, 60-75 cm high, found throughout the year, cultivated largely in southern India, especially in districts of Tinnevely, Madurai, Ramanathapuram and collected and dried in shade for 7-10 days, till they attain yellowish-green colour, graded and then packed into large bales. It contains not less than 0.2 per cent of *sennoside A* and 0.1 per cent of *sennoside B* when assayed.

**Synonyms:** Mārkaṇḍikā

**Other/Regional Language Names:** *Assamese:* Sonamukhi; *Bengali:* Svarnamukhi, Sonapata; *English:* Indian Senna, Tinnevely Senna; *Gujarati:* Mindhiaval; *Hindi:* Sanaya; *Kannada:* Nelavarike, Sonamukhi; *Kashmiri:* Sna; *Malayalam:* Sunnamukhi, Connamukhi; *Marathi:* Sonamukhi; *Oriya:* Sunamukhi; *Punjabi:* Sannamakhi, Sanapati, Sarnapatta; *Tamil:* Nilavarai, Nelavakai; *Telugu:* Sunamukhi; *Urdu:* Sena, Barg-e-Sana

### Description:

#### a) Macroscopic:

Leaflets 2.5-6 cm long and 7-15 mm wide at centre, pale yellowish-green, elongated lanceolate, slightly asymmetric at base, margins entire, flat, apex acute with a sharp spine; both surfaces smooth with sparse trichomes, odour, faint but distinct, taste mucilagenous and disagreeable

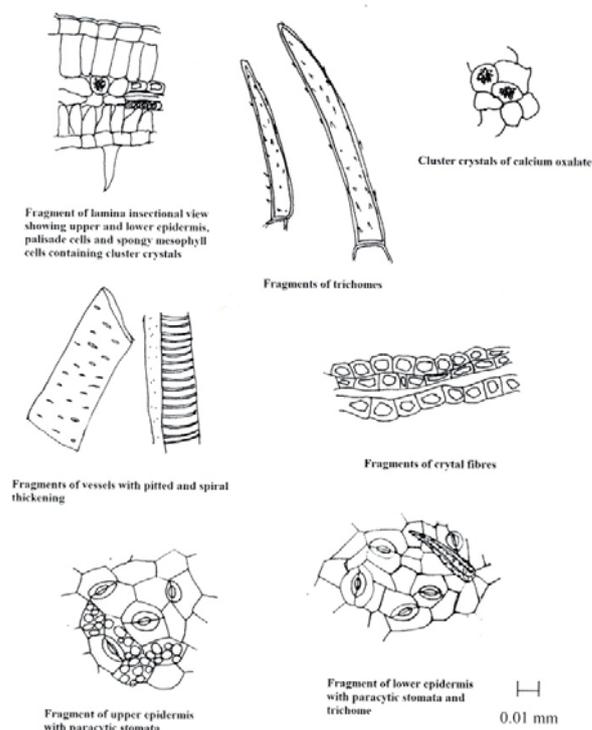
#### b) Microscopic:

Transverse section of a leaflet through midrib shows an isobilateral structure, epidermal cells on both surfaces straight walled, containing mucilage; both surfaces bear scattered, unicellular hair, often conical, curved near base, thick-walled, non-lignified, warty cuticle, stomata paracytic, numerous on both surfaces, mesophyll consists of upper and lower palisade

layers with spongy layer in between, palisade cells of upper surface longer than those of lower surface the latter having wavy anticlinal walls, prismatic crystals of calcium oxalate present on larger veins and clusters of calcium oxalate crystals distributed throughout the palisade and spongy tissues, midrib biconvex, bundles of midrib and larger veins, incompletely surrounded by a zone of pericyclic fibres and a crystal sheath of parenchymatous cells containing prismatic crystals of calcium oxalate

#### c) Powder:

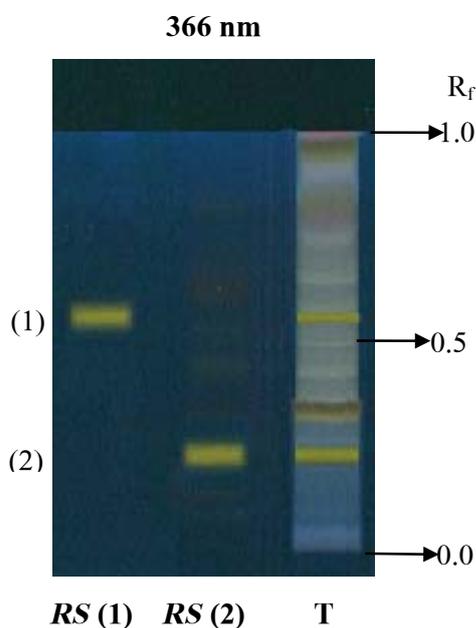
Powder shows covering type of trichomes which are short, thick, unicellular, warty and frequently curved near the base. Rubiaceous or paracytic type of stomata are characteristic. Calcium oxalate cluster crystals occur in the cells of the mesophyll and as prisms in a sheath of cells around the fibres and as well freely distributed in powder (Fig. 1.).



**Fig. 1: Powdered drug of SVARṆAPATRĪ  
(*Cassia senna* L.)**

**Identity, Purity and Strength:****Identification:***Thin-layer chromatography:*

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *sennoside A* and *sennoside B* as reference standards. *Test solution:* Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution:* Dissolve 5 mg each of *sennoside A RS* and *sennoside B RS* in about 10 ml of *methanol*. *Procedure:* Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase *ethyl acetate : methanol : water* (7.7 : 1.7 : 1.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 2).



**Fig. 2: Thin-Layer Chromatogram of Svarṇapatrī RS: (1) *sennoside A* and (2) *Sennoside B*; T: Test solution**

**Quantitative parameters:**

*Foreign matter:* not more than 1.0 per cent (Appendix 2.1.3); *Loss on drying:* not more than 8.0 per cent (Appendix 2.1.4); *Total ash:* not more

than 14.0 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 3.0 per cent (Appendix 2.1.8); *Water-soluble extractive:* not less than 25.0 per cent (Appendix 2.1.9)

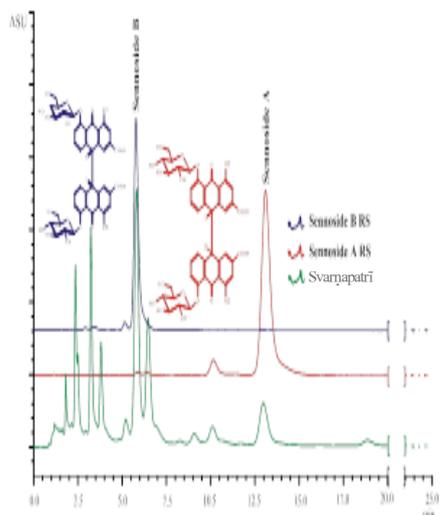
**Other requirements:**

*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 mixture of 0.05 M *potassium dihydrogen orthophosphate* and *water* (5 ml : 10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 50-ml volumetric flask, make up the volume with *water*. Filter through 0.42 µm membrane. *Standard solution:* Take about 4 mg, accurately weighed, each of *sennoside A RS* and *sennoside B RS* in a 25-ml volumetric flask, dissolve in about 5 ml of 0.05 M *Potassium dihydrogen orthophosphate* and 10 ml of *water* and make up the volume with *water*. Filter through 0.42 µm membrane. *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 83 volumes of 1 per cent *acetic acid* in *water* and 17 volumes of *acetonitrile*. *Injection volume:* 20 µl. *Flow rate:* 2 ml per min. *Detection:* UV 350 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 *sennoside A* and *sennoside B* in the substance being examined from the peak response

of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatograms of Svarṇapatrī with Sennoside A and Sennoside B as RS**

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standards:

API *Sennoside A RS* and *Sennoside B RS*

**Constituents:** Bioactive anthraquinone glycosides viz., sennoside A, B, C and D; total anthraquinone derivatives occur in the range of 2 to 3 per cent. Palmidin A, aloe-emodin dianthrone-diglycoside, rhein-anthrone-8-glycoside, rhein-8-diglucoside, aloe-emodin-8-glycoside, aloe-emodin-anthrone-diglucoside, a primary glycoside having greater potency than sennosides A and B, naphthalene glycoside tinnevellin glucoside, sennoside G; kaempferol, its glucoside kaempferin and isorhamnetin, water-soluble polysaccharides

**Properties and Action:** Rasa: Kaṭu, Tikta, Kaṣāya; Guṇa: Laghu, Rūkṣa, Tīkṣṇa; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Recana, Kuṣṭhaghna

**Important formulations:** Pañcasakāra cūrṇa, Sārivādyāsava

**Therapeutic uses:** Gulma (abdominal lump), Udararoga (disease of abdomen), Vibandha (constipation)

**Dose:** Cūrṇa (powder): 0.5 to 2 g

## SVARŅAPATRĪ HYDRO-ALCOHOLIC EXTRACT

Svarņapatrī Hydro-Alcoholic Extract is a dried and powdered extract prepared from Svarņapatrī (appropriately powdered). The extract contains not less than 2.0 per cent of sum of *sennoside A* and *sennoside B* when assayed.

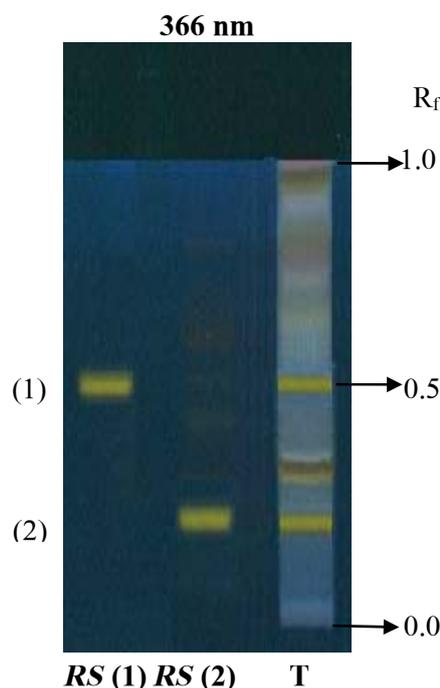
### Method of Preparation:

Take Svarņapatrī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 per cent. Mill the mass and sieve the powder through 500 μm mesh to obtain the extract and pack. The yield obtained is about 21 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *sennoside A* and *sennoside B* as reference standards. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of *sennoside A RS* and *sennoside B RS* in about 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *water* (7.7 : 1.7 : 1.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Svarņapatrī hydro-alcoholic extract**  
**RS: (1) Sennoside A and (2) Sennoside B**  
**T: Test solution**

### Quantitative parameters:

*Loss on drying*: not more than 5.0 per cent (Appendix 2.1.4); *Total ash*: not more than 13.0 per cent (Appendix 2.1.5); *Acid-insoluble ash*: not more than 1.0 per cent (Appendix 2.1.7); *pH*: 3.5-5.5 (Appendix 2.1.10); *Total soluble solids*: not less than 80.0 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:

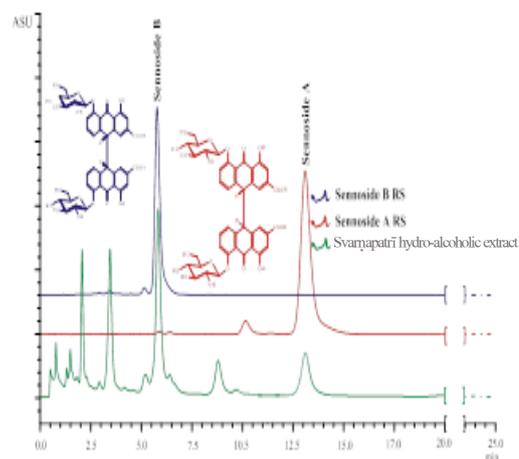
Carry out the assay by *liquid chromatography* (Appendix 3.6). *Test solution*: Take about 0.4 g,

accurately weighed, of the substance being examined and reflux with mixture of 0.05 M *potassium dihydrogen orthophosphate* and water (5 ml : 10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 50-ml volumetric flask, make up the volume with water. Filter through 0.42 µm membrane.

**Standard solution:** Take about 4 mg, accurately weighed, each of *sennoside A RS* and *sennoside B RS* in a 25-ml volumetric flask, dissolve in about 5 ml of 0.05 M *Potassium dihydrogen orthophosphate* and 10 ml of water and make up the volume with water. Filter through 0.42 µm membrane.

**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 83 volumes of 1 per cent *acetic acid* in water and 17 volumes of *acetonitrile*. **Injection volume:** 20 µl. **Flow rate:** 2 ml per min. **Detection:** UV 350 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 *sennoside A* and *sennoside B* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Svarṅapatrī hydro-alcoholic extract with Sennoside A and Sennoside B as RS**

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standards:**

API *Sennoside A RS* and *Sennoside B RS*

## SVARṆAPATRĪ WATER EXTRACT

Svarṇapatrī water Extract is a dried and powdered extract prepared from Svarṇapatrī. The extract contains not less than 1.0 per cent of sum of *sennoside A* and *sennoside B* when assayed.

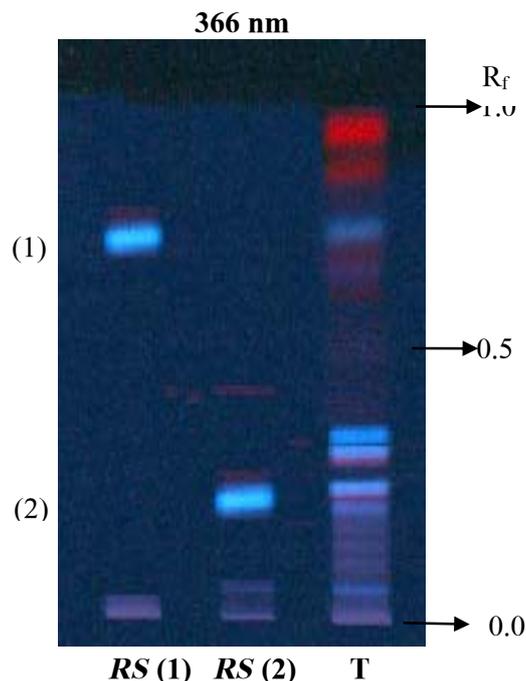
### Method of Preparation:

Take Svarṇapatrī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *sennoside A* and *sennoside B* as reference standards. *Test solution*: Extract 0.2 g of substance by refluxing with 50 ml of *methanol* for a period of 10-15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg each of *sennoside A RS* and *sennoside B RS* in about 10 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *ethyl acetate* : *methanol* : *water* (7.7 : 1.7 : 1.0). Dry the plate in air and examine under UV 366 nm. The chromatographic profile of the test solution shows bands corresponding to that of the standard solution (Fig. 1).



**Fig. 1: Thin-Layer Chromatogram of Svarṇapatrī water extract**  
**RS: (1) Sennoside A and (2) Sennoside B**  
**T: Test solution**

### Quantative 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 1.0 per cent (Appendix 2.1.7); *pH*: 3.5-5.5 (Appendix 2.1.10); *Total soluble solids*: not less than 80.0 per cent (Appendix 2.1.11) (Method-II)

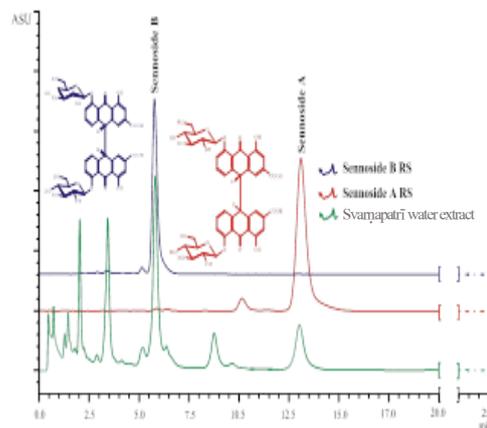
### Other requirements:

*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 mixture of 0.05 M

*potassium dihydrogen orthophosphate* and water (5 ml : 10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates and transfer to a 50-ml volumetric flask, make up the volume with water. Filter through 0.42 µm membrane. *Standard solution*: Take about 4 mg, accurately weighed, each of *sennoside A RS* and *sennoside B RS* in a 25-ml volumetric flask, dissolve in about 5 ml of 0.05 M *potassium dihydrogen orthophosphate* and 10 ml of water and make up the volume with water. Filter through 0.42 µm membrane. *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 83 volumes of 1 per cent *acetic acid* in water and 17 volumes of *acetonitrile*. *Injection volume*: 20 µl. *Flow rate*: 2 ml per min. *Detection*: UV 350 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 *sennoside A* and *sennoside B* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Svarṇapatrī water extract with Sennoside A and Sennoside B as RS**

#### **Additional requirements:**

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### **API reference standards:**

API *Sennoside A RS* and *Sennoside B RS*

## TULASĪ

Tulasī consists of dried leaf of *Ocimum tenuiflorum* L. syn. *O. sanctum* L. (Fam. Lamiaceae), an erect, 30-60 cm high, much branched annual herb, found throughout the country and cultivated in sacred groves. It contains not less than 0.4 per cent of sum of *oleanolic acid* and *ursolic acid* when assayed.

**Synonyms:** Surasā, Surasa

**Other/Regional Language Names:** *Assamese:* Tulasī; *Bengali:* Tulasī; *English:* Sacred Basil, Holy Basil; *Gujarati:* Tulasī, Tulsi; *Hindi:* Tulasī; *Kannada:* Tulasī; *Malayalam:* Tulasī; *Marathi:* Tulas; *Punjabi:* Tulasī; *Tamil:* Thulasi, Tulasī; *Telugu:* Tulasī; *Urdu:* Raihan, Tulsi

### Description:-

#### a) Macroscopic:

Leaves 2.5-5 cm long, 1.6-3.2 cm wide, elliptic-oblong, obtuse or acute, entire or serrate, pubescent on both surfaces, petiolate, thin, petiole 1.5-3 cm long, hairy; odour aromatic; taste, characteristic

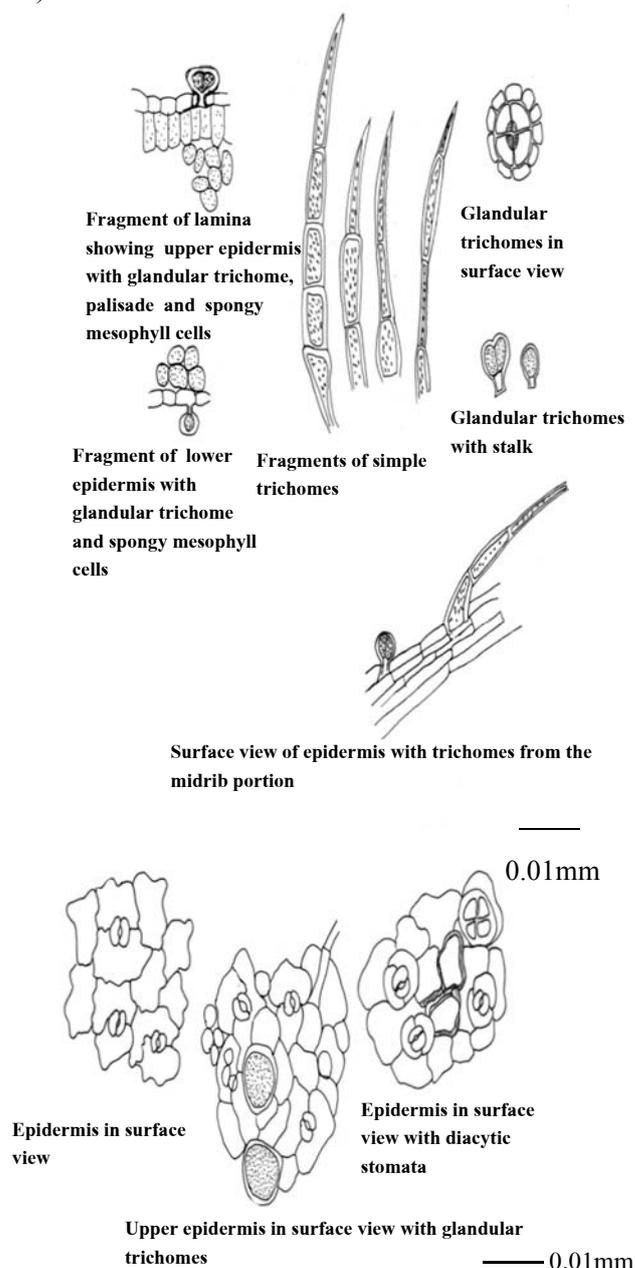
#### b) Microscopic:

**Leaf- Petiole** - shows cordate outline, consisting of single layered epidermis composed of thin walled, oval cells having a number of covering and glandular trichomes; covering trichomes multicellular, uniseriate 1-8 celled long, rarely slightly reflexed at tip; glandular trichomes short, sessile or with 1-2 celled stalk, and 2-8 celled, balloon-shaped head, enclosed in a cuticular bladder, measuring 22-27  $\mu$  in dia., upper epidermis, followed by 3-4 layers of collenchymatous and 1-2 layers of parenchymatous cells; lower epidermis followed by 1-3 layers of collenchymatous and 2-3 layers of parenchymatous cells; three vascular bundles situated centrally, middle one larger than the other two, consisting of xylem and phloem

**Midrib** - epidermis, trichomes and vascular bundles similar to those of petiole, except reduced in cortical layers towards apical region of midrib

Lamina - epidermis and trichomes similar to those of petiole on both surfaces; stomata anomocytic and diacytic present on both surfaces and slightly raised above the level of epidermis; palisade single layered followed by 4-6 layers of closely packed spongy parenchyma with chloroplasts and oleo-resin; stomatal index 10-13-15 on upper surface and 14-15-16 on lower surface; palisade ratio 3.8; vein islet number 31-33

#### c) Powder:



**Fig. 1: Powdered drug of Tulasī (*Ocimum tenuiflorum* L.)**

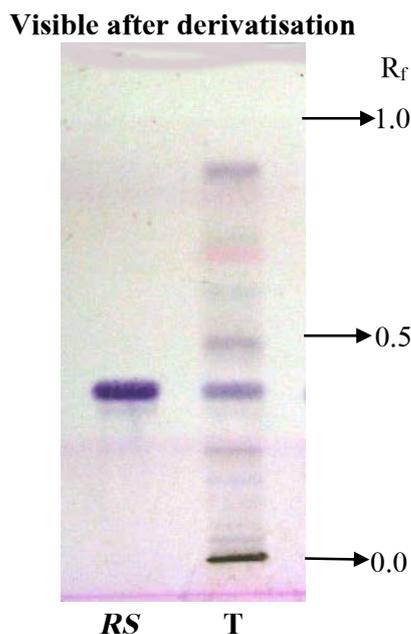
Light-green; shows fragments of polygonal, wavy to sinuous walled upper and lower epidermal cells in surface view, covering and glandular trichomes as a whole or in pieces, palisade and spongy parenchyma, anomocytic and diacytic stomata (Fig 1.)

## Identity, Purity and Strength:

### Identification:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a precoated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *ursolic acid* as a reference standard. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg of *ursolic acid RS* in about 25 ml of *methanol*.



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

*Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *acetic acid* (8.0 : 2.0 : 0.1). Dry the plate in air. Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the

spots/bands appear without charring. The chromatographic profile of the test solution shows a band corresponding to that of the standard solution (Fig. 2).

### 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 19.0 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 13.0 per cent (Appendix 2.1.9).

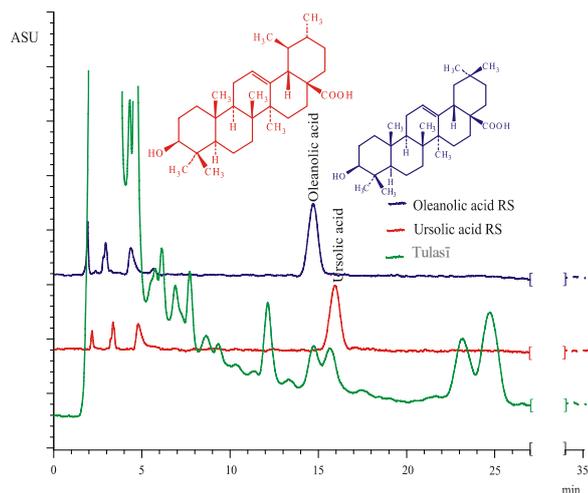
### Other requirements:

*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 *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. Filter through 0.42 µm membrane. *Standard solution*: Take about 5 mg, accurately weighed, each of *oleanolic acid RS* and *ursolic acid RS* in a 25-ml volumetric flask and dissolve in about 15 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42 µm membrane. *Chromatographic system*: High performance liquid Chromatography. *Column and stationary phase*: C18 (250 mm x 4.6 mm). *Mobile phase*: Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *methanol*. *Injection volume*: 20 µl. *Flow rate*: 1 ml per min. *Detection*: UV 210 nm. *Procedure*: Inject 20 µl of

the standard solution and record the chromatogram. Inject 20  $\mu$ l of the test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *oleanolic acid* and *ursolic acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 3: HPLC chromatograms of Tulasī with *Oleanolic acid* and *Ursolic acid* as RS**

#### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

#### API reference standards:

API *Oleanolic acid* RS and *Ursolic acid* RS

**Constituents:** Essential oil containing eugenol,  $\beta$ -caryophyllene; apigenin, apigenin-7-*O*-glucuronide, vicenin, vicenin-2, luteolin, luteolin-7-*O*-glucuronide, galuteolin, orientin, molludistin, cirsilineol; gallic acid and its methyl and ethyl esters; ursolic acid.  $\beta$ -carotene; rosmarinic, protocatechuic, vanillic, 4-hydroxybenzoic, caffeic, chlorogenic acids; fatty acids; vanillin, 4-hydroxybenzaldehyde, cholesterol, campesterol, stigmasterol,  $\beta$ -sitosterol

**Properties and Action:** Rasa: Kaṭu, Tikta; Guṇa: Laghu, Rūkṣa, Tīkṣṇa; Vīrya: Uṣṇa; Vipāka: Kaṭu; Karma: Dīpana, Hṛdya, Jvaraghna, Kāśahara, Kṛmighna, Kaphahara, Rasāyana, Śvāsahara, Vātahara

#### Important formulations:

Mānasamitra vaṭaka, Mahājvarāṅkuśa rasa, Muktaṭpañchāmṛta rasa, Tribhuvanakīrti rasa

#### Therapeutic uses:

Apasmāra (epilepsy), Aruci (tastelessness), Gulma (abdominal lump), Kṣata (wound), Hikkā (hiccup), Kāśa (cough), Kṛmiroga (helminthiasis / worm infestation), Kṣaya (pthisis), Kuṣṭha (disease of skin), Pārśvaśūla (intercostal neuralgia and pleurodynia), Plīhā (splenic disease), Pratiśyāya (coryza), Śvāsa (dyspnoea), Śoṣa (cachexia)

#### Dose:

Cūrṇa (powder): 1-3 g

## TULASĪ HYDRO-ALCOHOLIC EXTRACT

Tulasī Hydro-Alcoholic Extract is a dried and powdered extract prepared from Tulasī (appropriately powdered). The extract contains not less than 0.2 per cent of sum of *oleanolic acid* and *ursolic acid* when assayed.

### Method of Preparation:

Take Tulasī 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<sup>0</sup> 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<sup>0</sup> till the moisture is below 5 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 per cent.

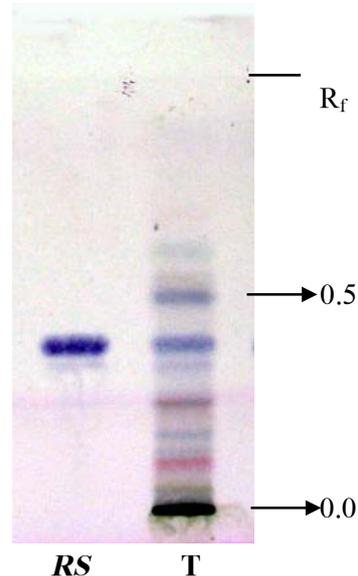
### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *ursolic acid* as a reference standard. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg of *ursolic acid* RS in about 25 ml of *methanol*. *Procedure*: Apply 10 μl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *acetic acid* (8.0 : 2.0 : 0.1). Dry the plate in air. Spray the plate with *anisaldehyde - sulphuric acid reagent* and heat at 105<sup>0</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of

the test solution shows a band corresponding to that of the standard solution (Fig. 1).

#### Visible after derivatisation



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

### Quantative parameters:

*Loss on drying*: not more than 5.0 per cent (Appendix 2.1.4); *Total ash*: not more than 12.0 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-I)

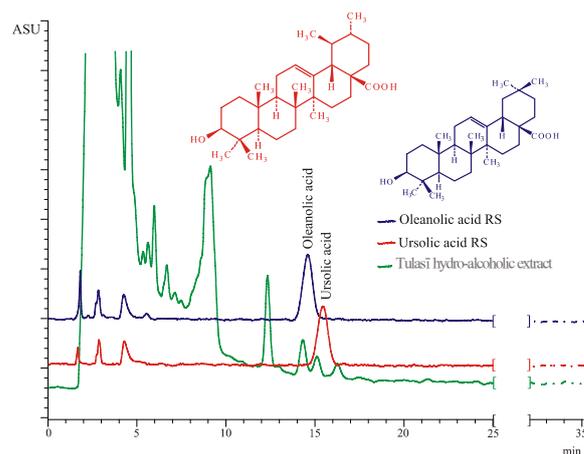
### Other requirements:

*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 *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter. Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 5 mg, accurately weighed, each of *oleanolic acid RS* and *ursolic acid RS* in a 25-ml volumetric flask and dissolve in about 15 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid Chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm). **Mobile phase:** Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *methanol*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1 ml per min. **Detection:** UV 210 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *oleanolic acid* and *ursolic acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.



**Fig. 2: HPLC chromatograms of Tulasī hydro-alcoholic extract with *Oleanolic acid* and *Ursolic acid* as RS**

### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *Oleanolic acid RS* and *Ursolic acid RS*

## TULASĪ HYDRO-ALCOHOLIC EXTRACT

Tulasī Water Extract is a dried and powdered extract prepared from Tulasī. The extract contains not less than 0.02 per cent of sum of *oleanolic acid* and *ursolic acid* when assayed.

### Method of Preparation:

Take Tulasī 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<sup>o</sup> 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<sup>o</sup> till the moisture is below 5 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 per cent.

### Identity, Purity and Strength:

#### Thin-layer chromatography:

Carry out *thin-layer chromatography* on a pre-coated silica gel 60F<sub>254</sub> plate (Appendix 3.5) using *ursolic acid* as a reference standard. *Test solution*: Extract 1 g of substance by refluxing with 50 ml of *methanol* for a period of 15 min. Filter and concentrate the extract to 25 ml. *Standard solution*: Dissolve 5 mg of *ursolic acid* RS in about 25 ml of *methanol*. *Procedure*: Apply 10 µl each of the test and standard solutions as bands at a height of 10 mm from the base of a 10 x 5 cm TLC plate and develop up to 8 cm from the base of the plate using the mobile phase: *toluene* : *ethyl acetate* : *acetic acid* (8.0 : 2.0 : 0.1). Dry the plate in air. Spray the plate with *anisaldehyde-sulphuric acid reagent* and heat at 105<sup>o</sup> till the colour of the spots/bands appear without charring. The chromatographic profile of

the test solution shows a band corresponding to that of the standard solution (Fig. 1).

#### Visible after derivatisation

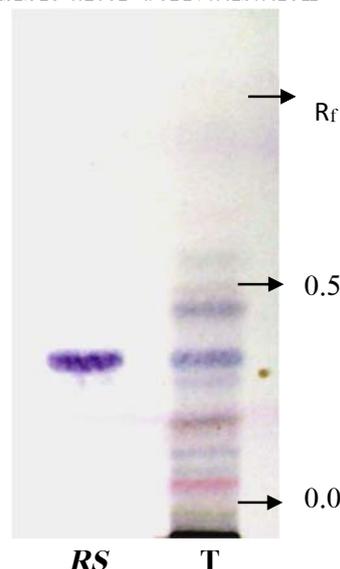


Fig. 1: Thin-Layer Chromatogram of water extract  
RS: *Ursolic acid*, T: Test solution

### 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 1.0 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 0.4 g, accurately weighed, of the substance being examined and reflux with *methanol* (10 ml x 3) on a water bath for 15 min each, cool and filter.

Combine all the filtrates, concentrate and transfer to a 25-ml volumetric flask and make up the volume. Filter through 0.42  $\mu\text{m}$  membrane. **Standard solution:** Take about 5 mg, accurately weighed, each of *oleanolic acid RS* and *ursolic acid RS* in a 25-ml volumetric flask and dissolve in about 15 ml of *methanol* and make up the volume with *methanol*. Filter through 0.42  $\mu\text{m}$  membrane. **Chromatographic system:** High performance liquid Chromatography. **Column and stationary phase:** C18 (250 mm x 4.6 mm). **Mobile phase:** Filtered and degassed mixture of 1 volume of *water* and 9 volumes of *methanol*. **Injection volume:** 20  $\mu\text{l}$ . **Flow rate:** 1 ml per min. **Detection:** UV 210 nm. **Procedure:** Inject 20  $\mu\text{l}$  of the standard solution and record the chromatogram. Inject 20  $\mu\text{l}$  of the test solution, record the chromatogram and measure the response for the analyte peaks. Calculate the content of *oleanolic acid* and *ursolic acid* in the substance being examined from the peak response of analytes. The test is not valid unless the relative standard deviation for replicate injections is not more than 2.0 per cent.

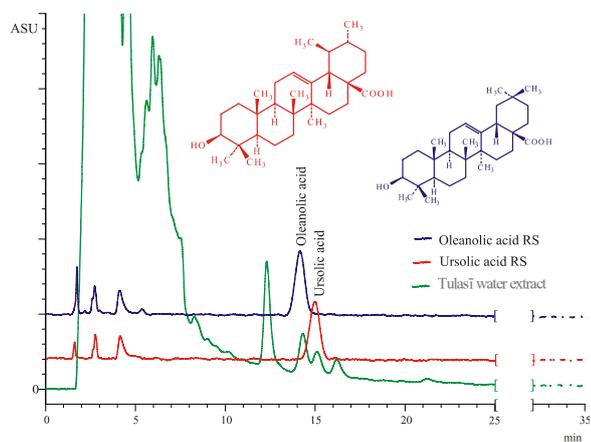
### Additional requirements:

**Storage:** Store in well closed container protected from heat, light, moisture and against attack by insects and rodents.

**Labelling:** The label states the official name, followed by the Latin binominal name and the part of the plant contained in the article.

### API reference standards:

API *Oleanolic acid RS* and *Ursolic acid RS*



**Fig. 2: HPLC chromatograms of Tulasi water extract with *Oleanolic acid* and *Ursolic acid* as 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 -**

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 millimeters (mm) and for aperture sizes smaller than 1 mm, these are expressed in micrometers (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  $\mu$ l 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  $\mu$ l 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°. However, the temperature generally specified for measurements of volume in the analytical operations of the pharmacopoeia, unless otherwise stated, is 25°. The discrepancy is inconsequential as long as the room temperature in the laboratory is reasonably constant and is around 27°.

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.

#### 1. 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.

**Bremer'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 min grains of starch otherwise undetectable.

**Chlorzinciodine (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 min 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.*

**Cuoxam (Ammoniacal Solution of Copper Oxide):** 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 1 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 min 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:** 1g of *phloroglucinol* dissolved in 100 ml of 90 per cent *ethyl alcohol*; mount debris in a few drops, allow to react for a min, 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 chloral 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*.

**Schweitzer'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. *sulphuric 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 per cent 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 unaided eye or using 6 x or 10 x 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 min 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 unpowdered 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<sup>0</sup> 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<sup>0</sup> until free from carbon, cool in a desiccator for 30 min 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<sup>0</sup>. 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 min with 25 ml of *water*; collect insoluble matter in a Gooch crucible, or on an ashless filter paper (Whatman 41), wash with hot *water*, and ignite for 15 min at a temperature not exceeding 450<sup>0</sup>. Subtract 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 min 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 24 hours, shaking frequently during 6 hours and allowing to stand for 18 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<sup>0</sup>, 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 pH Value:

The *pH* 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 *pH* is defined as the value given by a suitable, properly standardized, *pH* meter capable of reproducing *pH* values to 0.05 *pH* 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 *pH* 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 *pH* reading through a “temperature” and/or “slope” control. Measurements are made at 25 ± 2<sup>0</sup>, unless otherwise specified.

To standardize the *pH* meter, select two Buffer Solutions whose difference in *pH* does not exceed 4 units such that the expected *pH* of the material under test falls between them. Commercially available buffer solutions for *pH* 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 *pH* value identical with that of the declared *pH*. 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 *pH* of the second buffer solution is within  $\pm 0.07$  *pH* 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 *pH* values within 0.05 *pH* 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 *pH* value. Use carbon dioxide-free *water* for solution or dilution of test material in *pH* determinations. In all *pH* 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 *pH*.

#### 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 min, 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 min 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<sup>0</sup> 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.

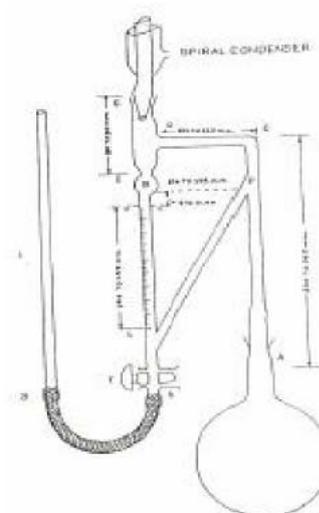
#### Determination of Total Soluble Solids: Method-II

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. 1). 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.



**Fig. 1: Apparatus for Volatile oil Content determination**

**(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 milliliters graduated into fifths and fiftieths of a milliliter.

**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 min and the tap T is opened and the tube L<sub>1</sub> 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<sub>1</sub> 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 1: 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 3 g 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° 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 2: 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	<i>Staphylococcus aureus</i> /g	Absent	-
2	<i>Salmonella</i> sp./g	Absent	Absent
3	<i>Pseudomonas aeruginosa</i> /g	Absent	-
4	<i>Escherichia coli</i>	Absent	10
5	Total microbial plate count (TPC)	10 <sup>5</sup> */g	10 <sup>7</sup> /g
6	Total Yeast & Mould	10 <sup>3</sup> /g	10 <sup>5</sup> /g

\*For topical use, the limits shall be 10<sup>7</sup>/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 pH 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<sup>0</sup> for 15 min. 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 pH in the medium when it is ready for use. Determine the pH at 25<sup>0</sup> ± 2<sup>0</sup>.

#### 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
Glycerin	12.0 g
Sodium pyruvate	10.0 g
Water to	1000 ml

Heat with frequent agitation and boil for 1 min. Sterilize, cool in between 45<sup>0</sup>-50<sup>0</sup>, 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 pH after sterilization to  $6.8 \pm 0.2$ .

### **Bismuth Sulphite Agar Medium**

#### Solution (1)

Beef extract	6.0 g
Peptone	10.0 g
Agar	24.0 g
Ferric citrate	0.4 g
Brilliant green	10.0 mg
Water to	1000 ml

Dissolve with the aid of heat and sterilize by maintaining at  $115^{\circ}$  for 30 min.

#### Solution (2)

Ammonium bismuth citrate	3.0 g
Sodium sulphite	10.0 g
Anhydrous disodium hydrogen phosphate	5.0 g
Dextrose monohydrate	5.0 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  $55^{\circ}$  and pour.

Bismuth Sulphite Agar Medium should be stored at  $2^{\circ}$  to  $8^{\circ}$  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
Sodium chloride	5.0 g
Water to	1000 ml

Mix, allow to stand for 15 min, sterilize by maintaining at  $115^{\circ}$  for 30 min and mix before pouring.

### **Buffered Sodium Chloride - Peptone Solution**

#### **pH 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  $121^{\circ}$  for 15 min.

### **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 pH 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 min with shaking. Adjust the pH 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
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 min. Gently boil with continuous stirring and continue boiling until solution is complete. Cool to  $80^{\circ}$ , 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	20.0 g
Soya lecithin	5.0 g
Polysorbate 20	40.0 ml
Water to	1000 ml

Dissolve the pancreatic digest of casein and soya lecithin in *water*, heating in a water-bath at 48<sup>0</sup> to 50<sup>0</sup> for about 30 min 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 pH 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 pH after sterilization to 6.9 ± 0.2.\*

**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	65.0 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 pH 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	30.0 mg
Crystal violet	1.0 mg
Water to	1000 ml

Boil the mixture of solids and *water* for 1 min to effect solution. Adjust the pH 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	10.0 mg
Water to	1000 ml

Adjust the pH after sterilization to 7.3 ± 0.2\*.

**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 min to effect solution. Adjust the pH 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	5.0 mg
Water to	1000 ml

\* Sterilize at 121° for 15 minutes in an autoclave

Dissolve with the aid of heat. Adjust the *pH* to 8.0 to 8.4 with 5M *sodium hydroxide* and boil for 10 min. Filter and sterilize by maintaining at 115<sup>0</sup> for 30 min and adjust the *pH* 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 min to effect solution. Adjust the *pH* after sterilization to 7.2 ± 0.2\*.

#### **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 min to effect solution. Adjust the *pH* after sterilization to 7.2 ± 0.2\*.

#### **Sabouraud Dextrose Agar Medium**

Dextrose	40.0 g
Peptic digest of animal tissue and pancreatic digest of casein (1:1)	10.0 g
Agar	15.0 g
Water to	1000 ml

Mix, and boil to effect solution. Adjust the *pH* 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	5.0 g
Lactose	4.0 g
Disodium hydrogen phosphate	10.0 g
Sodium hydrogen selenite	4.0 g
Water to	1000 ml

Dissolve, distribute in sterile containers and sterilize by maintaining at 100<sup>0</sup> for 30 min.

#### **Fluid Selenite - Cystine Medium**

Pancreatic digest of casein	5.0 g
Lactose	4.0 g
Sodium phosphate	10.0 g
Sodium hydrogen selenite	4.0 g
l-Cystine	10.0 mg
Water to	1000 ml

Mix and heat in flowing steam for 15 min. Adjust the final *pH* 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	70.0 mg
Water to	1000 ml

Heat just to boiling; do not reheat. Adjust the *pH* so that after heating it is 7.0 ± 0.2.

\* Sterilize at 121<sup>0</sup> for 15 minutes in an autoclave

**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	24.0 mg
Water to	1000 ml

Mix, allow standing for 15 min, bringing to boil and maintain at boiling point until solution is complete, mix, distributing in tubes and sterilising by maintaining at 121<sup>0</sup> for 15 min. 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	10.0 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 min. Sterilize, cool to between 45<sup>0</sup>-50<sup>0</sup> and add 20 ml of 1 per cent w/v sterile solution of *potassium tellurite*. Adjust the pH 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	80.0 mg
Agar	13.5 g
Sodium desoxycholate	2.5 g
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<sup>0</sup> and pour into plates as soon as the medium has cooled. Adjust the final pH 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 pH 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 pH to about 7.

\* Sterilize at 121<sup>0</sup> for 15 minutes in an autoclave

**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 pH 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 wetttable substances. If necessary, adjust the pH 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°. Mix carefully while maintaining the temperature in the water-bath or in an oven. Add 85 ml of buffered *sodium chloride-peptone* solution pH 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° if necessary. Maintain this temperature for the shortest time necessary for formation of an emulsion and in any case for not more than 30 min. If necessary, adjust the pH 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° to 35° in the test for bacteria and 20° to 25° 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°. 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° to 35° 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° to 25° 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  $\mu$ l) and 10 mg (or 10  $\mu$ l) 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 3, indicate the most probable number of microorganisms per g or per ml of the test specimen.

**Table 3: 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 $\mu$ l)	10 (10 $\mu$ l)	1 (1 $\mu$ l)	
3	3	3	>1100
3	3	3	1100
3	3	2	500
3	3	1	200
3	3	0	290
3	2	3	210
3	2	2	150
3	2	1	90
3	2	0	160
3	1	3	120
3	1	2	70
3	1	1	40
3	1	0	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 pH 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<sup>0</sup> 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 36-38<sup>0</sup> 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<sup>0</sup> 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 min; 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<sup>0</sup> for 24 hours.

**Table 4: 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<sup>0</sup> 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<sup>0</sup> for 18 to 24 hours. Upon examination, if none of

the colonies conforms to the description given in Table 4, the sample meets the requirements of the test for the absence of the genus *Salmonella*. If any colonies conforming to the description in Table 4 are produced, carry out the secondary test.

**Secondary test:** Subculture any colonies showing the characteristics given in Table 4 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<sup>0</sup> to 38<sup>0</sup> 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<sup>0</sup> to 37<sup>0</sup> 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<sup>0</sup> to 37<sup>0</sup> for 18 to 24 hours. If, upon examination, none of the plates contains colonies having the characteristics listed in Table 5 for the media used, the sample meets the requirement for freedom from *Pseudomonas aeruginosa*. If any colonies

conforming to the description in Table 5 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<sup>0</sup> to 37<sup>0</sup> 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 5 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 5: Tests for *Pseudomonas aeruginosa***

Medium	Characteristic colonial morphology	Fluorescence in UV light	Oxidase test	Gram stain
Cetrimide agar	Generally greenish	Greenish	Positive	Negative rods
<i>Pseudomonas</i> agar medium for detection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
<i>Pseudomonas</i> 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 6 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 6 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<sup>0</sup> 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 6: 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<sup>0</sup> to 35<sup>0</sup> for 18 to 24 hours or, for *Candida albicans*, at 20<sup>0</sup> for 48 hours.

<i>Staphylococcus aureus</i>	(ATCC 6538; NCTC 10788)
<i>Bacillus subtilis</i>	(ATCC 6633; NCIB 8054)
<i>Escherichia coli</i>	(ATCC 8739; NCIB 8545)
<i>Candida albicans</i>	(ATCC 2091; ATCC 10231)

Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 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 pH 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<sup>0</sup> to 35<sup>0</sup> for 18 to 24 hours. Dilute portions of each of the cultures using buffered sodium chloride-peptone solution pH 7.0 to make test suspensions containing about 10<sup>3</sup> viable microorganisms per ml. Mix equal volume of each suspension and use 0.4 ml (approximately 10<sup>2</sup> 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 7: 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 <i>cis</i> -, <i>trans</i> - 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
Dichlorvos	1.0
Dithiocarbamates (as CS <sub>2</sub> )	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 $\gamma$ )	0.3
Lindane ( $\gamma$ -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.

$$\frac{ADI \times M}{MDD \times 100}$$

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:

$$\frac{\text{ADI} \times \text{M} \times \text{E}}{\text{MDD} \times 100}$$

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-D):** To 10 g of the substance being examined, add 100 ml of *acetone* and allow to stand for 20 min. Add 1 ml of a solution containing 1.8 µg/ml of *carbophenothion* in *toluene*. Homogenise using a high-speed blender

for 3 min. 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<sup>0</sup> 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 150<sup>0</sup> 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 300 ml of *acetonitrile : water* (3 : 1) and homogenise using a high-speed blender for 5 min. 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 8: Permissible Limit of Aflatoxins**

Aflatoxin	Permissible Limit
B1	< 2 ppb
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 B 1, 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 min 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 min. 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 min. 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 min. 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 min. 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 min; 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 min, 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 B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub> 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 B<sub>1</sub> and G<sub>1</sub>, 0.2 µg/ml each for aflatoxins B<sub>2</sub> and G<sub>2</sub>.

**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<sub>f</sub> 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  $\mu\text{m}$  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  $\mu\text{l}$  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<sup>0</sup> to 105<sup>0</sup> for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 min 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 visualize 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.

#### **Visualization:**

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<sub>f</sub> Value:**

Measure and record the distance of each spot from the point of its application and calculate the R<sub>f</sub> 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**

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 exact 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 $\mu$ l 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 rates, 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.

$$A (1 \text{ per cent, } 1 \text{ cm}) = A/c_l$$

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<sup>0</sup> to 26<sup>0</sup>. 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<sup>0</sup> and 26<sup>0</sup>. 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  $\pm 2$  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  $\pm 3$  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 vapourisation 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 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 $\mu$ m to 5.0 $\mu$ m thick.

Packed columns, made of glass or metal, are usually 1 m to 3 m in length with an internal diameter 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  $\mu$ m to 180  $\mu$ m and 125  $\mu$ m to 150  $\mu$ m.

**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 min 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 1 g, of the substance being examined, dissolve in 15 ml of *dimethylformamide*. Heat the flask and collect the exactly 10 ml 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  $\mu\text{m}$ , 1.8  $\mu\text{m}$  or 3  $\mu\text{m}$ .

**Temperature:** Column 34<sup>0</sup> to 100<sup>0</sup> @ 15<sup>0</sup>/min., then increase to 180<sup>0</sup> @ 25<sup>0</sup>/min then increase to 225<sup>0</sup> @ 40<sup>0</sup>/min. Injection port temperature 250<sup>0</sup>; detector temperature 275<sup>0</sup>.

**Carrier Gas:** Nitrogen for chromatography at an appropriate flow

**Procedure:** Inject 1  $\mu\text{l}$  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. On the product label, 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°). 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 as, 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 system 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, pH 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 antimicrobial 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 6 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 month 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

Study	Storage condition	Minimum time
Accelerated	40° ± 2° / 75 % RH ± 5 %	6 months
Long term	30° ± 2° / 60 % RH ± 5 %	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<sup>0</sup> 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°.

### 3.9.8 Evaluation

The purpose of stability is to establish, based on testing a minimum of at least 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 analyzed for its marker). A + or - 15 per cent change from the initial assay value (If the drug is analyzed for its active compound).
2. Appearance of new spots in Identification by TLC (when compared with the sample stored in less than 10<sup>0</sup>) 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.

Failure to meet acceptance criteria for appearance (Physical attributes, and functionality tests *eg.*, 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  $25^0$  about 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 min 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 min, 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 0.1 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 min.

*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<sup>0</sup>; 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<sup>0</sup>, flammable. *Alcohol* containing not less than 94.85 per cent v/v and not more than 95.2 per cent v/v of C<sub>2</sub>H<sub>5</sub>OH at 15.56<sup>0</sup>.

*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<sup>0</sup>

*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<sup>0</sup> for thirty min; 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 min 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<sup>0</sup> for ten min; 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 150 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 150 for five min; 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 min.

*Aldehydes and ketones* - Heat 100 ml of *hydroxylamine hydrochloride* solution in a loosely stoppered flask on a water-bath for thirty min, 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 min in a loosely stoppered flask. Cool, transfer to a *Nessler 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 105<sup>0</sup> 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 min 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.

**0.1 M ammonia** - Solution of any molarity  $x$  M may be prepared by diluting  $75x$  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** -  $\text{Br}_2 = 159.8$  (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 0.05 M *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;  $\text{C}_4\text{H}_{10}\text{O} = 74.12$  (71-36-3).

Analytical reagent grade of commerce.

A colourless liquid; boiling point,  $116^0$  to  $119^0$ ;  $d_{20}^{20}$ , about 0.81

**Butan-1-ol** - n-Butyl alcohol; butanol;  $\text{C}_4\text{H}_{10}\text{O} = 74.12$  (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;  $\text{CHCl}_3 = 119.4$  (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** -  $\text{C}_6\text{H}_8\text{O}_7, \text{H}_2\text{O} = 210.1$  (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 10 M ammonia and add sufficient *water* to produce 20 ml. No pink colour is produced.

**Ether** -  $\text{C}_4\text{H}_{10}\text{O} = 74.12$  (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 min. No colour is produced.

Store protected from light at a temperature not exceeding 15<sup>0</sup>. The name and concentration of any added stabiliser are stated on the label.

**1,4-Dioxane** - 1,4-Dioxan; Diethylene Dioxide:  
C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> = 88.11

Analytical reagent grade of commerce.

Colourless liquid with an ethereal odour; bp, about 101<sup>0</sup>; 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-stoppered cylinder about 1.5 cm in diameter. Fill completely with the reagent under examination, shake vigorously and allow to stand protected from light for 30 min; no colour is produced.

#### Dragendorff's Reagent

*Solution A* - 0.85 g Basic *Bismuth nitrate* is dissolved in a mixture of 10 ml *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<sub>2</sub>H<sub>6</sub>O.

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<sub>2</sub>H<sub>6</sub>O.

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	Volume of Ethanol (96 per cent) (Approx.) ml	Weight per ml (g)
v/v		
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<sub>4</sub>H<sub>8</sub>O<sub>2</sub> = 88.1 (14 1-78-6)

Analytical reagent grade of commerce

A colourless liquid with a fruity odour; boiling point, about 76<sup>0</sup> to 78<sup>0</sup>; d<sub>20</sub><sup>20</sup> 0.90 1 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<sup>0</sup>). It should be golden in colour. Do not use it if it turns olive green.

**Phosphomolybdotungstic Reagent (Folins reagent)** Folin Ciocalteu 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 min), 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<sup>0</sup> to 8<sup>0</sup>.

**Formic Acid, Anhydrous** -  $\text{CH}_2\text{O}_2 = 46.03$ 

Analytical reagent grade formic acid of commerce containing not less than 98.0 per cent w/w of  $\text{CH}_2\text{O}_2$ .

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  $\text{CH}_2\text{O}_2$ .

**Hydrochloric Acid** -  $\text{HCl} = 36.46$  (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  $\text{HCl}$  and about 11.5 M in strength.

A colourless, fuming liquid

Solutions of molarity x M 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** -  $\text{Li}_2\text{SO}_4 \cdot \text{H}_2\text{O} = 128.0$  (10102-25-7)

Analytical reagent grade of commerce.

**Methanol** - Methyl alcohol;  $\text{CH}_4\text{O} = 32.04$  (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;  $\text{H}_3\text{PO}_4 = 98.00$  (7664-38-2)

Analytical reagent grade of commerce containing not less than 84 per cent w/w of  $\text{H}_3\text{PO}_4$  and about 15.7 M in strength.

A corrosive liquid; wt. per ml, about 1.75 g

**Potassium Dihydrogen Orthophosphate** - Potassium dihydrogen phosphate;  $\text{KH}_2\text{PO}_4 = 136.1$  (7778-77-0)

Analytical reagent grade of commerce, Colourless crystals

**Potassium Iodide** -  $\text{KI} = 166.0$  (7681-11-0)

Analytical reagent grade of commerce, A white, crystalline powder

**Sodium Carbonate** -  $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O} = 286.2$  (6132-02-1)

Analytical reagent grade of commerce. Melting point, greater than  $300^0$ .

**Sodium Molybdate** -  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O} = 242.0$  (10 102-40-6)

Analytical reagent grade of commerce

**Sodium Tungstate** -  $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O} = 329.9$  (10213-10-2)

Analytical reagent grade of commerce

**Sulphuric Acid** -  $\text{H}_2\text{SO}_4 = 98.08$  (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 18 M in strength; an oily, corrosive liquid; wt. 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 10 ml of *sulphuric acid* to 90 ml of ice- cold methanol.

**Sodium Sulphate, Anhydrous** -  $\text{Na}_2\text{SO}_4 = 142.0$  (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**

30 g of *sodium carbonate* is dissolved in *water* and make up to 100 ml.

**Tetrahydrofuran** - Tetramethylene oxide;  $\text{C}_4\text{H}_8\text{O} = 72.11$  (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 min 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;  $\text{C}_7\text{H}_8 = 92.14$  (108-88-3)

Analytical reagent grade of commerce

A colourless liquid with a characteristic odour; wt. per ml, 0.865 to 0.870 g; boiling point, about  $110^0$

**Vanillin**- 4-Hydroxy-3 - methoxybenzaldehyde;  $\text{C}_8\text{H}_8\text{O}_3 = 152.2$  (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 90 ml of *ethanol*; add drop wise 10 ml 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 min 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			II. LINEAR MEASURES		
Classical Unit		Metric Equivalent	Classical Unit	Inches	Metric Equivalent
1 Rattī or Guñjā		= 125 mg	Yavodara	1/8 of 3/4"	0.24 cm
8 Rattī or Guñjā	= 1 Māṣa	= 1 g	Aṅgula	3/4"	1.95 cm
12 Māṣas	= 1 Karṣa (Tolā)	= 12 g	Vitasti	9"	22.86 cm
2 Karṣas	= 1 Śukti	= 24 g	Aratni	10 1/2"	41.91 cm
2 Śukti	= 1 Palam	= 48 g	Hasta	18"	45.72 cm
2 Palas	= 1 Prasṛti	= 96 g	Nṛpahasta	22"	55.88 cm
2 Prasṛtis	= 1 Kuḍava	= 192 g	(Rājahasta)		
2 Kuḍavas	= 1 Mānikā	= 384 g	Vyāma	72"	182.88 cm
2 Mānikās	= 1 Prastha	= 768 g	III. MEASUREMENT OF TIME		
4 Prasthas	= 1 Āḍhaka	= 3 kg 72 g	Unit	Equivalent (in hours, min & seconds)	
4 Āḍhakas	= 1 Droṇa	= 12 kg 288 g	2 Kṣaṇas	= 1 Lava	
2 Droṇas	= 1 Śūrpa	= 24 kg 576 g	2 Lavas	= 1 Nimeṣa	
2 Śūrpas	= 1 Droṇī (Vāhī)	= 49 kg 152 g	3 Nimeṣas	= 1 Kāṣṭhā	= 4.66 seconds
4 Droṇīs	= 1 Khārī	= 196 kg 608 g	1 Ghaṭīs		= 24 min
100 Palas	= 1 Tulā	= 4 kg 800 g	30 Kāṣṭhās	= 1 Kalā	= 2 min
20 Tulās	= 1 Bhāra	= 96 kg			20 seconds
In case of liquids, the metric equivalents would be the corresponding litre and milliliter.			20 Kalās + 3 Kāṣṭhās	= 1 Muhūrta	= 48 min
			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	= 1 Yuga	= 5 Years
			1 Ahorātra of Devas		= 1 Year
			1 Ahorātra of Pitaras		= 1 Month

## 5.2. Metric System

### Measures of Mass (Weights)

- 1 Kilogram (Kg) - is the mass of the International Prototype Kilogram
- 1 Gram (g) - the 1000<sup>th</sup> part of 1 Kilogram
- 1 Milligram (mg) - the 1000<sup>th</sup> part of 1 gramme
- 1 Microgram ( $\mu$ g) - the 1000<sup>th</sup> part of 1 milligram

### Measures of capacity (Volumes)

1 Litre (l) 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<sup>th</sup> 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<sup>o</sup> 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<sup>th</sup> part of 1 metre
- 1 Millimetre (mm) - the 1000<sup>th</sup> part of 1 metre
- 1 Micron ( $\mu$ ) - the 1000<sup>th</sup> part of 1 millimetre
- 1 Milliimicron ( $m\mu$ ) - the 1000<sup>th</sup> part of micron