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Physicochemical and Phytochemical Investigation of the Roots of *Paederia Foetida Linn.*

Arun B. Joshi[1*], Gleason M. Ferrao[1]

1.Department of Pharmacognosy, Goa College of Pharmacy, Panjim, Goa, India

ABSTRACT

The present study was undertaken for the development of physicochemical and phytochemical parameters of the roots of *Paederiafoetida* [Linn] Belonging to the family, Rubiaceae. The plant commonly known as skunk vine is a perennial climber, in Sanskrit as Prasarni, in Marathi as Hiranvel, in Hindi as Gandha Prasarini, in English as Chinese flower plant. The physicochemical and phytochemical investigation confirms the purity and authenticity of *Paederiafoetida*roots by using standard methods. The physicochemical studies revealed the presence of moisture content as 6% w/w, total ash as 8.75 %w/w, acid insoluble ash as 1.1%w/w, water soluble ash as 1.8%w/w, alcohol soluble extractive as 9.8%w/w, water soluble extractive as 9.2%w/w, ether soluble extractive as 2.4% w/w, foaming index as less than 100 and swelling index as 1.7cm. The fluorescence analysis in short wavelength, long wavelength and day light is also reported, which is a tool to determine the chemical nature of crude drug. Preliminary phytochemical screening of the ethanolic extract of the roots revealed the presence of alkaloids, glycosides, carbohydrates, triterpinoids, steroids, tannins and resins. All these methods will help in setting down pharmacopoeial standards in determining the quality and purity of the *Paederiafoetida* roots.

Keywords: *Paederiafoetida*, Rubiaceae, Fluorescence, Physicochemical, Phytochemical.

*Corresponding Author Email: visitkk@rediffmail.com

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INTRODUCTION

Paederiafoetida[Linn] is a perennial climber belonging to the Family, Rubiaceae.[1,2,3,5]

Figure 1: Left: Leaves and Flowers of Plant, Right: roots of the plant:

It is found in Central and Eastern Hymalayas, upto 5000ft form Calcutta (Kolkata) and Malay Peninsula- Saim, Malay, Archpelago to Borneo[4]. *P. foetida* is cultivated and naturalized in the southern United States and Hawai'i. In the United States, itis located in the central and northern regions of Florida, as well as in Texas, Georgia, North Carolina, South Carolina, Mississippi and Alabama.*P. foetida* is an aggressive weed on Christmas Island and Mauritius[7].

The major classes of chemical constituent present in this plant are iridoid glycosides, sitosterol, stigmasterol, alkaloids, carbohydrates, protein, amino acid and volatile oil. Iridiod glycosides, paederolone, paederone, paederine and paederenine were the phytochemicals identified in this plant. Previous studies also identified a number of steroids and terpenoids and 77 constituents in the volatile oils of the leaves, stems and flowers of *P. foetida*. Upon distillation, a volatile oil is obtained with the offensive odor of the fresh crushed leaves and two alkaloids are obtained: a- and b-Paederine. The leaves yield an indole. Leaves are rich in carotene and vitamin C. Plant yields friedelan-3-1, beta-sitosterol and epifriedelinol. Leaves yield iridoid glycosides, asperuloside, paederoside and scandoside; sitosterol, stigmasterol, campesterol, ursolic acid, palmitic acid and methyl mercaptan. The methyl mercaptan is responsible for the foetid odor of the plant.[6,8]A pregnane ester oilgo glycoside (oxysine), a pregnanetriglycoside (culentin), a cardinolide (oxyline), two more cardinolides, oxystelmoside and oxystelmine have been isolated from roots[9].

Traditionally the plant is used as a bitter tonic, indigestible, aphrodisiac, cures “vata” and “kapha”, inflamation, piles, fever, good for night blindness, laxative (Ayurveda)[8]. The whole

plant is regarded specific for rheumatic infection which is administered both internally and externally. Roots are used by the hindus as an emetic. The juice of the leaves is considered as astringent and given to children when suffering from diarrhea. The decoction of the leaves is considered as nutritive for the sick and convalescent. It is also used in treating urinary dysuria, dyspepsia, gastritis and enteritis [4]. From the literature survey it was reveled that no substantial work had been carried out in physiochemical, fluorescence analysis and phytochemical screening form the roots of *P. foetida*. Hence an effort was made to carry out physicochemical, fluorescence analysis and phytochemical screening from the roots of *P. foetida.*

MATERIALS AND METHOD

Plant Collection

Fresh roots were collected from the plants of *Paederiafoetida* from Vittla, Bontwal Taluka, Manglore, Dakshina Kannada, Karnataka- India in the month of September 2013. It was authenticated by Dr. K. Gopalkrishna Bhatt, Dept. of Botany, Poornaprajna College, Udupi, Karnataka and by Mr. DineshNayak (Sasyashymala) Advisor green belt Mangalore[10, 11].

Preparation of Ethanolic Extract.

The roots were then washed thoroughly to remove the soil and adhering materials and dried in shade. The dried roots were then powdered and used for physicochemical evaluation, fluorescence analysis and preparation of ethanolic extract. The dried roots powder (450g) was exhaustively extracted by maceration with ethanol (95%) for three days. After three days ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using Rotary vacuum evaporator (super fit) and the concentrate was evaporated to dryness (35g) and then used for the preliminary phytochemical investigation[12,13, 14].

Physicochemical Evaluations

Determination of swelling index

The specified quantity (1cm) of dried root powder of *Paederiafoetida* was taken which was previously reduced to the required fineness. It was introduced in a 25 ml glass stoppered measuring cylinder. The internal diameter of the cylinder should be about 16mm, the length of the graduated cylinder should be 125mm and it was subdivided in 0.2ml and marked from 0 to 25 ml in an upward direction.25ml of water was added to the powdered roots and mixture was shaken thoroughly at an interval of every 10 min for 1 hour. It was allowed to stand for 3 hour at room temperature. The volume was measured in ml occupied by the plant material, including any sticky mucilage. The mean value of the individual determinations was calculated [15].

Determination of foaming index

1g of the plant material was taken and was reduced to a coarse powder (sieve no. 1250). Then weighed and transferred to a 500ml of conical flask containing 100ml of boiling water. It was maintained at moderate boiling for 30 min, cooled and filtered into 100ml volumetric flask. Sufficient amount of water was added to make up the volume to 100ml. The above decoction was poured in 10 stoppered test tubes (height 16cm, diameter 16mm) in series of successive portions of 1ml, 2ml, 3ml, etc. up to 10ml and volume of the liquid in each test tube was adjusted to 10ml with water. The tubes were stoppered and shaken lengthwise motion for 15 seconds. (2shake per second). Then it is allowed to stand for 15 min and the height of the foam was measured.

The results were assayed as follows

If the height of foam in every test tube is less than 1cm, the foaming index is less than 100. If in any test tube a height of foam of 1cm is measured, the dilution of the plant material in this test tube is the index sought. If this tube is the first or second tube in the series, it is necessary to have an intermediate dilution prepared in the similar manner to obtain a more precise result.

If the height of the foam is more than 1cm in every test tube, the foaming index is over 1000. In this case the determination needs to be made on a new series of dilution of the decoction in order to obtain the result [15].

Determination of extractive value

Method: cold maceration

4.0g of coarsely powdered air dried material was weighed accurately in a glass stoppered conical flask. This was macerated with 100ml of 90% alcohol for 6 hours, shaking frequently and then allowed to stand for 18 hours. It was filtered rapidly and 25ml of the filtrate was transferred to tarred flat bottom dish and evaporated dryness on water bath. Later it was dried at 105^0C for 6 hours, cooled in a desiccator for 30 minutes and weight was recorded. Percentage of alcohol soluble extractive was calculated with the reference to the air dried sample.

For water soluble extractive value the same procedure was followed and % was calculated by using water as solvent.

For ether soluble extractive value the same procedure was followed and % was calculated by using ether as solvent[15].

Determination of moisture content

2g of dried root powder of *Paederiafoetida* was taken into glass dish was previously weighed. This glass dish was kept into the hot air oven at 100-105^0C. The weight was noted every hour till

two successive readings comes same. At last the weight of drug was calculated and percent yield was determined [16].

Determination of ash values

Total ash:

2g of the powdered drug was accurately weighed and was taken in a tarred platinum or silica dish previously ignited and weighed. The powdered drug was spread in a fine even layer at the bottom of the dish and then ignited by gradually increasing the heat to 500-600^{0}C until free from carbon. It was cooled in a desiccator and weighed .The % of ash (total ash) was calculated with reference to the air dried drug.

Acid insoluble ash:

To the crucible containing total ash, 25ml of HCL (approx.70g/l) test solution was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5ml of hot water which was then added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was then transferred to original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 min and weighed without delay. The % of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash:

To the crucible containing the total ash 25 ml of water was added and boiled for 5 mins. The insoluble matter was collected on an ash less filter paper. It was washed with hot water and ignited for 15 mins, at a temperature not exceeding 450^{0}C. The weight of this residue obtained was subtracted from the weight of total ash .The % of water soluble ash was calculated with reference to the air dried drug [15].

Determination of fluorescence analysis

Powdered roots of the plant *Paederiafoetida* was subjected to analysis under ultra violet light (254 and 366nm) and visible light after treatment with various chemical and organic reagents as shown in the table no.2[14, 16, 17, 18, 20].

Preliminary phytochemical screening

The preliminary phytoconstituents studies were performed for testing the different phyto-constituents present in the ethanolic extract of the stem bark of *Paederiafoetida*. The phytochemical tests were performed as per the standard procedure[16, 17, 18, 19, 20].

ALKALOIDS

Dragendroff's reagent

To 2 mg of the ethanolic extract, 5ml of distilled water was added; 2 M Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendroff's Reagent was added. Formation of orange – red precipitate indicated the presence of alkaloids.

Mayer's test

To 2 mg of ethanolic extract, a few drops of Mayer's Reagent was added. Formation of white or yellow precipitate indicated the presence of alkaloids

Wagner's test

To 2 mg of ethanolic extract, 1 ml of dilute Hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicated the presence of alkaloids.

Hager's test

To 2 mg of the ethanolic extract is taken in a test tube, a few of Hager's reagent was added. Formation of yellow precipitate confirmed the presence of alkaloids.[16, 17, 18]

CARBOHYDRATES

Molisch's test

In a tube containing 2ml of extract, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2ml of Conc. Sulphuric acid was added so as to form a layer below the mixture. Red-violet ring appeared, indicating the presence of carbohydrates, which disappeared on the addition of excess of alkali.

Benedict's test

To 0.5 ml of extract, 5 ml of benedict's solution was added and boiled for 5 min. Formation of brick red colored precipitate indicated the presence of carbohydrates.

Fehling's Test

To 2ml of extract, 1ml mixture of equal parts of Fehling's solution A and B were boiled for few minutes. Formation of red or brick coloured precipitate indicated the presence of reducing sugar.[16, 17, 18]

FLAVONOIDS

Shinoda test

In a test tube containing 0.5 ml of extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

Lead acetate test

To 2mg of plant extract, 1ml of lead acetate solution was added. Formation of yellow precipitate indicted presence of flavonoids.

Vanillin- hydrochloric acid test

Vanillin HCl was added to the alcoholic solution of drug, formation of pink colour indicated presence of flavonoids.[16, 17, 18]

TRITERPINOIDS AND STEROIDS

Libermann- Burchard's test

2 mg of dry extract was dissolved in acetic anhydride, heating to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Brown ring was formed at junction of2 layers and Formation of a deep red colour in the upper layer indicated the presence of triterpenoids.

Salkowski test

2 mg of dry extract was shaken with chloroform, to the chloroform layer; sulphuric acid was added slowly by the side of test tube. Formation of red colour indicated the presence of steroids.[16, 17, 18]

Tannins and phenolic compounds

To 1-2 ml of the extract, few drops of 5% w/v $FeCl_3$ solution were added. A green colour indicated the presence of gallotannins; blue colour indicated the presence of hydrolysable tannins while brown colour indicated the presence of pseudotaninns

To 1-2 ml of extract add lead acetate solution. White precipitate indicated presence of tannins and phenolic compounds.

Resins

1ml of extract was dissolved in acetone and the solution was poured in distilled water. Turbidity indicated the presence of resins.

PROTEINS

Biuret's test

To 1 ml of hot extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops of 3% w/v copper sulphate solution were added. Formation of violet red colour indicated the presence of proteins.

Millon's test

1ml of extract was dissolved in 1ml of distilled water and 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.[17, 18]

Glycosides

Free sugar content of the extract was determined and hydrolyzed with mineral acids (dil.HCl /dil.

H_2SO_4. The total sugar content of hydrolysed extract was again determined. Increase in the sugar content indicated the presence of glycosides in the extract.

TEST FOR CARDIAC GLYCOSIDES

Baljet's test

A thick section showed yellow to orange colour with sodium picrate.

Legal's test

To the aqueous or alcoholic extract, 1ml pyridine and 1ml of sodium nitroprusside were added. Pink to red colour indicated presence of cardiac glycosides

Test for deoxysugars (killer-killiani test)

To 2ml extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc.H_2SO_4 were added. Reddish brown colour appeared at the junction of the two liquid layers and upper layer appeared bluish green indicated presence of deoxysugars.

Liebermann's test (test for bufadienoloids)

3ml extract with 3ml of acetic anhydride were mixed .it was heated and cooled. Few drops conc.H_2SO_4 were added. Blue colour appeared indicated presence of bufadienoloids.

Test for Anthraquinone glycosides

Borntrager's test for anthraquinone glycosides

To 3ml extract, 5%dil H_2SO_4 was added. It was boiled and filtered. To the cold filtrate; equal volume of benzene or chloroform was added and shaken well. The organic solvent was separated and then ammonia was added ammoniacal layer turned pink or red indicated the presence of anthraquinone glycosides.

Modified Borntrager's test for C- glycosides

To 3ml extract, 5ml 5%dil.HCl, few drops of $FeCl_3$ were added. It was then heated for five minutes in boiling water bath and cooled. to the cold filtrate equal volume of benzene or organic solvent was added. It was shaken well. The organic solvent was separated and ammonia was added. Ammoniacal layer turned pink or red indicated the presence of C-glycosides.

Test for Coumarin glycosides

Powder was moistened and taken in a test tube. The tube was covered with filter paper soaked in dilute NaOH and kept in water bath. Later the filter paper was exposed to UV light. Yellowish – green fluorescence indicated the presence of coumarin glycosides.

Test for Iridoid glycosides

Trim – hill reagent test

To the concentrated methanolic plant extract was added 1 ml of Trim-Hill reagent. It was then

heated for a few minutes. The presence of Iridoids was inferred when the solution turned to blue, green or red.

Test for coumarin glycosides

$FeCl_3$ test

To the conc. Alcoholic extract of the drug few drops of alcoholic $FeCl_3$ solution was added. Formation of deep green colour, which turned yellow on addition of conc.HNO_3, indicated the presence of coumarins.[16, 17, 18, 19]

Test for Saponins

Foam test

The drug extract or dry powder was shaken vigorously with water. Persistent foam observed indicated the presence of saponins.

Test for Starch

0.01g of Iodine and 0.075g of potassium iodide were dissolved in 5ml of distilled water and 2-3ml of extract was added. Formation of blue colour indicated the presence of starch.[16, 17, 18]

RESULTS AND DISCUSSION

The roots of *Paederiafoetida* were subjected to systemic physicochemical, fluorescence and preliminary phytochemical analysis. The data generated is helpful in determining the quality and the purity of the crude drug, especially in the powdered form. In this study the parameters included for the evaluation of *P.foetida* roots were moisture content, ash values (total ash, water soluble ash and acid insoluble ash), extractive values using alcohol, water and ether as solvents, swelling index and foaming index(Table 1).

Table.1: Results of all above physicochemical test on the powdered roots of *Paederiafoetida*

Sr. No.	Physicochemical Test	Results
1	Determination of Swelling Index	1.7cm
2	Determination of Foaming Index	Less than100
3	Determination of extractive value	
	• Alcohol soluble extractive of sample	9.8%w/w
	• Water soluble extractive of sample	9.2%w/w
	• Ether soluble extractive of sample	2.4%w/w
4	Determination of moisture content	6%w/w
5	Determination of ash values	
	• Total ash	8.75%w/w
	• Acid insoluble ash	1.1%w/w
	• Water soluble ash	1.8%w/w

The extractive values are, however, moderate but will be useful for the further extraction of phytoconstituents from the plant. The alcohol soluble extractive indicated the presence the

presence of polar constituents like phenols, flavanoids etc. The total ash is particularly important in the evaluation of purity of drugs i.e. the presence or absence of foreign matter such as metallic salts or silica. The fluorescence analysis performed showed a wide range of fluorescent colours for the crude drug with different reagents (Table 2). Fluorescence study of root powder helps in qualitative evaluation which can be used for its identification. The preliminary phytochemical screening of the ethanolic extract of roots was performed and it was found to contain Alkaloids, Glycosides, Carbohydrates, Triterpinoids, Steroids and Tannins (Table 3).

Table .2: Determination of fluorescence analysis of powdered roots of *Paederiafoetida* using various solvents.

Sr.no	Drug +reagent	Visible light	Short wavelength 254nm	Long wavelength 366nm
1	Powder	Black	Black	Black
2	Powder +50%NaOH (aqueous)	Light Brown	Light Brown	Bluish Green
3	Powder +50%NaOH (alcoholic)	Light Brown	Dark Brown	Green
4	Powder + Ammonia	Light Brown	Blue	Aqua Blue
5	Powder +Picric acid	Dark Brown	Light Brown	Light Brown
6	Powder +10%HCl	Brown	Brown	Sky Blue
7	Powder +10% H_2SO_4	Light Brown	Light Brown	Light Blue
8	Powder + Conc. HCl	Dark Brown	Dark Brown	Dark Green
9	Powder +Conc.H_2SO_4	Dark Brown	Dark blue	Dark Green
10	Powder +Conc. HNO_3	Orange Red	Brown	Orange Brown
11	Powder +10% NaOH	Dark Brown	Dark Brown	Dark Greenish blue
12	Powder + dist H_2O	Light Brown	Purple	Purple
13	Powder+ Methanol	Light Brown	Brown	Light Blue
14	Powder +Pet.Ether	Dark Brown	Colourless	Light purple
15	Powder +$CHCl_3$	Light Brown	Navy Blue	Purple
16	Powder+Ethyl Acetate	Light Brown	Light Brown	Violet
17	Powder +Acetone	Dark Brown	Dark Brown	Greyish
18	Powder+Solvent Ether	Light Brown	Light Brown	Light Blue
19	Powder+Ethanol	Light Brown	Light Brown	Light Brown
20	Powder+Toluene	Light Brown	Light Brown	Light Blue

Table 3: Phytochemical analysis of ethanolic extract of *Paederiafoetida* roots

Sr.No.	Chemical test	Result
1.	Alkaloids	++
2.	Carbohydrates	+
3.	Flavanoids	-
4.	Triterpinoids	+
5.	Steroids	+
6.	Tannins	+
7.	Resins	-
8.	Glycosides	+

9.	Cardiac glycosides	++
10.	Anthraquinone glycosides	-
11.	Iridoid glycosides	+
12.	Saponins	-
13.	Coumarins	-
14.	Proteins	-
15.	Starch	+

CONCLUSION

Paederiafoetida is widely available wild plant and used in traditional medicines having tremendous medicinal potential owing to its multiple biological functions. A lot of research has been done on the Pharmacological activity of the plant with so many activities reported and some yet to be done. Since the material traded mostly as roots, bark, twigs, flowers, leaves and fruits visible authentification of the material is difficult so there is need to set standards for such material in order to avoid adulteration of crude drugs. Despite the modern techniques, identification of plant drugs by pharmacognostic method is more reliable. The physicochemical parameters, fluorescence analysis and chemical test performed in the study will further guide in pharmacological and therapeutic evaluation of the species and will assist in standardization for quality, purity, and identification of drugs. In conclusion the parameters reported in the study will be useful in the development of further studies on the plant.

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