Regular articles

Pharmacognostical studies on *Ehretia microphylla* Lamk

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Abstract

***Ehretia microphylla* Lamk. is a member of the Boraginaceae family and finds its use in the Siddha System of Medicine. Aim of this study is to investigate the morphology, exo and endomorphic characters, powder analysis, preliminary phytochemical analysis, pharmacopoeial standards such as physico-chemical parameter, TLC photo documentation and HPTLC finger print of aerial parts. The results revealed the presence of an arc shaped collateral vascular bundles in the centre of the midrib and discontinuous sclerenchyma fibres below the vascular bundle. The adaxial and abaxial epidermii are nearly identical in structure but differ in their sizes. Presence of aerenchymatous spongy tissue, tanniniferous sacs and cluster crystals of calcium oxalate in the mesophyll and 2 or 3 layered hypodermis and isolated strands of pericyclic fibres in the stem are characteristic features. The powder showed abundant unicellular trichomes, foliar epidermal cells with anomocytic (ranunculaceous) stomata and druses of calcium oxalate crystals which are diagnostic characters to identify the drug. HPTLC finger printing showed 16 peaks at 254 nm; the TLC photo documentation showed four visible spots under 254 nm, 8 spots under 366 nm and 4 spots after derivatization.**

Key words: *Ehretia buxifolia*; *Carmona retusa*; Kuruvichi poondu; Boraginaceae; microphyllone

1 Introduction

Ehretia microphylla Lamk. syn. *E. buxifolia* var. *microphylla* (Lam.) DC.; *Carmona retusa* (Vahl) Masam. is a small shrub, common in the dry scrub forests of the Deccan peninsula and is sometimes cultivated in gardens [1].

This plant is recorded as Kuruvichi, or Kuruvichi poondu in Siddha Materia Medica [2, 3]. It is used for leprosy, eczema due to venereal diseases, chronic dysentery, infertility and toxic diarrhea in children [2-4]. The roots are used as an alterative in cachexia and syphilis and as an antidote to vegetable poisoning. It is widely used in the Philippines as herbal medicine. A decoction of the dried leaves is used for cough and stomach troubles; the leaves are used as a substitute for tea [1].

Literature survey on phytochemical constituents revealed the presence of microphyllone [5-8], baurenol, ursolic acid [5], dehydromicrophyllone, hydroxymicrophyllone, cyclomicrophyllone, allomicro-phyllone [6], kaempferol-3-*O*-

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glucoside (astragalin), kaempferol-3-*O*-rutinoside (nicotiflorin), rosmarinic acid [9], baurenol, α, β-amyrin [9, 10], ehretianone, stigmasterol, stigmastanol, campesterol, α-spinasterol, cholesterol, β-sitosterol and daucosterol [11].

The ethyl acetate-soluble portion of the methanol extract showed inhibitory activity on exocytosis in antigen-stimulated rat basophils [6]. 4-Hydroxy-7, 8, 11, 12, 15, 7', 8', 11', 12', 15' -decahydro-beta,psi-carotene (microphyllone) was found to reduce by approximately 68.4% the number of micronucleated polychromatic erythrocytes induced by tetracycline, a known mutagen in a *in vivo* study [7, 8]. Rosmarinic acid isolated from the leaves exhibited rat peritoneal mast cell histamine release inhibitory activity induced by the compound 48/80 [9].

Ehretianone together with known sterols, exhibited anti snake venom property against *Echis carinatus* venom in mice [11].

Microphyllone and ehretianone isolated from root bark showed antibacterial activity against all bacteria except *Escherichia coli* and *Pseudomonas aeruginosa* at the dose level of 1.25, 2.5, 5 and 10 mg/ml [12]. The aqueous extract showed activity against *S. aureus, B. subtilis, P. aeruginosa* and no activity against *E. coli*. [13]. Petroleum ether, chloroform and methanol extracts exhibited moderate to appreciable anti-bacterial activities against *Bacillus subtilis, Klebsiella pneumoniae, Shigella flexneri* and *Pseudomonas aeruginosa* [14]. The mixture of α and β-amyrin, baurenol exhibited analgesic activity (51%), anti-inflammatory activity (20%), anti-diarrheal activity (29%) and moderate antimicrobial activity [15]. The ethanolic extract of stem of *Carmona retusa* showed anti-inflammatory activity in human red blood cell membrane stabilization method, heat induced hemolysis and proteinase inhibitory activity as 55.72%, 56.37% and 61.75% respectively at 400 µg/ml concentration [16].

Tablets prepared from the dried leaves exhibited antimutagenic activity [17]. Significant stimulatory effect of this plant on reproductive function and ovarian folliculogenesis in rats was reported [18].

2 Material and methods

Fresh plants were collected from Adyar, Chennai. The botanical identification was carried out using floras [19] and authenticated by the Botanist, Siddha Central Research Institute, Arumbakkam, Chennai and the voucher specimen was deposited in the Pharmacognosy department.

Stem, leaf and fruit were fixed in FAA solution (70% ethyl alcohol, formalin and acetic acid in the ratio of 90 ml: 5 ml: 5 ml) for histological studies. Free hand as well as wax embedded microtome sections were prepared, stained with safranin and fast green [20, 21]. Quantitative microscopy such as stomatal index, palisade ratio and vein islet number was carried out and values were determined [22, 23]. Powder was analyzed as per standard procedure [22]. Photomicrographs were taken with the help of Nikon Eclipse E200 Microscope.

Qualitative phytochemical tests, physico-chemical parameters, thin layer chromatographic photo documentation and high performance thin layer chromatographic finger print profile documentation studies were carried out using the shade dried powder by adopting the procedures mentioned in the standard books [24-26]. Linomat IV applicator for spraying the extracts on TLC plate as bands, TLC twin trough chamber for developing the plate, visualizer for photo documentation under UV-visible conditions, TLC scanner 030618 programmed with WINCATS 4 software (CAMAG, Muttenz, Switzerland) for finger print development were the instruments used for the study. Aluminium plate precoated with silica gel $60F_{254}$ (E Merck) of 0.2 mm thickness was used for the chromatography. The chloroform extract was applied as 6 mm bands

with 6 mm distance in between tracks and the plate was developed in a solvent system of toluene: ethyl acetate (7:1.5, *v/v*). The developed plate was photo-documented under UV at wavelengths of 254 nm and 366 nm. Then scanned at UV 254 nm to develop the finger prints. Later, the plate was dipped in vanillin-sulphuric acid reagent, heated at 105°C till the development of colour of the separated compounds and the image was photodocumented.

3 Results

3.1 Plant morphology

A small shrub reaching 3-4 ft high with fascicled very coriaceous small leaves, flowers white (Fig. 1-A), axillary, solitary or two together on slender hairy peduncles and a scarlet, globose drupe [22].

3.2 Macroscopical characters

Stem: Branches numerous, slender, divaricate, bark brown, cracked.

Leaf: Leaves coriaceous, small 1–2.5 cm by 4–8 mm, sessile, fascicled, on suppressed branchlets, obovate, cuneate at base, rounded and often 3–5 lobed at apex, coriaceous above with short bristly hairs with a white spot at the base of each when dried, shining and polished above, paler and with conspicuous venation beneath.

Fruit: An orange to scarlet, globose shining small drupe 4 mm in dia., apiculate, endocarp hard consisting of 1 four celled pyrene. Dried fruits black in colour. Calyx persistent, hairy lobes large, acute, lanceolate, spathulate – oblong.

3.3 Microscopical characters

Transverse section of stem is circular in outline (Fig. 1-B). The outermost layer epidermis is made up of small rectangular cells, covered with thick cuticle. Most of the epidermal cells bear unicellular trichomes measuring 330-350 µm in length and 32-35 µm across. The hypodermis is 2 or 3 layered, composed of large, radially elongated thick walled cells (Fig. 1-C). Each cell measuring 12-18 µm in length and 4-9 µm in breadth. It is followed by cortex consists of 8 to 10 rows of round to slightly oval parenchyma cells. Isolated strands of pericyclic fibres separates the vasculature and cortex. The vasculature is represented by outer phloem composed of sieve tubes, companion cells and phloem parenchyma. Inner xylem region consists of usual elements. The xylem vessels are arranged in definite radial rows (Fig. 1-C). The central pith is large and composed of round to polygonal, thin walled, closely arranged parenchyma cells (Fig. 1-B).

Transverse section of midrib shows a small depression on the adaxial side and convexity on the abaxial side. The midrib measures 260-330 µm in vertical plane and 240-300 µm in horizontal plane. The adaxial epidermal cells are larger in size measuring 17-30 µm × 30-32 µm. The abaxial epidermal cells are measuring 8-12 µm × 8-14 µm. In the centre an arc shaped collateral vascular bundle is seen (Fig. 2-H). The sclerenchyma fibres are discontinuous and present below the vascular bundle. The ground tissue is parenchymatous.

A mature lamina measure 240-270 µm in thickness (Fig. 1-D, E). The adaxial and abaxial epidermii are nearly identical in structure that both are composed of square to rectangular cells, though those on the abaxial side are smaller in dimensions. The adaxial epidermal cells measure 20-30 µm × 22-30 µm, while the abaxial epidermal cells measure 15-22 µm × 7-11 µm. The cuticle covering the epidermis is thick. Some of the adaxial epidermal cells elongate to form covering trichomes. Trichomes are unicellular with bodies resembling cystoliths are present in the swollen basal part and sometime in the adjacent epidermal cells (Fig. 1-G). The structure of

these hairs and mode of deposition of the calcareous infiltration are constant for any given species. The palisade mesophyll consists of 2 rows of columnar, closely arranged cells. The spongy mesophyll is made up of aerenchymatous tissue. Small vascular bundles are situated between the palisade and spongy mesophyll (Fig. 1-F). Sclerenchyma fibres are seen below the vascular bundles. Tanniniferous sacs are also seen in the mesophyll. Cluster crystals are widely distributed (Fig. 2-K).

In surface view, the adaxial foliar epidermis is made up of penta-hexagonal cells with straight walls. Stomata are totally absent. Unicellular trichomes are seen (Fig. 2-I). Abaxial foliar epidermis is made up of smaller cells when compared to adaxial epidermal cells with slightly wavy contour. It is perforated by numerous ranunculaceous stomata measures 16–18 µm long and 10–14 µm across (Fig. 2-J). Stomatal index for abaxial surface 9–12, vein islet number 18–22; palisade ratio 2–4.

Longitudinal section of tender fruit shows a globose structure with persistent calyx and pedicel (Fig. 2-L). The calyx is made up of adaxial and abaxial epidermis made up of small rectangular cells. Some cells elongate to form unicellular trichomes. The ground tissue is made up of 6 rows of thin walled parenchyma cells. Vascular strands are traversed in this region (Fig. 2-M).

Fruit wall or pericarp shows two or three distinct layers. The exocarp or epicarp is composed of small transversely elongated parenchyma cells. The mesocarp is made up of outer larger rectangular cells and inner region composed of polygonal closely arranged parenchyma cells. The vascular strands ramify in the tissues of the mesocarp. In mature fruits hard endocarp is produced by the formation of one or more layers of sclereids in the hypodermal region. The gynoecium is divided into 4 locules by thin septa. Each locules contains a solitary embryo. The basal portion of the fruit is attached with the pedicel. Epidermis of the pedicel also bears unicellular trichomes. The ground tissue is parenchymatous, interspersed with vascular tissue. Distal region of the tender fruit is composed of thick walled cells (Fig. 2-L).

3.3.1 Powder analysis

The powder is pale brown colour, odourless with astringent taste. Under microscope, the powder showed abundant unicellular trichomes, fragments of epidermal cells with ranunculaceous stomata, druses of calcium oxalate crystals, palisade cells, parenchyma cells, fibres and vessels.

3.3.2 Histochemical tests

The sections of the leaf when treated with phloroglucinol and dilute hydrochloric acid gave red colour indicating the presence of lignin; with ferric chloride bluish black colour developed showing the presence of tannins; with con. HCl effervescence was observed showing the presence of calcium oxalate crystals; when treated with ruthenium red and sudan III no red colour was observed indicating the absence of mucilage and oil respectively.

4 Preliminary phytochemical tests

Preliminary phytochemical tests conducted in the extracts successively extracted with hexane, chloroform, ethyl acetate and methanol showed the presence of different types of secondary metabolites and the results are shown below in Table 1.

5 Physico-chemical studies

The foreign organic matter free, bulk sample was collected for the study. All the parameters were conducted with powdered samples. The moisture content was 10.38%. The determined total ash was 10.19%. The water soluble ash was calculated as

2.44% and the alkalinity was found to be 1.6 ml of 0.1 N HCl/g of the sample. The acid insoluble ash was 0.17%. The ethanol and water soluble extractives were found to be 15.24% and 9.71% respectively. The successive extractive values with hexane, chloroform, ethyl acetate and ethanol were to the extent of 3.60%, 2.50%, 3.40% and 4.10% respectively. The physico-chemical values are presented in Table 2.

Table 1. Preliminary phytochemical test results of *E. microphylla* aerial part

S. No	Phytochemical Tests	Hexane	Chloroform	Ethyl acetate	Methanol
1	Steroid	+	+	-	-
2	Triterpene	+	+	+	-
3	Phenol	-	-	+	+
4	Flavonoid	-	-	+	+
5	Tannin	-	-	-	+
6	Quinones	-	+	+	-
7	Furans	-	-	-	-
8	Acids	-	+	+	+
9	Alkaloids	-	+	+	+
10	Sugars	-	-	-	+
11	Saponin	-	-	-	+

Table 2. Physico-chemical values of *E. microphylla* aerial part

S. No	Parameter	Mean±SD
1	Foreign organic matter (%)	Nil
2	Loss on drying at 105°C (%)	10.38±0.04
3	Total ash (%)	10.19±0.03
4	Water soluble ash (%)	2.44±0.03
5	Alkalinity (ml of 0.1N HCl/g)	1.60±0.002
6	Acid insoluble ash (%)	0.17±0.05
7	Water soluble extractive (%)	15.24±0.02
8	Alcohol soluble extractive (%)	9.71±0.25
9	pH (10% solution)	6.88±0.01
10	Successive Hot Extractive values (%)	
	Hexane	3.60±0.04
	Chloroform	2.50±0.05
	Ethyl acetate	3.40±0.02
	Ethanol	4.10±0.50
11	Heavy metals	
	Lead	Absent
	Cadmium	Absent
	Mercury	Absent
	Arsenic	Absent

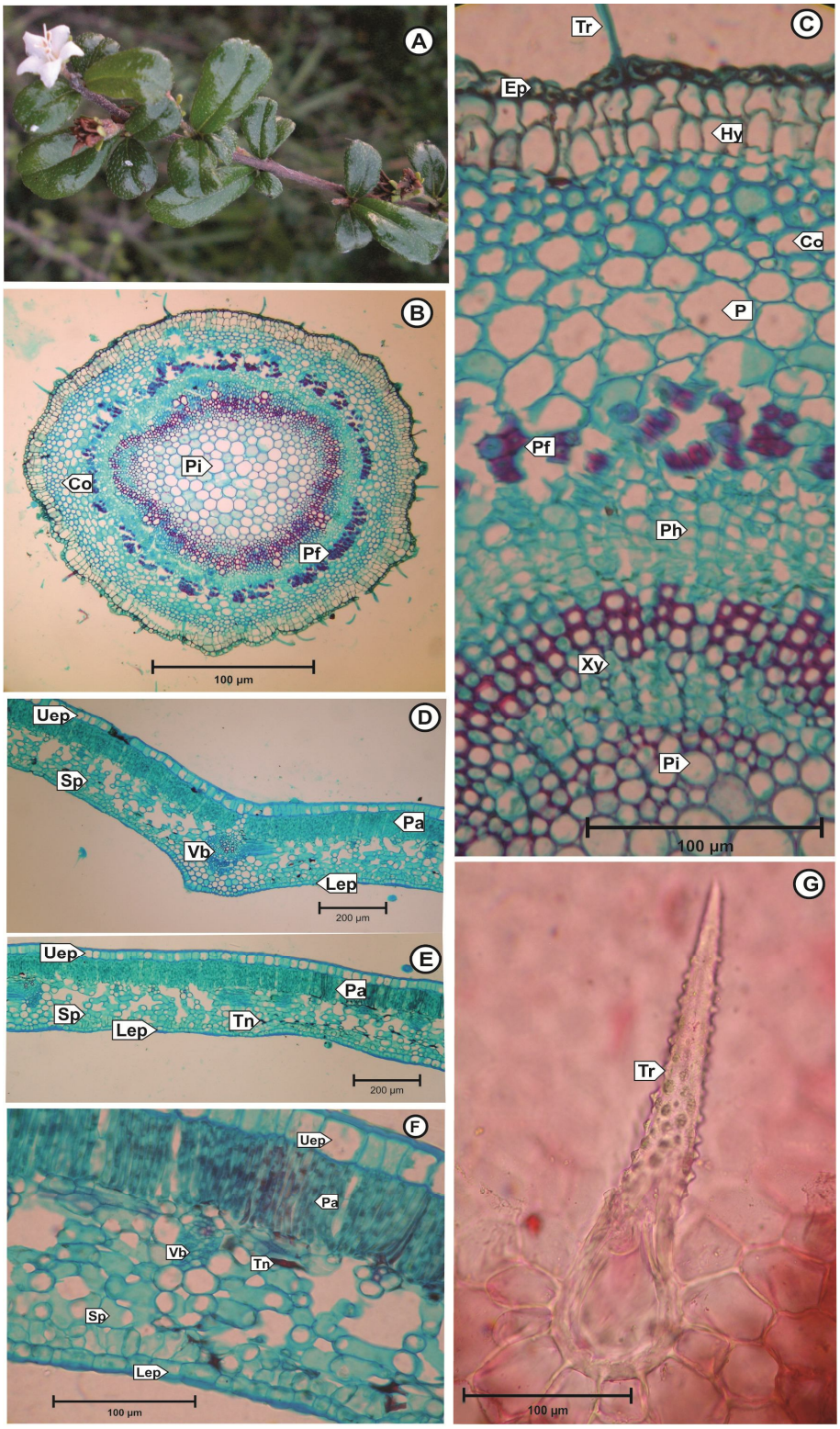


Fig. 1. A: Plant; B: T.S. of stem– Ground plant; C: T.S. of stem – Enlarged; D: T.S. of Leaf; E: T.S. of lamina; F: T.S. of lamina – Enlarged; G: Unicellular trichome – Enlarged. Co: Cortex; Ep: Epidermis; Hy: Hypodermis; Lep: Lower (abaxial) epidermis; P: Parenchyma; Pa: Palisade tissue; Pf: Pericyclic fibre; Ph: Phloem; Pi: Pith; Sp: Spongy tissue; Tn: Tanniniferous sacs; Tr: Unicellular trichome; Uep: Upper (adaxial) epidermis; Vb: Vascular bundle; Xy: Xylem

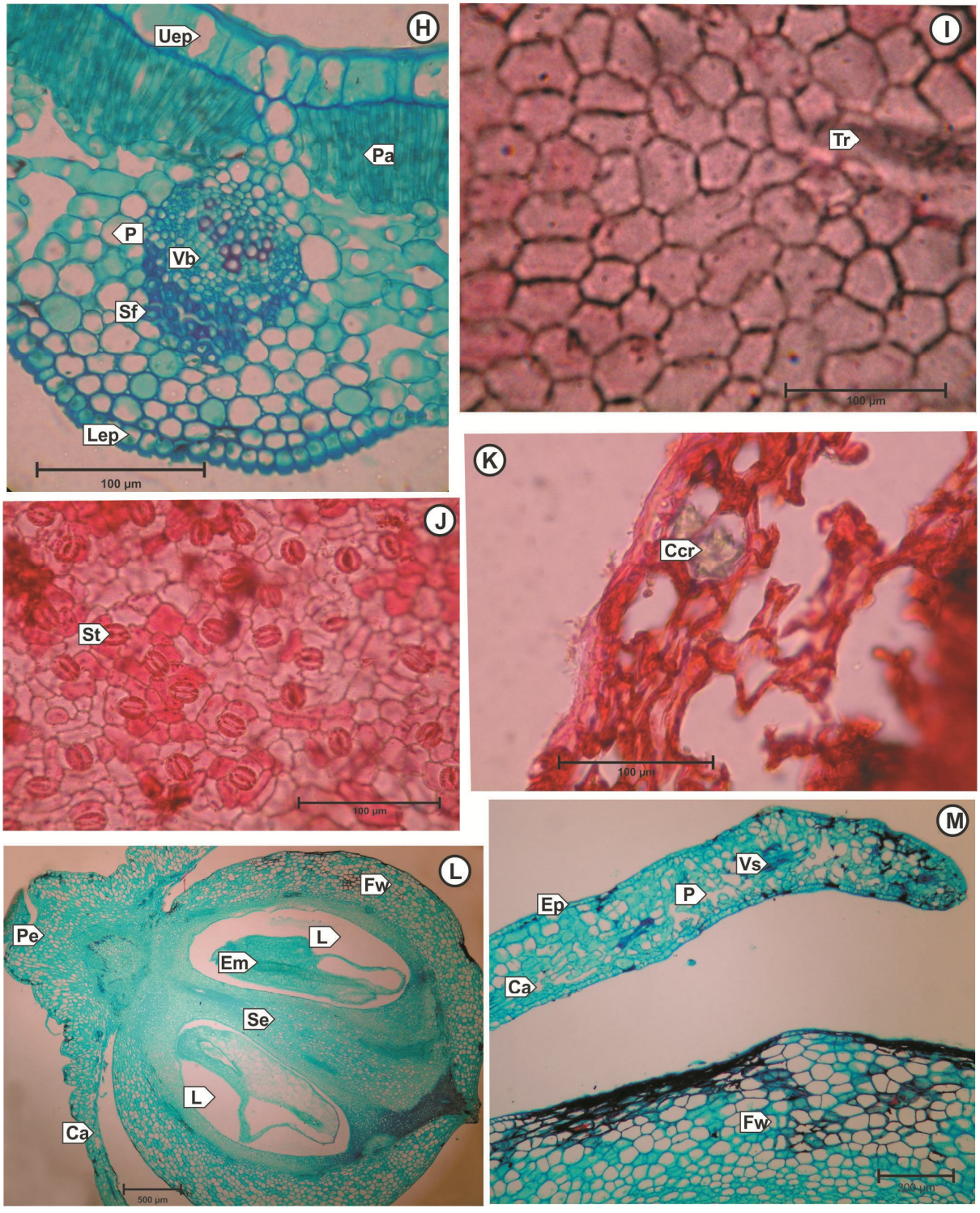
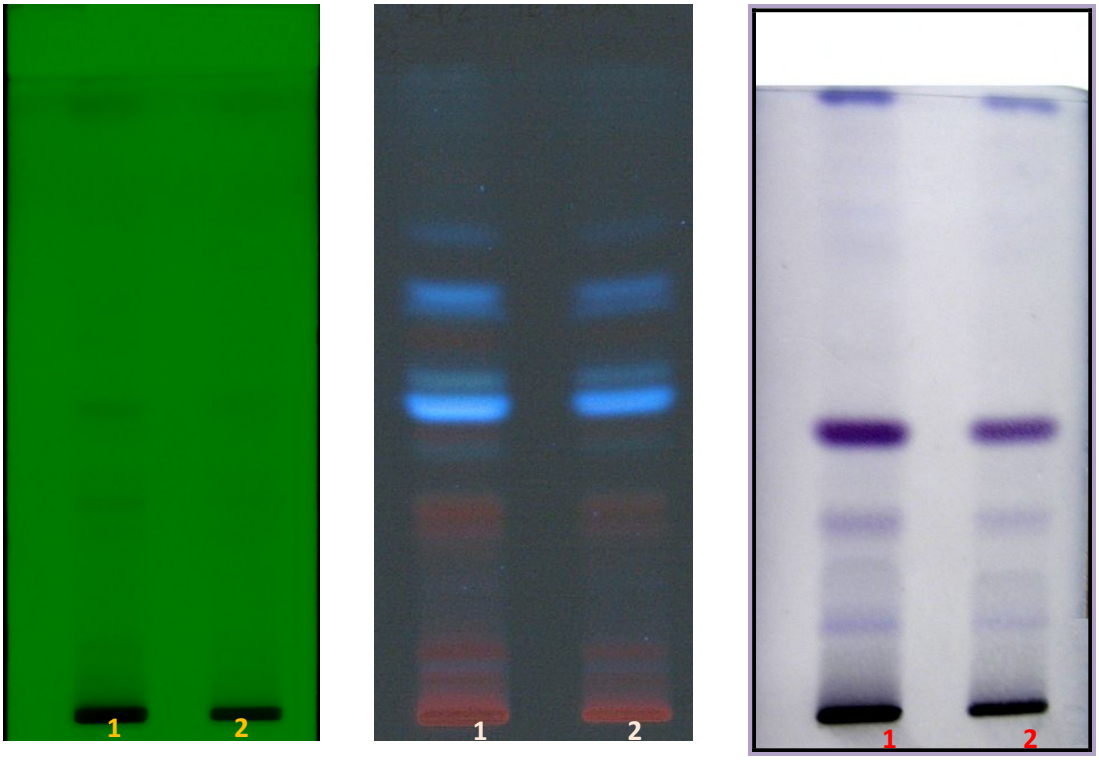


Fig. 2. H: T.S. of midrib; I: Adaxial foliar epidermis; J: Abaxial foliar epidermis; K: Cluster crystal of calcium oxalate; L: T.S. of tender fruit; M: T.S. of tender fruit showing calyx and fruit wall. Ca: Calyx; Ccr: Cluster crystal of calcium oxalate; Em: Embryo; Ep: Epidermis; Fw: Fruit wall; L: Locule; Lep: Lower (abaxial) epidermis; P: Parenchyma; Pa: Palisade tissue; Pe: Pedicel; Se: Septa; Sf: Sclerenchyma fibre; St: Stoma; Uep: Upper (adaxial) epidermis; Vb: Vascular bundle; Vs: Vascular strand.

6 Chromatographic studies

HPTLC finger print profile of the chloroform extract of aerial part was carried out. The chloroform extract showed 4 visible spots when viewed under wavelength 254 nm; 8 spots under 366 nm and 4 visible spots when it was dipped in vanillin-sulphuric acid reagent subsequently heated at 105°C till the coloured spots appearance. The chromatograms are presented in Fig. 3. The R_f values and colour of spots of TLC chromatograms recorded under UV 254 nm, 366 nm and after derivatization with vanillin-sulphuric acid are presented in Table 3. The HPTLC finger print profile at UV 254 nm is presented in Fig. 4. The R_f values and the percentage of all peak areas of the HPTLC finger print are shown in Table 4. The HPTLC 3D chromatogram of both tracks is presented in Fig. 5.



a. UV 254 nm b. UV 366 nm c. Post Derivatized

Fig. 3. TLC profile of chloroform extract of *E. microphylla* aerial part. Track 2: 10 µl; Track 1: 5 µl

Table 3. R_f values and colour of spots under UV 254 & 366 nm and after derivatization

S. No.	UV 254 nm		UV 366 nm		Post Derivatization	
	R_f value	Colour	R_f value	Colour	R_f value	Colour
1	0.11	Green	0.06	Pink	0.14	Purple
2	0.33	Green	0.11	Pink	0.24	Purple
3	0.37	Green	0.33	Pink	0.33	Purple
4	0.48	Green	0.41	Pale blue	0.46	Purple
5	-	-	0.48	Pale blue	-	-
6	-	-	0.52	Pale blue	-	-
7	-	-	0.65	Pale blue	-	-
8	-	-	0.75	Pale blue	-	-

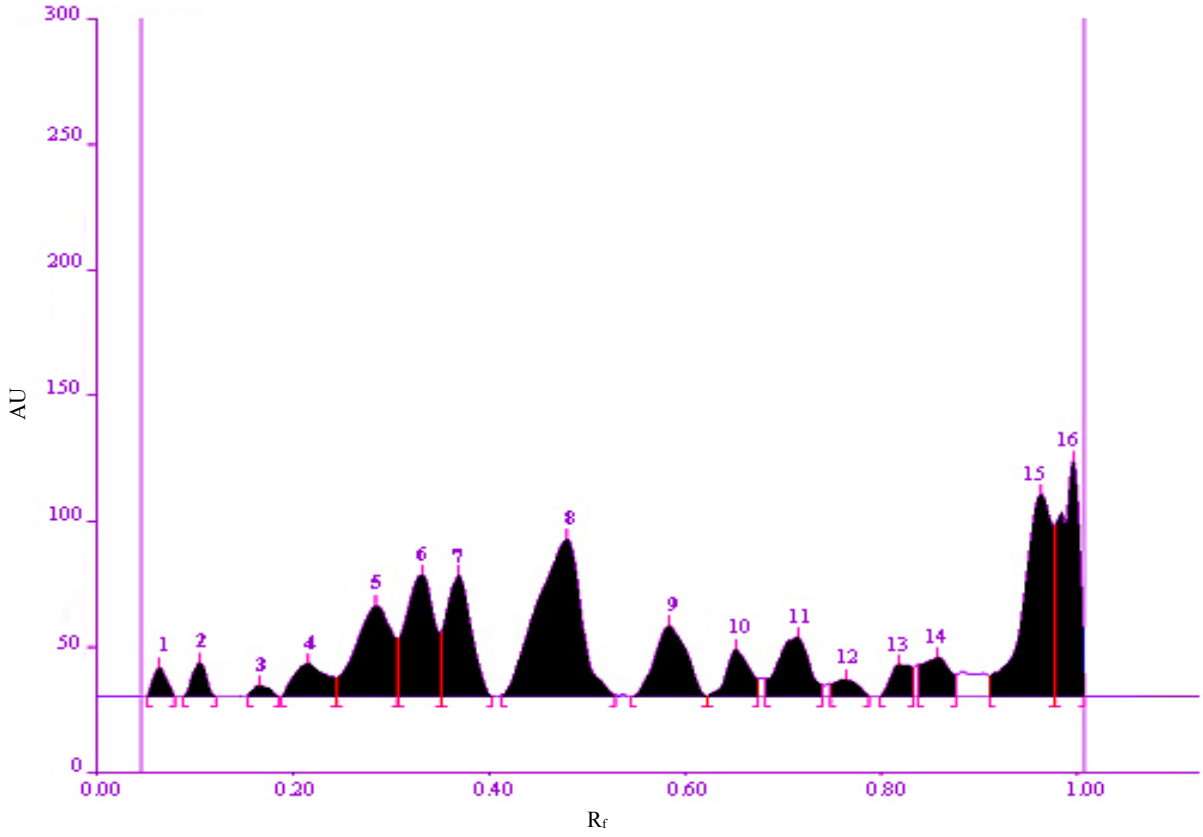


Fig. 4. HPTLC finger print profile of chloroform extract of *E. microphylla* aerial part at UV 254 nm

Table 4. R_f value and % peak areas of chloroform extract of *E. microphylla* aerial part at UV 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	0.05	0.1	0.06	11.6	2.24	0.08	0.2	151.4	1.10
2	0.09	0.2	0.11	13.7	2.64	0.12	0.1	172.3	1.26
3	0.16	1.1	0.17	4.6	0.88	0.19	0.0	70.0	0.51
4	0.19	0.1	0.22	13.2	2.54	0.24	7.5	402.0	2.93
5	0.25	7.6	0.28	36.3	6.98	0.31	23.3	1216.7	8.87
6	0.31	23.3	0.33	48.5	9.31	0.35	25.5	1325.7	9.66
7	0.35	26.2	0.37	48.3	9.29	0.40	0.5	1110.6	8.10
8	0.41	0.9	0.48	62.9	12.09	0.53	0.8	2607.2	19.01
9	0.55	0.0	0.58	28.3	5.44	0.62	0.3	851.2	6.20
10	0.62	0.4	0.65	18.7	3.59	0.68	7.1	413.7	3.02
11	0.68	7.1	0.71	23.9	4.58	0.74	4.9	695.6	5.07
12	0.75	5.1	0.76	7.0	1.34	0.79	0.2	156.8	1.14
13	0.80	0.1	0.82	12.9	2.49	0.83	11.8	249.6	1.82
14	0.84	12.6	0.86	15.7	3.02	0.88	8.7	420.8	3.07
15	0.91	8.6	0.96	80.7	15.50	0.98	68.3	2146.8	15.65
16	0.98	68.6	1.00	94.0	18.07	1.01	0.0	1727.4	12.59

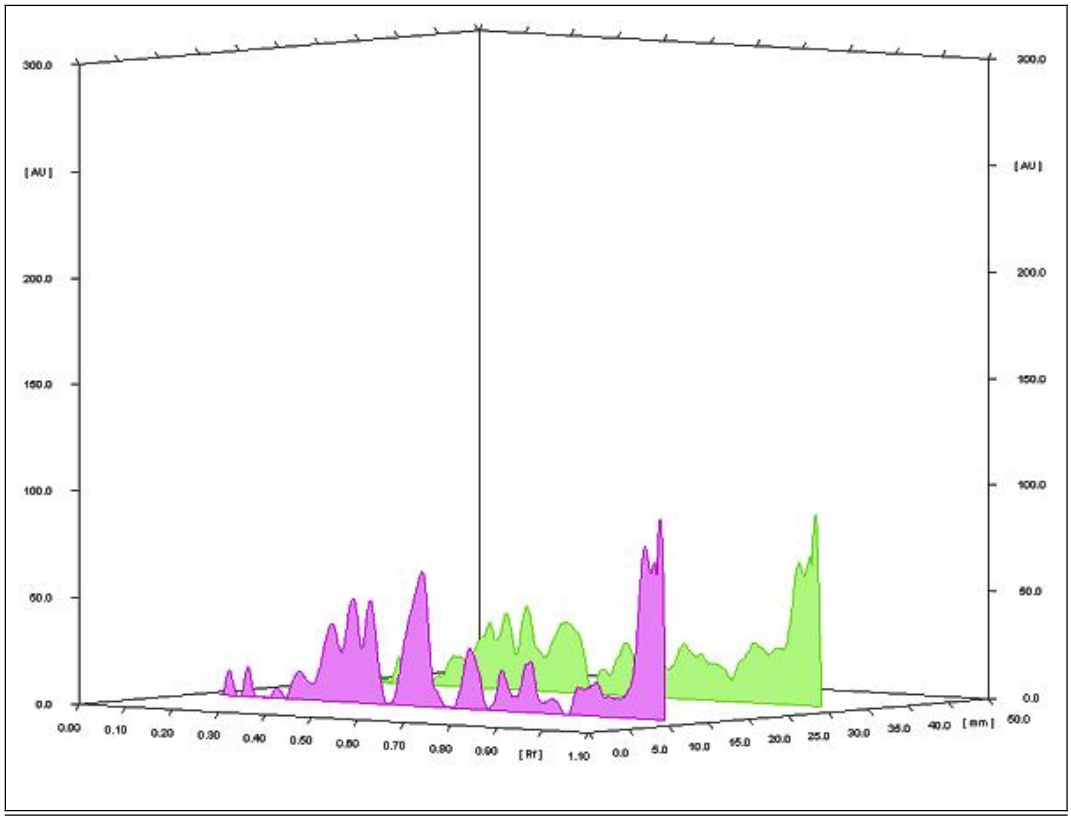


Fig. 5. HPTLC 3D chromatogram of chloroform (10 & 5 µl) extract under UV 254 nm

7 Discussion

Ehretia microphylla is known as Kuruvichi poondu in Siddha system of medicine. Numerous unicellular trichomes with bodies resembling cystoliths are present in the swollen basal part and sometimes in the adjacent epidermal cells, anomocytic (ranunculaceous) stomata, 2 layered palisade, aerenchymatous spongy, tanniniferous sacs, druses of calcium oxalate crystals in the leaf and presence of pericyclic fibres and 2 or 3 rows of hypodermal cells in the stem and 2 or 3 layers of fruit wall and four locules in the gynoecium separated by thin septa are important characters in the pharmacognostic determination of the drug.

The 10.19% of the drug amounts to total inorganic content present in it; 2.44% of the inorganic content are water soluble and the alkalinity of water soluble inorganic content was found to be 1.6 ml of 0.1N HCl/g of the sample. The content of acid insoluble siliceous matter was calculated as 0.17%. The ethanol and water soluble extractives were found to be 15.24% and 9.71% respectively revealing the presence of high polar compounds. The successive extractive values with hexane, chloroform, ethyl acetate and ethanol were to the extent of 3.60%, 2.50%, 3.40% and 4.10% respectively and the percentage of total extraction is 13.60 and the successive hot extraction method may be better one than cold extraction method. The pH of 10% solution was found to be slightly acidic. The qualitative inorganic analyses revealed the absence of heavy metals viz., lead, cadmium, mercury and arsenic.

Preliminary phytochemical study revealed the presence of different secondary metabolites which

include steroid, triterpene, flavonoid, quinone, alkaloids, glycosides, saponin, tannin, *etc*. and absence of furans. Presence of these secondary metabolites demonstrates that the plant may be more active and exhibit good antioxidant properties. All other peaks individually contributed to <5%.

8 Conclusion

It can be concluded that the anatomical and quantitative microscopic studies of the aerial portion, powder microscopy, analytical characters and thin layer chromatographic finger print profile and TLC photo documentation studies will be useful to identify the drug in dried condition.

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References

[1] Anonymous. The Wealth of India. Vol. 3. New Delhi: CSIR, 1952, 129.

[2] Murugesa Mudaliyar KS. Gunapadam. Part-I. Chennai: Tamil Nadu Siddha Medical Board, 1956, 274.

[3] Kannusamy Pillai C. Pathartha Guna Vilakkam. Chennai: B Rathina Nayakar & Sons, 1939, 265-266.

[4] Anonymous. Pathartha Guna Chinthamani. Chennai: B Rathina Nayakar & Sons, 1932, 103.

[5] Agarwal SK, Rastogi RP, Van Koningsveld H, *et al*. The molecular structure of 4a,5,8,8a–tetrahydro-11,14-dimethoxy-7-methyl-4a-(3-methyl-2-butenyl)-5,8a-*O*-benzeno-1,4-naphthoquinone.Tetrahedron, 1980, 36: 1435-1438.

[6] Yamamura S, Simpol LR, Ozawa K, *et al*. Antiallergic dimeric prenylbenzoquinones from *Ehretia microphylla*. Phytochemistry, 1995, 39: 105-110.

[7] Villasenor IM, Edu DA, Bremner JB. Structure of an antimutagen from *Carmona retusa* leaves. Carcinogenesis, 1993, 14: 123-125.

[8] Villasenor IM, Edu DA. Antimutagen from leaves of *Carmona retusa* (Vahl) Masam. Mutat Res/Genet Toxicol, 1993, 298: 215-218.

[9] Rimando AM, Inoshiri S, Otsuka H, *et al*. Screening for mast cell histamine release inhibitory activity of Philippine medicinal plants active constituent of *Ehretia microphylla*. Shoyakugaku Zasshi, 1987, 41: 242-247.

[10] Villasenor IM, Edu DA, Bremner JB. Triterpenoids of *Carmona retusa* (Vahl) Masam. Philips Res Sci, 1992, 121: 411-418.

[11] Selvanayagam ZE, Gnanavendhan SG, Balakrishna K, *et al.* Ehretianone, a novel quinonoid xanthenes from *Ehretia buxifolia* with anti-snake venom activity. J Nat Prod, 1996, 59: 664-667.

[12] Hamsaveni GR, Balakrishna K, Ramkumar V, *et al*. Antibacterial activity of *Ehretia buxifolia*. Fitoterapia, 1997, 68: 85-86.

[13] Penecilla GL, Magno CP. Antibacterial activity of extracts of twelve common medicinal plants from the Philippines. J Med Plants Res, 2011, 5: 3975-3981.

[14] Chandrappa CP, Govindappa M, Anil Kumar NV. Phytochemical screening and antibacterial property of *Carmona retusa* (Vahl.) Masam. Int J Pharm Med & Bio Sci, 2012, 1: 91-98.

[15] Villasenor IM, Canlas AP, Faustino KM, *et al*. Evaluation of the bioactivity of triterpene mixture isolated from *Carmona retusa* (Vahl.) Masam. Leaves. J Ethnopharmacol, 2004, 92: 53-56.

[16] Chandrappa CP, Govindappa M, Anil Kumar NV, *et al*. *In vitro* anti-inflammatory activity of *Carmona retusa* (Vahl.). World J Pharm Pharmaceut Sci, 2013, 2: 3991-3997.

[17] Lim-Sylianco CY, Blanco FRB, Lim CM. Mutagenicity, elastogenicity and antimutagenicity of medicinal plant tablets produced by the NSTA pilot plant IV, Tsaang gubat tablets. The Philippine Journal of Science, 1987, 116: 273-280.

[18] Aarthi V, Anbu J, Nazeer ahamed KFH, *et al*. Effect of *Ehretia microphylla* Lamk. on stimulation of

reproductive function and ovarian folliculogenesis in rats. Int J Pharm Bio Sci, 2012, 3: 273-280.

[19] Gamble JS. Flora of the Presidency of Madras. Vol.2. Calcutta: Botanical Survey of India, 1967, 627.

[20] Johansen DA. "Plant Microtechnique". New York: McGraw Hill Book Co. Ltd., 1940.

[21] Sass JE, Elements of Botanical Microtechnique. New York and London: Mc Graw Hill Book Co. Inc, 1940, 222.

[22] Wallis TE. Text book of Pharmacognosy. Shahdara, Delhi-32: CBS Publisher & Distributors, 1985.

[23] Trease GE, Evans WC. Pharmacognosy. 13th ed. London: Bailliere Tindal, 1989, 799-803.

[24] Harborne JB. Phytochemical Methods. London: Chapman and Hall, 1973.

[25] Anonymous. Quality Control Methods of Medicinal Plant Materials. Geneva: World Health Organization (WHO), 1998.

[26] Wagner H. Bladt S. Plant Drug Analysis. 2nd ed. Berlin: Springer, 1996.