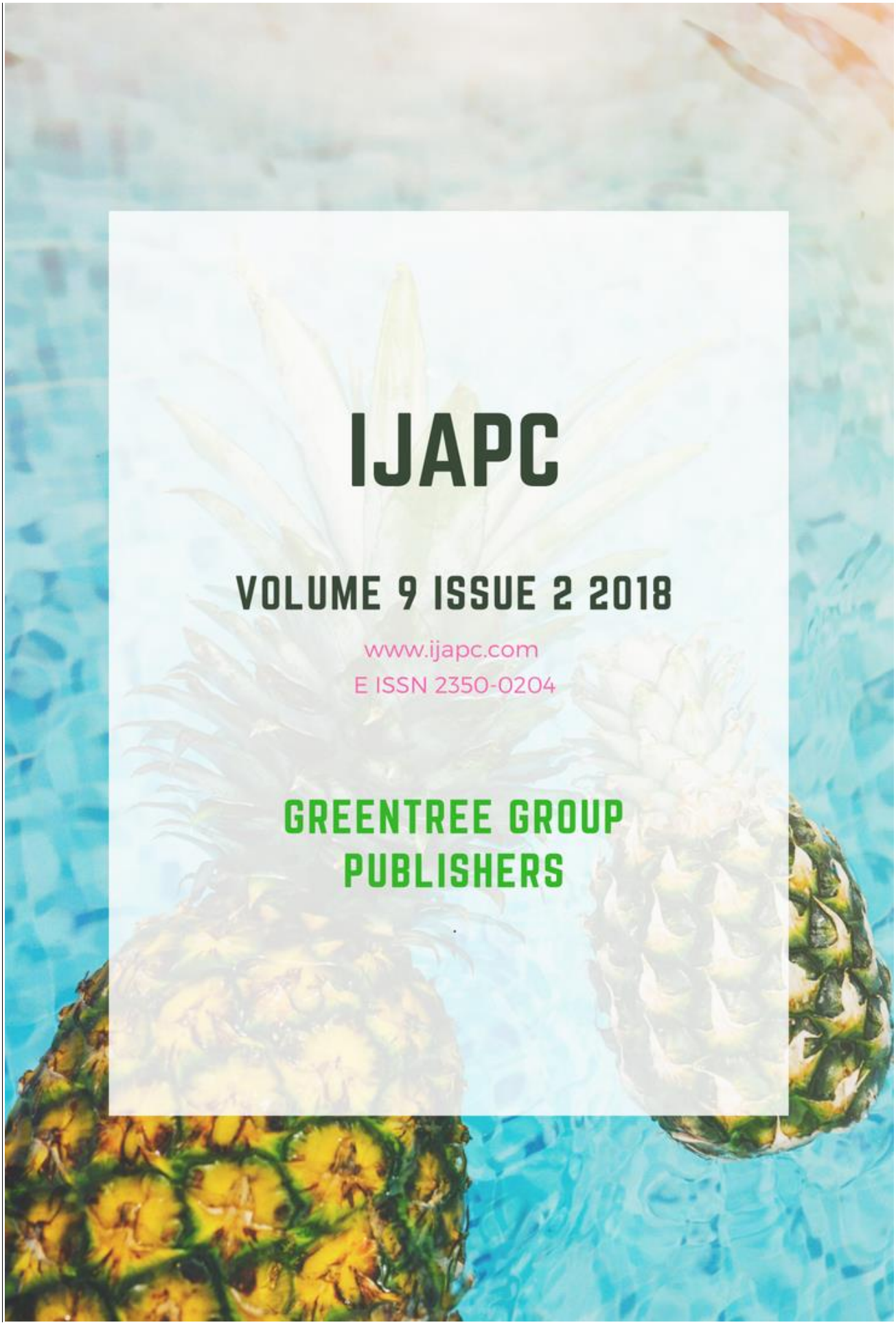

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Pharmacognostic and Chromatographic evaluation of *Ficus glomerata* Roxb (Stem bark)

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ABSTRACT

Udumbara (*Ficus glomerata* Roxb.) is a moderate sized tree, which is found all over India. The plant is mainly used for external application in burns, and internally in skin inflammation diarrohea and leucoderma. *Udumbara* (*Ficus glomerata* Roxb.) sample which was used for the study was collected from natural habitats in Hassan. In this study preliminary pharmacognostic, phytochemical evaluation and HPTLC analysis of sample of *Ficus glomerata* Roxb were done. The study revealed presence of alkaloids, steroid, tannins, saponin,flavanoids, carbohydrate, phenols. These secondary metabolites are responsible for its pharmacological actions.

KEYWORDS

Udumbara (*Ficus glomerata* Roxb.), Pharmacognistic evaluation, HPTLC, Pharmacological actions

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INTRODUCTION

Ficus glomerata Roxb. (Family – Moraceae) is also known as Cluster fig, it is named as *Udumbara* or *Oudumbara* in Ayurveda. The stem bark, leaves and fruits are used parts in Ayurveda. Stem bark of *Udumbara* is one of the constituents of *Panchavalkala Kwatha* which is commonly practiced in Ayurveda for *Vrana prakshalana* (cleaning of wound). Stem bark is also used as *Churna* (powder), *Kalka* (paste) and *Ghrita*(medicated ghee) [1-3]. Current researches have shown wide range of pharmacological activities including

anti-diabetic, anti-ulcer, hepatoprotective, anti-diarrheal, anti-bacterial and anti-inflammatory, analgesic and larvicidal activities [1].

Properties and uses of *Ficus glomerata* Roxb. [4]

The roots are useful in treating dysentery. The bark is astringent, anti-diabetic, refrigerant, and useful for washing wounds. It is highly efficacious in threatened abortion and also recommended in Uropathy. A decoction of the leaves, is a good for washing the wounds and ulcers. Tender fruits are used in diarrhoea, dyspepsia and haemorrhages. The ripen fruit are astringent, stomachic, refrigerant, carminative and are useful in menorrhagia and haemopysis. The latex is aphrodisiac and is administered in haemorrhoids and diarrhoea.

The present study is focused on pharmacognostic evaluation of stem bark of *Udumbara* (*Ficus glomerata* Roxb.) for determining the phytoconstituents and HPTLC fingerprinting.

MATERIALS AND METHODS

Collection and Authentication:

The bark of the drug *Udumbara* (*Ficus glomerata* Roxb.) was collected from the natural habitat of Hassan, Karnataka. The period of collection was the month of October -2017. The drug authentication was done by Department of Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan (Authentication no. SDMCAH-DG/2017/18, dated 15-06-2017). The obtained bark was cut into pieces of variable length and diameter. They were kept for drying approximately for a period of seven days under sunlight.

Pharmacognostical Standardization-

Organoleptic evaluation

The freshly peeled and dried stem bark of *Udumbara (Ficus glomerata* Roxb.*)* were spread on clean white paper sheet and investigated for different organoleptic features like, thickness, colour, taste, odour,

and fracture by repeated observations using magnifying lens (where necessary) and where recorded.

Powder microscopy

Fine powder of bark of *Udumbara* (*Ficus glomerata* Roxb.) was prepared in *Khalwa yantra* and a pinch of the sample was mounted on a microscopic slide with a drop of glycerin-water. Characters were observed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the pre-calibrated scale-bars using Zeiss Axio Vision software**.**

Physiochemical analysis-

The physicochemical parameters like loss on drying, total ash, acid-insoluble ash, alcohol and water-soluble extractive values were carried out as per the standard procedures mentioned in API (Ayurvedic pharmacopoeia of India).

Preparation of water and alcohol extract[5]-

Water extract and alcohol extract of both the samples were extracted by cold maceration method. For preparation of water extract, 4gm of coarse powder of the sample was taken and kept in conical flask having 100 ml of water and frequently shaken for 8 hrs and left for 16hrs then filtered and filtrate were vaporized on water-bath and then extract was collected. Similar process was done for alcoholic extract but instead of 100 ml of water, 100ml of ethanol was used.

Preliminary phytochemical tests[5]

Water and alcoholic extracts of *Udumbara* (*Ficus glomerata* Roxb.) were subjected to qualitative evaluation for the presence or absence of different groups of phytoconstituents such as alkaloids, flavonoids, saponins, carbohydrates, carboxylic acid, coumarins, phenol, quinine, resins, steroid, tannin, terpenoid, and amino acids according to standard procedures.

HPTLC

Udumbara (Ficus glomerata Roxb) raw drug powder 1gm, was extracted with 10 ml of ethanol. 4, 8 and 12µl of the above extract was applied on a pre-coated silica gel F 254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (12:1). The developed plates were visualized under short UV, long UV and then derivatised with vanillin sulphuric acid and scanned under UV 254nm, 366nm and 620nm. R_f, colour of the spots and densitometric scan were recorded.

RESULT AND DISCUSSION

The standardization of a crude drug is an integral part for establishing its correct

identity. Organoleptic evaluation, macroscopic and microscopic evaluations, physiochemical and phytochemical evaluations along with chromatographic studies are part of pharmacognostic standardization and quality control of herbal drugs. The macroscopic / organoleptic and microscopic study of a medicinal plant is the first step towards establishing its identity and purity. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of crude herbal drugs and these are the useful diagnostic criteria for identification and standardization. Physical parameters such as, foreign matters, ash values and extractive values can be used as reliable aid for detecting adulteration in drugs. Ash values of drug give an idea of inorganic composition of crude drugs and adulteration of earthy materials and other inorganic impurities. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. It helps to evaluate the chemical constituents present in the crude drug and also help in estimation of specific phytoconstituents soluble in particular solvents[6].

In present study organoleptic/macroscopic evaluation was done for determining its standardization. The results of organoleptic/macroscopic are mentioned in Table 1.

Table 1 Organoleptic Evaluation of *Ficus glomerata* Roxb. stem bark

Bark of *Udumbara(Ficus glomerata* Roxb.*)*		
Characters	**Fresh**	**Dry**
Condition	Moist, soft, sticky,thick,fissured. When wounded bark exudates gum	Hard and contracted
Shape of Bark	Slightly curved but not contracted	Recurved and channelled
Thickness	Varies,0.8-1.4cm	Varies,0.6-1.5cm
Colour	Outer surface pale greenish white flakes, Inner surface light brown	Outer surface;blackish brown inner surface; brownish buff coloured
Surface	Outer surface of the bark had scattered lenticels and small and large scars left by the prickles and branches. Outer surface was marked by wavy longitudinal striations; inner surface also had longitudinal striations. The cork was found frequently exfoliated	Outer surface of the bark had scattered lenticels and small and large scars left by the prickles and branches. Outer surface was marked by wavy longitudinal striations; inner surface also had longitudinal striations. The cork was found frequently exfoliated
Taste	Bitter astringent	Bitter astringent
Odour	Odourless	Slight woody odour
Fracture	Fibrous	Splinter

For microscopic evaluation powder microscopy was done which showed the presence of Parenchyma cells with cell

content and with thick walls, fibres, calcium oxalate crystals, latex containing cells with granular content, stone cells, sclereids, starch grain and tannin containing cells (Plate 1).

Fig. 7 Latex containg cells with granular

Fig. 8 Stone cells

Fig. 9 Sclereids

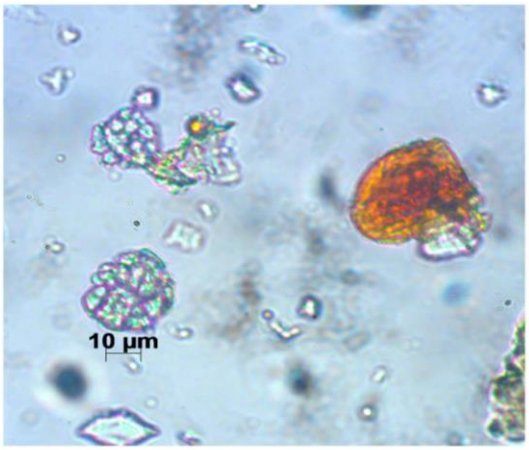


Fig. 10 Starch grain and tannin containing

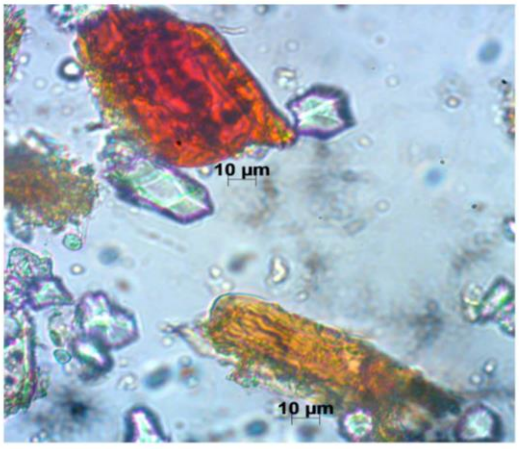


Fig. 11 Cells with content

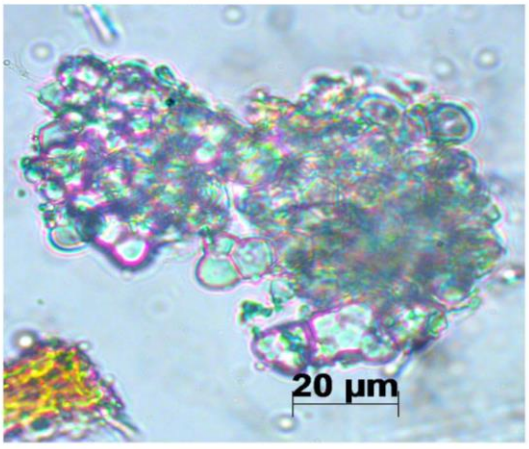


Fig. 12 Bunch of starch grains

Plate 1 (Figs 1-12) Powder microscopy of bark of *Udumbara* (Ficus glomerata Roxb.)

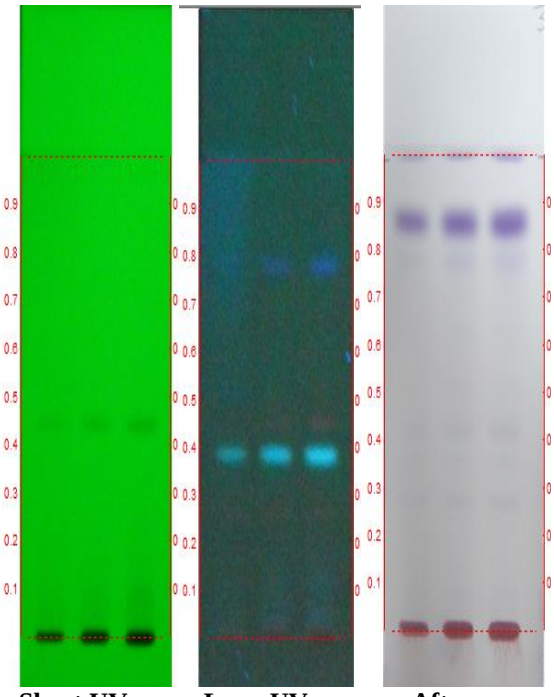
Physiochemical reference values for bark aren't mentioned in Ayurvedic pharmacopoeia of India, so the obtained value mentioned in Table 2, may be considered as standard.

Table 2 Physiochemical analysis of *Udumbara (Ficus glomerata* Roxb.*)*

Physio-chemicalanalysis of *Udumbara* (*Ficus glomerata* Roxb.) Bark	
Parameter	**Results n = 3%w/w**
Loss on drying	16.21
Total Ash	17.17
Acid Insoluble Ash	0.80
Water soluble Ash	5.85
Alcohol soluble extractive value	3.73
Water soluble extractive value	6.91

In present study, the stem bark of *Udumbara (Ficus glomerata* Roxb.), alcoholic extract showed the presence of steroids, carbohydrate, tannins, flavanoids, saponins, terpenoids, phenols and resins. Alkaloids, carbohydrates, tannins, saponins, phenols, resin and quinone are present in water extract (Table 3 and Table 4.). There are variations of presence of phytoconstituents between alcoholic extract and aqueous extract due to solubility of phytoconstituents in appropriate media. The phytoconstituents found in *Udumbara* bark, are responsible for its different pharmacological activities like wound healing, anti-inflammatory, analgesic, anti-diabetic, antifungal and others[7,8].

Photo documentation of HPTLC (Plate 2 and Table 5) on short wave revealed that 2 bands having light to deep green colour of R_f value 0.08 and 0.43 while under long U.V florescent red and florescent blue bands were visualized having R_f value of 0.28, 0.38, 0.46 and 0.78 after derivatisation five bands were visualized having light purple to deep purple colour of R_f value 0.28,0.36,0.51, 0.78,and 0.80. R_f values of 0.28 and 0.78 are common in both under long U.V and after derivatisation.



Short UV **Long UV** **After derivatisation**

Track 1-***Udumbara (Ficus glomerata* Roxb.*) raw drug***– 4µl

Track 2- ***Udumbara (Ficus glomerata* Roxb.*) raw drug***– 8µl

Track 3-***Udumbara (Ficus glomerata* Roxb.*) raw drug***– 12µl

Solvent system – **Toluene: Ethayl acetate (12.0: 1.0)Plate 2** HPTLC photo documentation of ethanolic extract of *Udumbara (Ficusglomerata*Roxb.) raw drug

Table 3 Phytochemical analysis of *Udumbar (Ficus glomerata* Roxb.*)* alcoholic extract and aqueous extract

Sl No	Tests	Colour if positive	*Udumbara (Ficusglomerata*Roxb.*)* alcoholic extract	*Udumbara (Ficusglomerata*Roxb.*)* aqueous extract
1.	**Alkaloids**			
	Dragendrof's test	Orange precipitate	Orange colour	Orange colour
	Wagner's test	Red precipitate	Red colour	Red colour
	Mayer's test	Dull white precipitate	Brown colour	Brown colour
	Hager's test	Yellow precipitate	Brown colour	Brown colour
2.	**Steroids**			
	Liebermann-buchard test	Bluish green	Bluish green	Brown colour
	Salkowski test	Bluish red to cherry red	Bluish red to cherry red	Brown colour
3.	**Carbohydrate**			
	Molish test	Violet ring	Violet ring	Violet ring
	Fehling's test	Brick red precipitate	Ink blue solution	Ink blue solution
	Benedict's test	Red precipitate	Dark green solution	Dark green solution
4.	**Tannin**			
	With $FeCl_3$	Dark blue or green or brown	Brown colour	Brown colour
5.	**Flavanoids**			
	Shinoda's test	Red to pink	Pink colour	Brown colour
6.	**Saponins**			
	With $NaHCO_3$	Stable froth	Stable froth	Stable froth
7.	**Triterpenoids**			
	Tin and thionyl chloride test	Red	Red colour	Brown colour
8.	**Coumarins**			
	With 2 N NaOH	Yellow	Brown colour	Red colour
9.	**Phenols**			
	With alcoholic ferric chloride	Blue to blue black, brown	Brown colour	Brown colour
10.	**Carboxylic acid**			
	With water and $NaHCO_3$	Brisk effervescence	No effervescence	No effervescence
11.	**Resin**			
	With aqueous acetone	Turbidity	Turbidity	Turbidity
12.	**Quinone**			
	5% NaOH	Pink/purple/red	Brownish green	Red colour
13.	**Amino acids**			
	Ninhydrine reagent	Purple colour	Brown solution	Pink colour

HPTLC densitometric study revealed (Plate 3) that there are 12 peaks at 254 nm and one peak at 366 nm. These peaks showed 12 phytoconstituents among which R_f 0.03 occupied the maximum percentage (50.76% under 254 nm and 100% 366nm) while R_f0.51 (22.12%) occupied the second position, while the least percentage of phytoconstituents is R_f0.05 occupying 1.60% under 254 nm (Fig. 12a and 12b). This study revealed the major phytoconstituents of *Udumbara*(*Ficusglomerata*Roxb.) bark is of 0.03 R_f value. According to previous study,Friedelin and leupol are the major chemical constituents found in bark of *Udumbara* (*Ficusglomerata*Roxb.)[9].

Table 4 Result of phytochemical analysis of *Udumbara (Ficus glomerata* Roxb.*)* alcoholic extract and aqueous extract

Test	***Udumbara (Ficus glomerata* Roxb.) alcoholic extract**	***Udumbara (Ficus glomerata* Roxb.) aqueous extract**
Alkaloid	-	+
Steroid	+	-
Carbohydrate	+	+
Tannin	+	+
Flavanoids	+	-
Saponins	+	+
Terpenoid	+	-
Coumarins	-	-
Phenol	+	+
Carboxylic acid	-	-
Resins	+	+
Quinone	-	+
Amino acids	-	-

Table 5 R_f values of sample of *Udumbara*(*Ficus glomerata* Roxb.) raw drug

Short UV	**Long UV**	**After derivatisation**
0.08 (L. green)	-	-
-	0.28 (F. red)	0.28 (L. purple)
-	-	0.36 (L. purple)
-	0.38 (F aqua. blue)	-
0.43 (D. green)	-	0.43 (L. purple)
-	0.46 (F. red)	-
-	-	0.51 (L. purple)
-	0.78 (F. blue)	0.78 (L. purple)
-	-	0.86 (D. purple)

***F –fluorescent; D – dark; L - light**

CONCLUSION

Udumbara (*Ficus glomerata* Roxb.) which belongs to moraceae is a well known medicinal plant used in Ayurveda and in folklore practices. The present study revealed that the bark of *Udumbara* contains alkaloids, flavonoids, tannins, steroids, saponins, carbohydrate, phenols, terpenoids and quinone as active phytoconstituents and HPTLC study revealed the presence of 12 phytoconstituents according to peak

observed, among which R_f0.03 is the major phytoconstituents occupying the maximum percentage.

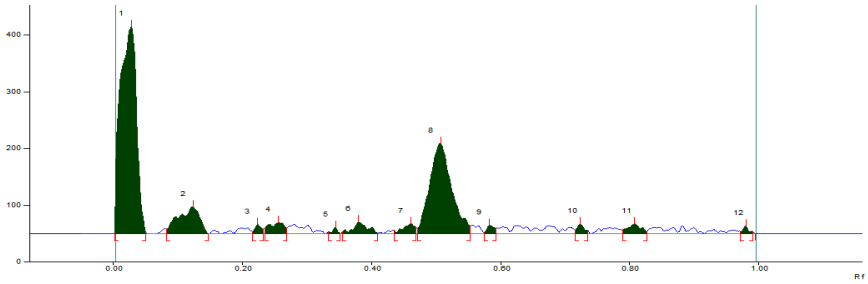
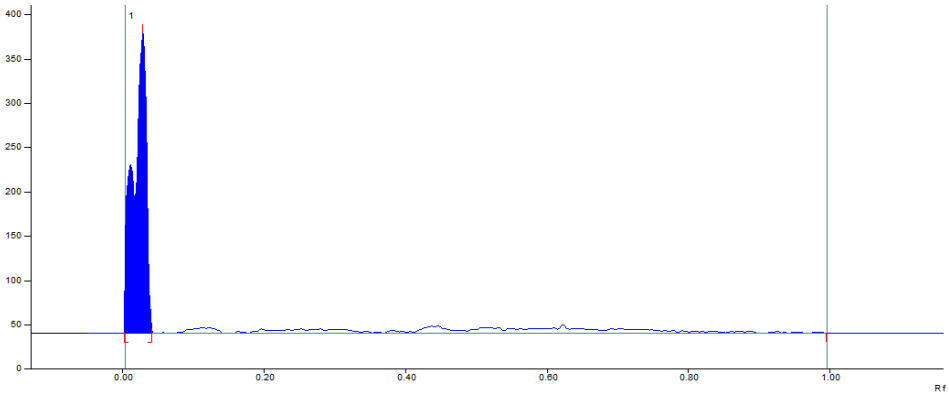


Fig. 1 Peaks observed

Track 3, ID: Udumbara raw drug

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	364.4 AU	50.76 %	0.05 Rf	2.0 AU	6556.2 AU	49.65 %
2	0.08 Rf	8.5 AU	0.12 Rf	47.4 AU	6.60 %	0.15 Rf	0.1 AU	1107.5 AU	8.39 %
3	0.22 Rf	3.4 AU	0.22 Rf	16.1 AU	2.25 %	0.23 Rf	8.4 AU	120.8 AU	0.91 %
4	0.24 Rf	9.6 AU	0.26 Rf	19.5 AU	2.71 %	0.27 Rf	10.0 AU	337.0 AU	2.55 %
5	0.33 Rf	3.7 AU	0.35 Rf	11.5 AU	1.60 %	0.35 Rf	0.6 AU	60.7 AU	0.46 %
6	0.36 Rf	3.7 AU	0.38 Rf	20.5 AU	2.86 %	0.41 Rf	1.2 AU	327.3 AU	2.48 %
7	0.44 Rf	6.5 AU	0.46 Rf	16.8 AU	2.34 %	0.47 Rf	11.9 AU	229.3 AU	1.74 %
8	0.47 Rf	9.3 AU	0.51 Rf	158.8 AU	22.12 %	0.55 Rf	13.5 AU	3852.1 AU	29.17 %
9	0.58 Rf	2.0 AU	0.58 Rf	14.4 AU	2.01 %	0.59 Rf	9.4 AU	123.2 AU	0.93 %
10	0.72 Rf	6.2 AU	0.72 Rf	17.0 AU	2.36 %	0.74 Rf	5.8 AU	141.4 AU	1.07 %
11	0.79 Rf	7.2 AU	0.81 Rf	17.9 AU	2.49 %	0.83 Rf	3.7 AU	261.2 AU	1.98 %
12	0.97 Rf	4.1 AU	0.98 Rf	13.6 AU	1.90 %	0.99 Rf	3.6 AU	87.9 AU	0.67 %

Fig. 2a At 254nm



Track 3, ID: Udumbara raw drug

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.03 Rf	338.7 AU	100.00 %	0.04 Rf	4.8 AU	4498.1 AU	100.00 %

Fig. 2b At 366nm

Plate 3 (Figures 1, 2a and 2b) Densitometric scan of *Udumbara(Ficusglomerata*Roxb.*) raw drug*

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