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STANDARDIZATION AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *FICUS BENJAMINA* AERIAL ROOTS

Divya Sharma, Anuja Verma, Gagandeep Kaur and Rakesh K. Sindhu*
Chitkara College of Pharmacy, Chitkara University, Punjab, India
Email ID: rakeshsindhu16@gmail.com

Abstract

Ficus benjamina also locally known as the Pilkhan. It has a good medicinal potential. Its latex and fruit extracts are used by some local communities to treat inflammation, skin disorders, piles, malaria, vomiting etc. Generally it is used as a tonic. It also possesses good anti-hypertensive, anti-microbial, antipyretic and anti-dysentery properties. Current study has been performed to investigate the phytochemical and pharmacognostic properties of aerial roots of *Ficus benjamina.* The parameters evaluated were: Morphology, Microscopy, Histochemical colour reactions with various agents, Ash value (7.25), Water soluble ash (4.35), acid soluble ash (5.8), sulphated ash (3.91), Swelling index, Extractive value and phytochemical investigation. The microscopy showed presence of lignified and non lignified fibres, parenchymatous cells and pitted vessels. The presence or absence of alkaloids, glycosides, carbohydrates, saponins, phenolic compounds and terpenoids detected by performing various tests with reagents like acetic acid, phloroglucinol, picric acid, millon's reagent, wagner reagent, dragondroff's reagent, etc. Powder was treated with different chemicals and analysed in short U.V., long U.V. and visible light for observing fluorescence. This was first ever investigations on aerial roots of *Ficus benjamina* and can be used for future standardization of this plant.

Keywords: *Ficus benjamina*, Extractive value, Phytochemical screening, Standardization.

Introduction

Ficus benjamina is a species belonging to family Moraceae which usually grow in tropical and subtropical region (Liu *et al*., 200). *Ficus benjamina* is an ornamental plant grows in mild temperature, due to its higher growth and it is tolerable to low and high temperature conditions (Kwang *et al*., 2008). It is a rapidly growing tree, up to 30 meter tall, multiple-stemmed, spread, strangler with multiple aerial roots, stem with drooping foliage, white latex, and strongest root system. The thick, shiny, alternate, simple, entire, elliptic, two to five-inch-long, evergreen leaves generously clothe the long branches. The branches weep toward the ground forming a canopy so dense that nothing grows beneath them. The axillary, unisexual, monoecious flowers are borne in the fig body. The fruits are fleshy, obovoid or subglobose, turn yellow via orange and dark red (Quattrocchi, 2012). Plant image shown in figure 1.

Fig. 1 : Plant Image of *Ficus benjamina*

Taxonomical Classification:

Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Mangoliopsida
Order	:	Rrosales
Family	:	Moraceae
Genus	:	*Ficus*
Species	:	*benjamina*

The phytochemistry has revealed the presence of various active constituents from different parts of plants. The leaves, bark, stems, roots and fruits contain cinnamic acid, lactose, naringenin, quercetin, caffeic acid and stigmasterol, lutein, β-sitosterol, chlorophyll-A, phytol, (E)-3-alkenoic acid, fatty alcohols triglycerides, and fatty acids, essential oils, flavone glycosides, chlorogenic, *p*-coumaric, isoflavonoids and syringic acidschlorogenic *p*-coumaric and ferulic acids (Ali *et al*., 2017; Hanelt, 2001).

Various part of the plant like bark, leaves, fruits and latex are medicinally important. The plant is well known due to its medicinal potential. Its latex and some fruit extracts are used treat skin disorders, inflammation, vomiting, leprosy, malaria and cancer. The plant is also used as antimicrobial, antipyretic, hypotensive and anti-dysentery remedy. The leaves and twigs are used as insecticides and hepatoprotective (Kanaujia *et al*., 2011).

Pharmacognostical study is the ground work in the standardization of herbal drugs. The detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. Pharmacognostic studies have been done on many important drugs, and the resulting

observations have been incorporated in various pharmacopoeias (Sindhu and Arora, 2014).

There is no information in the litrature regarding the pharmacognistical evaluation of *Ficus benjamina* aerial roots. The present study includes morphology, microscopy, powder study, ash values, loss on drying, foaming index, extractive value, swelling index, fiber content and phytochemical screening of the aerial roots of *Ficus benjamina*.

Materials and Methods

The plant *of Ficus benjamina* aerial roots were collected during the month of the October 2018 from Chitkara University Punjab Campus. The plant material was taxonomically identified and authenticated by Dr. Hamid Raja, Head, department of Horticulture, Chitkara University Punjab Campus. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division Chitkara College of pharmacy, Chitkara university, Panjab for further reference. The aerial root was dried under shade, sliced into small pieces, pulverised using a mechanical grinder and stored in an air tight container for further use.

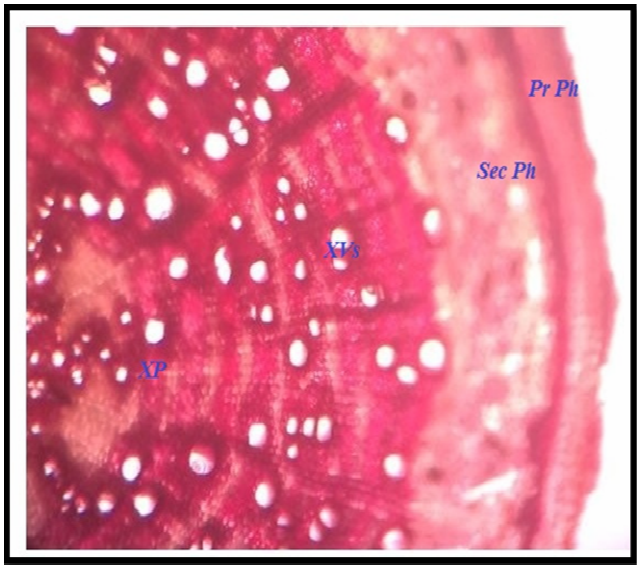
Morphology

The crude drug was evaluated for organoleptic properties shape, size. colour, odour, taste, fracture and texture were noted [Figure no.2].

Fig. 2: Morphological Characters of Aerial roots

Microscopy of Root

Microscopy of plant material is performed to distinguish it from the allied drugs and adulterant. The dried root was soaked overnight in water to make it smooth enough for transverse section. Paraffin wax embedded specimens were sectioned using the rotatory microtom. The thickness of section was 10-12 µm. The very fine section was selectively subjected to staining reaction with staining reagent Phloroglucinol and HCl mounting with glycerine. Photomicrographs were taken using trinocular microscope (Olympus). [Figure 3]



PrP: Primary Phloem; **SP:** Secondary Phloem ;
XVs : Xylem vessels; **XP** : Xlyem parenchyma

Fig. 3: Microscopical Characters of transverse section of Aerial roots

Histochemical Colour Reaction

Presence of different organic compounds in root of the plant is confirmed by using various histochemical tests. Care was taken to ascertain relative concentration of these chemicals by degree of colour produced in different tissues. The transverse section of fresh root was treated with different chemical reagents for colour tests viz. phloroglucinol, millon's reagent, iodine solution followed by sulphuric acid, dragendroff's reagent, wagner's reagent, sulphuric acid solution, libberman-burchard reagent, acetic anhydride and sulphuric acid solution, ferric chloride, iodine solution, caustic alkali, aqueous potassium hydroxide, chloroform with sulphuric acid, aniline sulphate and sulphuric acid.

Powder studies

Microscopic study

The shade dried root was mechanically pulverized to coarse powder and sifted through 40 mesh sieve. To study the ingredients of powder, a pinch of powder was taken on slide and mounted with phloroglucinol, hydrochloric acid and glycerine. The slide was observed under microscope.

Colour reactions

To study the behaviour of root powder with different chemical reagents, a pinch of powder was treated with different chemical reagents as 1N hydrochloric acid, sodium hydroxide, acetic acid, 5% ferric chloride, picric acid, nitric acid with ammonia solution, 5% iodine, 1N nitric acid and powder as such were performed, change in colour was observed.

Fluorescence behaviour of powder

Many herbs show fluorescence behaviour when cut surface or powder is exposed to UV light and this can help in their identification. To study the fluorescence nature of root powder, a pinch of powder was treated with different chemical reagents viz.1N hydrochloric acid, 1N sodium hydroxide, 1N sodium hydroxide in methanol, picric acid, 1N nitric acid, acetic acid, acetone, 50% sulphuric acid, nitric

acid in ammonia solution and observed under day light, long UV (365 nm) and short UV light (254 nm).

Ash Values

Total ash

Total ash is produced by incinerating the drug at the temperature possible to remove all of the carbon. A higher temperature may result in the conversion of carbonates to oxides. The total ash usually consists of carbonates, phosphates, silicates and silica which includes both physiological ash, which is derived from the plant tissue itself and non-physiological ash which is the residue of the adhering material to the plant, e.g., sand and soil. About 2 g of air-dried powdered drug was accurately weighed and taken in a silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed. The percentage of total ash was calculated with reference to the air dried drug.

Water soluble ash

Water-soluble ash is that part of the total ash content which is soluble in water. The total ash obtained was boiled for 5 min with 25 ml of water, the insoluble matter was collected in an ashless filter paper, incinerated at a temperature not exceeding 450°C, subtracted the weight of the insoluble matter from the weight of the ash and calculated the percentage of water soluble ash with reference to the air dried drug.

Acid-insoluble ash

Acid insoluble ash is determined by treating the total ash with dilute hydrochloric acid and weighing the residue. This limit particularly indicated contamination with siliceous materials such as earth and sand by comparison with the total ash value for the same sample differentiation can be made between contaminating material and in the natural ash of the drug. The total ash obtained was boiled with 25 ml of 2 N hydrochloric acid for 5 min, the insoluble matter was collected in an ashless filter paper, washed with hot water, ignited, cooled in dessicator and weighed. The percentage of acid-insoluble ash with reference to the air dried drug was calculated.

Suphated ash

About 1 g of air dried powder drug was treated with dilute sulphuric acid before ignition in a tared silica crucible to a constant weight. The ash obtained was weighed. Percentage of sulphated ash was calculated with reference to the air-dried drug (Sindhu *et al*., 2010).

Extractive Value

Extractive value is used as a means of evaluating crude drug which are not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist.

Petroleum ether extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of petroleum ether (60-80°) in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of petroleum ether soluble extractive was calculated with reference to air dried material [Table 5].

Chloroform extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of chloroform in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of chloroform soluble extractive was calculated with reference to air dried material.

Ethanol extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of ethanol in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried material.

Water extractive

Accurately weighed 5 g of the air dried powdered drug was macerated with 100 ml of water in a stoppered flask for 24 h. The mixture was vigorously shaken at regular intervals. After 24 h the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The percentage of water soluble extractive was calculated with reference to air dried material (Sindhu and Arora, 2012). [Table 6]

Determination of Crude Fiber Content

2 g of powdered drug was extracted with diethyl ether and added 200 ml of boiling dilute sulphuric acid (1.25%) to the ether exhausted marc in a 500 ml flask. The mixture was refluxed for 30 min, filtered through filter paper and the residue was washed with boiling water until the effluent washing was acid free. Rinsed the residue and placed back into the flask with 200 ml of boiling sodium hydroxide solution (1.25%) and refluxed the mixture again for 30 min., filtered through ashless filter paper and washed the residue with boiling water until the last washing was neutral. It was then dried at 110°C to constant weight and then ignited to constant weight. The ash was cooled in dessicator, weighed.

Calculations as follows

$$\% \text{ Crude Fibre} = \frac{\text{Weight of the ash obtained}}{\text{Weight of the drug sample}} \times 100$$

The results are represented in Table no. 6.

Loss on Drying

This parameter is used to determine the amount of moisture present in a particular sample. The powder drug (2 g) sample was placed on a tared evaporating dish. The tared evaporating dish was dried at 105 ± 1°C until constant weight and weighed. The drying was continued until two successive readings match each other.

Determination of Swelling Index

Swelling properties of many medicinal plants shows specific therapeutic or pharmaceutical utility e.g. gums, pectin, or hemicellulose. One g of plant material was accurately weighed, placed into 25 ml glass stoppered measuring cylinder. 25 ml water was added and shaken the mixture thoroughly in every 10 min for one h, allow standing for 3 h at room temperature. Measured the volume in ml occupied by plant material calculated the mean value of individual determination, related to one gm of plant material..

Determination of Foaming Index

The medicinal plant materials contain saponins that cause the persistent foam formation when an aqueous decoction is shaken. The foaming ability of plant material and their extract is measured in term of foaming index. 1 g of powdered root was accurately weighed and transferred in to a 500 ml conical flasks containing 100 ml water and boiled for 30 min, cooled and filtered into 100 ml volumetric flask and made the volume with water. The decoction was poured into 10 stoppered test tubes in successive portion of 1 ml, 2 ml, 3 ml, etc up to 10 ml and adjusted the volume of each test tube with water to 10 ml and shaken them in lengthwise motion for 15 sec. Allowed to stand for 15 min and measured the height of the foam. The results were assessed as follows:

If height of foam in every tube was less than 1 cm the foaming index was considered less than 100. If height of the foam was more than 1cm in every tube the foaming index was over than 1000. In such case repetitions was done by using a new series of dilutions of decoction in order to obtain the result. If height of foam in any test tube was 1 cm, the volume of the plant material decoction in that tube (a) was used to determination of index.

Formula used for calculation of foaming index = $\frac{1000}{a}$

a = Volume of decoction was used for preparing the dilution in tube where foaming height was 1cm measured. (Sindhu *et al*., 2010).

Preliminary Phytochemical

Preparation of the extract

About 20 g of air dried powdered root was extracted with ethanol in a soxhlet extractor for 72 h. the aqueous extract was prepared by maceration with distilled water for 24 h to obtain the aqueous extract. Concentrated ethanol and aqueous extract in rotary vaccum evaporator. The extracts were screened for the presence of various phytoconstituents [Table 7].

Test for alkaloids

Stirr a small portion of the solvent free petroleum ether, chloroform, ethyl acetate, alcohol and water extracts separately with a few drops of dilute hydrochloric acid and filter. The filtrates were tested with various alkaloidal reagents such as mayer's reagent (cream precipitate), dragendorff's reagent (orange brown precipitate), and wagner reagent (reddish brown precipitate). Mayer's reagent: Few drops of mayer's reagent were added in each extract and observed formation of the white or cream colored precipitates.

Dragendorff's reagent: Few drops of dragendorff's reagent were added in each extract and observed formation of the orange yellow or brown colored precipitates.

Wagner reagent: Few drops of wagner reagent were added in each extract and observed formation of the reddish brown precipitates.

Test for carbohydrates

Dissolve small quantities of alcoholic and aqueous extracts, separately in 4 ml of distilled water and filter. The filtrate may be subjected to varios tests to detect the presence of carbohydrates.

Molisch's Test: To about 2 ml of extract few drops of α-naphthol (20% in ethyl alcohol) were added. Then about 1 ml of concentrated sulphuric acid was added along the side of the tube. Reddish violet ring appeared at the junction of two layers. Indicates the presence of carbohydrates.

Fehlings Test: 1ml of fehling's reagent (copper sulphate in alkaline conditions) was added to the filtrate of the root extract in distilled water and heated in a steam bath. Brick red precipitates appeared which confirm the presence of carbohydrates.

Test for glycosides

Hydrolysed another small portion of the extract with dilute hydrochloric acid for few hours in water bath and subjected the hydrolysate with liebermann-burchard's, keller-killani, and borntrager's tests to detect the presence of different glycosides.

Keller-Killani Test: 1ml of glacial acetic acid containing traces of $FeCl_3$ and 1 ml of concentrated H_2SO_4 was added to the extract carefully. Colour appeared which confirm the presence of glycosides in the root extracts.

Borntrager's test: 1ml of benzene and 0.5 ml of dilute ammonia solution were added to the extract. A black brown colour was obtained which show the presence of glycosides in the root extracts.

Test for phenolic compound and tannins

Take small quantities of alcohol and aqueous extracts separately in water and test for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%) and lead acetate test.

Ferric chloride test: On addition of ferric chloride solution (5%), colour was observed in all the three portions due to the presence of phenolic compounds. colour appeared which show the presence of phenolic compound.

Lead acetate test: Few drops of lead acetate solution (5%) were added to the alcoholic extract of the root. White precipitate was appeared which confirm the presence of phenolic compounds.

Test for flavonoids

Ammonia test: Filter paper strips were dipped in the alcoholic and aqueous solutions of the extract and ammoniated. The filter paper changed its colour to yellow which indicates the presence of flavonoids.

Pew test for flavonoids: To 1ml of the each extracts, a piece of metallic magnesium/zinc was added followed by addition of 2 drops of concentrated hydrochloric acid. A

brownish colour confirmed the presence of flavonoids in all the extract.

Test for proteins and free amino acids

Added a few ml of alcoholic and aqueous extracts in a few ml of distilled water and subjected to million's, biuret and ninhydrin tests.

Millon's test: To 2 ml of filtrate, 5-6 drops of million's reagent (solution of mercury nitrate and nitrous acid) was added. A red colour precipitate appeared which confirms the presence of proteins and free amino acids.

Biuret test: To the ammoniated alkaline filtrate 2-3 drops of 0.02% copper sulphate solution was added. A red colour was obtained which confirms the presence of proteins and free amino acids.

Ninhydrin test: To each of the filtrate, lead acetate solution was added to precipitate tannins and filtered. The Filtrate was spotted on a paper chromatogram, sprayed with ninhydrin reagent and dried at 110^{o} C for 5 minutes. Violet spots were seen which confirm the presence of proteins and free amino acids.

Test for saponin

Foam test: Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponin.

Sodium bicarbonate test: To the few milligrams of extract few drops of sodium bicarbonate were added and shaken well. Formation of honey comb like frothing indicates positive test for saponins.

Test for phytosterol and triterpenes

Liebermann-Burchard's test: The hydro-alcohlic extract was shaken with chloroform and few drops of acetic anhydride were added chloroform extract along with a few drops of concentrated sulphuric acid from the side of the tube. The appearance of blue to brick red colour indicates the presence of sterol and triterpenes.

Hesse's reaction: The residue was dissolved in chloroform (4 ml) and an equal quantity of concentrated sulphuric acid was then along the side of the tube. The formation of the pink colored ring, which is on shaking diffused in both the layers, indicating the presence of sterols in the extract (Rajalakshmi *et al*., 2019).

Results and Discussion

Morphology

The mature aerial roots was 11-19 mm thick, grey, light green, slightly spiral, thickness varies with the age of the tree. Surface was slightly rough, fracture short and fibrous, light weight and odour characteristic as shown in figure 2.

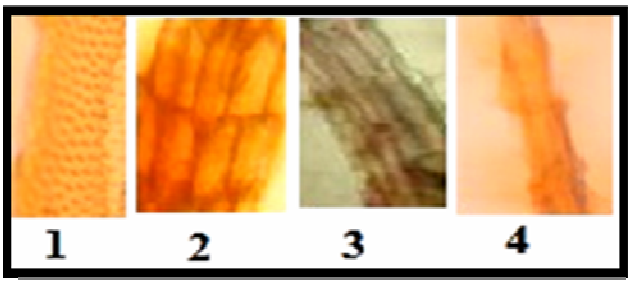
Microscopic Study of Transverse Section of Aerial Root

The transverse section of aerial roots Primary and secondary Phloem. Primary phloem shows the presence of paranchymatous cells. Then followed by xylem vessels and xylem parenchyma. Anatomical investigations of root of showed the following features in figure 3.

Histochemical Colour Reactions of transverse section with different chemical reagents as shown in Table 1.

Powder Microscopical study

The root powder was studied under microscope shows the presence of [Figure 4].



1: Pitted vessles; **2**: Parenchymatous cells;
3: Lignified fiber **4** : Non-lignified Fiber

Fig. 4: Microscopic character of powder

Behaviour of aerial root powder with different chemical reagents and under UV

The powdered plant material may be recognized from their adulterants on the basis of powder treated with different chemical reagents as shown in table 2. Fluorescence nature of powdered aerial root was treated with different chemical reagents and observed under UV, results are reported in Table 3.

Ash and Extractive values of aerial roots results shown in table 4 and table 5 respectively.

Quantitative studies: The other quantitative parameters for studies like crude fiber content, foaming index, swelling index, and loss on drying were performed. The results are shown in table 6.

Preliminary phytochemical screening

The different extracts of powdered aerial roots like petroleum ether, ethanol and aqueous extracts were subjected for evaluation of phytoconstituents. The Preliminary phytochemical screening revealed the presence of various phytoconstituents, like alkaloids, carbohydrate, glycosides, flavonoid, amino acids, protein, sterol and triterpenoid and phenolic compounds in different extracts as shown in Table 7.

Conclusion

The researcher and scientists from different disciplines are very open and dedicated to evaluate many pharmacologically importance of medicinal plants, due to their particular ethanomedicinal potential, and having minimum toxicity. The aerial roots of *Ficus benjamina* is used in treatment of different diseases. It also possesses good anti-hypertensive, anti-microbial, antipyretic and anti-dysentery properties. The standardization and phytochemical evaluation of this plant gives the idea about authentication, identification and standardization.

Table 1 : Behaviour of transverse section with different chemical reagents

Sr. No.	Reagents	Test for	Nature of change in histochemical zone	Degree of change
1.	Phloroglucinol+HCl	Lignin	Xylem vessels become pink	+
2.	Millon's reagent	Proteins	Yellow colour	+
3.	Iodine solution followed by H_2SO_4	Cellulose	Cellulose wall become violet	++
4.	Dragendroff's reagent	Alkaloid	Brown colour	++
5.	Wagner's reagent	Alkaloid	Dark yellow colour	-
6.	H_2SO_4 solution	Sterol	Red colour	+
7.	Libberman – Burchard reagent	Terpenes	Pink colour	+
8.	Acetic anhydride and H_2SO_4 solution	Strerol	Black colour	+
9.	$FeCl_3$ solution	Tannins	Dark green to black colour	+
10.	Iodine solution	Starch	Light bluish	++
11.	Caustic alkali+HCl	Calcium oxalate	No change	-
12.	Aqueous. KOH solution 10%+ H_2SO_4	Suberin	Light brown	-
13.	Chloroform+ H_2SO_4	Sterol	Red colour	+

++ High; + Moderate; - Absent

Table 2: Behaviour of aerial root powder with different chemical reagents

Sr. No	Treatment	Color of powder
1	Powder as such	Light Brown
2	Powder + 1 N HCl	Brown
3	Powder + 1N NaOH	Dark brown
4	Powder + Acetic Acid	Light reddish
5	Powder + 5% Ferric chloride	Creamish
6	Powder + Picric acid	Light yellow
7	Powder + HNO_3 + Ammonia solution	Black brown
8	Powder + 5% Iodine	Brown
9	Powder + 1N HNO_3	lightBrown

Table 3: Fluorescence nature of root powder under ultra violet (UV) and visible radiations

Sr. No.	Treatment	Long UV (365 nm)	Short UV (254 nm)	Visible
1	Powder as such	Reddish brown	red	cream
2	Powder + 1N HCl	Dark brown	brown	Light brown
3	Powder + 1N NaOH	Blue	brown	Light brown
4	Powder + 50% HNO_3	Violet	Reddish brown	Brown
5	Powder + Acetic acid	Deep blue	Reddish brown	Brown
6	Powder + Picric acid	Deep blue	Dark brown	Brown
7	Powder + 1N NaOH in methanol	Deep blue	Reddish brown	Light brown
8	Powder + $FeCl_3$	Brown	Light Brown	Creamish

Table 4: Total ash, water soluble ash, acid insoluble ash and sulphated ash of roots powder

S.No.	Ash	Percentage
1	Total ash	7.25%
2	Acid insoluble ash	5.80%
3	Water Soluble Ash	4.35%
4	Sulphated ash	3.91%

Table 5: Extractive values of roots powder with different solvents

Sr. No.	Solvent	Extractive value (%) w/w
1.	Alcohol soluble	8.2
2.	Water soluble	4.8
3.	Chloroform soluble	7.9
4.	Petroleum ether soluble	7.5

Table 6: Crude fiber content, Loss on drying, Swelling index, Foaming index

Parameter	Observation
Swelling index	0.00 ml/ gm
Loss on drying	6.45%
Foaming index	No significant result
Crude fiber content	22.03 %

Table 7: Preliminary phytochemical screening

Sr. no.	Plant Constituents Test / Reagent	Petroleum Ether extract	Ethanol extract	Aqueous extract
1.	**ALKALOIDS**			
	Mayer's reagent	-	-	-
	Dragendroff's reagent	+	+	-
	Wagner's reagent	+	+	-
2.	**GLYCOSIDES**			
	Killer-Killani test	+	+	-
	Sodium nitropruside test	+	-	-
	Borntrager test	+	+	-
3.	**CARBOHYDRATES**			
	Molisch's reagent	+	+	+
	Fehling solution	-	+	+
4.	**STEROLS**			
	Liebermann-Burchard's test	+	+	-
	Salkowski test	+	+	-
	Hesses reaction	+	+	-
5.	**SAPONINS**			
	Foam test	+	+	-
	Sodium bicarbonate test	-	+	-
6.	**PHENOLIC COMPOUNDS & TANNINS**			
	Ferric chloride solution	+	+	-
	Lead acetate solution	+	+	-
7.	**PROTEINS & AMINO ACIDS**			
	Biuret test	-	_	+
	Millon's reagent	-	+	+
	Ninhydrin reagent	+	+	+
8.	**FLAVANOIDS**			
	Shinoda/Pew test	+	+	_
	Ammonia test	_	+	+

+ve: Present; -ve: Absent

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