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Preliminary phytochemical screening and HPTLC finger printing of root extract of *Crataeva magna* LOUR (D.C).

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ABSTRACT

To study the Physico and phyto chemical characters of root bark of *Crataeva magna* Lour DC (family Capparidaceae) and also to carry out the chromatographic fingerprint. Phytochemical investigations and fluorescence analysis were carried out as per the standard techniques. Various quantitative parameters like ash values, extractive values and flavonoid content can be used as quality control parameters for root bark of *Crataeva magna* Lour DC were determined. The root bark powder was extracted with ethanol and fingerprinting pattern was developed by using High Performance Thin Layer Chromatography (HPTLC) technique. CAMAG HPTLC system equipped with pre coated Silica plate of 12cm height using automatic CAMAG Applicator, TLC scanner 3 and CAMAG software were used. Preliminary phytochemical studies confirmed the presence of alkaloid, carbohydrate, glycoside, protein, tannin, flavonoid and phenol. HPTLC finger printing of ethanol extract of root bark revealed 7 peaks with Rf values in the range of 0.5 to 0.89, in which highest concentration of the phytoconstituents was found to be 36.56 % and its corresponding Rf value was found to be 0.89. HPTLC fingerprint analysis of root bark of *Crataeva magna* Lour DC can be used as a diagnostic tool for the correct identification of the plant and it is useful as a Phytochemical marker and also a good estimator of genetic variability in plant populations.

Keywords: Root bark of *Crataeva magna* Lour DC, Phytochemical screening, Physico chemical character, HPTLC fingerprinting.

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INTRODUCTION

Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system, (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some of are still to be explored. Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [1,2].HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time.[3] Thus, HPTLC 'fingerprint analysis' may be a powerful tool for the quality control of raw plant material and may be an alternative technique, particularly in the analysis of crude plant extracts.

Crataeva magna Lour DC (family Capparidaceae) is known as three leaved caper in English, Varuna in Sanskrit and Baruna in Hindi, a small tree with a much branched head, found to be distributed mainly in the warmer (tropical) parts of the world. In folk medicine, its stem pith in the tribal peoples of Kandhamal district of Orissa known as Eastern Ghats of India that the bark is used for lactation after child birth, treat urinary disorders, kidney bladder stones, fever, vomiting and gastric irritation [4, 5, 6]. Leaves are deciduous three foliolate; petioles 3.8–7.6 cm long; leaflets 5–15 ovate, lanceolate or obovate, acute or acuminate, attenuate at the base, entire, glabrous on both surfaces, pale beneath, and reticulately veined[7]. The traditional plant used to treat various ailments in particular to Urolithiasis [8], Hepatoprotective [9], Cardio protective [10], anti arthritic and rubifacient [11-13]. Bark juice of this plant is given orally to prevent childhood diseases among the inhabitants of the Kanyakumari district [14]. The literature revealed that wide variety of medicinally important compounds including friedelin, diosgenin, sitosterol, butulic acid, dodecanoic anhydride, methyl pentacosanoate, kaemferol-3-O-α-D-glucoside and quercitin-3-O-α-D-glucoside have been reported from *C. magna* [15]

In this present study the Preliminary Phytochemical screening of *Crataeva magna* Lour DC root bark extraction has been done to identify the chemical constituents and HPTLC fingerprinting of *Crataeva magna* Lour DC root extract has been performed.

MATERIALS AND METHODS

Root bark of *Crataeva magna* Lour DC were collected in and around local forest area of Kanyakumari, Tamilnadu and authenticated by the Botanist Prof.Chelladurai, Department of Botany, Govt. Siddha Medical College, Tirunelveli. A voucher herbarium specimen number KMCP/CM/01/2015 was also preserved in the K.M.College of Pharmacy, Madurai.

Preparation and Extraction of Plant material

The root bark is collected were subjected to dried in shade and then coarsely powdered. The 800 gms of powdered root bark of *Crataeva magna* Lour DC were defatted with petroleum ether and extracted successively with chloroform and ethanol using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extract was filtered through a cotton plug, followed by whattman filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator.

PHYSICOCHEMICAL PARAMETERS

Physico chemical constants such as percentage of total ash, acid insoluble ash, water soluble ash, water soluble and alcohol soluble extractive values were calculated according to the described methods [16].Fluorescence analysis was conducted by using methods [17,18].

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DETERMINATION OF TOTAL FLAVONOID CONTENT [19]

Total flavonoids were estimated using the prescribed method. To 0.5ml of sample, 0.5ml of 2% AlCl3 ethanol solution was added. After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presences of flavonoid. Total flavonoid content were calculated as quercetin equivalent (mg/g) using the regression equation of the calibration curve: y = 0.41x + 0.087, R2 = 0.425

PHYTOCHEMICAL SCREENING

The Phytochemical investigation of the root bark of *Crataeva magna* Lour DC was carried out with standard protocol [20]. The extraction of plants material was carried out with petroleum ether, chloroform and ethanol. The results were presented in Table 1.

Table 1: Preliminary Phytochemical screening of different extracts of *Crataeva magna* Lour DC

Phytochemical Tests	Root bark of *Crataeva magna* Lour DC extracts		
	Petroleum ether	Chloroform	Ethanol
Test for Phytosterols			
a)Salkowski test	+	+	+
b) Liebermann-burchard test	+	+	-
Test for Glycosides			
a) Legal test	-	-	-
b) Keller killani test	-	-	-
c) Modified Borntrager's test	-	+	+
Test for Saponins	-	-	-
a) Foam test	-	+	-
b) Haemolysis test			
Test for Oils and fats	+	+	+
Stain test			
Test for Resins	-	-	-
Acetone water test			
Test for Phenols	+	+	+
Phenol test			
Test for Tannins	+	-	+
a) 5% Ferric chloride test	+	+	+
b) Gelatin test	+	-	+
c) Potassium dichromate test			
Test for Protein	+	+	+
a) Biuret test	+	+	+
b) Million reagent test			
Test for Flavonoid	+	+	+
a) Shinoda test	+	+	+
b) Zinc/Hcl reduction test			
Test for Carbohydrates	-	+	+
a) Molisch's test	-	-	+
b) Barfoed's test	-	-	-
c) Benedicts test	-	+	+
d) Fehling solution test			
Test for Coumarins	-	+	-
Test for Amino acid	+	+	+
Ninhydrin test			

HPTLC PROFILE (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

HPTLC studies were carried out following the method of Harborne [21] and Wagner [22] *et al.,*

Sample preparation

Ethanol extract was evaporated under reduced pressure using rotovac evaporator. 20 micro liters of the extract was taken in the CAMAG syringe. The extract in the syringe was applied in the Pre coated Silica plate of 12cm height using automatic CAMAG Applicator.

Developing solvent system

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent Ethyl acetate: Hexane; formic acid (7:3:0.5).

Sample application

Application of bands of extract was carried out (Plate Height: 12 cm; width: 3cm**)** using spray technique. Sample was applied in duplicate on Aluminum coated Silica Gel – MerkF254 with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through CAMAG software.

Development of chromatogram

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x 10 cm saturated with solvent Ethyl acetate: Hexane; formic acid (7:3;0.5) for 15 min.

Detection of spots

The air-dried plates were viewed in ultraviolet radiation to mid day light. Figure 1.The chromatograms were scanned by densitometer at 200- 400 nm after spraying with anisaldehyde sulphuric acid The Rf values and finger print data were recorded by CAMAG software.

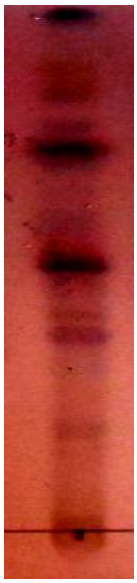

Fig 1: HPTLC plate seen at 400nm for ethanolic extract *Crataeva magna* Lour DC

RESULTS AND DISCUSSION

The Phytochemical test on petroleum ether, chloroform and ethanolic extracts of *Crataeva magna* Lour DC showed the presence of various Phytoconstituents like alkaloid, carbohydrate, tannin, Saponin, terpenoid, flavonoid, Coumarin and phenol are present .Table 1.

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. Therefore percentage of the total ash, acid insoluble ash and water soluble ash were carried out. The determinations such as loss on drying and ash values indicate the status of air-dried drugs used for studies. The total ash values when comes in acceptable range it simply shows that no inorganic adulteration is present. 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. Total ash value, acid insoluble ash and water-soluble ash were determined and results were in acceptable limits. Ash value, Extractive value and Loss on drying is the loss of mass expressed as percent w/w results are tabulated in Table 2.

Table 2: Physico-chemical studies of root bark of *Crataeva magna* Lour DC

S.NO	PARAMETER	OBSERVATION
1	**Ash values**	
	Total ash (%)	11.32±0.80
	Acid insoluble ash (%)	1.01±0.67
	Water soluble ash (%)	4.34±0.23
2	**Extractive values (%)**	
	Ethanol soluble	19.46±0.46
	Chloroform soluble	15.67±0.83
	Petroleum ether soluble	10.08±0.45
3	**Loss on drying (%)**	73.46±2.34

The powdered root bark of *Crataeva magna* Lour DC 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. The results obtained were comparable and satisfied the standard literature.

Table 3: Fluorescence analysis of root bark of *Crataeva magna* Lour DC

S.NO	PARAMETER	OBSERVATION		
		Ordinary light	**UV light**	
			254nm	**366nm**
1	Powder as such	Brown	Brown	Brownish green
2	Powder+ Nitrocellulose	Brown	Brown	Brown
3	Powder+1N NaOH in methanol	Dark green	Brown	Green
4	Powder+1N NaOH in methanol+ Nitrocellulose in amyl acetate	Brown	Black	Green
5	Powder+1NHCl	Brownish green	Black	Brownish green
6	Powder+1NHCl+ Nitrocellulose in amyl acetate	Black	Brown	Brownish red
7	Powder+1N NaOH in water	Greenish brown	Black	Green
8	Powder+1N NaOH in water dried and mounted in Nitrocellulose in amyl acetate	Greenish brown	Black	Reddish brown
9	Powder+HNO_3	Reddish brown	Brown	Reddish black
10	Powder+H_2SO_4	Dark brown	Reddish black	Black

The powdered root bark of *Crataeva magna* Lour DC flavonoid content for petroleum ether, chloroform and ethanol extract were found and in the ethanolic extract only significantly higher ($p<0.001$) depicts in the Table 4.

Table 4: Total flavonoid content extract of root bark of *Crataeva magna* Lour DC

Extract	Total Flavonoid content(mg of quercetin/g)
Ethanolic extract	6.2352 mg/g
Chloroform extract	3.0423 mg/g
Petroleum ether extract	9.251 mg/g

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Ethanolic root extract of *Crataeva magna* Lour DC showed there are nine polyvalent Phytoconstituents and corresponding ascending order of Rf values start from 0.5 to 0.89 in which highest concentration of the Phytoconstituents was found to be 36.56 % and its corresponding Rf value was found to be 0.89 respectively and was recorded in Table 5 & 6. The corresponding HPTLC chromatogram was presented in Figure 2.

Table 5: HPTLC profile of the ethanolic extract of *Crataeva magna* Lour DC

Lane Data for Plate							
ID	**X Coordinate**	**Y Coordinate**	**Width**	**Height**	**Number of Anchors**	**Number of Bands**	**Notes**
1	2197	400	253	2616	1	6	
Band Data for Plate							
ID	**Rf**	**Top Coordinate**	**Bottom Coordinate**	**Area**	**Volume (Scaled)**	**Volume (Real)**	**Notes**
1	0.89	533	823	73370	2706.9	270689001	
2	0.658	1192	1357	41745	116.92	11691636	
3	0.574	1417	1568	38203	882.71	88271447	
4	0.54	1577	1657	20240	366.08	36607582	
5	0.5	1677	1784	27071	181.03	18102656	

Table 6: HPTLC profile of the ethanolic extract of *Crataeva magna* Lour DC

Rf	Area	Area Percentage
0.89	73370	36.56%
0.65	41745	29.80%
0.57	38203	19.04%
0.54	20240	10.08%
0.5	27071	13.49%

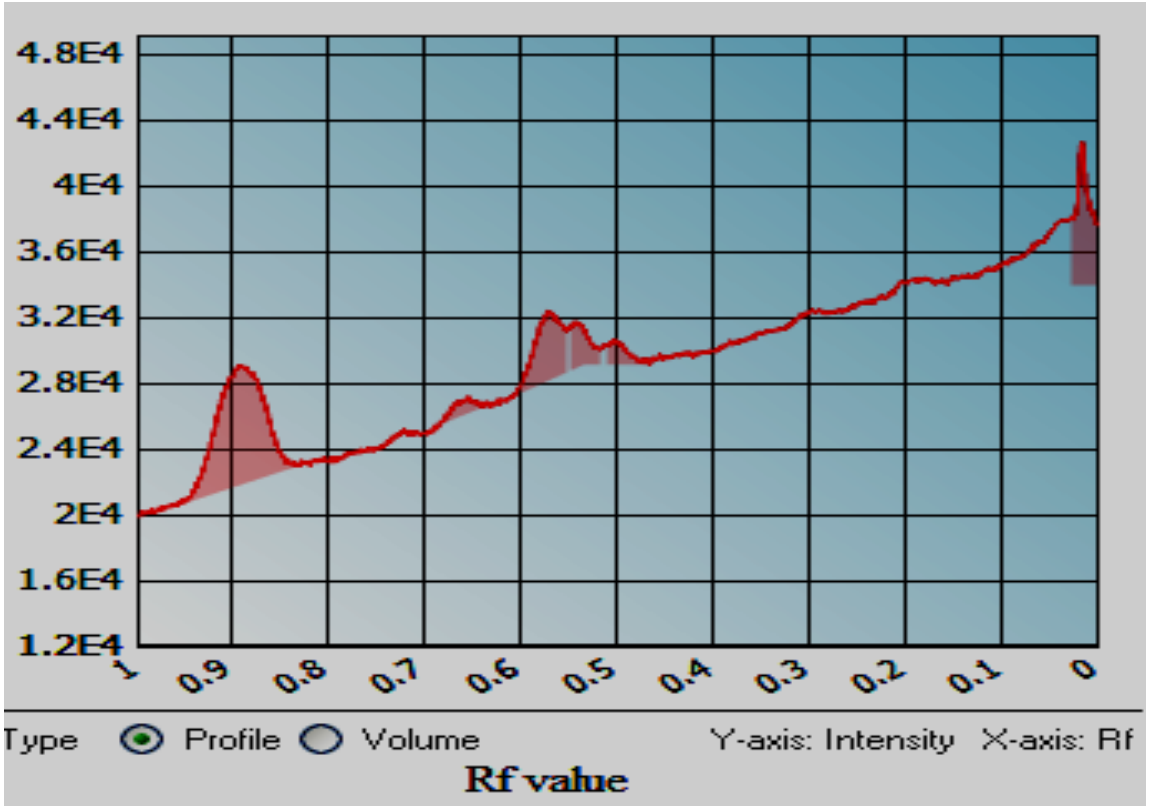

Fig 2: HPTLC Chromatogram of *Crataeva magna* Lour DC ethanolic root extract showing different peaks of phytoconstituents.

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CONCLUSION

Thus the physicochemical, fluorescence study, preliminary phytochemical screening and HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. The ethanolic root extract of *Crataeva magna* Lour DC has a significant flavonoid content it is useful for further antioxidant and anticancer studies. The adulterants if any in this plant material can be easily identified by using these results.

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