International Journal of Pharma and Bio Sciences V1(2)2010

PHARMACOGNOSTICAL STANDARDIZATION AND HPTLC FINGERPRINT OF *CRATAEVA TAPIA* LINN. SSP. *ODORA* (JACOB.) ALMEIDA LEAVES.

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ABSTRACT

Crataeva tapia Linn. ssp. *odora* (Jacob.) Almeida, a traditional medicinal plant, which is valued for its benefits in the management of urinary and inflammatory disorders. Leaves are externally rubefacient and used in rheumatism; internally they are given as febrifuge and tonic. The leaves also act as anti-periodic. The current study was therefore carried out to provide requisite pharmacognostic details about the leaves of *C. tapia*. Pharmacognostic evaluation included examination of morphological and microscopical characters; physicochemical properties, phytochemical analysis, fluorescence study and HPTLC finger print. The powder microscopy showed the presence of anomocytic stomata and spiral thickening. Phytochemical screening reported the presence of tannins, steroidal compounds, cardiac glycosides and alkaloids. The R*f* value of 0.35 detected at 300 nm and 600 nm by qualitative densitometric HPTLC fingerprint, can be used as identifying marker for methanolic extract. The present study will provide the information with respect to identification and authentication of crude drug.

KEYWORDS

Crataeva tapia Linn. ssp. *odora* (Jacob.) Almeida [syn. *C. religiosa* var. *nurvala* Hook. f.], leaves, pharmacognosy, HPTLC fingerprint.

INTRODUCTION

Crataeva tapia Linn. ssp. *odora* (Jacob.) Almeida [syn. *C. religiosa* var. *nurvala* Hook. f.], is a small much branched tree indigenous to South India. Although it is known by different names throughout India it is commonly identified by its Sanskrit name, Varuna. In Ayurvedic medicine, Varuna is valued for its benefits in the management of urinary and inflammatory disorders [1, 2]. A wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, butulinic acid and betulinaldehyde have been reported from *C. tapia* [3-6]. Pharmacological studies using the root bark and stem bark of *C. tapia*, in recent years, have confirmed its beneficial effects in supporting the management of urinary disorders, including urolithiasis and revealed its potential benefits in supporting the management of inflammatory conditions, such as arthritis [7]. Radicle and bark are

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laxative and lithontripic and increase appetite and biliary secretion [5]. In Ceylon the leaves are used for gouty swelling and heal wound. In Bombay the leaves are used as remedy for swelling of the feet and the burning sensation in sole of feet. In Konkan the juice is given in the rheumatism. In caries of the bones of the nose, the leaf is smoked and the smoke is exhaled through the nose [8, 9]. The leaves also act as anti- periodic [10]. Leaves are externally rubefacient and used in rheumatism; internally they are given as febrifuge and tonic [11, 12]. Treatment with *C. tapia* shifted the relative proportion of urinary electrolytes, particularly those that participate in calculus formation, towards the non-lithogenic zone [13]. Flavonoids, rutin, quercetin and isoquercetin have been isolated from the leaves [7]. Therefore, present investigation of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida leaves is taken up to establish pharmacognostic profile of the leaves which will help in crude drug identification as well as in standardization of the quality and purity.

MATERIALS AND METHODS

Herbarium of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. Fresh leaves of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida were collected from Kalyan, M.S., India, washed under running tap water and blotted dry for further studies. The leaves were dried in preset oven at 40 ± 2˚C for about two weeks, ground into powder and used for further analysis. Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described by Mukherjee[14]. Preliminary phytochemical analysis of powdered leaf was performed as described by Khandelwal [15] and Kokate [16]. Fluorescence analysis was conducted using methods of Kokoski [17] and Chase and Pratt [18] and histo-chemical analysis was carried using methods described by Madhavan *et al.* [19].

A qualitative densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile using different solvents according to polarity *viz.* Benzene, Petroleum Ether, Ethanol, Methanol and Water, which may be used as markers for quality evaluation and standardization of the drug. 10 µl of leaf extract was spotted on precoated silica gel G60 F_{254} TLC plates (Merck) with the help of CAMAG Linomat V applicator. The plates were then developed in glass twin trough chamber (20cm x 10 cm) presaturated with mobile phase. The developed plates were scanned using TLC Scanner 3 (CAMAG).

RESULTS

Macroscopic Characters of the leaf

Leaves of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida are deciduous, trifoliate and petiolate. Leaves are pale green in colour. Leaflet is 5-15 by 3-6.5 cm and petiole 3.5 -7 cm long. Leaves are ovate, acute at the apex, entire, glabrous on both the surfaces with reticulate venation (Plate No. 1: A, B, C).

Microscopic characters of the leaf

Surface preparation, of leaf revealed presence of anomocytic type of stomata on adaxial surface (Plate No. 1: I), whereas abaxial surface is devoid of stomata (Plate No. 1: H).

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Transverse section of Leaf

It is a dorsiventral leaf. In transverse section of midrib and lamina following tissues were observed:

Midrib: Section passing through midrib shows hump on adaxial side. Midrib shows 3-5 layers of collenchyma below the upper epidermis. The conducting tissue system (xylem and phloem) is situated at the centre of the midrib in the form of a crescent shape. Parenchymatous cells are usually found in the centre of the ring. The inner part of the ring is composed of xylem (towards the upper epidermis) and phloem (towards the lower epidermis). Sclerenchymatous cells as bundle caps adjacent to the phloem were also observed. Below vascular tissue it shows presence of paranchymatous cells, 3-4 layer of collenchyma and lower epidermis (Plate No. 1: D).

Lamina: The lamina of the leaf shows upper epidermis, mesophyll and lower epidermis. Upper epidermis is composed of flat, single layer of rectangular cells. Mesophyll is differentiated into palisade and spongy parenchyma. Palisade tissue lies just internal to upper epidermis. Palisade parenchyma consists of 3-4 layers of cells and occurs as a continuous band. The cells of bundle sheath extension situated above the vascular bundles interrupt the continuity. Spongy parenchyma is present towards the lower epidermis. The cells are more or less isodiametric, loosely arranged with intercellular spaces. Vascular bundles are collateral with mesarch xylem. A layer of bundle sheath made up of parenchyma surrounds the vascular bundle. Lower epidermis consists of flat, single layer of rectangular cells (Plate No. 1: E).

Transverse section of petiole

Transverse section of petiole of *C. tapia* leaf petiole shows single layered, compact epidermis. Epidermis is followed by hypodermis made up of 4-5 layers of collenchymatous cells without intercellular spaces. Just beneath the hypodermis ground tissue is found. It consists of thin walled parenchyma cells. The vascular bundles are found arranged in a complete ring in ground tissue. The vascular bundle consists of xylem and phloem. Sclerenchymatous cells as bundle caps adjacent to the phloem were also observed (Plate No. 1: F).

Transverse section of petiolule

Transverse section of petiolule of *C. tapia* leaf shows two winged projections at the upper side whereas the lower side is round. Epidermis is followed by ground tissue comprising parenchymatus cells. Vascular tissue is in the form of a crescent shaped ring. The inner part of the ring is composed of xylem (towards the upper epidermis) and phloem (towards the lower epidermis). Sclerenchymatous cells as bundle caps adjacent to the phloem were also observed (Plate No. 1: G).

Powder microscopy

Leaf powder is green in colour with characteristic odor and coarse texture.

Powder microscopy of *C. tapia* leaf shows abundant number of anomocytic stomata, spiral thickening, fragments of epidermal layer and parenchymatous cells. It also showed presence of starch grains when stained with iodine (Plate No. 1: J, K).

Quantitative determination

The number of stomata, vein islet number and measurement of stomatal index and size of

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stomata were done with the help of calibrated ocular micrometer and results are tabulated in Table - 1.

Table 1

Quantitative leaf microscopy of Crateava tapia Linn. ssp. odora (Jacob.) Almeida

No.	Parameter	Value
1.	Stomatal index	Lower surface: 18.4 %
		Upper surface: Nil
2.	Stomatal Number	Lower surface: 35-45
		Upper surface : Nil
3.	Veinislet number	06-08
4.	Stomatal size	
	Length (µm)	0.8-1.2
	Breadth (µm)	0.5-0.9

Physico-chemical Parameters

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs [14]. Therefore percentage of the total ash, acid insoluble ash and water soluble ash were carried out. The extraction of any crude drug with a particular solvent yields a solution containing different phyto-constituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction [15]. Loss on drying is the loss of mass expressed as percent w/w [14] results are tabulated in Table -2.

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Table 2

Physico-chemical studies of Crateava tapia Linn. ssp. odora (Jacob.) Almeida leaves

No.	Parameter	Observation
1.	**Ash values**	
	a. Total ash (%)	12.35± 0.50
	b. Acid insoluble ash (%)	0.91±0.38
	c. Water soluble ash (%)	5.45± 0.02
2.	**Extractive values (%)**	
	a. Water soluble	27.80±0.20
	b. Ethanol soluble	19.46±0.46
	c. Methanol soluble	16.26±1.66
	d. Petroleum Ether soluble	9.10±0.41
	e. Benzene soluble	8.60±0.52
3.	**Loss on drying (%)**	71.60±1.44

The leaf powder of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida was treated with various chemical reagents and examined under long UV (254 nm), short UV (366 nm) and visible light. The changes in colour are presented in Table – 3.

Table 3

Fluorescence analysis of Crateava tapia Linn. ssp. odora (Jacob.) Almeida leaves

No.	Treatment	Observation under		
		Ordinary light	UV light	
			254 nm	366 nm
1.	Powder as such	Green	Brown	Brownish Green
2.	Powder + Nitrocellulose	Green	Green	Green
3.	Powder + 1N NaOH in methanol	Dark Green	Brown	Green
4.	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Dark Green	Black	Brown

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5.	Powder + 1N HCl	Brownish green	Black	Brownish Green
6.	Powder + 1N HCl + Nitrocellulose in amyl acetate	Brown	Black	Brownish Red
7.	Powder + 1N NaOH in water	Greenish Brown	Black	Brown
8.	Powder + 1N NaOH in water, dried and mounted in Nitrocellulose in amyl acetate	Greenish Brown	Black	Brownish green
9.	Powder + HNO_3	Reddish Brown	Black	Black
10.	Powder + H_2SO_4	Reddish Black	Black	Greenish Black

The histochemical colour reactions on the leaf were performed for the identification of major cell inclusions. The results are tabulated in Table – 4.

Table 4
Histochemical colour reactions for Crateava tapia Linn. ssp. odora (Jacob.) Almeida leaves.

Reagents	Constituents	Colour	Histochemical Zone		
			Midrib + Lamina	Petiole	Petiolule
Phloroglucinol + Hydrochloric acid	Lignin	Pink	Xylem	Xylem	Xylem
Aqueous Ferric chloride	Tannins	Black	Epidermis	Ground tissue	Collenchyma
Weak Iodine Solution	Starch	Blue	Parenchyma	Parenchyma	Parenchyma
Conc. Hydrochloric Acid	Crystals	No change	-	-	-
Sudan III solution	Oil globules	No change	-	-	-

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Preliminary Phytochemical studies

The leaf powder was extracted with various solvents *viz.* water, methanol, ethanol, petroleum ether and benzene. These extracts were tested for presence of different phytoconstituents.
The results of phytochemical analysis are tabulated in Table – 5

Phytochemical Evaluation

Table 5
Preliminary phytochemical screening of *Crateava tapia* Linn. ssp. *odora* (Jacob.) Almeida Leaves

No.	Tests for Phytoconstituents	WE	EE	ME	PE	BE
1.	Carbohydrate	+	+	+	-	+
2.	Proteins	+	+	+	-	-
3.	Amino acid	+	+	+	-	-
4.	Saponins	-	-	-	-	-
5.	Tannins	+	+	+	-	+
6.	Hydrolysable Tannins	-	-	-	+	+
7.	Flavanoid	+	-	-	-	-
8.	Steroid	-	-	+	+	+
9.	Glycosides	-	-	+	+	+
10.	Cardiac glycosides	+	+	+	-	-
11.	Anthraquinone	-	-	-	-	-

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12.	Volatile oil	-	-	-	-	-
13.	Alkaloid	+	-	+	+	+

WE: Water Extract, EE: Ethanolic Extract, ME: Methanol Extract, PE: Petroleum Ether Extract, BE: Benzene Extract, + : Present, - : Absent

HPTLC fingerprint

A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile, which may be used as marker for quality evaluation and standardization of the drug. R*f* values and the relative percentage of the separated compounds are recorded in Table -6 (Fig 1a, 1b). The R*f* value of 0.35 detected at 300 nm and 600 nm can be used as identifying marker for methanolic extract of *C. tapia*.

Table 6

Rf values and relative percentage of the separated phytoconstituents by HPTLC fingerprint.

No. of Peaks	300 nm		600 nm	
	Max. R*f*	Relative %	Max. R*f*	Relative %
1.	0.20	5.65	0.20	1.87
2.	0.24	4.64	0.24	0.87
3.	0.29	5.42	0.28	0.58
4.	0.32	3.26	0.30	1.85
5.	0.35	0.57	0.35	1.03
6.	0.54	4.24	0.56	8.54
7.	0.59	7.35	0.65	0.85
8.	0.68	4.79	0.67	1.21
9.	0.75	4.87	0.70	1.15

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10.	0.83	9.10	0.82	5.17
11.	0.89	3.03	0.89	8.53

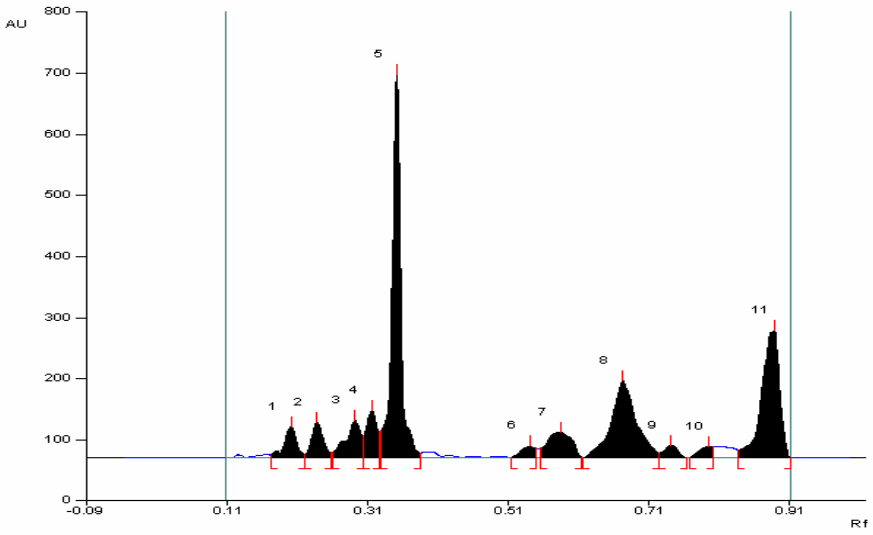

Fig 1a.
HPTLC finger print profile of methanol extract of *Crataeva tapia* Linn. ssp. *odora* (Jacob.) Almeida at 300 nm.

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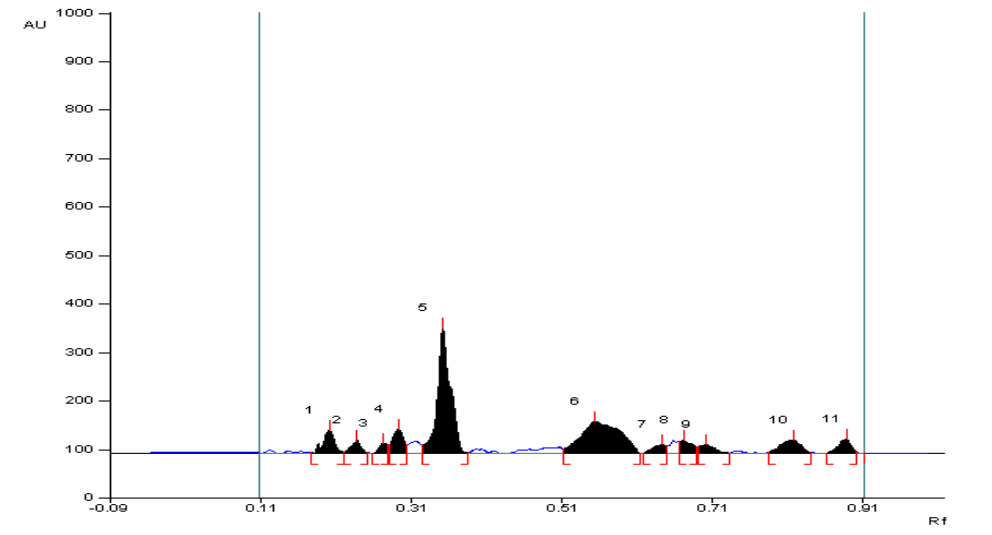

Fig 1b.
HPTLC finger print profile of methanol extract of *Crataeva tapia* Linn. ssp. *odora* (Jacob.) Almeida at 600 nm.

DISCUSSION

Crateava tapia Linn. ssp. *odora* (Jacob.) Almeida is currently being used in the treatment of various disease conditions without standardization. The standardization of a crude drug is an integral part of establishing its correct identity. For inclusion of a crude drug in Pharmacopoeia, pharmacognostic parameters and standards must be established. The results of these investigations could, therefore, serve as a basis for proper identification, collection and investigation of the plant.

As observed in the transverse section of lamina mesophyll cells are distinguishable into upper palisade and lower spongy cells which are the characteristic of dorsiventral leaves. The stomata are present on the lower epidermis only. The anatomical markers of leaf are presence of anomocytic stomata and spiral thickening. The powder microscopy of leaf shows presence of stomata, spiral thickening and parenchymatous cells. Presence of collenchyma and vascular bundle are few of the characteristics of the petiole and petiolule.

Equally important in the evaluation of the crude drugs, is the ash value, water soluble ash value and acid insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign organic matter such as metallic salts and/or silica [20]. The total ash value, water soluble ash value and acid insoluble ash value of *C. tapia* leaf is 12.35 %, 0.91 % and 5.45 % respectively. Since the ash value is constant for the given drug, this value is one of the diagnostic parameter of the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The aqueous extractive value was found to be higher (27.80%) than the other solvents used *viz.* benzene, petroleum ether,

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ethanol and methanol, revealing presence of large amount of water soluble constituents in the leaves. By conventional procedure, loss on drying was performed showing 71.60 % loss on drying.

The fluorescent method is adequately sensitive and enable the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [21]. Kalidas *et al.* [22] suggested that a non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. Therefore, the results obtained from the present fluorescent studies will also help to check any impurities present in leaf powder of *C. tapia.*

The histochemical test reveals presence of lignins, tannin and starch in different cellular components. However, presence of crystals and oil globules were not evident.

Presence or absence of certain important compounds in an extract is determined by colour reaction of the compound with specific chemicals. This procedure is a simple preliminary prerequisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds [24]. Different chemical compounds such as carbohydrate, protein, amino acids, tannin, hydrolysable tannin, flavonoids, steroids, glycosides, cardiac glycosides and alkaloids are detected in *C. tapia* leaf extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs for human use. HPTLC fingerprint profile along with their R*f* values and percentage proportion were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant.

The various morphological, microscopical, physico-chemical and phytochemical standards developed in this study will help for botanical identification and standardization of the drug in crude form.

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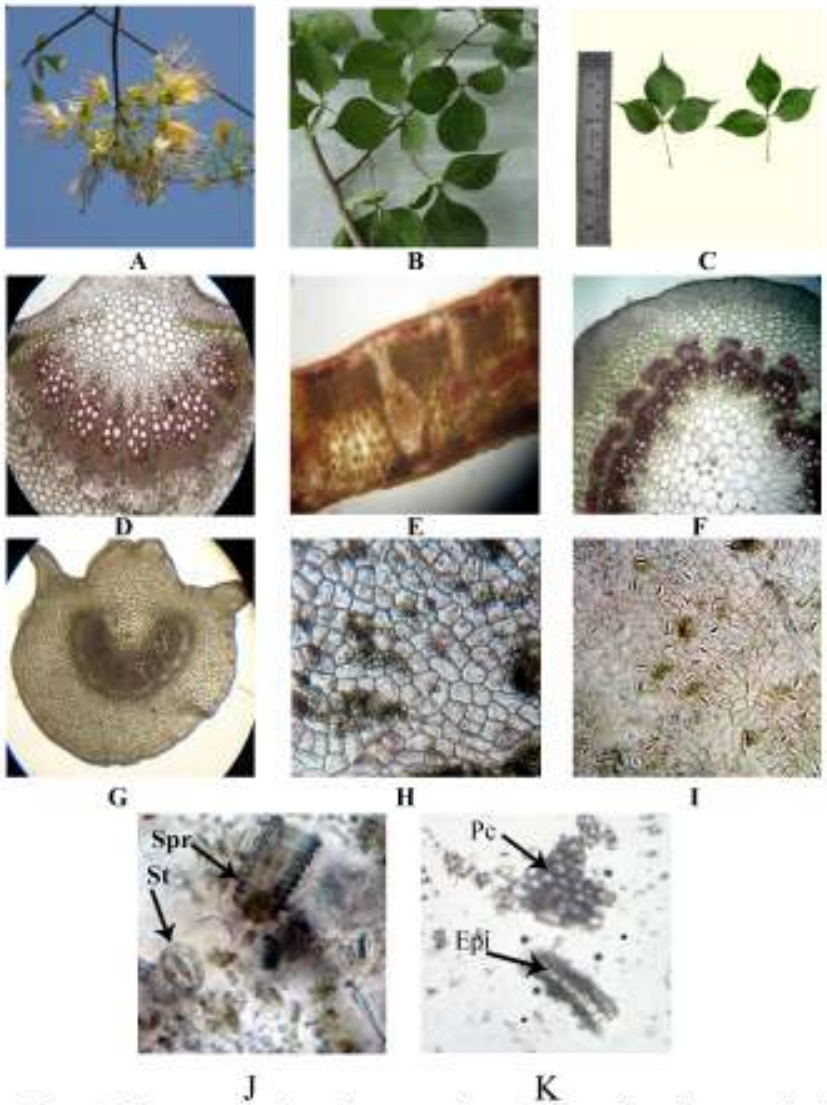

Plate No. 1: Macroscopic, microscopic and powder characteristics of *Crataeva tapia* Linn. ssp. *odora* (Jacob.) Almeida leaves.

A: Flowers, B:Twig, C: Leaves, D:T. S. of midrib, E: T. S. of lamina, F: T. S. of petiole, G: T. S. of petiolule, H: Surface preparation of upper epidermis, I : Surface preparation of lower epidermis showing stomata, J: Powder microscopy showing stomata (St) and spiral thickening (spr), K: Powder microscopy showing parenchyamatous cells (Pc) and epidermal fragment (Epi).

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CONCLUSION

Thus the organoleptic, microscopic characters, physico-chemcial, fluorescence study, preliminary phytochemical screening and HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. The adulterants if any in this plant material can be easily identified by using these results.

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