

TECHNOCRATS

Lab Work Book of

Pharmacognosy I
(BP - 409 P)

Department of Pharmacy

Lab Manual of **Pharmacognosy I** (BP-409 P)

Price : ₹ 110/-

Edition :

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Printed & Published by :



TECHNOCRATS
PUBLICATIONS

Arera Colony, Bhopal.

e-mail : technocratspublications@gmail.com



TECHNOCRATS
PUBLICATIONS

Lab Work Book
of
Pharmacognosy I
(BP-409 P)

(Strictly According to RGPV Syllabus)

Name :

Enrollment No. :

Institute :

Academic Session :

Department of Pharmacy



TECHNOCRATS
PUBLICATIONS

Vision of the Institute

To grow as an institute of Excellence for Pharmacy Education and Research and to serve the humanity by sowing the seeds of intellectual, cultural, ethical, and humane sensitivities in the students to develop a scientific temper, and to promote professional and technological expertise.

Mission of the Institute

M 1: To inculcate ethical, moral, cultural and professional values in students

M 2: To provide state of art infrastructure facilities to the staff and students so as to enable them to learn latest technological advancements

M 3: State of art learning of professionalism by the faculty and students

M 4: To produce well learned, devoted and proficient pharmacists

M 5: To make the students competent to meet the professional challenges of future

M 6: To develop entrepreneurship qualities and abilities in the students

PROGRAM OUTCOMES (POs)

- 1. Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
- 2. Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
- 3. Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
- 4. Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
- 5. Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
- 6. Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
- 7. Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
- 8. Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
- 9. The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
- 10. Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
- 11. Life-long learning:** Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

PEOs

PEO 1: To inculcate quality pharmacy education and training through innovative Teaching Learning Process.

PEO 2: To promote professionalism, team spirit, social and ethical commitment with effective interpersonal communication skills to boost leadership role assisting improvement in healthcare sector.

PEO 3: To enhance Industry-Institute-Interaction for industry oriented education and research, which will overcome healthcare problems of the society.

PEO 4: To adapt and implement best practices in the profession by enrichment of knowledge and skills in research and critical thinking

PEO 5: To generate potential knowledge pools with interpersonal and collaborative skills to identify, assess and formulate problems and execute the solution in closely related pharmaceutical industries and to nurture striving desire in students for higher education and career growth.

Course Outcomes (COs):

Student will be able to:

- CO1: Describes about essential oil content in Eucalyptus by Clevenger's apparatus.
- CO2: Demonstrates about extraction procedure of crude drugs.
- CO3: To interpret the R_f values of chemical constituents.
- CO4: To distinguish about microscopical characters of leaf constituents.
- CO5: Understands and differentiate spotting of crude drugs.

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Experiments No:-01

OBJECT

Analysis of given crude drug by chemical test

REFERENCE

Deore S L , Khadabadi S.S&Baviskar B A , Pharmacognosy&Phytochemistry, 1st edition 2014, Pharmamed press Hyderabad.

REQUIREMENTS

Test tube & other chemicals

TRAGACANTH GUM

Biological source – It is dried gummy exudation of stem & branches of *Astragalus gummifer*

Family- Leguminosae

Definition- Tragacanth Gum mainly consists of polysaccharides, obtained from the secretion of tragacanth trees .

Description Tragacanth Gum occurs as a white to whitish powder or white to light yellowish white translucent flattened or laminar flake, and it is odorless .

Identification Test

(1) To 1 g of powdered Tragacanth Gum, add 50 ml of water. An almost uniform, somewhat turbid viscous solution is formed.

(2) Place about 1.0 g of powdered Tragacanth Gum into a watch glass containing 2-3 drops of a mixture of water and glycerol (1 : 1) and 1 drop of Iodine TS. Mix well with a top of small glass rod cautiously, being careful to avoid air bubbles in it. Allow to stand for 10 minutes or more and swell it. Apply small amount of swelled sample to a slide glass with a top of small glass rod, add 1 drop of a mixture of water and glycerol (1 : 1). Cover it with a cover glass, being avoid air bubbles in, and observe it by an optical microscope. A little blue granular of starch is found. For microscopic observation, use 10 or 40 times scale as an objective and 10 times scale as an eyepiece.

Honey

Biological source- It is a sweet secretion found in honey comb secreted by honey bees such as *Apis dorsata*, *Apis indica* & other species of *Apis*

Family-Apidae

IDENTIFICATION TEST

1. The Thumb Test
2. The Water Test
3. The Flame Test, and
4. The Water-vinegar mix Test

Thumb Test:

Procedure

1. Put a small drop of the honey you have on your thumb
2. Check to see if it spills or spreads around
3. If it does, it is not pure
4. Pure honey will stay intact on your thumb

The Water Test to Spot Fake Honey:

Procedure

1. Fill a glass with water
2. Add one tablespoon of honey into the glass
3. Adulterated or artificial honey will dissolve in water and you will see it around the glass
4. Pure honey on the other hand will settle right at the bottom of your glass

The Flame Test to Check Purity of Honey:

Procedure

1. Take a dry matchstick
2. Dip its tip right into the honey
3. Strike the stick on the matchbox as if to light it
4. If the honey is pure, the matchstick will light with ease
5. The flame will also keep burning off the honey
6. However, if it is with impurities, it will not light because fake honey contains moisture as one of the impurities

Acacia

Biological source- It is a dried gummy exudation from the stem and branches of *Acacia Arabica* & *Acacia Senegal*, Family- leguminosae

IDENTIFICATION TEST-

Chemical Tests

1. **Lead Acetate Test:** An aqueous solution of acacia when treated with lead-acetate solution it yields a heavy white precipitate.
2. **Borax Test:** An aqueous solution of acacia affords a stiff translucent mass on treatment with borax.
3. **Blue Colouration due to Enzyme:** When the aqueous solution of acacia is treated with benzidine in alcohol together with a few drops of hydrogen peroxide (H_2O_2), it gives rise to a distinct-blue colour indicating the presence of enzymes

Test: Hydrolysis of an aqueous solution of acacia with dilute HCl yields reducing sugars whose presence are ascertained by boiling with Fehling's solution to give a brick-red precipitate of cuprous oxide.

4. **Specific Test:** A 10% aqueous solution of acacia fails to produce any precipitate with dilute solution of lead acetate (a clear distinction from Agar and Tragacanth); it does not give any colour change with Iodine solution (a marked distinction from starch and dextrin); and it never produces a bluish-black colour with $FeCl_3$ solution (an apparent distinction from tannins).

Agar-

Biological source : Hydrophillic colloidal polysaccharide complex extracted from the red algae

Gelidium amansii .

Family- Gelididaceae

Identification test- 1.Boil about 1.5gm agar with 100ml. water.Cool the solution to room temperature. It forms a stiff jelly. 2.To 0.2% solution of agar in water,add solution of tannic acid;no precipitate is produced.

OBSERVATION –

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RESULT-

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VIVA QUESTION

Q.-1. Write the chemical test for agar.

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Q.-2. Discuss lead acetate test to identify crude drug.

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Q.-3. Give the chemical test for acacia.

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Q.-4. The water vinegar mix test used for which crude drug.

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Experiment No:-02

OBJECT

Determination of stomatal number

REFERENCE

Deore S L , Khadabadi S.S&Baviskar B A , Pharmacognosy&Phytochemistry, 1st edition 2014, Pharmamed press Hyderabad.

REQUIREMENTS

A fresh leaf and camera lucida with stage microscope.

THEORY

Stomatal number – Stomatal number is average number of stomata per sq. mm of the epidermis of the leaf

Stomata was discovered by Pfeffer & name ‘stomata’ was given by Malpighii. Stomata cover 1-2% of leaf area. It is minute pore present in soft aerial parts of the plant. Algae, fungi and submerged plants do not possess stomata.

Stomata are minute pores of elliptical shape, consists of two specialized epidermal cell called guard cells.

PROCEDURE

Clear the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by mean of forceps. Keep it on slide And mount in glycerin water. Arrange a camera lucida and drawing board for making the drawings to scale. Draw a Square of 1 mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage.Trace the epidermis cell and stomata present in area of 1 sq. mm. Include the cell if at least half of its area lies within the square. Record the result for each of the ten fields and calculate the average number of stomata per sq mm.

OBSERVATION

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RESULT (DRAW)

VIVA QUESTION

Q.-1. What are stomata.

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Q.-2. Write the functions of Stomata.

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Q.-3. What is the shape of Guard Cells.

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Q.-4. Discuss the use of Chlorophyll in the plant.

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Experiment No:-03

OBJECT

To determine stomatal index (S. I.)

REFERENCE

DeoreSL, khadabadi S S & Baviskar B A ,Pharmacognosy & Phytochemistry ,1stedition 2014, Pharmamed press Hyderabad.

REQUIREMENTS

A fresh leaf and camera lucida with stage microscope.

THEORY

Stomatal index. Stomatal density (SD) is a function of both the number of stomata plus the size of the epidermal cells. Thus, SD is affected both by the initiation of stomata and the expansion of epidermal cells.

$$S.I.=S/E+S\times 100$$

Where ,

S.I.:Stomatal Index

S:No.of stomata per unit area

E:No.of epidermal cells in the same unit area

PROCEDURE

Clear the piece of the leaf (middle part) by boiling with chloral hydrate solution or alternatively with chlorinated soda. Peel out upper and lower epidermis separately by means of forceps. Keep it on slide and mount in glycerin water. Arrange a camera lucida and drawing board for making the drawings to scale. Draw a square of 1mm by means of stage micrometer. Place the slide with cleared leaf (epidermis) on the stage. Trace the epidermis cell and stomata. Count the number of stomata, also the number of epidermis cells in each field. Calculate the stomatal index using formula which is given above.

OBSERVATION

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RESULT (DRAW)

VIVA QUESTION

Q.-1. Name the cells that forms Stomata.

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Q.-2. What is Transcription.

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Q.-3. Which side of the leaf contains more number of stomata per square unit area.

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Q.-4. What will be the rate of Transcription during day and night.

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Experiment No:- 04

OBJECT

To determine the number of starch grain by lycopodium spore method

REFERENCE

DeoreSL, khadabadi S S & Baviskar B A ,Pharmacognosy & Phytochemistry ,1stedition 2014, Pharmamed press Hyderabad.

REQUIREMENTS

A fresh leaf and camera lucida with stage micrometer.

THEORY:

Validated modified lycopodium spore method has been developed for simple and rapid quantification of herbal powdered drugs. Lycopodium spore method was performed on ingredients of Shatavaryadi churna, an ayurvedic formulation used as immunomodulator, aphrodisiac and rejuvenator

PROCEDURE

Lycopodium Spore method:

Lycopodium spore method is composed of the spores of lycopodium clavatum L. each spore is tetrahedral in shape, the base is rounded and the three flat sides meet to form three wall marked covering ridge which join one another at the apex the whole surface of the spore is covered with minute reticulation and interior is filled with fixed oil. The spores are exceptionally uniform size (25 μ m), so that one can always know that a definite number of spores represent a particular weight of lycopodium. The whole process can be simplify as 1 mg of spore contain averagly 94000 spore by this figure one can calculate the weight of any number of spore under any condition under the microscope. If the lycopodium has been fixed with a definite proportion of another substance one can find immediatly how much of the second substance has been added .When examined microscopically it is admixed with any fine partilce like pollen grains starch etc. with characterstics countable particles it is possible to calculate the number of such charaterstics particles per mg. In this way it is possible to have a standerd figure that represent any such material the number of charactersitics particle per unit weight which is often constant and is useful in assuming the quality of a sample to be used in this method the number of particle in a good quality sample must either be known or first determined .

$$\% \text{ Purity} : \frac{N \times W \times 94000}{S \times M \times P} \times 100$$

where,

N = number of characteristics particle of sample in 25 fields.

W = weight of lycopodium spores taken in mg.

S = number of lycopodium spores in 25 fields.

M = weight of sample in mg.

P = standard value of number of characteristics sample per mg in taken sample material (e.g. 1 m.g. ginger powder contains 286000 starch grains)

OBSERVATION

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RESULT

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VIVA QUESTION

Q.-1. What is lycopodium spore method.

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Q.-2. Lycopodium spore method performed on which of the ingredients in Ayurvedic Formulations

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Q.-3. Write the procedure to determine starch by lycopodium spore method.

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Q.-4. Write the calculation of Percentage purity of spore method.

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Experiment No:- 05

OBJECT:

Determination of Foaming index.

REFERENCE:

“kokate ck” practical pharmacognosy ,4th edition 1994 vallabh prakashan ,new delhi.

THEORY:

The foaming ability of an aqueous decoction of plant materials & their extracts is measured in terms of a foaming index.

REQUIREMENT:

Measuring cylinder, stoppered test tube, volumetric flask

PROCEDURE:

Weighed accurately about 1 g of coarsely powdered drug and transferred to 500 ml conical flask containing 100 ml of boiling water maintained at moderate boiling at 80-90°C for about 30 mins .

Then made it cold, filtered into a volumetric flask and added sufficient water through the filter to make the volume up to 100 ml (V1).

Cleaned 10 stopper test tubes were taken and marked with 1 to 10. The successive portions of 1, 2 ml up to 10 ml drug was taken in separate tubes and adjusted remaining volume with the liquid up to 10 ml in each. After closing the tubes with stoppers, Shook them for 15 seconds and allowed to stand for 15 mins then measured the height.

If the height of the foam in each tube is less than 1 cm, the foaming index is less than 100(not significant). Here, if the foam is more than 1cm height after the dilution of plant material in the sixth tube, then corresponding number of the test tube was the index sought.

If the height of the foam in every tube is more than 1cm, the foaming index is more than 1000. In this case, 10ml of the first decoction of the plant material needs to be measured and transferred to a 100ml volumetric flask (V2) and volume is to be maintained up to 100ml and follow the same procedure.

CALCULATION:

Foaming Index was calculated by using this formula

Foaming Index = $1000/a$ in case of V1

Foaming Index = $1000 \times 10/a$ in case of V2

Where, a = Volume (ml) of decoction used for preparing the dilution in the tube where exactly 1 cm or more foam was observed.

OBSERVATION

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RESULT

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VIVA QUESTION

Q.-1. Define Foaming Index.

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Q.-2. What is automated foam index test.

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Q.-3. Discuss the procedure to determine foam and index.

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Q.-4. What do you mean by AEA.

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Experiment No:- 06

OBJECT:

Determination of Swelling index.

REFERENCE:

“Kokate CK” Practical Pharmacognosy ,4Th Edition 1994 Vallabh Prakashan ,New Delhi.

REQUIREMENT:

Stoppered measuring cylinder, stoppered test tube, volumetric flask,

THEORY:

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums containing an appreciable amount of mucilage, pectin or hemicellulose.

PROCEDURE:

It was carried out simultaneously no fewer than three determinations for any given material.

Introduce the specified quantity of the plant material concerned, previously reduced to the required fineness and accurately weighed 1g of plant material into a 25 ml glass-stopper measuring cylinder.

The internal diameter of the cylinder was about 16 mm, the length of the graduated portion about 125mm, marked in 0.2 ml divisions from 0-25 ml in an upwards direction.

25 ml of water was added and shake the mixture thoroughly every 10 minutes for 1 hr. Allowed to stand for 3 hr at room temperature.

Measure the volume in ml occupied by the plant material, including any sticky mucilage.

OBSERVATION:

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VIVA QUESTION

Q.-1. Define swelling index.

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Q.-2. Procedure for optimization of method for determination of swelling factor.

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Q.-3. Enlist any five gums which are used to determine swelling index.

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Q.-4. Write down the application of swelling index

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Experiment No:- 07

OBJECT:

To determine the ash value of crude drug.

REFERENCES:

Evans W.C., "Pharmacognosy", fifteenth edition, 2008, Elsevier Health Sciences Education, Noida.

Tailing M. and Sharma A., "Phytochemistry theory and practical", first edition, 2008-09, Birla publications, Delhi.

THEORY:

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination. On incineration, crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.

REQUIREMENTS:

Porcelain crucible (50mL), Muffle furnace (600±20), weighing machine, desiccators.

PROCEDURE:

Take about 2 or 3 g, accurately weighed, of the ground drug in a tarred platinum or silica dish previously ignited and weighed. Scatter the ground drug in a fine even layer on the bottom of the dish. Incinerated by gradually increasing the heat-not exceeding dull red heat- until free from carbon, cool and weigh.

If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, increase the residue and filter paper, add the filtrate, evaporate to dryness and ignite at low temperature. Calculate the percentage of ash with reference to the air dried drug.

OBSERVATIONS:

Data and observation sheet for water content determination

S.No.	Sample No.	1	2	3
1	Weight of silica dish W_1 gm			
2	Weight of silica dish + sample W_2 gm			
3	Weight of silica dish + ash W_3 gm			
4	Wt of ash $W = (W_3 - W_1)$			

CALCULATION:

Total ash % by weight = $100 \times (W_3 - W_1) / W_1$

OBSERVATION

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RESULT

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VIVA QUESTIONS

Q.-1. What is ash?

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Q.-2. Write note on acid insoluble ash.

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Q.-3. Write note on total ash.

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Q.-4. Define ash value.

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Q.-5. Write note on importance of ash.

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Experiment No:- 08

OBJECT:

To determine the extractive value of crude drug.

REFERENCES:

Evans W.C., "Pharmacognosy", fifteenth edition, 2008, Elsevier Health Sciences Education, Noida.

Tailing M. and Sharma A., "Phytochemistry theory and practical", first edition, 2008-09, Birla publications, Delhi.

THEORY:

Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. The amount of extractive a drug yields to a given solvent is often an approximate measure of a certain constituent or group of related constituents the drug contains. In some cases the amount of a certain constituent or group of related constituents the drug contains, in some cases the amount of drug soluble in a given solvent is an index of its purity. The solvent used for extraction should be in a position to dissolve quantities of substances desired.

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values were determined to indicate the nature of the constituents present in a crude drug.

REQUIREMENTS:

90% ethanol , sample, distilled water.

PROCEDURE:

Determination of Alcohol Soluble Extractive Value:-

10 gm. of the air-dried coarse powder of leaves were macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs.

Determination of Water Soluble Extractive Value:-

Coarsely powdered drug (10 gm) was weighed accurately and macerated with 100 ml of water in a closed flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand. After 18 hours it was filtered rapidly. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of water-soluble extractive value was calculated with reference to the air-dried drug.

[illegible]

This image shows a full page of a worksheet designed for handwriting practice. It features multiple sets of horizontal dashed lines spaced evenly across the page, providing a guide for letter height and placement. The background is plain white, and there are no other markings or text present.

VIVA QUESTIONS

Q.-1. What are different physical evaluation parameters?

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Q.-2. Differentiate between Soxhlet apparatus and Clevenger apparatus.

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Q.-3. Write a note on different extraction procedures.

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Q.-4. Define extractive value.

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Q.-5. Write a note on extraction.

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Experiment No:- 09

OBJECT:

To determine the moisture content of crude drug.

REFERENCES:

Evans W.C., "Pharmacognosy", fifteenth edition, 2008, Elsevier Health Sciences Education, Noida.

Tailing M. and Sharma A., "Phytochemistry theory and practical", first edition, 2008-09, Birla publications, Delhi.

THEORY:

Definition: Amount of water (in any form) in a material or substance.

Moisture content influences the taste, texture, weight, appearance, and shelf life of foodstuffs.

The methods of determining moisture content in coffee can be divided into three broad categories:

Direct measurement: Water content is determined by removing moisture and then by measuring weight loss;

Indirect measurement: An intermediate variable is measured and then converted into moisture content. Building up calibration charts before applying indirect measurements is a prerequisite;

Empirical measurement: Refers to methods such as biting, shaking, crunching, commonly used by both producers and small traders. These empirical measurements are both indirect and subjective. Surveys carried out during the 'Enhancement of Coffee Quality through the Prevention of Mould Formation' project have shown that these subjective methods of moisture determination to be insensitive over the range 12–20% moisture, content, and therefore unsuitable for determining the end of drying (i.e. when coffee has a maximum of 12% moisture), or for verifying that coffee in the marketing chain is at a safe moisture content.

REQUIREMENTS:

1. Non-corrodible air-tight container.
2. Electric oven, maintain the temperature between 105⁰ C to 110⁰ C.
3. Desiccator.
4. Balance of sufficient sensitivity.

PROCEDURE:

1. Clean the container with lid dry it and weigh it (W1).
2. Take a specimen of the sample in the container and weigh with lid (W2).

3. Keep the container in the oven with lid removed. Dry the specimen to constant weight maintaining the temperature between 105° C to 110° C for a period varying with the type of soil but usually 16 to 24 hours.
4. Record the final constant weight (W₃) of the container with dried soil sample. Other organic soils are to be dried at lower temperature (60°) possibly for a longer period.

Certain soils contain gypsum which on heating loses its water if crystallization. If it is suspected that gypsum is present in the soil sample used for moisture content determination it shall be dried at not more than 800 C and possibly for a longer time.

OBSERVATIONS:

Data and observation sheet for water content determination

S.No.	Sample No.	1	2	3
1	Weight of container with lid W ₁ gm			
2	Weight of container with lid +wet soil W ₂ gm			
3	Weight of container with lid +dry soil W ₃ gm			
4	Water/Moisture content $W = [(W_2 - W_3) / (W_3 - W_1)] \times 100$			

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What do you mean by moisture content in reference to crude drug.

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Q.-2. Write a note on physical parameters for analysis of crude drug.

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Q.-3. Write a note on drying.

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Q.-4. Define moisture content.

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Q.-5. What do you understand by the term crude drug.

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