TECHNOCRATS

Lab Work Book of

Herbal Drug Technology

(BP - 609 P)

Department of Pharmacy

Lab Manual of

Herbal Drug Technology

(BP - 609 P)

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TECHNOCRATS
PUBLICATIONS

Lab Work Book
of

Herbal Drug Technology

(BP-609 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 thelimitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivationissues, 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: Perform preliminary phytochemical screening of crude drugs.
- CO2: Incorporation of prepared and standardized extract in cosmetics formulations.
- CO3: Analysis of herbal drugs from recent Pharmacopoeias.
- CO4: Determination of Aldehyde content.
- CO5: Determination of phenolic content.

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Experiment No:- 01

OBJECTIVE:

To perform preliminary phytochemical screening of crude drug.

REFERENCE:

Khandelwal K.R., 'Practical Pharmacongnosy Techniques and Experiments', 21st edition, Aug 2011, Nirali prakashan, Pune, Page no. 25.1-25.6

MATERIALS REQUIRED:

Powder crude drug, Beaker, test tube, glass rod and reagents.

PROCEDURE:

1. To take a pinch of powder in test tube, this consists of chemical reagent.
2. After addition of powder in test tube, it shows the change in color.
3. The changed color of solution shows the presence of drug.

Phytochemical screening for alkaloids

- **Mayer's test:** Alkaloids give cream colour precipitate with Mayer's reagent (Potassium mercuric iodide solution).
- **Dragandroff's test:** Alkaloids give orange brown precipitate with Dragandroff's reagent (Potassium bismuth iodide solution).
- **Hager's test:** Alkaloids give yellow color precipitate with Hager's reagent (Saturated solution of picric acid).
- **Wagner's test:** Alkaloids give a reddish brown precipitate with wager's reagent (Solution of iodide in potassium iodide).

Phytochemical screening for Steroids and Triterpenoids:

- **Liebermann test –** Powder sample was mixed with few drops of acetic anhydride, boiled and cooled.Concentrated sulphuric acid was then added from the sides of the test tube blue color appears.

Phytochemical screening for Glycosides:

- **Keller Killiani Test –** Sample was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

- **Borntrager's test –** To the sample add dil sulphuric acid. Boil and filter. To cold filtrate add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer turns pink or red.

Phytochemical screening for Saponins:

- **Foam Test –** Test sample was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Phytochemical screening for Flavonoids:

- **Ferric chloride test –** Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.
- **Alkaline reagent Test –** Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.
- **Lead acetate solution Test –** Test solution when treated with few drops of lead acetate (10%) solution would result in the formation of yellow precipitate.

OBSERVATION:

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RESULT:

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VIVA QUESTIONS

Q.-1. What is the importance of secondary metabolites?

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Q.-2. Give some special characteristics of drugs?

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Q.-3. Give the Biological source of the given drugs?

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Q.-4. Write the Chemical test for alkaloid?

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Q.-5. What is phytochemical screening?

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Experiment No:- 02

OBJECT:

To determine the alcohol content in asava and arista.

REFERENCES:

1. Deore S.L., Khadabadi S.S., and Baviskar B.A., "Pharmacognosy and Phytochemistry", Pharmamed Press, 1st Edition, 2014, Page no. 602

THEORY:

Asava and Arishta are ayurvedic preparations that contain self-generated alcohol content and herbal extracts soluble in both water as well as alcohol.

Asava are ayurvedic medicines, which are prepared with natural fermentation process using herbs, water and sugar. Almost all Asava medicines do not include preparation of decoctions, but it has a very few exceptions.

Arishta are type of ayurvedic medicines, which are prepared with natural fermentation process using herbal decoctions, Dhataki (Woodfordia Fruticosa) flowers and sugar. Arishta means to have a long shelf life.

REQUIREMENTS:

Apparatus: The apparatus consists of a round- b ottomed flask fitted with a distillation head with a steam trap and attached to a vertical condenser. A tube is fitted to the lower part of the condenser and carries the distillate in to t he lower pail of a l00-ml or 250-ml volumetric flask. The volumetric flask is immersed in a beaker. Containing a mixture of ice and water during the distillation, A disc with a circular aperture, 6 cm in diameter, is placed under the distillation flask to reduce the risk of charring of any dissolved substances. ,

The GC system was used in the present study. The detection was performed by means of FID. Separation was achieved using a packed column with the dimensions of 2 m length and 3.170 mm diameter. Stationary material was made up of WHP having 0.14 mm in mesh size range. The column used was packed, carrier gas was nitrogen, detector used was FID and the data were acquired through Total Chrome Navigator software (version 6.3.10504). This developmental work involved the use of various simple laboratory chemicals of HPLC grade including ethanol, DMSO, benzene, toluene (Merck) and water (Double distilled). Samples of Khadirarishta, Drakshkumari and Saraswatarishta were purchased from Ayurvedic stores.

PROCEDURE:

There are 2 methods for determination of alcohol content in asava and arishta

Method 1

Transfer 25 ml of the preparation being examined, accurately measured at 24.9° to 25.1°C, to the distillation flask. Dilute with 150ml of water and add a little pumice powder. Attach the distillation head and condenser. Distil and ceiled not less than 90ml of the distillate into a 100-ml volumetric flask. Adjust the temperature to 24.9° to 25.1nCand dilute to volume with distilled water al 24.9 ° to 25.1 ° C. Determine the specific gravity at 25 ° C, read off percentage of ethyl alcohol corresponding to the specific gravity from the table.

If the specific gravity is found to be between two values, the percentage of ethanol should he obtained by interpolation. After calculation of the ethanol content, report the result to one decimal place.

Method 2

Preparation of standard

Toluene was selected as standard and sample diluent based on its ability to dissolve wide variety of substances and high boiling point that does not interfere with more volatile solvents tested by GC for method involving analysis of high boiling point solvents. Standard stock of ethanol was prepared by diluting with toluene in 10 ml volumetric flask.

Preparation of sample

Accurately weighed 1.2 ml of Khadirarishta, Drakshkumari and Saraswatarishta sample was transferred in 10 ml volumetric flask and volume was made up with toluene. The supernatant obtained contained alcohol in extracted form and was injected in the chromatographic system. Gas chromatographic conditions The experimental conditions used were: 0.01 µl volume of either standard or sample solutions was injected in GC injection port maintained at 150°C. Nitrogen was used as a carrier gas at the flow rate of 25 ml/min. Temperature of detector was set at 200°C with temperature gradient maintained initially at 85°C for 10 min. Further, trails were carried out to optimize the final method for estimation of alcohol content.

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are asava?

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Q.-2. Write note on arishta?

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Q.-3. Write classification of dosage form in ayurveda?

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Q.-4. Write test for asava?

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Q.-5. Write test for arishta?

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Experiment No:- 03

OBJECT:

To prepare and evaluate herbal cream.

REFERENCES:

1. Deore S.L., Khadabadi S.S., and Baviskar B.A., "Pharmacognosy and Phytochemistry", Pharmamed Press, 1st Edition, 2014, Page no. 617

THEORY:

Herbal cosmetics are the preparation which represent cosmetics associated with active bio-ingredients, neutraceuticals and pharmaceuticals.

Cosmetics are products that are used to cleanse and beautify the skin. The first recorded use of cosmetics is attributed to Egyptians in 4000 B.C. Pharmaceuticals are essentially drug products and are defined as products that prevent, mitigate, treat or cure disease and affect the structure or function of the body.

REQUIREMENTS:

Herbs, water, sweetner and alcohol.

PROCEDURE:

Preparation of extracts: Air dried and coarsely powdered (100gm) of crude drug placed in Soxhlet extractor separately and run for 6 hours at 500 c using distilled water. The extracts were as a solvent filtered and concentrated to dryness.

Preparation of extracts

S.No.	Herbal extract	Quantity (%)
1	Neem extract	1
2	Aloe juice	2
3	Haldi extract	0.5
4	Amba haldi extract	0.5
5	Khas	1
6	Rose water	0.5

Preparation of cream base

Oil in water (O/W) emulsion-based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, almond oil) were dissolved in the oil phase (Part A) and heated to 75° C. The preservatives and other water soluble components (Methyl paraban, Propyl paraban, Triethanolamine, Propylene glycol) were dissolved in the aqueous phase (Part B) and heated to

75° C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place.

S.No	Ingredients	F1	F2
1	Stearic acid	18	16
2	Cetyl alcohol	3	4
3	Castor oil	4	4
4	Glycerol	3	3
5	Methly paraben	0.02	0.02
6	Triethanolamine	qs	qs
7	Water	(qs)	100

Drug formulation

The appropriate base was selected and two different creams were formulated. The emulsifier (stearic acid) and other oil soluble components (Cetyl alcohol, almond oil) were dissolved in the oil phase (Part A) and heated to 75° C. The preservatives and other water soluble components (Methyl paraban, Propyl paraban, Triethanolamine, Propylene glycol, all extracts) were dissolved in the aqueous phase (Part B) and heated to 75° C. After heating, the aqueous phase was added in portions to the oil phase with continuous stirring until cooling of emulsifier took place.

Evaluation parameter

- **pH of the Cream**: The pH meter was calibrated using standard buffer solution. About 0.5g of the cream was weighed and dissolved in 50.0 ml of distilled water and its pH was measured.
- **Viscosity**: Viscosity of the formulation was determined by Brookfield Viscometer at 100 rpm, using spindle no.4.
- **Homogeneity** The formulations were tested for the homogeneity by visual appearance and touch.
- **Appearance** The appearance of the cream was judged by its colour, pearlscence and roughness and graded.
- **After feel** Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.
- **Type of smear** :After application of cream, the type of film or smear formed on the skin were checked.
- **Removal:** The ease of removal of the cream applied was examined by washing the applied part with tap water.
- **Saponification value** Introduce about 2 gm of substance refluxed with 25 ml of 0.5 N alcoholic KOH for 30 minutes, to this 1 ml of phenolphthalein added and titrated immediately, with 0.5 N HCL.

  Saponification value = (b-a)×28.05/w

  a - volume in ml of titrant,

b - Volume in ml of titrant,

w - Weigh of substance in gm

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are herbal cream?

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Q.-2. What are advantage of herbal cream?

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Q.-3. Write evaluation parameter of herbal cream?

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Q.-4. What are herbal lotion?

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Q.-5. What are herbal shampoo?

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Experiment No:- 04

OBJECT:

To prepare and evaluate herbal syrup.

REFERENCES:

1. Deore S.L., Khadabadi S.S., and Baviskar B.A., "Pharmacognosy and Phytochemistry", Pharmamed Press, 1st Edition, 2014, Page no. 582

THEORY:

Herbal Syrups offer powerful and concentrated medicine and nutrition. Syrups are concentrated infusions ordecoctions of herbs in a food-like medium, such as molasses, honey, vinegar and honey, black cherry concentrate. Syrups can be taken in a tonic fashion for their nutritive and preventative qualities in doses of 1-2 Tablespoons a day. When taken with food, you optimize your assimilation of herbal nutrients. Or syrups can be taken as needed for specific complaints. Syrups are pleasant to take, which can make them especially useful for children.

REQUIREMENTS:

Herbs, water, sweetner and alcohol.

PROCEDURE:

Methods for Syrup Making

1**. Create a strong tea by decocting or infusing the herbs:**A strong tea is the basis of your syrup. This can be accomplished by long slow simmering (infusion), a method which works well for things such as Elderberries, other berries and fruits or leafy material. With roots and barks you can pour boiling water over the herbal material and set aside overnight (decoction). In the morning you can drain the decoction

from the herbs and add the liquid to the pot and proceed. Alternatively you can gently simmer hard herbal material for a longer time.

2. Reduce volume:After you have strained and discarded the herbal material you will simmer the infusion/decoction and concentrate the mixture as desired.

3. Add sweetener and supplemental ingredients: Cook gently until well combined. Simmer for a

longer time to reduce volume if desired.

4. When cool, add alcohol if desired.

5. Bottle and refrigerate

EVALUATION PARAMETER

Color examination:

5 ml final syrup was taken into watch glasses and placed against white back ground in white tube light. It was observed for its color by naked eye.

Odor examination:

2 ml of final syrup was smelled individually. The time interval among 2 smelling was kept 2 minutes to nullify the effect of previous smelling.

Taste examination:

A pinch of final syrup was taken and examined for its taste on taste buds of the tongue.

Determination of pH:

Placed an accurately measured amount 10 ml of the final syrup in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

Determination of Viscosity:

The viscosity of syrup can be determined by Ostwald 's U-tube Viscometer.

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are herbal syrup?

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Q.-2. What are advantage of herbal syrup?

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Q.-3. Write evaluation parameter of herbal syrup?

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Q.-4. What are herbal mixture?

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Q.-5. What are herbal tablet?

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Experiment No:- 05

OBJECT:

To determine monograph analysis of herbal drug from pharmacopoeias.

REFERENCES:

'Indian Pharmacopoeia', Vol I, page no 19

THEORY:

Pharmacopoeia is an authoritative book containing a list of medicinal drugs with their uses, preparation, dosages, formulas, etc.

The herbal drug standardization is indeed bustle in Ayurveda. There are many contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental function. here are many challenges in front of Ayurveda drug industry which are raw drug standardization, product analysis, along with drug safety issues which include heavy metal contain, negative criticism of Ayurvedic formulations and increased toxicity reports. Hence, to tackle these issues there is need to develop evaluative data by using sophisticated modern techniques of standardization of Ayurvedic formulations.

REQUIREMENTS:

acacia, acetic acid, lead acetate solution, Porcelain crucible (50mL), Muffle furnace (600±20), weighing machine, desiccators

PROCEDURE:

Acacia

Gum Acacia; Indian Gum

Acacia is the air-hardened, gummy exudation from the stem and branches of Acacia nilotica (Linn.) Del. subsp. indica (Benth.) Brenan (syn. A. arabica Willd. var. indica Benth.) (Fam. Leguminosae), or other species of Acacia.

It is available as pieces (tears) or in the form of a powder.

Description:

Tears — Irregular and broken pieces of varying size, yellowishwhite**,** yellow or amber in colour, with numerous minute fissures; brittle fractured surface, glassy and occasionally iridescent; odourless.

Powder — A white or yellowish-white powder; odourless; on treatment with water it dissolves to give a mucilaginous liquid which is colourless or yellowish, dense, viscous, adhesive and translucent.

Identification:

A. An aqueous solution is gelatinised by the addition of lead subacetate solution.

B. To 5 ml of a 10 per cent w/v solution add gradually, while shaking, 10 ml of ethanol (95 per cent). The cloudy liquid, on addition of 0.5 ml of acetic acid, gives a white precipitate.

Filter and add to the clear filtrate 50 ml of ammonium oxalate solution; the filtrate becomes cloudy.

C. A 10 per cent w/v solution is either dextro-rotatory or slightly laevo-rotatory.

Tests:

Sterculia gum and agar: To 50 mg of the powdered substance under examination add 0.2 ml of freshly prepared ruthenium red solution and examine microscopically; the particles do not acquire a red colour after irrigation with water.

Agar and tragacanth: To 10 ml of a 10 per cent w/v solution add 0.2 ml of lead acetate solution; no precipitate is produced.

Starch and dextrin: Boil 10 ml of a 10 per cent w/v solution and cool, add 0.1 ml of 0.05 M iodine; no blue or brown colour is produced.

Tannins.: To 10 ml of a 10 per cent w/v solution add 0.1 ml of ferric chloride test solution; a gelatinous precipitate is formed, but neither the precipitate nor the liquid shows a dark blue colour.

Sucrose and fructose: To 1 ml of a 10 per cent w/v solution add 4 ml of water, 0.1 g of resorcinol and 2 ml of hydrochloric acid and heat on a water-bath; no yellow or pink colour develops.

Water-insoluble matter: Dissolve 5 g, in fine powder, in 100 ml of water in a 250-ml flask, add 10 ml of dilute hydrochloric acid and boil gently for 15 minutes. Filter by suction while hot through a sintered-glass crucible, previously tared, wash thoroughly with hot water, dry at 105º and weigh; the residue does not exceed 50 mg.

Microbial contamination : 1.0 g is free from Escherichia coli.

Sulphated ash : Not more than 5.0 per cent.

Acid-insoluble ash :

Determination of Total Ash Value

Weigh accurately 2gm of feed sample prepared in a tarred crucible.

Char at low temp. first and then incinerate the material in a muffle furnace for 4 hrs or more until free from all carbonaceous materials and ash is white or grayish white.

Cool the crucible and ash partly on asbestos sheet and then in a desiccators and weight.

Repeat the process of ignition in the muffle furnace cooling and weighing at half an hour interval until the different between two successive weighing is less than 1mg.

Note the lowest weight.

Calculation

Total ash % by weight = 100x (W2-W)/W1

Where, W2= weight in gram of the crucible with ash

W1=weight in gram of the sample taken

W=weight in gram of the empty crucible

Acid Insoluble Ash:

To the ash obtained in the total ash determination, add 2mL of HCl and heat on asbestos sheet for 10min.

Allow to cool and filter the contents of the crucible through Whatman no. 42 filter paper or its equivalent.

Wash the residue and the crucible with water until the washings are free from acid.

Return the residue to the crucible and collected the filtrate in a volumetric flask for mineral estimation. Keep in an electric air-oven maintained at 135 ±2°C for about 3hrs.

Ignite in a muffle furnace at 500±20 for 3hrs and cool the dish in desiccators and weigh.

Return the process of ignition in the muffle furnace, cooling and weighing at half an hour interval until the different between two successive weighing is less than 1mg.

Note the lowest weight.

Calculation

Acid insoluble ash, % by weight= 100x (W2-W)/W1

Where, W2= weight in gram of the crucible and acid insoluble ash

W1=weight in gram of the sample taken for analysis

W=weight in gram of the empty crucible

Not more than 1.0 per cent, determined on 1.0 g by Method C.

Loss on drying:

Procedure Dry a weighing bottle for about 30 minutes under the prescribed conditions, allow to cool it in a desiccator if heated, and weigh it accurately. If the sample is large crystals or lumps, promptly crush it into particles not larger than about 2 mm in diameter and, unless otherwise specified, place 1 to 2 g into the weighing bottle, spread the sample so that the layer is not thicker than 5 mm, and weigh it accurately. Place the bottle in the drying oven, remove the stopper (placing it nearby), dry under the specified conditions, stopper again, take the bottle out of the oven, and weigh it again. If heated, unless otherwise specified, allow to cool it in a desiccator, and weigh it accurately. If the sample melts at a temperature lower than the specified drying temperature, dry it at a temperature 5-10 lower than the

melting temperature for 1 to 2 hours, and dry it under the specified conditions Not more than 15.0 per cent, determined on 1.0 g by drying in an oven at 105º.

Storage: Store protected from heat, moisture and against attack by insects and rodents**.**

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are pharmacopoeia?

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Q.-2. What is monograph?

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Q.-3. Write is ash value?

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Q.-4. What is acid insoluble ash?

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Q.-5. What is loss on drying?

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Experiment No:- 06

OBJECT:

To determine the aldehyde content in given crude drug.

REFERENCES:

1. Kokate C.K., " Practical Pharmacognosy", 4rth edition, 2002, Vallabh prakashan, page no. 133.

THEORY:

Volatile oil is distilled oil, especially one obtained from plant tissue, as distinguished from glyceride oils by their volatility and failure to saponify.

A rapidly evaporating oil of plant derivation, especially an essential oil,that is capable of distillation and that does not leave a stain. Also called ethereal oil.

REQUIREMENTS:

Citronella oil , hydroxylamine hydrochloride solution,

PROCEDURE:

" 2 grms. Of citronella oil are carefully weighed into a flash, and to these are added 20 c.c. of a 5 per cent. solution of hydroxylamine hydrochloride in 80 per cent alcohol, made neutral to methyl orange. On the addition of the hydroxylamine hydrochloride solution to the oil the yellow colour of the methyl orange becomes pink, and N/2 alcoholic alkali is then added, drop by drop, with constant shaking, until the pink colour no longer returns, and a permanent yellow shade is established."

The percentage of citronellal is then calculated by the following formula:

$$\text{Per cent. of citronellal} = \frac{100 \times 0.077 \times \text{c.c. N/2 alkali}}{\text{Weight of oil taken}}$$

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are vol oil?

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Q.-2. Write test for vol oil?

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Q.-3. Write classification of vol oil?

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Q.-4. Define aldehyde vol oil ?

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Q.-5. Write about isolation of vol oil?

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Experiment No:- 07

OBJECT:

To determine the phenol content in given crude drug.

REFERENCES:

1. Evans W.C., " Pharmacognosy", fifteenth edition, 2008, Elsevier Health Sciences Education, Noida, page no. 299-302.

THEORY:

A class of chemical compounds in organic chemistry which consist of a hydroxyl group (-OH) directly bonded to an aromatic hydrocarbon group is known as phenols or phenolics. The first member of this class is phenol (C6H5OH) commonly called as carbolic acid. All other members of phenolic family are known as derivatives of phenol.

Generally phenolic compounds have strong antiseptic and antibacterial properties and act as nerve stimulants and immunostimulants. They can cause hepatotoxicity as well as irritating for the skin. Phenolic compounds are basically involving plant metabolic system and widely spread throughout the plant kingdom. Phenolic compounds have potential against oxidative damages diseases, therefore play a protective role through ingestion of fruits and vegetables. These compounds are very much essential for the growth of plant and involve in reproduction process of plants. These compounds produced during the response process against pathogens for defending injured plants. Because of their antioxidant activities, they widely used in processed foods as a natural antioxidant.

REQUIREMENTS:

UV-Spectrophotometer, sample drug, Soxhlet apparatus, Folin–Ciocalteu reagent.

PROCEDURE:

Plant material and preparation of extract

Roots of plant were collected. The roots were dried in shade at room temperature, then chopped and ground to a fine powder in a mechanical blender. Dried root powder (20 g) was packed into a Soxhlet apparatus and extracted with 300 mL methanol at 60–65 ◦C for 3–4 h. The extract was filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure at 40 ◦C. The extract was dried, weighed (2.6 g) and stored at 4 ◦C in storage vials for experimental use.

Total phenolic content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method. Briefly, 200L of crude extract (1 mg/mL) were made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate. The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 650 nm. The total phenolic contentwas calculated fromthe calibration curve, and the results were expressed as mg of

gallic acid equivalent per g dry weight.

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are crude drug?

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Q.-2. What is Folin–Ciocalteu reagent?

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Q.-3. Write the chemical test for secondary metabolites?

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Q.-4. What are phenolic compound ?

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Q.-5. Write about isolation methods for secondary metabolites?

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Experiment No:- 08

OBJECT:

To determine the total alkaloids in herbal drug.

REFERENCES:

Tailing M. and Sharma A., "Phytochemistry theory and practical", first edition, 2008-09, Birla publications, Delhi, page no. 200-201.

THEORY:

The herbal drug standardization is indeed bustle in Ayurveda. There are many contradictory theories on the subject of herbal medicines and their relationship with human physiology and mental function. here are many challenges in front of Ayurveda drug industry which are raw drug standardization, product analysis, along with drug safety issues which include heavy metal contain, negative criticism of Ayurvedic formulations and increased toxicity reports. Hence, to tackle these issues there is need to develop evaluative data by using sophisticated modern techniques of standardization of Ayurvedic formulations.

The Alkaloids are naturally occurring nitrogen-containing pharmacologically active organic compounds present in plant kingdom. These have made major impact on plant medicine because of its vast application. A research in 1985 suggested that there are more than 140 Angiospermic plant families and 20,000 genuses are rich with alkaloids

REQUIREMENTS:

UV-Spectrophotometer, Chitrakadivati, Dragendroff's reagent.

PROCEDURE:

Preparation of Chitrakadivati

The Churna (powder) was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients (except Citrus limon (Linn.) Burm) were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformly blended churna. 500 mg Vati (tablets) was prepared by adding quantity sufficient of Citrus limon (Linn.) Burm. Juice, then dried and stored in air tight bottle.

Extraction

The 100 gm of each plant material was ground and then extracted with methanol for 24 hours in a continuous extraction (Soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness.

Qualitative estimation (Test for alkaloids)

Presence of alkaloid was confirmed by Dragendroff's method. A part of extract was dissolved in dilute HCL and 2 drops of Dragon drop's was added, a crystalline precipitate indicates presence of alkaloid. The sample

which showed positive alkaloid was then subjected to further quantitative evaluation.

Preparation of reagents

1. Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water.

2. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na2HPO4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water).

3. Atropine standard solution was made by dissolving 1 mg of pure Atropine (AR-grade procured from Sigma Company) in 10 ml distilled water.

Seperation of Alkaloid

A part of extract residue was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

Preparation of standard curve

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform.

The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU UV-1800) against the blank prepared as above but without Atropine.

RESULT AND DISCUSSION:

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CONCLUSION:

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VIVA QUESTIONS

Q.-1. What are herbal drug?

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Q.-2. What is dragondroff reagent?

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Q.-3. Write the chemical test for alkaloids?

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Q.-4. What are alkaloides?

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Q.-5. Write about pseudo alkaloids?

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