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PHARMACOGNOSTICALANDPHYTOCHEMICALANALYSIS OF*CAYRATIAPEDATA*VAR. *GLABRA*–AVITACEAEMEMBER

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ABSTRACT: To evaluate a meticulous pharmacognostic cram of *Cayratiapedata*(Lam.)Gagnep.var.*glabra*Gamble(Vitaceae),animportantendemic and endangered medicinal plant in Thiashola, Manjoor. The studygenusisusedintheSiddha,AyurvedaandFolkmedicinefortreatingdiarrhoea, refrigerant, hysteria, astringent and ulcers. However, the detailedscientificinformation of*Cayratiapedata*var.*glabra*isnotavailabletoidentify 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 aheightof8-12 m with nodesand internodes.In presentcommunication,morphology,anatomicalandphysico-chemicalcharactersalongwithphytochemical screening and fluorescence analysis of powdered crude drugwere carried out for systemic identification and authentication of aerial plantparts.Thisstudyprovidesreferentialinformationforidentificationandcharacterizationof*C. pedata*var. *glabra*andits extracts.

Keywords: *Cayratiapedata*var.*glabra,* Pharmacognostical,Phytochemical, crudedrug

INTRODUCTION: Substitution and adulteration are common problems in the Indian herbal pharma sector. Perfumery, pharmaceutical, and biopesticide raw material specifications have recently seen an uptick in local demand 1, 2. Because of the scarcity and/or high cost of prescriptions, it is common for genuine medications to be mixed up (intentionally or unintentionally) or replaced with comparable alternatives. Evidence from studies of crude narcotics suggests that a combination of macro and microscopic features is often useful for accurate drug identification. It is possible to use quantitative microscopy to identify a leaf medicine that is contaminated with a related species that is hardly distinguishable morphologically. Wide differences in quality parameters are seen in the phytopharmaceutical sector since no quality standards for the raw materials have been defined today. 3.

Beloved as kattuppirandai in Tamil, ainthilaikodi (5-pedata) in Hindi, godhapadi in Sanskrit, and veluttasorivalli in Malayalam, Cayratiapedata (Lam.) Gagnep.var. glabra Gamble belongs to the Vitaceae family and is a weak climber (Fig. 1). This species is known to scramble over trees and hedges in the Thiashola and Korakundah ranges. Most of the fourteen genera and nine hundred species that make up the vitaceae family live in tropical areas of the neotropics, Asia, Africa, Australia, and the Pacific islands. 4.

Grapes, wine, and resins are the family's bread and butter. Uterine and other fluxes may be treated using the leaf decoction of the species under investigation. 5. Ear drops made of lukewarm leaf juice may be used to treat fungal infections. 6. The leaves have astringent, cooling, and ulcer-curing properties. 7. A paste made from the stems may help mend bone fractures. The whole plant has bitter and cooling properties, and it helps with hysteria, skin burning, and diarrhea.

Up until the 1990s, scientists considered the vast majority of plant chemicals to be innocuous byproducts. Different from the main metabolites, such as glucose and amino acids, which are necessary for the plant to operate, these waste products are known as secondary metabolites. It was only later that scientists discovered the myriad of roles played by these secondary metabolites. 8. Many different sectors, including the food, chemical, and pharmaceutical industries, make use of secondary metabolites. Extensive research on secondary metabolites as a potential source of therapeutic drugs has been conducted, despite their

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previously unknown pharmacological activity. 9. Verification of authenticity, purity, and assay is necessary for quality assurance and standardization purposes.

Unfortunately, there is a lack of comprehensive scientific data on Cayratiapedata var. glabra. Therefore, we conduct its pharmacognostic assessment in this work to try to standardize Cayratiapedata var. glabra.

MATERIALSAND METHODS:

Chemicalsandinstruments: Formalin,aceticacid, ethylalcohol,HCl,concentratedH_2SO_4,concentrate dHNO_3,aceticacidandallotherchemicalsusedinthes tudywereofanalyticalgrade.

Plant material: Aerial part of *Cayratiapedata*var.*glabra*werecollectedfromThia shola,Manjoor,Nilgiris South Division, Western Ghats before thatwe got proper permission from the Principal ChiefConservator ofForests,Chennai and the DistrictForestOfficer,OotyunderSection28(i)ofWi ldlifeProtectionAct,1972,inthemonthofOctober.Si ncethestudysholaisdeclaredasreserveforest,theentr yisrestrictedanddirectimpactofmanontheforestisn egligible.Thevoucherherbariumspecimenwaspres ervedbystandard methods Jain and Rao, 1970 [10]. The plantspecies is initially identified with the help of theexistinglocalFloras[11,12,13]andtheidentityisauthe nticatedbymatchedwithtypespecimensavailable in the herbarium of Botanical Survey ofIndia,SouthernCircle,TNAUCampus,Coimbato re, Tamil Nadu (No. BSI/SRC/5/23/2010-11/Tech. 1300). The type specimen was depositedforfurtherreferenceinVellalarCollegefor Women,Erode.

Macroscopicandmicroscopicanalysis: Themac roscopicalandmicroscopicalstudiesoftheplant were carried out according to the method ofJohansen, 1940 [14] and Wallis, 1985 [15]. Fresh leaves,stem and tendrils were separated from the plant andthoroughly washed with running water to removetheadherentimpurities.Somequantitiesof theleaves were air dried, powdered and stored in air–tight containers for powder analysis. Fresh leaveswere used for free hand section cutting and werefixed in FAA and dehydrated with TBA as per theschedulegiven bySass,1940[16].

The paraffin embedded specimens were sectionedwith the help of Rotary Microtome. Dewaxing ofthesectionswasdonebycustomaryprocedureJo hansen, 1940 [14]. The sections were stained withToluidine blue, Safranin andIKI -Lugol's iodineas per the method of O'Brien *et al.,* 1964 [17]. Afterclearing the T.S various microscopical studies werecarried out in the study plant. For studying the leafconstants like stomatal morphology and trichomedistribution Jeffrey's maceration fluid Sass, 1940 [16]wereprepared.Differentcellcomponentswerest udiedandmeasured.Photographsofdifferentmag nifications(40xand100x)weretakenwithNIKON ALPHAPHOTO-2microscopicunit.Descriptivetermsoftheanatom icalfeaturesaretakenfromthestandardanatomybo okEsau,1964 [18].

Physico-chemicalanalysis: Physicochemicalvaluessucha sorganolepticcharactersofplantpowder and the successive extracts, behaviour ofplantpowderwithdifferentchemicalreagents,fl uorescenceanalysis,moisturecontentandashvalu esweredeterminedaccording totheofficialmethod [19,20,21] and the WHO guidelines on qualitycontrolmethods formedical plantsmaterial[22].

Behaviourofpowderwithdifferentchemicalre agents: Thebehaviourofpowderedplantmaterialt reatedwithdifferentchemicalreagentssuchasconc entratedHCl,concentratedH_2SO_4,concentratedH NO_3andaceticacidwasobserved [20].

Fluorescence analysis: Fluorescence behaviour ofthe powdered plant material with different chemicalreagents like 1N NaOH in H_2O and 1N NaOH inethanol was observed under day light and UV lightat 254 nm[20].

Determinationofmoisturecontent(Lossondryin g): The loss in weight and the percentage ofloss on drying were calculated as per the method

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ofAnonymous[21].

Total ash: The ash values were determined as perTrease and Evans [19]. The ash values are helpful indeterminingthequalityandpurityoftheplantsample.

Extractivevalues(Solubilitypercentage): Extractivevalueswheredeterminedfollowingtheprocedureof TreaseandEvans[19].

Successivesolventextraction: Theairdried,powdered plant material was extracted in Soxhletapparatussuccessivelywithdifferentsolventsinthe increasing order of polarity [Acetone (56.5°C),Ethanol (78.5°C) and Water (99.98°C)]. Each time,beforeextractingwiththenextsolvent,thepowdered material was dried in a hot air oven at40°C. Finally, the material was macerated using hotwater with occasional stirring for 16 hrs and thewaterextractwasfiltered.Thedifferentsolventextractswereconcentrated,vacuumdriedandweighed. The percentage yields were expressed interms of air dried sample. The extracts were driedoveranhydroussodiumsulfate,storedinsealed vialsin refrigerator (5-8°C) untilanalysis[21].

Preliminaryphytochemicalscreening: Phytochemical screening of different successive solventextractswascarriedoutusingthestandardprocedureddescribed byKokate[23].

Quantitative phytochemical studies:Determinationoftotalphenolicsandtannins: Determinationoftotalphenolicsandtanninswere determinedfollowingtheprocedureofSiddhurajuand Becker [24].

RESULTS AND DISCUSSION: Standardizationisanessentialmeasureofquality,purityandauthenticity.Microscopicmethodisoneofthesimplestandcheapestmethodstostartwithestablishingthecorrectidentificationofthesourcematerials Kokoshi[20].Formerlythereisnophamacognosticworkwasrecordedonthismedicinallypotentplant,thepresentworkwasundertaken to lay down the standards which couldbeusefulforestablishingitsauthenticity.Microscopicexaminationoffreshleafandleafpowderisone oftheidentifyingparameterstosubstantiateandauthenticatethedrug.Botanicaldetectionofaphytodrugin volves two steps.

Oneisidentificationoftheplantbyitsfloralcharacters and the other is diagnosis of the plantwithitsmicroscopiccharacters.Thelatterprocedureisusefulforidentificationoffragmentaryplantspecimens.Earlyplantmorphologist Robert Hook [25] clearly demonstratedthateachkindofplanthasitsowndistinctivestructurebymeans of which it can berecognized.

Macroscopiccharacteristics: Themacroscopiccharacters 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, fragileclimber grows up to a height of 8-12 m with nodesandinternodes.Terminalbudsofplantsdevelopintotendrils.Thepresentmorphologicalinvestigations revealed that the stem is hirsute andgrowsuptoaheightof12m.Theleavesarealternate,pedatelylobed,lobesoblongandacuminatewithsmoothsurfaceandtexture.Thesize is3 to6cm.The stemandleavesshowedcharacteristic odour and bitter taste. Fruit shape isthe important differentiable characterization amongthe other genus (**Fig. 1**). Several earlier workershavedescribedmorphologicalfeaturesas oneofthe effective parameters for the pharmacognosticalidentification ofcrudedrugs[26,27].

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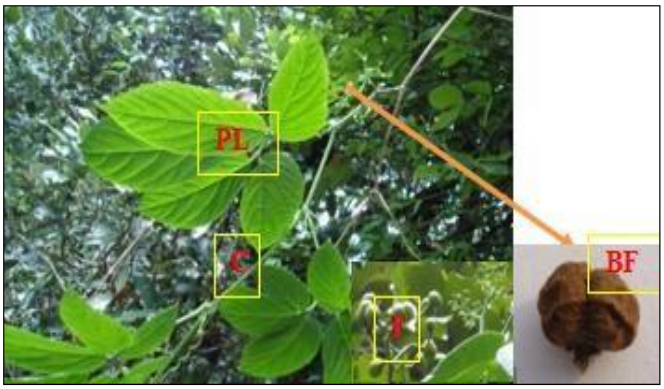

FIG.1:*CAYRATIA PEDATA*(LAM.)GAGNEP.VAR.GLABRAGAMBLE,IN ITSNATURALHABIT
F:Fruit;PL:PedateLeaf;C: Climber;BF:BilobedFruit

TABLE1:MACROSCOPICANALYSISOFAERIAL PLANTPARTSOF*C.PEDATA*VAR. *GLABRA*

S.no.	Macroscopiccharacters
1.	**Stem:**HirsuteHeight: Up to 12 m in heightSurface:Bearingdistinct hairs Texture: SmoothTaste:Bi tter Odour: CharacteristicColour:Dar kgreen
2.	**Leaves:** Alternate, pedately5foliolate, 8 - 15 cm longLeaflets: Elliptic, oblong, apex acuminate, serrateNumberofleaflets:7-12 Size:3-6cm Surface:QuiteglabrousexceptontheveinsunderneathTex ture:Smooth Taste: BitterOdour: CharacteristicColour: Darkgreen
3.	**Tendril:**Leafopposed,branched,wiry,coiled
4.	**Flowers:**Atfirstyellowlaterwhite
5.	**Fruit:**Berry,bilobed

Microscopiccharacteristics:

Plantmicrotechnique is a branch of botanical science that dealswith the internal structure and organization of plantorgans.Thismicroscopicstructurestudiesarecl oselycorrelatedwiththefunctionofanorganAsokan[2 8]. The present anatomical study provides aset of characters specific for *C. pedata*var. *glabra*withwhichonecanestablishtheidentityofthe plant in fragmentary form. Free hand section of theleaf observed under the microscope revealed thatthe leaf has thick midrib and lateral veins and thindorsiventral lamina. The midrib is characteristic inhaving thick (1.15 mm), flat and wide adaxial (500µm in horizontal plane and 350 µm in height) humpand thick and wide semicircular abaxial part (1.2mmwide) (**Fig. 2**).

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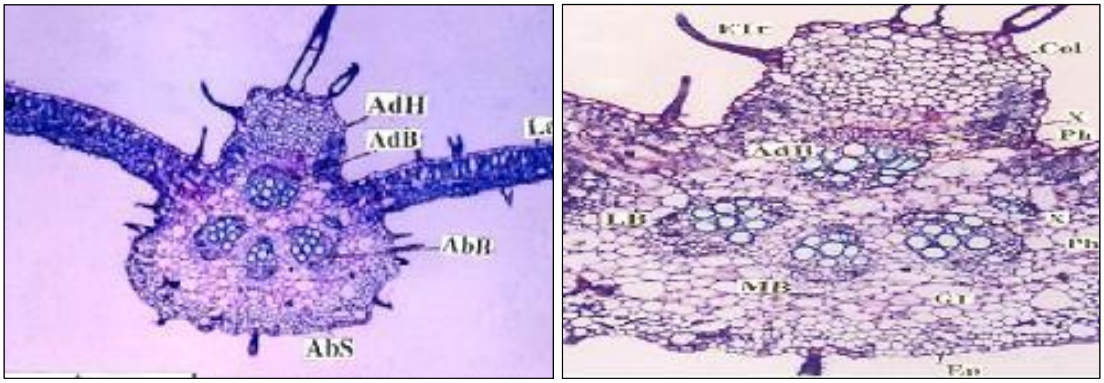

FIG.2:T.S.OFLEAFTHROUGHMIDRIBENLARGEDSECTIONAbS: AbaxialSide;**AbB:**AbaxialBundle;**La:**Lamina;**AdB:**AdaxialBundle;**AdH:**AdaxialHump;**LB:**LateralBundle;**MB:**MiddleBundle;**X:**Xylem;**Ph:**Phloem;**GT:**GroundTissue;
Ep: Epidermis;**ETr:**EpidermalTrichome;**Col:**Collenchyma

Inlaminaportioncalciumoxalatecrystalsofraphidetypearefairlyabundantinthepalisadezone; the raphide bundles are vertical in position,parallel to the palisade cells (**Fig. 3**). The adaxialepidermal cells are wide, angular and thick walledwithslightlywavyanticlinalwalls.Theadaxiallayer is apostomatic (**Fig. 4**). The abaxial epidermishas smaller cells with thin walls and is more wavy.Thestomataareanomocytic,havingnospecificsubsidiarycells.Theguardcellsarecircularmeasuring15-20µmindiameter.Thestomatalporeisdistinctandelliptic(**Fig.4**).Calciumoxalatecrystalsareabundantinthemesophyll,veins and trichomes. Druses or sphaero-crystals areseeninverticalrowswithintheveins.Onthesurfaceoftheepidermaltrichomesareseenasminuteprismaticcrystalsindensemasses.Raphides are thick cylindrical bundles of severalneedle shaped crystals aggregated together (**Fig. 5**).This is in corroboration with the work of SutapaChoudry[29].

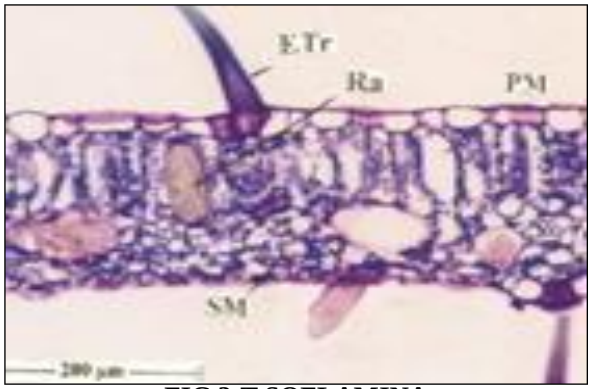

FIG.3:T.SOFLAMINA
ETr: EpidermalTrichome;**Ra:**Raphide;**PM:**PalisadeMesophyll;**SM:**SpongyMesophyll

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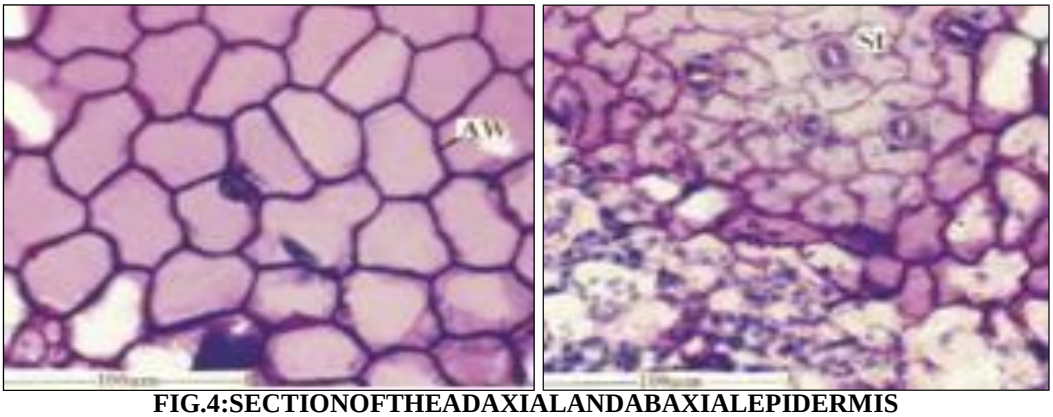

FIG.4:SECTIONOFTHEADAXIALANDABAXIALEPIDERMIS
AW: AnticlinalWalls;**St:**Stomata; **EC:**EpidermalCells

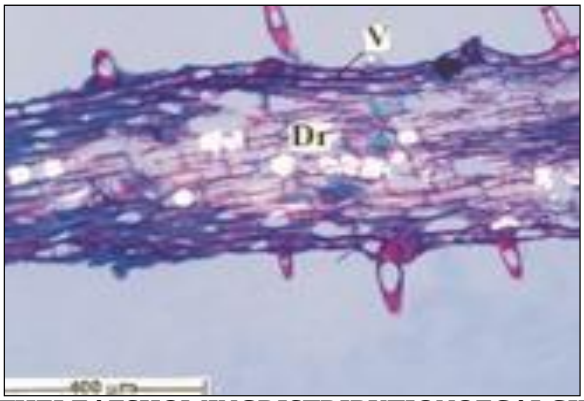

FIG.5:L.SOFAVEINOFTHELEAFSHOWINGDISTRIBUTIONOFCALCIUMOXALATEDRUSES
Dr: Druses;**V:**Vein

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Powder characteristic: The organoleptic evaluationof the aerial plant powder revealed the followingcharacteristics.Theplantpowdershowedcharacteristic odour and bitter taste. Upon dryingandpoweringthe colourofthepowder changedfromdarkgreentogreenishblackasshowninTable2.Theorganolepticcharacterssuchascolour, consistency andodour were noted in thesuccessive aerial plant extracts of *C. pedata*var.*glabra*(**Table 3**).

TABLE2:ORGANOLEPTICCHARACTERSOFAERIALPLANTPOWDEROF*C.PEDATA*VAR.*GLABRA*

S.no.	Characters	Observations
1.	Colour	Greenishblack
2.	Texture	Finesmoothpowder
3.	Taste	Bitter
4.	Odour	Characteristicsmell

TABLE3:ORGANOLEPTICCHARACTERSOFAERIALPLANTSUCCESSIVEEXTRACTSOFC. *PEDATA* VAR. *GLABRA*

S.no.	ExtractionMedium	Colour	Consistency	Odour
1.	Acetone	Yellowishgreen	Semisolid	Characteristicsmell
2.	Ethanol	Brownishblack	Semisolid	Characteristicsmell
3.	Water	Brownishblack	Solid	Characteristicsmell

Powdertreatedwithdifferentchemicalreagents: Thebehaviourofaerialplantpowderwith various reagents were observed and presentedin**Table4**.Slightdifference(palegreentodarkgreen) was noted in the powder as such and treatedwithconcentratedHClwhencomparedtootherreagentsused.

TABLE4:BEHAVIOUROFAERIALPLANTPOWDERWITHDIFFERENTCHEMICALREAGENTS

S.no.	Powder+Reagentsused	Colourofthepowder
1	Powderassuch	Palegreen
2	Powder+ConcentratedHCl	Darkgreen
3	Powder+ConcentratedH_2SO_4	Greenishbrown
4	Powder+ConcentratedHNO_3	Woodbrown
5	Powder+Aceticacid	Greenishyellow

Fluorescence analysis: Flouorescence analysis ofplant powder as such showed pale green to darkgreen in visible and UV light. Powder treated withNaOHinwaterandNaOHinethanolshowedslightcolourdifferenceslikepalegreenishyellowtoyellowish green to light green. There is no distinctcolour differences was seen in visible and UV light(**Table5**).

TABLE5:FLUORESCENCEBEHAVIOUROFAERIALPLANTPOWDEROF*C.PEDATA*VAR.*GLABRA*

S.no.	Reagents	Behaviourofpowder	
		Visiblelight	UVlight
1.	Powderassuch	Palegreen	Darkgreen
2.	Powder+1NNaOHinwater	Palegreenishyellow	Yellowishgreen
3.	Powder+1 NNaOHinethanol	Yellowishgreen	Greenishyellow

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Ash values and extractive values: The results ofash values determination indicate that the samplecontained 67.32% moisture. The total ashcontentofthesamplewas5.40%.Thepercentageofextractive value was maximum in ethanol extract(15%)followedbyacetoneextract(7.50%)respectively.

The extractive values are primarily useful for thedetermination of the exhausted or adulterated drug(**Table6**).

TABLE 6: PHYSICO-CHEMICAL AND EXTRACTIVEVALUESOFAERIALPLANTPOWDEROF*C.PEDATA*VAR.*GLABRA*

S.no.	Physico-chemicalproperties	Values inpercentage
1	Moisture content(Lossond rying)	67.32
2	Total ash	05.40
3	**Extractivevalues**	
	a. Acetone	07.50
	b. Ethanol	15.00

Preliminaryphytochemicalscreening:

Acompound or a group of compounds present canserveasa"Biomarker"andthepresenceandconcentrationofthesamecanbefollowedtodecideonthegenuinenessofthedrug/formulation [24]. To investigate the chemical constituents of plantpowderof*C.pedata*var.*glabra*,thesuccessivesolventextractsweresubjectedtoqualitativephytochemicalscreening.Thepreliminaryphytochemical screening revealed the presence ofcarbohydrates,proteins,aminoacids,alkaloids, anthroquinones,flavonoids,glycosides,phenolsand tannins, steroids and sterols, triterpenoids andvolatileoil.Theacetoneextractionwasmoreefficient than ethanol and water extracts (**Table 7**).All extracts showed negative response for saponins.Theresultsobtainedfromthepreliminary phytochemicalscreeningwillrevealtheusefulfindings about the chemical nature of the drug. Inaddition total ash values, fluorescence analysis andextractivevalueswillbehelpfulinidentificationandauthentificationoftheplantmaterial[30].Theextractive values are useful to evaluate thechemicalconstituentsofcrudedrug

TABLE7:QUALITATIVEPHYTOCHEMICALSCREENINGOFAERIALPLANTPOWDEREXTRACTSOF*C.PEDATA*VAR.*GLABRA*

S.no.	Chemicalconstituents	ChemicalTests	Acetone extract	Ethanol extract	Water extract
1	Carbohydrates	Molisch'stest	-	-	+
		Barfoed'stest	-	-	+
2	Proteins	Warmingtest	-	+	+
		Testwithtrichloroaceticacid	-	+	+
3		Biurettest	-	+	+
4	Aminoacids	Millon'stest	-	+	+
		Ninhydrintest	-	+	+
5	Alkaloids	Dragendorff'sreagent	+	-	-
		Mayer'sreagent	+	-	-
		Wagner'sreagent	+	-	-
6	Anthroquinones	Borntrager'stest	+	-	-
7	Flavonoids	Alkalinereagenttest	+	+	+
		Zinchydrochloridetest	+	+	+
8	Glycosides	Borntrager'stest	+	+	+
9	Phenolsandtannins	Ferricchloridetest	+	+	+
10	Saponins	Foamtest	-	-	-
11	Steroidsandsterols	Salkowskitest	+	-	-

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		Sulfurtest	+	-	-
12	Triterpenoids	Libermann-Burchardtest	+	+	+
13	Volatileoil	Sudantest	+	-	-

Note:'+','-'indicatesthepresence/absenceofcompounds

Totalphenolicsandtannincontent: Phenoliccompoundsarecommoninplantsandhavemultiplebiologicaleffects.Manyofthepolyphenols have been identified as anticancer [31]andcardioprotective[32].Tanninsarenaturallyoccurringphenoliccompoundswhichprecipitateprotein [33]. In the current study, the total phenolicsandtannincontentofdifferentsolventextractsof *C.pedata*var.*glabra*werestudiedandexpressedas tannic acid equivalent. As shown in Table 8, thetotalphenoliccontent wasmaximum inethanolicextract(131.7±3.6mg/g)followedbyacetoneextract(56.8±0.8mg/g).Theminimumwasrecorded in water extract (54.1 ± 1.8 mg/g). Whencomparedwithothersolventextracts,ethanolextract registered higherlevelsoftannincontent(52.8±12.9mg/g)followedbyacetoneextract(24.1 ± 4.5 mg/g). The minimum was recorded inwaterextract(10.5±3.2mg/g).Thisisinagreement with the reports of Yen and Chen [34,35]who reported that ethanol is an effective solvent forextraction ofantioxidants[36].

TABLE8:ESTIMATIONOFTOTALPHENOLICSANDTANNINCONTENTOFDIFFERENTSOLVENTEXTRACTSOF*C. PEDATA* VAR.*GLABRA* PLANTPOWDER

S.no.	ExtractionMedium	Totalphenolics(mgTAE/gextract)[#]	Tannin(mgTAE/gextract)[#]
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

[#]Valuesaremeansofthreeindependentanalysis±StandardDeviation**TAE**-Tannicacid equivalent

CONCLUSION: In conclusion, detailed anatomicalperspectives of the plant organs has been unequallyestablishedthatcertainanatomicalcharactersofplant organs sustain the environmental stress andremain little modified. Such features can be relieduponfordiagnosis of theplant. Thetotalsummation of macroscopic features coupled withmicroscopicparameterswillfurnishtheprotocolfor clinical or pharmaceutical investigations. Thepresent work was undertaken with a view to laydown standards which could be useful to detect theauthenticityofthismedicinallyusefulplant.

Pharmacognosticevaluationcanbeusefultosubstantiate and authenticate the plant. On the basisof the results obtained in the present study, it isconcluded that the ethanolic extract of *C. pedata*var.*glabra*,whichcontainslargeamountsofphenolicsandtannins,exhibitshighantioxidantandfreeradical scavengingactivities.

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