International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 02-06-2020; Accepted: 17-06-2020: Published: 03-07-2020
www.botanyjournals.com
Volume 5; Issue 4; 2020; Page No. 52-61

Phyto: pharmacognostical screening of stem and stem bark of *cipadessa baccifera* (Roth.) Miq

Minautee R Patel[1], Harisha CR[2], VJ Shukla[3], Rabinarayan Acharya[4]
[1, 4] Department of Dravyaguna, IPGT & RA, Gujrat Ayurved University, Jamnagar, Gujarat, India
[3] pharmaceutical chemistry department, IPGT and RA, Gujrat Ayurved University, Jamnagar, Gujarat, India
[2] Pharmacognocy Laboratory, IPGT and RA, Gujrat Ayurved University, Jamnagar, Gujarat, India

Abstract
The stem and stem bark of *Pittamari* (*Cipadessa baccifera* (Roth.) Miq of family Meliaceae) are traditionally has been reported for its use in treating psoriasis, chicken pox, fever etc. Present study reports the pharmacognostical and analytical study of its stem and stem bark as per standard procedures. The result shows, externally, the stem is brownish grey in colour with presence of red tint, fibrous, while bark was brownish red in colour. Transvers section of stem shows continuous rings of pericyclic fibers while stem bark is having uniseriate medullary rays. Powder microscopy of both parts shows brown and tannin contain, crystals, and stone cells. Qualitative and quantitative estimation reports the presence of phenol, flavonoid and tannin contain, higher in stem bark than the stem. HPTLC of stem and stem bark reports five (5) and four (4) different R_f value at short and long ultra-violate, respectively. The above reported microscopical, morphological characters and analytical profile of *C. baccifera* may help for identification, future validation and for new drug development.

Keywords: ethnomedicinal, *cipadessa baccifera*, *nalbila*, microscopy, physicochemical, HPTLC

1. Introduction
In Ayurveda, different dosage forms from appropriate parts of plants are made and used for treatment of various ailments.[1] Ethnomedicines/herbal medicines are at the present time much in demand as they are affordable and have much less side effects.[2] Scientific evaluation of ethnomedicinal plants, provides evidence-based alternative medicines in the herbal drug and pharmaceutical industry.[3] It may be put importance on here that the usage of ethnomedicinal plants in manufacturing of ayurvedic medicines/other herbal drugs or for traditional medical treatment, when supported by scientific evidences can ensure safe and more effective utilization of natural product drugs universally.

Cipadessa baccifera (Roth.) Miq from the family Meliaceae is native to tropical and subtropical regions, commonly found in Odisha and south India. It is a branched shrub or small trees up to 5 m height, distributed in the Indo-Malaysian region. It has imparipinnate leaves, elliptic lanceolate leaflets, small white flowers in axillary corymbose panicles, and red globose berries. It is known *Pittamari* and *Nalbila* in Odia and Hindi respectively.[4] As per tribal claims, it has been used to treat wide range of diseases such as malaria, dysentery, diabetes, snake poison, rheumatism and also used as a natural insecticide.[5] The paste of *C. baccifera* along with the goat milk used to treat wounds.[6] One formulation is also available in the market made from the extract of *C.*

baccifera and is use for the treatment of dark circles.[7] Though scientific data are available on the different parts of plant but for the identification and quality standard, macroscopy and microscopy characters have not been reported till yet.

In present study, an attempt has been made to study the pharmacognostical and preliminary phytochemical characters of stem and stem bark of *Cipadessa baccifera*.

2. Material and Method
2.1 Collection and Preservation of the Sample
Cipadessa baccifera (Roth.) Miq was identified from its natural habitat Paikmal, Odisha, during January 2017. Stem and bark were collected and authenticated by local taxonomist with the help of botanical flora[4] (Figure 1 (a)). A sample specimen was preserved in pharmacognosy laboratory of the institute (SPECIMEN NO-. Phm. 6303/19-20) and the sample was preserved in a solution prepared from 70% ethyl alcohol: glacial acetic acid: formalin (AAF) in the ratio of 90:5:5.[8]

2.2 Chemical and reagents
Chemicals utilized for study were procured from Sigma-Aldrich. Mumbai, India and other reagents or chemicals used were of standard ~~grade or~~ analytical grade.

2.3 Macroscopic Study
Macroscopic observations were made with naked eyes and with the help of dissecting microscope. The samples were cleaned properly and macroscopic study of the stem and stem bark were carried out with help of Quasmo binocular compound microscope.[9] Organoleptic Raw samples were evaluated for their various characters like, colour, texture, odour, taste etc.

2.4 Microscopic evaluation
Free hand sections of various parts of stem and stem bark were taken. First observed in distilled water and then stained with phloroglucinol and Conc. HCl[10]. Photographs were also taken by using microscope, attached with Kodak easy share C140, 8.2 megapixels 3x optical/5x digital zoom HD camera.

2.5 Powder microscopy
Powder of stem and stem bark were scrutinized with and

without staining[10] and microphotographs were taken under Carl Zeiss trinocular microscope attached with Kodak easy share C140, 8.2 megapixels 3x optical/5x digital zoom HD camera.

2.6 Physicochemical parameters and qualitative analysis
The powder of stem and stem bark were evaluated for physico-chemical that are pH, loss on drying, total ash value, water soluble extractive value and alcohol soluble extractive value, following protocols recommended by Ayurvedic pharmacopoeia of India.[11] For qualitative analysis, the presence of various secondary metabolites dissolved in water and alcohol extract was carried out following standard procedures.[12-14]

2.7 Quantitative analysis
Preparation of test samples: A stock solutions of the test samples were prepared by dissolving 10 mg of dried water extract in 10 ml of same solvent in concentration of 1 mg/ml.

Total phenolic content
Estimation of total phenol content in the selected plant extracts was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as gallic acid equivalent (GAE) per gram of sample. Different concentrations (0.01-1 mg/ml) of gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test samples and each sample of the standard solution were taken, mixed with 2 ml of Folin–Ciocalteu reagent (10 % v/v) and 5 min later, 4 ml of saturated solution of sodium carbonate (7.5% w/v) was added. The tubes were covered with silver foils and incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (gallic acid).[15]

Total flavonoid content
The flavonoid content was determined by 0.5 ml aliquots of the extracts and standard solutions (0.01-1.0 mg/ml) of quercetin were added to 2 ml of distilled water and subsequently, 0.15 ml of sodium nitrite (5% NaNO2 w/v) solution was added and mixed. After 6 minutes, 0.15 ml of (10% AlCl3 w/v) solution was added. The solutions were allowed to stand for further 6 min and after that, 2 ml of sodium hydroxide solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture but, without extract as a blank. Total flavonoid content was determined as mg quercetin equivalent per gram of sample with the help of calibration curve of quercetin.[16]

Total Tannin content
Total tannin content was determining by the 1107µl of sample were taken and 365 µl of $K_3Fe(CN)_6$ was added. Then, 365 µl $FeCl_3$ (0.02 M $FeCl_3$ in 0.10 N HCL) and 1661 µl satbilizer (25 µl H_3PO_4 + water) was added. Incubate at room temperature for 15 min. tannin in the plant extracts were calculated as per method given by Graham.[17] Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentration of 0.0001M Gallic acid.

2.8 High Performance Thin Layer Chromatography (HPTLC)
Methonalic extract of stem and stem bark were exposed to HPTLC study. The solvent system used for the study is Toluene: Eathyl acetate (9:1)

2.9. Chromatographic conditions
Application mode was Camag Linomat V, Development Chamber used was of Camag Twin Trough Chamber. Precoated Silica Gel 60F 254 plates were used. Chamber Saturation was done for 30 min. Development Time was 10 min. the plate was scanned in CAMAG TLC Scanner 3 with D2 and W lamp, Tungstan Lamp as detectors and Win. cats software was used for data analysis.

3. Observations and Result
3.1 Stem
Macroscopy: Stem is measures 20-7 cm in length and 0.3-1.5 in cm width, cylindrical in shape, brownish grey in colour with no characteristic odour. Fracture shows outer surface hard while inner surface fibrous. In mature condition externally it is brownish grey in colour and present of red colour dots on it. (Figure 1 (c)).
Organoleptic characters: Externally, it is brownish grey in colour in fresh condition and internally it is light cream; no characteristic in odour; bitter in taste.
Transvers section: Diagrammatic section of stem shows epidermis, cortex, vascular bundle parenchyma cell followed by phloem and xylem pith is filled with bundle of calcium oxalate crystal.
Epidermis: Detail transverse section shows epidermis cell is covered with colouring matter and with cuticle. It is made up of single layered compactly arranged barrel shaped cells without any inter cellular spaces. The cells are loaded with brown colouring matter.
Cortex: cortex is made up of 15-20 layers of parenchymatus cells along with the green chlorophyll pigment. Cortical cells are loaded by prismatic and cluster crystals.
Parenchyma cells: Here and there some of parenchyma cells are filled with dark brown contain may be tannin. Formed lysigenous cavity followed by continuous ring of 2-5 layered pericyclic fiber is lead in to the vascular bundle.
Vascular bundle: vascular bundle is radially arranged open and collateral. Meta-xylem is towards the epidermis and proto-xylem is towards the pith. Xylem is made up of xylem parenchyma and its fiber. Phloem is situated above the xylem. Phloem is made up with phloem fiber and sheave elements. Medullary rays are uniseriate most of filled with brown contain.
Pith: Centrally located wide pith is made up of parenchymatus cells observing ample amount of lysigenous cavity, calcium oxalate crystals that is to say cluster and prismatic crystal. (figure no. 2)
Powder microscopy:
Organoleptic characters: The powder of Stem is cremish brown in colour. characteristic in odour, astringent in taste and fibrous in touch.
Diagnostic powder characters of bark show presence of simple trichome filled with brown contain, cork cells, Prismatic crystal, compound starch grain, lignified pitted vessel and crystal fiber. (Figure 3).

3.2 Stem bark

Macroscopy: Quelled piece of bark is measuring about 7-14 cm in length and 0.1-0.4 cm in diameter. outer surface is brownish red and inner surface is reddish brown in colour. Inner surface is fibrous and quilling outward channel.

Organoleptic characters: Outer surface is brownish red and inner is reddish brown in colour; odour is specific; bitter in taste.

Microscopic study of stem bark: Diagrammatic section of bark shows outer cork and inner wide cortex cork made up of several layered tangentially elongated compressed compactly arranged consisting brown contain all over the cork zone.

Cortex: Widely wide zone of cortex is made up of parenchyma cell, some of the parenchyma cells loaded by the prismatic crystals and brown contain.

The cortical cells are heavily loaded by simple and compound starch grains.

Medullary rays are uniseriate, consisting starch grains and brown contain. In the cortical zone pericyclic fiber and secondary phloem are distributed throughout the cortical zone. (Figure 4).

Powder microscopy of Stem Bark powder

Organoleptic characters: Powder is reddish brown in colour; odour is characteristic; bitter- astringent in taste; texture is fibrous.

Diagnostic powder characters of bark show presence of cork cells, crystal fiber, rhomboidal crystal and rosette crystal of calcium oxalate, brown content and lignified crystal fiber; (Figure 5).

3.3 Physico-chemical analysis

The results of the physico chemical analysis is being presented in Table 1. Percentage of Loss on drying and extractive values are higher in stem bark than the stem. pH values are acidic in nature of both the stem and stem bark.

Table 1: Physicochemical parameters of *C. baccifera*

Powder parameters	Stem	Bark
Loss on drying (%w/w)	5.340 ± 0.0611	7.013 ±0.00882
Alcohol soluble extractive (%w/w)	7.867 ± 0.0267	13.080 ± 0.960
Water soluble extractive (% w/w)	8.240 ± 0.554	13.040 ±0.0462
Ash value at 450°C (% w/w)	2.533 ± 0.0926	5.770 ± 0.106
Acid insoluble Ash at 450°C (% w/w)	0.227 ± 0.0448	0.213 ± 0.0731
pH of 10% w/v aqueous solution	5.5	5.5

(n=3) Mean ± SD

3.4 Qualitative and Quantitative tests

Details of the result after qualitative analysis of stem and stem bark are being presented in Table 2. Presence of tannin, carbohydrate, phenolic, flavonoid and glycosides compounds in aqueous as well as methanolic extract of stem and stem bark. whereas saponin only present in the aqueous extract of stem. Quantitative results of tannin, phenol and flavonoid are given the quantity present in µg/mg of water extract of stem and stem bark (Table 3).

Table 2: Qualitative parameter of *C. baccifera*

Qualitative test	Water Extract		Methanol Extract	
	Stem	Bark	Stem	Bark
Carbohydrate	+	+	+	+
Steroids & terpinoids	+	+	+	+
Amino acid	-	-	-	-
Flavonoids	+	+	+	+
Tannin & phenol	+	+	+	+
Saponin	+	-	-	-
Glycoside	+	+	+	+
Proteins	-	-	-	-

+ = Present, - = Absent

Table 3: Quantitative parameter of *C. baccifera*

Test	Water Extract	
	Stem	Bark
Phenol (µg/mg)	56.653±13.015	75.320 ±0.661
Flavonoids (µg/mg)	61.840±0.000	79.587±0.716
Tannin (µg/mg)	151.033± 92.425	969.793 ±1251.16

(n=3) Mean ± SD

3.5 HPTLC

The obtained R_f values are mentioned in Table 4. The methanol extract of stem showed 21 and 18 peaks at 254 and 366 nm respectively, whereas, the methanol extract of stem bark shows 23 and 19 peaks at 254 and 366 nm respectively. HPTLC analysis of stem and bark at 254 nm having five (5) different R_f value 0.10, 0.32, 0.43, 0.72 and 0.87 while at 366 nm they are having four (4) 0.21, 0.34, 0.41and 0.75 different R_f values are founded and other values are similar in both the plant parts. (Figure 6).

Table 5: HPTLC analysis of *E. paniculata*

Solvent system	Sample (MeOH extract)	254 nm (short UV)		366 nm (long UV)	
		Number of spot	Rf value	Number of spot	Rf value
Toluene: Eathyl acetate (9:1)	Stem	21	0.03, 0.13, 0.23, 0.24, 0.27, 0.28, 0.34, 0.39, 0.47, 0.54, 0.60, 0.63, 0.67, 0.70, 0.74, 0.79, 0.83, 0.84, 0.87, 0.89, 0.95	18	0.03, 0.10, 0.13, 0.23, 0.32, 0.38, 0.43, 0.47, 0.54, 0.60, 0.67, 0.70, 0.72, 0.74, 0.79, 0.84, 0.89, 0.95
	Bark	23	0.03, 0.12, 0.16 0.21, 0.24. 0.34, 0.39, 0.41, 0.44, 0.47, 0.50, 0.54, 0.60, 0.63, 0.65, 0.72, 0.75, 0.80, 0.83, 0.87, 0.92, 0.94, 0.95	19	0.03, 0.12, 0.16, 0.24, 0.39, 0.44, 0.47, 0.50, 0.54, 0.61, 0.63, 0.65, 0.72, 0.80, 0.83, 0.87, 0.92, 0.94, 0.95

Fig.1 (a)

Fig.1 (b)

Fig.1 (c)

Fig 1: (a) *Cipadessa baccifera* (Roth.) Miq- shrubs or small tree in its natural habitat; (b) Authentication and storage of plant; (C) Measurement of stem;

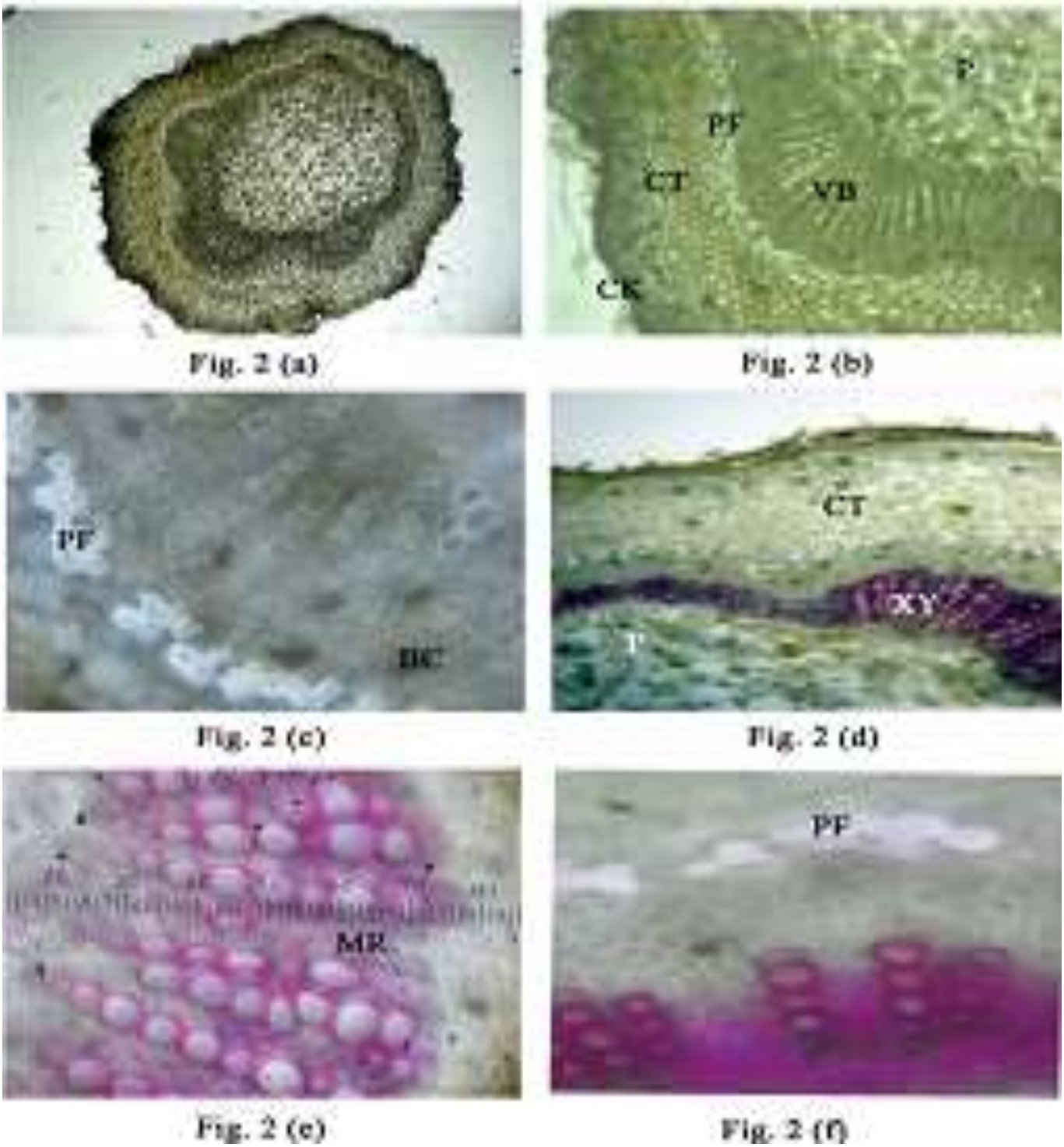

Fig. 2 (a) Fig. 2 (b) Fig. 2 (c) Fig. 2 (d) Fig. 2 (e) Fig. 2 (f)

Fig 2: (a) Diagrammatic transvers section of stem with cork, cortex, pith (10 x); (b) Cork, cortex, pericyclic fiber, vascular bundle and pith (CO- cork, CT- cortex, PF- pericyclic fiber, VB- vascular bundle, P- Pith); (c) Pericyclic fiber, phloem, xylem, brown content (PF- pericyclic fiber, BC- brown content); (d) Wide cortex along with lignified xylem elements and pith (CT- cortex, XY- xylem, P- Pith); (e) Medullary rays uniseriate along with brown content (MR- medullary rays); (f) Lignified pericyclic fiber, phloem and xylem (PF- pericyclic fiber).

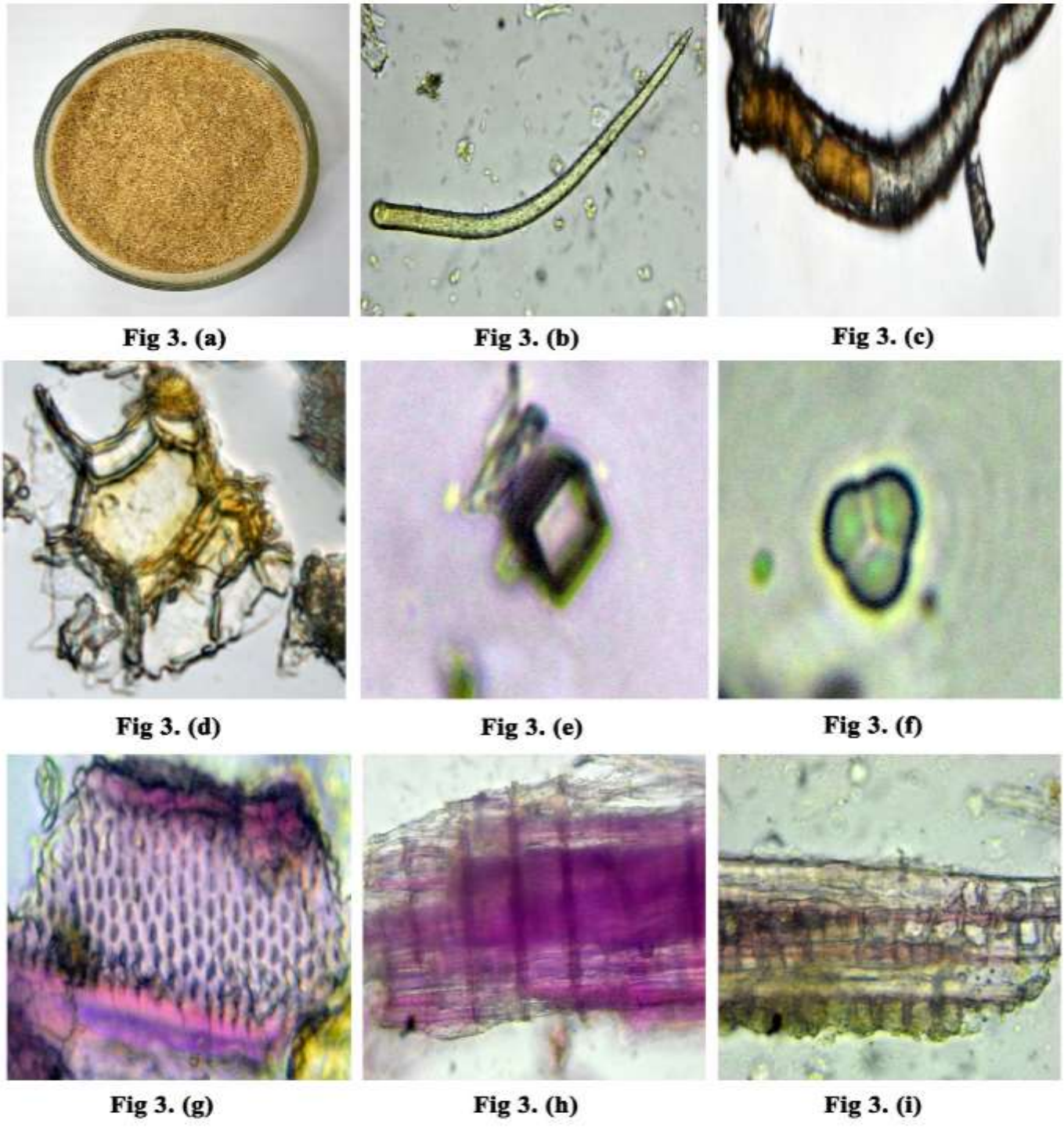

Fig 3: (a) Powder of stem; (b) simple trichome; (c) trichome filled with brown contain; (d) cork cells; (e) Prismatic crystal; (f) compound starch grain; (g) lignified pitted vessel; (h) sclerieds; (i) lignified crystal fiber.

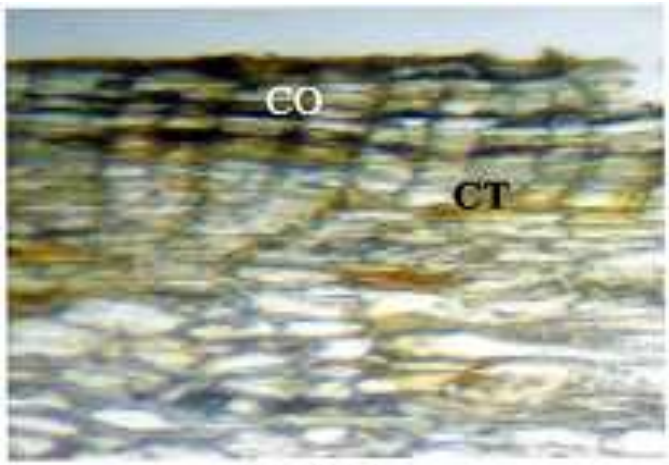

Fig. 4 (a)

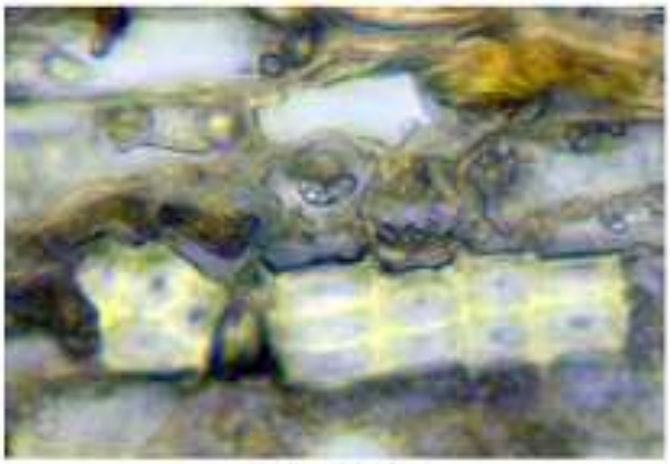

Fig. 4 (b)

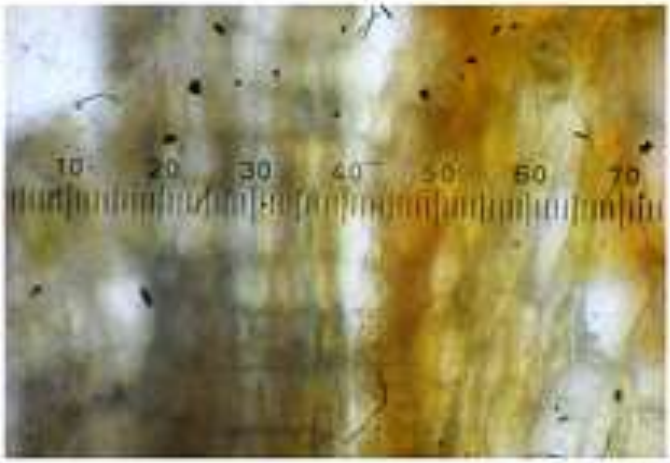

Fig. 4 (c)

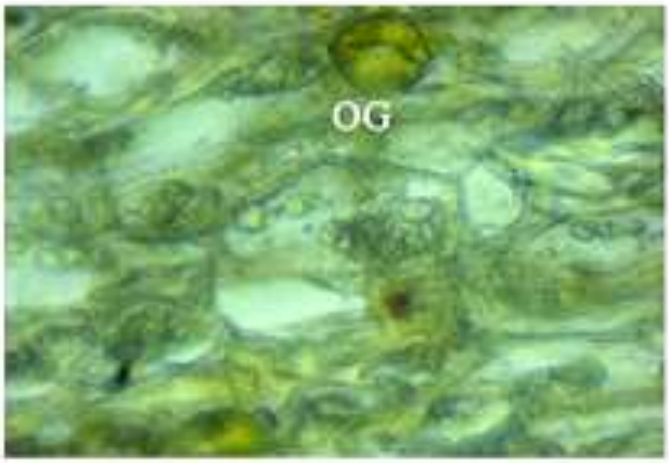

Fig. 4 (d)

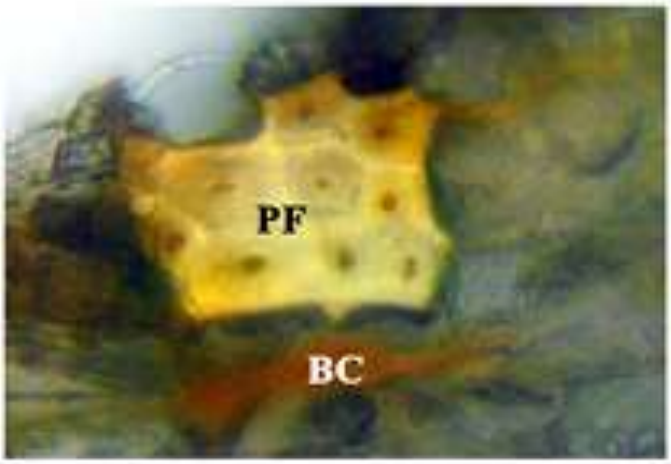

Fig. 4 (e)

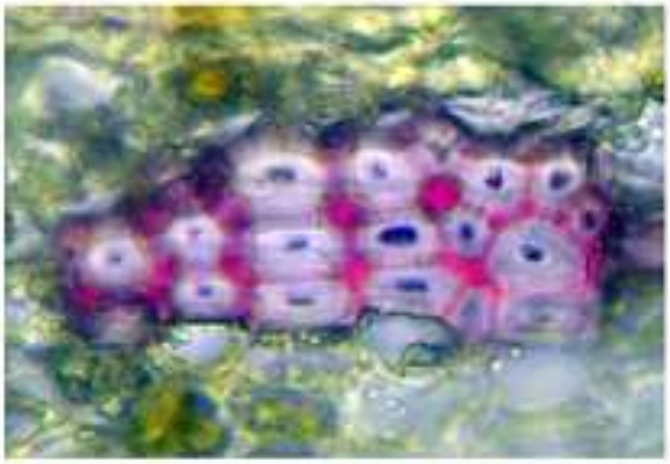

Fig. 4 (f)

Fig 4: (a) cork and cortex (CO- cork, CT- cortex); (b) Outer and inner cork with crystals; (c) Cork layers with brown content; (d) Cortical cells with oil globules and crystals (OG- oil globules); (e) Lignified pericyclic fiber and brown content (PF- pericyclic fiber, BC- brown content); (f) Lignified pericyclic fiber along with secondary phloem.

Fig 5. (a)

Fig 5. (b)

Fig 5. (c)

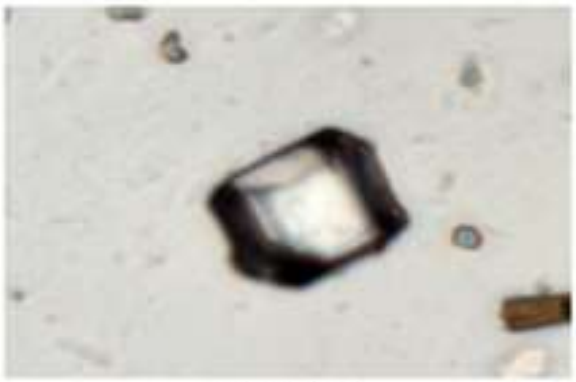

Fig 5. (d)

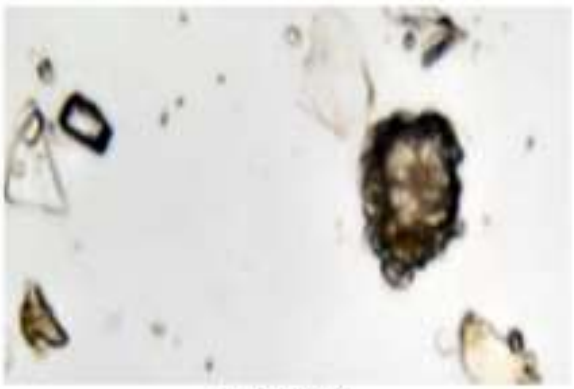

Fig 5. (e)

Fig 5. (f)

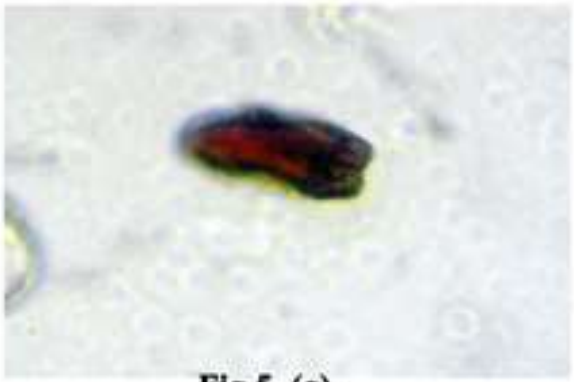

Fig 5. (g)

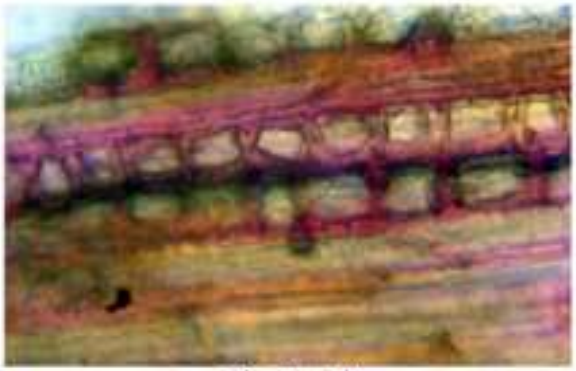

Fig 5. (h)

Fig 5: (a) powder of stem bark; (b) cork cells, (c) crystal fiber; (d) rhomboidal crystal; (e) rosette crystal of calcium oxalate; (f) Sclarieds; (g) brown content; (h) lignified crystal fiber;

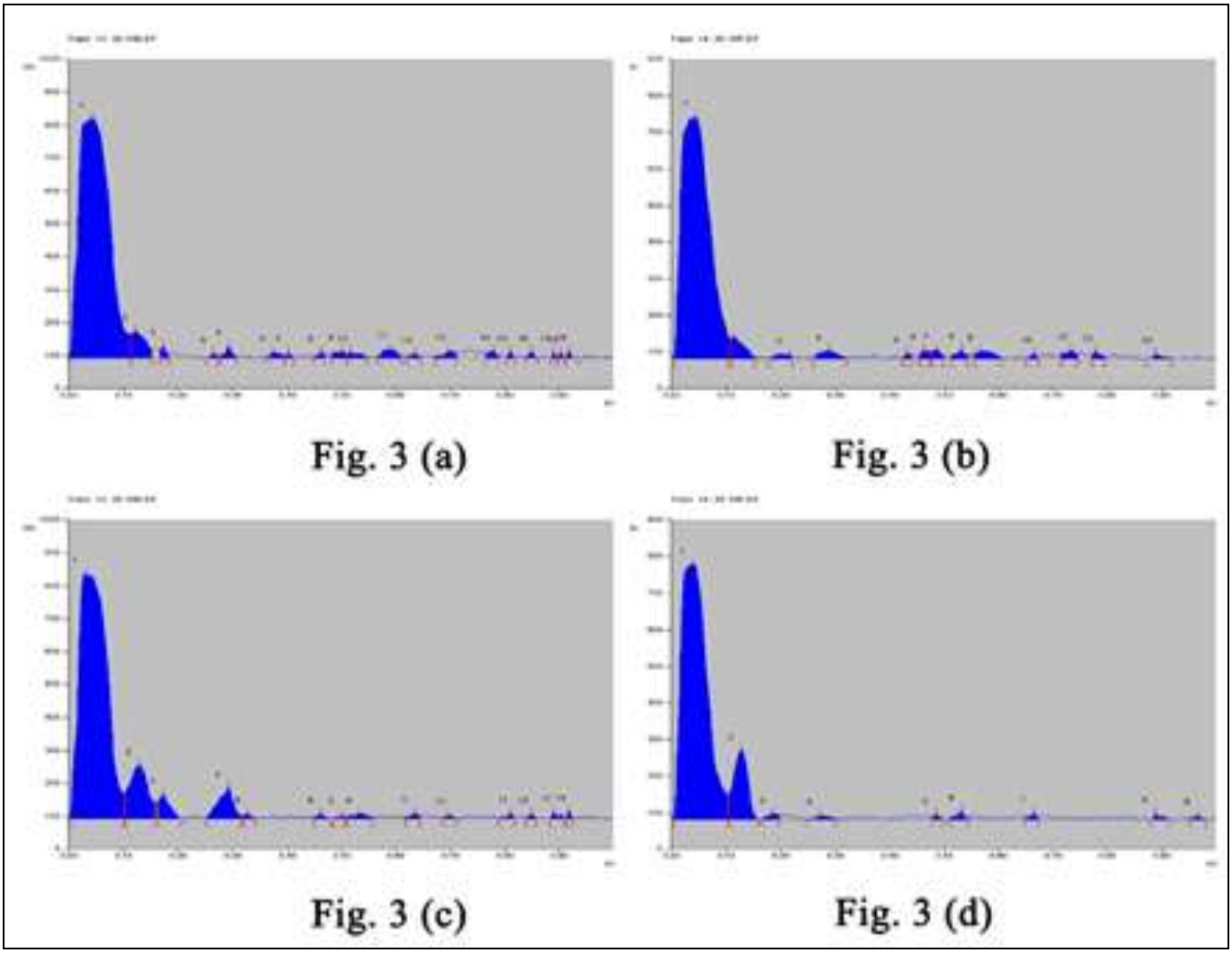

Fig 6: (a) Densitogram of stem at 254 nm; (b) Densitogram of stem at 365 nm; (c) Densitogram of stem bark at 254 nm; (d) Densitogram of stem bark at 365 nm

4. Discussion

Cipadessa baccifera (Roth.) Miq a much branched shrubs or small trees. Leaflets toothed and opposite, 3-5 in pairs, this key field identification character of the plant may help to identify the plant in its natural habitat. Externally, the stem is brownish grey in colour in its young condition while mature, it is brownish red in colour with present of red colour tint. Stem bark brownish red in colour, inner reddish brown in colour, fibrous and quilling outward channel. Transvers section of stem and stem bark shows group of pericyclic fiber nearly always containing isolated strands of fibers, but a composite and continuous rings which is special character of Meliaceae family.[18] Transverse section of stem bark shows terminal type of parenchyma and crystals presents in chambered cell, medullaruy rays exclusively uniseriate, varying from heterogenous to homogenous, they all are typically present in *Cipadessa* genus.[18] Presence of large amount of brown and tannin contain in stem and stem bark are important characters for identification. In powder microscopy stem and stem bark of *C. baccifera* shows crystal fibers.

Physicochemical parameters are useful for standardization as well as for developing the quality standards of crude drugs as well as finished product. The loss on drying is indicative of moisture content and volatile oil content. In present study, moisture content in the stem powder was lesser than the stem bark powder, which may indicate presence of less constituents of absorbent in the Stem. Thus, no spoilage of drug due to microbial growth. Pharmacognosy study and qualitative tests conform that the presence of large amount of tannin content in plant. Quantitative analysis conforms the presence of secondary metabolite like tannin, phenol and flavonoid that could be useful for further standardization of this plant and this phenolics (Phenol, flavonoid and tannin) also promising that the plant may having antioxidant, insecticidal, anthelmintic, antidotes in poisoning and anti-dysentery activities.[19] HPTLC analysis of stem and bark at 254 nm having five (5) different spectra while at 366 nm they are having four (4) different spectra. On the basis of this different peak present in stem and bark, may they both having some different chemical moiety and this data may useful for further study alike structural isolation and also compare with standard/marker compound.

5. Conclusion

Cipadessa baccifera (Roth.) Miq. is a much branched shrubs or tree, Leaflets toothed and opposite, fresh stems are present with red tint, they are key field identification characters of the plant. Presence of typical microscopic characters of stem and stem bark of *C. baccifera* like pericyclic fiber ring, uniseriate medullary rays, brown and

tannin content, crystal fibers helps for standardization and identification of the plant. Phytochemical data concluded that the, presence of secondary metabolites such as tannin, phenol and flavonoid in both the plant parts. HPTLC results will help in further validation and act as standards for assurance of quality.

6. Reference

1. RK S, Pralhad P. Double-blind, placebo-controlled clinical evaluation of an Ayurvedic formulation (GlucoCare capsules) in non-insulin dependent diabetes mellitus. J. Ayurveda Integr. 2010; 1:45-51.
2. Biplobkumar M, Partha G, Raghunath D, Anuradha M. Tradition in treating taboo: Folkloric medicinal wisdom of the aboriginals of Purulia district,West Bengal, India against sexual, gynecological and related disorders. J. Ethnopharmacol. 2015; 169:370-386.
3. Bhushan P. Ethnopharmacology and drug discovery. J. Ethnopharmacol. 2005; 100:50-52.
4. Saxena HO, Brahmam M. The flora of Orissa, Orissa forest development co.ltd., Bhubaneswar, Vol-I, 1995, 275.
5. Malarvannan S, Giridharan R, Sekar S, Prabavathy VR, Sudha N. Ovicidal activity of crude extracts of few traditional plants against *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera), J. Biopest. 2009; 2:64-71.
6. Jeevan Ram A, Bhakshu LM, Venkata Raju RR. *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J. Ethnopharmacol. 2004; 90:353-7.
7. Dhanlaxmi UR, Pralhad SP. Evaluation of the clinical efficacy and safety of under eye cream the treatment of under eye dark circles and pigmentation. Indain medical journal. 2009; 103(4):115-120.
8. Donald JA. Plant micro techniques. New York: London: Macgrow Hill Book Company, 1940, 105.
9. Trease, Evans. Pharmacognosy. 15th Ed., W.B. Sunders Company Ltd, 1996, 521-47.
10. Wallis TE. Text book of Pharmacognosy. Edition 5th, CBS Publishers, New Delhi, 1985, 572-578.
11. Anonymous. The Ayurvedic Pharmacopoeia of India. Part-II. 1st ed, Ministry of Health and Family Welfare, Department of AYUSH, New Delhi, Government of India, 2008, 2.
12. Kasture AV, Mahadik KR, More HN, Wadodkar SG. Pharmaceutical analysis. Nirali Prakashan, 2009, 2(18).
13. Vinay S, Ushnesh B. Methods of qualitative testing of some ayurvedic formulations. Gujarat Ayurvedic University, Jamnagar, 2001, 5-10.
14. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Amer Pharm Assoc. 1949, 324-31.
15. Singleton VL, Orthofer R, Lamuela-Raventós MR. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999; 299:152-78.
16. Elija K, vaishali A, Manik K, Nirmala D, Rajashree K. Spectroscopic determination of total phenol and flavonoid contents of *Ipomoea carnea*. Int J Chemtech Res. 2010; 2(3):1698-1701.
17. Graham HD. Stabilization of the Prussian blue color in the determination of polyphenols. Journal of Agricultural and Food Chemistry. 1992; 40(5):801-5.
18. Metcalfe CR and chalk L. Anatomy of the Dicotyledons. oxford University Press, Great Britain. 1950; 2:352-355.
19. Rehab A. Hussein and Amira A El-Anssary. Chepter 2. Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants, 2018, 13-16.