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PHARMACOGNOSTICAL PROFILE OF *PAEDERIA FOETIDA* LINN. LEAVES

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ABSTRACT

The leaves of *Paederia foetida* Linn. (*P. foetida)* are commonly known as skunk vine or Chinese fever vine, are used for various ailments medicinally throughout Asia and other tropical parts of the world by traditional healers. The plant is mainly used for arthritis and rheumatic disorders. The whole plant shows tonic, astringent and antiphlogistic actions and has been used in tenesmus. This present work presents a detailed pharmacognostical study of the leaf of the crude drug *P. foetida*. The samples were studied using procedures of light, confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this study may be proposed as parameters to establish the authenticity of *P. foetida* and may possibly help to differentiate the drug from its adulterants.

INTRODUCTION: Herbal drugs play an important role in the healthcare programs. Ancient literature incorporates a remarkable broad definition of medicinal plants and considers all parts of the plant to be potential source of medicinal substances. The main hindrance in the acceptance of herbal medicines is the lack of documentation and quality control. So it becomes extremely important to make an effort towards standardization of the plant material to be used as medicine [1].

Paederia foetida Linn., (Rubiaceae), is known as Prasarini in Sanskrit in India. It is an extensive foetid smelling perennial climber. The plant is found in most parts of India and all through the Malayan Archipelago, extending from Mauritius northward to China and Japan. The whole plant is specifically recommended for the treatment of rheumatism.

The root has been used as an emetic; its juice is useful for the treatment of piles, pain in chest and inflammation of spleen and liver. The leaves are said to be tonic, styptic, vulnerary and useful in ear-ache. It is reported to possess diuretic properties and helps to dissolve vesical calculi. The leaves, in the form of poultice, are used in herpes infection and are applied to the abdomen to relieve distension due to flatulence. The seeds are alexipharmic, used in piles and leucoderma. The fruits are used to prevent toothache [2].

A variety of curative properties have been attributed to the plant in folk medicine. It is used to treat enteromegaly, enterosis, flatulence, gastromegaly, rheumatism, rhinosis, sapraemia, sore, stomachache and toothache [3]. The plant is also reported to have anti-inflammatory [4] and Hepatoprotective [5] activities.

The drug has gained attention to some extent from phytochemical point of view. The phytochemical studies of the plant showed it contains iridiod glycosides, paederolone, paederone, paederine and paederenine [5]. Therefore, the present investigation on leaves of *P. foetida* is taken up to evaluate pharmacognostical standards which would help in crude drug identification as well as to determine the adulteration, if any. This study will further help to check the quality of finished herbal products.

MATERIALS AND METHODS:

Collection and authentication of Plant Material: The leaves of *P. foetida* were collected from the nursery of Mr. Manjunath Gooli, from Karje in Karnataka, India in the month of August 2010 ($30^{o}C \pm 2^{o}C$, rainy weather, and located 200m above mean sea level). The plant was identified by Dr. Gopalkrishna Bhat, Botanist, Poorna Prajna College, Udupi, Karnataka, India. A reference voucher specimen (PP 567) has been deposited in the herbarium of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Pharmacognostical Investigation: The different materials used for the study include basic microscopical equipments *viz*; compound microscope, glass slides, coverslips, watch glass and other common glass wares. The microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS 32 camera. Common solvents *viz*; ethanol (95%), hexane, petroleum ether ($60\text{-}80^{o}C$), solvent ether, n-butanol, chloroform and reagents *viz*; phloroglucinol, glycerin, hydrochloric acid, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals, Mumbai (India).

The macroscopy and morphology of the plant were studied according to the method developed by Brain and Turner [6]. Cross-sections for the microscopical studies were prepared and stained as per the procedure of Johansen [7]. The micro-powder analysis was also carried out using standard methods [6, 8].

Physicochemical values such as the percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopoeia [9] and WHO guidelines on quality control methods for medicinal plant materials [10].

Preliminary phytochemical screening was done for different extractives which were prepared by successive exhaustive solvent extraction of the powdered drug with different known solvents in increasing order of polarity *viz*; hexane, petroleum ether, solvent ether, n-butanol, chloroform, ethanol and finally with chloroform water [11, 12].

Histochemical tests [13] and Fluorescent analysis [14] of crude powder were also performed as per the standard methods. All the other standards *viz;* swelling index, bitterness value, foaming index, mucilage content and moisture content were done in compliance with the prescribed quality control methods for medicinal plant materials [10].

RESULTS AND DISCUSSION:

Morphological description of the plant: It is a slender, perennial herb. Its stinking and twining branches are 1.5-7m long. The young stems are purplish or reddish-brown, almost hairless to densely hairy. The old stems are yellowish-brown to grayish in colour, and it is smooth and shiny.

The leaf is simple, broadly egg-shaped and elliptical-oblong to linear, with sizes about 2-21cm x 0.7-9cm. The leaf base is heart-shaped, rounded or sometimes hastate, while the apex is acute to acuminate. The whitish to golden yellow-brown surface is hairless to variably hairy. The petiole size is 0.5-6(-9) cm long. Stipules are present in interpetiolar, rounded or ovate to triangular form in sizes ranging between 1.5-5mm x 2-3mm. It is usually entire, hairless or hairy.

The inflorescence consists of a terminal or axillary cymose panicle that is extremely variable. It grows from widely branched paniculate over 1m long to rather reduced size, normally 10cm long. The bracts are either leaf-like or small and linear, with few to numerous flowers, often in lax coiled cymes with peduncle that is 2-30mm long.

The flowers are bisexual, usually 5-merous; in dirty pink or lilac or purplish colour. The corolla lobes are pinkish to whitish on the inside while the throat is dark purple. The sepal is bell-shaped, with 5 normally smooth triangular-lobed with sizes up to 1mm x 0.6mm. The petal is cylindrical to bell-shaped, and sizes 5-17mm x 2-5mm.

The throat and the inside of the long tube are densely hairy with 5 oblong to triangular lobes and sizes between 1-3mm x 1.5-3mm. The margins are wavy and flexed. It has 5 stamens that are inserted in the middle of the tube which includes 2-2.5mm long anthers. The 2-celled and 2 ovuled ovary is inferior with a small disk and 4-15mm long style. The stigmas joined the style up to 2mm of its length. The 2 stigma branches are thread-like and irregularly twisted.

The sub-spherical fruit is a drupe at 4-6mm in diameter. The fruit walls are thin, dry and brittle. It is crowned by the persistent sepals, shiny pale brown to yellowish-or reddish-brown in color. The 2 semi-orbicular or semi-ellipsoidal kernels are flat on one side and convex or compressed on the other. It is normally slightly smaller than the fruit, without conspicuous wings, black in color and often conspicuously covered with needle-shaped crystals. The seedling is germinated above the ground, with cotyledons broadly rounded. The veins are prominent while the first pair of leaves form is elliptical and apex is acuminate (**Figure 1**).

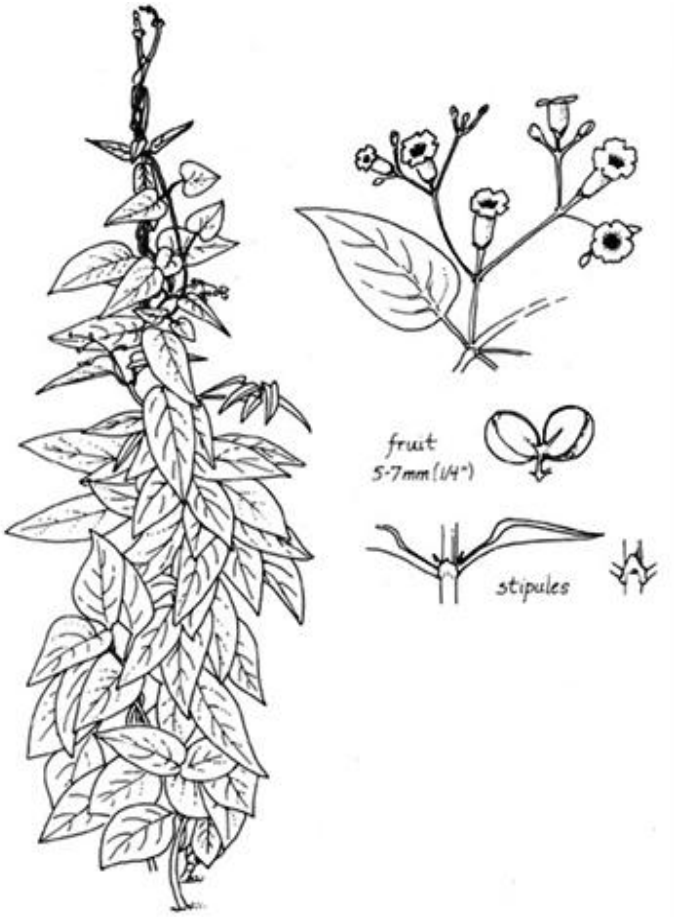

FIG. 1: MORPHOLOGY OF THE AERIAL PARTS OF *P. FOETIDA*

Anatomical characteristics of the leaf:

Macroscopy: The leaves are simple, petiolate, and stipulate and are 10-15cm long and 5-6cm broad; somewhat glabrous and mostly ovate; margin entire; base narrow or broad; apex acute or cuspidate. The veins are reticulate. The petiole is 1.2-6cm long. The stipules are ovate, lanceolate, bifid, entire, acute, base broad with hairy surface and thin texture (Figure 1). The taste is indistinct with foetid odour which is well marked in a fresh sample.

Microscopy: The leaf presents a dorsi-ventral structure. The epidermis is single layered covered externally with thin-ridged cuticle. The cells of upper epidermis, in surface view, have straight walls and the cuticular striation appear to radiate from the center of each cell as well as the outer wall of each subsidiary cell, while the cells of lower epidermis have sinuous walls and the cuticular striation diverge from the outer walls and the cuticular striation diverge from the outer walls of gaurd cells of stomata. Some of the epidermal cells of both the surface elongate to produce uni-seriate covering trichomes.

The mesophyll is composed of single layered palisade of cylindrical cells and 3 to 4 layers of spongy tissue having oval, triangular to irregular cells with distinct inter-cellular spaces. The margin of the leaf has a few thick-walled cells replacing the mesophyll. The veins are usually surrounded by bundle sheath; the larger ones are transcurrent while the smaller ones are embedded.

The presence of a large ground tissue, consisting of 2-5 layers of collenchyma and the rest of parenchyma following the externally cutinized single layered epidermis; and a large median cresent-shaped vascular bundle in both mid-rib and petiole are common anatomical features. But the latter differs from the former in possession of trichomes comparatively smaller in length as well as two more small and somewhat spherical accessory bundles, one flanking on each side of median vascular bundle close to the lateral extensions where they further split after reaching the distal end (**Figure 2 - 4**).

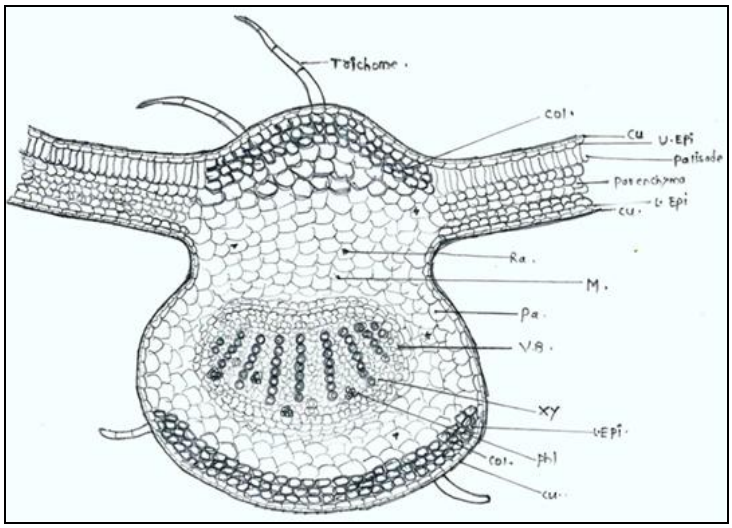

FIG. 2: TRANSVERSE SECTION OF THE LEAF OF *P. FOETIDA*

Col-Collenchyma, Cu-Cuticle, U.Epi-Upper epidermis, Pa-Palisade, M-Mesophyll, L.Epi-Lower epidermis, Ra-Raphides, V.B.-Vascular Bundles, Xy-Xylem, Phl-Phloem

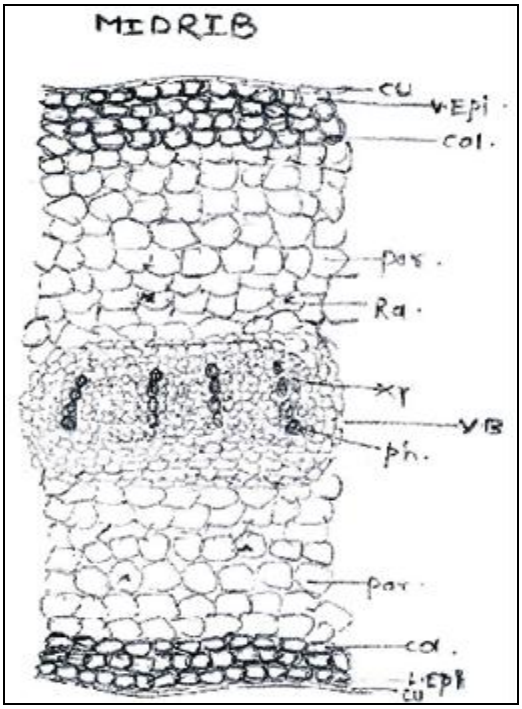

FIG.3: TRANSVERSE SECTION OF MIDRIB

Col-Collenchyma, Cu-Cuticle, U.Epi-Upper epidermis, Par-Parenchyma, Ra-Raphides, L.Epi-Lower epidermis, M-Mesophyll, V.B.-Vascular Bundles, Xy-Xylem, Phl-Phloem

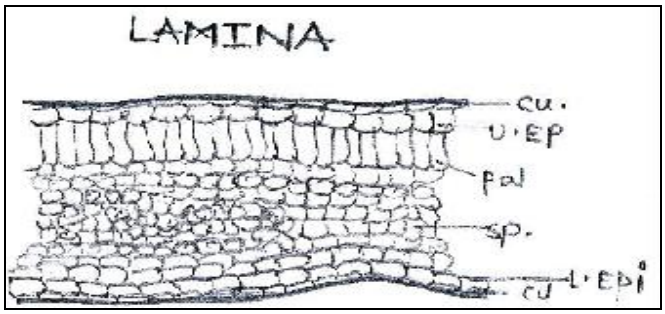

FIG.4: TRANSVERSE SECTION OF LAMINA

Cu-Cuticle, U.Epi-Upper epidermis, Sp-Spongy parenchyma, L.Epi-Lower epidermis, Pal-Palisade

Measurements of leaf constants: Leaf constants namely palisade ratio, stomatal index and vein islet number values were determined and are presented in **Table 1**.

TABLE 1: LEAF CONSTANTS OF *P. FOETIDA*

Name	Base	Middle	Apex	Mean
Palisade ratio	10.2-14.1	07.0-12.2	06.75-10.25	10.28
Stomata1 index	14.8-20.1	14.1-21.0	15.3-18.0	17.13
Vein-islet	06.0-09.0	06.0-08.0	05.0-10.0	07.41

Cell contents: Starch grains, oil globules and raphides of calcium oxalate are present in some of the cells of phelloderm, phloem, xylem and medullary rays of the root and the stem and also in the pith cells of latter. They are also present in the parenchymatous cells of the cortex of petiole and midrib and in the mesophyll cells of the leaf.

The starch grains are present as granular masses and are present more abundantly in the starch sheath, particularly in the young stem and they are usually abundant in the cells near the vascular bundles of the leaf. The oil globules are present as small circular body. The raphides are usually composed of spindle-shaped, thin crystals of calcium oxalate but rarely thick, spindle-shaped crystal of calcium oxalate are also found which sometimes appear as prismatic crystals when cut transversely.

Study of powder: The leaves dried in shade were finely powdered and passed through a sieve No.180 and a sieve No.125, separately, to obtain fine and very fine powder respectively and then subjected for microscopic examination.

The sample was treated with following reagents and studied for their components of diagnostic value (50% glycerine as temporary mountant; phloroglucinol (2% W/V) in ethanol (90%) and Conc. HCl (1:1) for lignified cork cells, parenchyma cells and phloem fibres; 5% W/V of alcoholic ferric chloride for tannin; 2% Iodine solution for starch grains; and Ruthenium red (0.08%) in 10% lead acetate for mucilage and results of histochemical tests are tabulated in **Table 2**.

TABLE 2: HISTOCHEMICAL TEST OF LEAF OF *P. FOETIDA*

Reagent	Test for	Color change	Result
Iodine	Starch	Blue	+
$FeCl_3$ sol. (10%)	Tannin	Black	+
Sudan III sol.	Oil globules	No change	-
Dil. HCl + pinch of phloroglucinol	Lignin	Magenta color	+
Con. HCl	Calcium oxalate crystals	Slight effervescence	+

+ - Present, - - Absent

The leaf powder is greenish in color with a foetid-odour and is slightly bitter. On microscopical examination the powder showed needle shaped crystals of calcium oxalate, a number of stone cells which are lignified are seen isolated or in groups, phloem fibres in single and groups, sieve elements. There are also a few lignified xylem vessels and non-lignified phloem cells (**Fig. 5**).

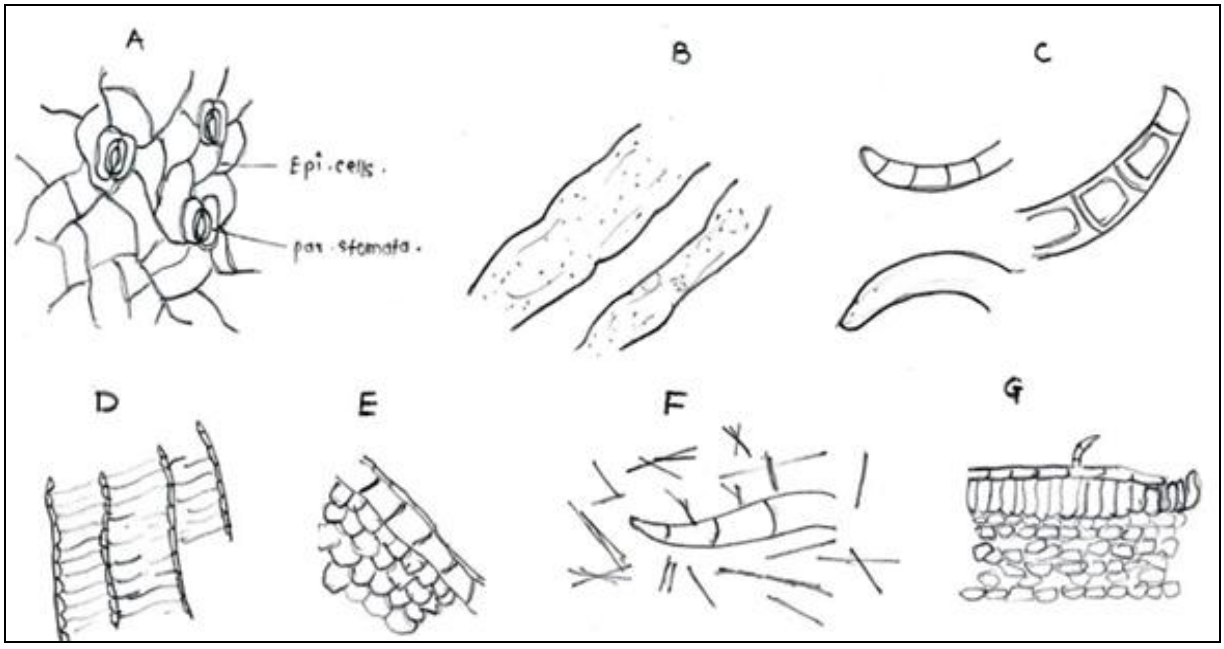

FIG. 5: POWDER MICROSCOPY OF THE LEAF POWDER OF *P. FOETIDA*

4A- Stomata, B- Cystoliths, C- Stomata, D- Xylem vessels, E- Epidermal cells, F-Calcium oxalate, G- Lamina portion.

Preliminary Phytochemical Screening: Preliminary phytochemical screening of the powder showed the presence of alkaloids, flavonoids, tannins, fixed oils and fats, volatile oils and phytosterols. The results are depicted in **Table 3.**

TABLE 3: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE LEAF POWDER OF *P. FOETIDA*

Test	Pet. ether	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	+	+	-	-	-	-
Carbohydrates	-	-	-	-	-	-
Phytosterols	+	-	-	+	+	-
Fixed oils and fats	+	+	-	-	-	-
Saponins	-	-	-	-	-	-
Phenolic compounds and tannins	-		-	+	+	+
Proteins	-	-	-	-	-	-
Gums and mucilage	-	-	-	-	-	-
Volatile oil	-	-	-	-	-	+
Flavonoids	-	-	-	-	+	+

+ - Present, - - Absent

Physicochemical Constants: The various physic-chemical parameters were determined and are represented in **table 4**. Moisture content of the leaf powder was found to be 11%; foaming index, swelling index and mucilage content value was found to be nil. No bitterness was detected.

TABLE 4: PHYSICOCHEMICAL CONSTANTS OF THE LEAF POWDER OF *P. FOETIDA*

Parameters	Values
Moisture content	11%
Bitterness value	Nil
Foaming index	Less than 100
Swelling index	0.5
Mucilage content	Nil
Volatile oil content	Less than 0.1 % v/w
Foreign matter	0.05%
Tannin content	1.24%
Total phenol content	2.35mg/g of Ferulic acid equivalent
Total flavonoid content	0.494mg/g Quercetin equivalent

An ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values of the powdered *P. foetida* leaves revealed a high concentration of Sulphated ash (10.75%) and Nitrated ash (10.45%), whereas acid insoluble ash was found to be very low since the drug was collected a fresh (**Table 5**).

TABLE 5: ASH VALUES OF THE LEAF POWDER OF *P. FOETIDA*

Parameters	Values %w/w
Total ash	10.67
Acid insoluble ash	1.10
Water soluble ash	6.02
Sulphated ash	10.75
Carbonated ash	5.22
Nitrated ash	10.45

The extractive values which is of equal importance for the quality control of any crude drug is primarily useful for the determination of exhausted drug. The water soluble extractive was high in *P. foetida* (**Table 6**).

TABLE 6: EXTRACTIVE VALUES OF THE LEAF POWDER OF *P. FOETIDA*

Parameters	Values % w/w
Hot extraction	28.84
Cold maceration:	
a) water soluble extractive	21.34
b) Ethanol soluble extractive	15.6
c) Nonvolatile ether soluble extractive	2.84

Successive Solvent Extractive Constants: Of the total extractive value, the major portion was found to be aqueous extract (18.4%) followed by ethanol, pet ether, benzene, acetone and chloroform (**table 7**).

TABLE 7: SUCCESSIVE SOLVENT EXTRACTIVE CONSTANTS OF THE LEAF POWDER OF *P. FOETIDA*

Solvents	Consistency and color	Average value of extractive (%w/w)
Pet. Ether	sticky semisolid/ Dark green	10.25
Benzene	Sticky semisolid/yellowish green	1.87
Chloroform	Sticky semisolid/Dark green	0.64
Acetone	Sticky semisolid/Green	0.50
Ethanol	Sticky semisolid/Green	15.6
Water	Non sticky Solid/ Dark brown	18.4

Fluorescence Analysis: Fluorescence analysis of crude drug powder was carried out in various solvents. The results are presented in **Table 8**.

TABLE 8: FLUORESCENCE ANALYSIS OF LEAF POWDER OF *P. FOETIDA*

Parameters	Visible light	UV light	
		Short wave (254nm)	Long wave (365nm)
Powder as such	Light Green	Green	Light brown
In Methanol	Light green	Green	Light green
In Methanol NaOH	Green	Dark green	Greenish brown
In ethanol	Light Green	Dark green	Green
In ethanol NaOH	Green	Dark green	Dark yellowish green
In dil. HCl	Light brown	Green	Dark green
In dil. H_2SO_4	Light green	Dark green	Dark green

CONCLUSION: The present study concluded that the various morphological, microscopic and physic-chemical standards developed will beneficial for plant identification and standardization of the crude plant, *Paederia foetida* Linn. Further, these findings lead the importance for the standardization of this plant leaf material to ensure the quality of the herbal formulations.

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