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R Selvarani
Regional Research Institute of Unani Medicine, Royapauram, Chennai, Tamil Nadu, India

Jaculin Raiza A
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

Meera Devi Sri P
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

Mageswari S
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

Wasim Akram SA
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

John Christopher J
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

Rampratap Meena
Central Council for Research in Unani Medicine, Janakpuri, New Delhi, India

Zaheer Ahmed N
Regional Research Institute of Unani Medicine, Royapuram, Chennai, Tamil Nadu, India

Corresponding Author:
R Selvarani
Regional Research Institute of Unani Medicine, Royapauram, Chennai, Tamil Nadu, India

Physico-chemical properties and HPTLC finger print profile of *Alangium salvifolium* L. F. Wang.: Leaves

R Selvarani, Jaculin Raiza A, Meera Devi Sri P, Mageswari S, Wasim Akram SA, John Christopher J, Rampratap Meena and Zaheer Ahmed N

Abstract
The analysis and quality control of herbal based medicines are moving a step ahead towards an integrative and comprehensive direction in order to tackle the complex nature of herbal medicinal treatment. High-performance thin layer chromatography (HPTLC) is one of the most sophisticated instrumental techniques for the qualitative and quantitative analysis of the herbs and herbal products. This article emphasizes on standardization of *Alangium salviifolium* L.f. (Wang). The physicochemical analysis and HPTLC finger print method is useful for quantitative identification and characterization of the leaf which may lead to correct selection of drug ingredient in any formulations.

Keywords: *Alangium salviifolium* L. f. (Wang), HPTLC finger print, physico-chemical parameters

1. Introduction
Ancient humans recognised their reliance on nature for a healthy life and since that time humankind has relied on a variety of plant resources for food, shelter, clothing and medicine to treat a wide range of diseases. Medicinal plants have a significant impact on global health [1]. *Alangium salviifolium* L. f. (Wang) is a well-known and widely used medicinal plant [2]. It is a one of the species of *Alangiaceae* family and commonly known as Sage Leaved Alangium. It is distributed widely in South East Asia, from India to China, Thailand, Philippines, Indonesia, Africa, tropical Australia, etc., In India, it can be found in Hyderabad forests and Rajasthan's Sitamata wildlife sanctuary [3]. *Alangium salviifolium* is a deciduous, rambling shrub or tree that grows up to 10 metres tall and has a maximum girth of 1.2 metres [4]. The leaf has an entire margin, simple, petiolate, lanceolate, narrowly oblong or ovate, base rounded or acute pubescent, alternate, reticulate venation, upto 15cm long petiole and glabrescent [5].
Alangium salviifolium leaves extract contains various bioactive chemical components such as tannins, alkaloids, flavonoids, terpenoids and steroids [6]. Moreover, ankorine salviifosides A-C, salicin, kaempferol and kaempferol 3-O-b-D-glucopyranoside were also isolated from the leaves of *Alangium salviifolium* [7]. The secondary metabolites are usually the ones responsible for the therapeutic action [8]. *Alangium salviifolium* leaves extract has biological activities such as antiulcer, analgesic, anti-inflammatory, antimicrobial, antioxidant, diuretic, antifertility, anthelmintic, anti-arthritic, hypoglycemic, anti-diabetic, anti-protozoal, anti-cholinesterase antiepileptic, antifungal [2], anticancer and antispasmodic properties [5] and used as a laxative, astringent, pungent and purgative [4]. It is being used for the treatment of asthma [3], diabetes [9], rheumatism and also have wound-healing properties [7].
Standardization is one of the most essential tasks in developing a consistent biological activity, chemical profile, or simply a quality assurance process for the production and manufacturing of herbal drug. It's the process of constructing and implementing technical standards. Specific standards are developed by experimentation and observations, which would lead to the process of providing a set of characteristics indicated by the specific herbal medicine. Therefore standardization is a tool in the quality control process. HPTLC is a modern adaptation of TLC with better and advanced separation efficiency and detection limits [10]. It is used to expand chromatographic fingerprints in order to detect the key active constituents of medicinal plants seems rational. It has the primary advantage in qualitative and quantitative assessments using scanning densitometry when combined with digital scanning profiling. Additionally, the colourful pictorial HPTLC image provides extra, simple visible colour and fluorescence data for parallel evaluation on the same plate [11].
The present study was designed to develop the physico-chemical standards and HPTLC finger print profile of *Alangium salviifolium* leaf to assess the genuine quality, safety, efficacy, repeatability.

2. Materials and Methods

2.1. Collection of plant material

The leaves of the plant *Alangium salviifolium* were collected from Tiruvallur district, Tamil Nadu. The leaves were shade dried and made it into a coarse powder using electric blender and kept in an airtight container for further use.

2.2. Physicochemical analysis

The coarse powder of *Alangium salviifolium* leaves were studied for various physico-chemical parameters like foreign matter (%), loss on drying at 105 °C (%), alcohol soluble extractive (%), water-soluble extractive (%), hexane soluble extractive (%), total ash (%), acid-insoluble ash (%) and pH as per World Health Organization (WHO) guidelines [12].

2.3. HPTLC analysis

2.3.1. Preparation of sample

The powdered leaves were successively extracted with chloroform and ethanol using a cold percolation technique. Exact 2g of coarse powder of leaves were soaked in 20ml of each solvent for 18 hrs. The solvent was filtered and concentrated to carry out the thin layer chromatography.

2.3.2. Development of HPTLC Finger print profile

- **Instrument:** CAMAG HPTLC instrument
- **Sample applicator:** CAMAG ATS4 with N_2 gas flow
- **Photo documentation system:** Digi store-2 documentation system with Win Cats software
- **Scanner:** CAMAG HPTLC scanner-3 (030618) with Win Cats-IV
- **Development chamber:** CAMAG TLC 10x10, 10x20 twin trough linear development chamber
- **Stationary phase:** Aluminium plate (thickness 0.2mm) pre-coated with silica gel 60 F254 (E. Merck)
- **Scanning wavelength:** 254nm and 366nm
- **Laboratory condition:** 26±5 °C and 53% relative humidity.

HPTLC analyses were carried out using spray techniques in ATS4 on aluminium plates pre-coated with silica gel 60 F254 (Merck, Germany). Alcohol extract and chloroform extract and 6µl each were applied as band of 8mm on TLC plate. The plate was developed using Toluene: Ethyl acetate: Formic acid (7.6: 2.4: 0.1) as mobile phase in a CAMAG twin-trough chamber previously equilibrated with a mobile phase for 30mins. The plates were developed upto 8cm, air dried, observed and scanned at wavelength of 254 and 366nm using CAMAG TLC Scanner. After the chromatogram recorded, the plates were derivatized with Vanillin Sulphuric acid and heated at 110 ºC on TLC hot plate till the development of colour of bands and observed under white light.

3. Result and Discussion

3.1. Physicochemical analysis

Physicochemical parameters have to be analysed to establish the quality and purity in herbal medicines. The ash value shows the degree of 'cleanliness,' and high values may be the result of improper sample collecting process. Total ash value i.e., 7.76% indicated that the amount of minerals and earthy material present with the plant material. Acid insoluble ash value indicates the presence of amount of siliceous matter and was found to be 0.89%. The extractive values are useful for determining the chemical constituents contained in the herbal drug, as well as for estimating the amount of specific constituents soluble in a suitable solvent. Alcohol-soluble extractive values (5.59%) are important method to estimate the different components like phenol, flavanoids, steroids, tannins, saponins, alkaloids etc. Water-soluble extractive value (31.12%) indicates the presence of water soluble active component such as sugar, glycosides, tannins, acids, inorganic compounds, mucilage etc. Hexane soluble extractive value (1.95%) indicates presence of volatile and non-volatile substances. Loss on drying is a measure of both water and volatile matter. The value of loss on drying was found to be 5.29%, high value of moisture content encourages the growth of bacteria, fungi and yeast during storage and deterioration following hydrolysis. The pH of this plant material was found to be 7.1. The value of foreign matter (1.42%) indicates the presence of any soil, sand, dust, stone and other inorganic matter with plant material.

Table 1: Physico-chemical parameters of *Alangium salviifolium* leaves

Parameters analyzed	Result
Foreign Matter (w/w,%)	1.42%
Loss on drying (w/w,%)	5.29%
Total ash content (w/w,%)	7.76%
Acid-insoluble ash content (w/w,%)	0.89%
Alcohol-soluble extractive matter (w/v,%)	5.59%
Water-soluble extractive (w/v,%)	31.12%
Hexane soluble extractive (w/v,%)	1.95%
pH values (5% Aqueous solution)	7.1

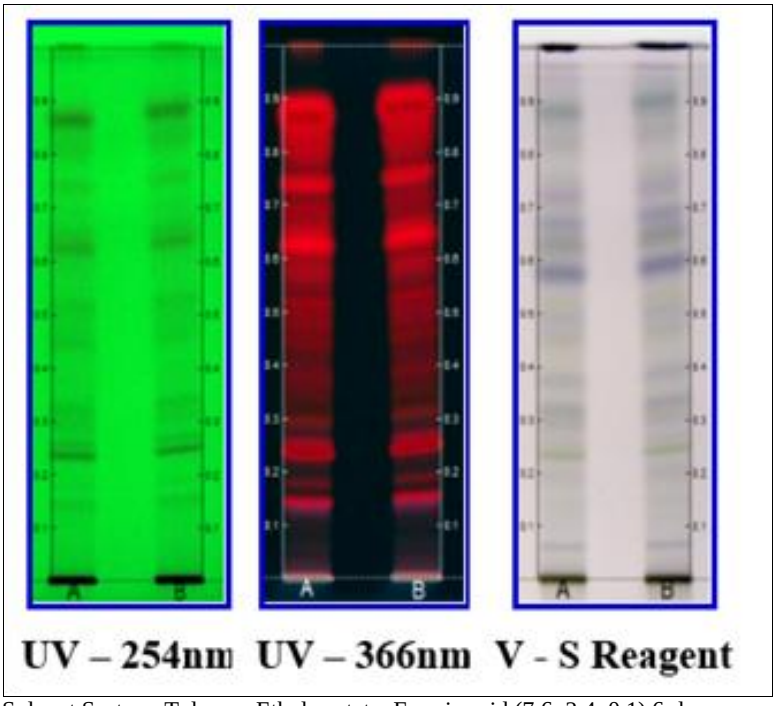
3.2. HPTLC analysis

HPTLC is one of the most flexible, reliable and cost-efficient separation technique ideally suited for the analysis of botanicals and herbal drugs. It is used for the identification of constituents, determination of impurities, qualitative and quantitative determination of active substances [13]. HPTLC profiling has resulted in directing towards the presence of a number of phytochemicals in plant extracts. In different solvent systems, different phytochemicals have distinct Rf values. Different Rf (Retention factor) values of various phytochemicals provide useful information about their polarity and solvent selection for phytochemical separation [14].

Chloroform and alcohol extract of *Alangium salviifolium* leaves on TLC plate showed many spots under 254nm, 366nm and under visible light after derivatization with vanillin-sulphuric acid (Fig. 1 & 8). The Rf of the spots and its colours are given in the table (Table. 2 & 3). In chloroform extract, HPTLC profiling gives 11 peaks when scanned at wavelength of 254nm and 15 peaks when scanned at wavelength of 366nm (Fig. 2, 3, 5 & 6). In alcohol extract, HPTLC profiling gives 15 peaks when scanned at wavelength of 254nm and 12 peaks when scanned at wavelength of 366nm (Fig. 9, 10, 12 & 13). The presence of various constituents is evidenced by the multiple numbers of peaks. Every peak has different Rf value which indicates the qualitative variation of phyto constituents in chloroform and alcohol extract of *Alangium salviifolium* leaves. Densitometric chromatogram of chloroform and alcohol extract at wavelength of 254nm and 366nm are also depicted (Fig. 4, 7, 11 & 14).

Table 2: Chloroform extract of *Alangium salviifolium* – leaves

UV - 254 nm	UV – 366nm	Vanillin-sulphuric acid
0.85 (Dark green),	0.88 (Fluorescent red),	0.87 (Light grey),
0.80 (Green),	0.80, 0.75 (Dark red),	0.81 (Green),
0.74, 0.62, 0.33, 0.26,	0.69, 0.65 (Brown),	0.71, 0.62, 0.57, 0.49, 0.38,
0.24, 0.18, 0.15 (Light	0.63 (Dark red),	0.32 (Light grey),
green)	0.55, 0.50, 0.44, 0.34, 0.29	0.23 (Green),
	(Brown),	0.06 (Light grey).
	0.26 (Dark red),	
	0.18, 0.16 (Brown)	



Solvent System: Toluene: Ethyl acetate: Formic acid (7.6: 2.4: 0.1) 6µl
Track 1. Batch - I; Track 2. Batch – II

Fig 1: HPTLC image of chloroform extract of *Alangium salviifolium* - leaves

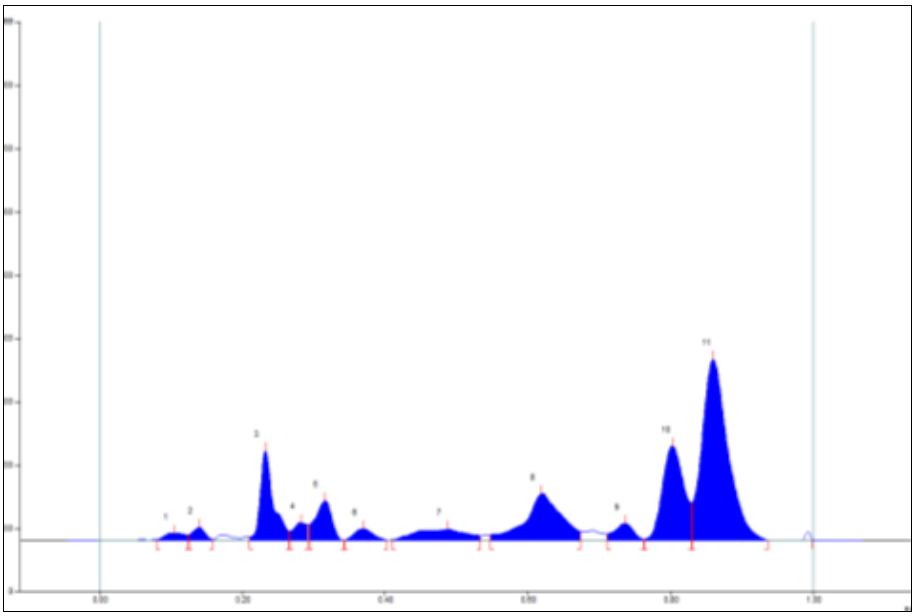


Fig 2: HPTLC finger print of *Alangium salviifolium* in Chloroform extract at 254nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	1.0 AU	0.11 Rf	11.5 AU	1.37 %	0.13 Rf	8.6 AU	276.2 AU	1.26 %
2	0.13 Rf	8.9 AU	0.14 Rf	20.3 AU	2.43 %	0.16 Rf	1.7 AU	305.8 AU	1.40 %
3	0.21 Rf	5.2 AU	0.23 Rf	142.7 AU	17.05 %	0.27 Rf	14.4 AU	2187.0 AU	9.98 %
4	0.27 Rf	14.5 AU	0.28 Rf	27.7 AU	3.31 %	0.29 Rf	24.6 AU	440.3 AU	2.01 %
5	0.29 Rf	24.9 AU	0.32 Rf	62.7 AU	7.49 %	0.34 Rf	0.1 AU	1204.2 AU	5.50 %
6	0.34 Rf	0.4 AU	0.37 Rf	18.2 AU	2.18 %	0.40 Rf	0.1 AU	398.2 AU	1.82 %
7	0.41 Rf	1.0 AU	0.49 Rf	17.5 AU	2.09 %	0.53 Rf	7.4 AU	1039.6 AU	4.74 %
8	0.55 Rf	8.9 AU	0.62 Rf	74.1 AU	8.85 %	0.68 Rf	14.4 AU	3074.7 AU	14.03 %
9	0.71 Rf	9.6 AU	0.74 Rf	26.4 AU	3.16 %	0.76 Rf	1.6 AU	588.3 AU	2.68 %
10	0.77 Rf	1.9 AU	0.80 Rf	149.5 AU	17.86 %	0.83 Rf	59.4 AU	3741.6 AU	17.08 %
11	0.83 Rf	60.3 AU	0.86 Rf	286.4 AU	34.21 %	0.94 Rf	0.3 AU	8657.1 AU	39.51 %

Fig 3: R_f values of *Alangium salviifolium* in Chloroform extract at 254nm (Absorbance mode)

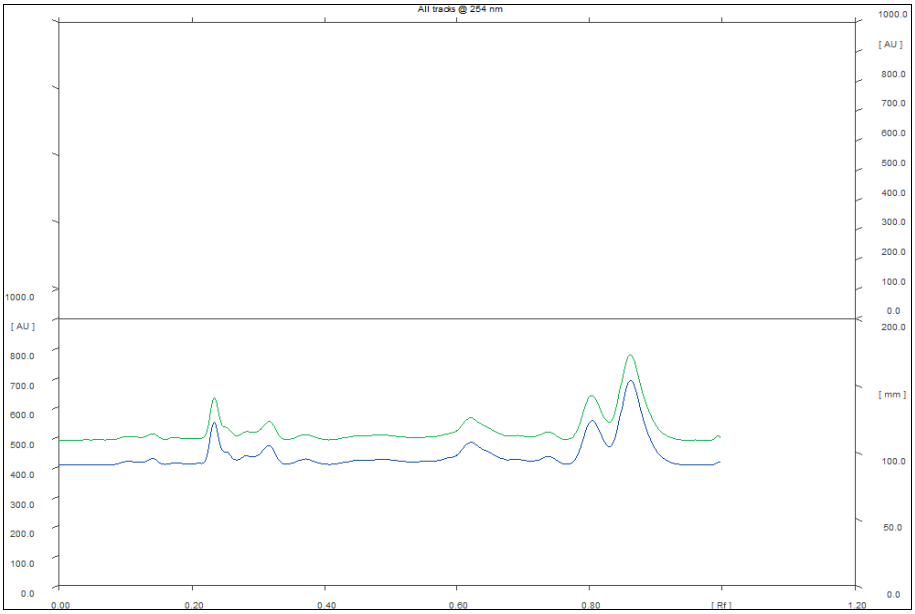


Fig 4: Densitometric chromatogram of *Alangium salviifolium* in Chloroform extract at 254nm (Absorbance mode)

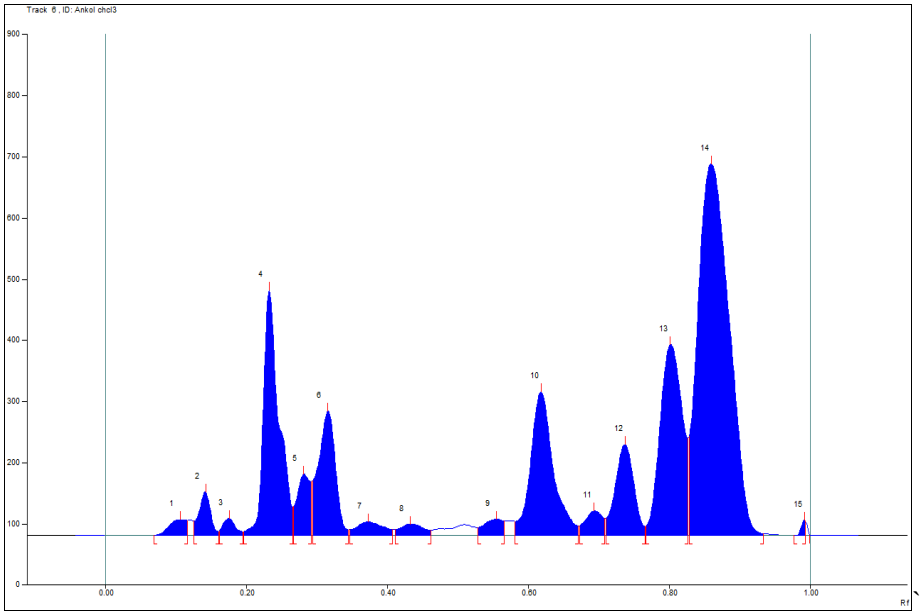


Fig 5: HPTLC finger print of *Alangium salviifolium* in Chloroform extract at 366nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.11 Rf	25.8 AU	1.14 %	0.12 Rf	24.3 AU	525.3 AU	0.90 %
2	0.13 Rf	23.3 AU	0.14 Rf	70.8 AU	3.13 %	0.16 Rf	6.3 AU	975.8 AU	1.68 %
3	0.16 Rf	7.4 AU	0.18 Rf	27.4 AU	1.21 %	0.20 Rf	5.9 AU	410.5 AU	0.71 %
4	0.20 Rf	6.1 AU	0.23 Rf	401.1 AU	17.72 %	0.27 Rf	46.1 AU	7373.7 AU	12.70 %
5	0.27 Rf	47.2 AU	0.28 Rf	99.8 AU	4.41 %	0.29 Rf	88.2 AU	1565.0 AU	2.70 %
6	0.29 Rf	89.3 AU	0.32 Rf	202.8 AU	8.96 %	0.35 Rf	8.9 AU	4138.3 AU	7.13 %
7	0.35 Rf	9.1 AU	0.37 Rf	22.6 AU	1.00 %	0.41 Rf	10.0 AU	706.7 AU	1.22 %
8	0.41 Rf	9.8 AU	0.43 Rf	18.6 AU	0.82 %	0.46 Rf	8.3 AU	512.7 AU	0.88 %
9	0.53 Rf	12.8 AU	0.56 Rf	26.8 AU	1.18 %	0.57 Rf	23.6 AU	607.2 AU	1.05 %
10	0.58 Rf	22.8 AU	0.62 Rf	235.2 AU	10.39 %	0.67 Rf	15.5 AU	6306.7 AU	10.86 %
11	0.67 Rf	15.7 AU	0.69 Rf	40.1 AU	1.77 %	0.71 Rf	27.7 AU	826.1 AU	1.42 %
12	0.71 Rf	27.8 AU	0.74 Rf	148.4 AU	6.56 %	0.77 Rf	14.5 AU	3269.5 AU	5.63 %
13	0.77 Rf	15.1 AU	0.80 Rf	312.0 AU	13.78 %	0.83 Rf	58.6 AU	8239.5 AU	14.19 %
14	0.83 Rf	161.4 AU	0.86 Rf	607.7 AU	26.84 %	0.93 Rf	2.7 AU	22484.2 AU	38.72 %
15	0.98 Rf	0.0 AU	0.99 Rf	24.9 AU	1.10 %	0.99 Rf	24.1 AU	126.1 AU	0.22 %

Fig 6: R_f values of *Alangium salviifolium* in Chloroform extract at 366nm (Absorbance mode)

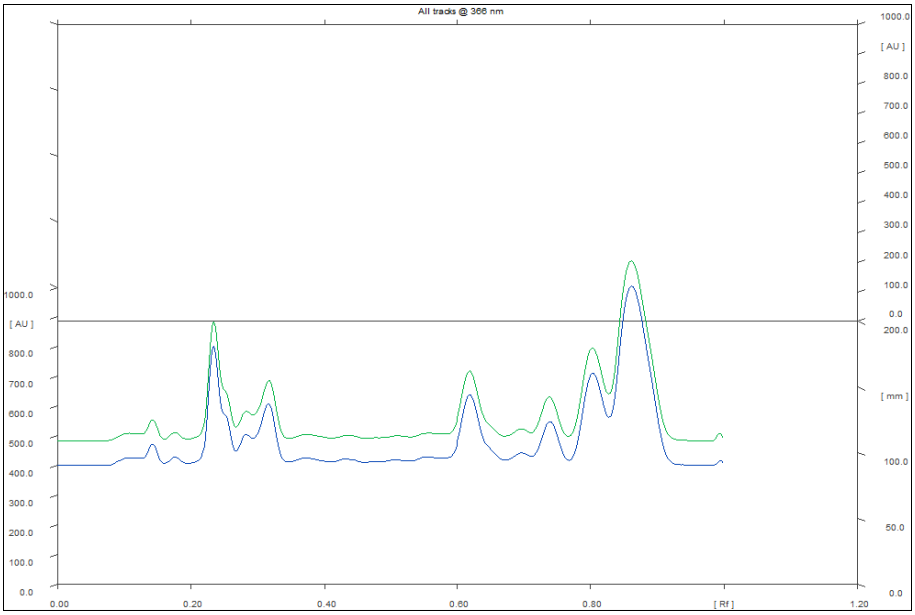
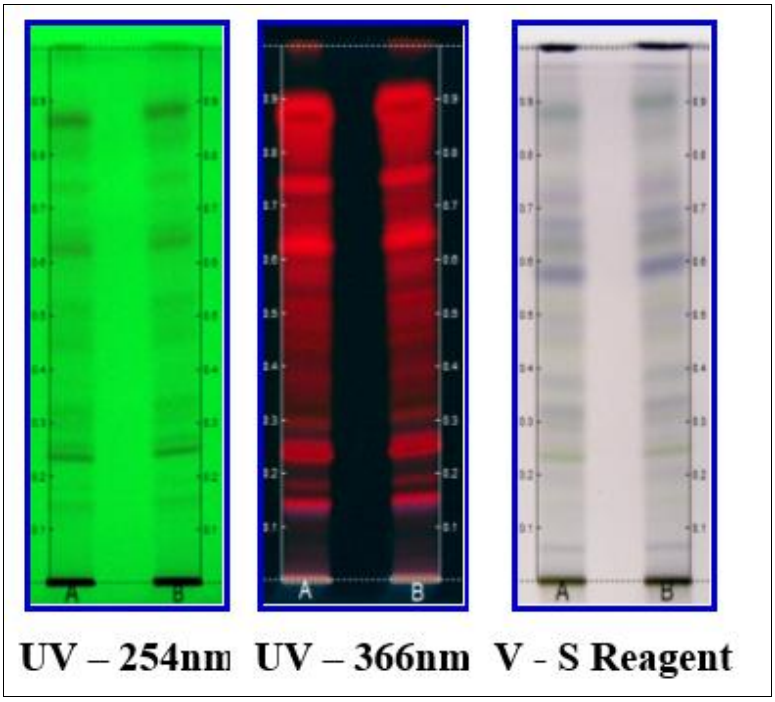


Fig 7: Densitometric chromatogram of *Alangium salviifolium* in Chloroform extract at 366nm (Absorbance mode)

Table 3: Alcohol extract of *Alangium salviifolium* – leaves

UV - 254 nm	UV – 366nm	Vanillin-sulphuric acid
0.87 (Green),	0.81 (Dark red),	0.88 (Green),
0.81, 0.74, 0.70, 0.63,	0.74 (Fluorescent red)	0.82 (Light grey),
0.51, 0.45, 0.32, 0.29,	0.70 (Dark red),	0.71 (Violet),
0.25, 0.24, 0.18,	0.62 (Fluorescent red),	0.67, 0.63 (Light grey),
0.15 (Light green).		0.58 (Blue),
		0.50, 0.46, 0.37, 0.33, 0.28 (Light grey),
	0.52, 0.48, 0.38 (Brown),	0.24 (Green),
	0.26 (Dark red),	0.20, 0.14, 0.05 (Light grey).
	0.18 (Brown),	
	0.14 (Dark red),	
	0.13 (Violet)	



Solvent System: Toluene: Ethyl acetate: Formic acid (7.6: 2.4: 0.1) 6µl
Track 1. Batch - I; Track 2. Batch - II

Fig 8: HPTLC image of alcohol extract of *Alangium salviifolium* – leaves

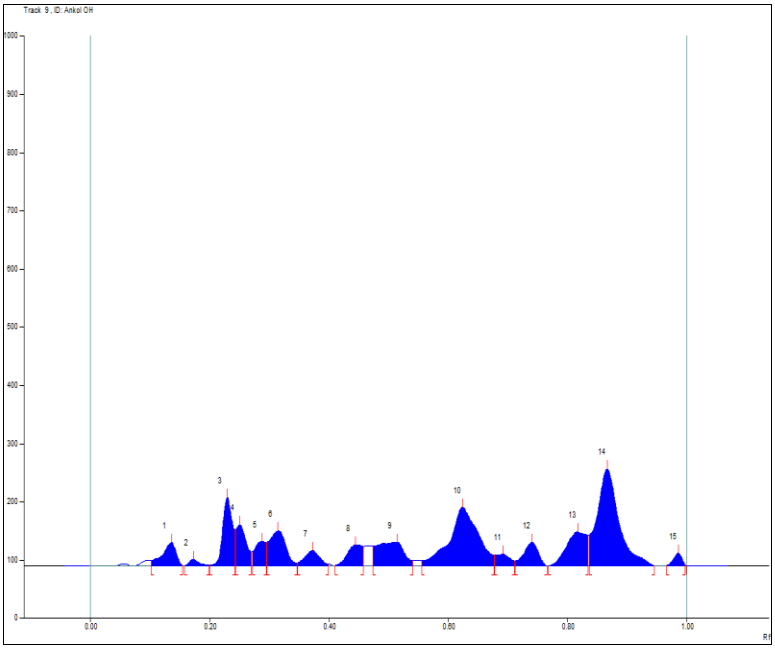


Fig 9: HPTLC finger print of *Alangium salviifolium* in alcohol extract at 254nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.10 Rf	9.0 AU	0.14 Rf	39.9 AU	4.68 %	0.16 Rf	0.3 AU	779.5 AU	3.78 %
2	0.16 Rf	0.1 AU	0.17 Rf	11.3 AU	1.33 %	0.20 Rf	0.8 AU	146.6 AU	0.71 %
3	0.20 Rf	0.9 AU	0.23 Rf	117.8 AU	13.84 %	0.24 Rf	61.7 AU	1593.9 AU	7.72 %
4	0.24 Rf	62.0 AU	0.25 Rf	70.8 AU	8.31 %	0.27 Rf	23.7 AU	1022.8 AU	4.96 %
5	0.27 Rf	24.3 AU	0.29 Rf	41.3 AU	4.85 %	0.30 Rf	40.6 AU	652.8 AU	3.16 %
6	0.30 Rf	40.9 AU	0.32 Rf	59.9 AU	7.03 %	0.35 Rf	4.8 AU	1371.4 AU	6.64 %
7	0.35 Rf	5.3 AU	0.37 Rf	26.2 AU	3.08 %	0.40 Rf	3.0 AU	543.5 AU	2.63 %
8	0.41 Rf	0.4 AU	0.45 Rf	36.0 AU	4.23 %	0.46 Rf	33.1 AU	754.7 AU	3.66 %
9	0.47 Rf	33.5 AU	0.52 Rf	40.9 AU	4.81 %	0.54 Rf	9.5 AU	1550.7 AU	7.51 %
10	0.56 Rf	9.0 AU	0.63 Rf	100.7 AU	11.83 %	0.68 Rf	18.5 AU	4016.5 AU	19.46 %
11	0.68 Rf	18.5 AU	0.69 Rf	19.9 AU	2.34 %	0.71 Rf	8.3 AU	402.0 AU	1.95 %
12	0.71 Rf	8.4 AU	0.74 Rf	40.2 AU	4.72 %	0.77 Rf	0.1 AU	816.2 AU	3.95 %
13	0.77 Rf	0.1 AU	0.82 Rf	58.4 AU	6.86 %	0.84 Rf	53.2 AU	1660.9 AU	8.05 %
14	0.84 Rf	53.4 AU	0.87 Rf	166.7 AU	19.58 %	0.95 Rf	0.0 AU	5072.0 AU	24.58 %
15	0.97 Rf	0.5 AU	0.99 Rf	21.4 AU	2.52 %	1.00 Rf	1.9 AU	254.6 AU	1.23 %

Fig 10: R_f values of *Alangium salviifolium* in alcohol extract at 254nm (Absorbance mode)

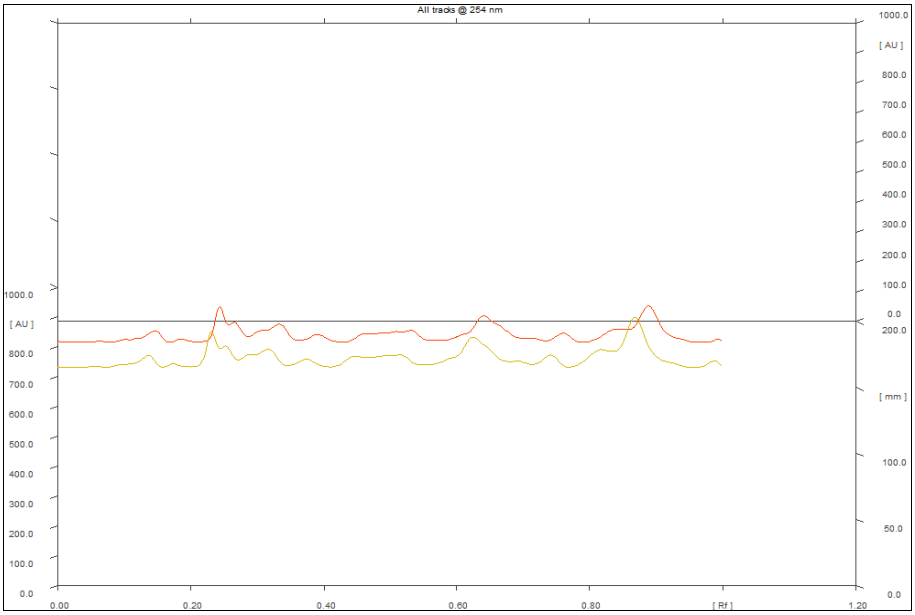


Fig 11: Densitometric chromatogram of *Alangium salviifolium* in alcohol extract at 254nm (Absorbance mode)

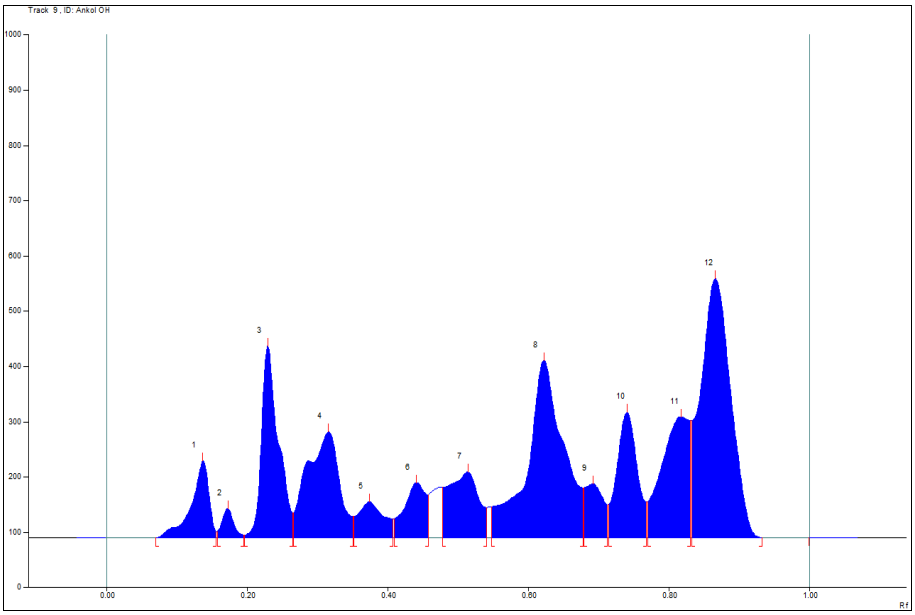


Fig 12: HPTLC finger print of *Alangium salviifolium* in alcohol extract at 366nm (Absorbance mode)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.07 Rf	0.1 AU	0.14 Rf	138.7 AU	5.91 %	0.16 Rf	11.3 AU	2923.8 AU	4.05 %
2	0.16 Rf	11.6 AU	0.17 Rf	52.7 AU	2.25 %	0.20 Rf	4.7 AU	732.6 AU	1.01 %
3	0.20 Rf	4.8 AU	0.23 Rf	347.4 AU	14.80 %	0.27 Rf	44.5 AU	6950.9 AU	9.63 %
4	0.27 Rf	44.7 AU	0.32 Rf	191.3 AU	8.15 %	0.35 Rf	38.9 AU	7296.4 AU	10.11 %
5	0.35 Rf	39.0 AU	0.38 Rf	65.7 AU	2.80 %	0.41 Rf	34.4 AU	1962.1 AU	2.72 %
6	0.41 Rf	34.6 AU	0.44 Rf	99.8 AU	4.25 %	0.46 Rf	77.9 AU	2546.0 AU	3.53 %
7	0.48 Rf	91.3 AU	0.52 Rf	118.6 AU	5.05 %	0.54 Rf	54.5 AU	4303.6 AU	5.96 %
8	0.55 Rf	56.3 AU	0.62 Rf	321.2 AU	13.68 %	0.68 Rf	90.2 AU	13847.1 AU	19.18 %
9	0.68 Rf	90.3 AU	0.69 Rf	97.6 AU	4.16 %	0.71 Rf	59.5 AU	2119.1 AU	2.94 %
10	0.72 Rf	59.9 AU	0.74 Rf	227.0 AU	9.67 %	0.77 Rf	64.4 AU	5515.7 AU	7.64 %
11	0.77 Rf	65.1 AU	0.82 Rf	218.7 AU	9.32 %	0.83 Rf	11.8 AU	7238.1 AU	10.03 %
12	0.83 Rf	211.8 AU	0.87 Rf	469.0 AU	19.98 %	0.93 Rf	0.2 AU	16763.0 AU	23.22 %

Fig 13: R_f values of *Alangium salviifolium* in alcohol extract at 366nm (Absorbance mode)

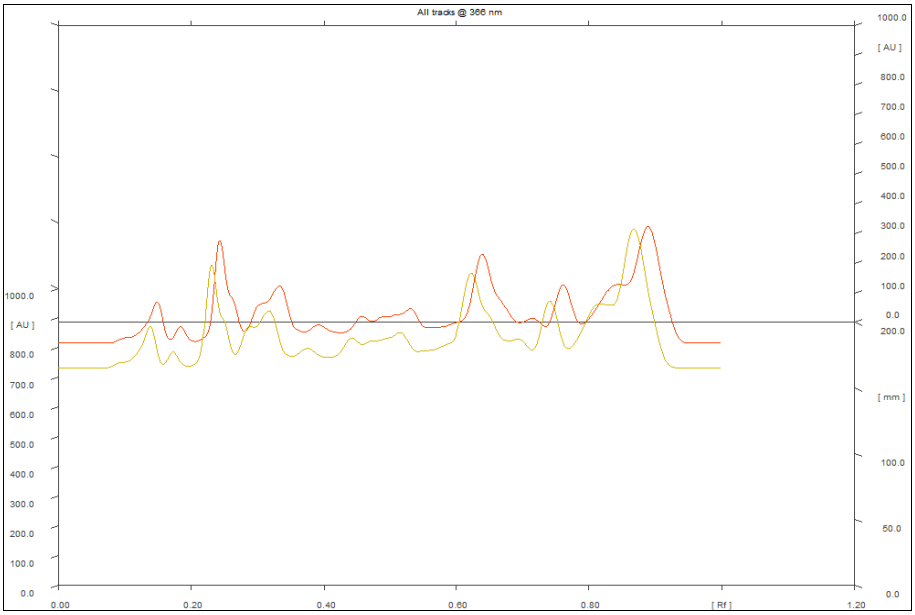


Fig 14: Densitometric chromatogram of *Alangium salviifolium* in alcohol extract at 366nm (Absorbance mode)

4. Conclusion

A novel method for HPTLC analysis of *Alangium salviifolium* L. f. (Wang) has been presented along with results that show the presence of secondary metabolites such as steroids, terpenoids and glycosides in the alcohol and chloroform leaf extract. The essences of these metabolites are helpful in maintaining human health and preventing chronic degenerative illnesses. The physicochemical characterization of the leaf can serve as quality characters and criteria for the evaluation of the identity and authenticity of the plant.

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