



PRACTICAL LAB MANUAL

Herbal Drug Technology

B. Pharm VIth Semester

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1. PRELIMINARY PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT OF *Azadirachta indica* (NEEM)

Aqueous extract of *Azadirachta indica* (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols as per Harborne 1998.

Extraction of crude drug

- Take 50 gm of powdered crude drug and macerate it with 500 ml of water for 24 hrs.
- Then occasionally shake with 6hr time period and allow it to stand for 18 hr.
- After filtration evaporate the filtrate to dryness in a tare flat bottom shallow dish.

Preparation of test solution

- Take 500 mg of extract and dissolve it in 100 ml of water. stir the solution till the extract is completely soluble in water.
- The sample solution is then subjected to various qualitative test to reveal the presence or absence of common phytopharmaceuticals.

TEST FOR ALKALOIDS

About 2 gm of the powdered material was mixed with 1gm of calcium hydroxide and 5 mL of water into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. To this 200 mL of chloroform was added, mixed well and refluxed for half an hour on a water bath. Then it was filtered and the chloroform was

evaporated. To this 5 mL of dilute hydrochloric acid was added followed by 2 mL of each of the following reagents.

MAYER'S TEST:

A small quantity of the extract was treated with Mayer's reagent. Cream colour precipitate indicates the presence of alkaloids.

DRAGENDORFF'S TEST:

A small quantity of the extract was treated with Dragendorff's reagent. Orange brown precipitate indicates the presence of alkaloids.

WAGNER'S TEST:

A small quantity of extract was treated with Wagner's reagent. Reddish brown precipitate indicates the presence of alkaloids.

HAGER'S TEST:

A small quantity of extract was treated with Hager's reagent. Yellow precipitate indicates the presence of alkaloids.

TEST FOR PURINE GROUP (MUREXIDE TEST)

The residue obtained after the evaporation of chloroform was treated with 1 mL of hydrochloric acid in a porcelain dish and 0.1 gm of Potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapour of dilute ammonia solution. No purple Colour was obtained indicating the absence of purine group of alkaloids.

TEST FOR INDOLE

To the test solution, add acetic acid and trace amount of anhydrous FeCl_3 , under – Lay / H_2SO_4 intense blue at interface.

TEST FOR QUINOLINE (Thalleioquin Test)

To the extract, add 1 drop of dilute sulphuric acid and 1ml of water. Add bromine water drop wise till the solution acquires permanent yellow colour and add 1mL of dilute ammonia solution, emerald green colour is produced. The powdered drug when heated with glacial acetic acid in dry test tube, evolves red fumes, which condense in the top portion of the tube. The bark, when moistened with sulphuric acid and observed under ultraviolet light shows a blue fluorescence due to the methoxy group of quinine and quinidine.

TEST FOR CARBOHYDRATES

MOLISCH'S TEST

The extract of the powdered drug was treated with 2-3 drops of 1% alcoholic α naphthol and 2mL of concentrated sulphuric acid was added along the sides of the test tube. A purple colour indicating the presence of carbohydrates.

FEHLING'S TEST

The extract of the powdered leaf was treated with Fehling's solution I and II and heated on a boiling water bath for half an hour. Red precipitate was obtained indicating the presence of free reducing sugars.

BENEDICT'S TEST

The extract of the powdered leaf was treated with equal volume of Benedict's reagent. A red precipitate was formed indicating the presence of reducing sugar.

TEST FOR ANTHRAQUINONE GLYCOSIDES

BORNTRAGER'S TEST

The powdered drug was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The organic layer was separated to which ammonia solution was added slowly. No pink colour was observed in ammoniacal layer showing the presence of anthraquinone glycosides.

MODIFIED BORNTRAGER'S TEST

About 0.1 g of the powdered drug was boiled for 2 minutes with dil.HCl and few drops of FeCl₃ solution, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dil.NH₃ solution was added to the benzene extract. No pink colour was observed in ammoniacal layer showing the presence of glycosides.

TEST FOR CARDIAC GLYCOSIDES (FOR DEOXY SUGAR)

KELLER KILIANI TEST

About 1 g of the powdered leaf was boiled with 10 mL of 70 % alcohol for 2 minutes, cooled and filtered. To the filtrate 10 mL of water and 5 drops of solution of lead subacetate were added and filtered, evaporated to dryness. The residue was dissolved in 3 mL of glacial acetic acid. To these 2 drops of ferric chloride solution was added. Then 3 mL of concentrated H₂SO₄ was added to the sides of the test tube carefully and observed. No reddish brown layer was observed indicating the absence of deoxysugars.

RAYMOND TEST

Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

LEGAL'S TEST

Test solution when treated with pyridine made alkaline by sodium nitro prusside solution gives pink to red colour.

TEST FOR CYANOGENETIC GLYCOSIDES

Small quantity of the powder was placed in a stoppered conical flask with just sufficient water, to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2 hours in a warm place. Brick red colour was produced on the paper indicating the presence of cyanogenetic glycosides.

TEST FOR COUMARIN GLYCOSIDES WITH AMMONIA

Take a drop of ammonia on a filter paper; to this add a drop of aqueous extract of leaves. Development of fluorescence shows positive test for coumarins.

WITH HYDROXYLAMINE HYDROCHLORIDE

To ethereal extract, added one drop of alcoholic KOH. It was then heated, cooled and acidified with 0.5N hydrochloric acid. Violet colour developed upon addition of a drop of 1 % w/v FeCl_3 indicated presence of coumarins.

TEST FOR STEROLS

The powdered drug was first extracted with petroleum ether and evaporated to a residue. Then the residue was dissolved in chloroform and tested for sterols.

SALKOWSKI'S TEST

A few drops of concentrated sulphuric acid was added to the above solution, shaken well and set aside. The lower chloroform layer of the solution turned red in colour indicating the presence of sterols.

TEST FOR LIBBERMANN – BURCHARD'S

To the chloroform solution a few drops of acetic anhydride and 1 mL of concentrated sulphuric acid were added through the sides of the test tube and set aside for a while. At the junction of two layers a brown ring was formed. The upper layer turned green indicating the presence of sterols.

TEST FOR SAPONINS

FROTH TEST

0.1 g of powder was vigorously shaken with 5ml of distilled water in a test tube for 30 seconds and was left undisturbed for 20 min, persistent froth indicated presence of saponins.

TEST FOR TANNINS

FERRIC CHLORIDE

Small quantity of the powdered drug was extracted with water. To the aqueous extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

GOLD BEATER'S SKIN TEST

Add 2 % hydrochloric acid to all small piece of g old beater's skin, rinses it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to a 1% ferrous sulphate solution. A brown or black colour on the skin indicates presence of tannin.

TEST FOR PHENOLIC COMPOUNDS

FERRIC CHLORIDE

A small quantity of the powdered drug was extracted with water. To the alcoholic extract few drops of ferric chloride solution was added. Bluish black colour was produced indicating the presence of tannins.

TEST FOR FOLIN COICALTEU REAGENT

To a drop of methanolic extract of a few drop of Folin Ciocalteu reagent was added, development of bluish green colour showed presence of phenol.

TEST FOR FLAVONOIDS

SHINODA'S TEST

Little of the powdered drug was heated with alcohol and filtered. To the test solution magnesium turnings and few drops of concentrated hydrochloric acid were added. Boiled for five minutes. Red colour was obtained indicating the presence of flavonoids.

ALKALI TEST

To the small quantity of test solution 10% aqueous sodium hydroxide solution was added. Yellow orange colour was produced indicating the presence of flavonoids.

LEAD ACETATE

To the test solution add a mixture of 10 % lead acetate in few drops added. It gives white

precipitate.

TEST FOR ACID

To the small quantity of test solution, few drops of concentrated sulphuric acid were added.

Yellow orange colour was obtained indicates the presence of flavonoids.

TEST FOR PROTEIN AND AMINO ACIDS

MILLON'S TEST

Small quantity of acidulous – alcoholic extract of the powdered drug was heated with Millon's reagent. White precipitate turned red on heating indicate the presence of proteins.

BIURET TEST

To one portion of acidulous – alcoholic extract of the powdered drug one ml of 10% sodium hydroxide solution and one drop of dilute copper sulphate solution were added. Violet colour was obtained indicating the presence of proteins.

NINHYDRIN TEST

To the test solution add Ninhydrin solution, boil, violet colour indicates presence of amino acid.

TEST FOR SULPHUR CONTAINING AMINO ACID

5 ml test solution is mixed with 2 ml 40 % sodium hydroxide and 2 drops of 10% lead acetate solution. Then boil the solution turned black or brownish due to PLS formation.

TEST FOR TERPENOIDS

Little of the powdered drug was extracted with chloroform and filtered. The filtrate was warmed gently with tin and thionyl chloride. Pink colour solution appeared which indicated the presence of terpenoids.

TEST FOR CAROTENOIDS (Carr-Price reaction)

Extract treated with concentrated sulphuric acid and with a chloroform solution of antimony trichloride. Deep blue colour appeared which indicated the presence of carotenoids.

TEST FOR VOLATILE OIL

Weighted quantity (250 gm) of fresh leaves were extracted and subjected to hydro distillation using volatile oil estimation apparatus.

TEST FOR FIXED OIL

A small amount of the powder was pressed in between in the filter paper and the paper was heated in an oven at 105⁰ C for 10 minutes. A translucent greasy spot appeared indicating the papers.

TEST FOR GUM

The small quantity of extract was added with few drops of alcohol to form white precipitate which indicates the presence of gum.

TEST FOR MUCILAGE

Few ml of aqueous extract was prepared from the powdered crude drug was treated with ruthenium red. Red colour was produced indicating the presence of mucilage.

TEST FOR BETACYANINS

To 1 ml of plant extract, 1 mL of 2N NaOH was added and heated for 5 minutes at 100⁰C. Formation of yellow colour indicated the presence of betacyanins.

TEST FOR ANTHOCYANIN

About 0.2g of plant extract was weighed in separate test tube, 1ml of 2N sodium hydroxide was added, and heated for 5 minutes. Observed for the formation of bluish green colour which indicates the presence of anthocyanin.

TEST FOR LEUCOANTHOCYANINS

To 1 ml of plant extract, 1 ml of isoamyl alcohol was added. Formation of red colour indicated the presence of leucoanthocyanins.

TEST FOR QUINONES

To 1 ml of plant extract, 1 mL of conc. H_2SO_4 was added. Formation of red colour indicated the presence of quinones.

TEST FOR EMODINS

The dry extract was added to 25% ammonia solution. The formation of a cherry red colour solution indicated the presence of emodins.

TEST FOR COUMARINS

To 1 ml of plant extract, 3 ml of NH_4OH and 2mL of benzene was added. Formation of red colour indicated the presence of coumarin.

TEST FOR RESINS

The extracts were treated with acetone. A small amount of water was then added and shaken. Appearance of turbidity indicates the presence of resins.

TEST FOR PHLOBATANNINS

About 2 ml of aqueous extract was added to 2ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was an evidence for the presence of phlobatannins.

REPORT

Preliminary phytochemical screening of the aqueous extract of *Azadirachta indica* shows the presence of _____

Preliminary phytochemical screening of the aqueous extract of *Azadirachta indica* shows the absence of _____

DETERMINATION OF THE ALCOHOL CONTENT OF ASAVA AND ARISTA

AIM: To determine the alcohol content of asava and arista.

REQUIREMENTS:

- Digital weighing balance
- Beaker
- Measuring cylinder
- Distillation flask
- Separating funnel
- Heating metal
- Water bath
- Specific gravity bottle
- Water

PROCEDURE:

Measure 25 ml of formulation by measuring cylinder and transfer to distillation flask having capacity of 500 ml. Wash the measuring cylinder with 150 ml of water and add it to the flask. Add some porcelain pieces to the flask and distill it. Collect about 90 ml of distillate from this take 25 ml of distillate and dilute it to 100 ml with water and with the help of specific gravity bottle determine the specific gravity of liquid at 25°C. Follow the alcohol content table with specific gravity and determine the V/V alcohol content in sample.

Calculate the % alcohol content by using multiplication factor.

DETERMINE THE SPECIFIC GRAVITY:

PROCEDURE:

A tube of known weight (W) was filled first with essential oil and then with water and the respective weight w₁ and w₂ was determined. Then, the specific gravity was calculated using the following formula:

$$d = \frac{w_1 - w}{w_2 - w}$$

RELATIVE DENSITY WITH PERCENT ETHANOL CONTENT:

Relative density at 25°C	Per cent ethanol content w/w at 15.56°C	Per cent ethanol content v/v at 15.56°C
0.8158	90	93.3
0.8146	90.5	93.6
0.8131	91	94
0.8118	91.5	94.3
0.8104	92	94.7
0.8090	92.5	95
0.8076	93	95.4
0.8062	93.5	95.8
0.8048	94	96.1
0.8034	94.5	96.5
0.8020	95	96.8
0.8006	95.5	97.1
0.7992	96	97.5
0.7977	96.5	97.8
0.7962	97	98.1
0.7957	97.5	98.4
0.7932	98	98.8
0.7917	98.5	99.1
0.7902	99	99.4
0.7886	99.5	99.7
0.7871	100	100

REPORT:

Alcohol content of Asava and Arista was found to be _____

EVALUATION OF EXCIPIENTS OF NATURAL ORIGINS

Chemical test for Tragacanth.

Aim: To identify the chemical characters of given excipient.

REQUIREMENTS Hydrochloric acid Sodium hydroxide solution Fehling's solution Barium chloride solution Lead acetate Ruthenium red Iodine Caustic potash.

Biological source

A dried exudation obtained from the stems and branches of *Astragalus gummifer* and other Asiatic species of Asragalus. **Family:** Leguminosae

Description

Color: white to slight yellow color

Odour: odourless

Taste: mucilaginous taste

Shape: The gum seeps from the plant in twisted ribbons or flakes that can be powdered

Solubility: 1 gm of the sample in 50 ml of water swells to form a smooth, stiff, opalescent mucilage; insoluble in ethanol and does not swell in 60% (w/v) aqueous ethanol.

Chemical constituents: Tragacanthin (water soluble part), Bassorin (water insoluble part)

Uses: Demulcent, emollient, thickening agent, emulsifying agent, binding agent

PROCEDURE

- 1). To 4 ml of 0.5% w/v solution, add 0.5 ml of hydrochloric acid and heat for 30 minutes on a water bath. Divide the liquid into two parts. (a). To one part, add 1.5 ml of sodium hydroxide solution and Fehling's solution, warm on water bath: red precipitate is produced. (b). To the second part, add barium chloride solution (10%): No precipitate is obtained (distinction from agar)
- 2). To a 0.5% w/v solution of the gum, add 20% w/v solution of lead acetate: A voluminous flocculent precipitate is obtained (distinction from acacia)
- 3). Mount a small quantity of powder in ruthenium red and examine microscopically: Particles do not acquire pink colour (distinction from Indian tragacanth)
- 4). To 0.1 g of powder, add N/50 Iodine: The mixture acquires an olive green colour (distinction from acacia and agar).

5). Powder is warmed with 5% aqueous caustic potash: Canary yellow colour will obtain. Indian tragacanth It is obtained from *Sterculiaurens* Roxburgh; (Fam: Sterculiaceae). It is insoluble in alkali. It has acetous (acetic acid like) odour and starch is absent. It gives brownish colour when boiled with aqueous KOH. It is stained pink by solution of Ruthenium red.

REPORT

From the above morphological characters and chemical tests the given excipient is identified as Tragacanth.

Chemical test for Acacia

Aim: To identify the chemical characters of given excipient acacia.

Biological source: Indian gum is the dried gummy exudation obtained from the stem and branches of *Acacia Arabica* **Family:** Leguminosae

Description

Color: white to slight yellow color

Odour: odourless

Taste: Bland and mucilaginous taste

Shape: Tears are mostly spheroidal or ovoid in shape with approx. diameter of 2.5-3.0 cm

Solubility: soluble in water, insoluble in alcohol.

Constituents:

Gum Arabic consists almost entirely of glycosidal acid named Arabic acid, combined with potassium, magnesium and calcium. By hydrolysis Arabic acid yields 1 molecule of l-rhamnose, 2 molecules of D – galactose and 3 molecules of l– arabinose and an aldobionic acid. It also contains diastase and an oxidase enzyme.

Uses: Demulcent, emollient, thickening agent, emulsifying agent, binding agent also used to form coacervates for microencapsulation of drug.

PROCEDURE:

- 1) Dissolve about 0.25 gm of the coarsely powdered drug in 5 ml of distilled water by shaking in the cold. Add 0.5 ml of hydrogen peroxide and 0.5 ml of benzidine solution, shake and allow to stand for few minutes; a deep blue color or greenish blue color is formed due to the presence of oxidase enzyme.
- 2) A 10% aqueous solution of acacia fails to produce any precipitate with dilute solution of lead acetate (a clear distinction from Agar and Tragacanth); it does not give any colour change with Iodine solution (a marked distinction from starch and dextrin); and it never produces a bluish-black colour with FeCl_3 solution (an apparent distinction from tannins).
- 3) Hydrolysis of an aqueous solution of acacia with dilute HCl yields reducing sugars whose presence are ascertained by boiling with Fehling's solution to give a brick-red precipitate of cuprous oxide

REPORT

From the above morphological characters and chemical tests the given excipient is identified as Acacia.

Chemical test for starch

Biological source:

Starch consist of polysaccharide granules obtained from the grains of maize *Zea mays L.* or of rice *Oryza sativa L* or of wheat *Triticum sativum L.* Family: Graminae or from the tubers of the potato *solanum tuberosum L.* **Family:** solanaceae

Description:

Color: white (Rice and maize starch) , cream (wheat), slight yellow (potato)

Odour: odourless

Taste: mucilaginous taste

Shape: Fine powder or irregular, angular masses

Solubility: It is sparingly soluble in cold water and mostly soluble in hot water after cooling it forms gel

Chemical constituent: Amylose, Amylopectin

Uses: Dusting powder, Pharmaceutical aid, protective and demulcent, Tablet disintegrating agent and diluents

Chemical Test for Starch or Iodine

Amylose in starch is responsible for the formation of a deep blue color in the presence of iodine. The iodine molecule slips inside of the amylose coil. Iodine - KI Reagent: Iodine is not very soluble in water; therefore the iodine reagent is made by dissolving iodine in water in the presence of potassium iodide. This makes a linear triiodide ion complex with is soluble that slips into the coil of the starch causing an intense blue-black color.

REPORT

From the above morphological characters and chemical tests the given excipient is identified as starch.

Chemical test for Honey

Biological source: Honey is the saccharine liquid prepared from the nectar of the flowers by the hive bee *Apis mellifera*, *Apis dorsata* and bees of other species of *Apis*. **Family:** Apidae

Description:

Color: slight yellow to brown yellow

Odour: pleasant

Taste: sweet

Solubility: soluble in water and insoluble in alcohol

Chemical constituents: Glucose 35% ($\pm 3\%$), fructose 45% ($\pm 5\%$), sucrose 2-3% and water (14-20%). Dextrin, maltose, gum, traces of succinic acid, acetic acid, volatile oil, amino acid, protein, coloring matters etc.

Uses: Demulcent, nutritive, mild laxative. It is used as an important component of linctuses and cough mixture, sweetening agent, antiseptic and bactericidal. Used as a vehicle in ayurvedic and unani preparations.

Sl.No.	Test	Observation	Inference
1.	<p>Fiehe's test</p> <p>Take about 3ml of honey + 2ml of ether and shake thoroughly and allow the 2 layers to separate and evaporate to dryness. The upper etherial layer is separated and put in a china dish and evaporate, to the residue add 1% resorcinol and HCl.</p>	<p>Transient pink colour</p> <p>Permanent red colour</p>	<p>Pur honey</p> <p>Adulterated honey(Invert sugar)</p>
2.	<p>Molisch's Test</p> <p>Honey is treated with alpha Naphthol and concentrated sulphuric acid</p>	Purple colour	Presence of carbohydrate
3.	<p>Reducing Sugar Test</p> <p>Heat honey to this add a drop of mixture of Fehling's solution A & B</p>	Brick red colour of cuprous oxide	Presence of monosaccharide

REPORT

From the above morphological characters and chemical tests the given excipient is identified as Honey.

PREPARATION AND EVALUATION OF TURMERIC CREAM

AIM:

To prepare and evaluate 10gm of Turmeric – Aloe Vera Gel Herbal Cream.

PRINCIPLE:

Creams are viscous semi solid emulsions which are meant for external application. They usually contain water soluble base so that it can easily be removed from the skin. When applied to the skin, creams leave no visible evidence of their presence on skin.

Creams consist of medicaments dissolved or suspended in water removable or emollient bases. Creams are of two types,

1. Aqueous creams (O/W)
2. Oily creams (W/O)

Therefore, combining immiscible compounds is possible by mechanical agitation or heat.

HERBAL CREAM FOR 10gms:

- White beeswax – 5gm
- Liquid paraffin – 7ml
- Borax – 2gm
- Water – 5ml
- Perfume – 2 drops
- Curcumin (Turmeric) – 200mg
- Aloe Vera gel – 1gm
- Methyl paraben – 0.02gm
- Ethyl alcohol – 2ml (Q.S to dissolve curcumin)

NOTE: Curcumin content is 3.14% by dry weight basis.

PROCEDURE:

Melted the bees wax with mineral oil by heating on a water bath at the temperature of 70°C. Here, curcumin is not soluble in water. So it was mixed with minimum quantity of ethyl alcohol. This was added to the borax water mixture and heated to the same temperature. Both the temperature were attained at a temperature of 70°C added the aqueous phase with rapid constant stirring until cool. Filtered it into a container and labeled.

EVALUATION PARAMETERS OF TURMERIC HERBAL FORMULATION:

- **DETERMINATION OF PH:**

1gm of prepared tooth paste and add 9ml of freshly boiled and cooled water.

Stir well and make a suspension, PH was determined by using PH meter.

- **SPREADABILITY TEST:**

About 1gm of sample was weighed and placed at the center of the glass plate and another glass plate was placed over it carefully above the glass plate 100 gm weight was placed up on upper slide so that the formulation between two slides was pressed uniformly to form a thin layer, the weight was removed and the excess of formulation adhering to the slides was scrapped off. One of the slides was fixed on which the formulation was placed, the time in which upper slide moves over the lower plate was taken as measure of spreadability. Spreadability is calculated by using the formula:

$$\text{SPREADABILITY} = m \cdot l / t$$

Where,

m – Weight on the slide

l – Length moved on the slide

t – Time taken.

- **SKIN IRRITATION TEST:**

The ointment was placed on patches and covered with gauze for 4 hrs, skin was observed for any signs of redness, inflammation and weeping of scabs.

- **RATION TPENETEST:**

0.5gm of ointment was rubbed over definite area of skin for a given time then unabsorbed was collected from skin and weighed.

USES:

- Anti wrinkle properties of herbal ingredients,
- Anti ageing properties,
- Skin protective properties.

REPORT:

Curcumin – Aloe Vera gel skin Herbal Cream prepared and evaluated.

5.0 PREPARATION AND STANDARDIZATION OF HERBAL LOTION

Method of Extraction

All the drugs were weighed accurately & aqueous extraction had been done 10 times of the weight of the drug i.e. 5g in 50 ml of water on water bath at 80 -100°C. As the solution concentrated up to 20 ml, filtration was done. Residue had been taken & volume was making up to 40 ml, again was boiled. After remaining 20 ml was filtered and the same procedure was followed again.

Composition of lotion

S.NO.	INGREDIENTS	FORMULA % (W/W)	
		F1	F2
1.	Extract	1	2
2.	Glycerin	2.0	2.0
3.	Water	q.s	q.s
4.	Sunflower oil	4.0	4.0
5.	Mineral oil	2.0	2.0
6.	Petroleum jelly	1.0	1.0
7.	Cetyl alcohol	1.5	1.5
8.	Glyceryl monostearate	2.0	2.0
9.	Methyl paraben	0.2	0.2
10.	Propyl paraben	0.2	0.2
11.	Fragrance	0.1 - 1	0.1 -1

Drug formulation

The formulation components used were listed in table. Oil in water emulsion of 1 and 2% of drugs were formulated. The emulsifier (glyceryl monostearate) and other oil soluble components (sunflower oil, mineral oil, petroleum jelly, cetyl alcohol) were dissolved in oil phase (part A) and heated up to 80°C. Extract and water soluble components (glycerin, methyl paraben, propyl paraben) were dissolved in (part B) and heated up to 80°C. After heating, the aqueous phase was added in portions to the oil phase with constant stirring until cooling of emulsifier took place. Perfume was added when the temperature dropped to 45°C ± 50°C.

EVALUATION OF LOTION

Test for thermal stability

Thermal stability of the formulation was determined by the humidity chamber controlled at 60 -70%RH and 37 ± 1°C.

Determination of PH

5 ± 0.01g of the lotion was weighed accurately in a 100ml beaker, 45 ml of water was added & dispersed the lotion in it. The pH of the suspension was determined at 27°C using the pH meter.

Determination of total fatty matter

2g of the sample was weighed in a conical flask, added 25ml of dil. HCL (1% v/v) & refluxed. Poured this into the separating funnel and 50ml of ethyl ether were added into it. The separating funnel was shaken well until two layers were separated. The aqueous layer was separated out and added 50ml portion of ether twice. All the ether extracts were combined and filter through the filter paper containing dried sodium sulphate on it. Distilled off the ether (filtrate) & dried the material remaining in the flask at temperature 60 ± 2°C to constant mass.

Calculation

$$\text{Total Fatty Matter} = 100 \times M1/M2$$

Microbial examination of lotion

1g of material was weighed and aseptically transferred into the conical flask containing 50ml of dil. Phosphate buffer at pH 7.2 and pipette out 1ml portion into 3 sterile plates. Melted soya bean casein digest agar (SCDA) where, M1 = mass in gram of residue M2 = mass in gram of material taken for test.

Determination of water content

10g of the material was weighed and transferred it into the flask. 200ml of toluene and few pieces of pumice stone was added and connected the apparatus with condenser. The flask was heated until toluene was begin to boil and refluxed. When the H₂O was distilled over source of heat was removed.

Calculation

$$\text{Water \% by mass} = V \times D \times 100 / M$$

Where,

V = volume of water in ml at room temperature collecting in receiving tube

D = density of water at room temperature

M = mass in gm of the material taken for the test

GENERAL EVALUATION OF LOTION:

Microbial Examination of lotion

1g of material was weighed and aseptically transferred into the conical flask containing 50ml of dil. Phosphate buffer at pH 7.2 and pipette out 1ml portion into 3 sterile plates. Melted soya bean casein digest agar (SCDA) medium was poured over it (at 45°C) and cooled. After that plates were rotated to mix properly. Then the plates were incubated at 30 ± 40°C for 74 hrs in an inverted portion. Average number of colonies was determined by multiplying the dilution factor.

Patch test

About 1 -3gm of material to be tested was placed on a piece of fabric or funnel and applied to the sensitive part of the skin e.g. skin behind ears. The cosmetic to be tested was applied to an area of 1 sq.m. of the skin. Control patches (of similar cosmetic of known brand) were also applied. The site of patch is inspected after 24 hrs. As there was no reaction the test was repeated three times. As no reaction was observed on third application, the person may be taken as not hypersensitive.

Accelerated stability testing

Accelerated stability testing of prepared formulations conducted at $40 \pm 2^{\circ}\text{C}$ temperature and $75 \pm 5\%$ relative humidity and studied for 90 days.

Report:

Herbal lotion was prepared and evaluated

PREPARATION AND STANDARDIZATION OF METHI-SHIKAKAI SHAMPOO

AIM:

To prepare and standardize Methi-shikakai shampoo.

REQUIREMENTS:

Methi, shikakai, orange peel, distilled water, beaker, glass rod, measuring cylinder, weighing balance, pH meter, evaporating dish, canvas paper and stop watch.

PRINCIPLE:

Herbal shampoos are the cosmetic preparations that with the use of traditional ayurvedic herbs are meant for cleansings the hair and scalp just like the regular shampoo. They are used for removal of oils, dandruff, dirt etc. Methi's protein, nicotinic acids and large amounts of lecithin are highly effective against hair fall and provide strength from the roots. The seed contains a special hormone that enhances hair growth and helps repair the hair structure.

Shikakai is excellent for hair as it does not have side-effects unlike shampoo which are loaded with chemicals to add more lather. It does not strip your hair's natural oils, which means that they are stronger from within and do not look rough and dry. It helps in controlling hair fall and also in reducing dandruff naturally due to its antibacterial action.

PROCEDURE:

Formulation of Methi-shikakai shampoo

- Methi - 250g
- Shikakai - 1g
- Orange peel - Handful
- Water Q.S - 2 liters

Crush all ingredients into powder form. Add sufficient quantity water to produce 2 liters.

STANDARDISATION:

Physical Appearance/Visual Inspection

The formulation prepared was evaluated for the clarity, color, odor and foam producing ability.

Determination of pH

The pH of 10% v/v shampoo solution in distilled water was measured by using pH meter at room temperature.

Determination of physico-chemical parameters

Moisture content at 105°C

Weight about 1gm of material into large weighing bottle and heat on a steam bath under a jet of air for 30 min. Continuous heating at 105°C in oven for 2 hrs, cool in desiccator, weight and report non-volatile matter.

Ash content at 600°C

Weigh 5ml of material place in a flat bottom platinum dish and heat on a steam bath under a jet of air for 1 hr. Remove and add 1gm of ash less cellulose powder, keep the material in dish and heat in a 1k heating lamp till 600°C in muffle furnace. Note the difference in weight.

Determination of % of solid content

4 gram of shampoo were placed in a previously clean dry and weighted evaporating dish. The dish and shampoo was weighed again to confirm the exact weight of shampoo. The liquid portion of the shampoo was evaporated by placing the evaporating dish on the hot plate. The weight and thus % of the solid contents of shampoo left after complete drying was calculated.

Quantitative Estimation of selected phytoconstituents

Foam test

Shake the drug/ sample extract vigorously with water. Persistent foam observed, confirms the presence of saponins.

Hemolytic test

Add drug/ sample extract or dry powder to one drop of blood placed on glass slide.

Hemolytic zone appears.

Saponification test

- Add few drop of 0.5N alcoholic KOH to a small quantity of various extract along with a drop of phenolphthalein separately and heat on a water bath for 1 hour the formation of alkali indicate the presence of fixed oil and fats.
- 5 drop of sample, add pinch of sodium hydrogen sulphate, pungent odour indicate presence of glycerin.

EVALUATION OF SHAMPOO:

Net content

At the beginning of experiment mark the outside of bottle at the surface level of liquid, at the end of experiment empty the bottle and note the volume of water required to fill it to the mark.

Dirt Dispersion

Two drops of shampoo were added in a large test tube contain 10ml of distilled water. 1 drop of India ink was added; the test tube was Stoppard and shakes it ten times. The amount of ink in the foam was estimated as none, light, moderate or heavy.

Wetting Time

The canvas was cut into 1 inch diameter discs having an average weight of 0.44g. The disc was floated on the surface of shampoo solution of 1% w/v and the stop watch started. The time required for the disc to begin to sink was measured acutely and noted as the wetting time.

Surface Tension measurement

Measurements were carried out with a 10% shampoo dilution in distilled water at room temperature. Thoroughly clean the stalagnometer using chronic acid and purified water, because surface tension is highly affected with grease or other lubricants. The data calculated by following equation given below:

$$R_2 = (W_3 - W_1) n_1 \times R_1 (W_2 - W_1) n_2$$

Where,

W1 is weight of empty beaker.

W2 is weight of beaker with distilled water.

W3 is weight of beaker with shampoo solution.

n 1 is no. of drops of distilled water.

n 2 is no. of drops of shampoo solution.

R1 is surface tension of distilled water at room temperature.

R2 is surface tension of shampoo solution.

Specific Gravity

The two methods are commonly used for determination the specific gravity of liquid one method use the hydrometer and instrument that gives a specific gravity reading directly. A second method called a bottle method uses a specific gravity bottle that is a flask makes to hold a known volume of liquid at a specified temperature usually 20°C. The bottle is weighed filled with the liquid. Whose specific gravity is to be found and weight again. The different weight is divided by the weight of equal volume of water to give the specific gravity of the liquid.

Test to evaluate foaming ability and foam stability

Foaming ability was determined by using cylinder shake method. Briefly 50 ml of the 1% commercial or formulated shampoo solution was placed into a 250ml graduated cylinder, it was covered with one hand and shaken 10 times. The total volume of the foam content after 1 minute of shaking was recorded.

Wetting time test

A canvas paper was cut into one inch diameter discs having an average weight of 0.44g. The smooth surface of disc was placed on the surface of 1% v/v shampoo solution and the stop watch started. The time required for the disc to begin to sink was noted down as the wetting time.

Determination of water by Toluene Distillation

Transfer 10 -20gm sample to 250ml round bottom flask, add 50 ml of toluene and 2gm of lamprosin and few glass heads, connect to distillation unit. Distil until no more water is collected in the receiver. Cool, read the volume of water under the toluene at room temperature and calculate % water content.

$$\text{Water content} = \text{volume of water (ml)} \times 100 / \text{weight of sample}$$

REPORT:

Methi-shikakai shampoo was prepared and evaluated.

Standardization of the shampoo was performed by determining the physical parameters such as pH, visual appearance, % of solid contents, foaming ability, wetting time test.

7.0 PREPARATION AND EVALUATION OF ORANGE SYRUP B.P.C

AIM:

To prepare and evaluate 20ml of Orange Syrup B.P.C

REQUIREMENTS:

- ✓ Beaker,.
- ✓ 90% alcohol,
- ✓ Fresh orange peel 250 gm.

PRINCIPLE:

Tincture of orange is an alcoholic extract of fresh bitter orange peel and is prepared by maceration process. Fresh peel is used for this preparation because it is stable and contains a higher proportion of volatile oil than the dried peels. Drying dissipates part of the oil and fresh peels are more aromatic. Alcohol (90%) is used as the menstrum for the maceration process. This preparation contains flavoring oils which are volatile in nature and should therefore be stored in tightly closed containers in a cool place.

PROCEDURE:

PREPARATION OF ORANGE TINCTURE:

Take fresh orange peel and cut into thin slices. Weigh the required quantity of thin slices and macerate it with whole quantity of alcohol in a covered vessel. Allow to macerate for seven days with occasional stirring. Strain the liquid press the marc and mix the expressed liquid to the strained liquid. Clarify the combined liquids by filtration.

I.P. FORMULA:

- Fresh orange peel in thin slices – 250gm
- Alcohol – 1000ml

PREPARATION OF SIMPLE SYRUP:

A 100 ml empty beaker was weighed and the weight was noted. Half the quantity of purified water was placed into the beaker. Calculated quantity of sucrose was weighed and added to the water. Sucrose was dissolved by heating with occasional stirring. After cooling, purified water was added to make up the required volume.

I.P. FORMULA:

- Sucrose – 667gm
- Purified water (Qs) – 1000ml

PREPARATION OF ORANGE SYRUP B.P.C:

The measured quantity of orange tincture was mixed with 3/4th quantity of simple syrup. The volume was then made up with remaining syrup.

I.P. FORMULA:

- Tincture of orange – 60 ml
- Simple syrup – 1000ml

STANDARDISATION:

- **PHYSICAL APPEARANCE/ VISUAL INSPECTION:**

The formulation prepared was evaluated for the clarity, color, and odour.

- **DETERMINATION OF PH:**

The PH of 10%v/v of syrup was measured by using PH meter at room temperature.

REPORT:

Orange Syrup B.P.C was prepared and standardized.

8.0 PREPARATION AND EVALUATION OF CHURNA MIXTURE

AIM:

To prepare and evaluate 50gm of Churna mixture.

PRINCIPLE:

Churna is the best known example of mixture. Churna is a powdered form of medications used in the treatment of disorders in the Ayurvedic system of medicine. It is the most basic and simplest form of Ayurvedic medicine containing a fine powder of herbs.

Churna may contain a single herb or a mixture of several herbs.

GENERAL METHOD OF PREPARATION OF CHURNA:

All the ingredients required for the Churna should be cleaned thoroughly and dried well in the shade or in the sun separately to ensure the complete absence of moisture in the same. Each ingredient is powdered finely and then, sieved to remove any coarse particles. The ingredients are weighed separately and then, mixed together. It should be noted that some herbs contain a fibrous matter. Hence, the weight of such herbs may vary before and after drying. Hence, it is important to powder and weigh them separately so that the correct quantity of each herb can be present in the final product.

FORMULATION FOR 50 gm OF CHURNA:

- Zingiber officinale (powder) – 16.7 gm
- Foeniculum vulgare (powder) – 16.7 gm
- Cinnamomum zeylanicum (powder) – 8.7 gm
- Trachyspermum ammi (powder) – 8.7 gm

PROCEDURE:

- ❖ Take all the ingredients in given proportion.
- ❖ Mix the powders thoroughly.
- ❖ Pass the mixture through sieve number 60.
- ❖ Pack it in well close container and store it in dry place.

EVALUATION TESTS:

1. PHYSICAL PARAMETERS:

- Color examination
- Odor examination
- Taste examination
- PH determination
- Determination of Moisture Content
- Determination of Total Ash
- Acid – insoluble Ash
- Water – soluble Ash
- Sulphated Ash
- Water Soluble Extractive Value
- Alcohol Soluble Extractive Value

REPORT:

50 gm Churna was prepared and evaluated.

9.0 PREPARATION AND EVALUATION OF TABLET

AIM:

To prepare and evaluate the 400mg tablet

PRINCIPLE:

A tablet is a pharmaceutical dosage form. Tablet may be defined as the solid unit dosage form of medicament or medicaments with or without suitable excipients and prepared by either by molding or by compression .it comprises a mixture of active substance and excipients usually in powder form, pressed or compacted from a powder into a solid dose.

The compressed tablet is the most popular dosage form use in today. About two-thirds of all prescription are dispensed as solid dosage forms, and half of these are compressed tablets. A tablet can be formulated to deliver an accurate dosage to a specific site; it is usually taken orally, but can be administered sublingually, buccally, rectally, or intra vaginally.

REQUIREMENTS:

INGREDIENTS(mg)	F1	F2	F3	F4
Clove	10	–	100	–
Cinnamon	–	100	–	100
Lactose	290	290	–	–
Mannitol	–	–	290	290
Sodium Sachrine	2	2	2	2
Talc	4	4	4	4
Magnesium stearate	4	4	4	4

PROCEDURE:

- Weigh all the excipients along with API as shown in table.
- Passed through sieve no.20.
- Mix all ingredients following geometric mixing excluding glidant and lubricant thoroughly for 15 min.
- Mix the powder blend was thoroughly with talc and magnesium stearate .
- Compress 400 mg tablet using single rotary punching machine.

EVALUATION OF TABLET:

1. General Appearance: The general appearance of a tablet, its identity and general elegance is essential for consumer acceptance, for control of lot-to-lot uniformity and tablet-to-tablet uniformity. The control of general appearance involves the measurement of size, shape, colour, presence or absence odour, taste etc.

2. Content uniformity Test: Randomly select 30 tablets. 10 of these assayed individually. The tablet pass the test if 9 of the 10 tablets must contain less than 85% and not more than 115% of the labeled drug content and the 10th tablet may not contain less than 75% and more than 125% of the labeled content. If these conditions are not met, remaining 20 tablets assayed individually and none may fall outside of the 85 to 115% range.

3. Thickness: Dimension of the tablets are measured by using a calibrated dial caliper. Five tablets sample formulation are picked out randomly and its thickness is measured individually. Mean value of thickness is observed.

4. Weight variation: Twenty tablets were selected at random and weighed individually. The individual weights were compared with the average weight for determination of weight variation. The percentage deviation was calculated and then compared with IP limit.

5. Hardness: Five tablets are randomly selected from each batch and hardness of tablet is determined by using Monsanto hardness tester. The mean values for each batch are calculated and compared to IP standard.

6. Friability: Friability indicates the ability of a tablet to withstand mechanical shocks while handling. Friability of the tablets were determined using Roche Friabilator

and is expressed in (%). Twenty tablets were initially weighed and placed into the friabilator. The friabilator was operated at 25rpm for 4 minutes or run upto 100 revolutions and then the tablets are weighed again. The loss in tablet weight due to abrasion or fracture is measured as a tablet friability.

7. Disintegration time: The disintegration time for all formulations is carried out using tablet disintegration test apparatus. Six tablets are placed individually in each tube of disintegration test apparatus and discs are placed. The water is maintained at temperature of $37 \pm 2^\circ \text{C}$ and the time taken for the entire tablet to disintegrate completely is noted.

8. Dissolution study: Dissolution study of tablet is carried out using phosphate buffer 6.8 as a dissolution media. The samples are withdrawn for 8 hours at the interval of 45 min. The absorbance of sample measured on UV spectrophotometer and percentage release are calculated.

REPORT:

The 400mg of tablet are prepared and evaluated.

MONOGRAPH ANALYSIS OF CASTOR OIL

BIOLOGICAL SOURCE:

Castor oil is the fixed oil obtained from the seeds of *Ricinus communis* linn.

FAMILY:

Euphorbiaceae.

DESCRIPTION:

COLOR: Pale yellowish in color or almost colorless.

ODOR: Slight.

TAST: At first bland but afterwards slightly acrid and usually nauseating.

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

IDENTIFICATION:

- Mixes completely with half its volume of light petroleum and it is only partially soluble in two volumes (boiling point 40-60°C).
- Add to an equal volume of alcohol "a clear liquid is obtained", cool to 0° for 3hrs the liquid remains clear. Distinction from other fixed oil.

✓ **Wt./ml – At 25°C – 0.945-0.965 per gram**

✓ **Acetyl value – Not less than 143**

✓ **Acid value – Not more than 2**

✓ **Iodine value – 82-90**

✓ **Refractive index at 25°C – 1.4758-1.4798**

✓ **Optical rotation – Not less than +3.5**

✓ **Saponification value – 177-185**

STORAGE:

Preserve castor oil in a well closed container in a cool place.

CATEGORY: Laxative, pharmaceutical aid.

DOSE: 4-16ml

PROCEDURE:

DETERMINATION OF Wt/ml OF CASTOR

OIL Weight per millilitre

The wt per ml of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25°C unless otherwise specified.

Method

Select a clean and dry pycnometer. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25°C and weighing the contents. Assuming that the weight of 1 ml of water at 25°C when weighed in air of density 0.0012 g per ml is 0.99602g. Calculate the capacity of the pycnometer (ordinary deviations in the density of air from the value given do not affect the result of a determination significantly). Adjust the temperature of the substance to be examined to about 20° and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25°, remove any excess of substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the wt per ml by dividing the weight in air expressed in g of the quantity of liquid which fills the pycnometer at the specified temperature by the capacity expressed in ml of the pycnometer at the same temperature.

ACID VALUE OF CASTOR OIL

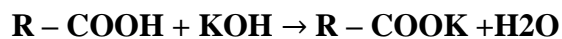
AIM:

To determine the acid value of the given sample of castor oil and report the purity with the standard level given by Indian pharmacopoeia.

PRINCIPLE:

- ✓ Acid value is the number which express in mg of amount of KOH necessary to neutralize the free acid present in (oil) given substance.

- ✓ Acid value can be determined by titrating with an ethereal alcoholic solution of fixed oil with 0.1 M KOH using phenolphthalein as an indicator. End point is the appearance of pink colour.



Increased acid value can be determined by using the following formula

$$\mathbf{Acid\ value = \eta \times 5.61 / weight\ of\ oil}$$

η = Strength of KOH \times titre value

PROCEDURE:

Dissolve about 10 g of castor oil in 50 ml mixture of equal volume of ether and ethanol previously neutralized with 0.1 M KOH to phenolphthalein. If the sample does not dissolve and boil solvent. Connect a flask to reflex condenser and warm slowly with frequent shaking until the sample dissolved. Add phenolphthalein solution and titrate with 0.1 M KOH until the solution remains faintly pink after shaking for 30 seconds. Calculate the acid value from the following formula:

$$\mathbf{Acid\ value = 5.61 \times Titre\ value\ (KOH) \times Strength\ of\ KOH / Wt\ of\ sample\ taken}$$

DETERMINATION OF SAPONIFICATION VALUE OF CASTOR OIL

AIM: To determine the saponification value of given sample of castor oil.

- Saponification value is the number which express in mg of amount of potassium hydroxide to neutralize free acids and present in 1 gm of the substance.

PROCEDURE:

Introduce about 2 gms of substance accurately weigh to a 200 ml conical flask of borosilicate glass fitted with a reflex condenser. Add 25 ml of 0.5 N alcoholic potassium hydroxide and boil in a reflex water bath for 30 minutes. Frequently rotating the content. Cool and add for 2 drops of phenolphthalein and titrate immediately against 0.5 N HCL.

Saponification value of castor oil =

$$= (b - a) \times 28.05 \times \text{strength of N/2 HCL} / \text{weight taken}$$

b – Blank value

a – Titre value

REFRACTIVE INDEX OF CASTOR OIL

AIM:

To determine the refractive index of the given sample of castor oil.

PRINCIPLE:

- ✓ The refractive index (n) of a substance with reference to air is the ratio of the sin of the angle of incidence to the sin of the angle of refraction of a beam of light passing from air into the substance.
- ✓ It varies with the wavelength of light used in its measurement. The refractive index is usually measured at 25°(±0.5) with reference to the wavelength of the sin of sodium ($\lambda = 589.3\text{nm}$). The temperature should be carefully adjusted as refractive index varies significantly with temperature.
- ✓ The Abbe's Refractometer is convenient for most refractive index measurements. To achieve accurately the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25°C.

PROCEDURE:

- Clean the nickel prism by means of cotton wool, dipped in ether, acetone or chloroform. Clamp the lower prism with the upper prism and view through the eye piece to get a clean bright field.
- Without disturbing the light arrangements remove the lower prism and place 2-3 drops of the given sample of castor oil, between the two prisms and clamp it again. Remove excess of liquid if any, using cotton wool. View for a clear light field arrange

Cross wire such that to the bright field. Look for exact reading on a graduated scale and not down the refractive index. Repeat the same for concordant value.

USES:

Refractive index can be used to identify a substance, to measure its purity and to determine concentration of one substance dissolved in another. Refractometer is used to determine refractive index.

REPORT:

- The Wt/ml of water =
- The Wt./ml of castor oil =
- Acid value of castor oil =
- Saponification value of castor oil =
- Refractive index of water =
- Refractive index of castor oil = (I.P Limit = 1.4758 – 1.4798).

11.0 DETERMINATION OF ALDEHYDE CONTENT IN LEMON OIL

AIM: To determine the total aldehyde content of given sample of lemon oil.

REQUIREMENTS:

- ✓ Stoppered tube
- ✓ Hydroxylamine hydrochloride reagent in alcohol 60%
- ✓ 0.5 N potassium hydroxide in alcohol (60%)
- ✓ Methyl orange
- ✓ Lemon oil

PROCEDURE:

- Weigh accurately about 10 g of lemon oil in a stoppered tube. Add to it, 7 ml of hydroxylamine hydrochloride reagent in alcohol (60%) and a drop of solution of methyl orange.
- Titrate the liberated acid with 0.5 N potassium hydroxide in alcohol (60%) until the red color changes to permanent yellow in the lower layer.
- Calculate the aldehyde content as follow:
- 1ml of 0.5N potassium hydroxide in alcohol (60%) is equivalent to 0.07672 of citrus.

PREPARATION OF HYDROXYLAMINE HYDROCHLORIDE REAGENT IN ALCOHOL 60%.

Dissolve 3.475 g of hydroxylamine hydrochloride in 95 ml of 60% alcohol; add 0.5 ml of 0.2% w/v solution of methyl orange in 60% alcohol and 0.5N potassium hydroxide in alcohol 60% until yellow color is produced. Make volume with sufficient alcohol to 100ml.

REPORT:

The Aldehyde content of given sample of lemon oil is found to be _____

DETERMINATION OF PHENOL CONTENT

Determination of Gallic Acid Equivalent In (HAECB)

Principle

Total phenolic content of the various concentrations of HAECB was determined by Folin-ciocalteu reagent method. The hydroxyl group (OH) of phenolic compounds reduce the phosphomolybdic acid to molybdenum blue in the presence of alkaline medium (present in Folin reagent). The blue coloured complex was then spectrophotometrically measured at 760nm.

Instrument

UV Visible spectrophotometer, Shimadzu (Model 1800).

Reagents required

- Folin-Ciocalteu Reagent (1N)
- Sodium carbonate solution (10%)
- Standard Gallic acid solution

Procedure

About 1 mL (1mg/ml and 0.5 mg/mL) of hydroalcoholic extract of *Commelina benghalensis L.* (Leaf) (HAECB), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectrophotometrically at 760nm wavelength.

The calibration curve was generated by preparing gallic acid at different concentration (5, 10, 15, 20 and 25 $\mu\text{g/mL}$). The reaction mixture without sample was used as blank. Total phenolic content of HAECB extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g).

Report:

Hydroalcoholic extract of *Commelina benghalensis L.* (HAECB) was found to contain _____
of GAE (Gallic acid equivalent)

DETERMINATION OF TOTAL ALKALOIDS

Aim: To determine the total alkaloid content of cinchona extract

Theory

Alkaloids are basic nitrogen containing compounds obtained from plants, animals and microorganism having a marked physiological action. Alkaloids have diverse and important physiological effects on humans and other animals. The term Alkaloids is derived from the word alkali-like and they have some of the characters of natural amines. The definition of alkaloid is the organic compounds from natural or synthetic origin which are basic in nature and contain one or more nitrogen atoms normally in heterocyclic ring and possess specific physiological action on human animal body when used therapeutically.

Alkaloids found in cinchona bark still play an important role in medicine for example as antimalarial and anti-arrhythmic drugs. Six respective derivatives (dihydro quinidine, dihydro quinine, quinidine, quinine, cinchonine and cinchonidine) has been quantified in crude plant extract.

Total alkaloids are determined volumetrically by acid – base titration and calculated as quinine.

Determination of total alkaloids of cinchona extract

1. Introduce 10 ml of the cinchona extract into a separating funnel, add 1N sulphuric acid (10 ml) and water 10 ml.
2. Shake with 10 ml of chloroform and allow to separate the mixture. Shake and allow to separate and discarded the chloroform layer.
3. Transfer the acid wash to the mother liquor and basify with about 5 ml of strong ammonia (test with litmus paper)
4. Shake with successive portions of chloroform (30, 20, 20 and 10 ml). Test for completion of the extraction with Mayer's reagent.
5. Wash the combined chloroform extract with 10ml of water. Transfer the extract to a distillation flask to remove the solvent on a boiling water bath.

6. Add 5 ml of alcohol to the residue and evaporate the alcohol on a water bath.
7. Dissolve the residue in 2 ml of chloroform and add 10 ml standard N/10 hydrochloric acid. Heat on a water bath to remove the chloroform and back titrate the excess acid against N/10 sodium hydroxide using 3-5 drops of methyl red as indicator.

Calculation

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

Percentage total alkaloids= $[(10 \text{ ml of N/10 NaOH}) \times 0.0391 \times 100] / 10$

= _____ % w/v calculated as quinine

Report

The total alkaloid content of cinchona sample is _____