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PHARMACO GNOSTICAL AND PRELIMINARY PHYTOCHEMICAL EVALUATION OF ROOT OF *ECBOLIUMVIRIDE*[FORSK.]ALSTON.

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ABSTRACT

In ethnomedicinal practices the traditional healers use the roots of *Ecbolium viride* in the treatment of various ailments.Scientific information on their pharmacognosy is very scant. Scientific parameters are not yet available to identify the true plantmaterialand to ensure its quality. Therefore the present work has beenundertaken to establish preliminary phytochemical profileandthenecessarypharmacognosticstandardsforevaluatingtheplantmaterial.Variousparameterslikemorphology,microscopy,powderanalysis,fluorescence characteristics andphysico-chemical constantsof the rootswere studied and thesalient diagnosticfeaturesare documented. Obviousmorphologicalfeatures and the microscopic characteristicswerefound inthe tissue structures of the roots, many diagnosticelementsandpreliminary phytochemical profile were found to be usefulevidencesforfurtherscientificinvestigations ofthismedicinalplant.

KEYWORDS:*Ecboliumviride*,Ethnomedicine,Microscopy,PharmacognosticalParameters,PreliminaryPhytochemical.

INTRODUCTION

Ecboliumviride(Forsk)Alston.(Acanthaceae)locallyknownas"*Nilambari*",isaperenialwoodyundershrubfoundoccasionalinplainsofIndiaandalsofoundinArabia,SrilankaandtropicalAfrica.Infolkmedicine, aqueousextractsofdriedrootsoftheplantareused for menorrhagia, rheumatism and jaundice (Datta andMaiti, 1968; Kirtikar and Basu, 1987). The rural people inTirunelvelidistrictofTamilnaduareusedjuiceoftherootofthisplanttothetreatmentofjaundicebythevaidhiyars.Mostofthecasesofaccidental herbalmedicinemisusestartwithwrong identificationofamedicinalplantprescribed.Manyofthetraditionalsystemshaverecordswhereonecommonvernacularissuppliedinplaceoftwoormoreentirely different species. However, no scientificparametersareavailabletoidentifythetrueplantmaterialandtoensureitsquality.Forthisallreasonswetakeaplanttobringoutanofficialmannerbythethroughinvestigationonthisplantsuchaspharmacognosticalandphytochemicalstudiesof roots of *Ecbolium viride*,whichcouldserveas avaluablesourceofinformationandprovidesuitablestandardsforthefutureidentificationofthisplant.

MATERIALSANDMETHODS

Plantmaterials

Fresh plant was collected from Wastelands of Kadyanallur,Tirunelveli(District),Tamilnadu,India.TheplantspecimenwasauthenticatedbyDr.P.Jayaraman,M.Sc.,Ph.D,PlantAnatomyResearchCentre(PARC),ChennaiTamilNadu,India(VoucherspecimenNo.PARC/2008/495). All the reagents used were of analyticalgradeobtained fromSigma ChemicalCo,St. Louis,USAorFineChemicalsLtd.,Mumbai,India.

CollectionofSpecimens

The roots of this plant were cut and removed from the plantandfixedinFAA(Formalin5ml+Aceticacid5ml+70%Ethylalcohol90ml)forhistologicalstudies;transverse sections (T.S) of the different organs of the plantmaterial.After24hoursoffixing,thespecimensweredehydratedwithgradedseriesoftertiary–butylalcohol(TBA) asper the schedulegiven by Sass,1940.Infiltrationofthespecimenswascarriedoutbygradualadditionofparaffinwax(meltingpoint58-68˚C)untilTBAsolutionattainedsupersaturation.Thespecimenswerecastintoparaffinblocks.

Sectioning

Theparaffinembeddedspecimensweresectionwiththehelp of rotary microtome. The thicknesses of the sectionswere10-12µm.Dewaxingofthesectionswasperformedbycustomaryprocedure(Johansen,1940).Thesectionswere stained with toludine blue as according to the methodprescribed by O'Brien *et al.,* 1964. Wherever necessary, thesectionswerealsostainedwithsaffraninandFast-green.Themicrophotographsofthesectionsweremadeusing Olympus BX 40 microscope attached with Olympus DP12digitalcamera.

Physico-chemicalconstants

Physico-chemicalconstantssuchasconsistencyandorganolepticcharacters(PrattandChase,1949),fluorescence (Kokashi*et al.,* 1958)and the percentage oftotalash,acid-insolubleash,water-solubleashandalkalinity ofwater solubleashvaluesandlossondrying(LOD)werecalculatedaspertheIndianPharmacopoeia(Anonymous,1985)**.**

MethodofextractionandPreliminaryphytochemicalscreening

Therootsweredriedinshadeatroomtemperatureandscreened for the presence of foreign matter. The roots weregroundtoamoderatelycoarsepowderinamechanicalgrinder.About200gofthepowderwasextractedsuccessively withpetroleumether(60- 80ºC),benzene,chloroform and ethanol (95%) using soxhlet apparatus. Theextraction witheach solvent was carried for24 h. Finally,themarcleftwasextractedwithwaterbydigestingona boiling water bath. The extraction was continued till a fewdrops of the last portion of the extract left no residue ondrying. The extracts were taken in a tarred porcelain dishesandevaporated to drynesson a water bath and dried at 105ºCtoaconstantweight.Thepercentageextractiveswerecalculatedwithreferencetoairdrieddrug.Thephytochemicalexaminationofeachextractofwasperformedbythestandardmethods(Harbone,2005).

Powdermicroscopy

The root of the plant *Ecbolium viride* were powdered welland then powder was passed through sieve No: 60 and thenproceededforpowderanalysis(Wallis,1985;TreaseandEvan1985).

RESULTSANDDISCUSSION

TheExternalfeaturesoftheplant;(fig1)

The plant is a shrub growing up to 2.5m height. The leavesareelliptic-ovatetoovate.Thelaminaisthinandcoriaceous;leafapexisgraduallyacute.Spikesterminalandaxillary(fig1.1-3).Bractsandbracteolesleafy.Calyx:5 sepals imbricate; petals: 5 lobed, bluish green. 2 -lipped,upper lip two lobed, lower lip three lobed and spreading.Stamens: 2, attached at the base of the upper lip; Anthers -two lobedlobes unequal. Ovary:Bicarpellary syncarpous,2-ovuled;Fruits-2seededCapsules;seedscircularflat.Root:thethinrootmeasures1.2mmthick,circularwithdark fissured surface. The thick root is more than 2mm indiameter.Thegeneralstructureissimilartothatofthinroot.Ithas dark,roughandfissuredsurface

Microscopicalfeaturesoftheroot

Boththinandthickrootswerestudied.

Thinroot;Theepidermalandsubepidermallayersarecrushed into dark surface layer. The cortex is100µmwide.It consists of four or five layers of radially oblong elliptical,loosely arranged parenchyma cells with small air-chambers.Some of the cortical cells are dilated and posses cylindricalcystoliths(fig.2).Thecystolithcontainingcellsareidioplastsandare150-200µminsize.Thecystolithsare50-150µminsize.

Phloem occurs in narrow, continuous zone around thexylemcylinder(fig.2).Thephloemelementsarenarrow,angular, thinwalledandarearrangedinthisradialfiles.Thephloemzoneis30µmwide.

Secondary xylem is in the form of a circle with evenline. It is 650µm in diameter. It consists of vessels, fibresandnarrowstraightrays(fig 2,2.1and2.2).Thevesselsoccur in uni seriate radial lines which are widely separatedfromeachother.They arecircular,solitary,thickwalledand are 15-20µm in diameter. Xylem fibres are thick walledand lignified with wide lumen. They occur in regular radiallines.Xylemraysarenarrowsandlessprominent.

Thick root: The thick root is more than 2mm in diameter.The general structure is similar to thatof thin root.It hasdark, rough and fissured surface followed by a thin layer ofperidermarewideaerenchymatouscortex.Thecorticalzone is 350µm wide and comprises of tangentially stretchedcylindricalcellsandthecellshaveundergoneradialdivisious,wide,irregularair-chambersareseeninthecortex (fig.3, 3.1). Some of the corticalcellsaredilatedintocystolithbearing idioplasts.

*Secondary phloem*consistsofnarrow continuouscylinderof radial files of small phloem elements (fig.3.2). The sieveelementsarerectangularwithlateralcompanioncells.Secondary xylem is a dense, solid, smooth circle. It exhibitsless prominent growth-ring which is demarcated by narrowthickwalledfibres(fig.3).Thevesselsarediffuseindistribution. They are solitary and are in radial chains. Thevesselsarecircularand thick walled,measuring 20µm indiameter.Xylemfibresthickwalledandlignified.Thelumen of the fibres is wide and angular. The fibres are inradial rows.Xylemraysarethinandstraight.

Powdermicroscopy(fig.4)

RootPowderincludesfibres,vesselelementsandxylemparenchyma. The fibres are bibriform type; they are needlelikewithtaperingends.Someofthefibreshavewidelumen and others have narrow lumen (fig. 4a and 4b). Thewide fibres (fig. 4a) are 20µmwide and up to 400 long. Thenarrowfibres [shown by asterisk in fig.1.1are 10µm thickand500µmlong.Thelateralpitsarenotevident(fig.4b).

Xylemparenchyma[Rayparenchyma]cellsarecommon in the powder. The parenchyma cells narrow andoblong;theyarescale-likeinoutlinewithbluntorconical,semicircular ends (fig. 4c). These cells have thin walls anddense cell inclusions. The cells are 40µm wide and 240µmlong.

Vesselelements(fig.4dand4e).Thevesselselementsarenarrowandcylindrical.Theyhavelongorshorttails.Whenthevesselelementhaslongtails,itisgraduallytaperingintothetail.Thelateralwallshavedense, circular pits. The perforation plate is simple, circularand slightly oblique. The vessel elementsrange in lengthfrom150-320µm.Thelongandnarrowvesselelementswithlongtailsresembleverymuchtothewidefibre,excepting that the vessel elements have dense elliptical orcircularborderedpitsandperforationsattheendwalls.**Physico-chemicalconstantsofrootpowder**

Thepowderoftherootwasanalyzedforvariousphysico-chemicalconstantsandlossondrying(LOD).

Organolepticcharacters

Therootpowderwastestedwithvarioussolventsandchemicalstodetermineconsistencyandorganolepticcharactersaregiven inTable1.

Ashvalues

Total ash, water-soluble ash, alkalinity of water soluble ashand acid-insoluble ash values of the root powder was doneandtheresultsaretabulatedin Table2.

Fluorescenceanalysisofroot*powder*

Thepowderofrootisexaminedindaylight,short(at254nm) and long UV (at 365nm) to detect the fluorescentcompoundsandtheobservationsaregiveninTable3.

Preliminaryphytochemicalscreening

Theresultsofphytochemicalexaminationofeachextractare giveninTable 4.

Table1:Organolepticcharactersof*Ecboliumviride* (Forsk)Alston.rootpowder

1.Colour: Palebrownishyellow	
2.Appearance:Coarsepowder	
3.Odour: Nocharacteristicodour	
4.Taste: Nocharacteristictaste	

Table2:Ashvaluesof*Ecboliumviride*(Forsk)Alston.rootpowder

1.Totalashvalue:	20.08**%**
2.Water-solubleashvalue:	12.36**%**
3.Alkalinityofwatersolubleashvalue:	1.89ml
4.Acid-insolubleashvalue:	0.451**%**

Table3:Fluorescencecharacteristicsof*Ecboliumviride*(Forsk)Alston.rootpowder

Treatment	Daylight	UVlight	
		254nm	365nm
Powder	Palegreen	Palegreen	Black
Powder+1NNaOH(aqueous)	Pale-brown	Palegreen	Black
Powder+1NNaOH(alcoholic)	Pale-brown	Pale-brown	Black
Powder+1NHydrochloricacid	Pale-brown	Pale-brown	Black
Powder+50%Sulphuricacid	Brown	Yellow	Black
Powder+50%Nitricacid	Brown	Palegreen	Yellow
Powder+Picricacid	Palegreen	Green	Black
Powder+Aceticacid	Brown	Palegreen	White
Powder+Ferricchloride	Brilliantgreen	Greenishyellow	Black
Powder+Con.Nitricacid	Yellowishgreen	Green	Black
Powder+Nitricacid+Ammonia	Greenishbrown	Greenishyellow	Black

Table4:Phytochemicalprofilesofextractsofrootof*Ecboliumviride*(Forsk)Alston.

Solvent extracts	Chemicalconstituents							
	Alkaloids	Sterols	Protein& Amino acids	Carbohydrate	Glycosides	Tannins	Saponins	Flavonoids
Petroleumether (60-80°C)	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	-	-	-	-
Ethyl alcohol	+	-	-	+	-	+	-	-
Water	+	-	-	+	+	+	+	-

(+)=Present;(-)=Absent

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Figure1:Atwigof*Ecbolium viride*(Forsk)Alston.

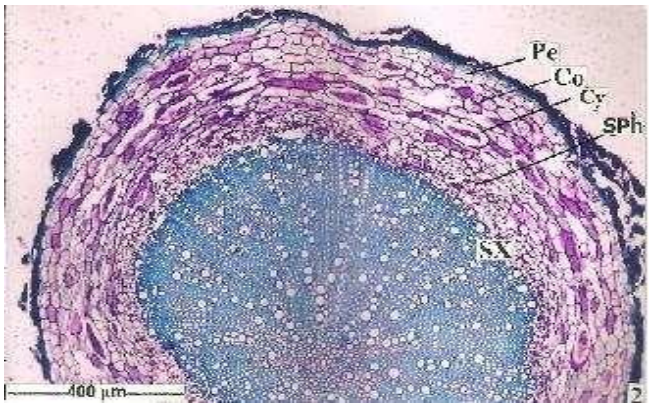


Fig.2:T.Softhin-root(Halfsectionenlarged): ***viride*(Forsk)Alston. Co,**Cortex;**Cy,**Cystolith;**Pe,**Periderm;**SPh,** Secondaryphloem;**SX,**Secondaryxylem.

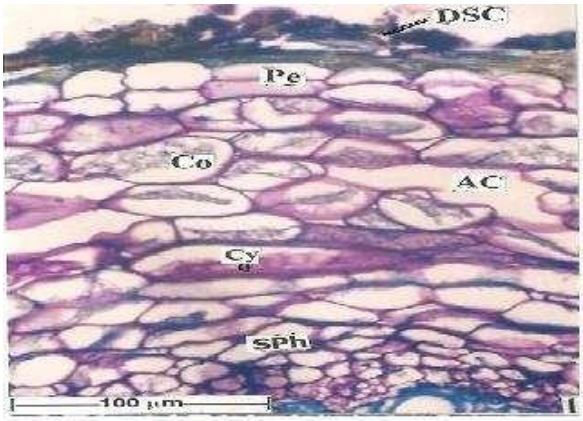


Fig2.1:T.Softhin-rootshowing Periderm:Co,Cortex;**Cy,**Cystolith;

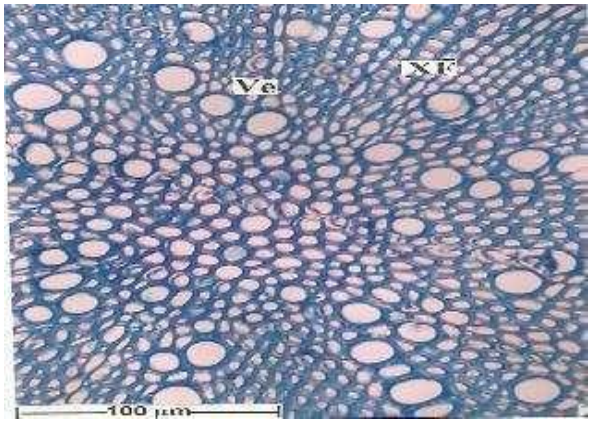
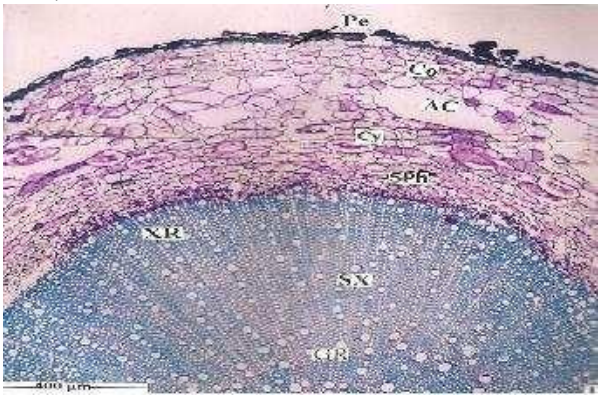


Fig2.2:T.Softhin-rootshowing Secondaryxylem:XF,Xylemfiber;

Pe,Periderm;**SPh,**Secondaryphloem;
AC,Air-chambers

Ve,Vessel.



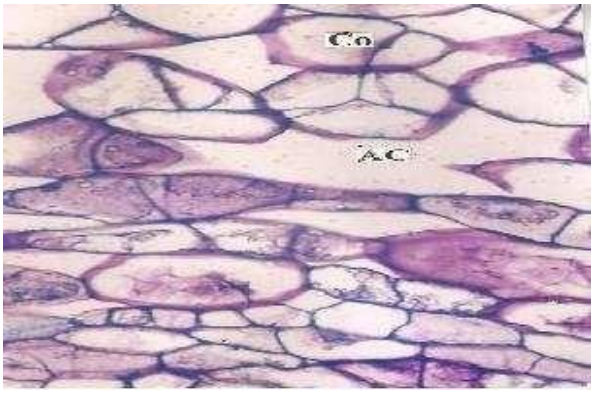


Fig.3:T.Softhick-root(Halfsectionenlarged):Co,Cortex;**Cy,**Cystolith;**Pe,**Periderm;**AC,**Air-chambers;**SPh,**Secondaryphloem;**SX,**Secondaryxylem;**XR,**Xylemrays;**GR,**Growth-ringboundary.

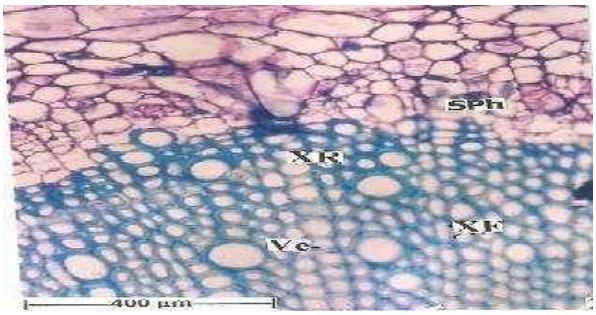
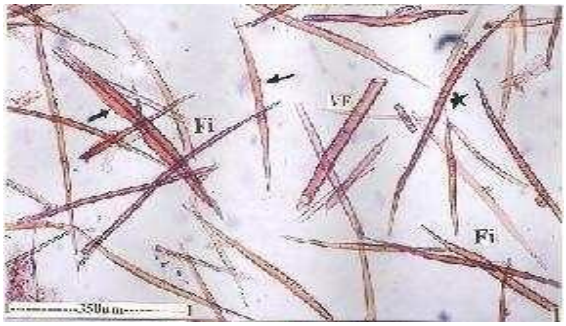
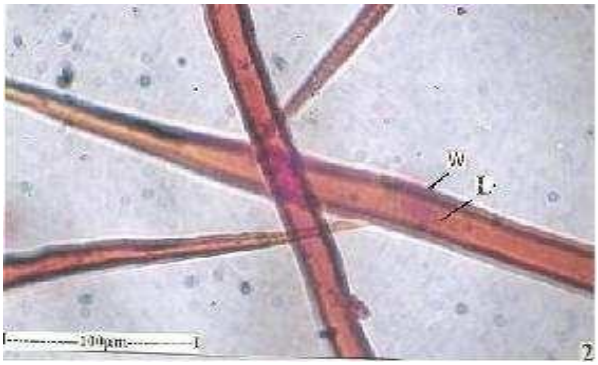


Fig3.2:T.Softhick-rootshowingSecondaryphloem andSecondaryxylem:
SPh,Secondaryphloem;**XF,**Xylemfibre;
XR,Xylemrays;**Ve,**Vessel

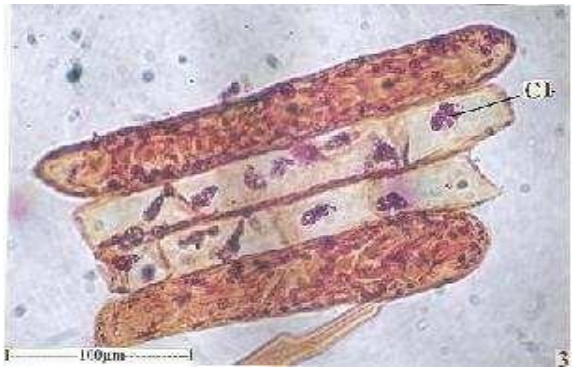
Fig 3.1: T.S ofthick-root showingAerenchymatouscortex:Co,Cortex;**AC,**Air-chambers.



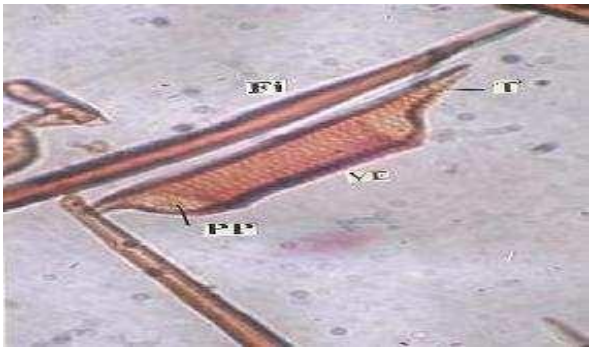
(A)



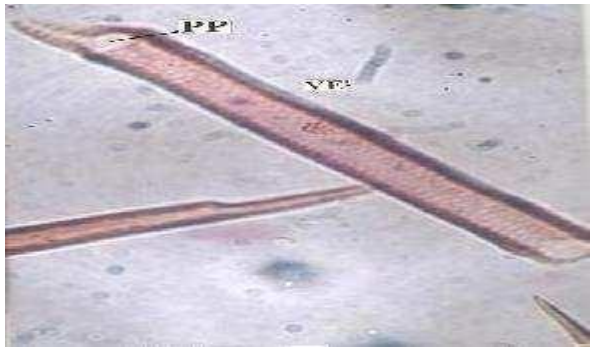
(B)



(C)



(D)



(E)

Fig .4: Diagnostic features forthe powder microscopy ofthe rootof\ *Ecboliumviride*: Vesselelementsand fibres (fig.4a);Fibresenlarged(fig.4b);Parenchymacellswithcellinclusions(fig.4c);Tailedvesselelementswithfibres(fig.4dand4e).**CI,**Cellinclusions;**Fi,**Fibre;**L,**Lumen;**Ve,**Vesselelement;**W,**Wall;**PP,**Perforationplate;**T,**Tail.

CONCLUSION

In ethnomedicinal practices the traditional healers use*Ecboliumviride*intreatmentofvariousailments,menorrhagia,rheumatismand jaundice.

AsperWHOnorms,botanicalstandardsaretobeproposed as a protocol for the diagnosis of the herbal drug.Macroscopicaswellasmicroscopicalstudiesofanyphytodrugareindispensabletoolforidentificationofmedicinalherbstoestablishitsbotanicalqualitycontrolbeforegoingtootherstudies.Theabovementionedparametersarehelpfulforthefutureidentificationandauthentification of the plant in the herbal industry and infactories.Thephysico-chemicalstandards,suchasashvaluesandfluorescenceanalysis,willbeusefultoidentifytheauthenticityofthedrugevenfromthecrushedorpowderedplantmaterials.Itwillserveasastandarddatafor the quality control of the preparations containing root ofthisplantinfuture.Thepowderconstantscanbeincludedas microscopical standards in Indian herbal pharmacopoeia.Phytochemicalstudyisalsousefultoisolatethepharmacologicallyactiveprinciplespresentinthedrug.
Inconclusion,thepresentstudyonpharmacognosticalcharacters and phytochemical profiles of root of *Ecboliumviride*(Forsk) Alston.willbe providing usefulinformationforthe futureidentificationofthisplant.

REFERENCES

Anonymous.IndianPharmacopoeia,Vol.II,3rded.,GovernmentofIndia,MinistryofHealth,ControllerofPublications,NewDelhi,India, 1985,74.

DattaPC,MaitiRK.Pharmacognosticstudyon*Ecboliumlinneanum*Var.Dentata.*JournalofCrudeDrugResearch*,8,1968,1189–1192.

HarboneJB.*Phytochemicalmethods*,3rded,ChapmanandHall,London,2005,49-52.JohansenDA.PlantMicrotechnique.NewYork,McGraw-Hill,1940,523.

KirtikarKR,BasuBD.*IndianMedicinalPlants.*InternationalBookDistributors,BookSellersandPublishers,Dehradun,1987,1904.

KokashiCJ,KokashiRJ,SharmaM.Fluorescenceofpowderedvegetabledrugsinultra-violetradiation.*J.Am.Pharm.Assoc*,47,1958, 715-717.

O'BrienTP,FederN,McCullyM.PolychromaticstainingofplantcellwallsbyToluidineBlue-O.*Protoplasma*,1964,368-373.PrattRT,ChaseER.Fluorescencepowdervegetabledrugsinparticulartodevelopmentsystemofidentification.*J.Am.Pharm. Assoc*,38,1949,324-331.

SassJE.ElementsofBotanicalMicrotechnique.McGrawHillBookCo;NewYork;1940,222.TreaseGEandEv anWC.*Pharmacognosy*(ELBSPublications,London),1985,344,539-540.

WallisTE.TextBookofPharmacognosy,CBSPublishersandDistributors,Shahdara,Delhi,India,1985,104-105.