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PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF *CAYRATIA PEDATA* VAR. *GLABRA* – A VITACEAE MEMBER

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ABSTRACT: To evaluate a meticulous pharmacognostic crum of *Cayratia pedata* (Lam.) Gagnep. var. *glabra* Gamble (Vitaceae), an important endemic and endangered medicinal plant in Thiashola, Manjoor. The study genus is used in the Siddha, Ayurveda and Folk medicine for treating diarrhoea, refrigerant, hysteria, astringent and ulcers. However, the detailed scientific information of *Cayratia pedata* var. *glabra* is not available to identify the plant material and to ascertain its quality and purity. The plant *C. pedata* var. *glabra* is an herbaceous, large, fragile climber grows up to a height of 8-12 m with nodes and internodes. In present communication, morphology, anatomical and physico-chemical characters along with phytochemical screening and fluorescence analysis of powdered crude drug were carried out for systemic identification and authentication of aerial plant parts. This study provides referential information for identification and characterization of *C. pedata* var. *glabra* and its extracts.

INTRODUCTION: Indian herbal pharma industries normally face lot of problem for adulteration and substitution. There is an increase in domestic stipulate for raw materials used for perfumeries, pharmaceutical and biopesticidal units^{1, 2}. Due to the paucity of drugs and / or the high cost, the authentic drugs are often adulterated (intentional and unintentional) or substituted with similar drugs. In many instances research on crude drugs indicate that both macro and microscopic characters often help in correct identification of the drug. When a leaf drug is adulterated with its allied species hardly differing morphologically, the quantitative microscopy may serve the purpose of identification.

In the phytopharmaceutical industry presently no quality standards have been fixed for the raw materials and as such one finds wide variations in the quality specifications³.

Cayratia pedata (Lam.) Gagnep. var. *glabra* Gamble (Family: Vitaceae) (**Fig. 1**) is a weak climber commonly known as kattuppirandai, ainthilai kodi (5-pedata) in tamil, goalilata in hindi, godhapadi in sanskrit and velutta sori valli in malayalam. This species can be found in Thiashola and Korakundah range and scrambling over the hedges and trees. Vitaceae consist of approximately 14 genera and about 900 species primarily distributed in tropical regions in Asia, Africa, Australia, the neotropics and the Pacific islands⁴.

The family is well known economically for grapes, wine and resins. The leaf decoction of the study genus is used for treating uterine and other fluxes⁵; lukewarm leaf juice is used as ear drops for fungal infections⁶; leaves are astringent, refrigerant and also used to cure ulcers⁷; stem paste is applied for

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healing bone fracture; whole plant is useful in acid, refrigerant and beneficial in hysteria, burning of the skin and diarrhoea.

Until 1990s scientists thought that most of the compounds produced by plants were useless waste products. These waste compounds are called as secondary metabolites to distinguish from primary metabolites including sugars and amino acids that are essential for plant functions. But later found that these secondary metabolites perform a huge array of functions⁸. Secondary metabolites have a wide range of application in pharmaceuticals, chemicals and food industries. Secondary metabolites, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents⁹. For the standardization and quality assurance purpose, the following three attributes (authenticity, purity, assay) must be verified.

However, the detailed scientific information of *Cayratia pedata* var. *glabra* is not available. Hence, in this present study we make an attempt for the standardization of *Cayratia pedata* var. *glabra* by carrying out its pharmacognostic evaluation.

MATERIALS AND METHODS:

Chemicals and instruments: Formalin, acetic acid, ethyl alcohol, HCl, concentrated H₂SO₄, concentrated HNO₃, acetic acid and all other chemicals used in the study were of analytical grade.

Plant material: Aerial part of *Cayratia pedata* var. *glabra* were collected from Thiashola, Manjoor, Nilgiris South Division, Western Ghats before that we got proper permission from the Principal Chief Conservator of Forests, Chennai and the District Forest Officer, Ooty under Section 28 (i) of Wildlife Protection Act, 1972, in the month of October. Since the study shola is declared as reserve forest, the entry is restricted and direct impact of man on the forest is negligible. The voucher herbarium specimen was preserved by standard methods Jain and Rao, 1970¹⁰. The plant species is initially identified with the help of the existing local Floras^{11, 12, 13} and the identity is authenticated by matched with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus,

Coimbatore, Tamil Nadu (No. BSI/SRC/5/23/2010-11/Tech. 1300). The type specimen was deposited for further reference in Vellalar College for Women, Erode.

Macroscopic and microscopic analysis: The macroscopical and microscopical studies of the plant were carried out according to the method of Johansen, 1940¹⁴ and Wallis, 1985¹⁵. Fresh leaves, stem and tendrils were separated from the plant and thoroughly washed with running water to remove the adherent impurities. Some quantities of the leaves were air dried, powdered and stored in air-tight containers for powder analysis. Fresh leaves were used for free hand section cutting and were fixed in FAA and dehydrated with TBA as per the schedule given by Sass, 1940¹⁶.

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. Dewaxing of the sections was done by customary procedure Johansen, 1940¹⁴. The sections were stained with Toluidine blue, Safranin and IKI - Lugol's iodine as per the method of O'Brien *et al.*, 1964¹⁷. After clearing the T.S various microscopical studies were carried out in the study plant. For studying the leaf constants like stomatal morphology and trichome distribution Jeffrey's maceration fluid Sass, 1940¹⁶ were prepared. Different cell components were studied and measured. Photographs of different magnifications (40x and 100x) were taken with NIKON ALPHA PHOTO-2 microscopic unit. Descriptive terms of the anatomical features are taken from the standard anatomy book Esau, 1964¹⁸.

Physico-chemical analysis: Physicochemical values such as organoleptic characters of plant powder and the successive extracts, behaviour of plant powder with different chemical reagents, fluorescence analysis, moisture content and ash values were determined according to the official method^{19, 20, 21} and the WHO guidelines on quality control methods for medical plants material²².

Behaviour of powder with different chemical reagents: The behaviour of powdered plant material treated with different chemical reagents such as concentrated HCl, concentrated H₂SO₄, concentrated HNO₃ and acetic acid was observed²⁰.

Fluorescence analysis: Fluorescence behaviour of the powdered plant material with different chemical reagents like 1N NaOH in H₂O and 1N NaOH in ethanol was observed under day light and UV light at 254 nm²⁰.

Determination of moisture content (Loss on drying): The loss in weight and the percentage of loss on drying were calculated as per the method of Anonymous²¹.

Total ash: The ash values were determined as per Trease and Evans¹⁹. The ash values are helpful in determining the quality and purity of the plant sample.

Extractive values (Solubility percentage): Extractive values were determined following the procedure of Trease and Evans¹⁹.

Successive solvent extraction: The air dried, powdered plant material was extracted in Soxhlet apparatus successively with different solvents in the increasing order of polarity [Acetone (56.5°C), Ethanol (78.5°C) and Water (99.98°C)]. Each time, before extracting with the next solvent, the powdered material was dried in a hot air oven at 40°C. Finally, the material was macerated using hot water with occasional stirring for 16 hrs and the water extract was filtered. The different solvent extracts were concentrated, vacuum dried and weighed. The percentage yields were expressed in terms of air dried sample. The extracts were dried over anhydrous sodium sulfate, stored in sealed vials in refrigerator (5-8°C) until analysis²¹.

Preliminary phytochemical screening: Phytochemical screening of different successive solvent extracts was carried out using the standard procedure described by Kokate²³.

Quantitative phytochemical studies:

Determination of total phenolics and tannins: Determination of total phenolics and tannins were determined following the procedure of Siddhuraju and Becker²⁴.

RESULTS AND DISCUSSION: Standardization is an essential measure of quality, purity and authenticity. Microscopic method is one of the simplest and cheapest methods to start with establishing the correct identification of the source

materials Kokoshi²⁰. Formerly there is no pharmacognostic work was recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Microscopic examination of fresh leaf and leaf powder is one of the identifying parameters to substantiate and authenticate the drug. Botanical detection of a phytodrug involves two steps.

One is identification of the plant by its floral characters and the other is diagnosis of the plant with its microscopic characters. The latter procedure is useful for identification of fragmentary plant specimens. Early plant morphologist Robert Hook²⁵ clearly demonstrated that each kind of plant has its own distinctive structure by means of which it can be recognized.

Macroscopic characteristics: The macroscopic characters of fresh aerial plant parts of *C. pedata* var. *glabra* are presented in **Table 1**. The plant *C. pedata* var. *glabra* is an herbaceous, large, fragile climber grows up to a height of 8-12 m with nodes and internodes. Terminal buds of plants develop into tendrils. The present morphological investigations revealed that the stem is hirsute and grows up to a height of 12 m. The leaves are alternate, pedately lobed, lobes oblong and acuminate with smooth surface and texture. The size is 3 to 6 cm. The stem and leaves showed characteristic odour and bitter taste. Fruit shape is the important differentiable characterization among the other genus (**Fig. 1**). Several earlier workers have described morphological features as one of the effective parameters for the pharmacognostical identification of crude drugs^{26,27}.

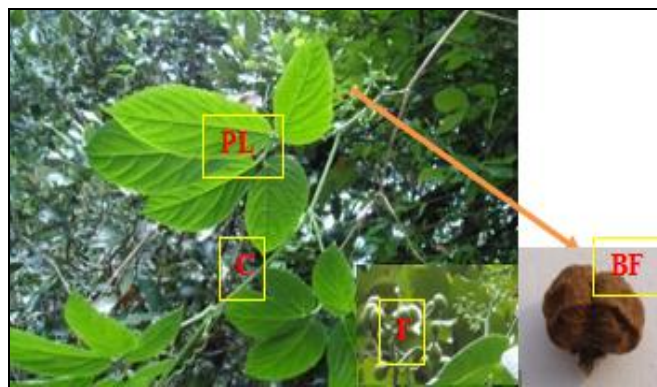


FIG. 1: CAYRATIA PEDATA (LAM.) GAGNEP. VAR. GLABRA GAMBLE, IN ITS NATURAL HABIT
F: Fruit; PL: Pedate Leaf; C: Climber; BF: Bilobed Fruit

TABLE 1: MACROSCOPIC ANALYSIS OF AERIAL PLANT PARTS OF *C. PEDATA* VAR. *GLABRA*

S.no.	Macroscopic characters
1.	Stem: Hirsute Height: Up to 12 m in height Surface: Bearing distinct hairs Texture: Smooth Taste: Bitter Odour: Characteristic Colour: Dark green
2.	Leaves: Alternate, pedately 5 foliolate, 8 - 15 cm long Leaflets: Elliptic, oblong, apex acuminate, serrate Number of leaflets: 7 - 12 Size: 3-6 cm Surface: Quite glabrous except on the veins underneath Texture: Smooth Taste: Bitter Odour: Characteristic Colour: Dark green
3.	Tendrils: Leaf opposed, branched, wiry, coiled
4.	Flowers: At first yellow later white
5.	Fruit: Berry, bilobed

Microscopic characteristics: Plant micro technique is a branch of botanical science that deals with the internal structure and organization of plant organs. This microscopic structure studies are closely correlated with the function of an organ Asokan²⁸. The present anatomical study provides a set of characters specific for *C. pedata* var. *glabra* with which one can establish the identity of the

plant in fragmentary form. Free hand section of the leaf observed under the microscope revealed that the leaf has thick midrib and lateral veins and thin dorsiventral lamina. The midrib is characteristic in having thick (1.15 mm), flat and wide adaxial (500 μ m in horizontal plane and 350 μ m in height) hump and thick and wide semicircular abaxial part (1.2 mm wide) (**Fig. 2**).

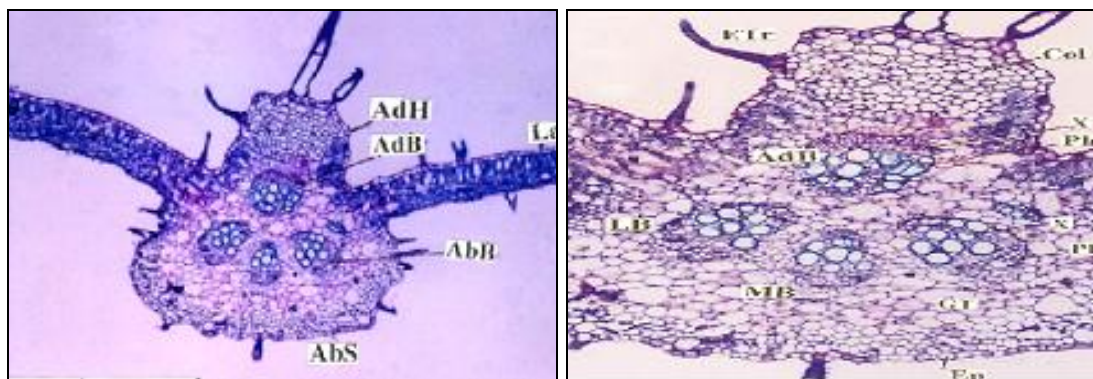


FIG. 2: T.S. OF LEAF THROUGH MIDRIB ENLARGED SECTION AbS: Abaxial Side; AbB: Abaxial Bundle; La: Lamina; AdB: Adaxial Bundle; AdH: Adaxial Hump; LB: Lateral Bundle; MB: Middle Bundle; X: Xylem; Ph: Phloem; GT: Ground Tissue; Ep: Epidermis; ETr: Epidermal Trichome; Col: Collenchyma

In lamina portion calcium oxalate crystals of raphide type are fairly abundant in the palisade zone; the raphide bundles are vertical in position, parallel to the palisade cells (**Fig. 3**). The adaxial epidermal cells are wide, angular and thick walled with slightly wavy anticlinal walls. The adaxial layer is apostomatic (**Fig. 4**). The abaxial epidermis has smaller cells with thin walls and is more wavy. The stomata are anomocytic, having no specific subsidiary cells. The guard cells are circular measuring 15-20 μ m in diameter. The stomatal

pore is distinct and elliptic (**Fig. 4**). Calcium oxalate crystals are abundant in the mesophyll, veins and trichomes. Druses or sphaero-crystals are seen in vertical rows within the veins. On the surface of the epidermal trichomes are seen as minute prismatic crystals in dense masses. Raphides are thick cylindrical bundles of several needle shaped crystals aggregated together (**Fig. 5**). This is in corroboration with the work of Sutapa Choudry²⁹.

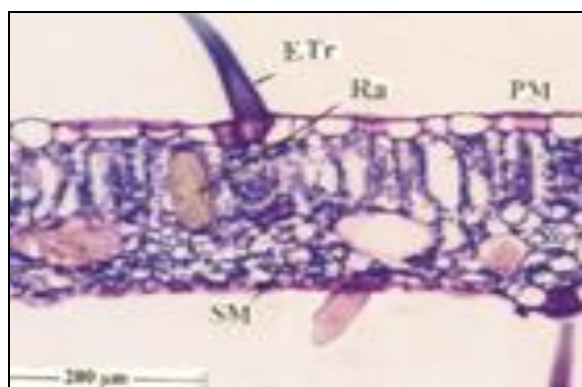


FIG. 3: T.S OF LAMINA

ETr: Epidermal Trichome; **Ra:** Raphide; **PM:** Palisade Mesophyll; **SM:** Spongy Mesophyll

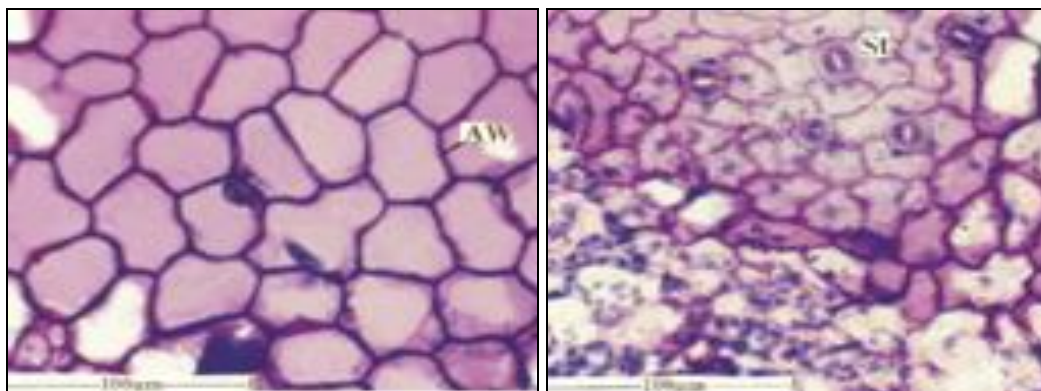


FIG. 4: SECTION OF THE ADAXIAL AND ABAXIAL EPIDERMIS

AW: Anticlinal Walls; **St:** Stomata; **EC:** Epidermal Cells

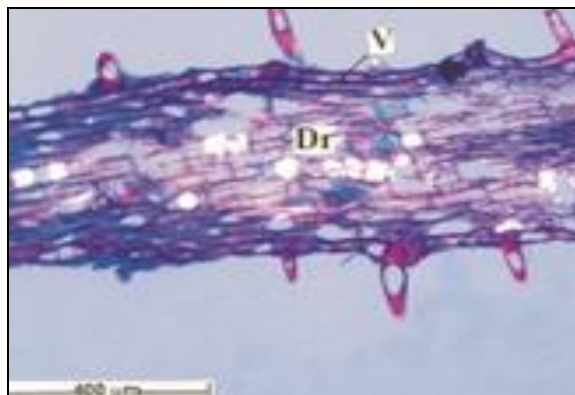


FIG. 5: L.S OF A VEIN OF THE LEAF SHOWING DISTRIBUTION OF CALCIUM OXALATE DRUSES

Dr: Druses; **V:** Vein

Powder characteristic: The organoleptic evaluation of the aerial plant powder revealed the following characteristics. The plant powder showed characteristic odour and bitter taste. Upon drying and powdering the colour of the powder changed

from dark green to greenish black as shown in **Table 2**. The organoleptic characters such as colour, consistency and odour were noted in the successive aerial plant extracts of *C. pedata* var. *glabra* (**Table 3**).

TABLE 2: ORGANOLEPTIC CHARACTERS OF AERIAL PLANT POWDER OF *C. PEDATA* VAR. *GLABRA*

S.no.	Characters	Observations
1.	Colour	Greenish black
2.	Texture	Fine smooth powder
3.	Taste	Bitter
4.	Odour	Characteristic smell

TABLE 3: ORGANOLEPTIC CHARACTERS OF AERIAL PLANT SUCCESSIVE EXTRACTS OF *C. PEDATA* VAR. *GLABRA*

S. no.	Extraction Medium	Colour	Consistency	Odour
1.	Acetone	Yellowish green	Semi solid	Characteristic smell
2.	Ethanol	Brownish black	Semi solid	Characteristic smell
3.	Water	Brownish black	Solid	Characteristic smell

Powder treated with different chemical reagents: The behaviour of aerial plant powder with various reagents were observed and presented in **Table 4**. Slight difference (pale green to dark

green) was noted in the powder as such and treated with concentrated HCl when compared to other reagents used.

TABLE 4: BEHAVIOUR OF AERIAL PLANT POWDER WITH DIFFERENT CHEMICAL REAGENTS

S. no.	Powder + Reagents used	Colour of the powder
1	Powder as such	Pale green
2	Powder + Concentrated HCl	Dark green
3	Powder + Concentrated H ₂ SO ₄	Greenish brown
4	Powder + Concentrated HNO ₃	Wood brown
5	Powder + Acetic acid	Greenish yellow

Fluorescence analysis: Fluorescence analysis of plant powder as such showed pale green to dark green in visible and UV light. Powder treated with NaOH in water and NaOH in ethanol showed slight

colour differences like pale greenish yellow to yellowish green to light green. There is no distinct colour differences was seen in visible and UV light (**Table 5**).

TABLE 5: FLUORESCENCE BEHAVIOUR OF AERIAL PLANT POWDER OF *C. PEDATA* VAR. *GLABRA*

S. no.	Reagents	Behaviour of powder	
		Visible light	UV light
1.	Powder as such	Pale green	Dark green
2.	Powder + 1 N NaOH in water	Pale greenish yellow	Yellowish green
3.	Powder + 1 N NaOH in ethanol	Yellowish green	Greenish yellow

Ash values and extractive values: The results of ash values determination indicate that the sample contained 67.32% moisture. The total ash content of the sample was 5.40%. The percentage of extractive value was maximum in ethanol extract (15%) followed by acetone extract (7.50%) respectively.

The extractive values are primarily useful for the determination of the exhausted or adulterated drug (**Table 6**).

TABLE 6: PHYSICO-CHEMICAL AND EXTRACTIVE VALUES OF AERIAL PLANT POWDER OF *C. PEDATA* VAR. *GLABRA*

S. no.	Physico-chemical properties	Values in percentage
1	Moisture content (Loss on drying)	67.32
2	Total ash	05.40
3	Extractive values	
	a. Acetone	07.50
	b. Ethanol	15.00

Preliminary phytochemical screening: A compound or a group of compounds present can serve as a “Biomarker” and the presence and concentration of the same can be followed to decide on the genuineness of the drug / formulation²⁴. To investigate the chemical constituents of plant powder of *C. pedata* var. *glabra*, the successive solvent extracts were subjected to qualitative phytochemical screening. The preliminary phytochemical screening revealed the presence of carbohydrates, proteins, aminoacids, alkaloids, anthroquinones, flavonoids, glycosides, phenols and tannins, steroids and sterols, triterpenoids and volatile oil. The acetone extraction was more efficient than ethanol and water extracts (**Table 7**). All extracts showed negative response for saponins. The results obtained from the preliminary phytochemical screening will reveal the useful findings about the chemical nature of the drug. In addition total ash values, fluorescence analysis and extractive values will be helpful in identification

and authentication of the plant material³⁰. The chemical constituents of crude drug extractive values are useful to evaluate the

TABLE 7: QUALITATIVE PHYTOCHEMICAL SCREENING OF AERIAL PLANT POWDER EXTRACTS OF *C. PEDATA* VAR. *GLABRA*

S. no.	Chemical constituents	Chemical Tests	Acetone extract	Ethanol extract	Water extract
1	Carbohydrates	Molisch's test	-	-	+
		Barfoed's test	-	-	+
2	Proteins	Warming test	-	+	+
		Test with trichloroacetic acid	-	+	+
3	Aminoacids	Biuret test	-	+	+
		Millon's test	-	+	+
4	Alkaloids	Ninhydrin test	-	+	+
		Dragendorff's reagent	+	-	-
5	Anthroquinones	Mayer's reagent	+	-	-
		Wagner's reagent	+	-	-
6	Flavonoids	Borntrager's test	+	-	-
		Alkaline reagent test	+	+	+
7	Glycosides	Zinc hydrochloride test	+	+	+
		Borntrager's test	+	+	+
8	Phenols and tannins	Ferric chloride test	+	+	+
		Foam test	-	-	-
9	Saponins	Salkowski test	+	-	-
		Sulfur test	+	-	-
10	Steroids and sterols	Liebermann-Burchard test	+	+	+
		Sudan test	+	-	-
11	Triterpenoids				
12	Volatile oil				
13					

Note: '+', '-' indicates the presence / absence of compounds

Total phenolics and tannin content: Phenolic compounds are common in plants and have multiple biological effects. Many of the polyphenols have been identified as anticancer³¹ and cardioprotective³². Tannins are naturally occurring phenolic compounds which precipitate protein³³. In the current study, the total phenolics and tannin content of different solvent extracts of *C. pedata* var. *glabra* were studied and expressed as tannic acid equivalent. As shown in Table 8, the total phenolic content was maximum in ethanolic

extract (131.7 ± 3.6 mg/g) followed by acetone extract (56.8 ± 0.8 mg/g). The minimum was recorded in water extract (54.1 ± 1.8 mg/g). When compared with other solvent extracts, ethanol extract registered higher levels of tannin content (52.8 ± 12.9 mg/g) followed by acetone extract (24.1 ± 4.5 mg/g). The minimum was recorded in water extract (10.5 ± 3.2 mg/g). This is in agreement with the reports of Yen and Chen^{34, 35} who reported that ethanol is an effective solvent for extraction of antioxidants³⁶.

TABLE 8: ESTIMATION OF TOTAL PHENOLICS AND TANNIN CONTENT OF DIFFERENT SOLVENT EXTRACTS OF *C. PEDATA* VAR. *GLABRA* PLANT POWDER

S. no.	Extraction Medium	Total phenolics (mg TAE/g extract) [#]	Tannin(mg TAE/g extract) [#]
1	Acetone	56.8 ± 0.8	24.1 ± 4.5
2	Ethanol	131.7 ± 3.6	52.8 ± 12.9
3	Water	54.1 ± 1.8	10.5 ± 3.2

[#] Values are means of three independent analysis \pm Standard Deviation TAE - Tannic acid equivalent

CONCLUSION: In conclusion, detailed anatomical perspectives of the plant organs has been unequally established that certain anatomical characters of plant organs sustain the environmental stress and remain little modified. Such features can be relied upon for diagnosis of the plant. The total

summation of macroscopic features coupled with microscopic parameters will furnish the protocol for clinical or pharmaceutical investigations. The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant.

Pharmacognostic evaluation can be useful to substantiate and authenticate the plant. On the basis of the results obtained in the present study, it is concluded that the ethanolic extract of *C. pedata* var. *glabra*, which contains large amounts of phenolics and tannins, exhibits high antioxidant and free radical scavenging activities.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest

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