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Research Article

STANDARDIZATION OF *KADAMBA* (*ANTHOCEPHALUS CADAMBA(ROXB.)MIQ* LEAF W.S.R. TO ITS PHARMACOGNOSTICAL EVALUATION

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KEYWORDS: *Kadamba* leaf, *Anthocephalus cadamba* (Roxb.) Miq, Standardisation, Pharmocognostical evaluation.

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ABSTRACT

In today's world, the demand for herbal products is growing exponentially throughout the world due their potentiality and less side effects. Major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. Growing demand, the market value and the lack of quality of raw materials which affects the safety and efficacy created the need for standardization and critical evaluation of the herbal drugs. *Kadamba* is one of the important plant which is possessing many of medicinal properties. It *is* useful in disorders like *Medoroga, Prameha, Graharogas, Vrana, Atisara, Grahani, Ashmari, Mutrakrchra* etc. This article highlights the importance of standardization of *Kadamba* (*Anthocephalus Cadamba* (Roxb.) Miq) leaf considering Pharmacognostical evaluation including its Macroscopic identification, Microscopic, Organoleptic characters, Powder microscopy, Physicochemical analysis, Extractive values in solvents like aqueous, Ethanol, Methanol, Chloroform, Phytochemical analysis in various solvents, TLC and HPTLC for Aqueous and Alcohol extracts are done in Telangana State Level Drug Testing Laboratory and Dravyaguna department Dr.BRKR Govt Ayurvedic college, Hyderabad as per the guidelines laid down in Ayurvedic Pharmacopoeia of India. This study of *Kadamba* leaf can serve as a preliminary step towards its standardization emphasizing the need of further research.

INTRODUCTION

Kadamba is one of the important plants which possesses many of medicinal Properties and is considered as a mythic tree and explained in various Ayurvedic classics. Botanically it was identified as *Anthocephalus cadamba* (Roxb.) Miq belongs to Rubiaceae family. Its fruit and inflorescence reported as edible. Its flowers are also used in perfumes and its wood is useful in timber and paper making apart from its medicinal uses. The tree is frequently found in moist and warm type of deciduous and evergreen forests. It is found in the sub-Himalayan tract from Nepal eastwards on the lower hills of Darjeeling terai in West Bengal, in Karnataka, Kerala (on the west coasts) and in Western Ghats. In the Andamans, it is very common in damp places along the large steams. It is also frequently cultivated for ornament, and as a shade tree in plantations throughout the country.[1]

It is mentioned as one of the drug in *Charakasamhita Sukrasodhana* and *Vedanasthapana dasemani*[2] and in *Susruta Samhita Lodhradigana* and *Nyagrodhadigana.*[3] It was mentioned in *Amradivarga* in *Dhanvantarinighantu*[4] and *Sodhala nighantu;*[5] *Prabhadradi Varga in Raja Nighantu;*[6] *Oshadi Varga in Kaiyadevanighantu;*[7] *Guduchyadi Varga* in *Bhava prakashanighantu.*[8] In *Vrndha madhava Kadamba* is indicated in the diseases of - *Kaphajaprameha, Kushta, Gulma, Sotha, Vatarakta, Pleehodara, Arshas, Vidradhi, Apasmara, Unmada, Pitta-Raktaja Upadamsa, Utpata.* In *Chakradatta* it was indicated in *Gulma, Udara, Asthila, Kushta, Prameha, Sotha, Vatarakta, Pleehodara, Arshas, Vidradhi, Apasmara, Unmada Pramehapidaka, Pitta-*

Raktaja Upadamsa.[9] In *Bhaishajyaratnavali* it is indicated in various diseases like *Mandagni, Sula, Jwara, Chardi, Masurika, Swasa, Daha, Pliha-Yakrtrogas*, *Yakshma, Twakroga.*[10]

Kadamba possess *Tikta*, *Kashaya rasa*, *Rukshaguna*, *Sitavirya* and *Katuvipaka.*[6] It is having properties of *Kapha-vatahara* and *Medohara* and *Prabhava* as *Vedanasthapana. Kadamba* has some proven experimental research activities like its hypolipidaemic activity, Hypoglycemic activity, Anti-Hypercholesteremic activity, Anti-Dyslipidemic activity, Anti Inflammatory activity and Anti-Oxidant activity.

Growing demand of herbal products, increase of the market value, adulteration and mainly the lack of quality of raw materials which affects the safety and efficacy created the need for standardization and critical evaluation of the herbal drugs.

Aim and Objective

1. To standardize *Kadamba* (*Anthocephalus Cadamba* (Roxb.) Miq) Leaf.
2. To carry out Pharmacognostical study including Macroscopic & Microscopic examination, Organoleptic study, Powder microscopy, Physicochemical analysis and Phytochemical analysis, TLC, HPTLC study of *Kadamba* (*Anthocephalus Cadamba* (Roxb.) Miq) leaf.

Material and Methods

Kadamba leaves are collected from the Vikarabad Forest near Hyderabad, Telangana state. The Pharmacognostical study including Macroscopic & Microscopic examination, Organoleptic study, Powder microscopy, Physicochemical analysis and Phytochemical analysis, TLC, HPTLC were conducted at Telangana State Level Drug Testing Laboratory, Hyderabad and Department of *Dravyaguna,* Dr.B.R.K.R.Govt Ayurvedic College, Hyderabad as per the guidelines of Ayurvedic Pharmacopoeia of India.[11] For Microscopic study take pieces of leaf (margin and vein of leaves only), in test tube. Add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 minutes, pour the contents into a porcelain dish, drain off the liquid, wash the material with water and leave for sometimes. Remove the pieces of the material from the water with a spatula and put on the slide, add a few drops of the solution of glycerol or chloral hydrate. Crush the material with scalpel and cover with cover slip before examining. Photomicrographs were taken using Binocular Microscope (10×40) attached with camera. For examining characters of the powder take sufficient amount of powder in Chloral-hydrate solution on a slide and cover it with a cover slip, warm over a low flame for a short time.[12] Extracts are taken in four solvents Aqueous, Ethanol, Methanol, Chloroform by Soxhlet apparatus. TLC and HPTLC are carried out for aqueous and alcohol extracts.

Results and Discussion

Macroscopic: Leaves glossy green, opposite, simple more or less sessile to petiolate, ovate to elliptical, entire margin, pulvinus base, acute shortly acuminate.

Figure 1: Leaves of *Kadamba*

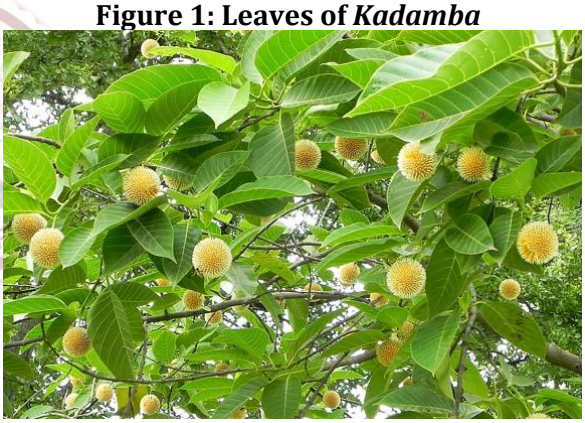


Figure 2: Leaves of *Kadamba* with Flowers

Microscopic

A transverse section through the midrib shows a dorsiventral structure with single layer of Palisade below upper epidermis and spongy parenchyma below palisade. The upper and lower epidermis is covered with a thick layer of striated cuticle. Lower epidermis shows plenty of rubiaceous stomata. At the midrib region, inner to the upper and lower epidermis, there are 2-3 layers of collenchymatous tissue, inner to the collenchyma is the parenchymatous tissue. Towards the centre is the circular vascular tissue with additional medullary bundles within the circle.

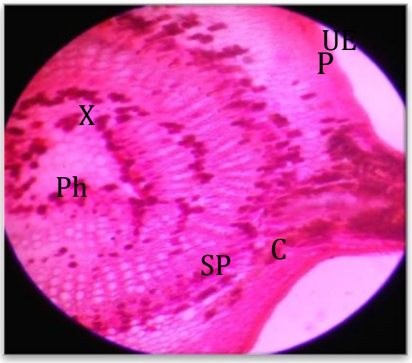


Figure 3: T.S of *Kadamba* Leaf through Midrib

UE- Upper Epidermis; P – Palisade parenchyma; X- Xylem; Ph – Phloem; SP – Spongy Parenchyma; C - Cuticle

Organoleptic characters of *Kadamba* leaf powder

Kadamba Leaf Powder is in Fine texture, with Strong Odour, Pungent taste, in Dark green colour.

Figure 4: *Kadamba* Leaf Powder

Powder microscopy

Isolated fragments of uniseriate conical hairs either whole or broken are found. Few, whole unicellular conical hairs, pieces of epidermis of lower surface with wavy anti clinical walls and stomata, few pieces of isolated stomata and prismatic crystal of calcium oxalate, annular rings are found in the microscopy.

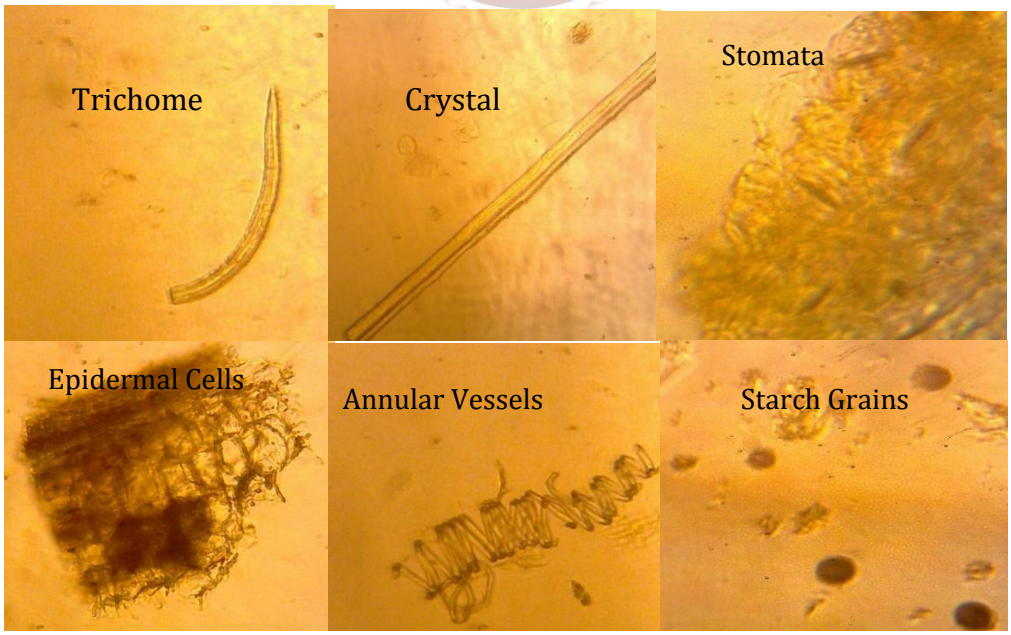


Figure 5: Powder Microscopy of *Kadamba* Leaf Powder

Physico-chemical values

Foreign matter 1%
Moisture content 4.0%
Total Ash 6.29%

Acid Insoluble Ash 0.79%

Figure 6: Determination of acid insoluble ash Figure 7: Determination of Ash value

Figure 8,9: Determination of Ash value

Table 1: Extractive Values of *Kadamba Bark* & Leaf in Different Solvents

Extract	Bark	Colour	Leaf	Colour
Aqueous	16.32	Brown	14.87	Reddish brown
Alcohol	17	Thick brown	20	Thick green
Ethanol	16.39	Brownish red	14.57	Thick green
Methanol	38.5	Reddish brown	13.88	Green
Chloroform	5.1	Brown	4.05	Thick green

Table 2: Preliminary Phytochemical Analysis of *Kadamba* Leaf in Various Solvents

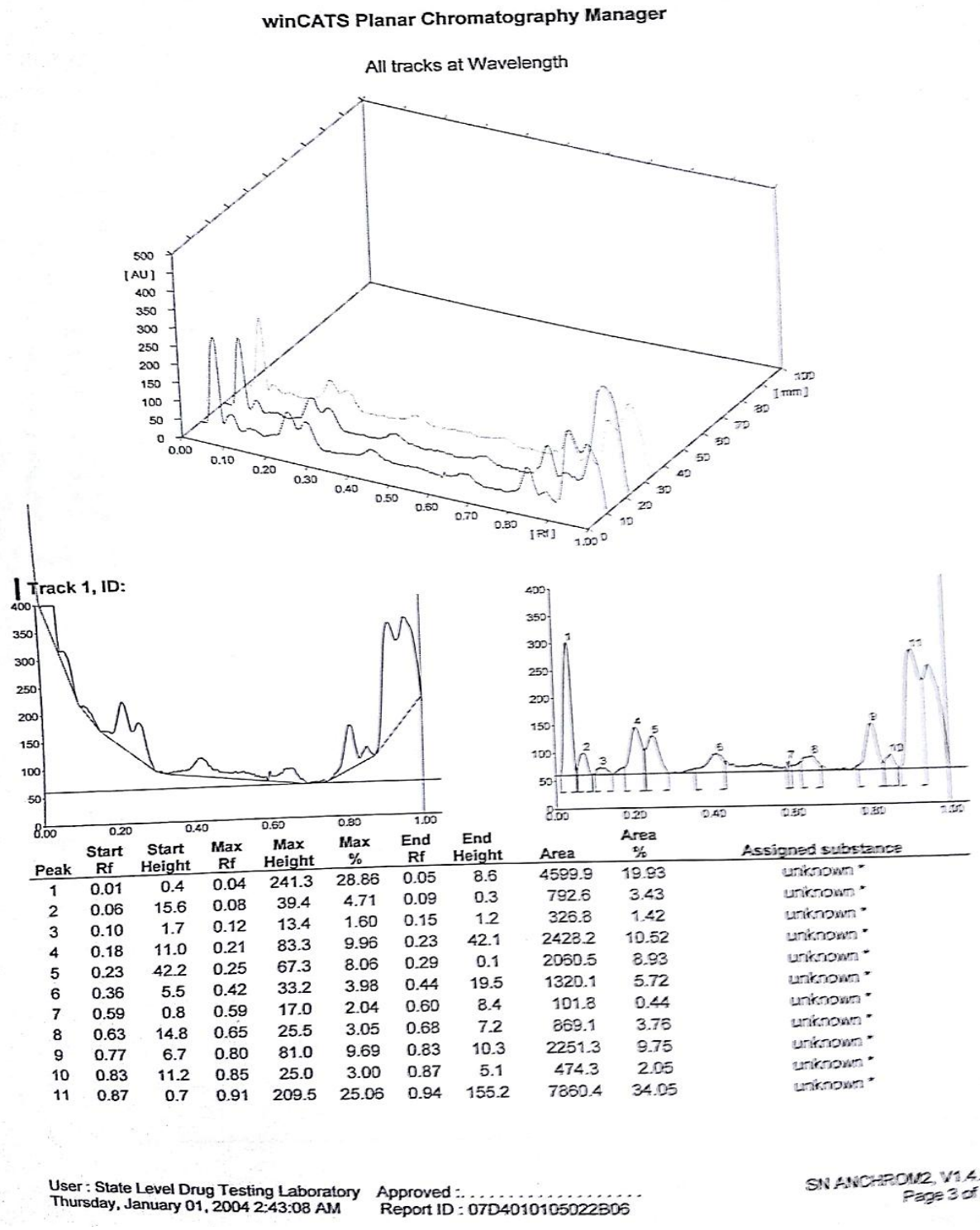
Chemical constituents	Aqueous	Ethanol	Methanol	Chloroform
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Carbohydrates	+	+	+	+
Glycosides	-	-	+	-
Tannins	+	+	+	+
Saponins	-	-	+	-
Phenols	+	+	+	+
Proteins	-	-	+	+
Alkaloids	-	+	+	+

+ indicates present; - indicates absent

The Preliminary Phytochemical studies of *Kadamba* Leaf revealed the presence of. Flavanoids. Carbohydrates, Phenols, Alkaloids are present in aqueous extract, Ethanolic extract, Methanolic, Chloroform extracts. Proteins, Glycosides, steroids are present in Methanol and Chloroform extracts. Flavanoids are present in Aqueous and Ethanolic extracts.

TLC: Chromatogram of leaf sample carried out with mobile phase Toluene: Ethyl acetate. For alcoholic extract Rf values of short waves 0.05, 0.47, 0.58, 0.68 and of long waves at 0.05, 0.22, 0.48, 0.67, 0.75, 0.85. For water extract Rf values of short waves at 0.03, 0.11, 0.21 and of long waves at 0.03, 0.11, 0.31, 0.91 are observed.

HPTLC: HPTLC Study of Alcoholic extract of *Anthocephalus cadamba* (Roxb.) Miq leaf is carried out at 366nm wavelength with mobile phase Ethyl Acetate: Methanol: Water (10:1.35:1). In Track-1 11 peaks are observed. In Track-2 8 Peaks and in Track-3 11 peaks are observed.



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.4	0.04	241.3	28.86	0.05	8.6	4599.9	19.93	unknown *
2	0.06	15.6	0.08	39.4	4.71	0.09	0.3	792.6	3.43	unknown *
3	0.10	1.7	0.12	13.4	1.60	0.15	1.2	326.8	1.42	unknown *
4	0.18	11.0	0.21	83.3	9.96	0.23	42.1	2428.2	10.52	unknown *
5	0.23	42.2	0.25	67.3	8.06	0.29	0.1	2060.5	8.93	unknown *
6	0.36	5.5	0.42	33.2	3.98	0.44	19.5	1320.1	5.72	unknown *
7	0.59	0.8	0.59	17.0	2.04	0.60	8.4	101.8	0.44	unknown *
8	0.63	14.8	0.65	25.5	3.05	0.68	7.2	869.1	3.76	unknown *
9	0.77	6.7	0.80	81.0	9.69	0.83	10.3	2251.3	9.75	unknown *
10	0.83	11.2	0.85	25.0	3.00	0.87	5.1	474.3	2.05	unknown *
11	0.87	0.7	0.91	209.5	25.06	0.94	155.2	7860.4	34.05	unknown *

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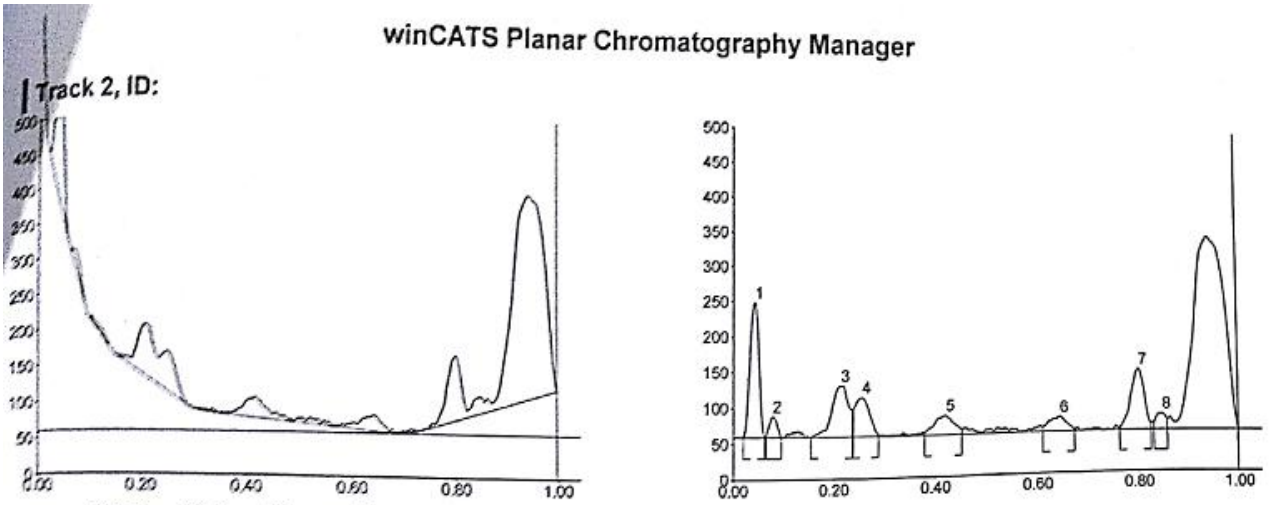
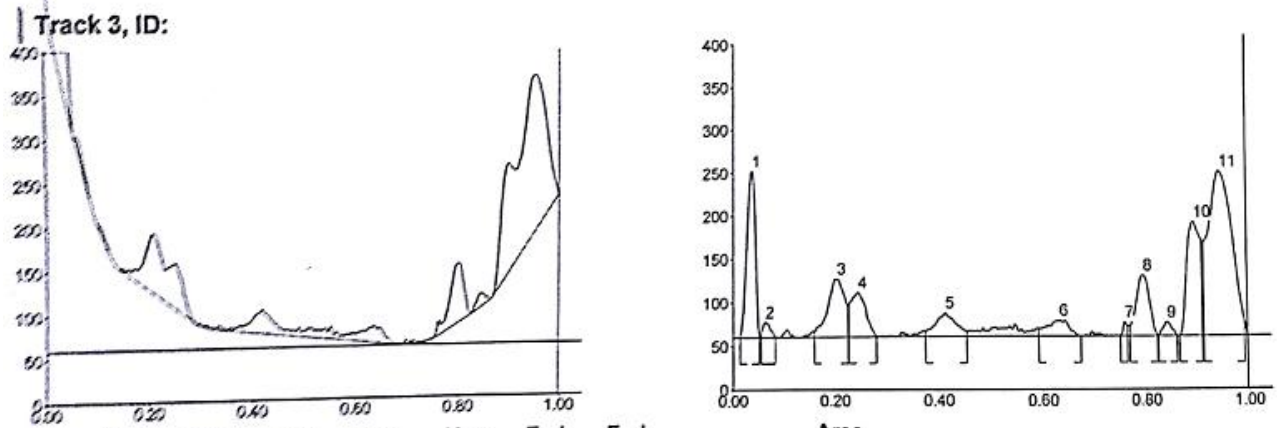


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.02	0.6	0.04	189.4	37.42	0.06	0.2	3289.8	26.66	unknown *
2	0.06	0.8	0.08	28.6	5.65	0.10	0.3	419.3	3.40	unknown *
3	0.15	0.4	0.21	71.7	14.16	0.24	38.1	2384.8	19.33	unknown *
4	0.24	38.5	0.26	54.7	10.80	0.29	2.4	1574.6	12.76	unknown *
5	0.38	4.1	0.42	27.9	5.51	0.45	7.5	1070.7	8.68	unknown *
6	0.61	6.6	0.64	21.3	4.21	0.67	1.5	741.3	6.01	unknown *
7	0.76	3.0	0.80	89.0	17.59	0.82	9.1	2390.5	19.37	unknown *
8	0.83	11.0	0.85	23.6	4.65	0.86	14.3	467.8	3.79	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	1.5	0.04	192.2	24.44	0.05	0.1	3227.4	14.05	unknown *
2	0.06	5.1	0.06	17.4	2.21	0.08	0.8	261.5	1.14	unknown *
3	0.16	6.2	0.21	66.7	8.48	0.23	36.2	2204.6	9.59	unknown *
4	0.23	36.8	0.25	50.8	6.47	0.28	0.9	1492.6	6.50	unknown *
5	0.38	5.5	0.41	27.1	3.45	0.45	5.9	1050.5	4.57	unknown *
6	0.59	8.3	0.63	18.1	2.30	0.68	0.2	762.6	3.32	unknown *
7	0.75	1.4	0.76	15.9	2.02	0.77	10.7	114.6	0.50	unknown *
8	0.77	10.6	0.80	68.3	8.69	0.82	0.8	1741.1	7.58	unknown *
9	0.83	0.2	0.84	15.5	1.97	0.86	0.5	259.6	1.13	unknown *
10	0.87	0.1	0.90	128.6	16.36	0.91	108.2	3249.4	14.14	unknown *
11	0.91	105.0	0.95	185.7	23.61	0.99	18.1	8613.6	37.49	unknown *

Figure 10, 11: Showing Peaks in Track I, II & III of alcoholic extract of *Kadamba* leaf in HPTLC study

CONCLUSION

The present study explores Pharmacognostical, physico-chemical and phytochemical study of *Kadamba* leaf which can serve as a preliminary step towards its standardization. Further study is necessary to explore other parameters related to standardization to set the limit for the reference standards for the Quality Control and Quality Assurance.

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