A HANDBOOK ON PRACTICAL PHARMACOGNOSY AND PHYTOCHEMISTRY

Kaveti Vamshi Sharathnath

Dr. B. Sree Giri Prasad

Narender Boggula

Kripa Drishti Publications, Pune.

A HANDBOOK ON PRACTICAL PHARMACOGNOSY AND PHYTOCHEMISTRY

Kaveti Vamshi Sharathnath

Dr. B. Sree Giri Prasad

Narender Boggula

Kripa-Drishti Publications, Pune.

Book Title: **A Handbook on Practical Pharmacognosy and Phytochemistry**

Authored by: **Kaveti Vamshi Sharathnath, Dr. B. Sree Giri Prasad, Narender Boggula**

1st Edition

ISBN: **978-93-90847-76-1**

Published: **JUNE 2021**

Publisher:

Kripa-Drishti Publications
A/ 503, Poorva Height, SNO 148/1A/1/1A,
Sus Road, Pashan- 411021, Pune, Maharashtra, India.
Mob: +91-8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

© **Copyright KRIPA-DRISHTI PUBLICATIONS**
All Rights Reserved. No part of this publication can be stored in any retrieval system or reproduced in any form or by any means without the prior written permission of the publisher. Any person who does any unauthorized act in relation to this publication may be liable to criminal prosecution and civil claims for damages. [The responsibility for the facts stated, conclusions reached, etc., is entirely that of the author. The publisher is not responsible for them, whatsoever.]

PREFACE

People with open minded will always try to seek for the new things and information from many sources.

It gives immense pleasure to place before a large community of pharmacy students, my humble work on **A Handbook on Practical Pharmacognosy and Phytochemistry,** written in accordance with the recent syllabus prescribed for the B. Pharmacy students. My aim in writing this book is to present the fundamental principles **of A Handbook on Practical Pharmacognosy and Phytochemistry** for the pharmacy students on modern lines. Keeping in view the requirement of the students and the teachers, this book has been written to cover all the topics with the desired limits of the prescribed syllabus. I hope the book will be useful and meets the requirements of students and academicians.

It is a matter of my duty and delight to bring out the book **"A Handbook on Practical Pharmacognosy and Phytochemistry".** The idea of writing this book was convinced when our own students found difficulty in getting a book suited to their academic needs.

I feel immense pleasure to introducing **'A Handbook on Practical Pharmacognosy and Phytochemistry'** to the students of pharmaceutical sciences. Primarily this book has been written for the D. Pharmacy, B. Pharmacy, M. Pharmacy and Pharm D students. The major thrust has been to make the book more students friendly. The practical contents of the book are vital in the field of pharmaceutical sciences. Experiments have been explained according to the syllabus.

The importance of a laboratory course attached to a theory course is undisputable in science subjects. Every effort has been made to include these recent trends and knowledge in this book.

Most of the information in this book has represented in a very simple manner. The aim of this book is to make the subject easy and understandable to the students.

We have made every possible effort to make this book informative and useful to the teachers and students. I will be grateful to all the teachers and students who will be kind enough to point out my mistakes that have escaped my attention. Suggestions for future improvement are always welcome.

It is hoped that the book will be received favourably as an effective practical book by both students and faculty of pharmacy.

The structure of this book is simple, self-explanatory and easy for the readers to grasp the subject.

Kaveti Vamshi Sharathnath,

Dr. B. Sree Giri Prasad,

Narender Boggula

ACKNOWLEDGEMENTS

To write a book of this magnitude, it needs lot of patience, skills and expertise over the subject, which I have gained because of opportunity given to me by my teachers and friends. Writing a book is harder than we thought and more rewarding than we could have ever imagined. We express our immense gratefulness to almighty for will-power, patience, courage and dedication towards work has made me possible for successful completion of this handbook.

Preparing this book was a collective adventure and I am most grateful to all authors for their cooperation and for the time and the effort they spent to write their respective contributions. I appreciate also their patience, especially as the editing process took much more time than initially expected.

Last but not least, we would also express a special thanks to publishers for their encouragement and publishing the book.

"The greatest challenge in life is discovering who you are. The second greatest is being happy with what you find."

Kaveti Vamshi Sharathnath,

Dr. B. Sree Giri Prasad,

Narender Boggula

INDEX

Exp 1: Introduction to Microscope 1

Exp 2: Introduction to Extraction Techniques 7

Exp 3: Isolation of Piperine from Black Pepper 20

Exp 4: Isolation of Eugenol from Clove 21

Exp 5: Isolation of Trimyristin from Nutmeg 23

Exp 6: Isolation of Azelaic Acid from Castor Oil 24

Exp 7: Isolation of Curcumin from Turmeric 26

Exp 8: Isolation of Sennosides from Senna Leaves 27

Exp 9: Isolation of Lycopene from Tomatoe 28

Exp 10: Isolation of Pectin from Orange Peel 29

Exp 11: Isolation of Hesperidin from Dried Orange Peels 31

Exp 12: Isolation of Ammonium Glycyrrhizinate from Liquorice Powder ... 33

Exp 13: Isolation of Solanine from Potatoes 34

Exp 14: Isolation of Naringin from Grape Fruit Peels 35

Exp 15: Isolation of Embelin from Embelia Ribes 36

Exp 16: Isolation of Quinine from Cinchona Bark 37

Exp 17: Isolation of Caffeine from Tea Powder 38

Exp 18: Isolation of Lactose from Milk 39

Exp 19: Isolation and Formulations of Nutrient-Rich Carotenoids 40

Exp 20: Isolation of Volatile Oil by Hydro Distillation 42

Exp 21: Isolation of Volatile Oil by Steam Distillation 43

Exp 22: Extraction of Calcium Citrate from Lemon 44

Exp 23: Isolation of Caffeine from Tea Powder...45

Exp 24: Isolation of Tannic Acid from Galls..46

Exp 25: Isolation of Volatile Oil from Caraway...47

Exp 26: Isolation of Volatile Oil from Clove..48

Exp 27: Aseptic Seed Germination..49

Exp 28: Tracer Technique 50

Exp 29: Preparation of Different Alkaloidal Testing Reagents.......................55

Exp 30: Reagents Used..56

Exp 31: Preliminary Phytochemical Screening...57

Exp 32: Thin Layer Chromatography of Alkaloids..61

Exp 33: Introduction of Leaf Constants..62

Exp 34: Determination of Stomatal Number and Stomatal Index of Vinca Leaf...64

Exp 35: Determination of Stomatal Number and Stomatal Index of Senna Leaf...65

Exp 36: Determination of Stomatal Number and Stomatal Index of Datura Leaf...66

Exp 37: Morphology of Acacia 67

Exp 38: Morphology of Isabgol...69

Exp 39: Morphology of Starch..71

Exp 40: Morphology of Tragacanth 73

Exp 41: Morphology of Honey..75

Exp 42: Morphology of Castor Oil 77

Exp 43: Morphology of Linseed..78

Exp 44: Morphology of Beeswax 79

Exp 45: Morphology of Olive Oil...80

Exp 46: Morphology of Wool Fat ... 81

Exp 47: Morphology of Cinnamon ... 82

Exp 48: Microscopy of Cinnamon Bark ... 83

Exp 49: Morphology of Fennel ... 85

Exp 50: Microscopy of Fennel Fruit ... 86

Exp 51: Morphology of Coriander ... 88

Exp 52: Microscopy of Coriander Fruit ... 89

Exp 53: Morphology of Clove ... 91

Exp 54: Microscopy of Clove Bud ... 93

Exp 55: Morphology of Cinchona ... 95

Exp 56: Microscopy of Cinchona Bark ... 96

Exp 57: Introduction to Section Cutting ... 98

ISBN: 978-93-90847-76-1

Exp 1: Introduction to Microscope

A microscope (micro-small and scope-to view) is an optical instrument consisting of a lens or combination of lenses. It helps in magnification (enlargement) of the image of an object, which is too small to be viewed by the naked eye. There are three principal kinds of microscopes: Simple, Dissecting and compound.

Simple Microscope: It consists of a single lens or magnifying glass, fixed on a suitable frame to view any object e.g., a hand lens. It is useful where only a low magnification is required e.g., for examination of external characters of crude drugs. Here the magnification is obtained is approximately two times. A simple microscope is a magnifying glass that has a double convex lens with a short focal length. The examples of this kind of instrument include the hand lens and reading lens. When an object is kept near the lens, then its principal focus with an image is produced which is erect and bigger than the original object. The formed image is virtual and cannot be projected on a screen like a real image. A simple microscope works on the principle that when a tiny object is placed within its focus, a virtual, erect and magnified image of the object is formed at the least distance of distinct vision from the eye held close to the lens.

Uses:

- It is used in pedology (a study of soil particles)
- It is used by a dermatologist to find out various skin diseases.
- It is used in microbiology to study samples of algae, fungi etc.
- It is used by the jewellers to get a magnified view of the fine parts of the jewellery.

Figure 1.1: Simple Microscope

Dissecting Microscope:

This is nothing but the simple microscope with additional features of a stage, on which a dissection can be carried out, a suitable hand rest for convenience, and a mirror to focus the light on the object.

The lens can be raised or lowered by rack-and-pinion arrangement or moved horizontally for proper focusing. The magnification in this case is about five times.

To reveal the anatomical characters of plant parts (like leaves, fruits, seeds, flowers etc.) or animals (like cockroach, mosquito, house fly, etc.), a simple instrument used in the laboratory is known as the dissecting microscope (Figure 1.2).

Its arrangement is similar to that of a compound microscope. It has a powerful bi-convex lens, capable of enlarging the object to 50 times its original size. It also consists of a mirror, a stage or platform made up of thick plain glass, clips, a course adjustment screw and an eye piece.

All these are fixed with a vertical rod on a strong steel base. The mirror is meant for reflection of light, the stage for placing the material under study and the clips to hold the material to be observed. The bi-convex lens is fixed in the eye piece, which can be moved up and down.

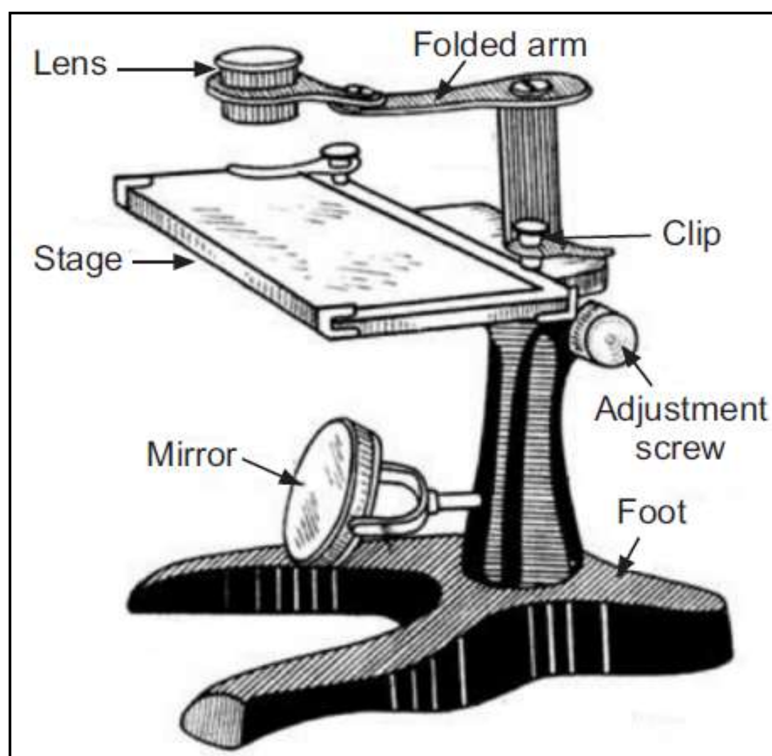

Figure 1.2: Dissecting Microscope

Compound Microscope:

It consists of two sets of lenses. Here one set of lenses of short focal length is used to produce an enlarged image of an illuminated object at a short distance, which is further enlarged by the second set of lenses placed approximately.

A compound microscope consists of the following parts:

- The base, usually U or V shaped, which rests on the table.
- The pillar, an upright bar supporting the rest of instrument on an inclination joint.
- The stage, a horizontal shelf with graduated mechanical slide holder with X and Y movement, for holding the slide to be examined. The stage bears a hole in the centre for transmitting light reflected up by the mirror.
- The mirror, situated below the stage, reflects the light upward through the hole in the stage. The mirror is usually double-faced. The plane face is for initial light intensity and the concave for concentrating the light on the object.
- The diaphragm, situated in between the hole on the stage and the mirror, regulates the amount of light reflected by the mirror.
- The body tube, a cylinder holding the draw tube and lenses and move up and down vertically above the hole in the stage. The tube is raised or lowered by the coarse adjustment and is used for finding the focus.
- The fine adjustment, which on being turned produces a very slow motion of the entire frame work which holds the body tube and is used for exact focusing of the higher power lenses.
- The ocular or eye piece is to be inserted into the upper end of the draw tube. It consists of two plane-convex lenses, the lower and large collective or field lens increasing the field of vision and the upper and the smaller eye lens. Ocular enlarges the image formed by the objective.

  Midway between the field lens and eye lens is a perforated diaphragm, which cut out edge rays from the image, determining the size of the field of view. Oculars are designated usually with magnification numbers as 5x, 10x, 15x etc. or by figures which represent focal lengths.

- The objectives are fitted into the bottom of the body tube or nosepiece. Each of these consists of a system of 2, 3 or more lenses. Objectives, like oculars are usually designated by magnification numbers as 10, 45 etc. or by focal lengths.

  The smaller the number of focal lengths, the greater is its magnifying power. If only two objectives accompany your microscope, the lower power objective is the shorter in length. Objective enlarges the object and projects them in the direction of eyepiece.

- Magnification and field view: Microscopes are usually fitted with two objectives, 16mm and 4mm, two or three eyepieces, and condenser. Different combinations of eyepieces and objective give different magnification and field of view as indicated in the following table.

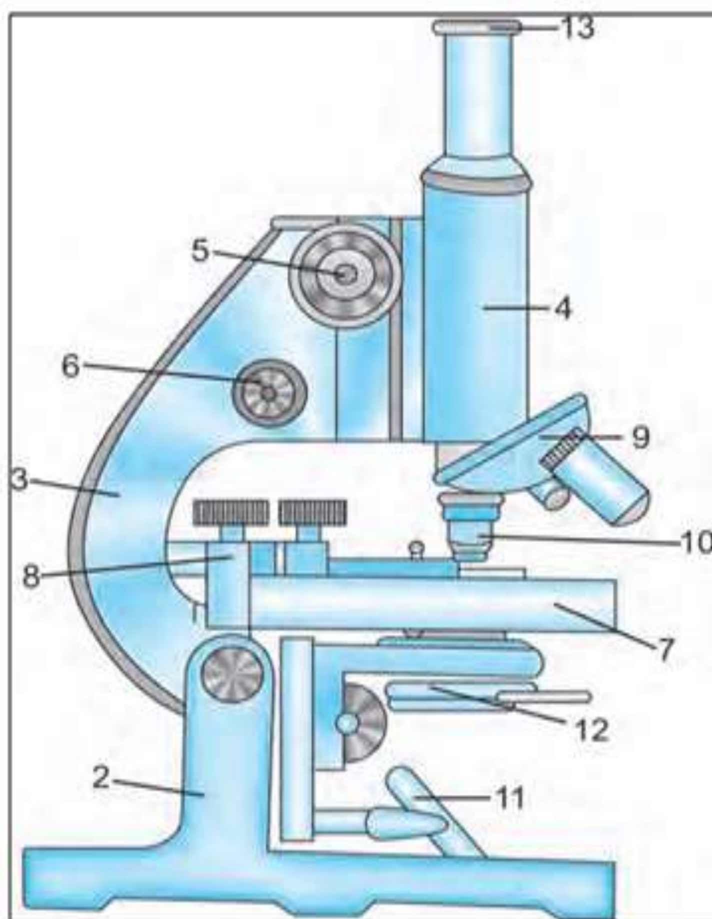

Figure 1.3: Compound Microscope

(1) Base, (2) Pillars, (3) Handle, (4) Body tube, (5) Coarse adjustment screw, (6) Fine adjustment screw, (7) Fixed stage, (8) Mechanical stage, (9) Fixed and revolving nose pieces, (10) Objective lenses, (11) Mirror, (12) Condenser and (13) Eye-piece

Table 1.1: Magnification and Field View

Focal length of objective	Initial magnifying power	Approximate magnification with eyepiece (field of view in bracket)		
		5x	10x	15x
16 mm	10	51 (1.65mm)	110 (1.1mm)	155 (0.89mm)
4 mm	45	238 (0.42mm)	490 (0.25mm)	690 (0.21mm)

When using the microscope, it is useful to have the knowledge about the size of the field of view, e.g., using a 4mm objective and a 5x eyepiece, field of view is approximately 0.42mm or 420μ. However, accurate measurements are made with eyepiece micrometer or camera lucida.

Objectives are of two types, dry lenses and immersion lenses. The lens is called a dry one, if an air space is present between the tip of the objective and the object: if a liquid is present, the lens is called an immersion lens (water immersion, if it is water or oil immersion, if it is oil). Some microscopes are fitted with a nosepiece capable of carrying 2, 3 or 4 objectives, which may be adjusted into place at the lower end of the body tube. Others have a condenser to concentrate the light upon the object to be examined. When using the condenser, use only the plane mirror.

How to Use Microscope:

- Place the microscope on the table with the arm or pillar nearest to you.
- Turn the lowest power objective into position.
- Find the light by looking into ocular (eye piece) and at the same time turn the mirror at such an angle that it reflects light from the window or lamp up through the hole in the stage to the objective.
- Place the prepared slide in the slide holder on the stage in the horizontal position with the object right in the centre of the whole through which light is reflected from the mirror.
- Make the lower power objective quite close to the slide by turning the coarse adjustment down. Then while looking through the eye piece move the coarse adjustment upward until the objective is seen distinctly. The object, if not under the lens, may now be brought into the field by moving the X-Y movements very slow while looking through the eye piece. Then slowly turn the fine adjustment to improve the focus.
- Regulate the quantity of light with the iris diaphragm to improve the clarity of the field.
- Raise the low power objective by means of coarse adjustment. Then turn the high-power objective into position and lower until the objective front lens nearly touches the cover glass. A slight movement of the fine adjustment should show the object clearly. Never try to focus with the high-power objective while looking through the eye piece because of the danger of breaking the cover glass and damaging the tissue lying underneath. It may also cause damage to the delicately mounted lenses.

Care of Microscope:

- While carrying the microscope from one place to another, hold it firmly by the arm in an erect position so that the ocular, which is fitted loosely in the draw tube, may not fall out.
- Do not allow the dry object or the liquid in which it is mounted to touch the lens.
- Do not touch the ocular or objective lenses with fingers or clothes.
- Do not clean the microscope or the stand with cleaning cloth soiled by reagents.

- Raise the body tube sufficiently while changing from low power to high power objective to avoid damage to the objective or the mounts.
- If the lens is soiled it may be cleaned with a clean cloth wetted with few drops of xylene.
- Never observe objects without putting a cover glass.
- For removing the slide from the stage, first raise the body tube and slowly slide it out of the stage.
- The slide must be prepared in such a manner that the stage is never wetted with any solvent or reagents.
- When not in use, microscope must be kept covered.
- Precautions:
- Select a stool or chair of suitable height so that your eyes are at a level slightly above the eyepiece. This will ensure comfortable working for long periods.
- Ensure that all the lenses are clean and free from dust and smudges. Do not touch them with your fingers, nor blow on them to remove dust.
- Check the position of the objective, condenser, and diaphragm, to ensure optimal illumination.
- Never lower any objective from any height while looking into the microscope.
- If the objectives are not parfocal, check the working distance of each objective separately by using fine focusing.
- Once a specimen has been focused, continuously "rack" the microscope.
- Cleaning the microscope. Never leave cedar wood oil on the OI lens, because it may seep into the body of the objective and damage the lens permanently. Dried oil is difficult to remove. Remove oil with lens paper, then xylene to clean the lens.
- Cover the microscope with the plastic cover after use.
- Microtome: Normally, a blade or a razor is used for taking the sections of the material under observation, but when a large number of sections are required for a detailed study, a hand operated machine "Microtome" shown in Figure 4 is used. With the microtome, even very thin and soft material can be used for taking transverse or longitudinal sections.

Figure 1.4: Microtome

ISBN: 978-93-90847-76-1

Exp 2: Introduction to Extraction Techniques

To achieve a suitable concentration of the active ingredients contained in the plants and that their action can be more effective, it is necessary to perform several procedures through which are extracted the active ingredients with the adequate solvents, selected according to the solubility and stability of the beneficial substances.

Extraction methods allow obtaining products in pharmaceutical forms suitable for oral or external dosage according to the place of action recommended. Extraction may be defined as the process of removal of desirable soluble constituents from a substance, leaving out those which are undesirable with the aid of solvent and standardized process. Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts and powdered extracts. Such preparations popularly have been called Galenicals, named after Galen, the second century Greek physician. The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as menstruum.

The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modern drugs. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

Galenicals: Decoctions, fluid extracts, infusions, solid and semi-solid extracts and tinctures are termed as Galenicals.

Menstruum: The solvent used for extraction is called menstruum.

Marc: The undissolved residue left behind is known as marc.

Expression: Extraction accomplished by mechanical mean is termed as expression.

Theory: The general procedure employed in extraction of organized drugs (those built up of natural cells and tissues) differs from that employed in extraction of un-organized drugs (mere exudates like gums, resins, and gum resin (or) deogum resin).

- Extraction implies that the desirable constituents should dissolve in the menstruum leaving behind the marc. Thus, all the factors which help in accomplishing, facilitating or enhancing the solubility of desired constituents in the menstruum explain the theory of extraction.

In case of organized drugs, the cells constituting the tissues contain various plant constituents in the fluid called the cell sap either in solution form or dispersed in a colloidal state on drying the drug, such dissolved or colloidally dispersed matter is precipitated, crystallized or deposited in an amorphous form in the cell structure. The drying or dehydration of natural drugs affects the strength of the cell wall but the contents of the dried cell sap are enclosed within and are prevented from being directly exposed to the solvent action of the menstruum. Thus, it is often necessary to comminute the drug prior to extraction so as to rupture it and expose the cell contents to the menstruum.

- The cell walls consist essentially of cellulose and these molecules being polar, there is natural affinity for polar solvents such as water, alcohol, etc. therefore, in presence of a polar solvent, the cellulose walls get hydrated or solvated and swell. This is why moistening of the drug with the menstruum is an important stage in extraction. Swelling or inhibition is maximum in polar solvents. Thus, water causes considerable swelling while there is lesser with ethanol.
- Mobility of liquids through crude is generally diffusion controlled which may be termed endosmosis. As the solution with in the cell wall becomes stronger, a differential concentration is set up within and outside the cell. This causes the stronger solution to diffuse out of the cell until equilibrium is attained foster through endosmosis and by ex-osmosis if the system is agitated.
- However, as the movement of liquid through the pores and capillaries present in natural systems is influenced by the surface tension and wetting properties of the liquid inside the cell. This displacement of air can be aided by employing a vacuum pump. In absence of agitation a significant stagnant diffusion layer exists around a given particle which acts as a barrier to diffusion away from the site. The entire process of drug extraction can summarily be divided into four essential steps.
- Penetration of the solvent into the drug.
- Dissolution of constituents.
- Outward diffusion of the solution from the cells and Separation of dissolved portion and the exhausted, upon the following
- Nature and properties of the drug and its extractable constituents,
- The particle size of the powdered drug;
- The nature of the solvent; and
- The state of contact between the solvent and drug particles.
- Gravitation, diffusion, osmosis, adhesion, capillarity, convection, solubility and surface tension are the physical factors that influence drug extraction. The optimum particle size of the crude drug powder will depend upon the botanical structure of the drug.

Treatment of the powdered drug with the menstruum of high temperature under pressure or passing a suspension of the drug powder in the menstruum through a colloid mill can alter the permeability of cell walls. Wetting property in the capillaries and also by using surface active agent. A surfactant can also increase the solubility of cellular contents in the menstruum by solubilization. Agitation brings about a faster equilibrium of the contents outside and inside the cell thus facilitating rapid dissolution of the desired constituents in the menstruum. Extraction at elevated temperatures whenever feasible has still many advantages.

E.g.:

- Decrease in viscosity reduces the boundary layers.
- Convection convents have an effect similar to agitation during extraction.
- Diffusion coefficients being directly proportional to absolute temperature and inversely.
- Proportional to viscosity, rate of diffusion is moderatory influenced by raising the temperature.
- In general, the solubility of the constituents in the menstruum increases as the temperature is raised.
- The solvent (menstruum) uses for extracting drugs should be chemically and physically inert material. Based on these considerations water, ethanol and their mixtures are most commonly used. Extraction implies separation of soluble matter from insoluble substances and thus differs from dissolution although the object of both the processes is the same.

Factors Affecting Choice of an Extraction Process:

- Character of the drugs
- Therapeutic value of the drug
- Stability of drug
- Cost of drug
- Solvent
- Concentration of the product

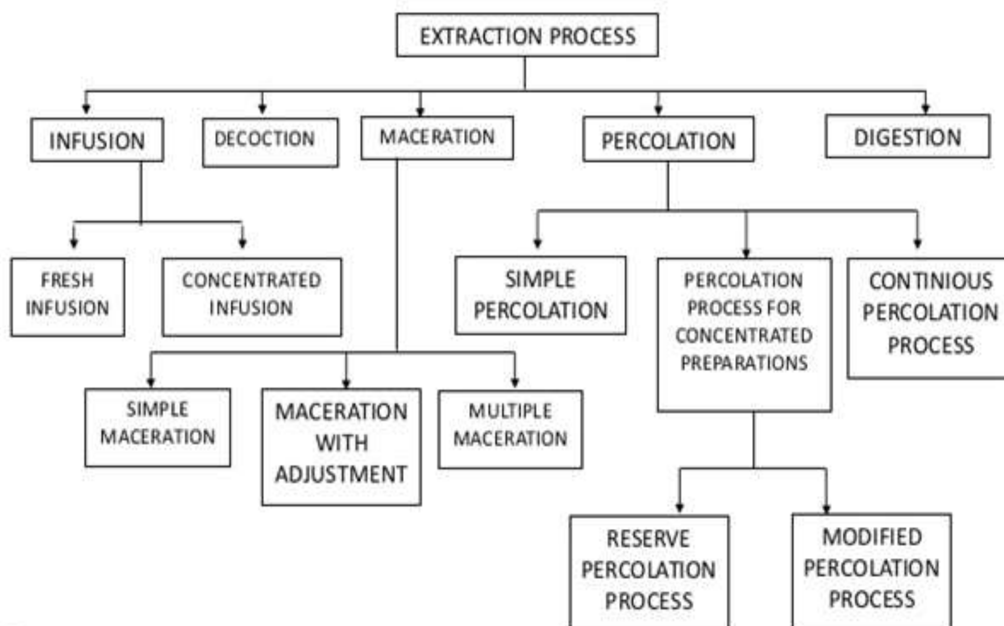

Figure 2.1: Schematic Representation of Different Types of Extraction Processes

The Main Extraction Methods are:

- Maceration
- Percolation
- Digestion
- Infusion
- Decoction

Methods of Extraction of Medicinal Plants:

Maceration:

In this process, the whole or coarsely powdered crude drug is place in a stoppered container with the solvent and allows standing at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. Strain the mixture, press the marc (the damp solid material), and clarify the combined liquids by filtration or decantation after standing.

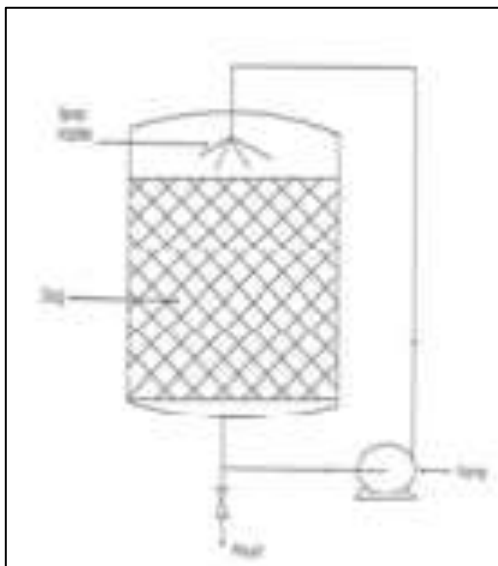

Figure 2.2: Circulatory extraction

Types of Maceration:

- Repeated maceration may be more efficient than a single maceration, since an appreciable amount of active principle may be left behind in the first pressing of the marc.
- The repeated maceration is more efficient in cases where active constituents are more valuable.
- Double maceration is used for concentrated infusions which contain volatile oil,

E.g., Concentrated compound gentian infusion.

- Triple maceration: Where the marc cannot be pressed, a process of triple maceration is sometimes employed. The total volume of solvent used is however large and the second and third macerates are usually mixed and evaporated before adding to the first macerates.

Infusion:

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

Digestion:

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

Decoction:

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat stable constituents. This process is typically used in preparation of Ayurvedic extracts called "Quath" or "Kawath".

The starting ratio of crude drug to water is fixed, e.g., 1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

Percolation:

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h.

The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

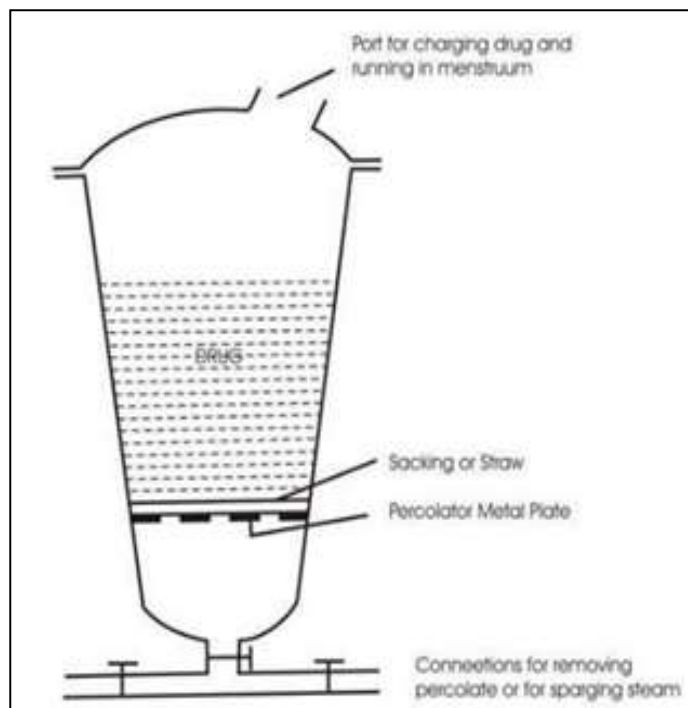

Figure 3: Percolator

Hot Continuous Extraction (Soxhlet):

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus.

The extracting solvent in flask A is heated, and its vapors condense in condenser D.

The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into flask A.

This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent.

This affects tremendous economy in terms of time, energy and consequently financial inputs.

At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

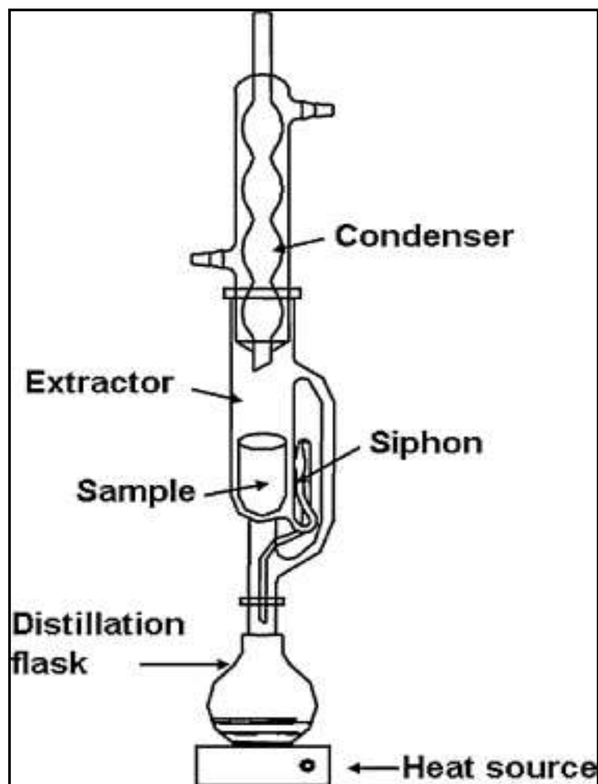

Figure 2.4: Hot Continuous Extraction

Ultrasound Extraction (Sonication):

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation.

Although the process is useful in some cases, like extraction of Rauwolfia root, its large-scale application is limited due to the higher costs.

One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

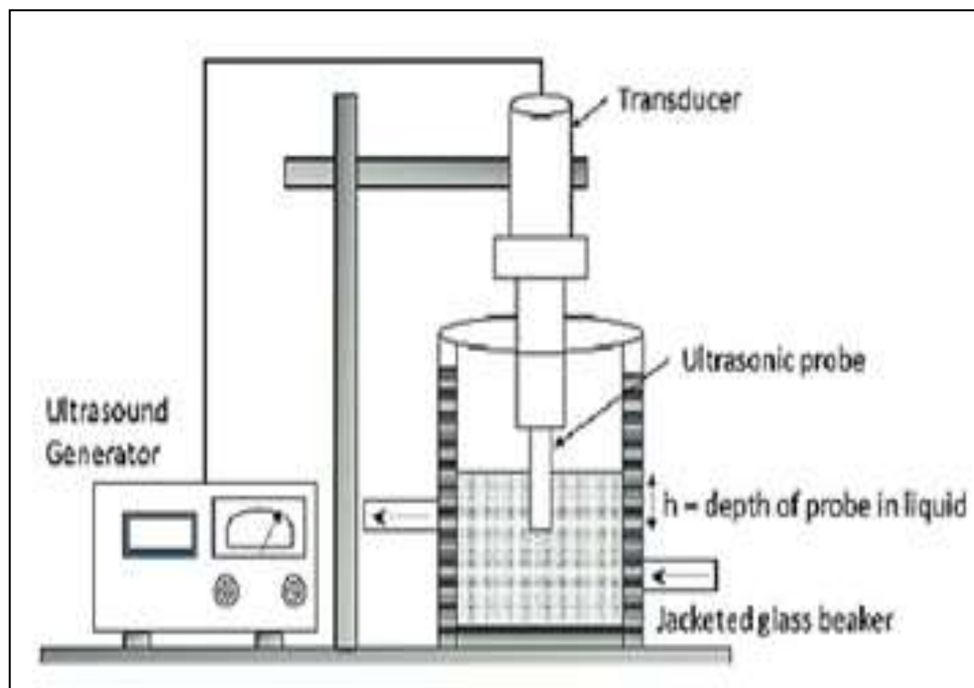

Figure 2.5: Ultrasound Sonicator

Supercritical Fluid Extraction:

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput.

The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (co-solvent) addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt.

The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor.

There are many advantages to the use of CO_2 as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations.

Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present.

Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations.

Of late, instead of carbon dioxide, argon is being used because it is inexpensive and more inert. The component recovery rates generally increase with increasing pressure or temperature: the highest recovery rates in case of argon are obtained at 500 atm and 150^0C.

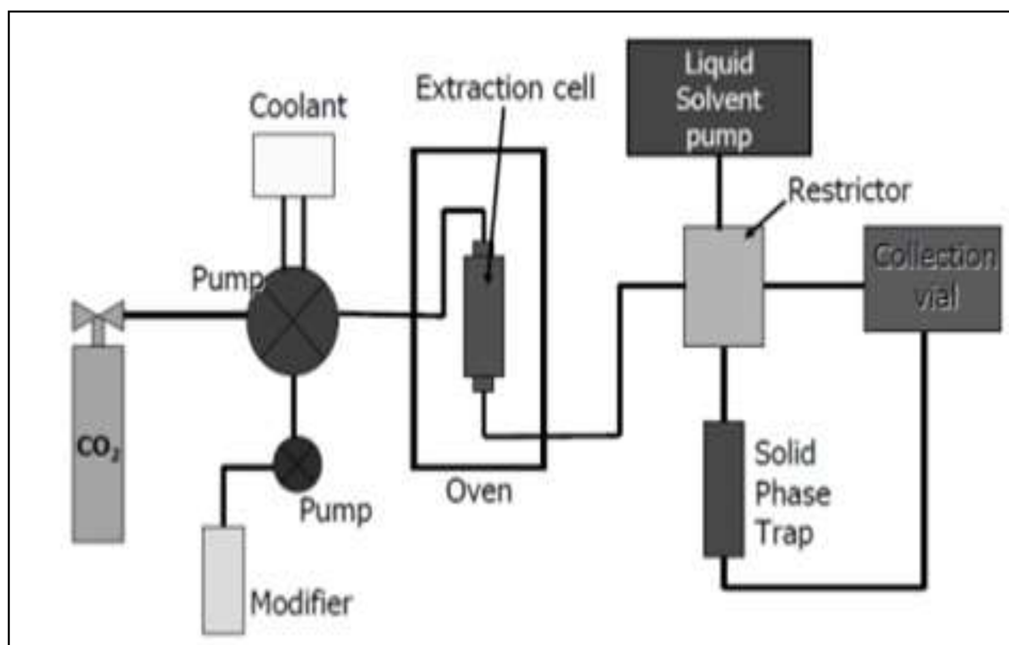

Figure 2.6: Supercritical fluid extraction

The Extraction Procedure Possesses Distinct Advantages:

a. The extraction of constituents at low temperature, which strictly avoids damage from heat and some organic solvents.
b. No solvent residues.
c. Environmentally friendly extraction procedure.

The largest area of growth in the development of SFE has been the rapid expansion of its applications. SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products. The major deterrent in the commercial application of the extraction process is its prohibitive capital investment.

Filtration:

Cake Filtration: It is the most frequently used model. Here it is assumed that the solids are deposited on the upstream side of the filter medium as a homogeneous porous layer with a constant permeability. As soon as the first layer of cake is formed, the subsequent filtration takes place at the top of the cake and the medium provides only a supporting function.

Thus, if the flow rate dV/dt is constant, the pressure drop will increase linearly, proportional to the quantity of solid deposited. This model can be applied especially for all hard, particulate solids.

Blocking Filtration:

The pressure drop is caused by solid particles blocking pores. Soft, gelatinous particles retained by a sieve exhibit such a behavior. If the flow rate dV/dt is constant, the pressure drop increases exponentially with the quantity filtered, the number of open pores asymptotically approaching zero.

The pores may belong to a filter medium (screen or filter layer) or it may be pores within a filter cake of coarse particles, which are blocked by migrating fine particles.

Aqueous Alcoholic Extraction by Fermentation:

Some medicinal preparations of Ayurveda (like Asava and Arista) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (Kashaya), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel.

In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are Karpurasava, Kanakasava, and Dashmularishta.

In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

Counter-Current Extraction:

In Counter-Current Extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce fine slurry. In this process, the material to be extracted is moved in one direction (generally in the form of fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent.

The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end.

This Extraction Process Has Significant Advantages:

a. A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction, and percolation.
b. CCE is commonly done at room temperature, which spares the thermolabile constituents from exposure to heat which is employed in most other techniques.
c. As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermolabile constituents from exposure to heat.
d. The extraction procedure has been rated to be more efficient and effective than continuous hot extraction.

Phytonics Process:

A new solvent based on hydrofluorocarbon-134a and a new technology to optimize its remarkable properties in the extraction of plant materials offer significant environmental advantages and health and safety benefits over traditional processes for the production of high quality natural fragrant oils, flavours and biological extracts. Advanced Phytonics Limited (Manchester, UK) has developed this patented technology termed "Phytonics process". The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment. The properties of the new generation of fluorocarbon solvents have been applied to the extraction of plant materials. The core of the solvent is 1, 1, and 2, 2-tetrafluoro ethane, better known as hydrofluorocarbon-134a (HFC-134a). This product was developed as a replacement for chlorofluorocarbons. The boiling point of this solvent is -25^{0}C.

It is not flammable or toxic. Unlike chlorofluorocarbons, it does not deplete the ozone layer. It has a vapour pressure of 5.6 bar at ambient temperature. By most standards this is a poor solvent. For example, it does not mix with mineral oils or triglycerides and it does not dissolve plant wastes.

The process is advantageous in that the solvents can be customized: by using modified solvents with HFC-134a, the process can be made highly selective in extracting a specific class of phytoconstituents. Similarly, other modified solvents can be used to extract a broader spectrum of components. The biological products made by this process have extremely low residual solvent.

The residuals are invariably less than 20 parts per billion and are frequently below levels of detection. These solvents are neither acidic nor alkaline and, therefore, have only minimal potential reaction effects on the botanical materials. The processing plant is totally sealed so that the solvents are continually recycled and fully recovered at the end of each production cycle. The only utility needed to operate these systems is electricity and, even then, they do no consume much energy. There is no scope for the escape of the solvents. Even if some solvents do escape, they contain no chlorine and therefore pose no threat to the ozone layer. The waste biomass from these plants is dry and "Eco friendly" to handle.

Advantages of the Process:

- Unlike other processes that employ high temperatures, the phytonics process is cool and gentle and its products are never damaged by exposure to temperatures in excess of ambient.
- No vacuum stripping is needed which, in other processes, leads to the loss of precious volatiles.
- The process is carried out entirely at neutral pH and, in the absence of oxygen; the products never suffer acid hydrolysis damage or oxidation.
- The technique is highly selective, offering a choice of operating conditions and hence a choice of end products.
- It is less threatening to the environment.
- It requires a minimum amount of electrical energy.
- It releases no harmful emissions into the atmosphere and the resultant waste products (spent biomass) are innocuous and pose no effluent disposal problems.
- The solvents used in the technique are not flammable, toxic or ozone depleting.
- The solvents are completely recycled within the system.

Applications:

The phytonics process can be used for extraction in biotechnology (e.g. for the production of anti-biotics), in the herbal drug industry, in the food, essential oil and flavour industries, and in the production of other pharmacologically active products. In particular, it is used in the production of top-quality pharmaceutical-grade extracts, pharmacologically active intermediates, antibiotic extracts and phytopharmaceuticals. However, the fact that it is used in all these areas in no way prevents its use in other areas. The technique is being used in the extraction of high-quality essential oils, oleoresins, natural food colours, flavours and aromatic oils from all manner of plant materials. The technique is also used in refining crude products obtained from other extraction processes. It provides extraction without waxes or other contaminants. It helps remove many biocides from contaminated biomass.

Parameters for Selecting an Appropriate Extraction Method:

a. Authentication of plant material should be done before performing extraction. Any foreign matter should be completely eliminated.
b. Use the right plant part and, for quality control purposes, record the age of plant and the time, season and place of collection.
c. Conditions used for drying the plant material largely depend on the nature of its chemical constituents. Hot or cold blowing air flow for drying is generally preferred. If a crude drug with high moisture content is to be used for extraction, suitable weight corrections should be incorporated.
d. Grinding methods should be specified and techniques that generate heat should be avoided as much as possible.
e. Powdered plant material should be passed through suitable sieves to get the required particles of uniform size.
f. Nature of constituents:

- If the therapeutic value lies in non-polar constituents, a non-polar solvent may be used. For example, lupeol is the active constituent of Crataeva nurvala and, for its extraction, hexane is generally used. Likewise, for plants like Bacopa monnieri and Centella asiatica, the active constituents are glycosides and hence a polar solvent like aqueous methanol may be used.
- If the constituents are thermolabile, extraction methods like cold maceration, percolation and CCE are preferred. For thermostable constituents, Soxhlet extraction (if non-aqueous solvents are used) and decoction (if water is the menstruum) are useful.
- Suitable precautions should be taken when dealing with constituents that degrade while being kept in organic solvents, e.g., flavonoids and phenyl propanoids.
- In case of hot extraction, higher than required temperature should be avoided. Some glycosides are likely to break upon continuous exposure to higher temperature.
- Standardization of time of extraction is important, as:
- Insufficient time means incomplete extraction.
- If the extraction time is longer, unwanted constituents may also be extracted. For example, if tea is boiled for too long, tannins are extracted which impart astringency to the final preparation.
- The number of extractions required for complete extraction is as important as the duration of each extraction.

g. The quality of water or menstruum used should be specified and controlled.
h. Concentration and drying procedures should ensure the safety and stability of the active constituents. Drying under reduced pressure (e.g., using a Rotavapor) is widely used. Lyophilization, although expensive, is increasingly employed.
i. The design and material of fabrication of the extractor are also to be taken into consideration.
j. Analytical parameters of the final extract, such as TLC and HPLC fingerprints, should be documented to monitor the quality of different batches of the extracts.

ISBN: 978-93-90847-76-1

Exp 3: Isolation of Piperine from Black Pepper

Aim: To isolate piperine from powdered black pepper.

Requirements: 95% Ethanol, alcoholic KOH, Soxhlet apparatus, vacuum distillation unit.

Discussion: Black pepper is obtained from dried unripe fruit of perennial climbing vine, *Piper nigrum* Linn, containing Family: Piperaceae. Pepper contains an alkaloid piperine (5-9%), volatile oil (1-2.5%), pungent resin (6%), piperidine and starch (about 30%).

The volatile oil is yellowish in colour and contains mainly l-phellandrene and caryophyllene.

It has a specific gravity of 0.898-0.900. Here piperine is extracted from black pepper with ethanol and may be converted to a red 1, 3, 5-trinitro benzene complex.

Procedure:

a. Extract the powdered drug (20g) with 250ml of ethanol (95%), in Soxhlet apparatus for 3 h.
b. Filter the concentrated solution under vacuum or water bath at 60^0C.
c. Add 20ml of 10% KOH with constant stirring to the concentrate extract and filter.
d. Allow the alcoholic solution to stand overnight, where upon needles of piperine separate out.
e. The yield of the yellowish crystals of piperine is approximately 2.5% w/w.

Uses: Stomachic, carminative, diaphoretic, gastric secretagogue, insecticide, to impart pungent taste to brandy.

ISBN: 978-93-90847-76-1

Exp 4: Isolation of Eugenol from Clove

Aim: To isolate eugenol from clove.

Requirements: Distillation unit, desiccator, solvent ether, KOH (10%), H_2SO_4.

Discussion: Essential oils are highly aromatic compounds extracted from a variety of botanical materials, including tree bark, flowers, stems, leaves, needles, plant roots, fruits and grasses. They are used in the production of perfumes, cosmetics, drinks, food flavouring, air fresheners, household cleaning products and aromatherapy oils. Essential oils also have a long history of use in traditional medicines.

Clove oil is the volatile oil obtained from the steam distillation of flower buds of *Eugenia caryophyllus*. Family: Myrtaceae.

It is heavier than water. Clove oil contains 70-80% eugenol acetate, caryophyllene and small quantity of esters, ketones and alcohols. Clove buds yield approximately 15% to 20% of a volatile oil that is responsible for the characteristic smell and flavour.

The bud also contains a tannin complex, a gum and resin, and a number of glucosides of sterols. The principal constituent of distilled clove bud oil (60-90%) is eugenol (4-allyl-2-methoxy phenol). The oil also contains about 10% acetyleugenol and small quantities of gallic acid, sesquiterpenes, furfural, vanillin, and methyl-n-amyl ketone.

Principle: Eugenol is a constituent of clove oil which is volatile in nature. The odourless volatile principles of the plant and animal origin are known as volatile oils. Chemically they are derived from terpenes and their oxygenated compounds. Volatiles are oil non-polar in nature, soluble in ether and other organic solvents and practically insoluble in water.

Clove oil is a phenol volatile oil and is extracted from clove by steam distillation. The clove oil is extracted from non-polar solvents like solvent ether for maximum miscibility. Then it is treated with 10% KOH to produce potassium eugenolate.

This potassium eugenolate is present in aqueous layer and eugenol is regenerated from aqueous layer by adding excess of sulphuric acid.

Procedure:

a. Dissolve 10ml of volatile oil in 100ml of solvent ether and shake with three successive quantities of 100ml 10% KOH.
b. Regenerate the eugenol by acidifying the aqueous layer with excess of sulphuric acid and extracting the acidified layer with three successive quantities of 50ml of solvent ether.
c. Distil off the solvent with care to ensure minimum loss of eugenol and dry the residue in desiccators.

Hydro Distillation:

Hydro distillation is also one of the mostly used methods for the extraction of essential oils. During hydro distillation method, powdered sample (100 g dried and ground clove buds) is soaked into water. To carry out hydro-distillation, dried clove sample is taken into 500 mL volumetric flask and subjected to hydro-distillation for 4-6 h. Subsequently, the volatile distillate is collected and saturated with sodium chloride following the addition of petroleum ether or other suitable organic solvent. Later, hydro and ether layers are separated and dehydrated by using anhydrous sodium sulphate. Eventually, the sample is heated in water bath at 60^0C for the recovery of ether and concentration of extract. The average yield of oil using hydro-distillation is about 11.5% whereas reported eugenol concentration is 50.5–53.5%. However, extraction yield can be increased by reducing the particle size of ground clove buds.

Uses: Analgesic, mainly dental analgesic, used in perfumery, as flavouring agent, dentifrices.

ISBN: 978-93-90847-76-1

Exp 5: Isolation of Trimyristin from Nutmeg

Aim: To isolate trimyristin from nutmeg powder.

Requirements: Nutmeg powder, diethyl ether, acetone, reflux assembly, distillation assembly.

Discussion: Nutmeg consists of dried kernels of seeds of *Myristica fragrans,* Family: Myristicaceae. The kernels are grayish brown or brown in colour, about 20-30mm in length and 20mm broad, ellipsoidal in shape, aromatic in odour and taste is pungent-aromatic. Nutmeg consists of 5-16% volatile oil and about 30% fat. The volatile oil consists about 4-8% myristicin, elemicin and safrole. The fatty acid constituents of fixed oils are myristic acid, palmitic acid, oleic acid, lauric acid and other fatty acids. The fat of nutmeg is also called as nutmeg butter. It also contains protein and starch. Trimyristin is isolated from crude nutmeg by simple extraction with diethyl ether.

Procedure:

a. Crush and grind whole nutmeg to coarse powder, weigh 15g of the powder and transfer it to 250ml RBF.
b. Add 15ml of diethyl ether (so that powder is covered with solvent), attach a reflux condenser and heat the mixture under a gentle reflux for 15 min on steam plate or water bath.
c. Allow the mixture to cool to room temperature without disturbing the settled solids.
d. Decant the supernatant layer of ether into conical flask.
e. Add another 10ml of ether to the solid residue and shake it vigorously for some time. Allow it to settle and decant the supernatant layer.
f. Add it to the previously collected contents. Add a porcelain piece to content and evaporate the ether fully on water bath.
g. Add sufficient acetone to dissolve the remaining crude solid product.
h. Collect the crystalline trimyristin by vacuum filtration and allow it to air dry.
i. Weigh the product and calculate the % trimyristin in the nutmeg.

Uses: Aromatic, anti-spasmodic, carminative, anti-emetic, anti-microbial, hypotensive.

ISBN: 978-93-90847-76-1

Exp 6: Isolation of Azelaic Acid from Castor Oil

Aim: To isolate azelaic acid from castor oil.

Requirements: Castor oil, potassium permanganate, ethanol, sulphuric acid, KOH, RBF, beaker.

Discussion: Castor oil is a glyceryl ester of ricinoleic acid. It is obtained from the isolated seeds of *Ricinus communis.* Family: Euphorbiaceae.

Castor seeds contain 40-60% of oil which is rich in triglycerides that is recinoline. It is a derivative of sebacic acid.

Azelaic acid is saturated dicarboxylic acid found in castor oil. Azelaic acid can be prepared by different methods.

a. By oxidation of castor oil with HNO_3.
b. By oxidation of recinoleic acid with nitric acid and alkaline $KMnO_4$.
c. By ozonization of methyl recinoleate.
d. By ozonization of oleic acid and decomposition of ozonide.
e. By oxidation of dihydroxystearic acid with dichromate in sulphuric acid.

Principle:

Castor oil is a glyceryl ester of ricinoleic acid. Castor oil is saponified in the presence of KOH and ethanol to yield potassium salt of ricinoleic acid, which is oxidized with $KMnO_4$ in presence of acid to give azelaic acid.

Procedure:

a. Weigh and add 11ml of castor oil to a solution of KOH in 120ml of alcohol and place in 250ml flask equipped with reflux condenser and boil it for 3 h.
b. Pour the solution into 60ml of water and acidify it by adding a solution of 2ml of conc. H_2SO_4 in 6ml of water, yields crude oily ricinoleic acid.
c. Dissolve the ricinoleic acid in 64ml of water containing 2g of KOH in a RBF, equipped with a powerful mechanical stirrer and add a solution of 25g of $KMnO_4$ in 300ml of water.
d. Stir the mixture to facilitate the solution of KMnO4 and if necessary, apply the heat to maintain the temperature at 35^0C.
e. Add the alkaline solution of ricinoleic acid in a single portion with vigorous stirring, when the permanganate was completely dissolved. Then rise the temperature to 75^0C.
f. Continue the stirring for 1 h until a test portion added to water shows no permanganate colour.
g. To the above mixture, add a solution of 9ml of conc. H_2SO_4 in 50ml of water.
h. Add the acid slowly and carefully to prevent too rapid evolution of CO_2 with consequent foaming.

i. Heat the mixture on a steam bath for 15 min to coagulate the manganese oxide which was filtered while still hot and the filtrate was added to the main portion.
j. Combine the filtrate and evaporate to $1/4^{th}$ and then cool the solution in ice.
k. Collect the crystals, which are separated, filter with suction and wash with cool water, then dry.

Uses: Cathartic, emollient, anti-neoplastic, in treatment of hair loss, acne treatment.

ISBN: 978-93-90847-76-1

Exp 7: Isolation of Curcumin from Turmeric

Aim: To isolate the curcumin from the rhizomes of turmeric.

Requirements: Turmeric powder, Soxhlet apparatus, distillation unit, 95% alcohol, benzene, dil. HCl, Conc. H_2SO_4, NaOH.

Discussion: Curcumin is a colouring matter present in the rhizomes of *Curcuma longa.* Family: Zingiberaceae. Curcumin is phenolic in nature and used as an indicator in analytical chemistry.

Principle: Curcumin the colouring matter present in turmeric is phenolic hydrocarbon. Turmeric contains various constituents like volatile oil, resins, etc. Turmeric is extracted with polar solvents (95% ethanol) in Soxhlet apparatus. The extract is treated with benzene to remove non-polar components and curcumin is isolated from the ethanol layer by acidification.

Procedure:

a. Weigh 50g of turmeric powder and extract with 95% alcohol in Soxhlet apparatus until all the colouring matter was completely extracted.
b. Distil the alcoholic extract till a semi solid brown colour mass is obtained.
c. Dissolve the crude extract in 60ml of benzene and extract twice with equal volume of 0.1% NaOH.
d. Combine the all extracts and acidify with HCl until the formation of a yellow colour precipitate, which is allowed to settle for few min.
e. After settling of precipitate, concentrate the extract in boiling water bath.
f. Dissolve the precipitate in boiling water, a resinous matter separates out and forms a lumpy mass.
g. Filter the solution in hot condition and concentrated filtrate, finally cool to get curcumin.

Uses: Anti-inflammatory agent, anti-oxidant, wound healing, anti-diabetic, colouring and flavouring agent.

ISBN: 978-93-90847-76-1

Exp 8: Isolation of Sennosides from Senna Leaves

Aim: To isolation of sennosides from senna leaves.

Requirements: Senna leaf powder, methanol, HCl, anhydrous $CaCl_2$, ammonia, P_2O_5.

Discussion: Sennosides are anthracene glycosides with anthraquinone nucleus. They are obtained from *Cassia angustifolia* and C. *acutifolia,* Family: Leguminosae. Sennosides A and B are sterioisomers of each other. They are dimeric glycosides with rheindianthron or glycon i.e., 10, 10'-bis (9, 10-dihydro 1, 8-dihydroxy) 9-oxanthracene 3-carboxylic acid. Sennoside A is trans in nature & sennoside B is meso in nature. Other constituents of senna are sennoside C and D, Rhein, Aloe emoedin dianthron diglycosides, Rhin anthron–8-glycosides, Rhin-8-diglycosides, aloe emoedin 8-glycoside. Senna also contains oxalate and free anthraquinones. There are two forms of senna leaves Alexandrian senna & Indian senna. Indian senna contains 1.5-2.5% of sennosides & Alexander senna contains 2.5-4.5% of sennosides. One of the methods of extraction of sennosides from senna leaves for commercial purpose involves extraction of calcium sennosides in different strength for the purpose of stability of compounds. The dry powder is either heated with 90% alcohol or 80% acetone for 6 h and then for three hours with cold water containing 17-18% sennosides. This method was established to extract 62% of sennosides.

Principle: The powdered drug is extracted with benzene to remove the benzene soluble non-polar constituents of senna. The dry marc is extracted with methanol to isolate the glycoside i.e., sennosides, the extraction is repeated for maximum isolation, and the extract is concentrated and then acidified to precipitate the sennosides. Calcium chloride is added and the pH is made basic by adding ammonia to get the calcium salt of sennosides.

Procedure:

a. Extract powdered leaves (200g) with benzene (600ml) for 2 h on electric shaker.
b. Filter under vacuum and distil off the solvent.
c. Dry the marc at room temperature and extract with 70% methanol (600ml) and shake for 4-6 h, filter under vacuum and extract the marc with (400ml), 70% methanol for 2 h.
d. Filter and combine methanolic extract. Concentrate methanolic extract to $1/8^{th}$ volume, acidify to pH 3.2 by the addition of HCl with constant stirring.
e. Set aside the mixture for 2 h at 5^0C, filter under vacuum and to the filtrate add anhydrous calcium chloride (2g) in 25 ml of denatured spirit with vigorous stirring.
f. Adjust the pH of the solution to 8 by ammonia solution and keep aside for 2 h.
g. Filter the solution under vacuum and dry the precipitate over P_2O_5 in desiccator.

Uses: Purgative, cathartic, eases biliousness, clean the system of worms.

ISBN: 978-93-90847-76-1

Exp 9: Isolation of Lycopene from Tomatoe

Aim: To isolate lycopene from tomatoes.

Requirements: Acetone, CH_2Cl_2, anhydrous $CaCl_2$.

Discussion: Lycopene was first isolated from *Tamus communis.* It has been prepared from a variety of fruits & berries. Lycopene is carotenoid, bright red in colour and lipid soluble. Structurally, it is tetraterpenoid containing 8 isoprene units and 11 conjugated bonds. Beta-carotene is yellow pigment which is an isomer of lycopene. Tomato paste is dehydrated with acetone and extracted with dichloromethane. This is the crude lycopene, recrystallize from appropriate solvent.

Procedure:

a. Take tomato paste 5g, add 7ml acetone and stir it well till gummy residue is obtained (Acetone removes water from cellular mixture).
b. Filter through funnel, discard the yellow filtrate, return the residue to the tube, and add 5ml dichloromethane and extract.
c. Shake the mixture and filter once again.
d. Repeat extraction 2-3 times with 5ml dichloromethane, collect the filtrate and dried over calcium chloride and collect the crude material.

Uses: Anti-oxidant, cardiovascular support, protect skin from sunburn, reduce the risk of cataracts.

ISBN: 978-93-90847-76-1

Exp 10: Isolation of Pectin from Orange Peel

Aim: To isolate pectin from fresh orange peels.

Requirements: Isopropanol, acetone, citric acid, demineralized water, HCl, NaOH, ethanol, vacuum drier, distillation unit, thermometer, nylon cloth, fresh orange peel.

Discussion: Pectin is a complex carbohydrate component found in the middle lamella of plant cell.

Chemically, pectin is a neutral methoxy ester of pectic acid. Pro-pectin (galacturonic acid) which cements the cell wall as fruit ripens. Propectin breaks to pectin, pectinic acid and pectic acid. Pectin forms gel with sugar under acidic condition which is the setting agent in jam. Orange peels contains about 2.5% of volatile oils, apart from volatile oils it also contains several compounds like pectin, hesperidin, isohesperidin, neohesperidin and vitamin C. Orange peel is dried or fresh outer part of the pericarp of the ripe or nearly ripe fruit of *Citrus aurantium* linn. Family: Rutaceae

Principle: Pectin is obtained by extraction of small piece of fresh orange peels with demineralized water by adjusting the pH to 4 with the addition of citric acid or tartaric acid or by addition of 3N H_2SO_4 and heating with stirring

Procedure:

Method 1:

a. Cut the peels into smaller pieces.
b. Weigh about 200g of the peels.
c. Wash thoroughly with water and immerse in 1 L of demineralized water.
d. Adjust the pH to 4-5 with addition of citric acid.
e. Heat with stirring at 85-95^{0}C for about 1 h adds filter aid and filter immediately while the solution is hot.
f. Cool the filtrate and pour it slowly into three volumes of acidic isopropanol or acetone.
g. Stir the solution thoroughly for the precipitation of pectin.
h. Filter through nylon sieve and wash with several times with small volumes of 70% isopropanol or acetone in order to make it free from acidic ions.
i. Dry the product in vacuum drier, weigh and store in a well closed container. The yield of pectin from fresh orange peel is 4-5% w/w.

Method 2:

a. Crush the pericarp of the orange peel is taken and 0.05M HCl is added.
b. Stir for 1 h and filter the suspension.
c. Adjust the pH 4-5 of the filtrate with 0.5M NaOH if necessary.
d. Filter once again and concentrate the filtrate on evaporator to 1/4th.

e. Add ethanol to the filtrate (2-3 volumes) so that precipitates will form.
f. Warm the precipitates with ethanol 2 times.
g. Collect the precipitates and dry.

Uses: Anti-diarrheal, plasma expander, as a haemostatic for internal and external haemorrhage.

ISBN: 978-93-90847-76-1

Exp 11: Isolation of Hesperidin from Dried Orange Peels

Aim: To isolate hesperidin from dried orange peels.

Requirements: Buchner funnel, petroleum ether, methanol, dil. acetic acid, aq. form amide, calcium hydroxide (10%), Con. HCl, celite, powdered orange peels.

Discussion: Hesperidin, a flavonoid compound was first isolated by Laberton in 1828 from the Albedo (The spongy inner portion of peel) of oranges of the family: Hesperides, and was given the name Hesperidin.

Pheffer detected its presence in lemons as early as 1874. Neohesperidin an isomer of hesperidin was isolated together with hesperidin from unripe sour orange cultivated in Europe, and isolated from *Citrus mitis* by Sartaj and Row.

Neohesperidin is a bitter compound present in bitter oranges; *Citrus aurantium* while hesperidin a non-bitter compound is predominant flavonoids in lemons and ordinary sweet orange-citrus sinensis. It was found that hesperidin prevents the fragility of blood capillaries.

On hydrolysis, hesperidin yields hesperetin, rhamnose and glucose.

Principle: Hesperidin can be isolated by two methods:

a. By extracting the dried peel successively with petroleum ether and methanol, the 1st solvent removing the essential oil and second glycosides.
b. By alkaline extraction of chopped orange peels and acidification of the extracts. It may be purified effectively by treatment of form amide, activated charcoal. Due to high insolubility, the crystalline nature makes hesperidin is easy to isolate.

Procedure:

Method 1:

a. Place 200g of sundried peel powder in 2 L RBF with reflux.
b. Add Pet. Ether 1 L and heat on a water bath (40 to 60 ^{0}C) for 1 h.
c. Filter the contents of the flask while hot through funnel.
d. Allow the powder to dry at room temperature.
e. Return the dried powder to the flask and add 1 L methanol.
f. Reflux for 3 h.
g. Filter while it’s hot and wash with hot methanol.
h. Concentrate the filtrate under reduced pressure, leaving a syrupy residue.
i. Crystallize it from dilute acetic acid to white needles.

Method 2:

a. Place 200g of chopped orange peel and 750ml of 10% calcium hydroxide in 2 L flask and mix thoroughly, keep it to overnight.
b. Filter the mixture thorough Buchner funnel containing a thin layer of celite on a filter paper.
c. Acidify the obtained yellow orange filtrate carefully to pH 4-5 using concentrated HCl.
d. Collect the amorphous powder of hesperidin through filtration.
e. Wash with water and recrystallize from aqueous form amide.

Uses: To prevent the fragility of blood capillaries, anti-oxidant, hypotensive, hypocholestermic agent.

ISBN: 978-93-90847-76-1

Exp 12: Isolation of Ammonium Glycyrrhizinate from Liquorice Powder

Aim: To extract ammonium glycyrrhizinate from liquorice powder.

Requirements: Liquorice, dil. nitric acid, dil. ammonia, acetone, pH paper, refrigerator, electrical oven.

Discussion: Liquorice is the commercial source for extraction *Glycyrrhiza* & 18 beta Glycyrrhizinic acid. It consists of roots and subterranean stems of various spices of Glycyrrhiza especially G. *glabra,* family-Leguminosae. It contains about 6% w/w of glycyrrhizin, the potassium & calcium salt of glycyrrhizinic acid. Glycyrrhizinic acid has triterpenoid structure. Liquorice's sweet taste is due to the presence of glycyrrhizin. The yellow colour of liquorice is due to presence of flavonoids.

Procedure:

a. Weigh about 20g of powdered drug; add 50ml of acetone and 2ml of dil. nitric acid.
b. Mix thoroughly, cork the flask & macerate it for 1 h, shake occasionally.
c. Filter the contents, to the marc add 20ml of acetone and warm it on water bath and filter.
d. Combine the filtrates, concentrate, preferably under vacuum.
e. To combined acetone extracts, add sufficient quantity of dilute ammonia solution for precipitation of ammonium glycyrrhizinate.
f. Separate the precipitation by filtration, wash it with 5ml of acetone twice, dry the product and weigh.
g. The yield of ammonium glycyrrhizinate should be approximately 6% w/w.

Uses: Addison's disease, demulcent, expectorant, pharmaceutical vehicle.

ISBN: 978-93-90847-76-1

Exp 13: Isolation of Solanine from Potatoes

Aim: To isolate solanine from potatoes.

Requirements: Glacial acetic acid, methanol, water bath, centrifuge, conc. ammonium hydroxide, potatoes, beaker.

Discussion: Solanine is a glycol alkaloid obtained from potato, which is the tubes of *Solanum Tuberosum*, Family: Solanaceae. Steroidal Alkaloidal glycosides like saponin have hemolytic properties. Solanine contains aglycon Solanidin, which is derived from Cholesterol and contains 27 carbon atoms. A sugar component is attached to C-3 and they are 1 to 4 in number and may be glucose, rhamnose and xylose.

Procedure:

a. Macerate tubes of potatoes with 20 parts of 5 % acetic acid for 24 h.
b. Warm to 70^0C and add concentrated NH_4OH solution drop wise and adjust the pH 10.
c. Centrifuge and wash the precipitates thrice with sufficient quantity of 1% NH_4OH solution and recentrifuge.
d. Collect, dry and weigh the crude solanine.
e. Purify by dissolving in boiling methanol (sparingly soluble) filter & concentrate until alkaloid starts crystalline.

Uses: Used as a starting material for the synthesis of sex hormones, in asthma, bronchitis and epilepsy it can be used.

ISBN: 978-93-90847-76-1

Exp 14: Isolation of Naringin from Grape Fruit Peels

Aim: To isolate naringin from fresh grapefruit peels.

Requirements: Celite, isopropanol, grape fruit peel.

Source: Grapefruit peel is dried or fresh outer part of the pericarp of the ripe or nearly ripe fruit of *Citrus paradisi*. Family: Rutaceae.

Principle: It involves the extraction of grape fruit peel with water at 90^0C which is repeated filtered and concentrated to about one-ninth the volume of the original volume, affords naringin is a octahydrate. Recrystallization from isopropanol gives the pure dihydrate.

Procedure:

a. Collect and heat one part chopped grape fruit peel and four-part water to 90 ^{0}C and maintain at this temperature for 5 min.
b. Filter the water extract. Add two parts water to the solid, repeat the extraction at 80^0C and immediately followed by filtration.
c. Combine the all extracts, boil with 1% celite, filter and concentrate in vacuum to approximately one-ninth the original volume.
d. The concentrated extract is allowed to crystallize in a refrigerator and is then filtering as an octahydrate of melting point 83^0C (needles).
e. Dissolve the naringin in 100ml boiling isopropanol and filter in hot condition.
f. Heat the filtrate to its boiling point to initiate crystallization, then allow to cool, filter on a Buchner funnel, and wash with cold isopropanol.

Uses: Anti-oxidant, anti-carcinogenic and cholesterol lowering activity.

ISBN: 978-93-90847-76-1

Exp 15: Isolation of Embelin from Embelia Ribes

Aim: To isolation and characterization of embelin from *Embelia ribes.*

Source: It consists of dried ripe fruits of *Embelia ribes*. Family: Myrsinaceae.

Chemical constituents: It consist of 2-3% hydroquinone *Embelia*, a dimer of *Embelia* (hydroquinone) is known as the vilangin an alkaloid christembine tannins, quencitol and minute amount of volatile oil. Chemically embelin is 2, 5-dihydroxy 3-undecyl 2, 5-cyclohexadiene 1, 4-benzoquinone. Embelin occurs as golden yellow needles, which is insoluble in water but soluble in alcohol, chloroform and benzene.

Principle: Embelin is extracted by using n-hexane/benzene in Soxhlet and then concentrating the extract, refrigerated and filtered.

Procedure:

Method 1:

a. Weigh 250g of fruit and extract with n-hexane.
b. Evaporate the extract to $1/4^{th}$ and collect the residue.
c. Add cold petroleum ether to the residue. Stir and filter under vacuum.
d. Wash the residue with ether, dry and recrystallize from chloroform.

Method 2:

a. Weight about 250g of *Embelia* ribes and subject to Soxhlet extraction with 300ml of benzene until the required constituent is extracted and the solvent I tube after extraction is colourless.
b. Distill off benzene, evaporate to $1/4^{th}$ and refrigerate to about 30 min.
c. Filter the products and dry.

Uses: Anxiolytic, anti-convulsant, anti-depressant, anti-diabetic, wound healing, anti-tumour.

ISBN: 978-93-90847-76-1

Exp 16: Isolation of Quinine from Cinchona Bark

Aim: To isolate quinine from powdered cinchona bark.

Source: It is obtained from the dried bark of *Cinchona officinalis*. Family: Rubiaceae.

Chemical constituents: The bark of trees in this genus is the source of a variety of alkaloids, the most familiar of which is quinine, an antipyretic (anti-fever) agent especially useful in treating malaria. It contains alkaloids like quinine, quinidine, cinchonine, cinchonidine, dihydroquinine and dihydroquinidine. Alongside the alkaloids, many cinchona barks contain cinchotannic acid, and particular tannin, which by oxidation rapidly yields a dark-coloured phlobaphene called red cinchonic, cinchono-fulvic acid, or cinchona red.

Procedure:

a. Weigh and mix 500g of cinchona bark with milk of lime 150ml and 3 to 5% caustic soda solution and keep the mixture for 10 to 12 h with proper mixing.
b. Extract the mixture with methanol (1.5 L) for 9 h at 70^0C.
c. Filter the extract. After filtration, acidify the extract with 1% HCl and the two layers were allowed to separate it out.
d. Adjust the aqueous layer pH to 4.5 to 5.5 with 1% NaOH solution to obtain crude precipitate.
e. After the filtration, dry the precipitate at 45^0C.
f. Dissolve the crude precipitate in distilled water (1:5).
g. Add activated charcoal to the mixture at 65^0C.
h. Stir the mixture gradually and continuously for 30 min and filter when it is hot.
i. Crystals will obtain at ambient temperature.

Uses: Anti-malarial agent, and also used to treat lupus and arthritis.

ISBN: 978-93-90847-76-1

Exp 17: Isolation of Caffeine from Tea Powder

Aim: To isolate caffeine from tea powder.

Apparatus: Soxhlet apparatus, beakers, glass rod, measuring cylinder, spatula, and porcelain dish.

Chemicals: Ethanol (95%), sulphuric acid, sodium hydroxide, chloroform, nitric acid, ammonium hydroxide.

Procedure:

a. Weigh 50g of tea powder, extract with 200ml of ethanol for 6 h in Soxhlet apparatus.
b. Transfer the extract in to a porcelain dish containing 30g of magnesium oxide in 200ml of water.
c. Evaporate with frequent stirring on a steam bath.
d. Boil residue thrice with 100ml of water, filter while hot on a Buchner funnel.
e. Add 30ml of 10% $H\neg_2SO_4$ to combined filtrate and concentrate under vacuum to about one third of the original volume.
f. Filter the solution while hot and extract with five 25ml portions of chloroform.
g. Treat chloroform extract with a few ml of 1% NaOH till the decolourization takes place. Add equal volume of water.
h. Evaporate the Chloroform extract and recrystallize the crude caffeine from a very small volume of hot water.
i. The yield is 1g approximately of silky needles of caffeine.

Uses: As CNS stimulant and is less potent diuretic.

ISBN: 978-93-90847-76-1

Exp 18: Isolation of Lactose from Milk

Aim: To isolate lactose from milk.

Apparatus: Beakers, glass rod, measuring cylinder, funnel.

Chemicals: Skimmed Milk, 10% acetic acid, calcium carbonate, 95% ethanol, charcoal.

Procedure:

a. Place 200ml of skimmed milk (non-fat) in 600ml distilled water and warm it to 40^0C.
b. Then add some 10% acetic acid until the significant precipitate of casein occurs (excess acid should not be add, acid may hydrolyze disaccharide lactose to monosaccharide glucose and galactose).
c. Stir the casein until it warms large amorphous mass and remove it from the mix.
d. Heat this mixture solution to a gentle boil for about 10 min.
e. This should result in the nearly complete precipitate of albumins, filter the hot mixture by vacuum to remove the precipitated albumin and once remaining calcium carbonate and concentrate the filtrate in a 600ml beaker using a water bath to about 30ml, do not boil to vigorously or excessive foaming and boiling over may occur.
f. Mix marble chips to prevent bumping, then add 175ml of 95% ethanol and 2g of decolorizing charcoal to hot solution, and mix well.
g. Filter the warm solution by vacuum through a layer of wet filtrate (using diatomaceous earth). Make some of the filtrate clear.
h. Transfer the precipitate to the flask and allow it to stand for several days.
i. This provides time for lactose to crystallize on the walls and bottom of the flask.
j. Discard the crystals and vacuum filter them, wash the product with 100ml of 25% ethanol.
k. Weigh the product after it is thoroughly dried.

Uses: Lactose is widely used as filter or diluent in tablets, capsules, in front feed formula and in dry powder inhalations. In combination with sucrose used to prepare coating solutions, as pharmaceutical aid.

ISBN: 978-93-90847-76-1

Exp 19: Isolation and Formulations of Nutrient-Rich Carotenoids

Aim: To isolation and formulations of nutrient-rich carotenoids from carrots.

Apparatus: Centrifuge, grinder, beakers, measuring cylinder, filter press.

Chemicals: Mannitol, ascorbic acid.

Principle:

The selected carrots are washed thoroughly with water and comminuted in an appropriate mill, typically a fruit mill which consists of a rotating stainless-steel blade whose speed of rotation is adjustable to between 100 to 1000 rpm and which is fitted with a sieve with apertures variable from 1 to 10 mm to control the particle size of the homogeneous comminution. The comminution is treated through a filter press or a coarse filter (50-150 microns) for the purpose of separating the pulp from the juice.

To the juice is added with stirring a mono-carboxylic acid such as ascorbic acid, and/or sorbic acid, and/or a dicarboxylic acid such as adipic acid, malic acid, fumaric acid or tartaric acid or mixtures of them, and/or a tricarboxylic acid such as citric acid, in solid form or as a saturated aqueous solution in an amount of acid equivalent to 0.03-3.0% of the liquid, such that a pH value of the resulting mixture of about 3.0-6.0, preferably 5.0 is reached. This helps to build up the particle size of colloidal carotenoid complexes enabling the further processing of the juice by filtration or centrifuging. The carboxylic acids also stabilize the juice during processing.

To the resulting solution or suspension is added one or more components of a monosaccharide such as fructose and/or dextrose, and/or a disaccharide such as sucrose, lactose, and/or hexitols such as mannitol, sorbitol, either in solid form or as a saturated aqueous solution, in an amount of saccharide ranging from 1-50% of the juice, in particular 20-30% of the carrot juice, which mixture is subjected to centrifugation to provide carotenoids-rich carrot paste containing 0.1-1.0% carotenoids.

It has been observed that the use of saccharides not only increases the stability of the carotenoid fraction but also is instrumental in extracting the carotenoid fraction bound to lipoproteins in combination with micronutrients such as the B-complex vitamins and minerals. In the absence of the saccharides step, the concentration of micro nutrients in the final composition is less than 50 per cent. Moreover, in the absence of the above, the carotenoids degrade within a few months storage.

Procedure:

a. Select fresh, hard and good quality reddish coloured carrots with a smooth surface, excluding those that were found defective, and wash thoroughly with water.

b. The sorted and wash carrots (1.0kg) are subject to crush in a fruit mill to provide a comminution which is subject to press through a filter press for the purpose of separating the pulp from the juice to provide a juice (ca. 600 ml).
c. To the juice, add 3g of ascorbic acid with stirring.
d. To the resulting mixture add 60g of mannitol and the mixture is subject to centrifuge to provide paste (ca. 17.2 g).
e. Dry the paste under high vacuum. Pulverize of the solid material and sieve the carotenoid powder of the invention (3.8g).

Uses: It is used to boost the activity of natural killer immune cells. It causes stimulation for the DNA repair enzymes. It provides cornea protection against the normal UV radiation.

ISBN: 978-93-90847-76-1

Exp 20: Isolation of Volatile Oil by Hydro Distillation

Aim: To isolate the volatile oil from eucalyptus by hydro distillation.

Apparatus: Clevenger apparatus.

Chemicals: Eucalyptus leaves, anhydrous sodium sulphate.

Procedure: Weigh about 100g of leaves of the eucalyptus species and air-dry it. Then subject it to hydro distillation for two hours using a Clevenger apparatus. Separate the oils from the water by decantation and dry it by filtration over anhydrous sodium sulphate. The oil yield is 2%.

ISBN: 978-93-90847-76-1

Exp 21: Isolation of Volatile Oil by Steam Distillation

Aim: To isolate the volatile oil from clove buds.

Apparatus: Motor & pestle, stand, condenser, beaker, steam can.

Chemicals: Clove buds, distilled water, saturated NaCl solution, anhydrous Na_2SO_4.

Back ground: Essential oils are the volatile components associated with the aromas of many plants. In this experiment, the essential oil eugenol (the main component of oil of cloves) will be isolated from ground cloves by steam distillation. The principle of steam distillation is based on the fact that two immiscible liquids will boil at a lower temperature than the boiling points of either pure component, because the total vapour pressure of the heterogeneous mixture is simply the sum of the vapour pressures of the individual components. Steam distillation can be carried out in two ways: the direct method and the live steam method. In the direct method, steam is generated by boiling a mixture of the source of the compound of interest and water. The live steam method is carried out by passing steam from an external source into the distillation flask.

Procedure:

a. Weigh 1g of clove buds. Grind them to a coarse powder using a motor and pestle. Reweigh the powder and record the mass.
b. Use a powder funnel to transfer the ground cloves to a 100ml round bottom flask.
c. Add 40ml of deionized or distilled water and a boiling chip to the flask.
d. Mix well with a glass stirring rod, and mark the level of water on the side of the flask.
e. Add 30ml of water to a 50ml round bottom flask. Mark the level of water on the side of the flask. Then discard the water from the flask.
f. Carry out the steam distillation. Stop the when approximately 30ml of distillate is has been collected.
g. Allow the receiver to cool to room temperature.
h. Carefully pour the distillate from the receiver into a 125ml separating funnel.
i. Add 10ml of saturated NaCl solution.
j. Add approximately 0.5g of anhydrous Na_2SO_4 to the flask containing the dichloromethane extracts.
k. Stopper the flask. Allow the extract to dry for 5 min.
l. Weigh a clean, dry 50ml round bottom flask to the nearest 0.001g and the mass.
m. Using a Pasteur filter pipe, take the dichloromethane layer making certain that no Na_2SO_4 is transferred with the solution.
n. Use two additional 0.5ml portions of dichloromethane to rinse the Na_2SO_4 and ensure complete transfer of the clove oil to the beaker.

ISBN: 978-93-90847-76-1

Exp 22: Extraction of Calcium Citrate from Lemon

Aim: To extract calcium citrate from lemon.

Requirements: Lemon, sodium hydroxide and calcium chloride.

Procedure:

a. To about 50ml of lemon juice, add 15% sodium hydroxide with constant stirring until the mixture is slightly alkaline as indicated by colour change on pH paper turning to green colour (pH 0-8).
b. Strain the mixture through muslin both to remove pulp particles and then through Buchner funnel. If the filter paper gets clogged, change it as required to complete filtration.
c. Add 5ml of10% sodium chloride solution for each 10ml of filtrate, heat to boiling and filter off white hot copious precipitate of calcium citrate through Buchner funnel. Wash the precipitate with small volumes of boiling water.
d. Resuspended the precipitate in minimum quantity of cold water, heat to boiling, filter and allow the salt to air dry. Calculate the yield on dry weight basis (about 5%).

ISBN: 978-93-90847-76-1

Exp 23: Isolation of Caffeine from Tea Powder

Aim: To isolate caffeine from the given tea powder.

Requirements: Tea powder (50g), 500ml beaker, distilled water, glass rod, china dish, muslin dish, heating mantle, $CHCl_3$, lead acetate, spatula, plunger, separating funnel.

Procedure:

a. Weigh 50g of tea powder.
b. Place it in 500ml beaker.
c. Fill the beaker up to 300ml with distilled water.
d. Boil the tea powder for 1 h maintain 300ml of water till 1 h of boiling.
e. Filter the decoction in hot condition using muslin cloth.
f. Collect the filtrate in separate beaker (500ml).
g. To this filtrate add two spatula of lead acetate and stir it vigorously to precipitate tannins.
h. Again, filter using muslin cloth to separate tannins.
i. Take the filtrate into separating funnel of 500ml capacity.
j. Add 20ml of chloroform in separating funnel.
k. Extract caffeine with $CHCl_3$ with slow agitation using separating funnel (3 times).
l. The chloroform fractions are pooled and then concentrated to get caffeine in china dish.
m. Caffeine is obtained.
n. The obtained caffeine is subjected to chemical test for its identification and the percentage yield is calculated.

ISBN: 978-93-90847-76-1

Exp 24: Isolation of Tannic Acid from Galls

Aim: To isolate tannic acid from galls.

Requirements: Powdered myrobalan, methanol, ethyl acetate, beakers (500ml) capacity, mantle, filter paper, refrigerator, separating funnel, china dish, desiccator.

Principle:

Galls is an important source of tannins. The tannins of myrobalan are of pyrogallol type (hydrolysable tannins), which on hydrolysis gives chebulic acid and α-galloyl glucose powdered galls is treated with methanol to extract the tannic acid, as tannic acid is very soluble in methanol. The above solution is filtered, the filtrate was concentrated under vacuum to recover methanol till the semisolid mass is obtained. The semisolid mass is diluted with water to remove interfering materials and is kept at 5^0C overnight, filtered. The filtrate is extracted with ethyl acetate in separating funnel. Ethyl acetate is concentrated to get the tannic acid.

Procedure:

a. Tannic acid is isolated using Soxhlet apparatus.
b. Weigh 50g of gall powder place in a muslin cloth bag.
c. Place muslin cloth containing powder into Soxhlet apparatus.
d. Extract the powdered material using 300ml of methanol in Soxhlet apparatus for about 10 to 12 cycles.
e. The extract obtained is collected and then filtered.
f. The excess solvent present is distilled off.
g. The extract is concentrated to a semisolid mass.
h. The semisolid mass is diluted with water in 1:50 ratio and kept at 5℃ overnight.
i. It is then filtered
j. The filtrate is extracted with equal volumes of ethyl acetate.
k. The ethyl acetate fractions are pooled and then concentrated to get tannic acid.

ISBN: 978-93-90847-76-1

Exp 25: Isolation of Volatile Oil from Caraway

Aim: To isolate volatile oil from caraway.

Apparatus: Clevenger apparatus, mantle, porcelain piece, burette, thermometer, RBF (500ml), beaker (100ml), simple distillation unit, caraway, simple distillation unit, tripod stand, mesh.

Principle: Volatile/Essential/Ethereal oil contains terpene and its oxygenated product terpenoids. The volatile oil present in different plant parts. Various isolation methods are used to isolate volatile oils which include.

- Distillation method (water distillation and steam distillation).
- Enfleurage (cold fat extraction method)
- Maceration
- Hot fat extraction method
- Solvent extraction method
- Expression method
- Super critical fluid extraction method
- Enfleurage and super critical fluid extraction methods are used to trap very delicate essential oils used in perfumes and which would be altered destroyed by distillation.
- Isolation of volatile oil from caraway is generally done in simple water distillation method using common distillation apparatus.
- In this method material to be distilled comes in direct contact with boiling water.
- It may float on water or may be completely immersed depending on density. The water used in isolation is boiled by application of heat or by direct fire.

Procedure:

a. Weigh about 75g of caraway.
b. Assemble distillation unit.
c. Place caraway in RBF of 500ml capacity.
d. Fill the RBF with water about 250ml.
e. Place porcelain piece in RBF.
f. Start the distillation process by boiling water of RBF by settling temperature 100^0C.
g. Volatile oils start coming out along with the distillate which is collected in beaker of 100ml capacity.
h. Transfer collected distillate into burette.
i. Volatile oil present in caraway is lighter than water hence start separating and remains on top in burette.
j. Remove aqueous portion from the burette and collect volatile oil in glass container and label it.

ISBN: 978-93-90847-76-1

Exp 26: Isolation of Volatile Oil from Clove

Aim: To isolate volatile oil from clove.

Apparatus: Clevenger apparatus, mantle, porcelain piece, thermometer, round bottomed flask (500ml), beaker (100ml), burette (150ml), simple distillation unit, tripod stand, mesh.

Principle: Volatile/Essential/Ethereal oil contains terpene and its oxygenated product terpenoids. The volatile oil present in different plant parts. Various isolation methods were used to isolate volatile oils which include;

- Distillation method
- Enfleurage (cold fat extraction method)
- Maceration
- Hot fat extraction method
- Solvent extraction method
- Expression method
- Super critical fluid extraction method
- Distillation has always been most widely practiced method of essential oil production.
- Enfleurage and super critical fluid extraction methods are used to trap very delicate essential oils used in perfumes and which would be altered destroyed by distillation.
- Solvent extraction and super critical fluid extraction are recent process.

It may float on the water and or may be completely immersed depending on the density differences. The water is boiled by application of heat or by using direct fire.

Procedure:

a. Weigh about 75g of clove.
b. Assemble the distillation unit.
c. Place 75gms of clove in the round bottomed flask of 500ml capacity.
d. Place a porcelain piece in round bottomed flask.
e. Start the distillation process by boiling water in RBF by setting temperature at 100^0C.
f. The volatile oil starts coming out along with distillate which is collected in beaker of 250ml capacity
g. Transfer, collect distillate into burette.
h. Volatile oil settles at bottom of burette.
i. Remove aqueous portion, half volume from burette and replace same volume with collected with fresh distillate.
j. This process is continued until the cloves are completely exhausted.
k. Collect isolated volatile oil in 5ml glass container and label it.

ISBN: 978-93-90847-76-1

Exp 27: Aseptic Seed Germination

Aim: To establish the callus cultures from the seeds of *Trigonella foenum-graecum*.

Procedure:

a. Perform all the operations under aseptic conditions.
b. Immerse the seeds in 70% ethanol for 2 min and rinse thrice with sterile distilled water carry the surface sterilization of seeds by submerging for 5 min in 2% (v/v) bromine solution or 2% aqueous solution of sodium hydrochloride.
c. Wash the seeds thrice with sterile water to totally remove the sterilizing agent.
d. Germinate the seeds in dark for 2 to 3 days on sterile filter paper or cotton wool.
e. Previously moistened with sterile distilled water in petri dishes at 26 ± 2^0C.
f. Remove the cotyledon portion by culturing with sterile scalpel and transfer the explant portion into solid state medium (25ml) in culture flask.
g. Incubate the culture at 26 ± 1^0C in darkness for three weeks. Transfer the cultures aseptically on sterile fresh medium on internal of 4 weeks. Calculate the growth rate in terms of growth index as follows

$$\text{Growth Index} = \frac{\text{Final weight of callus}}{\text{Initial weight of callus}}$$

h. Use these static cultures for detection of plant metabolites and precursor studies in bio-production of secondary products.

ISBN: 978-93-90847-76-1

Exp 28: Tracer Technique

Living plants considered as biosynthetic laboratory for production of primary as well as secondary metabolite. Various intermediate and steps are involved in biosynthetic pathway in plants that can be investigated by means of following techniques:

- Isolated organs
- Tissues grafting method
- Mutant strains
- Tracer techniques

Introduction:

Using Isolated Organ/Tissues: This method is based on using isolated parts of plant (e.g. Stem, roots etc.). This technique is useful in determination of site of synthesis of particular compound. Ex.: Roots and Leaves for study of *Nicotiana and Datura*, Petal disc for study of oil of rose, Tropane Alkaloids formed in roots of Solanaceae Family.

Grafting Methods: This method is used for study of alkaloid formation by Grafted Plants. Ex.: Tomato Scions grafted on Datura accumulate alkaloids, while Datura Scion grafted on Tomato contained very small amount of Alkaloids. This suggests that main site for formation of Datura Alkaloids is Root.

Use of Mutant Strains: In this method Mutant Strains of microorganisms are produced which lack certain enzymes. Ex.: Gibberella mutant is used to produce isoprenoid compounds, Lactobacillus acidophilus is used for Mevaloinic Acid Pathway of isoprenoid compound synthesis.

Tracer Technique: This technique utilizes labelled compound to find out/ trace different intermediates & various steps in Biosynthetic Pathway. It can be defined as technique which utilizes a labelled compound to find out or to trace the different intermediates and various steps in biosynthetic pathways in plants, at a given rate & time. Also, this technique utilizes the labelled compound which when introduced into plant system, they become part of general metabolic pool & undergo reactions associated with that particular plant system.

Significance: High sensitivity. Applicable to living system. Wide ranges of isotopes are available. More reliable, easy administration & isolation procedure.

Gives accurate result, if proper metabolic time & technique applied. Location & Quantity of compound containing tracer 14 C labelled glucose is used for determination of glucose in biological system. Different tracers can be used for different studies.

Ex. For studies on nitrogen and amino acid, Labelled nitrogen gives specific information than carbon.

Criteria for Tracer/Isotope Selection:

Two types of isotopes are generally used for labelling are

a. Radioactive isotopes.
b. Stable isotopes.

Following points must be considered before selection:

- The starting concentration of tracer must be sufficient enough to withstand dilution in course of metabolism.
- Physical & chemical nature of compound must be known for proper labelling.
- Higher Half-life.
- Should actively participate during synthesis.
- Should not damage the system.

Radioactive Isotopes:

[E.g. 1 H, 14 C, 24 Na, 42 K, 35 S, 35 P, 131 I] For biological investigation – C & H. For metabolic studies – S, P, and alkali and alkaline earth metals are used. For studies on protein, alkaloids, and amino acid - labelled N-atom.

Stable Isotopes:

[e.g., 2 H, 13 C, 15 N, 18 O] Used for labelling compounds as possible intermediates in biosynthetic pathways. Usual method of detection is Mass spectroscopy [15 N, 18 O] N.M.R spectroscopy [2 H, 13 C].

Steps Involved In Tracer Technique:

- Preparation of labelled compound.
- Introduction of labelled compound into a biological system.
- Separation & determination of labelled compound in various biochemical fractions.
- Methods for tracer technique.

Preparation of labelled compound: The labelled compound produces by growing them in atmosphere of 14 CO_2. All carbon compounds get 14 C labelled. The 3 H (tritium) labelled compound are commercially available. Tritium labelling is affected by catalytic exchange in aqueous media by hydrogenation of unsaturated compound with tritium gas. Tritium is pure β-emitter of low intensity & its radiation energy is lower than 14 C. By the use of organic synthesis

Introduction of Labelled Compound into Biological System:

- Root feeding
- Stem feeding
- Direct injection

- Infiltration
- Floating method
- Spray technique

Separation & Detection Techniques:

- GM Counters
- Liquid Scintillation Chamber
- Gas Ionization Chamber
- Mass Spectrophotometer
- NMR Spectrophotometer
- Auto-Radiography

Geiger–Muller (Gm) Counters:

A Geiger–Muller counter is a type of particle detector that measures ionizing radiation e.g., alpha, beta particles, or gamma rays-by the ionization produced in a low-pressure gas usually helium, neon or argon with halogens added in a Geiger–Muller tube which briefly conducts electrical charge when a particle or photon of radiation makes the gas conductive by ionization. This charge has been detected in form of current pulse.

Liquid Scintillation Chamber:

A scintillation detector or scintillation counter is obtained when a scintillator is coupled to an electronic light sensor such as a photomultiplier tube (PMT) or a photodiode. A scintillator is a material that exhibits scintillation-the property of luminescence when excited by ionizing radiation. Samples are dissolved or suspended in a "cocktail" containing a solvent (aromatic organics such as benzene or toluene), typically some form of a surfactant, and small amounts of scintillators.

Gas Ionization Chamber:

The ionization chamber is the simplest of all gas-filled radiation detectors, and is widely used for ionizing radiation; X-rays, gamma rays and beta particles. Conventionally, the term "ionization chamber" is used exclusively to describe those detectors which collect all the charges created by direct ionization within the gas through the application of an electric field.

Mass Spectrophotometer:

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles.

It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules, such as peptides and other chemical compounds.

NMR Spectrophotometer:

NMR spectroscopy is a research technique that exploits the magnetic properties of certain atomic nuclei to determine physical and chemical properties of atoms or the molecules in which they are contained.

It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules.

Autoradiography:

Autoradiography is a method for investigating the distribution of radioactive material in a plant object, e.g., histological tissues, a chromatography plate.

This technique uses a photographic film or emulsion as detector of ionizing radiation. The sample is in close contact with emulsion for a certain period of time (exposure period).

Methods for Tracer Technique:

- Precursor Product Sequences
- Double & Multiple Labelling
- Competitive Feeding
- Isotope Incorporation
- Sequential Analysis

Precursor Product Sequence:

In this technique, the presumed precursor of the constituent under investigation on a labelled form is fed into the plant and after a suitable time the constituent is isolated, purified and radioactivity is determined.

Application: Stopping of hordenine production in barley seedling after 15-20 days of germination. Restricted synthesis of hyoscine, distinct from hyoscyamine in *Datura stramonium.* This method is applied to the biogenesis of morphine & ergot alkaloids.

Double & Multiple Labelling:

This method gives the evidence for nature of biochemical incorporation of precursor arises double & triple labelling. In this method specifically labelled precursor and their subsequent degradation of recover product are more employed.

Application: This method is extensively applied to study the biogenesis of plant secondary metabolite. Used for study of morphine alkaloid. E.g., Leete, use doubly labelled lysine used to determine which hydrogen of lysine molecule was involved in formation of piperidine ring of anabasine in *Nicotiana glauca.*

Competitive Feeding:

This method provides the possible intermediates that plant normally used during biogenesis.

Application: This method is used for elucidation of biogenesis of propane alkaloids. Biosynthesis of hemlock alkaloids (coniine, conhydrine etc.) using 14 C labelled compounds.

Isotope Incorporation:

This method provides information about the position of bond cleavage & their formation during reaction. E.g., Glucose-1-phosphatase cleavage as catalyzed by alkaline phosphatase this reaction occurs with cleavage of either C-O bond or P-O bond.

Sequential Analysis:

The principle of this method of investigation is to grow plant in atmosphere of 14 CO_2 & then analyze the plant at given time interval to obtain the sequence in which various correlated compound become labelled.

Applications:

- 14 CO_2 sequential analyses have been very successfully used in elucidation of carbon in photosynthesis.
- Determination of sequential formation of opium hemlock and tobacco alkaloids.
- Tracing of bio-synthetic pathway of cyanogenetic glycoside "prunacine"; by incorporating 14 C into phenylaniline.
- Interrelationship among 4 – methyl sterols & 4, 4-dimethyl sterols, by use of 14 C acetate.
- Study of squalene cyclization by use of 14 C, 3 H labelled mevalonic acid.
- Terpenoid biosynthesis by chloroplast isolated in organic solvent, by use of 2- 14 C mevalonate.
- Study the formation of cinnamic acid in pathway of coumarin from labelled coumarin.
- Origin of carbon & nitrogen atoms of purine ring system by use of 14 C or 15 N labelled precursor.
- Study of formation of scopoletin by use of labelled phenylalanine.
- By use of 45 Ca as tracer, - found that the uptake of calcium by plants from the soil. (CaO & $CaCO_2$).
- By adding ammonium phosphate labelled with 32 P of known specific activity the uptake of phosphorus is followed by measuring the radioactivity as label reaches first in lower part of plant, than the upper part i.e., branches, leaves etc.

ISBN: 978-93-90847-76-1

Exp 29: Preparation of Different Alkaloidal Testing Reagents

Aim: To prepare and submit the different alkaloidal testing reagents.

Procedure:

Dragendorff's Reagent:

Solution A: 1.7g basic bismuth nitrate in 100ml water or acetic acid (4:1).

Solution B: 40g of potassium iodide in 100ml of water.

Mix together as follows:

5ml A + 5ml B + 20ml acetic acid + 70ml water.

In the presence of alkaloid, it shows reddish brown precipitate.

Mayer's Reagent: Dissolve 1.385g of mercuric chloride in 60ml of water. Dissolve 5g of potassium iodide in 100ml water. Mix the two solutions and dilute to 100ml with water. In the presence of alkaloid, it shows cream brown ppt.

Wagner's Reagent: Dissolve 2g of Iodine and 6g of KI in 100ml of water. In the presence of alkaloid, it shows reddish brown ppt.

Hager's Reagent: Saturated picric acid solution. In the presence of alkaloid, it shows yellow colour ppt.

Tannic Acid Test: 10g of tannic acid in 100ml of water. In the presence of alkaloid, it shows buff colour ppt.

Picrolonic Acid Test: 10g of picrolonic acid dissolve in 100ml water. Due to the presence of alkaloid, it shows yellow ppt.

ISBN: 978-93-90847-76-1

Exp 30: Reagents Used

Aim: To prepare and submit the different reagents used.

Procedure:

Chloral Hydrate Solution: Dissolve 25g of chloral hydrate in 10ml water.

Glycerin Water Solution: Prepare by adding equal portion of glycerin and distilled water.

Iodine Water: Add as much iodine to distilled water as it will dissolve

Phloroglucinol Solution: Dissolve 1g of phloroglucinol in 50ml of 95% alcohol. Use the solution with in a period of four months.

Hydrochloric acid.

ISBN: 978-93-90847-76-1

Exp 31: Preliminary Phytochemical Screening

Aim: To perform the preliminary phytochemical screening.

The different chemical tests are present for establishing profile of the plant.

a. **Test for Alkaloids:**

- **Dragendorff's Test:** In a test tube containing 1ml of extract, add few drops of Dragendorff's reagent.
- Appearance of orange colour indicates the presence of alkaloids.
- **Wagner's Test:** To the extract, add 2 ml of Wagner's reagent. Formation of a reddish-brown precipitate indicates the presence of alkaloids.
- **Mayer's Test:** To the extract, add 2 ml of Mayer's reagent, a dull white precipitate revealed the presence of alkaloids.
- **Hager's Test:** To the extract, add 2 ml of Hager's reagent; the formation of yellow precipitate indicates the presence of alkaloids.

b. Test for Amino Acids:

- **Ninhydrin Test:** Two drops of ninhydrin solution were added to the extract; formation of characteristic purple colour indicates the presence of amino acids.

c. Test for Anthocyanins:

- To the extract, add 10% sodium hydroxide. Formation of blue colour shows the presence of anthocyanins.
- To the extract, add conc. sulphuric acid. Formation of yellowish orange colour confirms the presence of anthocyanins.

d. Test for Carbohydrates:

- **Molisch's Test:** To the extract, add 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube.
  Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.
- **Fehling's Test:** To the extract, add equal quantities of fehling's solution A and B on heating, formation of a brick red precipitate indicates the presence of carbohydrates.
- **Benedict's Test:** To 5 ml of benedict's reagent, add the extract and boiled for two min and cool.
- Formation of red precipitate showed the presence of carbohydrates.

e. Test for Coumarins:

- To 1 ml of extract, add 1 ml of 10% sodium hydroxide. The presence of coumarins is indicated by the formation of yellow colour.

f. Test for Fixed Oils and Fats:

- **Spot Test:** A small quantity of extract is press between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

g. Test for Flavanones:

- To the extract, add 10% sodium hydroxide and the colour changes from yellow to orange, which indicates the presence of flavanones.
- To the extract, add conc. sulphuric acid and the colour changes from orange to crimson red, which indicates the presence of flavanones.

h. Test for Flavones:

- **Shinoda Test:** To the extract, add a few magnesium turnings and 2 drops of concentrated hydrochloric acid, formation of red colour showed the presence of flavones.
- To the extract, add 10% sodium hydroxide or ammonia; formation of dark yellow colour indicates the presence of flavones.

i. Test for Glycosides: For detection of glycosides about 50mg of extract is hydrolyse with concentrated HCl for 2 h on a water bath and filter. The hydrolyzed is subject to following tests

- **Borntrager's Test:** To 2ml of hydrolysate, add 3 ml of chloroform and shake well.
  To the separated chloroform layer, add 1ml of 10% ammonia solution and observe for colour.
  Formation of pink colour indicates the presence of anthraquinone glycosides.
- **Keller-Kiliani Test:** About 50mg of the extract is dissolve in 2 ml of glacial acetic acid and add 2drops of 5% ferric chloride solution and mix, to this add 1 ml of H_2SO_4, reddish brown colour appears at the junction of two liquid layers and the upper layer appears bluish green colour indicating the presence of steroidal glycosides.

j. Test for Phenols:

- **Ferric Chloride Test:** To the extract, add few drops of 10 % aqueous ferric chloride. Appearance of blue/green colour indicates the presence of phenols.

k. Test for Proteins:

- **Biuret Test:** To the extract, add 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution. Formation of violet colour indicates the presence of proteins.
- **Xanthoprotein Test:** To the extract, add 1 ml of concentrated nitric acid. A white precipitate will form; it is then boil and cool.
  Then add 20% sodium hydroxide or ammonia. Orange colour indicates the presence of aromatic amino acids.
- **Tannic Acid Test:** To the extract, add 10% tannic acid. Formation of white precipitate indicates the presence of proteins.

l. Test for Quinones:

- To 1 ml of the extract add 1 ml of concentrated sulphuric acid. Formation of red colour shows the presence of quinones.

m. Test for Saponins:

- To 1 ml of the extract, add 5 ml of water and shake it vigorously. Copious lather formation indicates the presence of saponins.

n. Test for Steroids:

- **Liebermann Burchard Test:** To 1ml of extract, add 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid.
  The solution become red, then blue and finally bluish green indicates the presence of steroids.

o. Test for Tannins:

- To few mg of extract, add ferric chloride, formation of a dark blue or greenish black colour showed the presence of tannins.
- The extract was mix with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

p. Test for Terpenoids:

- **Salkowski Test:** To 1 ml of extract, add tin (one bit) and thionyl chloride. Formation of pink colour indicates the presence of terpenoids.
- **Hirshon Reaction:** The extract is heat with trichloroacetic acid; formation of red to purple colour indicates the presence of terpenoids.

q. Test for Volatile Oils:

- To the extract, add alc. solution of Sudan III reagent. Formation of red colour indicates the presence of volatile oils.
- **Alkaline Test:** To the extract, add a drop of tincture alkaline. Formation of red colour indicates the presence of volatile oils.

ISBN: 978-93-90847-76-1

Exp 32: Thin Layer Chromatography of Alkaloids

Aim: To perform thin layer chromatography of given alkaloids.

Apparatus: TLC plates, beaker.

Chemicals: Benzene, ethyl acetate, anisaldehyde-sulphuric acid reagent, toluene, acetone, methanol, ammonia, Dragendorff's reagent.

Principle: In Thin layer chromatography the separation is carries on a glass or plastic which is coated with a thin uniform layer of finely divided inert adsorbent such as silica gel or alumina. The plates are activated; the solution of the sample in a volatile solvent is applied by using a capillary tube or a micropipette to a spot keeping 1-2cm from the bottom of the TLC plate.

The position of the sample spot is indicated by marking an 'origin line' on the plate with the lead pencil. When the spot has dried, the plate is placed vertically in a suitable tank with its lower edge immersed in selected mobile phase.

The solvent rises by capillary action, resolving the sample mixture into discrete spots. At the end of the run solvent is allowed to evaporate from the plate and the separated spots are located and identified by various physical and chemical methods.

Procedure:

TLC of Piperine: The crude extract is spotted on a thin layer plate (silica gel) and developed with benzene-ethyl acetate solvents in 2:1 ratio spraying with anisaldehyde-sulphuric acid reagent (Anisaldehyde-0.5ml, glacial acetic acid-10ml, CH_3OH-85ml, conc. H_2SO_4-5ml). This solution is sprayed on the plate which is then heated at 110^0C for 10 min. Piperine appears as yellow spots.

TLC of Nicotine:

Toluene: Acetone: Methanol: Ammonia - 4:4.5:1:0.5.

Spraying reagent-Dragendorff's reagent.

The crude extract is spotted on a thin plate (silica gel) and developed with toluene, acetone, methanol, ammonia, solvents in the ratio of 4:4.5:1:0.5 spraying with Dragendorff's reagent.

ISBN: 978-93-90847-76-1

Exp 33: Introduction of Leaf Constants

Epidermis of the leaf shows different characteristics. E.g.: cuticle, stomata, trichomes, water pores.

Stomata:

Stomata are a minute epidermal opening covered by two kidneys shaped guard cells in dicot leaves. These guard cells are surrounded by epidermal cells (subsidiary cells). Stomata perform the functions of gaseous exchange and transpiration plants.

It is defined as the average number of stomata per square mm of the epidermis of the leaf. The actual number of stomata per square mm may vary for the leaves of the same plant grown in different environmental conditions.

The leaves of the bryophytes and submerged leaves of aquatic plants do not contain stomata.

Stomatal Index:

It is the percentage of the number of stomata to the total number of epidermal cells. Each stomata is counted as one cell. Stomatal index can be calculated by using following equation:

$$S.I. = \frac{S}{E + S} X\ 100$$

Where,

S.I = Stomatal index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Types of Stomata:

Depending up on the type of the guard cell and arrangement of subsidiary cells stomata are divided into following types:

a. Parasitic stomata
b. Diacytic stomata
c. Anisocytic stomata
d. Anomocytic stomata
e. Actinocytic stomata

Parasitic Stomata:

This type of stomata contains two guard cells covered by two subsidiary cells. The long axis of which are parallel to that of stoma. E.g.: Senna, Coca leaves.

Diacytic Stomata:

The guard cells are covered by two subsidiary cells as in case of parasitic stomata. But the arrangement of subsidiary cells on the guard cell is at right angle to that stoma. E.g.: Vasaka and peppermint.

Anisocytic Stomata:

The number of guard cells is two, as in all other cases but the guard cells are covered by three subsidiary cells at which one is markedly smaller than the other two. E.g.: Datura and Belladonna.

Anomocytic Stomata:

In this type, stoma is surrounded by varying number of subsidiary cells resembling other epidermal cells. E.g.: Digitalis and Lobelia.

Actinocytic Stomata:

The two guard cells are surrounded by a circle of radiating subsidiary cells.

ISBN: 978-93-90847-76-1

Exp 34: Determination of Stomatal Number and Stomatal Index of Vinca Leaf

Aim: To determine the stomatal number and stomatal index of vinca leaf.

Materials and Reagents: Compound microscope, stage micrometer, camera lucida, drawing board, chloral hydrate solution, glycerine water.

Procedure:

a. Clean the piece of leaf by boiling with chloral hydrate solution.
b. Peel out the epidermis by means of forceps, keep it on slide and mount in glycerin water.
c. Arrange a camera lucida and drawing board for making the drawing scale.
d. Draw a square of 1mm by means of stage micrometer.
e. Place the slide with cleaned leaf on the stage.
f. Trace the epidermal cells and stomata.
g. Count the number of stomata, also the number of epidermal cells in each field, provided two adjacent sides are considered for the purpose of calculation.
h. Calculate the stomatal index and stomatal number.

The stomatal index can be calculated by using following equation:

$$\mathbf{S.I.} = \frac{\mathbf{S}}{\mathbf{E+S}}\ \mathbf{X\ 100}$$

Where,

S.I. = Stomatal index,

S = Number of stomata per unit area, and

E = Number of epidermal cells in the same unit area.

ISBN: 978-93-90847-76-1

Exp 35: Determination of Stomatal Number and Stomatal Index of Senna Leaf

Aim: To determine the stomatal number and stomatal index of senna leaf.

Materials and Reagents: Compound microscope, stage micrometer, camera lucida, drawing board, chloral hydrate solution, glycerine water.

Procedure:

a. Clean the piece of leaf by boiling with chloral hydrate solution.
b. Peel out the epidermis by means of forceps, keep it on slide and mount in glycerin water.
c. Arrange a camera Lucida and drawing board for making the drawing scale.
d. Draw a square of 1mm by means of stage micrometer.
e. Place the slide with cleaned leaf on the stage.
f. Trace the epidermal cells and stomata.
g. Count the number of stomata, also the number of epidermal cells in each field, provided two adjacent sides are considered for the purpose of calculation.
h. Calculate the stomatal index and stomatal number.

The stomatal index can be calculated by using following equation:

$$\mathbf{S.I. = \frac{S}{E + S} X\ 100}$$

Where,

S.I. = Stomatal index,

S = Number of stomata per unit area, and

E = Number of epidermal cells in the same unit area.

ISBN: 978-93-90847-76-1

Exp 36: Determination of Stomatal Number and Stomatal Index of Datura Leaf

Aim: To determine the stomatal number and stomatal index of datura leaf.

Materials and Reagents: Compound microscope, stage micrometer, camera lucida, drawing board, chloral hydrate solution, glycerine water.

Procedure:

a. Clean the piece of leaf by boiling with chloral hydrate solution.
b. Peel out the epidermis by means of forceps, keep it on slide and mount in glycerin water.
c. Arrange a camera Lucida and drawing board for making the drawing scale.
d. Draw a square of 1mm by means of stage micrometer.
e. Place the slide with cleaned leaf on the stage.
f. Trace the epidermal cells and stomata.
g. Count the number of stomata, also the number of epidermal cells in each field, provided two adjacent sides are considered for the purpose of calculation.
h. Calculate the stomatal index and stomatal number.

The stomatal index can be calculated by using following equation:

$$\mathbf{S.I.} = \frac{\mathbf{S}}{\mathbf{E + S}} \ \mathbf{X\ 100}$$

Where,

S.I. = Stomatal index,

S = Number of stomata per unit area, and

E = Number of epidermal cells in the same unit area.

ISBN: 978-93-90847-76-1

Exp 37: Morphology of Acacia

Aim: To study the morphological characters of given crude drug.

Synonyms: Gum acacia, gum Arabic, and acacia.

Biological Source: Indian gum is the dried gummy exudation obtained from the stem and branches of *Acacia arabica* wild, belonging to family Leguminosae.

Morphology:

Colour: Tears are cram-brown to red in colour, while powder is light brown in colour.

Odour: Odourless

Taste: Bland and mucilaginous

Size and Shape: Irregular brown bears of varying size

Chemical Constituents: It consists principally of arabin, which is a complex mixture of calcium, magnesium and potassium salts of Arabic acid. Arabic acid on hydrolysis gives L-arabinose, L-rhamnose, D-galactose and D-glucuronic acid. It also contains an enzyme oxidase.

Uses: Acacia is a demulcent. It is also administered intravenously in haemolysis. In the form of mucilage, it is used as a suspending agent, specifically in mixtures with resinous substance. Acacia is a good emulsifying agent for fixed oils, volatile oils and also for liquid paraffin. It is a good blinding agent and used in the preparation of lozenges, pastilles and compressed tablets in combination with gelatin. It is used to form coacervates for microencapsulation of drugs.

Adulterants: B.P. variety consists of gum obtained from acacia Senegal wild, a plant of African origin and grown in Africa. The tears are rounded or avoid and about 5-40mm in diameter. It can be used a substitute to Indian gum.

Identification Tests:

Solubility:

Sr. No.	Test	Observation	Inference
1.	Solubility in alcohol: To 1g of gum, add 2 ml of alcohol.	Insoluble in alcohol	May be acacia, tragacanth or gelatin
2.	Mount 0.5g of powder in 1 ml of	Particles dissolve	May be acacia

Sr. No.	Test	Observation	Inference
	alcohol and irrigate with water.	without swelling	
3.	Mount 0.5 gm of powder in 2 ml of N/50 iodine.	No colour reaction	May be acacia

From the above preliminary test it may be identified as acacia.

Sr. No.	Test	Observation	Inference
1.	Add 1 ml of strong lead sub acetate solution to 5 ml solution.	Formation of white precipitate	May be acacia
2.	Dissolve 0.25 gm in 5 ml of H_2O & add 0.5ml H_2O_2 solution & 0.5 ml of benzidine in alcohol and shake it and allow to stand.	Unstable deep blue colour (enzyme oxidase)	May be acacia
3.	Take small quantity of powder in ruthenium red solution.	Formation of red colour	May be acacia
4.	To 10 ml add 0.2ml of lead acetate solution.	No precipitate	May be acacia
5.	To 1 ml of solution add 4ml H_2O & dilute HCl & boil it for few min. hydrolysis takes place add fehling's solution.	No crimson colour (agar as tragacanth)	May be acacia

ISBN: 978-93-90847-76-1

Exp 38: Morphology of Isabgol

Aim: To study the morphological characters of given crude drug.

Synonyms: Ispaghula, isabgul, Indian psyllium, isabgof.

Biological Sources: It consist of the dried seeds of the plant known as *Plantago ovata Forskal family:*

Plantaginaceae in the farm field seed as well as dried seed coats (isapgol husk) are used.

Morphological Characters:

Colour: Pinkish – gray to brown

Odour: None

Size: 10-35 mm (length) & 1-1.75 mm (width)

Shape: Ovate cymbiform

Seeds: hard, transparent & smooth with gray (or) reddish brown oval spot in the centre of convex surface.

Concave surface has hilum covered with thin coating having 2 perforations 1g accommodates 500-600 seeds.

Chemical Constituents: Pentosum, mucilage, aldobionic acid.

Uses: Used as demulcent t, laxative, pharmaceutical aid

Identification Tests:

Test	Observation	Inference
Take 0.5g drug + water	Seeds well in water	May be isabgol

From the above preliminary test the drug may be identified as isabgol.

General Chemical Test:

Sr.No.	Test	Observation	Inference
1.	To the section of drug, add 2-3	Reddish pink	May be isabgol

Sr.No.	Test	Observation	Inference
	drops of ruthenium red solution.		
2.	Take a few seeds on slide & add little water.	Mucilage exudates forming thin layer	May be isabgol

Swelling Factor Test:

Sr. No.	Test	Observation	Inference
1.	Take 1 gm of seed in a 25 ml measuring cylinder & add H_2O, allow it to stand for 24 h with occasional stirring observe for swelling after 24 h.	Seeds & swelling factor is from 10-14	May be isabgol

ISBN: 978-93-90847-76-1

Exp 39: Morphology of Starch

Aim: To study the morphological characters of given crude drug.

Synonym: Amylum

Biological Source:

Starch consists of polysaccharide granules obtained from the grains of maize, rice or wheat, belonging to family Gramineae or from the tubers of potato, family Solanaceae.

Morphology: Starch occurs as fine powder or irregular, angular masses readily reducible to powder.

Colour: Rice and starch grains are white, wheat is cream

Odour: Odourless

Taste: Mucilaginous

Size and Shape: Starch grains vary depending upon the types which can be described as under.

Chemical Constituents: Starch contains chemically two different polysaccharides viz amylase and amylopectin in the proportion of 1:2 amylose is water soluble and amylopectin is water insoluble amylase gives blue colour with iodine.

Uses: It is used as a nutritive, demulcent, protective and as an absorbent. It is used in the preparation of dusting talcum powder for application over the skin.

Adulterants: Tapioca starch or carsava or Brazilian arrowroot.

Identification Tests:

Preliminary Test:

Colour, Rice & starch grains are white, wheat in cream colour, potato.

Sr. No.	Experiment	Observation	Inference
1.	Take 0.5g drug and add 1ml of alcohol.	Insoluble in alcohol	May be gelatin, agar, acacia
2.	Take 0.5g of drug and add 5ml of water & boil.	Formation of translucent gel	May be starch

From the above preliminary test, drug is may be starch.

General Chemical Tests:

Molisch's Test:

Test	Observation	Inference
Dissolve 1g of drug in 3 ml of water, 2 drops of alcohol solution of ∝-naphthol and carefully add 2 drops of conc.H_2SO_4 along the sides of test tube.	A deep violet colour ring is observed at the junction of two layers	May be carbohydrate is present

Tollens Test:

Test	Observation	Inference
To 0.5g of powder add 2 ml of $AgNO_3$ solution & add 2-3 drops of NaOH and 1 ml of NH_4OH is added until the ppt of Ag_2O dissolves and heat on water bath.	Formation of silver mirror	May be carbohydrates are present

Fehling's Test:

Test	Observation	Inference
To 0.05g drug add 1 ml of each of Fehling A & B and boil the solution for 5 min on water bath.	Formation of Reddish brown ppt of Cu_2O	May be reducing sugar

Iodine Test:

Sr. No.	Test	Observation	Inference
1	To 1 gm of starch, add 15ml of H_2O and boil for 2 min.	Formation of stiff jelly	May be starch is present
2	To the above viscous solution add 2-3 drops of iodine solution.	Blue colour appears & disappears on warming & reappears on cooling	May be starch is present

ISBN: 978-93-90847-76-1

Exp 40: Morphology of Tragacanth

Aim: To study the morphological characters of given crude drug.

Synonyms: Gum tragacanth and tragacanth.

Biological Source: It is the dried gummy exudation obtained by making incision from stems and branches of *Astragalus gummifer* and other species of Astragalus belongs to Leguminosae family.

Morphological Characters:

Colour: white or pale yellowish white

Odour: Odour less

Taste: Mucilaginous

Shape: Flattened ribbon like flakes, more or less curved

Size: Flakes are approximately 25 X 12 X 2 mm in size

Chemical Constituents: It contains carbohydrates, water soluble portion tragacanth and water insoluble portion bassorin.

Uses: Demulcent, emollient, thickening agent, suspending agent and emulsifying agent.

Identification Tests:

Solubility:

Sr. No.	Test	Observation	Inference
1.	Solubility in alcohol: To 0.1g of gum add 2 ml of alcohol.	Insoluble in alcohol	May be tragacanth, acacia, gelatin
2.	Mount 0.5g of powder in 1 ml of alcohol and irrigate with water.	Particles swell as ultimately dissolve (or) become diffused	May be tragacanth, gelatin
3.	Mount 0.5g of powder in 2ml of N/50 iodine.	Olive green colour	Tragacanth

General Chemical Test:

Sr. No.	Test	Observation	Inference
1.	To 4 ml of 0.5% solution add 0.5 ml of HCl & heat for 30 min on water bath. a) To one part add 1.5ml NaOH solution & Fehling's solution on water bath.	Red ppt	May be tragacanth
2.	To 0.5% of solution of gum add 20% solution of lead acetate.	Formation of flocculant ppt (distinct from acacia)	May be tragacanth
3.	To small quantity of powder add ruthenium red.	Do not acquire pink colour	May be tragacanth
4.	To 0.1 gm of powder add 0.5N iodine.	Olive green colour	May be tragacanth
5.	Powder is warmed with 5% aqueous caustic potash.	Canary yellow colour	May be tragacanth
6.	When solution of tragacanth is boiled with few drops add 10% aqueous ferric chloride solution.	Formation of deep yellow colour precipitate	May be tragacanth

ISBN: 978-93-90847-76-1

Exp 41: Morphology of Honey

Aim: To study the morphological characters of given crude drug.

Synonym: Madhu, honey purified, Mel.

Biological Source: Honey is a sugar secretion deposited in honey comb by the bees, Apis mellifera, *Apis dorsata and other species* of Apis, belonging to family Apidae, order Hymenoptera.

Description:

Colour: Pale yellow to yellowish – brown

Odour: Characteristic, pleasant

Taste: Sweet and faintly acid

Chemical Constituents: Honey is an aqueous solution of glucose 35% fructose 45% and sucrose about 2%.

The proportion of sugar may vary depending upon the source of nectar and the enzymatic activity responsible for converting nectar into the honey.

The other constituents of honey are maltose, gum, traces of succinic acid, acetic acid, dextrin, formic acid, colouring matters, enzymes and traces of vitamins. Proteins and pollen grain from various flowers are also found in honey.

Uses: Honey is used as a demulcent and sweetening agent it is readily assimilated and hence is a good nutrient to infants and patients.

It is antiseptic and applied to burns and wounds. It is common ingredient of several cough mixture; cough drops and vehicle for Ayurvedic formulations.

Recently, it is used in preparation of creams, lotions, soft drinks and cardies also.

Identification Tests:

Sr. No.	Test	Observation	Inference
1.	Add 1 ml of drug to 5ml of water.	Drug is completely miscible with water	May be honey

From the above preliminary test drug is identified as honey.

General Chemical Test:

Take 1: Part of Honey & Dilute with 5 Parts of H_2O & Solution is used for Performing Following Tests.

Sr. No.	Test	Observation	Inference
1.	Take 1ml of above solution add Fehling's A & B of 1 ml each and heat.	Formation of brick red ppt	May be carbohydrate

Fiehe's Test:

Test	Observation	Inference
To 5ml sample add 2.5 ml of diethyl ether, shake the solution and separated ether layer, transfer in to china dish and evaporate ether comp and to obtained residue add 1 drop of 1% w/v solution of resorcinol in concentrated HCl.	If no cherry red	Sample contains pure honey
	Observation of cherry red colour	Sample contain artificial invert sugar (or) adult rate honey

ISBN: 978-93-90847-76-1

Exp 42: Morphology of Castor Oil

Aim: To study the morphological characters of given crude drug.

Synonym: Ricinus oil

Biological Source:

Castor oil is the fixed oil obtained by the cold expression of the seeds of *Ricinus communis* family Euphorbiaceae.

Description:

Colour: Pale yellow or almost colourless liquids

Odour: Slight and characteristic

Taste: First it is bland but afterwards slightly acrid and usually nauseating.

It is a viscous and transparent liquid.

Chemical Constituents:

Castor oil chiefly contains triglyceride of ricinoleic acid other glycerides are also present in the drug, where the fatty acids are represented by isoricinoleic, linoleic, stearic and isostearic acids.

Uses:

Castor oil is cathartic. It is also used for lubrication commercially several other forms of the castor oil, such as dehydrated castor oil or hydrogenated castor oil are used industrially for several other purposes.

Identification Test:

a. Add to the oil an equal volume of ethanol; clear liquid is obtained. On cooling at 0^0C and on storage for 3 h; the liquid remains clear (distinction from other fixed oils).

ISBN: 978-93-90847-76-1

Exp 43: Morphology of Linseed

Aim: To study the morphological characters of given crude drug.

Synonym: Flax seed, linum.

Biological Source:

Linseed consists of dried ripe seeds of the plant known as *Linum usitatissimum Linn,* family Linaceae. It contains not less than 25% of fixed oil & not more than 1% of organic matter.

Morphological Characters:

Colour: Brown

Odour: None

Taste: Mucilaginous & bland

Size: Seeds are about 2 to 6mm in length as 2-2.5 in width.

Shape: Elongated, ovate, strongly flattened rounded at one end & obliquely pointed at their end.

Chemical Constituents:

It has 20-40% of fixed oil, 20% of proteins, 2-7% mucilage & a cyanogenetic glycoside linemarin and also 10% of pectin & enzyme linase.

Uses: Used as source of fixed oil, demulcent, linseed meal is used as poultice. Linseed oil is a drying oil.

Identification Test:

a. Mucilage of seed gives a red colour with ruthenium red and a blue colour with iodine.

ISBN: 978-93-90847-76-1

Exp 44: Morphology of Beeswax

Aim: To study the morphological characters of given crude drug.

Synonym: Beeswax, cera-flava

Biological Source: Yellow beeswax is purified wax and obtained from the honey combination of the bees *Apis mellifica* and other species of Apis, belonging to family Apidae.

Morphology:

Colour: Yellow to yellowish brown

Odour: Agreeable and honey like

Chemical Constituents: It consists of esters of straight – chain monohydric alcohols with straight chain acids. The chief constituents of the beeswax is myricin i.e., myricyl palmitate. Indian beeswax is characterized by its low acid values, while European beeswax has the acid value of 17 to 22.

Uses: It is used in preparation of ointments, plasters and polishes. It is used in ointment for hardening purposes and the manufacture of candles, moulds and in dental and electronic industries also used in cosmetics for preparation of lip-sticks and face creams pharmaceutically. It is an ingredient paraffin ointment I.P.

Adulterants: Very frequently beeswax is adulterated with colophony, hard paraffin, stearic acid, Japan wax spermaceti, carnauba wax and other substances. Adulteration can be detected on the basis of solubility and melting point.

Identification Tests:

a. Boil 0.5g of beeswax with 20ml of aqueous caustic soda solution for 10 min. Cool it. No turbidity is produced.
b. Treat 5 drops of sample with 1ml of 1% copper sulphate solution. Then add 10% sodium hydroxide solution, a blue colour solution is obtained showing glycerin present in the sample.

ISBN: 978-93-90847-76-1

Exp 45: Morphology of Olive Oil

Aim: To study the morphological characters of given crude drug.

Synonym: Oleum olivae.

Biological Source: It is the fixed oil expressed from the ripe fruit *of Olea europaea Linn,* belonging to family Oleaceae.

Description:

Colour: Pale yellow or greenish – yellow

Odour: Slight and characteristic

Taste: Bland, faintly acrid.

Solubility: It is slightly soluble in alcohol and miscible with carbon disulphide, chloroform and ether.

Chemical Constituents: The olive oil contains the triglycerides mainly in the form of olein, palmitin and linolein.

Uses: Externally, it is an emollient and soothing agent for inflamed surfaces. It is used to soften the skin and crusts in eczema and psoriasis. It is also used as an ingredient of ear wax.

Identification Tests:

a. Under radiation, it gives deep yellow colour while refined oil yet gives pale blue fluorescence decolouring with charcoal removes fluorescence.
b. Treat 5 drops of the sample with 1ml of 1% copper sulphate solution. Then add sodium hydroxide solution, and then a blue colour solution is obtained showing glycerin is present in the sample solution. The cupric hydroxide formed in the reaction does not precipitate as it is.

ISBN: 978-93-90847-76-1

Exp 46: Morphology of Wool Fat

Aim: To study the morphological characters of given crude drug.

Synonym: Lanolin, adeps lanae.

Biological Source: Hydrous wool fat is the purified fat like substance obtained from the wool of the sheep *Ovis Aries Linn.* Family Bovidae. It is the secretion of sebaceous glands of sheep deposited onto the wool fibres.

Morphological Characters:

Colour: Whitish – yellow

Odour: Faint & characteristic

Taste: Bland

Chemical Constituents: It is a complex mixture of esters & poly esters of 33 high M.W. alcohols & 36 fatty acids. Hydrous wool fat contains mainly esters of cholesterol & isocholesterol with caranubic, oleic, myristic, palmitic, lanocenic and lanopalmitic seeds.

Uses: Lanolin is mainly used as water absorbent ointment base used for cosmetic preparations.

Identification Test:

a. Dissolve 0.5g of hydrous wool fat in chloroform and to it add 1ml of acetic anhydride and 2 drops of sulphuric acid. A deep green colour is produced, indicating the presence of cholesterol.

ISBN: 978-93-90847-76-1

Exp 47: Morphology of Cinnamon

Aim: To study the morphological characters of given crude drug.

Synonyms: Cinnamon bark, kalmi-dalchini, Ceylon cinnamon.

Biological Source: It consists of the dried inner bark of the shoots of coppiced trees of *Cinnamomum zeylanicum Ness*. belonging to the family Lauraceae. It should contain NLT 10% of volatile oil.

Morphological Characters:

Colour: outer surface: dull – yellowish brown; Inner surface: dark – yellowish brown

Odour: Fragrant

Shape: Found in the form of compound quills

Size: 1m (length) 1 cm in diameter, the thickness of bark 0.5mm

Taste: Aromatic & sweet followed by warm sensation.

Fracture: Splintery

The outer surface of bark is marked by wavy longitudinal striations with small holes of scars left by the branches. The inner surfaces also show the longitudinal striations. Bark is free of cork

Chemical Constituents: Cinnamon bark contains 0.5-10% of volatile oil. 1.2% of tannins (phlobatannins) mucilage, Ca. oxalate, starch & a sweet substance mannitol.

Uses: It is used as carminative, stomachic & mild as astringent. It is also used as a flavouring agent, stimulant an aromatic & antiseptic commercially. It is used as a spice & condiment & also in the preparation of candy dentifrices and perfumes.

Identification Tests:

a. Alcoholic extracts of the drug treated with a drop of ferric chloride solution forms green colour.
b. Chloroform extract of the drug treated with 10% aqueous solution of phenyl hydrazine hydrochloride shows red shaped crystals of hydrazone of cinnamaldehyde.

ISBN: 978-93-90847-76-1

Exp 48: Microscopy of Cinnamon Bark

Aim: To identified the microscopical characters of given crude drug by section cutting.

Microscopical Characters: Transverse section shows broadly a periderm, cortex, a band of sclerenchyma and secondary phloem.

Periderm:

Cork: Few layers of which the outer ones are with thin walls and the inner ones are lignified and thick walled.

Phellogen and Phelloderm: Cannot be distinguished either from each other or form the cork.

Cortex: Consists of 10-15 layers of parenchyma in which are scattered sclereids either isolated or in groups. Each sclereid is more or less rectangular and pitted with thickened inner and radial walls; some of the parenchymatous cells contain minute acicular raphides and abundant starch.

Sclerenchymatous Band or Stone Cell Layer: A continuous well-developed belt of sclereids occurs in between the primary cortex and secondary phloem. The sclereids which are lignified and pitted are typical and characteristic of cassia bark.

As mentioned above, the inner and radial walls of the sclereids are thicker than the outer walls giving the appearance of the letter ‘U’. On the outer side of the sclerenchymatous band are found a few groups of small pericyclic fibres.

Secondary Phloem: This region comprises of phloem parenchyma, phloem fibres and medullary rays. Phloem parenchyma consists of thin-walled cells containing abundant starch and few number acicular raphides.

Numerous, big isolated oil cells are seen frequently in the phloem parenchyma which is again a characteristic feature of cassia bark. Phloem fibres occur mostly single and isolated, rarely in groups of 2 to 3 embedded in phloem parenchyma. The fibres are almost circular and lignified with stratification.

Medullary Rays: Divide radially several times the phloem parenchyma; 1-3 cells wide and externally extend up to the stone cell layer where they become wider. Ray ells also contain starch and acicular raphides.

Chemical Constituents: Cinnamon bark contains about 0.5 to 1.0% of volatile oil, 1.2% of tannins, mucilage, calcium oxalate, starch and a sweet substance known as mannitol. The volatile oil is the active constituents of the drug. It is light yellow in colour and changed to red on storage. Bark yields 14-16% of 90.0% alcohol-soluble extractive.

Cinnamon oil contains 60-70% of cinnamaldehyde, 5-10% eugenol, benzaldehyde, cuminaldehyde and other terpenes like phellandrene, pinene, cymene, caryophyllene etc. Cinnamon oil is yellow to red in colour with sp.gr.1.00 to 1.030; optical rotation 0 to 2; and refractive index 1.562-1.582. The export of cassia oil during 1994-95 was approximately Rs.16 lakhs.

Chemical Test:

a. On addition of a drop of ferric chloride solution to a drop of volatile oil, a pale green colour is produced with ferric chloride; cinnamic aldehyde gives brown colour and eugenol give blue colour, resulting in the formation of pale green colour.
b. In cassia oil, brown colour is obtained, as it contains only cinnamic aldehyde.

Uses: Bark is used as a carminative, stomachic and mild astringent. It is also used as a flavouring agent, stimulant, an aromatic, and antiseptic. Commercially, it is used as a spice and condiment and also in the preparation of candy, dentifrices and perfumes.

Adulterants:

a. Jungle cinnamon
b. Cinnamon chips
c. Saigon cinnamon
d. Java cinnamon

ISBN: 978-93-90847-76-1

Exp 49: Morphology of Fennel

Aim: To study the morphological characters of given crude drug.

Synonyms: Fennel fruits, fructus foeniculum

Biological Source: Fennel consists of dried ripe fruits of the plant known as *Foeniculum vulgare* miller, family Umbelliferae. It should contain NLT 1.4% of volatile oil.

Morphological Characters:

Colour: green to yellowish – brown

Odour: sweet aromatic

Taste: strongly aromatic

Size: 5-10 x 2-4 mm

Shape: Straight on slightly curved

It is 5-sided fruit in the form of cremocarps with pedicles and rarely found in the form of mericarps.

- Fruits are glabrous with straight, prominent, yellow coloured 5 primary ridges & bifid stylopod at the top.
- It is an orthospermous fruit.
- Transfer section shows 2 commissural vittae & 4 dorsal vittae.

Chemical Constituents: It has 3-7% of volatile oil, 20% of proteins & fixed oil.

- The chief active constituent of volatile oil is a ketone fenchone (20%) & a phenolic ether anethole (50%).
- The other constituents are phellandrene, limonene, methyl chavicol, anisic aldehyde etc.
- Fenchone – colourless pungent liquid with aromatic odour. Anethole – Sweet in odour & taste. Oil of fennel – pale yellow with sp. Gr.0.953 – 0.973. Refractive index of 1.526 – 1.538.

Uses: It is used as carminative, aromatic, stimulant & expectorant pharmaceutically as flavouring agent.

Adulterant: Fennel is commonly adulterated with exhausted fennel fruits.

ISBN: 978-93-90847-76-1

Exp 50: Microscopy of Fennel Fruit

Aim: To identify the microscopical characters of given crude drug by section cutting.

Synonym: Fennel fruits, fructus foeniculum.

Biological Source:

Fennel consists of dried ripe fruits of the plant known as *Foeniculum vulgare* miller, family Umbelliferae, obtained by cultivation. It should contain not less than 1.4% of volatile oil.

Procedure:

a. Take thin uniform transverse sections on to a clean glass slide with the help of brush.
b. Add 2 drops of chloral hydrate solution, 2 drops of phloroglucinol followed by 2 drops of conc. HCl excess of reagent is wiped off with filter paper and add 2 drops glycerin and place the cover slip with the help of needle and see that there should not be any entrapment of air bubbles.
c. Chloral hydrate removes the chlorophyll content from section especially in case of leaf drug phloroglucinol and conc. HCl stain lignified tissues to pink colour and glycerine prevents the drying of section.
d. Now mount the slide under microscope and observe first under low magnification (10x) followed by high magnification (45x).

Microscopical Characters:

Fennel exhibits features of a typical umbelliferous fruits cremocarp (schizocarp). It consists of two portions each of which is called a mericarp connected by carpophore. Transverse section of a mericarp shows 2 prominent surfaces.

- Commissural surface with two pronounced ridges and carpophore in the middle.
- Dorsal surface is also 3 ridged. Thus, in all the mericarp shows five primary rides.

Mericarp: Mericarp can broadly be divided into pericarp, testa and the bulky endosperm.

Pericarp:

The epicarp (or) the exocarp of the pericarp surrounding the entire mericarp consist of layer of polygonal, tangentially elongated cells with smooth cuticle.

Mesocarp:

- The bulk of mesocarp is made of parenchyma.
- Bicollateral vascular bundles appear below the primary ridges.

- Reticulate and lignified parenchyma, a characteristic feature of funnel appears surroundings the vascular bundles.
- Besides, yellowish brown & elliptical vittae, 4 on the dorsal surface between the ridges and two on commissural surface are important features of mesocarp.

Endocarp:

- Another typical umbelliferous feature is the presence of parquetry arrangement of cells of endocarp.
- It is seen as a single layer between merocarp and testa.

Testa: Single layered and yellowish in colour.

Endosperm:

- Thick walled, polygonal colourless parenchyma containing oil globules and aleurone grains.
- A crescent shaped is seen passing through the apical region of merocarp.
- Rephe, a ridge of vascular stand, appears in the middle of commissural surface in front of carpophore, as the ovule is anatropous.

Chemical Constituents:

- Fennel consists of volatile oil (3-7%), proteins and fired oils (20%).
- The chief active constituent of volatile oil is a ketone, fenchone (20%) and anethole (50%).
- Other constituents are phellandrene, limonene, methyl chavicol, anisic aldehyde etc.

Uses: It is used as carminative, aromatic and stimulant and expectorant pharmaceutically, it is used as flavouring agent.

Adulterants:

- Fennel is commonly adulterated with exhausted fennel fruits.
- The fennel which does not contain fenchone.
- The fruits which contain only trace of volatile oil & sink in water.

ISBN: 978-93-90847-76-1

Exp 51: Morphology of Coriander

Aim: To study the morphological characters of given crude drug.

Synonym: Coriander fruits.

Biological Source:

These are the fully dried ripe fruits of the plant known as *Coriandrum sativum* Linn. Family Umbelliferae. The fruits should contain not less than 0.3% of the volatile oil.

Morphology:

Colour: yellowish – brown to brown

Odour: aromatic

Taste: spicy and characteristic

Size: fruits are 2-4mm in diameter and 4-30mm in length.

Shape: coriander is a sub-globular cremocarp fruit about 10 primary ridges and 8 secondary ridges are present.

Chemical Constituents:

Coriander yield from 0.3 to 1% of volatile oil the fixed oil and proteins are the other contents of the drug volatile oil of the drug contains 90% of D-linalool and coriandryl acetate. Coriander leaves are rich in vitamin a content. This oil is pale yellow liquid having sp gravity of 0.863 – 0.875.

Uses:

The fruits as well as volatile oil are used as an aromatic, carminative, stimulant & flavouring agent. This oil is used along with purgatives to prevent gripping.

ISBN: 978-93-90847-76-1

Exp 52: Microscopy of Coriander Fruit

Aim: To identify the microscopical characters of given crude drug by section cutting.

Synonym: Coriander fruits.

Biological Source: These are the fully dried ripe fruits of the plant known as *Coriandrum sativum* Linn. Family Umbelliferae. The fruits should contain not less than 0.3% of the volatile oil.

Procedure:

a. Take thin uniform transverse sections on to a clean glass slide with the help of brush.
b. Add 2 drops of chloral hydrate solution, 2 drops of phloroglucinol followed by 2 drops of con. HCl excess of reagent is wiped off with filter paper, and add 2 drops of glycerin and place the cover slip with the help of needle and see that there should not be any entrapment of air bubbles.
c. Chloral hydrate removes the chlorophyll content from the section especially in case of leaf drugs. Phloroglucinol and conc. HCl stain lignified tissue to red colour glycerin prevents the drying of section.
d. Now mount the slide under microscope and observed first under low magnification (10x) followed by high magnification (45x).

Microscopical Characters: A transfer section of the mericarp shows two prominent surfaces the commissural and the dorsal.

The commissural surface is flat with two vittae and carpophore in the middle. The dorsal surface is ridged but with no vittae unlike *Fructus foeniculi*, here 5 less pronounced primary ridges, and 4 pronounced secondary ridges, each secondary ridge appearing between two primary ridges are seen. Pericarp, testa and a kidney shaped endosperm are the important tissues of mericarp.

Pericarp:

Epicarp:

Of the pericarp consists of a single row of small but thick-walled cells.

Mesocarp:

It can be differentiated into 3 zones.

a. Outer loosely arranged tangentially elongated parenchyma.
b. Middle compact sclerenchyma: Outer region of this sclerenchymatous band is represented by longitudinally running fibres whereas the inner region is made of

tangentially running fibres corresponding to secondary ridges vascular bundles crown the sclerenchymatous region of mesocarp below the primary ridges.

c. Inner, irregular, polygonal and lignified parenchyma.

Endocarp: Or inner pericarp is made of typical parquetry layer.

Testa: Single layered and yellowish in colour.

Endosperm: Thick walled, polygonal, colourless parenchyma containing fixed oil and aleurone grains. AN embryo is seen only in transfer section is taken from the apical region of mericarp.

Chemical Constituents: Coriander yields from 0.3 to 1% of volatile oil. The fixed oil and proteins are the other contents of the drug. Volatile oil of the drug contains 90% of D-linalool and coriandryl acetate, and small quantities of L-borneal, geranial and pinene. Coriander oil is pale yellow liquid having sp gr of 0.863-0.875, refractive index 1.462-1.472 and optical rotation of +80 to +150.

Uses: The fruits, as well as, volatile oils, are used as an aromatic, carminative, stimulant and flavouring agent. Coriander oil is used along with purgative to prevent gripping. It is an ingredient of compound spirit of orange and cascara elixir.

ISBN: 978-93-90847-76-1

Exp 53: Morphology of Clove

Aim: To study the morphological characters of given crud drug.

Synonyms: Caryophyllum, clove flower, clove buds

Biological Source: Clove consists of dried flower buds of *Eugenia caryophyllus*, family Myrtaceae. It should contain not less than 15% (v/w) of clove oil.

Morphological Characters:

Colour: Crimson to dark brown

Odour: Slightly aromatic

Taste: Pungent and aromatic followed by numbness

Size: About 10 to 17.5mm in length, 4mm in width, and 2mm in thick.

Shape: Hypanthium is surmounted with 4 thick acute divergent sepals surrounded by dome shape corolla. The corolla consists of unexpanded membranous petals with several stamens and single stiff prominent style cloves are heavier than water.

Chemical Constituents:

Clove contains about 15 to 20% of volatile oil, 10% to 13% of tannin (gallotannic acid), resin, chromone and eugenin.

The volatile oil of the drug contains eugenol, eugenol acetate, caryophyllenes and small quantities and esters, ketones and alcohols.

An oil of clove is colourless to pale yellow in colour. It becomes thick and darker in colour on storage. It has specific gravity of 1.038-1.06, refractive index of 1.527 to 1.535 and it boils at 250^0C.

Uses: Clove is used as a dental analgesic, carminative, stimulant, flavouring agent, an aromatic and antiseptic. It is also used in the preparation of cigarettes. The oil is used in perfumery and also in the manufacture of vanillin.

Adulterants:

a. Mother cloves
b. Blown cloves
c. Clove stalks
d. Exhausted cloves

Identification Tests:

a. Thick section of hypanthium with 5% potassium hydroxide; needle shaped crystals of potassium eugenolate are observed under microscope.
b. A drop of chloroform extract with 3% sodium hydroxide: crystals of sodium eugenolate are formed.
c. Alcoholic extract of drug treated with solution of ferric chloride shows blue colour.

ISBN: 978-93-90847-76-1

Exp 54: Microscopy of Clove Bud

Aim: To identify the microscopical characters of given crude drug by section cutting.

Procedure:

a. Take a thin uniform section on to a clean glass slide with the help of brush add 2 drops of concentrated HCl, chloral hydrate solution, 2 drops of phloroglucinol.
b. Excess of reagent is wiped off with filter paper and add 2 drops of glycerin and placed the cover slip with the help of needle and see that there should not be any entrapment of air bubbles.
c. Chloral hydrate removes the chlorophyll content from the section especially in case of leaf drugs. Phloroglucinol and conc. HCl stain lignified tissue to red colour glycerin prevents the drying of section.
d. Now mount the slide under microscope and observed first under low magnification (10x) followed by high magnification (45x).

Microscopical Characters:

Transverse section be taken both through the short upper portion containing bilocular and as well through the long solid, sub-cylindrical lower portion (hypanthium) of the bud.

Here in a detailed descriptive account of the transfer section through hypanthium only is given. Transfer section though hypanthium:

Following are the important tissues from the periphery to the centre. They are epidermis, cortex, and columella.

Epidermis:

Single layered small cells with straight walls and has a very thick cuticle. Epidermal layer gets intercepted by Ranunculaceous type of stomata.

Cortex: Three distinct zones or regions can be made out.

a. The peripheral region containing 2 to 3 layers of big ellipsoidal, schizolysigenous oil glands embedded in the radially elongated parenchymatous cells.
b. The middle region containing or 2 rings of bicollateral vascular bundles associated with a few pericyclic fibres, embedded in thick-walled parenchyma and
c. The inner region made of loosely arranged aerenchyma.

Columella:

Forms the central cylinder containing thick-walled parenchyma with a ring of bicollateral vascular bundles towards the periphery of the cylinder. Numerous cluster crystals are seen scattered throughout the columella and to a certain extent in the middle cortical zone.

Transfer section through the ovary region shows identical structures as that of hypanthium but for the absence of the central columella this central region here is occupied by a bilocular ovary with several ovules showing an axile placentation.

Chemical Constituents:

Clove contains about 15 to 20% of volatile oil, 10% to 13% of tannin, resin, chromone and eugenin. The volatile oil of the drug contains eugenol, eugenol acetate, caryophyllenes and small quantities of esters, ketones and alcohols. Oil of clove is colourless to pale yellow in colour. It becomes thick and darker in colour on storage. It has specific gravity of 1.038-1.06, refractive index of 1.527 to 1.535 and it boils at 250^0C.

Uses: Clove is used as a dental analgesic, carminative, stimulant, flavouring agent, an aromatic and anti-septic. It is also used in the preparation of cigarettes. The oil is used in perfumery and also in the manufacture of vanillin.

Adulterants:

Mother Cloves:

These are dark brown, ovate ripened fruits of clove tree. They are slightly aromatic and contain starch.

Blown Cloves:

These are expanded flowers of the clove tree. The stamens generally get detached. They also contain volatile oil and are similar in colour to the cloves.

Clove Stalks:

These are generally used to adulterate the powdered cloves and are detected by presence of isodiametric sclereids and prisms of calcium oxalate. Their ash value and crude fibre contents are also high.

Exhausted Cloves:

These are the cloves form which oil has been removed by distillation. They are dark in colour, more shrunken and when pressed with fingers nail, do not show the presence of oil. Exhausted cloves float on water.

ISBN: 978-93-90847-76-1

Exp 55: Morphology of Cinchona

Aim: To study the morphological characters of given crud drug.

Synonyms: Peruvian bark, Jesuit's bark

Biological Source: It consists of the dried bark of the *Cinchona officinalis* and other species of Cinchona belongs to Rubiaceae family.

Morphological Characters:

Colour: Outer surface is dull brownish grey or grey in colour, inner surface is pale yellowish to deep reddish brown in colour

Odour: Slight odour

Taste: Astringent and bitter taste

Size: Up to 30cm long and about 0.2 - 0.6cm thick. Cinchona bark occurs in quilled or curved pieces.

Chemical Constituents: It contains important alkaloids (Quinoline type alkaloids) like quinine, quinidine, cinchonine, cinchonidine, quinicine.

Uses: Bark is anti-malarial in nature. Cinchona tincture is used as bitter stomachic and anti-pyretic.

Adulterants:

a. Cuprea bark (remijia peduncunata)
b. False cuprea bark

ISBN: 978-93-90847-76-1

Exp 56: Microscopy of Cinchona Bark

Aim: To identify the microscopical characters of given crude drug by section cutting.

Synonyms: Peruvian bark, cinchona bark.

Biological Source:

It consists of the dried bark of the *Cinchona officinalis* and other species of Cinchona belongs to Rubiaceae family.

Procedure:

a. Take a thin uniform section on to a clean glass slide with the help of brush add 2 drops of concentrated HCl, chloral hydrate solution, 2 drops of phloroglucinol.
b. Excess of reagent is wiped off with filter paper and add 2 drops of glycerin and placed the cover slip with the help of needle and see that there should not be any entrapment of air bubbles.
c. Chloral hydrate removes the chlorophyll content from the section especially in case of leaf drugs. Phloroglucinol and conc. HCl stain lignified tissue to red colour glycerin prevents the drying of section.
d. Now mount the slide under microscope and observed first under low magnification (10x) followed by high magnification (45x).

Microscopical Characters:

Periderm:

Cork: Several layers of thin walled, flat polygonal cells with reddish brown content impregnated with suberin

Phellogen: 2-3 layers of thin-walled cells without any cellular content

Phelloderm: 6-8 layers of thin-walled rectangular cells without any cellular content.

Cortex: Several layers of thin walled tangentially elongated cells containing reddish brown matter. Sclereids are absent.

Secondary Phloem:

Sieve Tubes: The compact cells being about 200µ long and 50-20µ wide and having narrow companion cells; most of the sieve tubes are compressed and collapsed.

Fibers: Numerous, large, fusiform, lignified phloem fibers having straight walls and conspicuous tubular or funnel shaped pits, mostly isolated, sometimes in groups of 2-3 fibers.

Phloem Parenchyma: Thin, dark reddish-brown walls, some with micro prisms of calcium oxalate

Medullary Rays: 1-3 serate, extended up to cortex cells, radially elongated and contains starch grains.

ISBN: 978-93-90847-76-1

Exp 57: Introduction to Section Cutting

Sections can be obtained from bark, stem, root, stolon depending on the plane of cutting.

Transverse Section:

It is obtained by cutting along the radical plane of a cylindrical portion of a stem, root stolon perpendicular to the long axis.

The section when observed under microscope reveals the radial arrangement of tissues and shows concentric layers and vascular bundles. It reveals the structure and morphology of particular cell in all angles.

Section of Bark:

The case of bark transverse section was important as to reveal the horizontal arrangement of cells and also helps us to reveals arrangements & thickness of cells.

Section of Fruits and Seeds:

Transverse section of various parts of fruit and seeds are observed. In case of fruits like fennel transverse section of (microscope) mericarp is observed while in cardamom transverse section of pericarp and seed transverse section is observed.

Section of Leaf:

In case of leaf a section is to be taken through midrib in case of bilateral leaf either surface may be observed and in case of dorsiventral leaf lower epidermis is important which is bearing stomata, guard cells and epidermal cells.

Preparation of Sample for Section Cutting:

Hold the sample vertically between first, second fingers and thumb and move the blade back on forth from one end to other obtaining fine slices.

Take sufficient no. of sections. In case of leaf drug, the lamina is very thin and hence section cutting is very difficult. The surface area of the surface to be cut has to be increased.

This is done by embedding the sample in black of pith. This pith is obtained from red pumpkin raw papaya (or) potato.

A vertical portion of pith is cut off and bifurcated take the leaf sample in the size of pith and insert it. Now take the section by holding of it with leaf in between the fingers take uniform section of leaf discord at section of pith.

Staining and Mounting of Sections:

Staining is processes in which chemical dies are used to impart colour to various tissues in the section of sample which unable to distinguish arrangement of various tissues in a sample. A stain is a chemical dye which combines chemically (or) physically with cell content to impart colour to it.

Safranin + lignin (cell wall) ----------> Red colour

Iodine + starch grain ----------> Blue colour

Sudan red III + Oil globules ----------> Red colour

ABOUT THE AUTHORS

KAVETI VAMSHI SHARATHNATH, *presently working as Associate Professor at Nalla Narasimha Reddy Education Society's Group of Institutions, School of Pharmacy, Chowdariguda, Ghatkesar, Hyderabad, Telangana. He is having 9 years of experience in academics. He published 15 papers in various National and International Journals. He authored 1 book. He has attended 20 National and International conferences. He guided many projects to B.Pharmacy students.*

Dr. B. SREE GIRI PRASAD, *Assoc. Professor & H.O.D, in Dept. of Pharmaceutics at Nalla Narasimha Reddy Education Society's Group of Institutions, School of Pharmacy, Chowdariguda, Ghatkesar, Hyderabad, Telangana. He has 15 years of teaching experience and published 39 research papers in various National and International journals of repute and attended 48 National and International Conferences/Seminars/Workshops. He has guided many projects of both B.Pharmacy and M.Pharmacy students. He has successfully completed various NPTEL courses. He is life member of Association of Pharmacy Professionals (APP), Telangana Chapter and Association of Pharmaceutical Teachers of India (APTI). He received Best Achiever Award from Association of Pharmacy Professionals in the Year 2017.*

NARENDER BOGGULA*, is an Associate Professor at School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Hyderabad, Telangana. He is a dynamic, hardworking professional person in the Pharmaceutical Chemistry department. He has to his credit eight years of experience in Research and Academics and one year of Industrial Experience. He has 105 publications in various Journals of International and National repute. He has attended 60 National and International conferences, seminars, workshops and presented his research and development work. He has contributed 11 books and 3 chapters in his expertise area. He has 1 Indian Patent and 1 Australian Grant Innovation Patent. He is also an editorial board member and reviewer of some prestigious journals.*

He has successfully completed various NPTEL courses. He has guided many projects to UG students. He is a life member of Association of Pharmaceutical Teachers of India (APTI). Mr. Narender Boggula has an excellent track record in academics and actively engaged in teaching, research, administration and service to his profession.

Kripa-Drishti Publications
A-503 Poorva Heights, Pashan-Sus Road, Near Sai Chowk,
Pune – 411021, Maharashtra, India.
Mob: +91 8007068686
Email: editor@kdpublications.in
Web: https://www.kdpublications.in

ISBN: 978-93-90847-76-1