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Effect of extraction solvents on phytochemicals of various parts of *Salacia fruticosa* Wall. (Celastraceae) - An endemic medicinal plant

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Abstract
Nature has been a source of medicinal agents for thousands of years and a large number of modern drugs have been derived from natural sources. The medicinal plant industry is posing great threat due to the unavailability of genuine raw drugs thereby resulting in the use of several substitutes / adulterants as the source plant. In the near future, many species may be totally unavailable for the use of industry due to over exploitation. Solvent extraction is most frequently used technique for isolation of plant metabolites. However, the extract yields of the plant materials are strongly depend on the nature of extracting solvent, due to the different solubility of the chemical compounds present in it. Identification of most effective extraction solvents to increase the yield of raw materials is of great importance as it helps reducing the quantity required for medicine manufacture. *Salacia*, one among the over exploited medicinally valuable plant and many of its species are proven to be anti-diabetic. *Salacia fruticosa* Wall. One of the red listed medicinal plants belonging to the family Celastraceae is selected. In the present study the effect of extraction solvents on phytochemicals of various parts of *Salacia fruticosa* (root, stem, leaves) using various chromatographic techniques like HPTLC and HPLC is evaluated. HPLC studies revealed substantial variation in terms of phytoconstituents.

Keywords: *Salacia fruticosa*, Extraction solvents, Phytochemistry, HPTLC, HPLC

Introduction
The herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs [1]. The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant, although; it is usually not attributed to a single compound but a combination of the metabolites [2].One among the premier steps to utilize the biologically active compound from plant resources is extraction. Extraction methods involve separation of medicinally active fractions of plant tissue from inactive/inert components by using selective solvents and extraction technology [3, 4]. Continuous exploitation of several medicinal plant species and substantial loss of their habitats have resulted in the population decline of many high value medicinal plant species over the years at an alarming rate [5,6].
Salacia L. is a genus of tropical climbing shrubs comprising about 200 species world over belonging to the major family Celestraceae [7, 8]. The aerial parts and roots of *Salacia* are extensively used in Ayurvedic system of medicine, traditional Indian medicine and Unani for treating diabetes, gonorrhoea, rheumatism, itching, asthma, ear diseases, leukaemia and inflammations [9, 10, and 11]. All available species of *Salacia* are heavily extracted from their natural areas of distribution and the roots are sold in the raw drug market [12]. *Salacia fruticosa* Wall, one among the species was selected for the present study [13]. Phytochemical screening of the methanolic extract of *S. fruticosa* reveals the presence of alkaloids, carbohydrates, phytosterols, glycosides, saponins, and phenolic compounds.

The methanolic extract of *Salacia fruticosa* leaves exhibited significant antihyperglycemic activity in alloxan induced diabetic rats [14]. Identification of most effective extraction solvents to increase the yield of raw materials is of great importance as it helps reducing the quantity required for medicine manufacture**.** Extraction of phytochemicals from various parts (stem, leaf and root) of *Salacia fruticosa* using different solvents such as Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol+Water), Water and effect of extraction solvents on Phytochemicals of various parts of *S. Fruticosa* using HPTLC and HPLC have been evaluated in the present investigation. The possibilities of using organic solvents such as hydro alcohol instead of water for the preparation of Herbal drugs have been evaluated in the present investigation.

Materials and Methods

Collection, identification and drying of Plant Material

The plant used for the investigation *Salacia fruticosa* was collected in fresh condition from natural habitats of Kozhikode district and was identified and authenticated at Department of Botany, St. Joseph's College, Devagiri. The plant material was washed thoroughly with water. The root, stem and leaves were cut into small pieces and were shade dried until the chopped parts became dried for grinding. After drying, the plant materials were ground separately using mechanical blender into fine powder and transferred into airtight containers at ambient temperature.

Chemicals and reagents used in the study

Solvents such as Methanol, Ethanol, Chloroform and Hexane purchased from Merck and HiMedia were used. Mangiferin was procured from Sigma Chemicals Co. (Bangalore, India). All other chemicals employed were of standard analytical grade from Merck, India.

Preparation of extracts

The extracts of the stem, leaves and roots of *Salacia fruticosa* were prepared using different solvents such as Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol+Water) and Distilled water. The extraction was done using Reflex condenser. Dried stem, leaves and roots were taken in the RB flask and extracted using different solvents (200ml) at boiling temperature for 4 hours. The extracts filtered and concentrated to 5ml in a water bath.

High Performance Thin Layer Chromatography (HPTLC) studies

Aluminium backed pre-coated Merck silica gel plate 60 F_{254} plate (10×10 cm) was used as the stationary phase. Ethyl acetate: Formic acid: Acetic acid: Water (9: 1: 1: 0.3) solvent system was used and Mangiferin was used as the marker for comparison of samples. Toluene: Ethyl acetate: Formic acid (7: 3: 0.3) was used for profiling without marker compounds. Samples were applied on the plate using Camag automatic TLC sampler 4 attached to Camag HPTLC system. 10 µl of test solution applied on a precoated silica gel 60 F_{254} TLC plate (E. Merck, 0.2 mm) as bands of 8mm using Hamilton syringe (100µl). The plate developed in the solvent system in a twin trough chamber to a distance of 9 cm. The plate observed under UV light at 254 nm and 366 nm. Densitometric scanning of the plates was done by using Camag TLC scanner at 254 nm, 366 nm.

High Performance Liquid Chromatography (HPLC) studies

HPLC analysis was carried out using Shimadzu High Performance Liquid Chromatographic system equipped with LC-10ATVP pump, SPD M10AVP Photo Diode Array Detector in combination with CLASS-VP 6.12 SP5 integration software. Gradient elution was performed with methanol (solvent A) and 0.1 % acetic acid in water (solvent B) in a binary gradient flow by increasing the concentration of solvent B; 0-5 min 70%; 5-10 min 60%; 10-15 min 50%. 15-20; 40%, 20-25 30 % .The DAD signal was recorded at 275 nm. The total run time was optimized to 25 minutes.1 ml of the extract was evaporated to dryness on a water bath. The residue was dissolved in 10 ml of HPLC grade methanol and centrifuged at 1000 rpm for 10 minutes. 5 ml of this was filtered through PVDF membrane (0.45µm). Injection volume was 20 µl.

Results and Discussions

The chemical patterns of different extracts were compared using HPLC and HPTLC profiling. Variations were observed in terms of number of bands/peaks and band/peak intensity/area which indicate the qualitative and quantitative divergence in chemical constituents.

HPTLC profiling

The HPTLC profiles were developed for different parts, such as leaf, stem and root of *S. fruticosa* with various solvents like Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol+Water) and Water. Ethyl acetate: Formic acid: Acetic acid: Water (9: 1: 1: 0.3) was the best solvent system for the separation of Mangiferin (Tables 1, 2, 3 and Plates 2, 3, 4). Profiling of extracts from various parts in different solvents without using marker was best achieved in the solvent system containing Toluene: Ethyl acetate: Formic acid (7: 3: 0.3), (Tables 4, 5, 6 and Plates 5, 6, 7). Disparities were observed in terms of number of bands and band intensity of the HPTLC profiles developed for different parts, such as leaf, stem and root of *S. fruticosa* with various solvents like Hexane, Chloroform, Methanol, Hydro-alcohol (Ethanol+Water) and water which specify the qualitative and quantitative deviation in chemical constituents.

Maximum quantity of mangiferin (0.133µg/10µl) from *S. fruticosa* root sample was observed in Hydro-alcohol (Ethanol+Water) extract and the least (0.06µg/10µl) in methanol extract. Maximum quantity of mangiferin (0.16µg/10µl) from *S. fruticosa* leaf sample was also observed in Hydro-alcohol (Ethanol+Water) extract and the least (0.075µg/10µl) in methanol extract. Maximum quantity of mangiferin (0.09µg/10µl) from *S. fruticosa* stem sample was also observed in Hydro-alcohol (Ethanol+Water) extract and the least (0.05µg/10µl) in methanol extract. Hydro-alcohol (Ethanol+Water) was the best solvent as far as the quantity of mangiferin is concerned. The present results also showed that the aqueous based solvents are the most effective extraction solvent for phytochemicals. Similar results of superior effect of ethanol in combination with different proportions of water have been reported by Dai and Mumper (2009). Concomitant results of high antioxidant and free radical scavenging activities of Hydroalcoholic extract of three species of *Salacia* (*S. oblonga, S. prinoides, and S. reticulata*) have been reported by Subhasree *et al*. (2013). Kaliappan *et al.* (2014) have reported the LC-MS

Quantification of Mangiferin in hydroalcoholic extract of *Salacia oblongata* and *Salacia roxburghii*.

In contrast to the results obtained for the solvent system Ethyl acetate: Formic acid: Acetic acid: Water (9: 1: 1: 0.3) used for the separation of Mangiferin, maximum number of phytoconstituents indicated by more number of bands were observed in hexane and chloroform extracts of different parts and least number of bands were observed in hydro-alcohol and distilled water extracts in the solvent system containing Toluene: Ethyl acetate: Formic acid (7: 3: 0.3).

HPTLC study of *Salacia* using Mangiferin marker

Table 1: *Salacia* Root extract

Solvent sample	Rf value (Different bands formed)		Mangiferin Quantity (µg/10 µl)
	254nm	366nm	
Hexane	Nil	Nil	Nil
Chloroform	Nil	Nil	Nil
Methanol	0.33 (Mangiferin) 0.52 0.60	0.33 (Mangiferin)	0.06
Hydro-alcohol	0.33 (Mangiferin) 0.52 0.60	0.33 (Mangiferin)	0.133
Distilled water	0.33 (Mangiferin) 0.52 0.60	0.33 (Mangiferin)	0.07

Table 2: *Salacia* Stem Extract

Solvent sample	Rf value (Different bands formed)		Mangiferin Quantity (µg/10 µl)
	254 nm	366 nm	
Hexane	Nil	Nil	Nil
Chloroform	Nil	Nil	Nil
Methanol	0.36 (Mangiferin)	0.36 (Mangiferin)	0.075
Hydro-alcohol	0.36 (Mangiferin)	0.36 (Mangiferin)	0.16
Distilled Water	0.36 (Mangiferin)	0.36 (Mangiferin)	0.1

Table 3: *Salacia* Leaf Extract

Solvent Sample	Rf value (Different bands formed)		Mangiferin Quantity (µg/10 µl)
	254 nm	366nm	
Hexane	Nil	Nil	Nil
Chloroform	Nil	Nil	Nil
Methanol	0.31(Mangiferin) 0.52 0.60	0.31(Mangiferin) 0.52 0.60	0.05
Hydro-alcohol	0.31(Mangiferin) 0.52 0.60	0.31(Mangiferin) 0.52 0.60	0.09
Distilled water	0.31(Mangiferin) 0.52 0.60	0.31(Mangiferin) 0.52 0.60	0.08

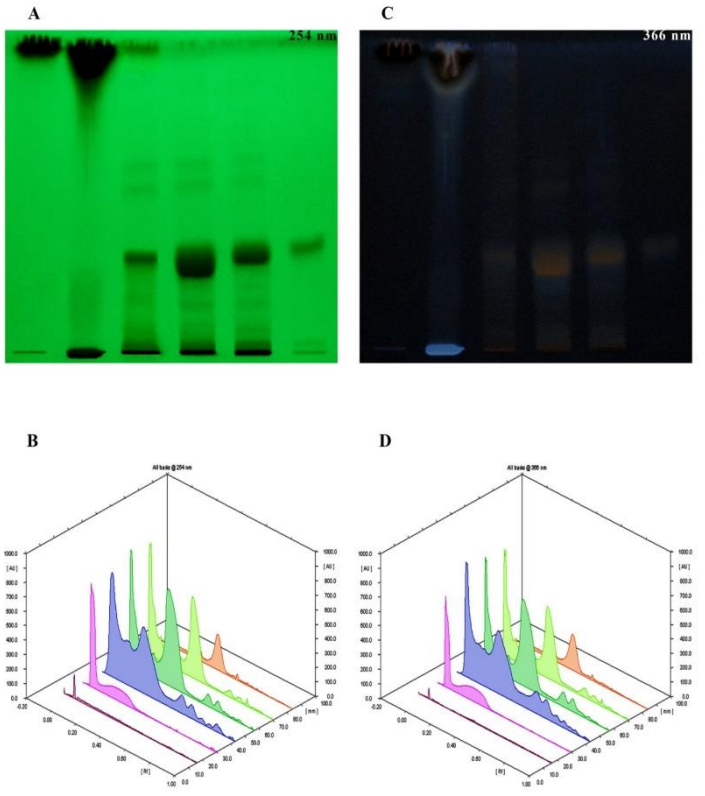

Plate 2: HPTLC profile of root extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm. Track-1: Hexane extract of root, Track-2: Chloroform extract of root, Track-3: Methanol extract of root, Track-4: Hydroalcohol extract of root, Track-5: Distilled water extract of root, Track-6: Mangiferin used as standard marker.

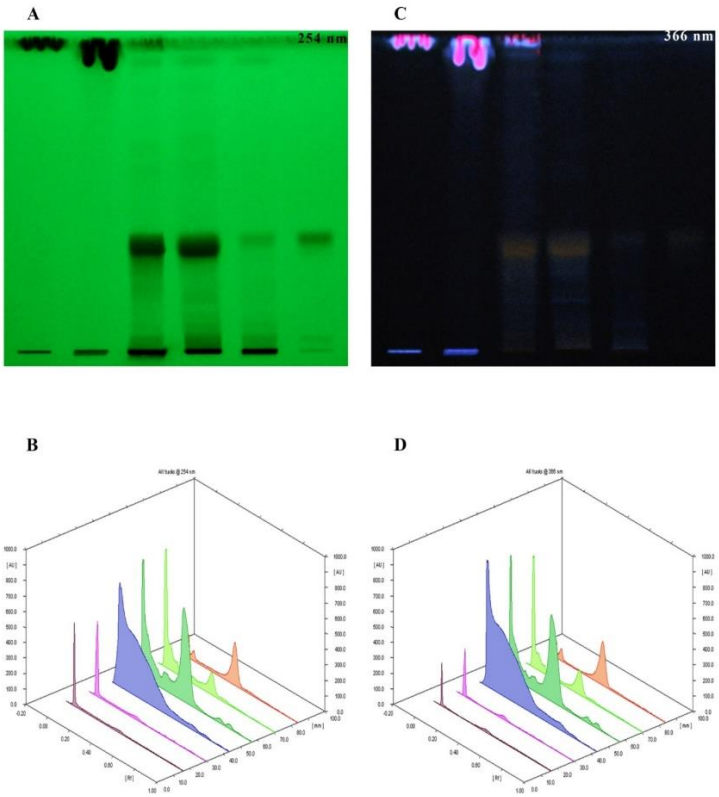

Plate 3: HPTLC profile of stem extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm. Track-1: Hexane extract of stem, Track-2: Chloroform extract of stem, Track-3: Methanol extract of stem, Track-4: Hydroalcohol extract of stem, Track-5: Distilled water extract of stem, Track-6: Mangiferin used as standard marker.

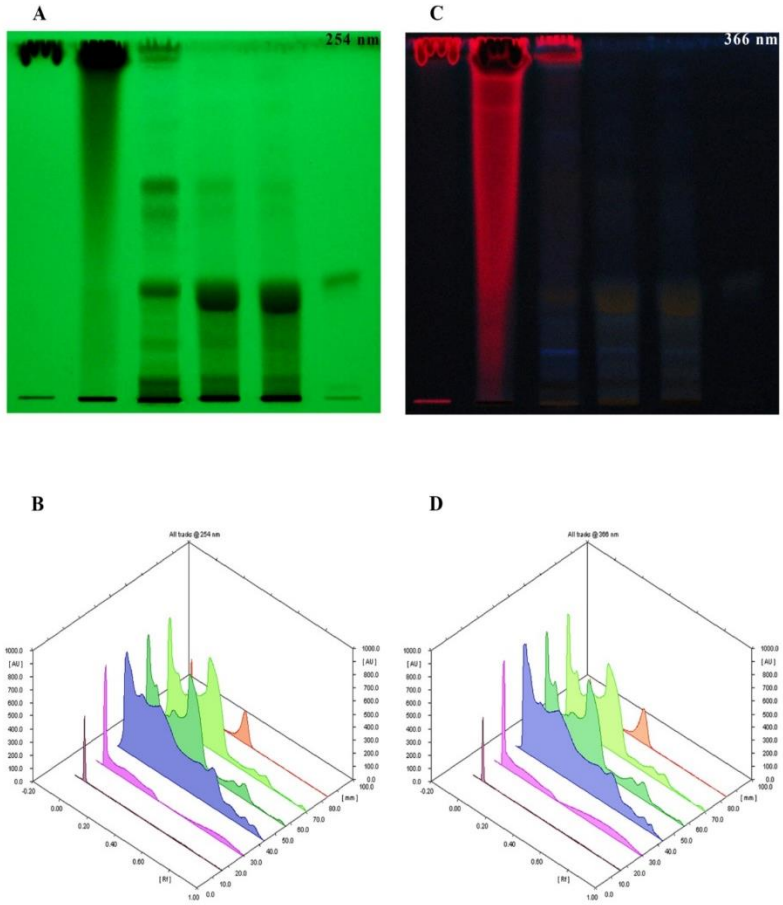

Plate 4: HPTLC profile of leaf extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm. Track-1: Hexane extract of leaf, Track-2: Chloroform extract of leaf, Track-3: Methanol extract of leaf, Track-4: Hydroalcohol extract of leaf, Track-5: Distilled water extract of leaf, Track-6: Mangiferin used as standard marker.

HPTLC studies in *Salacia* without marker compounds

Table 4: *Salacia* Root Extract

Solvent Sample	Rf value (Different bands formed)	
	254 nm	**366 nm**
Hexane	0.32, 0.38, 0.65, 0.74, 0.79, 0.87	0.16, 0.73, 0.79, 0.84
Chloroform	0.19, 0.61, 0.75, 0.78, 0.82, 0.86	0.12, 0.16, 0.18, 0.22, 0.34, 0.39, 0.45, 0.50, 0.68, 0.71
Methanol	0.19, 0.87	Nil
Hydro Alcohol	0.19	Nil
Distilled Water	0.19	Nil

Table 5: *Salacia* Stem Extract

Solvent Sample	Rf value (Different bands formed)	
	254 nm	**366 nm**
Hexane	0.90, 0.93	0.58, 0.69, 0.81, 0.85, 0.91
Chloroform	0.14, 0.18, 0.90, 0.93	0.15, 0.19, 0.23, 0.31, 0.39, 0.51, 0.60, 0.66, 0.81, 0.85, 0.91
Methanol	0.10, 0.93	0.84
Hydro Alcohol	0.10	Nil
Distilled Water	0.10	Nil

Table 6: *Salacia* Leaf Extract

Solvent Sample	Rf value (Different bands formed)	
	254 nm	**366 nm**
Hexane	0.62, 0.74, 0.83	0.19, 0.39, 0.58, 0.61, 0.68, 0.73, 0.78, 0.86, 0.96
Chloroform	0.62, 0.69, 0.77, 0.79	0.13, 0.16, 0.19, 0.50, 0.55, 0.58, 0.61, 0.64, 0.66, 0.72, 0.77, 0.81, 0.86, 0.93
Methanol	Nil	0.12, 0.13, 0.16, 0.19, 0.55, 0.58, 0.62, 0.75, 0.83, 0.88
Hydro Alcohol	Nil	Nil
Distilled Water	Nil	Nil

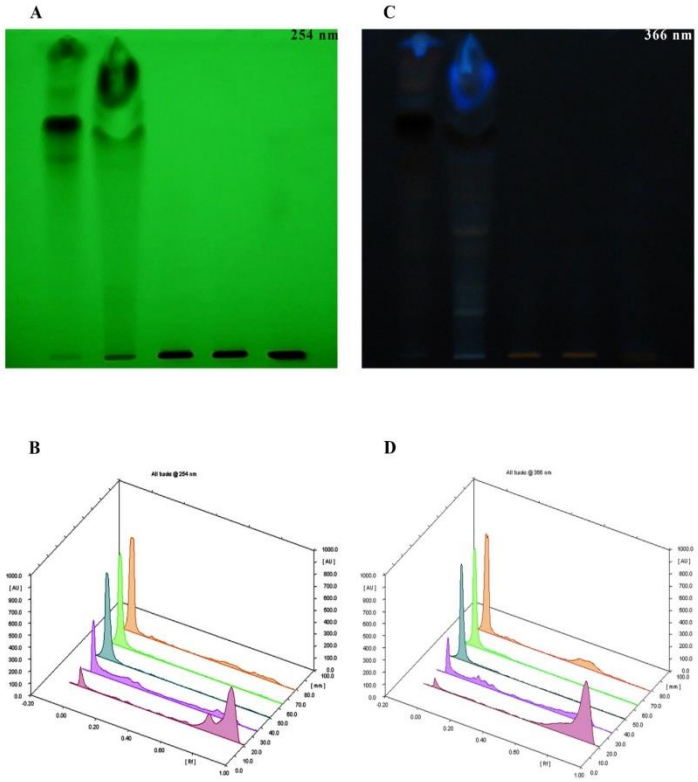

Plate 5: HPTLC profile of root extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm respectively. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm respectively. Track-1: Hexane extract of root, Track-2: Chloroform extract of root, Track-3: Methanol extract of root, Track-4: Hydro-alcohol extract of root, Track-5: Distilled water extract of root.

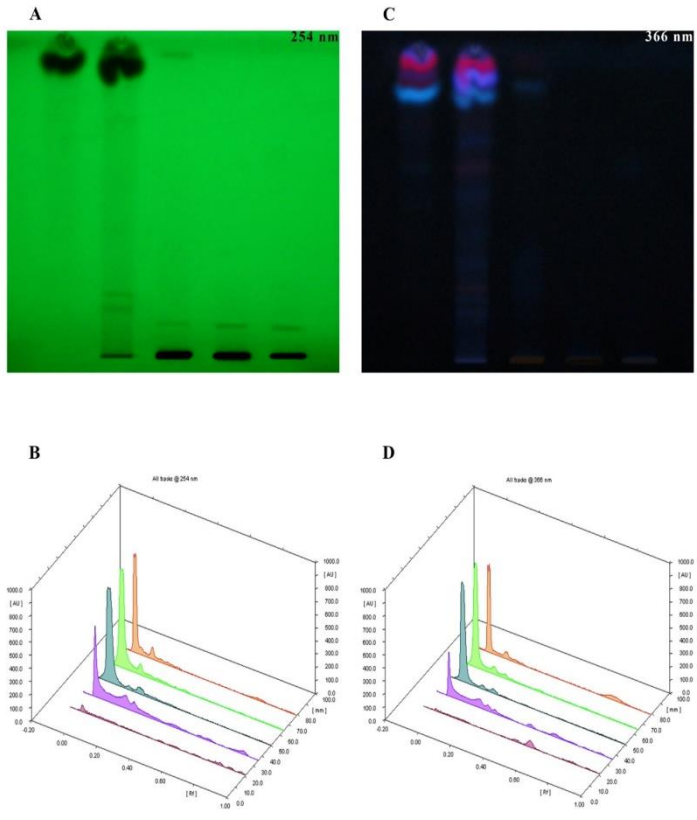

Plate 6: HPTLC profile of stem extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm. Track-1: Hexane extract of stem, Track-2: Chloroform extract of stem, Track-3: Methanol extract of stem, Track-4: Hydroalcohol extract of stem, Track-5: Distilled water extract of stem.

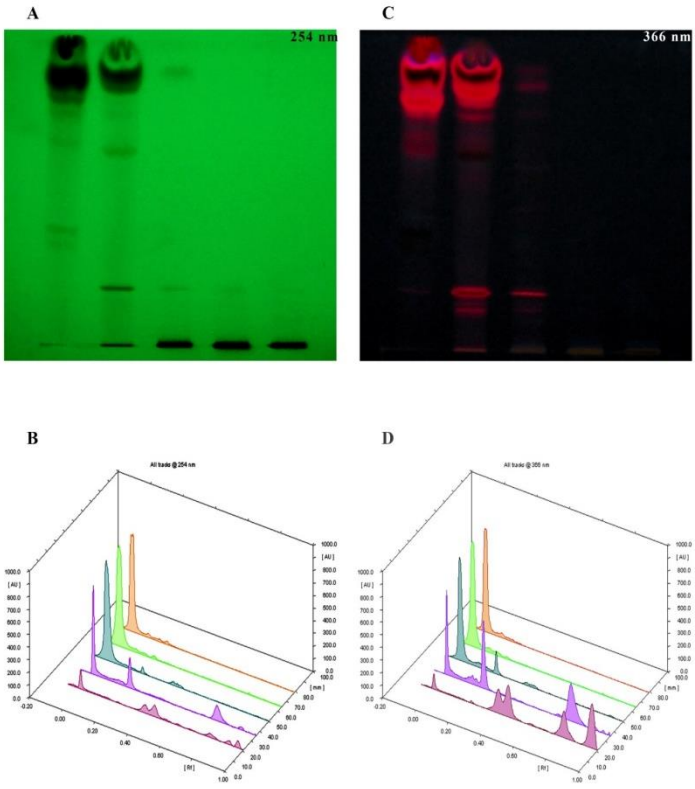

Plate 7: HPTLC profile of leaf extracts of *Salacia fruticosa*. **A&B:** HPTLC chromatogram and densitometric scanning image under 254 nm respectively. **C&D:** HPTLC chromatogram and densitometric scanning image under 366 nm respectively. Track-1: Hexane extract of leaf, Track-2: Chloroform extract of leaf, Track-3: Methanol extract of leaf, Track-4: Hydro-alcohol extract of leaf, Track-5: Distilled water extract of leaf.

The results of the present studies revealed that hydro alcoholic extract exhibited better yield with comparable chemical constituents with that of aqueous extract. Possibility of reducing the quantity of raw drugs can be explored if raw drugs are being extracted with hydro-alcohol (ethanol+water. 50:50) instead of water. Mangiferin at different quantities was detected in Methanol, Hydro alcohol and Distilled water extracts of various parts. Mangiferin was totally absent in Chloroform and Hexane extracts. Among the different solvents used for the extraction, hydro alcohol showed the maximum yield of mangiferin.

HPLC Profiling

The HPLC profiles were developed for Methanol extracts of different parts, such as leaf, stem and root of *S. fruticosa* (Figures 1, 2, 3). HPLC studies to compare the methanolic extracts of leaf, stem and root of *Salacia fruticosa* revealed substantial variation in terms of phytoconstituents. Maximum numbers of compounds indicated by peaks were more in root extracts and the least was in stem extract. In leaf and root extracts the maximum quantity (29.6% and 7.5% respectively) indicated by area percentage was for compound at retention time 11.3, whereas the maximum quantity of compounds in stem extract (42.1 %) was for compound at retention time 3.6.

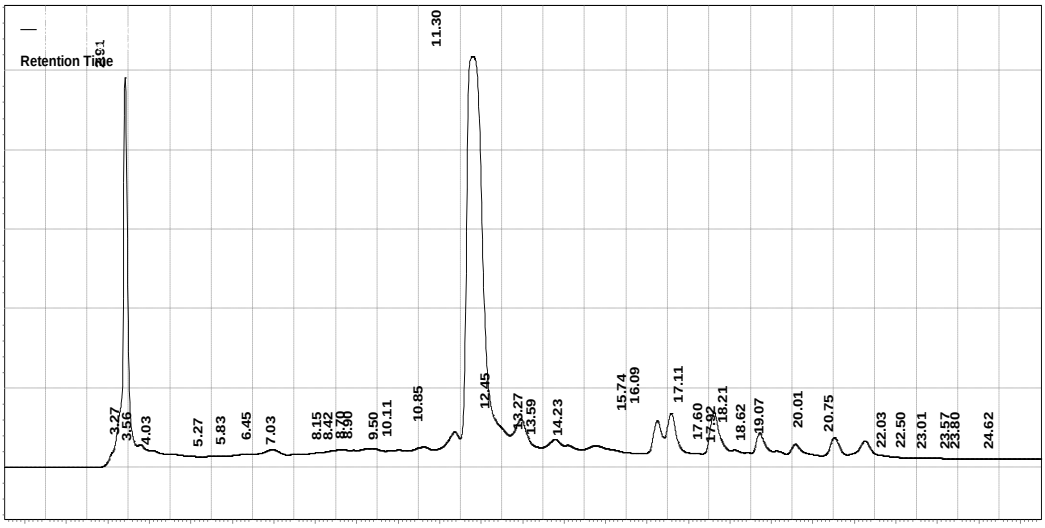

Fig. 1: HPLC Chromatogram of *Salacia* root extract

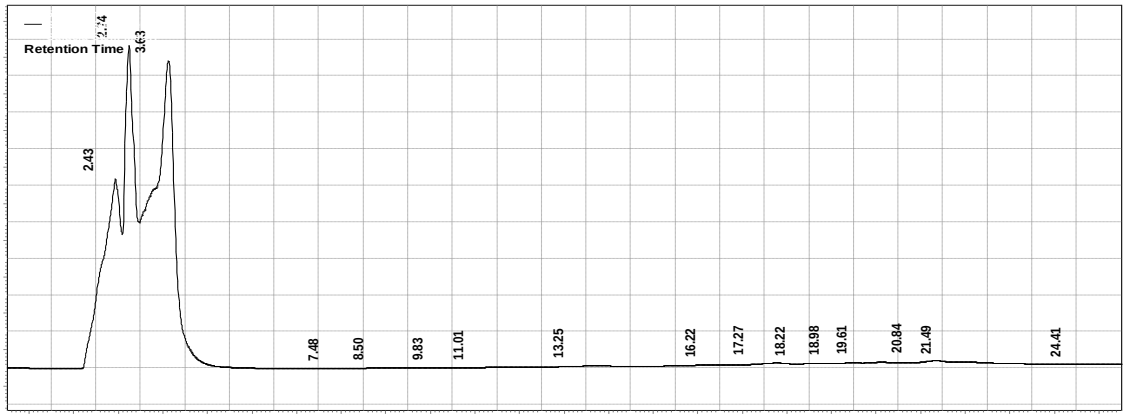

Fig. 2: HPLC Chromatogram of *Salacia* stem extract

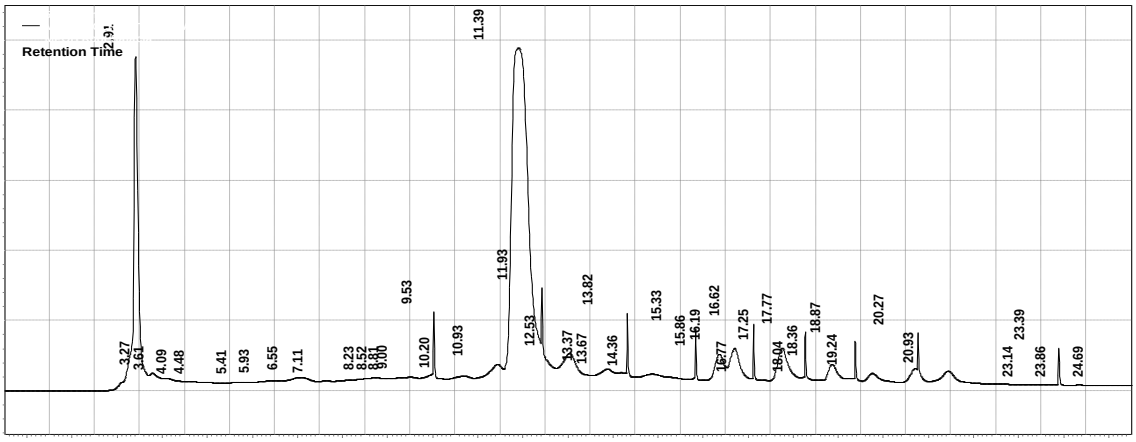

Fig. 3: HPLC Chromatogram of Salacia leaf extract

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