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It is our hope and expectation that this lab manual will provide an effective learning experience and refenced resource for the VI semester B. Pharm students studying Pharmacy in accordance with the syllabus of Pharmacy Council of India. This book is written in a simple, comprehensive and illustrative language for proper understanding of the subject. The manual is designed to provide students with hands-on experience in various laboratory techniques related to herbal drug technology, including extraction, phytochemical evaluation, quality control, and formulation aspects of herbal drugs.

This manual will serve as a practical guide for students to correlate theoretical concepts with laboratory work and to enhance their competence in the field of herbal drug technology. We hope that this laboratory manual will help students gain confidence and foster scientific curiosity, enabling them to meet the professional challenges of the pharmaceutical and herbal industry. The manual will be useful for the students to get a sure success in the examinations.

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HERBAL DRUG TECHNOLOGY LAB MANUAL

-As per PCI syllabus

-VI Semester B. Pharm

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Dr. Malarkodi Velraj
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As per PCI syllabus

VI Semester B. Pharm

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Dr. Malarkodi velraj

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Dr. P. Shanmugasundaram

LIST OF EXPERIMENTS

S. No.	Experiment
1.	Preliminary phytochemical screening of crude drug
2.	Evaluation of Excipients of Natural Origin
3.	Preparation and Evaluation of Herbal Lotion
4.	Preparation and Evaluation of Herbal Shampoo
5.	Preparation and Evaluation of Herbal Cream
6.	Preparation and Evaluation of Herbal Syrup
7.	Preparation and Evaluation of Herbal mixture
8.	Preparation and Evaluation of Herbal Tablets
9.	Monograph analysis of herbal drugs from recent Pharmacopoeia
10.	Determination of the alcohol content of Asava/Arista
11.	Determination of Aldehyde content
12.	Determination of Phenol content
13.	Determination of total Alkaloids

SAFETY IN THE LABORATORY (DO'S AND DON'TS)

The following precautions and care should be taken while working in the practical laboratory:

1. The students should be well aware of the exercise they are going to perform in the laboratory.
2. The instruments, glassware and any other equipment should be kept clean at its proper place before and after its use.
3. The microscope, reagents and other delicate instruments should be handled gently and properly and should be kept atleast 5 inches from the edge of the table to avoid it knocking off accidently.
4. Handle the inflammable solvents with extra care and do not bring them close to the flame.
5. Do not throw any broken glassware in the sink. It should be thrown in the dust bin.
6. Whenever working with the sharp instrument as blade/scalpel etc, be careful not to cut or puncture your skin.
7. Do not inhale, never taste or apply stain or any chemical as it may harm.
8. Never eat in the laboratory to avoid infection.

IDENTIFICATION OF PHYTOCOMPOUNDS IN THE GIVEN CRUDE EXTRACT BY PRELIMINARY PHYTOCHEMICAL SCREENING

Aim

To identify the phytoconstituents present in the herbal extract by preliminary phytochemical analysis.

Theory

Phytochemicals (from Greek *phyto*, meaning "plant") are chemicals produced by plants through primary or secondary metabolism. Phytochemicals are basically divided into two groups, viz., primary and secondary metabolites according to their functions in plant metabolism. **Primary metabolites** are organic compounds produced by living organisms that are directly involved in normal growth, physiological development and reproduction of the organism. Primary metabolites are widely distributed in nature and are needed for the survival of the organism. **Secondary metabolites** are organic compound, biosynthetically derived from primary metabolites but are more limited in distribution, usually being restricted to a taxonomic group. Primary metabolites comprise of carbohydrates, proteins and fats, while secondary metabolites consist of alkaloids, phenolic compounds, flavonoids, tannins, glycosides, terpenoids, saponins, and so on.

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently may lead to drug discovery and development. In this experiment, the given crude extract is subjected to a series of specific chemical tests that produce characteristic color changes or precipitates in the presence of particular phytoconstituents. Each test is based on the chemical reaction between the functional groups of the phytochemicals and the added reagents. The appearance of distinct colors, froth formation, or precipitates serves as an indicator of the presence or absence of the corresponding phytocompound.

Extraction of crude drug

Extraction of medicinal plants is a process of separating active plant materials or secondary metabolites such as alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides from inert or inactive material using an appropriate solvent and standard extraction procedure.

Several methods were used in the extraction of medicinal plants such as maceration, infusion, decoction, percolation, digestion, hot continuous extraction, counter current extraction, supercritical fluid extraction, ultrasound-assisted, and microwave-assisted extraction.

In general, polar solvents such as water, methanol, and ethanol are used in the extraction of polar compound, whereas nonpolar solvents such as hexane and dichloromethane are used in the extraction of nonpolar compounds. The following are 11 various solvents of extractions arranged according to the order of increasing polarity.

S. No.	Solvents	Polarity
1.	*n*-Hexane	0.009
2.	Petroleum ether	0.117
3.	Diethyl ether	0.117
4.	Ethyl acetate	0.228

5.	Chloroform	0.259
6.	Dichloromethane	0.309
7.	Acetone	0.355
8.	*n*-Butanol	0.586
9.	Ethanol	0.654
10.	Methanol	0.762
11.	Water	1.000

Successive solvent extraction: About 50gm of the air-dried powdered plant material was extracted successively with the non-polar and polar solvents in a Soxhlet extractor. Each time before extracting with the next solvent, the powdered material was dried in air-oven below 50^{o}C.

Aqueous/Alcohol extraction

The powdered drug was macerated with the water/ethanol for 24 hours to obtain the aqueous/alcohol extract. Each extract was concentrated by distilling off the solvent and then evaporated to dryness on the water bath. The extract obtained with each solvent was weighed and calculated its percentage in terms of the air-dried weight of the plant material. Also, the consistency of the extract was noted. The extracts obtained as above were then subjected to qualitative tests for the identification of various plant constituents as per following procedure.

Chemical Test

S. No.	Name of Test	Procedure	Observation	Inference
TEST FOR CARBOHYDRATE				
1	**Molish's Test (General test)**	To 2-3ml of the test solution, few drops of molish's reagent was added (α-naphthol solution in alcohol) and shaken. Conc. Sulphuric acid was added from sides of test tube.	Violet ring appeared at the junction of two liquids.	Presence of Carbohydrate.
2	**Fehling's Test**	To 1ml of the test solution, added equal quantities of Fehling solution A & B and heat.	Formation of brick red precipitate (Cuprous oxide)	Presence of reducing sugar.
3	**Iodine Test**	To the 3ml of test solution few drops of iodine solution was added.	Blue color appeared which disappeared on boiling and reappeared on	Presence of polysaccharide (Amylase)

			cooling.	
4	**Benedict's Test**	To 5ml of Benedict's solution, added 1ml of the test solution and shaken. The test tube containing the above sample was placed in a boiling water bath and heated for 3 minutes and cooled.	Formation of green-red or yellow precipitate	Presence of reducing sugar
5	**Barfoed's test**	Added equal volume of barfoed's reagent and test solution and heated for 1-2 min in boiling water bath and cooled.	Appearance of Red Precipitate	Presence of Carbonyl group in Monosaccharide
TEST FOR PROTEINS				
1	**Biuret Test (General test)**	To 3ml test solution, added 4% NaOH and few drops of 1% $CuSO_4$ solution.	Appearance of violet or pink colour	Presence of Proteins (Peptide bonds).
2	**Millon's Test**	To 2 ml of extract, added few drops of Millon's reagent	White precipitate turned red on heating indicates the presence of proteins.	Presence of Proteins.
3	**Xanthoproteic Test**	To test solution, added 1 ml of Con. Nitric acid along the sides of the tube	Appearance of yellow colour	Presence of Proteins (Aromatic Amino acids).
4	**Ninhydrin Test**	To about 3ml test solution, added 3 drops 5% Ninhydrin solution and heated in a boiling water bath for 10min.	Appearance of purple or bluish colour	Presence of Proteins (Amino acids).
TEST FOR FIXED OILS AND FAT				
1	**Solubility Test**	To the extract, added ether or benzene or chloroform or ethanol or water.	Soluble in ether, benzene, chloroform and insoluble in ethanol and water.	Presence of Fats and oils

2	**Stain Test**	Little quantity of plant extract was pressed in between two filter papers	Oil stain on the paper	Presence of Fats and oils
3	**Saponification Test**	To the extract, added few drops of 0.5N alcoholic KOH and a drop of phenolphthalein and heated on a water bath for 2hr.	Soap formation on partial neutralization of alkali	Presence of Fats and oils
4	**Sudan Red Test**	A thin section of drug was placed on glass slide. A drop of Sudan red III reagent was added. After 2 min. washed with 50% alcohol. Mounted in glycerin and observed under microscope.	Oil globules appear in red colour	Presence of Fats and oils
TEST FOR ALKALOIDS				
1	**Mayer's Test**	To the extract, added few drops of Mayer's regent (potassium mercuric iodide solution).	Appearance of cream-colored precipitate.	Presence of alkaloids.
2	**Dragendorff's Test**	To the extract, added few drops of dragendorff's reagent (potassium bismuth iodide solution).	Appearance of orange-brown precipitate.	Presence of alkaloids.
3	**Hager's Test**	To the extract, added few drops of Hager's reagent (saturated aqueous solution of picric acid).	Appearance of yellow-colored precipitate.	Presence of alkaloids.
4	**Wagner's Test**	To the extract, added few drops of Wagner's reagent (iodine in potassium iodide).	Appearance of reddish-brown color precipitate.	Presence of alkaloids.
TEST FOR SAPONINS				
1	**Foam Test**	The extract was shaken with few ml of distilled water vigorously.	Appearance of persistent foam.	Presence of Saponins.
2	**Emulsion Test**	The froth from the above reaction was taken and few drops of olive oil was added and shaken vigorously.	Formation of emulsion	Presence of Saponins.
TEST FOR STEROIDS AND TRITERPENOID TESTS				

1	**Salkowski Test**	Dissolved the extract in chloroform and added few drops of Con. Sulphuric acid to the side of the test tube and allowed to stand for some time.	The Appearance of red color in the lower layer. The Appearance of yellow color in lower layer.	Presence of Steroids. Presence of triterpenoids.
2	**Libermann Burchard Test**	To the extract, added 1 ml of glacial acetic acid, 1 ml of acetic anhydride and 1-2 drops of conc. H_2SO_4 (along the side of test tube)	The solution becomes red, then blue and finally bluish green.	Presence of Steroids.
3	**Noller's Test**	To 1 ml of extract, added tin (one bit), thionyl chloride and heated in a water bath.	Appearance of pink color	Presence of triterpenoids.
4	**Hirshonn reaction**	The extract was heated with trichloro acetic acid	Appearance of red to purple color	Presence of triterpenoids
TEST FOR GLYCOSIDES				
General test: To the pinch of extract in a watch glass, 2 drops of alcohol was added and dissolved the extract. Equal quantity of anthrone was added, mixed thoroughly and dried. Then added one drop of concentrated sulphuric acid, spreaded in a thin film with a glass rod in a watch glass, heated over the water bath. Formation of dark green colour confirms the presence of glycosides.				
1	**Borntrager's Test**	To about 3 ml extract, added dilute sulphuric acid, boiled and filtered. To the filtrate added equal volume of benzene or chloroform. To the organic layer (benzene or chloroform), ammonia solution was added.	Ammonical layer turns pink.	Presence of anthraquinone glycoside
2	**Keller-killiani Test**	To the extract, added glacial acetic acid, one drop 5% ferric chloride solution and conc. H_2SO_4	Appearance of two layer occur simultaneously, lower layer of reddish brown color	Presence of deoxy sugars

			and upper layer of bluish green color	
3.	**Legal test**	To a pyridine extract of the sample, added an alkaline solution of sodium nitroprusside	Formation of pink or red	Presence cardiac glycoside (aglycone)
4.	**Baljet test**	To the extract, added sodium picrate solution	Formation of a yellow to orange color	Presence cardiac glycoside (aglycone)
3	**Sodium Picrate Test**	A strip of filter paper was dipped in 10% aqueous solution of picric acid. Drained and re-dip in 10% sodium carbonate solution and drained again. The above paper (sodium picrate paper) was placed on the mouth of flask containing aqueous solution of the extract.	Hydrocyanic acid vapours from the extract turns the paper brick red or maroon-colour.	Presence of cyanogenetic glycoside
4	**Vanillin Test**	To alcoholic solution of extract added Vanillin HCl solution.	Appearance of pink colour	Presence flavonoid glycoside
5	**Ammonia vapour Test**	A filter paper was dipped in the alcoholic solution of the extract and exposed the paper to ammonia vapour.	Formation of Yellow spot on the filter paper	Presence flavonoid glycoside
TEST FOR PHENOLIC COMPOUNDS				
1	**Ferric chloride Test**	To the aqueous solution of the extract, added few drops 5% ferric chloride solution.	Appearance of dark green/bluish black color.	Presence of phenolic compound
2	**Gelatin Test**	To the extract, added few ml of 1% gelatin solution containing 10% sodium chloride.	Appearance of the white precipitate.	Presence of phenolic compound

3	**Lead acetate Test**	About few ml of 10% lead acetate solution was added to the test solution.	Appearance of a voluminous white precipitate.	Presence of phenolic compound
4	**Iodine Test**	To the extract, added few drops of dil. Iodine solution.	Appearance of a transient red colour.	Presence of phenolic compound
TEST FOR FLAVONOIDS				
1	**Ferric chloride Test**	To the aqueous solution of the extract, added few drops 10% ferric chloride solution.	Appearance of green color.	Presence of flavonoids
2	**Shinoda Test**	To the alcoholic solution of extract, added a small piece of magnesium turnings and few drops of Concentrated hydrochloric acid.	Appearance of pink color.	Presence of flavonoids
3	**Lead acetate Test**	To small quantity of extract added 10% lead acetate solution.	Appearance of yellow color precipitate.	Presence of flavonoids
4	**Ammonia Test**	To the extract, added 5 ml of dil. ammonia solution and conc. H_2SO_4	Appearance of yellow color	Presence of flavonoids
5	**Acid Test**	To the small quantity of test solution, added few drops of concentrated sulphuric acid.	Appearance of yellow orange colour.	Presence of flavonoids
6	**Sodium Hydroxide Test**	To the extract, sodium hydroxide was added.	Formation of yellow color, which decolorize on adding acid.	Presence of flavones
TEST FOR TANNINS				
1	**Ferric Chloride Test**	To the extract, added ferric chloride solution.	Appearance of dark blue or greenish black color.	Presence of tannins
2	**Gelatin Test**	To the extract, few ml of 1% gelatin solution containing 10% sodium chloride was added.	Appearance of the white precipitate.	Presence of tannins

3	**Lead Acetate Test**	Few ml of 10% lead acetate solution was added to the test solution.	Appearance of a voluminous white precipitate.	Presence of tannins
TEST FOR GUM AND MUCILAGE				
1	**Alcohol Test**	To the aqueous solution of the extract added few drops of alcohol.	Formation of white precipitate	Presence of gum
2	**Ruthenium red Test**	To the aqueous solution of the extract added ruthenium red.	Appearance of red color.	Presence of mucilage
TEST FOR MISCELLANEOUS COMPOUNDS				
1	**Test for Resin**	To the extract, added acetone, small amount of water and shaken.	Appearance of turbidity	Presence of resin
2	**Test for Quinone**	To the extract, added 1 ml of conc. H_2SO_4	Appearance of red color.	Presence of quinone
3	**Test for Coumarin**	To 1 ml of extract, 3 ml of NH_4OH and 2 ml of benzene was added.	Appearance of red color.	Presence of coumarin
4	**Test for Anthocyanin**	To the extract, 1ml of 2N sodium hydroxide was added and heated for 5 mins.	Appearance of bluish green color	Presence of anthocyanin

Report

Based on the above observations the given herbal extract showed the presence of the following phytoconstituents ----------.

EVALUATION OF EXCIPIENTS OF NATURAL ORIGIN

Aim

To evaluate the excipients of natural origin.

Theory

Excipients are defined as 'the inert and non-active ingredients that are mixed with therapeutically active compound(s) in the manufacture of various pharmaceutical dosage forms such as tablets, capsules, syrups, creams, lotions, shampoos, etc. They are mixed with the active component to make up the volume or improve their stability or mask the bitter taste or improve the appearance, odour and other characteristics of the dosage forms. The Herbal or natural excipients have a great advantage over their synthetic analogues as these are non-toxic, less expensive, bio degradable, bio compatible and easily available.

Excipients used in different dosage form

Excipient	Function	Examples
Diluents	To act as bulking agent and filling material.	Lactose, starch, mannitol, sucrose
Binding agent	Bind the tablet ingredients together giving form and mechanical strength for tableting.	Acacia, gelatin, tragacanth, starch
Disintegrants	To facilitate the breakup of tablet the in gastrointestinal tract.	Compounds which swell or dissolve in water e.g. starch, cellulose derivatives, Isapgol husk and alginates
Glidants	To improve powder flow**,** minimize friction between particles, and enhance manufacturing efficiency	Rice starch, Purified talc
Lubricants	To reduce the friction between the granules and die wall during compression and ejection of tableting process.	Rice bran oil, Castor oil, Cocoa butter
Viscosity builders	These are the aqueous solutions to increase its viscosity without altering properties	Pectin, tragacanth, cellulose, guar gum, gelatin
Tablet coatings and films	Protect tablet from the environment (air, light and moisture), increase the mechanical strength, mask taste and odour.	Sugar (sucrose) has now been replaced by film coating using natural or synthetic polymers. Shellac (natural resin), Pectin,
Colouring agents	Improve acceptability to patients, aid identification and prevent counterfeiting. Increase stability of light-sensitive drugs.	Henna, chlorophyll, caramel, amaranth, indigo, Saffron, Cochineal beetles, Beetroot
Sweetening agents	These are the substances which are added	Glycyrrhiza, honey, stevia

	to drug formulation to mask bitter taste to improve patient acceptance	
Flavouring agent	To improve the taste and palatability of oral pharmaceutical dosage forms such as syrups, chewable tablets, lozenges, and pediatric formulations	Cardamom, vanilla, lemon oil, orange oil, Mentha oil, fruit essence
Perfumes	Perfumes are used to mask unpleasant odour in topical and cosmetic formulations.	Essential oil: Rose, Lavender, Sandal wood, Jasmine, Lemon, Mentha
Suspending Agents	Keep particles dispersed	Gum ghatti, Gum karaya, Xanthan gum

GUM TRAGACANTH

B.S: Dried gummy exudate obtained by incisions from the stem and branches of *Astragalus gummifer* Labill. **Family**: Leguminosae

Description

Colour: The flakes are white or pale yellowish-white

Odour: Odourless

Taste: Mucilagenous

Shape: Thin flattened ribbon like flakes, more or less curved

The gum is horny, translucent with transverse and longitudinal ridges.

Solubility: Partly soluble in water, in which it swells to homogenous, adhesive and gelatinous mass. Insoluble in alcohol.

Chemical Constituents: Consists of water soluble portion-tragacanthin (8-10%) and a water insoluble portion bassorin (60-70%). It contains 15% of methoxy group which swells in water, responsible for its high viscosity.

Identification Test

- With 10% aq. $FeCl_3$, tragacanth solution produces deep yellow precipitate.
- Tragacanth and precipitated copper oxide in con. ammonium hydroxide, forms a stringy precipitate.
- With NaOH and warmed, develops a canary yellow colour.
- With strong iodine soln. gives green colour.

Uses:

- Demulcent, suspending agent, emulsifying agent, binding agent
- Tragacanth powder is used as an adhesive.
- Also used in lotions for external use and as stabilizer for ice-cream and also in sauces.

ACACIA (INDIAN GUM)

B.S: Dried gummy exudate from the stem and branches of *Acacia arabica* Wild. **Family**: Leguminosae

Description

Colour: Tears are cream brown to red in colour, while powder is light brown in colour

Odour: Odourless

Taste: Bland and mucilaginous

Size and Shape: Irregular brown tears of varying size. The tears are glossy and marked with minute fissures and are brittle in nature.

Solubility: Soluble in water but insoluble in alcohol. The aq. Solution is viscous and acidic.

Chemical Constituents: 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 and peroxidase.

Identification:

- Lead subacetate soln. gelatinizes the aq. solution of Indian gum
- With ruthenium red soln. no pink colour.
- Hydrogen peroxide and benzidine in alcohol to aqueous solution of gum, blue colour was produced due to oxidase enzyme.

Uses:

- Demulcent, suspending agent, emulsifying agent, binding agent.
- In combination with gelatin, used to form coacervates for microencapsulation of drugs.

STARCH

Biological source: Starch consists of polysaccharide granules obtained from the grains of *Zea mays* (maize) belongs to family Graminae or from the tubers of (potato) *Solanum tuberosum* belonging to family Solanaceae.

Macroscopical character

Shape: Irregular, angular masses or in form of white powder.

Solubility: It is insoluble in cold water and forms a colloidal solution on boiling. After cooling, the starch solution becomes translucent jelly.

Chemical constituents:

1. Starch contains generally a mixture of two polysaccharides, amylopectin (α-amylose) and amylose (β-amylose).

2. Amylopectin it is the main constituent of most of the starches (more than 80%) and is present in outer pats of granules. It contains both strait chained and branched glucose unit. It is insoluble in water and is responsible for gelatinizing property. It gives bluish black colour with iodine solution.

Uses: It is mainly used as dusting powder, as a pharmaceutical aid, used as an antidote for iodine poisoning, as a source of food for nutrition. It is also used as a tablet disintegrating agent and diluents as a protective and demulcent.

Evaluation of herbal excipients

1. **Organoleptic Evaluation:** Observed and recorded colour, odour, taste, and appearance of each excipient.
2. **Solubility Test:** The solubility of the excipients in water, alcohol, and other common solvents was determined.
3. **Swelling Index (for gums/mucilages):** It is defined as the volume in milliliters occupied by the swollen drug material after it has been allowed to swell in an aqueous medium under specified conditions.
   - ❖ About 1 g of sample was weighed and transferred into a 25 mL graduated cylinder.
   - ❖ About 25 mL distilled water was added and kept aside for 24 hours.
   - ❖ The increase in volume was noted and swelling index was calculated (ml/g).
4. **pH Measurement**
   - ➢ The pH meter was calibrated using standard buffer solution.
   - ➢ About 1% w/v solution of the excipient in distilled water was prepared.
   - ➢ The pH was measured using digital pH meter.
5. **Viscosity (for mucilage/gums)**
   - ➢ About 1% solution of the sample was prepared.
   - ➢ The viscosity was measured using a viscometer. The viscometer was filled with the excipient solution up to the mark
   - ➢ The liquid was sucked above the upper mark and allowed it to flow freely.
   - ➢ The time taken for the liquid to flow between the two marks was recorded using a stopwatch.
   - ➢ The procedure was repeated and calculated the average flow time.
   - ➢ Similarly, the flow time for distilled water was determined.

$$\eta 1 = \eta 2 \times \frac{\rho 1 t 1}{\rho 2 t 2}$$

Where:
$\eta 1$ = viscosity of excipient
$\eta 2$ = viscosity of water

ρ1 = density of excipient
ρ2 = density of water
t1 = flow time of excipient
t2 = flow time of water

6. **Loss on Drying:**

- ❖ About 1 g of sample was weighed, transferred in a crucible and weighed the crucible containing sample.
- ❖ The crucible was placed in an oven at 105°C until a constant weight was obtained.
- ❖ The procedure (drying and weighing) was repeated until the difference between two successive weights was not more than 0.5 mg.
- ❖ The percentage moisture content was calculated using the formula;

$$\text{Loss on Drying (\% w/w)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

Report:

Excipients	Colour	Odour	Solubility	pH	Swelling Index	Viscosity	Moisture Content (%)
Acacia gum							
Tragacanth gum							
Starch							

FORMULATION AND EVALUATION OF POLYHERBAL CREAMS, LOTIONS AND SHAMPOOS USING STANDARDISED EXTRACT IN COSMETICS

FORMULATION AND EVALUATION OF POLYHERBAL LOTION

Aim

To prepare and standardize herbal lotion.

Theory

A lotion is a low-viscosity topical preparation intended for application to the skin without friction. Lotions are applied to external skin with bare hands, a brush, a clean cloth, or cotton wool. Lotion also has an advantage in that they may be spread thinly compared to cream or ointment and readily applied to hairy skin. Herbal lotions are topical formulations prepared using plant-derived ingredients that possess therapeutic activities such as moisturizing, antimicrobial, antioxidant, anti-inflammatory, and skin-soothing effects. They are widely used due to their safety, minimal side effects, and consumer preference for natural cosmetics. Herbal lotions are O/W emulsions in which the oil phase (emollients, waxes) is dispersed as fine droplets throughout a continuous aqueous phase containing herbal extracts, emulsifiers and humectants. Emulsifiers stabilize the interface; controlled heating, homogenization, and gradual cooling produce a smooth, stable lotion.

Composition of Lotion

S.No.	Ingredients	Quantity (100 g)	Uses
Aqueous Phase			
1.	Aloe vera gel	2 g	Moisturizer, Anti acne
2.	Neem Extract	2 g	Anti microbial
3.	Honey	2 g	Anti septic
4	Glycerin	5 g	Humectant
5	Gum Acacia	2 g	Emulsifier
6	Rose water	2 ml	Perfume
7	Distilled water	70 ml	Vehicle
Oil Phase			
8.	Almond oil	3 g	Emollient
9.	Coconut oil	10 g	Emollient
10.	Beeswax	2 g	Thickener

Procedure for the preparation of lotion

Preparation of Oil Phase: Oil-phase ingredients (Almond oil, Coconut oil, Beeswax) were weighed. Heated to 70–75°C until completely melted and stirred continuously

Preparation of Aqueous Phase: Water-soluble ingredients (aloe vera gel, neem extract, honey, glycerin, gum acacia) were dissolved in distilled water. Heated to 70–75°C same as oil phase temperature.

Emulsification: The oil phase was added slowly into the aqueous phase with continuous stirring. Using a homogenizer/stirrer, stirred for 10–15 minutes until uniform lotion base was formed. Temperature was maintained during mixing to avoid phase separation. The mixture was cooled gradually to 40°C, added rose oils and mixed gently to avoid air bubbles. The prepared lotion was filled into sterilized containers while still flowable and labeled suitably.

EVALUATION OF LOTION

Organoleptic characters: Colour and appearance of the lotion was observed by visual examination and the odor of the lotion was tested by smelling

pH: The pH meter was calibrated using standard buffer solution. About 0.5gm of lotion was weighed and dissolved in 50 ml of distilled water and determined its pH using digital pH meter.

Viscosity: Viscosity has an important role in explaining and controlling many attributes like shelf-life ability and product aesthetics such as clarity, ease of flow, on removal from packing and reading when applied to face. The viscosity of the formulation was determined using a Brookfield viscometer, LV-64 spindle at 25 revolutions per minute by submerging the prepared lotion straight into the spindle to measure its viscosity.

Spreadability: About 1 g of the sample was applied between two glass slides and compressed to uniform thickness by placing 100 gm weight for 5 minutes. The weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower slide was noted to measure the spreadability. The value should be in between 9.0 to 31.02 g.cm/s. Calculated the spreadability using the formula:

$$S = M\times L / T$$

Where,

M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

4. **Irritancy test**: The lotion was applied to a specific area of the left- hand dorsal surface and checked for irritancy, erythema, oedema up to 24 hrs.

5. **Homogeneity:** The Homogeneity of the formulation was tested by visual appearance and by touching for the presence of any aggregates.

6. **Washability**: A small portion of lotion was applied over the skin of hand and allowed to flow under the force of flowing tap water for 10 minutes. The time required for the lotion to remove completely was noted.

7. Stability Test: To check the microbial growth, the formulation was placed in the center of the petri dish, and incubated the plates at 37°C for 72 hrs. to monitor the microbial growth.

Report:

Sr. No.	**Test**	**Observation**
1	Colour	
2	Odour	
3	pH	
4	Viscosity (Cps)	
5	Spreadability (g.cm/s)	
6	Irritancy Test	Eg. Non-irritable & non allergic on the skin
7	Homogenicity	Eg. Homogenous
8	Washablility	Eg. Easily washable from the skin by using water
9	Stability test	Eg. No microbial growth is observed after 5 months

FORMULATION AND EVALUATION OF HERBAL SHAMPOO

Aim

To formulate and evaluate the herbal shampoo.

Theory

Shampoo is basically a cleansing system designed to remove dirt, excess oil (sebum), sweat, environmental pollutants. The detergent action of shampoo is based on surfactants (detergents). A surfactant molecule has two ends, hydrophilic "head" (water-loving) and hydrophobic "tail" **(**oil-loving). The hydrophobic tail attaches to oils, dirt, and sebum on hair, while the hydrophilic head remains attracted to water. When the hair is rinsed with water, water pulls the hydrophilic heads away, carrying the trapped oils and dirt with them. The dirt/oil droplets are lifted off from the hair in the form of micelles, a tiny spherical structure containing the trapped oils. Surfactants also works through the process of emulsification in which these molecules surround grease (like tiny bubbles), allowing it to be suspended in water and washed away. Additives in shampoo includes conditioner, moisturizer and flavoring agent. Conditioners in shampoo reduces friction and breakage, smooth the hair shaft by coating the hair lightly to give a silky texture, and prevent static i.e. cationic ingredients neutralize static electricity. Moisturizers in shampoo help retain water (humectant) in the hair and scalp, preventing hair from becoming brittle, preventing dryness and improving elasticity.

Surfactants which are synthetic are added in synthetic shampoos increase foaming and cleaning property but continues usage of synthetic shampoo can cause various side effects such as dryness of hair, hair loss, scalp irritation etc, So, alternative to synthetic shampoo, herbal shampoos can be used which contains natural herbs.

Preparation of Shampoo

Extraction

The extracts were prepared by decoction method individually. Homogenized about 100 g of each powdered plant materials viz., powdered fenugreek seeds, amla fruit, hibiscus leaves, neem leaves, soap nut fruit, shikakai pods, and extracted with sufficient distilled water (1:10) by boiling for 4 h. Filtered, the filtrate was evaporated and dried the extract of each plant material.

To the gelatin solution (10% w/v), added all herbal extracts and mixed by stirring gently to prevent foam for 20 mins. To improve aroma of the formulation, added sufficient quantity of essential oil (rose oil) and finally adjusted the volume to 100 ml with gelatin.

Composition of Herbal Shampoo

S.No.	Ingredients	Biological Source	Quantity for 100g	Uses
1	Fenugreek seeds	*Trigonella foenum-graecum*	2g	Moisturizer
2	Neem leaves	*Azadirachta indica*	2g	Antibacterial and anti lice
3	Aleo vera juice	*Aloe vera*	2g	Moisturizer and conditioner
4	Amla fruit	*Emblica officinalis*	2g	Cooling agent, anti

				dandruff agent
5	Soap nut seed	*Sapindus trifoliatus*	5 g	Cleansing agent, Natural Surfactant
6	Hibiscus leaves	*Hibiscus rosa-sinensis*	1g	Promotes hair growth, prevent hair loss
7	Shikakai pod	*Acacia concinna*	5 g	Cleansing and Foaming agent
8	Rose oil	Essential oil from Rosa species	1 ml	Fragrance
9	Gelatin 10%	Protein extracted by partial hydrolysis of animal collagenous tissue	qs	Thickener
10	Water		qs	Solvent for extraction

Evaluation of formulated herbal shampoo

To evaluate the quality of prepared formulations, several quality control tests should be performed.

Physical appearance/ visual inspection

Evaluated the prepared formulation for the clarity, colour, odour, and foam producing ability.

Determination of pH

The pH meter was calibrated using standard buffer solution. The pH of 10 percentage v/v shampoo solution was determined at room temperature 25°C using digital pH meter.

Determination of percentage of solid contents

About 4 grams of shampoo was weighed and placed in a clean dried evaporating dish. Evaporated by placing it on hot plate until the liquid portion gets evaporated. Dried completely and the residue was weighed. The percentage of the solid contents present in the shampoo was calculated. To confirm the result, the procedure was repeated.

Wetting time

About 1-inch diameter discs of a canvas having an average weight of 0.44g was cut. The disc was placed on the surface of shampoo solution of 1%w/v and started the stopwatch. The time required for the disc to begin to sink was noted and wetting time was calculated.

Foaming ability and foam stability

The stability of the foam was determined using cylinder shake method. About 50 ml of formulated shampoo (1%) solution was taken in a graduated cylinder of 250 ml capacity and shaken for 10 times vigorously. After shaking, the foam volume was measured at 1 min and 4 min, respectively and foam stability was recorded.

Dirt dispersion test

About two drops of cleanser was added to 10 ml of distilled water in a wide-mouthed test tube. To this solution, one drop of Indian ink was added and shaken for 10 min after closing the test tube with a stopper. The volume of ink in the froth was measured and the result was graded in terms of none, slight, medium, or heavy. Shampoos that cause the ink to concentrate in the foam were considered poor quality. The dirt should remain in the water portion. Dirt that remains in the foam will be difficult to rinse away and will be redeposited on the hair.

Report

Sr. No.	Evaluation Test	Observation
1	Colour	
2	Odour	
3	pH	
4	Transparency	
5	Solid content (%)	
6	Wetting time	
7	Foam volume (ml)	
8	Foam type	
9	Dirt Dispersion	

FORMULATION AND EVALUATION OF POLYHERBAL CREAM

AIM

To prepare and evaluate the anti-acne herbal cream.

THEORY

Cream is a semisolid emulsion dosage form containing one or more active drug substances dissolved or dispersed in oil-in-water (non-greasy, washable) or water-in-oil (greasy, occlusive) emulsion base, intended for external application to the skin or mucous membranes. In oil-in-water creams (O/W), oil droplets are dispersed in a continuous water phase and are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. In water-in-oil creams (W/O), water droplets are dispersed in a continuous oil phase and drugs will be released more readily from a water-in-oil cream than an oil-in-water cream and provide better moisturizing effect. Useful for dry, cracked skin or where enhanced penetration is needed.

Creams can be formulated based on emulsion and rheology theory stabilized by emulsifying agents (surfactants). In emulsification process, two immiscible phases (oil and water) are heated separately to similar temperatures (usually 60 - 70°C). The aqueous phase is added to the oil phase (or vice versa), depending on the emulsion type. The emulsifying agents reduce interfacial tension between oil and water phases, enabling stable droplet formation and continuous mixing prevents separation of phases. Herbal creams are prepared by incorporating natural plant extracts, essential oils, or phytochemicals into an oil-in-water (O/W) or water-in-oil (W/O) emulsion base and are used for cosmetic purposes such as cleansing, beautifying, improving appearances, protective or for therapeutic function.

Acne is a chronic inflammatory disorder of pilosebaceous unit, which involves increased sebum production by sebaceous glands and abnormal desquamation of hair follicles occur in response to increasing androgen levels with the onset of puberty. Obstruction of follicles causes follicular distention which is often accompanied by the proliferation of the bacteria *Propionibacterium acnes* and the activation of an inflammatory response. The present study is aimed at formulating and evaluating topical herbal cream as a potential anti acne cream.

Composition of Anti acne herbal cream

S.No.	Ingredients	Biological Source	Quantity for 100g	Uses
Aqueous Phase				
1	Cinnamon extract	Dried bark of *Cinnamomum zeylanicum*		Antimicrobial and anti inflammatory

2	Neem extract	Dried leaves of *Azadirachta indica*	2g	Antibacterial and anti inflammatory
3	Turmeric extract	Dried rhizome of *Curcuma longa*		Antibacterial and anti inflammatory
4	Tulsi extract	Dried leaves of *Ocimum sanctum*	2g	Antimicrobial, anti-inflammatory, and antioxidant properties
5	Liquorice extract	Dried root of *Glycyrrhiza glabra*	5 g	Anti-inflammatory and reduces post-acne pigmentation
Oil Phase				
6	Bees wax	Wax obtained from the honey comb of honey bee *Apis mellifera*	3 g	Emulsifying, thickening, stabilizing, and protective properties.
7	Coconut oil	Fixed oil from the dried kernel of *Cocos nucifera*	10 g	Emollient, moisturizing
8	Rose oil	Essential oil from **Rosa species**	1 ml	Fragrance

PREPARATION OF HERBAL CREAM

Preparation of Oil Phase: The oil-phase ingredients (Coconut oil, Beeswax) were weighed. Heated to 70–75°C until completely melted and stirred continuously

Preparation of Aqueous Phase: The water-soluble ingredients (cinnamon extract, neem extract, turmeric extract, tulsi extract, liquorice extract) were dissolved in distilled water. Heated to 70–75°C same as oil phase temperature.

Emulsification: The oil phase was slowly added into the aqueous phase with continuous stirring using a homogenizer/stirrer for 10–15 minutes until smooth emulsion was formed. The temperature was maintained during mixing to avoid phase separation. The cream was cooled gradually to 40°C, added rose oils and mixed gently to avoid air bubbles. Filled into wide-mouth jars and labeled suitably.

EVALUATION PARAMETERS

Organoleptic characters

The colour and appearance of the lotion was observed by visual examination. The odor of the cream was tested by smelling

pH: The pH meter was calibrated using standard buffer solution. About 1 gm of cream was weighed and dissolved in 10 ml of distilled water and its pH was determined using digital pH meter. Ideal pH: 5–7 for skin compatibility

Viscosity: Viscosity has an important role in explaining and controlling many attributes like shelf-life ability and product aesthetics such as clarity, ease of flow, on removal from packing and reading when applied to face. The viscosity of the formulation was determined using a

Brookfield viscometer, LV-64 spindle at 25 revolutions per minute by submerging the prepared lotion straight into the spindle to measure its viscosity.

Spreadability: About 1 gm of the sample was placed between two glass slides and compressed to uniform thickness by placing 100 gm weight for 5 minutes. Weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower slide was noted to measure of spreadability. The value should be in between 9.0 to 31.02 g.cm/s. Calculated the spreadability using the formula:

$$S = M\times L / T$$

Where,

M = weight tied to upper slide

L = length of glass slides

T = time taken to separate the slides.

Phase separation: The prepared cream was kept in tightly closed container at room temperature away from sunlight and observed for 24 hours for phase

Irritancy test: The cream was applied to a specific area of the left- hand dorsal surface and checked for irritancy, erythema, oedema up to 24 hrs.

Homogeneity: The homogeneity of the formulation was tested by visual appearance and by touch for the presence of any aggregates.

Greasiness: This test is basically used to check nature of cream either oily or greased. The cream was applied on skin surface in the form of smear and checked the smear for oily or grease

Stability Test: To check the microbial growth, the formulation was placed in the center of the petri dish, and incubated the plates at 37°C for 72 hrs to monitor the microbial growth.

REPORT

Sr. No.	**Test**	**Observation**
1	Colour	
2	Odour	
3	pH	
4	Viscosity (Cps)	
5	Spreadability (g.cm/s)	
6	Phase separation	Eg. No phase separation
7	Irritancy Test	Eg. Non-irritable & non allergic on the skin
8	Homogenicity	Eg. Homogenous
9	Greasiness	Eg. Non greasy
10	Stability test	Eg. No microbial growth is observed after 5 months

FORMULATION AND EVALUATION OF POLYHERBAL SYRUPS, MIXTURES AND TABLETS USING STANDARDISED EXTRACT AS PER PHARMACOPOEIAL REQUIREMENTS

FORMULATION AND EVALUATION OF HERBAL COUGH SYRUP

AIM

To prepare and evaluate herbal cough syrup

THEORY

Cough syrup is a liquid medicinal preparation formulated to relieve coughing and associated symptoms. It typically contains one or more active ingredients such as antitussives (to suppress cough), expectorants (to loosen mucus), mucolytics (to thin mucus), antihistamines (to reduce allergic reactions), or decongestants, combined in a flavored, sweetened syrup base for easier consumption. Coughing is a protective reflex triggered when irritants (mucus, dust, microbes, allergens) stimulate receptors in the respiratory tract. These signals travel to the cough center in the medulla of the brain, producing the cough action.

Cough syrups work by modifying this reflex or clearing the irritants that trigger it. Antitussives act on the cough center in the brain to reduce the urge to cough. Expectorants, by thinning and hydrating mucus, loosen and increase the volume of bronchial secretions, making mucus easier to cough out. Mucolytics, break down the structure of thick mucus, reducing viscosity making mucus thinner. Antihistamines inhibit histamine release and reduce allergic cough, decreases nasal and throat irritation, reducing the reflex to cough. Decongestants, constrict swollen nasal passages to relieve congestion, easing breathing. The syrup contains sugar or sweeteners that improve taste and palatability, soothe the throat by creating a smooth coating, increase viscosity, helping the medicine stay longer on the mucosal surface.

Herbal cough syrup is a medicinal liquid preparation made from plant-based ingredients used to relieve cough and soothe respiratory irritation. It uses herbs with natural antitussive, expectorant, demulcent, anti-inflammatory, or antimicrobial properties.

COMPOSITION OF HERBAL COUGH SYRUP

S.No.	Ingredients	Quantity (for 100 ml)	Uses
1	Tulsi leaves (*Ocimum sanctum*)	1g	Antimicrobial and expectorant

2	Pepper fruit (*Piper nigrum*)	1g	Decongestant and expectorant
3	Kuppaimeni leaves (*Acalypha indica*)	1g	Expectorant, anti inflammatory, broncho dilator
4	Adhathoda leaves (*Adhatoda vasica*)	1g	Expectorant and anti tussive
5	Ginger rhizome (*Zingiber officinalis*)	1g	Anti inflammatory
6	Liquorice root (*Glycyrrhiza glabra*)	1 g	Demulcent and anti tussive
7	Lemon oil (*Citrus limon*)	1 ml	Flavouring agent, decongestant and expectorant
8	Sugar syrup (66.7 % w/v)/Honey (35% v/v)	q.s	Vehicle, preservative, sweetening agent

PREPARATION OF COUGH SYRUP

Preparation of extract

The dried plant material listed in the table was pulverized to a coarse powder. The weighed quantity of the coarsely powdered ingredients was extracted with distilled water in the ratio 1:10 plant to water by boiling until the volume was reduced to one-third. The decoction was filtered and the filtrate were concentrated till dryness.

Preparation of simple syrup

The simple syrup was prepared using 66.7 gram of sucrose and finally dissolving in sufficient quantity of distilled water with ambient boiling. The volume was adjusted up to 100 ml.

Procedure

The concentrated herbal extract was added slowly to the prepared syrup base and stirred continuously to ensure proper mixing. The final volume was adjusted with purified water and filtered through fine muslin cloth if clarity needed. Lemon oil (1 ml) was added dropwise and stirred. The formulation was then transferred into amber-colored or opaque bottles to protect from light and labelled.

STANDARDIZATION

Physical appearance /visual inspection

The formulated cough syrup was evaluated for the clarity, color, and odour.

Determination of pH

The pH meter was calibrated using standard buffer solution. About 10 ml of the cough syrup was taken in a 100 ml volumetric flask and made up the volume up to 100 ml with distilled water.

The pH of 10 percentage v/v of prepared cough syrup solution was determined at room temperature 25°C using digital pH meter.

Viscosity Determination

The viscosity of the cough syrup was determined by using Oswald's viscometer. The viscometer was filled with water up to the mark and noted the time (in sec) taken for the water to flow from the mark A to mark B and the procedure was repeated with cough syrup and noted the time (in sec) taken for the syrup to flow from the mark A to mark B. Viscosity was calculated as per the formula;

$$\text{Viscosity} = \frac{\text{Density of the test sample} \times \text{time required for the test sample to flow}}{\text{Density of the water} \times \text{time required for the water to flow}} \times 100$$

Determination of density: The density of syrup can be determined by using the specific gravity bottle (Pycnometer). The cleaned specific gravity bottle was filled with the distilled water, wiped the excess of water outside the bottle and weighed the bottle and distilled water along with the capillary tube stopper (w1). The specific gravity was filled with the cough syrup wiped the excess of syrup outside the bottle and weighed the bottle and cough syrup along with the capillary tube stopper (w2). Density was expressed in g/mL or g/cm³ and calculated using the formula;

$$\text{Density}(\rho) = \frac{\text{Mass of liquid}}{\text{Volume of liquid}}$$

Stability Testing: Stability Testing of the prepared herbal syrup was performed on keeping the sample at accelerated temperature conditions. Nine portions of the final herbal syrup were kept at accelerated temperature at 4°C, room temperature and 47°C respectively. The sample was tested for all the physicochemical parameters, turbidity and homogeneity at the interval of 24hr, 48hr and 72hr to observe any change.

REPORT

Sr. No.	Test	Observation
1	Colour	
2	Odour	
3	pH	
4	Viscosity (Cps)	
5	Density	
6	Specific gravity	
7	Stability test	

FORMULATION AND EVALUATION OF HERBAL MIXTURES (CHURNA)

AIM

To prepare Triphala churna using herbal ingredients and to evaluate it for standard quality parameters

THEORY

Herbal mixtures are formulations prepared by combining two or more powdered crude drugs in specific proportions to obtain synergistic therapeutic effects. Churna is a finely powdered herbal preparation obtained by pulverizing dried medicinal plant parts and passing them through a suitable sieve to ensure uniform particle size. Churna is one of the most commonly used Ayurvedic solid dosage forms. The therapeutic activity of herbal mixture churna is based on the synergistic action of combined herbs and fine particle size, which increases surface area and improves absorption.

COMPOSITION OF HERBAL MIXTURE

S.No.	Ingredients	Quantity	Uses
1	Amla fruit (*Emblica officinalis)*)	1g	Rich in Vitamin C, antioxidants, and is cooling in nature
2	Kadukkai fruit (*Terminalia chebula*)	1g	antimicrobial and expectorant, anti oxidant, anti diabetic,
3	Thandrikkai fruit (*Terminalia bellirica*)	1g	anti diabetic, anti oxidant, antibacterial and hepatoprotective

PREPARATION OF CHURNA

Crude drugs were cleaned and dried properly. Pulverized each ingredient separately using a grinder. The powders were passed through sieve No 80. Triphala churna was prepared by mixing an equal proportion (1:1:1) of powdered fruits. It was then kept in air tight containers in cool and dried place.

EVALUATION OF CHURNA

Organoleptic Evaluation

All the organoleptic properties viz. color, odor, taste, and texture of the drug to touch were performed as per standard procedure and noted down.

Physico-Chemical Evaluation

Physicochemical parameters like total ash, acid insoluble ash, water-soluble extractive, alcohol soluble extractive values, moisture content (Loss on Drying) and pH of the triphala churna was determined as per standard protocols.

Determination of Total Ash

The ash remaining after ignition of crude drug is determined by three different methods that are total ash, acid-insoluble ash and water-soluble ash. This includes both physiological ash and non-physiological ash. Total ash is useful in detecting the crude drugs that are mixed with various mineral substances like sand, soil, calcium oxalate, chalk powder, or other drugs with different inorganic contents to improve their appearance.

Procedure:

1. About 2 g of the accurately weighed powdered drug was taken in a tarred silica crucible and ignited at a temperature not exceeding 450ºC until it is white, indicating the absence of carbon.
2. The residue was cooled in a suitable desiccator for 30 minutes and weighed.

Calculation

Weight of empty silica crucible (A) = ---------- gm

Weight of silica crucible and powdered drug (B) = ---------- gm

Weight of powdered drug taken (B-A) = ---------- gm

Weight of silica crucible and ash (D) = ---------- gm

Weight of ash (D - A) = ---------- gm

Total ash value of the sample = $\frac{100 \times \text{Weight of the ash}}{\text{Weight of the powdered drug taken}}$

Acid insoluble ash

Total ash obtained from above process was boiled for 5 minutes in 25ml dil. hydrochloric acid. The solution was then filtered through ash less filter paper. The ash was dried and the percentage of acid insoluble ash was calculated with reference to air dried sample.

Acid insoluble ash, % by mass = $\frac{100 \times (W2-W1)}{W}$

Where,

W1 = weight in g of the empty dish

W2 = weight in g of the dish with acid-insoluble ash

W = weight in g of the sample.

Water Soluble Ash

The ash was boiled with 25 ml of water. The insoluble matter was then collected in an ash less filter paper. It was washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450oC. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water soluble ash with reference to the air-dried drug was calculated.

Alcohol/Water Soluble Extractive Value

About 5 g of coarsely powdered air-dried material was weighed accurately and macerated with 100 ml of 90% ethanol in a glass stopper closed flask for 24 h, with frequent shaking during the first 6 hours and allowed to stand for 18 hours. Filtered and 25 ml of filtrate was transferred to porcelain dish and evaporated the content to dryness in a porcelain dish and dried at 105˚ to a constant weight. Cooled in a desiccator for 30 min and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried plant material. The procedure was repeated with 100 ml of water to determine water soluble extractive value.

Calculation:

25 ml of alcoholic/water extract gives – A g of residue

100 ml of alcoholic/water extract gives – B g of residue

5 g of air dried drug gives B g of alcohol/water soluble residue

100 g of air dried drug gives (100 x B)/5 g of alcohol/water soluble residue

$$\text{Extractive value} = \frac{\text{Weight of the residue obtain} \times 100}{\text{Weight of the dried powder taken}}$$

Determination of Moisture Content/ Loss on Drying (LOD)

1. About 1 g of sample was weighed, transferred in a crucible and weighed the crucible containing sample.
2. The crucible was placed in an oven at 105°C for 3 hrs.
3. The drying and weighing were continued at half an hour interval until difference between two successive weighing corresponded to, not more than 0.25 per cent.
4. The percentage moisture content was calculated using the formula;

$$\text{Loss on Drying (\% w/w)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

Determination of pH Value: The powder sample of triphala churna was weighed to about 5g and immersed in 100 ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated digital pH meter.

PHARMACEUTICAL EVALUATION

Pharmaceutical parameters like Bulk density, Tapped density, Carr's Index, Hausner's Ratio and Angle of repose were determined as per standard protocols.

Bulk density: It is defined as ratio of total mass of the powder to the bulk volume of powder it occupies before tapping. About 15 g of the sample was added into a measuring cylinder of 100 ml and the measuring cylinder was not to tapped or shaken. The volume occupied by the powder sample was noted. The bulk density was expressed in gm/ml and calculated by using the formula,

$$Db = \frac{M}{Vo}$$

Where, M= Mass of powder, Vo = Bulk volume of powder

Tapped density: It is the ratio of the weight of powder to the minimum volume occupied in measuring cylinder.

About 15 g of the sample was added into a measuring cylinder of 100 ml and mechanically tapped the measuring cylinder containing the sample of 15 gm with a fixed drop of 14±2 mm at a nominal rate of 300 drops per mins until a constant volume was observed. The tapped density was expressed in gm/ml and calculated by using the formula,

$$\text{Tapped density (Dt)} = \frac{M}{Vt}$$

Where, M= Mass of powder, Vt = Tapped volume of powder

Carr's Index: Based on the apparent bulk density and the tapped density, the percentage compressibility of the powder mixture was determined by the following formula.

$$\text{Carr's index} = \frac{\text{tapped density- bulk density}}{\text{tapped density}} \times 100$$

Carr's index	**Type of flow**
5-15	Excellent
12-15	Good
15-22	Fair
23-30	Poor
33-38	Very poor
>40	Extremely Poor

Hausner's Ratio: It is an indirect index of ease of measuring of powder flow. Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25)

$$\text{Hausner's ratio} = \text{tapped density/ bulk density}$$

Angle of Repose: It is the maximum angle possible between surface of pile of powder and the horizontal plane, can be used to measure frictional forces in a powder. Angle of repose was determined by using the funnel method. A funnel was clamped with its tip 2cm above a graph paper placed on a flat horizontal surface. The powder was filled in the funnel by pouring the powders carefully through the funnel. The orifice of the funnel was blocked by thumb and the thumb was removed immediately. After emptying the powder from the funnel, the height of the pile and diameter was measured. Formula to calculate the angle of repose is as follows.

$$\Theta = \text{Tan-1}[h/r]$$

Where h = height of pile formed. r = radius of the powder/granule.

Angle of repose(θ)	**Type of flow**
<25	Excellent
25 - 30	Good
35-40	Passable
>40	Very poor

Report:

Sr. No.	**Test**	**Observation**
1	Total Ash	
2	Acid insoluble ash value	
3	Water soluble ash	
4	Alcohol soluble extractive value	
5	Water soluble extractive value	
6	Loss on drying	
7	pH	
8	Bulk density	
9	Tapped density	
10	Angle of repose	
11	Carr's Index	
12	Hausner ratio	

FORMULATION AND EVALUATION OF HERBAL TABLET

AIM

To prepare and evaluate herbal tablet

THEORY

According to USP, tablet is defined as a compressed solid dosage form containing medicaments with or without excipients. According to the Indian Pharmacopoeia, Pharmaceutical tablets are solid, flat or biconvex dishes, unit dosage form, prepared by compressing a drug or a mixture of drugs, with or without diluents. A tablet contains one or more active pharmaceutical ingredients (APIs) along with excipients, prepared by compression or molding. Any substance apart from the active ingredient can be classified as an excipient. The excipients that are used in tablets are diluents, binders, disintegrants, glidants and lubricants. A tablet can be designed to accurately deliver targeted cavities of the body. Tablets can be administered orally, buccally, intravaginally or rectally. The tablets may differ in size, shape, weight, thickness, dissolution and hardness depending on the use and manufacturing method.

The manufacturing process of tablets involves blending the active ingredient with pharmaceutical ingredients and dry powder excipients**,** followed by granulation or direct compression. Granulation is a wet granulation process that involves adding a liquid binder to the powder mixture to create granules, which are then dried, sieved, and compressed into tablets. Direct compression, on the other hand, involves directly compressing the powder mixture into tablets without the need for granulation.

A herbal tablet is a solid dosage form containing one or more plant-derived active ingredients (powder, extract, or standardized fraction), compressed with suitable pharmaceutical excipients.

PREPARATION OF HERBAL TABLET

Composition of Herbal tablet

Ingredients	Quantity for 1 Tablet (400 mg)	Uses
Triphala Churna	300 mg	Anti inflammatory, improve digestion,
Lactose	10 mg	Diluent
Mannitol	20 mg	Sweetening agent
Talc	30 mg	Glidant
Magnesium stearate	30 mg	Lubricant
Starch	10 mg	Binding agent

Procedure

- All the ingredients were weighed as per the formula in the table. Passed through sieve no.20.
- All the ingredients were mixed following geometric mixing excluding talc (glidant) and lubricant thoroughly for 15 min.
- The binding agent (1% starch mucilage) was added gradually until a coherent mass was formed.
- The coherent mass was passed through sieve no. 22 and dry the granules.
- The dried granules were mixed thoroughly with talc and magnesium stearate.

- The dried granules were compressed into a 400 mg tablet using single rotary punching machine.

EVALUATION OF TABLETS

The tablets were subjected to the following evaluation tests.

General appearance

The general appearance and color of tablets were found by visual determination.

Weight variation test

About 20 tablets were weighed individually and considered as X1, X2, X3,…. .X20. The average weight of 20 tablets X= (X1+X2+X3+….+X20)/20 were determined. The individual weight was compared with average weight for the weight variations. Not more than two of the tablets differs from the average weight by more than the % error listed, and no tablets differ by more than double that percentage. The following percentage deviation in weight variation is allowed (U.S.P).

Average weight	**% difference**
130 mg or less	10
130 – 324 mg	7.5
More than 324 mg	5

Hardness and thickness test

The tablet hardness is defined as the force required to break a tablet in a diametric direction. A tablet was placed between two anvils and applied force to anvils and recorded the crushing strength that causes the tablet to break. The hardness was measured using Monsanto hardness tester. For each formulation, the hardness and thickness of 20 tablets were determined. Hardness test was determined by Monsanto hardness tester and the thickness of tablets was determined by Vernier Calipers.

Friability

Friability was determined by Roche friabilator. About 10 or 20 tablets were weighed and revolved at 25 rpm for 4 min in a plastic chamber. The tablets were reweighed after removing the fines and calculated the weight loss and expressed in percentage (%). Acceptance criteria for % friability % weight loss should be less than 1%.

The % friability was calculated by the formula,

% Friability = initial weight- final weight/ initial weight.

Disintegration time testing: Determined using USP tablet disintegration test apparatus, using 900 ml of distilled water without disk at room temperature. The test was performed on 6 tablets. Placed one tablet each in all six tubes. The tubes were moved upward and downward in water at 37ºC±2ºC. The time taken for all the six tablets to break down and passes through the mesh at the

bottom of the tube was noted. The tablet passes the test if all the six tablets disintegrated within the prescribed time (Less than 30 mins for uncoated tablets as per U.S.P).

REPORT

The herbal tablet was prepared and evaluated.

Sr. No.	Test	Observation
1	General appearance	
2	Weight variation test	
3	Hardness	
4	Thickness test	
5	Friability	
6	Disintegration time	

MONOGRAPH ANALYSIS OF CRUDE DRUGS

AIM:
Monograph analysis of herbal drug from recent pharmacopoeias (castor oil)

DESCRIPTION
Castor oil is a fixed oil obtained from *Ricinus Communis* belonging to family Euphorbiaceae. *Ricinus communis* Linn, (family: Euphorbiaceae).

Colour: colourless or pale yellow viscous liquid

Odour: slight and characteristic

Taste: At first bland but afterwards slightly acrid and nauseating

Solubility: Soluble in alcohol, miscible with ethyl alcohol and with chloroform and with solvent ether.

Chemical constituents: The oil contains up to 87-90% ricinoleic acid, oleic acid 2-6%, linoleic acid 1-5%, isoricinoleic acid, along with other constituents such as stearic acid 0.5-1%, palmitic0.5-1%, dihydroxystearic, and eicosanoic acids. The purgative effect of castor oil is due to free ricinoleic acid which is liberated in the small intestine.

Identification test:

1. It mixes with half its volume of light petroleum ether.
2. To the oil, equal volume of alcohol was added, clear liquid was obtained. On cooling at $0^{o}C$ and on storage for three hours, the liquid remains clear.

Uses:

1. Castor oil is used as a purgative.

2. It is used as lubricant, emollient, in hair oil and hair fixers, in the preparation of paints, polishes and varnishes.

PHYSICAL ANALYSIS
Appearance: 5ml of oil was taken in clean 10 ml test tube and viewed under light for the appearance of its colour, odour and nature.

Solubility: Solubility of all the samples was observed in different solvents like alcohol, glacial acetic acid and chloroform. 1ml of oil was taken in a test tube and specified solvents like alcohol, glacial acetic acid and chloroform were taken respectively in different test tubes and their solubility were observed.

Weight per ml (or) Specific Gravity
The specific gravity is the ratio of the weight of the liquid in air at specified temperature to that of an equal volume of water at the same temperature.

Procedure: A clean, dry pycnometer was selected and calibrated whose empty weight was noted (B). Then the weight of pycnometer filled with water (C) and oils (A) was noted separately which was measured at 30˚C. The specific gravity was calculated using the formula.

$$\text{Specific Gravity} = \frac{\text{Weight of liquid}}{\text{Weight of equal volume of water}}$$

Refractive index

Refractive index is defined as the ratio of the velocity of light in a vacuum to its velocity in a specified medium. The refractive index of castor oil was measured using an Abbe refractometer, with values generally falling between 1.477 and 1.481 at 20°C–25°C. Two drops of the castor oil was placed on the prisms of an Abbe refractometer. Water at 30^{o}C was circulated round the lens to keep its temperature uniform. Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index which was read against the internal monochromatic source of light in the equipment. This was repeated 3 times and the mean value noted and recorded as the refractive index.

CHEMICAL ANALYSIS

Acid value

The acid value is the number which expresses in milligrams, the amount of KOH necessary to neutralize the free acids present in 1g of substance.

Procedure: 10 g of oil was weighed and mixed with 50 ml mixture of equal volumes of ethanol (95%) and ether, previously neutralized with 0.1M KOH to phenolphthalein solution. 1 ml of phenolphthalein solution was added and titrated with 0.1M KOH until the solution remained faintly pink for 30 sec (V). Blank titration was performed by repeating the procedure without the oil sample (using only the solvent and indicator) (Vb).

$$\text{Acid value} = \frac{N \times 5.61 \times (V - Vb)}{W}$$

Where,

V = Volume of 0.1M KOH consumed for sample.
Vb = Volume of 0.1M KOH used in blank titration
N = Normality of the 0.1M KOH solution
W = Weight in grams of the oil sample

Limits: Not more than 2

Preparation of 0.1M KOH

5.611 g of KOH was dissolved in little amount of water and the volume was made up to 1000 ml of water.

Standardization of 0.1M KOH

0.63 g of oxalic acid was weighed accurately in 100 ml volumetric flask and the volume was made up to 100 ml with distilled water (0.1 M). 10 ml of above solution was pipetted out into a conical flask and titrated against 0.1M KOH solution using phenolphthalein as indicator.

$$\text{Normality of KOH} = \frac{\text{Titre value} \times \text{Volume of oxalic acid (10 ml)}}{\text{Normality of oxalic acid (0.1 M)}}$$

Saponification value: The saponification value is the number of milligrams of potassium hydroxide required to neutralize the free acids and to saponify the esters present in 1 g of the substance.

Procedure

About 2 g of oil under examination was weighed in a 200 ml flask of borosilicate glass fitted with a reflux condenser and 25 ml of 0.5 M ethanolic KOH was added. The flask was boiled under the reflux on a water bath for 30 min. 1 ml of phenolphthalein solution was added and titrated immediately with 0.5 M HCl (Va) until the pink colour just disappears. A blank titration was performed omitting the substance under examination (Vb). Saponification value was calculated from the following expression

$$\text{Saponification value} = \frac{N \times 28.05 \times (Vb - Va)}{W}$$

Where,

Va = Volume of 0.5M HCl consumed for sample.

Vb = Volume of 0.5M HCl used in blank titration

N = Normality of the 0.5M HCl solution

W = Weight in grams of the oil sample

Limits: 176 – 187

Preparation of 0.5 M alcoholic KOH-

30 g of KOH was dissolved in 20 ml of water and finally the volume was made up to 1000 ml with 95% ethanol.

Preparation of 0.5 M HCl

42.5 ml of concentrated HCL was dissolved in little quantity of water and the volume was made up to 1000 ml with distilled water.

Standardization of 0.5 M HCl

About 0.5 g of anhydrous sodium carbonate was weighed accurately and dissolved in 20 ml of water, 0.1 ml of methyl orange solution was added and titrated with the 0.5 M Hydrochloric acid until the solution becomes reddish yellow. Boiled for 2 minutes and continued the titration until the reddish yellow colour was restored.

Each ml of 0.5 M Hydrochloric acid is equivalent to 0.02650 g of sodium carbonate.

$$\text{Molarity (M)} = \frac{0.5 \times \text{weight of } Na_2CO_3 \text{ (in g)}}{\text{Titre value} \times 0.0265}$$

Iodine Value: Iodine value is defined as the number of grams of iodine absorbed by 100 g of oil, which indicates the degree of unsaturation in the sample. Iodine reacts with the double bonds of unsaturated fatty acids. The unreacted iodine is then treated with potassium iodide, which liberates free iodine. This iodine is titrated against standard sodium thiosulphate solution using starch indicator.

Procedure

About 0.2 – 0.3 g of the oil was weighed accurately and transferred to a 500-mL Iodine flask, added 20 mL of CCl_4 and dissolved. About 20 ml of Iodine monochloride (Wijs solution) was added, stoppered the flask and kept in a dark place at the temperature between 15ºC to 25ºC for 30 minutes. Added 20 mL of 10% potassium iodide solution and 100 mL of distilled water to the above flask. The liberated iodine was titrated with 0.1 N sodium thiosulphate until pale yellow color has almost disappeared, added 1 to 2 mL of starch indicator solution, and continued the titration until the blue color has just disappeared. A blank titration was performed using the same procedure without the oil sample.

$$\text{Iodine value} = \frac{(B-S) \times N \times 12.69}{\text{weight of sample}}$$

where

B = Titre value of blank (mL)

S = Titre value of sample (castor oil)

N = Normality of sodium thiosulphate

Preparation of 0.1 N Sodium thiosulfate solution: 0.1 N $Na_2S_2O_3$ solution was prepared by dissolving 24.8 g of sodium thiosulfate in distilled water and diluted to 1000 mL.

Preparation of 0.1 N potassium dichromate solution: About 4.903 g of potassium dichromate was weighed, dissolved in distilled water and diluted to 1000 mL.

Standardization of the Sodium thiosulfate solution: 25 mL of the standardized potassium dichromate solution was pipetted into Erlenmeyer flask or bottle. Added 5 mL of hydrochloric acid, 10 mL of potassium iodide solution and kept the flask in the dark for 5 minutes to liberate iodine and then added 100 mL of distilled water. The liberated iodine was titrated with sodium thiosulfate solution, shaking continuously, until the yellow color has almost disappeared. Added 1 to 2 mL of starch mucilage indicator and continued titration, adding the thiosulfate solution slowly, until the blue color has just disappeared. The strength of the sodium thiosulfate solution is expressed in terms of its normality.

Calculation

$$N1V1 = N2V2$$

$$N2 = \frac{V1N1}{V2}$$

Where:

N1 = Normality of potassium dichromate (0.1 N)

V1 = Volume of potassium dichromate (mL)

N2 = Normality of sodium thiosulfate

V2 = Volume of sodium thiosulfate (mL)

Report

Monograph analysis of given herbal drug castor oil from recent pharmacopoeias was performed and observation was recorded.

S.NO	PARAMETERS	As per IP standard	Test value
1	Solubility		
2	Weight per ml (or) Specific Gravity	0.945 – 0.965	
3	Refractive Index	1.477 – 1.481	
4	Acid value	Not More Than 2	
5	Saponification value	176 – 187	
6	Iodine value	82 to 90	

DETERMINATION OF ALCOHOL CONTENT IN ARISTA AND ASAVA

AIM

To determine the alcohol content in the ayurvedic formulation asava and arista by specific gravity method.

THEORY

Asavas and *aristas* are alcoholic formulations prepared by soaking the drugs, either in coarse powder or in the form of decoction (*kasaya*), in a solution of sugar or jaggery, as the case may be, for a specified period of time, during which it undergoes a process of fermentation generating alcohol, thus facilitating the extraction of the active principles contained in the drugs. Arishtas are prepared when the herbs undergo the process of decoction in boiling water while Asavas are prepared by directly using fresh herbal juices. Alcohol, so generated, also serves as a preservative and improvement in the efficiency of extraction of drug molecules from the herbs. Both of these formulations contain up to 12% by volume of alcohol, these formulations are sweet, slightly acidic and they have agreeable aroma. However, the conc. of alcohol in the formulation should be in the range of 3–10%.

The ethanol content of a liquid is expressed as the number of volumes of ethanol contained in 100 volumes of the liquid, the volumes being measured at 24.9 º to 25.1ºC. This is known as the "percentage of ethanol by volume". The content may also be expressed in g of ethanol per 100 g of the liquid. This is known as the 'percentage of ethanol by weight".

Distillation of sample of Ayurvedic formulation

About 25 ml of accurately measured sample was transferred at 25°C to the distillation flask. Diluted with equal volume (25 mL) of water and added a little pumice powder. Attached the distillatory head and condenser and allowed to distillate. Collected 2 mL less than original volume of the test liquid (i.e., 23 mL). Adjusted the temperature to 25°C and diluted with water to 25 ml.

Determination of specific gravity of sample

Specific gravity method is easiest and simplest method to determine the concentration of ethanol volume/volume in the given sample. The specific gravity of 100% alcohol (ethanol) at 25°C is 0.7899. While specific gravity of distilled water at 25°C is 1. The mixture of water and alcohol (ethanol) will have specific gravity between these two limits, i.e., 0.7899 and 1. The more the water content in the given sample the specific gravity is near to 1. When the concentration of alcohol (ethanol) in the sample is higher, the specific gravity falls near to 0.7899 g. Hence, the specific gravity of mixture of water and ethanol cannot be more than 1 and cannot be <0.7899.

- The weight of empty specific gravity bottle (SGB) along with stopper was measured (W1)
- The specific gravity bottle was filled with pure distilled water up to its rim and put the stopper. The excess amount of water that comes out of the stopper must be wiped out and the specific gravity was determined at 25°C
- The percentage w/v of ethyl alcohol at 15.56°C in the preparation was calculated by reference to the accompanying table. If the specific gravity was found to be between 2 values the percentage of alcohol should be obtained by interpolation.
- The determined specific gravity and a standard relative density table at 25°C was used to find the corresponding percentage of alcohol (% w/w or % v/v).

Calculations:

Weight of empty specific gravity bottle (SGB) W1= ---------- g

Weight of specific gravity bottle + distilled water W2 = ---------- g

Weight of water (W2-W1) = ---------- g

Weight of specific gravity bottle and distillate (alcohol) W3 = ---------- g

Weight of distillate (W3 -W1) = ---------- g

Apparent Specific gravity = $\frac{\text{Weight of distillate}}{\text{Weight of water}}$

Determine the alcohol content by referring AOAC chart.

Report

The alcohol content of the given sample of Asava/Arista was found to be ----------.

AOAC Chart

Percentages by volume at 15.56°C (60°F) of ethyl alcohol corresponding to apparent specific gravity at various temperatures												
Apparent specific gravity	15.56/15.56	20/20	22/22	24/24	25/25	26/26	28/28	30/30	32/32	34/34	35/35	36/36
1.0000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.9999	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
98	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
97	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
96	0.27	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.24
95	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33
94	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
93	0.47	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46	0.46
92	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53
91	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
90	0.67	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	0.66
89	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
88	0.80	0.80	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79
87	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.86	0.86	0.86	0.86	0.86
86	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93	0.93
85	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99
84	0.07	0.07	0.07	0.07	0.07	0.07	1.06	1.06	1.06	1.06	1.06	1.06
83	0.14	0.14	0.14	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
82	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19
81	0.27	0.27	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.26	0.26	0.26
80	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.32	0.32	0.32	0.32	0.32
79	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.39	0.39	0.39	0.39	0.39
78	0.48	0.48	0.48	0.47	0.47	0.47	0.47	0.46	0.46	0.45	0.46	0.46
77	0.54	0.54	0.54	0.54	0.54	0.53	0.53	0.53	0.53	0.53	0.52	0.52
76	0.61	0.61	0.61	0.60	0.60	0.60	0.60	0.59	0.59	0.59	0.59	0.59
75	0.68	0.68	0.68	0.67	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66
74	0.75	0.75	0.75	0.74	0.74	0.73	0.73	0.73	0.73	0.72	0.72	0.72
73	0.82	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80	0.79	0.79	0.79
72	0.88	0.88	0.88	0.87	0.87	0.87	0.86	0.86	0.86	0.85	0.85	0.85
71	0.95	0.95	0.95	0.94	0.94	0.94	0.93	0.93	0.93	0.92	0.92	0.92
70	2.02	2.02	2.02	2.01	2.01	2.01	2.00	2.00	2.00	0.99	0.99	0.99
69	0.09	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.06	2.05	2.05	2.05
68	0.16	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.13	0.12	0.12	0.12
67	0.23	0.22	0.22	0.21	0.21	0.21	0.20	0.20	0.20	0.19	0.19	0.19

66	0.30	0.29	0.29	0.28	0.28	0.28	0.27	0.27	0.27	0.26	0.26	0.26
65	0.37	0.36	0.36	0.35	0.35	0.35	0.34	0.34	0.33	0.32	0.32	0.32
64	0.43	0.43	0.43	0.42	0.42	0.42	0.41	0.41	0.40	0.39	0.39	0.39
63	0.50	0.50	0.50	0.49	0.49	0.49	0.48	0.48	0.47	0.46	0.46	0.46
62	0.57	0.57	0.57	0.56	0.56	0.56	0.55	0.54	0.54	0.53	0.53	0.53
61	0.64	0.64	0.64	0.63	0.63	0.63	0.62	0.61	0.60	0.60	0.59	0.59
60	0.71	0.70	0.70	0.70	0.70	0.70	0.69	0.68	0.67	0.67	0.66	0.66
59	0.78	0.77	0.77	0.77	0.77	0.77	0.76	0.75	0.74	0.74	0.73	0.73
58	0.85	0.84	0.84	0.83	0.83	0.83	0.82	0.82	0.81	0.81	0.80	0.80
57	0.92	0.91	0.91	0.90	0.90	0.90	0.89	0.88	0.87	0.87	0.86	0.86
56	0.99	0.98	0.98	0.97	0.97	0.97	0.96	0.95	0.94	0.94	0.93	0.93
55	30.06	3.05	3.05	3.04	3.04	3.04	3.03	3.02	3.01	3.01	3.00	3.00
54	0.13	0.12	0.12	0.11	0.11	0.11	0.10	0.09	0.08	0.08	0.07	0.07
53	0.20	0.19	0.19	0.18	0.18	0.18	0.17	0.16	0.15	0.15	0.14	0.14
52	0.27	0.26	0.26	0.25	0.25	0.25	0.24	0.23	0.22	0.22	0.21	0.21
51	0.34	0.33	0.33	0.32	0.32	0.32	0.31	0.30	0.29	0.28	0.27	0.27
50	0.41	0.40	0.40	0.39	0.39	0.39	0.38	0.37	0.36	0.35	0.34	0.34
49	0.49	0.47	0.47	0.46	0.46	0.46	0.45	0.44	0.43	0.42	0.41	0.41
48	0.56	0.54	0.54	0.53	0.53	0.53	0.52	0.51	0.50	0.49	0.48	0.48
47	0.63	0.61	0.61	0.60	0.60	0.60	0.59	0.58	0.57	0.56	0.55	0.55
46	0.70	0.68	0.68	0.67	0.67	0.67	0.66	0.65	0.64	0.61	0.62	0.62
45	0.77	0.76	0.75	0.74	0.74	0.74	0.73	0.72	0.70	0.69	0.68	0.68
44	0.84	0.83	0.82	0.81	0.81	0.81	0.79	0.78	0.77	0.76	0.75	0.75
43	0.91	0.90	0.89	0.88	0.88	0.88	0.86	0.85	0.84	0.81	0.82	0.82
42	0.99	0.97	0.96	0.95	0.95	0.95	0.93	0.92	0.91	0.90	0.89	0.89
41	4.06	4.04	4.03	4.02	4.02	4.02	4.00	0.99	0.98	0.97	0.96	0.96
40	0.13	0.11	0.10	0.10	0.09	0.09	0.07	4.06	4.05	4.04	4.03	4.03
39	0.20	0.18	0.17	0.17	0.16	0.16	0.14	0.13	0.12	0.11	0.10	0.10
38	0.28	0.26	0.25	0.25	0.24	0.23	0.21	0.20	0.19	0.18	0.17	0.17
37	0.35	0.33	0.32	0.32	0.31	0.30	0.28	0.27	0.26	0.25	0.24	0.24
36	0.42	0.40	0.39	0.39	0.38	30.7	0.36	0.35	0.33	0.32	0.31	0.30
35	0.50	0.48	0.47	0.46	0.45	0.44	0.43	40.2	0.40	0.39	0.38	0.37
34	0.57	0.55	0.54	0.53	0.52	0.51	0.50	0.49	0.47	0.46	0.45	0.44
33	0.64	0.62	0.61	0.60	0.59	0.58	0.57	0.55	0.54	0.53	0.52	0.51
32	0.71	0.69	0.68	0.67	0.66	0.65	0.64	0.63	0.61	0.60	0.59	0.58
31	0.79	0.77	0.76	0.75	0.74	0.73	0.72	0.70	0.68	0.67	0.66	0.65

ESTIMATION OF ALDEHYDE CONTENT

AIM

To estimate the percentage of aldehyde content in the given sample of volatile oil.

PRINCIPLE

The determination of aldehyde content is important in determining the quality of oils. Aldehydes are mainly formed by antioxidation of lipids and are the most important contributors of rancid and unpleasant flavors in oxidized oils and some aldehydes are known to cause potential damage to biological system. The aldehyde content is estimated by hydroxylamine hydrochloride method. It involves the interaction of aldehyde with hydroxylamine hydrochloride with the formation of aldoxime with liberation of equal amount of hydrochloric acid. The liberated HCl is titrated against standard alkali (NaOH) and from this, the aldehyde content is calculated.

Reaction

$C_6H_5CH = CH.CHO + H_2NOH.HCl \longrightarrow C_6H_5CH = CH.CH=NOH + HCl + H_2O$

Cinnamic Aldehyde + Hydroxylamine hydrochloride Aldoxime (Cinnamaldoxime)

Cinnamon oil

To 1g of accurately weighed cinnamon oil, 5 ml of benzene and 10 to 15ml of hydroxylamine hydrochloric acid reagent in alcohol was added and titrated with 0.5M potassium hydroxide in alcohol (60%) using Methyl red as indicator.

Each ml of 0.5M potassium hydroxide in alcohol (60%) is equivalent to 0.07672g of cinnamaldehyde.

Lemon oil:

About 10g of the lemon oil was weighed accurately in to a stoppered tube approximately 25 mm in diameter and 150 mm in length; 7 mL of hydroxylamine hydrochloride reagent in alcohol (60%) was added and 1 drop of solution of methyl orange; shaken and neutralised the liberated acid with 0.5N potassium hydroxide in alcohol (60%) until the red colour changes to yellow. Titration was continued with the shaking and neutralising, until the full yellow colour of the indicator is permanent in the lower layer, after shaking vigorously for two minutes and allowing separation to take place. The reactions complete in about 15 mins.

Each mL of 0.5N potassium hydroxide in alcohol (60%) is equivalent to 0.07672g of Citral.

This procedure gives an approximate determination of Citral in the oil. A second determination was carried out in exactly the same manner, using, as the colour standard for the end point, the titrated liquid of the first determination with the addition of 0.5mL of 0.5N potassium hydroxide in alcohol (60%). The accurate value was calculated from the second determination.

NOTE: The volume of the hydroxylamine hydrochloride reagent in alcohol (60%) used is varied according to the Citral content of the oil, and must exceed by 1 to 2 mL the volume of 0.5N potassium hydroxide in alcohol (60%) required.

Lemon grass oil

Carry out the process described for lemon oil, using about 1g, accurately weighed, of the lemon grass oil, with 5 mL of benzene and 10 to 15mL of hydroxylamine hydrochloride reagent in alcohol (60%), according to the aldehyde content of the oil. The volume of hydroxylamine hydrochloride reagent in alcohol (60%) used must exceed by 1 to 2mL of the volume of 0.5N potassium hydroxide in alcohol (60%) required.

Each mL of 0.5N potassium hydroxide in alcohol (60%) is equivalent to 0.07672g of Citral.

Reagents

A solution containing in 1000 ml 28.05 g of potassium hydroxide prepared with alcohol (60 per cent) and standardised by means of 0.5 M HCl by running the alkali into the acid, until the full yellow colour of the indicator, a 0.2 per cent w/v solution of methyl orange in alcohol (60 per cent), is obtained.

Standardization of 0.5 M hydrochloric acid:

About 0.75g of anhydrous sodium carbonate was weighed accurately, previously heated at about 270°C for 1hr. Dissolved it in 50ml of water and added 0.1ml methyl red solution, titrated with 0.5M hydrochloric acid solution becomes faintly pink, heated the solution.

Each ml of 0.5 M HCL= 0.0265 of sodium carbonate solution.

Standardization of 0.5 alcoholic potassium hydroxide

About 15 g of potassium hydroxide was dissolved in ethanol (60%v/v) and make upto 100 ml of water. 20 ml of 0.5 M hydrochloric acid was pipetted into a conical flask, 0.1ml of phenolphthalein solution was added and titrated with ethanolic Potassium hydroxide solution until the permanent pale pink color was produced. Each ml of 0.5 M hydrochloric acid is equivalent to 0.02806 g of potassium hydroxide

Calculation:

Standardization of 0.5 M hydrochloric acid

Weight of sodium carbonate = 0.7501g

Sodium Carbonate Vs 0.5M hydrochloric acid

S.No	Content of the Flask	Burette reading			Volume of 0.5M HCl	Indicator	End point
		Initial		Final			

Molarity of 0.5M hydrochloric acid = $\frac{0.5 \times \text{wt. of sodium carbonate taken}}{\text{Titre value} \times \text{Eq. Factor (0.0265)}}$

0.5M Hydrochloric acid Vs 0.5M potassium hydroxide

S.No	Content of flask	Burette reading		Volume of 0.5M KOH	Indicator	End point
		Initial	Final			

Molarity of 0.5M potassium hydroxide (N_2) =

Volume of 0.5M potassium hydroxide (V_2) =

Molarity of 0.5M hydrochloric acid (V_1) =

Volume of 0.5M hydrochloric acid (N_1) =

Molarity of 0.5M potassium hydroxide (N_2) = $\frac{V_1N_1}{V_2}$

Assay of cinnamon oil:

Weight of cinnamon oil =

Cinnamon oil Vs 0.5M potassium hydroxide

S.No	Content of flask	Burette reading		Volume of 0.5M KOH	Indicator	End point
		Initial	Final			

$$\text{Aldehyde content} = \frac{(B - S) \times \text{Equivalent factor} \times \text{Molarity of KOH}}{\text{Weight of the Sample}} \times 100$$

B = Volume of 0.5M KOH used in blank titration

S = Volume of 0.5M KOH consumed for sample

Assay of Lemon oil:

Weight of Lemon oil =

Lemon oil Vs 0.5M potassium hydroxide

S.No	Content of flask	Burette reading		Volume of 0.5M KOH	Indicator	End point
		Initial	Final			

$$\text{Aldehyde content} = \frac{(B - S) \times \text{Equivalent factor} \times \text{Molarity of KOH}}{\text{Weight of the Sample}} \times 100$$

B = Volume of 0.5M KOH used in blank titration

S = Volume of 0.5M KOH consumed for sample

STANDARD VALUE

Lemon oil- not less than 4.0% w/w of aldehyde, calculated as Citral $C_{10}H_{16}O$.

Cinnamon oil- contains not less than 55.0% w/w and not more than 70.0% w/w of cinnamic aldehyde C_9H_8O.

Lemon grass oil- not less than 75.0% w/w of aldehyde, calculated as Citral $C_{10}H_{16}O$.

Report

The aldehyde content of the given sample of cinnamon oil was found to be ---------- and was found to be within the limit ----------

The aldehyde content of the given sample of Lemon oil was found to be ---------- and was found to be within the limit ----------

DETERMINATION OF PHENOL CONTENT

AIM

To determine the phenol content present in the given crude drug.

PRINCIPLE

Total phenolic amounts are determined as equivalent to gallic acid using the Folin-Ciocalteu reagent. The determination is based on the redox reaction in which phenolic compounds reduce the Folin-Ciocalteu reagent in an alkaline medium and turn into oxidized form. Phenolic compounds react with Folin–Ciocalteu reagent under alkaline conditions to form a blue-colored complex due to reduction of phosphomolybdic–phosphor tungstic acid complexes. The intensity of the blue color is proportional to the phenolic content and is measured spectrophotometrically at 760–765 nm**.** Results are expressed as gallic acid equivalents (GAE)**.**

Preparation of Standard Stock Solution: 100 µg/ml Gallic acid standard stock solution was prepared by dissolving 10mg Gallic acid in methanol and makeup volume up to 100 ml with methanol in volumetric flask.

Preparation of Standard Solution: About 2, 4, 6, 8, and 10 ml of standard stock solution, were transferred into 10 ml volumetric flask respectively and diluted to obtain concentrations of 20, 40, 60, 80, and 100 µg/mL**.**

Procedure

1. About 1 mL of standard or sample solution was pipette into a test tube and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken.
2. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water.
3. It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed.
4. After incubation, absorbance was measured at 750 nm using UV- visible spectrophotometer.
5. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard.
6. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of the test sample was expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass.
7. The concentration of Gallic acid in each sample was calculated from the regression equation using their absorbance. Finally, these results were converted to the total phenolic

content (TPC) as milligrams of Gallic acid equivalent per gram of dry extract (mg GAE/g) using Eq.

$$C = \frac{C1 \times V}{m}$$

where

C is total phenol content in mg/g, in GAE (Gallic acid equivalent)

C_1 is the concentration of Gallic acid established from the calibration curve in mg/mL

V is the volume of the extract in mL

m is the weight of the dry plant extract in g

REPORT

The phenol content of the given sample of crude drug was found to be ----------.

DETERMINATION OF TOTAL ALKALOIDS

AIM

To determine the total alkaloids, present in the crude drug Cinchona bark.

Chemical reagents required

Ammonia solution, chloroform, methanol, hydrochloric acid, sodium hydroxide, methyl red, bromocresol green, quinine

Theory

Alkaloid are organic compound, basic in nature containing one or more nitrogen atoms normally of heterocyclic nature derived from amino acids, with a pronounced physiological activity and optically active. Alkaloid molecules have a unique property, an ability to work as either hydrogen-acceptor or hydrogen-donor for hydrogen bonding, depending on the type of amine functionality present in alkaloids. This property is critically important for the interaction (binding) between targets (enzymes, proteins, and receptors) and drugs (ligands). It has been observed that the alkaloids invariably occur in the plant sources as the salt of acids, such as: oxalates, tannates etc. Therefore, when the plant substance is exposed to an alkaline medium (eg. dil. ammonia, sodium carbonate), the alkaloidal salts are readily converted to the corresponding alkaloid bases. The alkaloidal base can be extracted by three different types of solvents.

a) **Extraction with Water-Miscible Solvents**: A plethora of alkaloids and their respective salts are soluble in alcohols, such as: methanol, ethanol, isopropanol. The extraction of total alkaloids with alcohol is highly recommended because of its maximum efficiency.

b) **Extraction with Water-Immiscible Solvents:** The most widely used water-immiscible solvents for the extraction of alkaloids are: chloroform, diethyl ether (solvent ether) and isopropyl ether. Interestingly, chloroform is regarded as the choicest water-immiscible solvent for a broad-spectrum of alkaloids present in the plant kingdom and extracts them with varying degrees of ease.

c) **Extraction with Water**: The crude drug is subjected to extraction with water previously acidified with dilute solution of HCl, H_2SO_4 or CH_3COOH, which is subsequently rendered alkaline, preferably with dilute NH_4OH solution and finally extracted with a water-immiscible solvent viz., chloroform, diethyl ether (solvent ether) and isopropyl ether.

Extraction of Alkaloids

1. About 5 g of the coarsely powdered crude drug was weighed and extracted with methanol for 24 h in a continuous extraction apparatus at 260^{o} C.
2. The methanol extract was filtered and concentrated in a rotary evaporator under vacuum at temperature of 450^{o}C to dry.
3. A part of this residue was dissolved in 0.1N HCl and then filtered. To the filtrate added ammonia solution dropwise until it reaches pH 9-10.
4. The alkaline filtrate was extracted with 10 mL chloroform in a separating funnel (thrice).
5. The chloroform layer was evaporated to dryness to obtain crude alkaloids

DETERMINATION OF ALKALOID CONTENT BY TITRIMETRIC METHOD

The residue of crude alkaloid extract was dissolved in a measured amount of 0.02N HCl and titrated against 0.02N NaOH using methyl red as an indicator.

Each 1ml of N/10 hydrochloric acid – 0.03091 g of quinine

The total alkaloid content (%) was calculated using the formula:

$$\frac{\text{Volume of NaOH (Titre value)} \times \text{Normality of HCl} \times \text{Equivalent weight} \times 100}{\text{Weight of the powdered sample taken}}$$

DETERMINATION OF ALKALOID CONTENT BY SPECTROPHOTOMETRIC METHOD

Reagent Preparation

Bromocresol green (BCG) solution was prepared by heating 69.8 mg bromocresol green with 3 ml NaOH 2N and 5 ml distilled water until completely dissolved. The solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of sodium phosphate 2M (71.6 g Na_2HPO_4 in 1000 ml distilled water) to 4.7 with citric acid (42.02 g citric acid in 1000 ml).

Preparation of Standard solution

Quinine standard solution (100 µg/ml) was made by dissolving 1 mg pure quinine (Sigma Chemical, USA) in 10 ml distilled water.

About 0.4, 0.6, 0.8. 1.0 and 1.2 ml of quinine standard solution was measured accurately and transferred each to different separated funnels. Then, 5 ml pH phosphate buffer solution and 5 ml BCG solution was added and shaken the mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10 ml volumetric flask and then diluted to adjust the volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm blank prepared as above but without atropine.

Preparation of Test solution

The dried alkaloid residue was dissolved in a known volume of methanol. Then 5 ml of BCG solution and 5 ml phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, 4 ml chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. Compared with a standard calibration curve of known alkaloid (quinine) and determined the alkaloid content.

RESULT:

The total alkaloid present in the drug was found to be ---------- **.**

References

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