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Preliminary phytochemical study and HPTLC valuation of leaves of grey mangrove *Avicennia marina*

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Abstract

Mangroves are the chemical factories of nature and extensive sources of organic chemical matter on this planet. The conventional methods are poor, time consuming as well as less systematic, so there is a need to use appear technological knowledge and sophisticated scientific methods to fulfill this knowledge gap. Therefore, present study is an attempt to study the preliminary phytochemistry and distinguish HPTLC pattern of *Avicennia marina* leaf. To check presence or absence of photochemical compounds like alkaloids, flavonoids, tannins, terpenoids, saponins, reducing sugar, phenols, steroids and glycosides were identified by preliminary analysis. Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system is the best solvent system for measuring the establishment of phytochemicals of *A. marina*. HPTLC analysis of methanol extract showed nine peaks and the R_f values ranged in between 0.07 to 0.80 at 254nm and R_f values ranged in between 0.07 to 0.81 showing six peaks at 366 nm. It is used as a phytochemical identification tool to check genetic variability in various plant species.

Keywords: *Avicennia marina*, phytochemicals, HPTLC, extract, leaf

1. Introduction

The genus *Avicennia* belongs to mangrove family Avicenniaceae which is widely distributed in all intertidal areas Robertson & Alongi (1992) [1]. The plants absorb oxygen through pneumatophores, which is deficient in its habitat little (1983). The knowledge about compounds derived from mangrove is very scarce but many important classes of compounds like alkaloids, benzofurans, flavonoids, benzoquinones, tannins, triterpenes, aminoacids, carbohydrates, carotenoids, steroids, organicacids, glycoside, anthocyanides, procyanides, alcohols, sugars, lipids and nitrogen containing salts have previously reported Bandaranayake (2002) [3].

The leaves and roots possess aphrodisiac properties. The unripe seeds are used as poultice to hasten suppuration of boils and abscesses. It is used for small pox in Madras. The bark is astringent Kirtiker and Basu (1975) [4].

Among all true mangrove species of the world, *Avicennia marina* is a valuable mangrove because of its abundant distribution Duck *et al* (1998) [5], Availability and useful medicinal values Bandarnayake (1998) [6] of its different parts like leaves, stems, roots, flowers and also as a whole plant formulation Ishrak *et al* (2003) [7].

The main objective of this study is to HPTLC fingerprint pattern for *A. marina* leaf by methanol extracts of the leaf and its preliminary phytochemical analysis for detection of chemical compounds.

2. Materials and methods

2.1 Collection of plant materials

A. marina (leaves) were collected from Ayiramthengu (9° 7' N: 76° 29' E.) of Kollam district in Kerala state. The plant materials were authenticated from Botanical Survey of India.

2.2 Preliminary Phytochemical Screening

The collected leaves were washed with tap water and shade dried at room temperature. The dried leaves were powdered using electrical blender. 10 g. of material was stirred overnight in 70% methanol (100 ml) and then centrifuged at 10,000 rpm for 10 min. The resultant supernatant was collected and the methanol was removed by evaporation. This extract was used for further phytochemical analysis. Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, glycosides and phenols were carried out in the extract as per the method described Harborne (1973) [8], Sofowora (1993) [9] and Trease and Evans (1989) [10].

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2.2.1 Test for Tannins: A small portion of the extract was diluted with 20 ml of distilled water and boiled in a boiling tube. Then few drops of 0.1% ferric chloride were added. The appearance of brownish green or blue-black colour indicates the presence of tannins.

2.2.2 Test for Saponins: One ml of the extract was diluted with 20 ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins.

2.2.3 Test for Flavonoids: About 1 ml of the extract was mixed with few fragments of magnesium ribbon and concentrated hydrochloric acid. The appearance of pink or magenta-red colour indicates the presence of flavonoids.

2.2.4 Test for Phenols: A small portion of the extract was mixed with 2 ml of ferric chloride solution. The appearance of green or blue colour indicates the presence of tannins.

2.2.5 Test for Alkaloids: Two ml of the extract was mixed with 0.2 ml of 1% HCl. Then 1 ml of Mayer's reagent was added. Any precipitate or turbidity indicates the presence of alkaloids.

2.2.6 Test for Steroids: A small portion of the extract 2 ml of sulphuric acid was added by the sides of the test tube. The appearance of bluish-green or violet colour indicates the presence of steroids.

1.2.7 Test for Terpenoids: A small portion of the extract was mixed with 2 ml of chloroform. Then 3 ml of sulphuric acid was carefully added. The appearance of reddish brown or pinkish brown ring/colour indicates the presence of terpenoids.

1.2.8 Test for Glycosides: A small portion of the extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of ferric chloride solution. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. The appearance of brown ring indicates the presence of glycosides.

1.2.9 Reducing suger-fehling's test: Few drops of Fehling's solution A and B in equal volume were added in dilute extracts and heated for 30 min. and observed for the formation of brick red colored precipitate.

1.3 Preparation of Extract

Weight 1g. of sample and boil for 5-10 min. on a water bath. Concentrate the filtrate and make up to 10ml. in a volumetric flask.

1.4 Application of Extract

The extracts were applied with the help of linomat syringe using the Linomat applicator V on the HPTLC plates (10×10 cm). Wash syringe with test solution and fill the syringe with extract prepared above for the qualitative analysis.

1.5 Development of the Chromatogram

The principle of separation in HPTLC is same as like TLC. One mobile and one stationary phase were used. Silica gel on the percoated plates acts as stationary phase. The solvent system selected was same as that used in TLC analysis. The

plates were developed in CAMAG twin trough chamber. The sample travels through the stationary phase and elute the components according to the binding capabilities of components with stationary phase. Here the plates were developed up to a distance of 80 mm and after the run was completed, they were taken out of the chamber and dried in air.

2.6 Photo documentation

CAMAG HPTLC (Scanner 3) was used as a scanner in absorbance mode at both 254 and 366 nm, the scanned data was subjected for integration through the software win CATS Planar Chromatography Manager. The fingerprint so developed was used for the detection of phytoconstituents present in the samples and the chromatograms and R_f value were noted. Bands were resolved and their colour was noted. Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when TLC plates were sprayed with detection reagent (sulphuric acid reagent and plate was heated at 110°C for 1minutes).

3. Result and discussion

3.1 Photochemical Screening

Various phyto components like alkaloids, saponins, flavonoids, phenols tannins, alkaloids, reducing sugar were detected in the methanol extract of *A. marina* leaves (Table 3).

3.2 HPTLC Profile

The best results were shown using Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system. TLC plate of *Avicennia marina* methanol (leaf) extract scanned at 254 nm wavelength signified the existence of nine phytoconstituents whose R_f values ranged from 0.07 to 0.80. Peak one showing with an R_f value of 0.07 with area 10.34%. Peak two with an R_f value of 0.11, area of 1.95 %. Peak three with an R_f value of 0.14 and area 6.89%. Peak four showing R_f value of 0.24 with area 4.09%. Peak five showing an R_f value of 0.44 with an area of 2.83%. Peak six showing R_f value of 0.51 with 15.43 % area. Peak seven showing R_f value of 0.58 with area 11.16%. Peak eight showing R_f value of 0.76 with area 11.16%. Peak nine showed R_f value of 0.80 with area of 9.35%. The total peaks present in HPTLC profile of *Avicennia marina* is nine with an area of 9781.2(AU).

The methanol (leaf) extract scanned at 366 nm wavelength signified the existence of six phytoconstituents whose R_f values ranged from 0.07 to 0.81. Peak one showing with an R_f value of 0.07 with area of 11.58%. Peak two with an R_f value of 0.14 with area of 1.55 %. Peak three with an R_f value of 0.44 and area 6.02%. Peak four showing R_f value of 0.51 with area 21.36%. Peak five showing an R_f value of 0.58 with an area of 13.79%. Peak six showing R_f value of 0.81 with area of 45.70%. The total peaks present in HPTLC profile of *Avicennia marina* is six with an area of 17767 (AU). (Table 1, Table 2, Figure A&B, Plates: A, B, C, D & E.)

Dawane *et al* (2016) ^[11] phytochemical analysis of methanol extracts on stem of *A. marina*. Through this study they establish presence of (phenols, alkaloids, terpenoids, steroids, carbohydrates, proteins, amino acids, tannins, saponins, flavonoids, gums and mucilage) and also hptlc fingerprinting. The stem extract (methanol) revealed the presence of 4 components with R_f values in the range of 0.03 – 0.90.

Table 1: Phytochemical analysis of methanol extract of *A. marina* at 244 nm.

Peak No	R _f Value	AREA(AU)	Area(AU) %
1	0.07	1006.4	10.34
2	0.11	230.5	1.95
3	0.14	724.3	6.89
4	0.24	371.6	4.09
5	0.44	255.5	2.83
6	0.51	1507.3	15.43
7	0.58	3688.1	37.96
8	0.76	1086.5	11.16
9	0.80	911.0	9.35

Table 2: Phytochemical analysis of methanol extract of *A. marina* at 366 nm.

Peak No	R _f value	Area (AU)	% Area(AU)
1	0.07	2057.5	11.58
2	0.14	276.1	1.55
3	0.44	1070.0	6.02
4	0.51	3794.1	21.36
5	0.58	2449.6	13.79
6	0.81	8119.8	45.70

Table 3: HPTLC fingerprint analysis of *A. marina* leaves.

Phytoconstituents	Methanol
Saponins	+
Flavanoid	+
Phenols	+
Alkaloids	-
Steroids	-
Terpenoids	+
Glycosides	+
Tannins	+
Reducing suger	-

“+” present, “-“absent

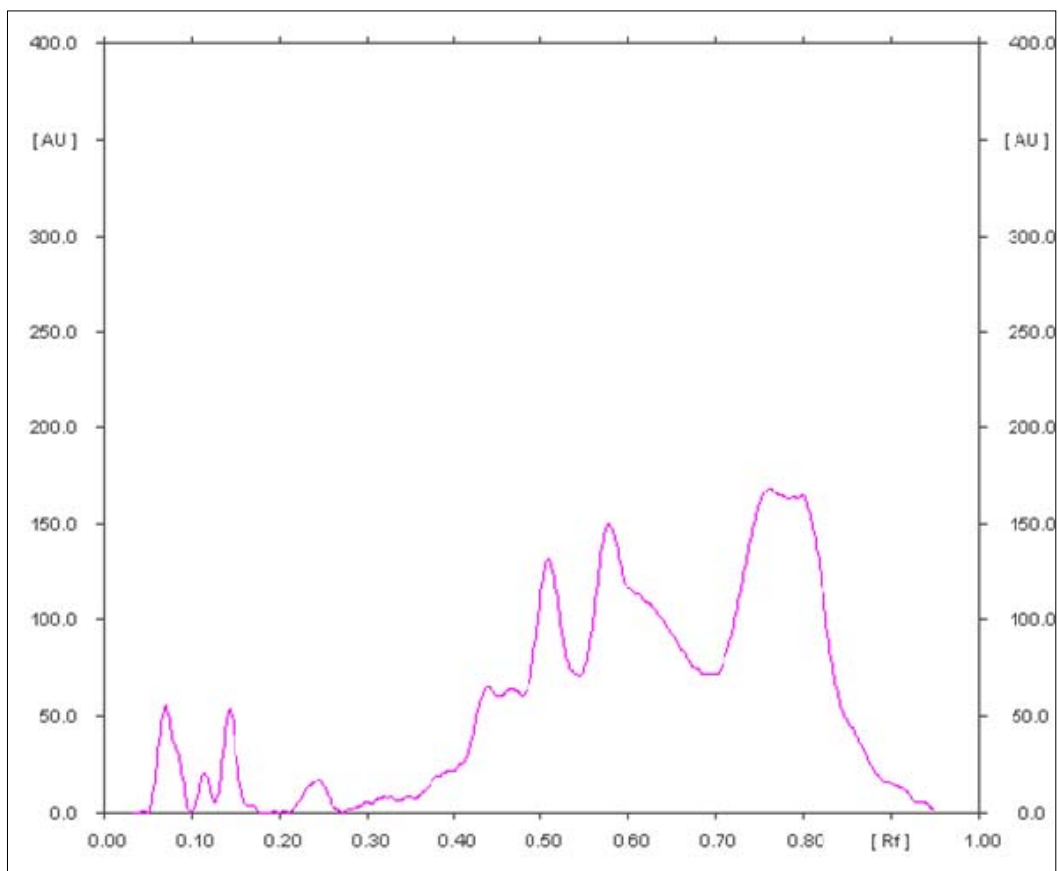


Fig A. An overview of *Avicennia marina* (Forssk.) Vierh. sample at 254 nm before derivatization

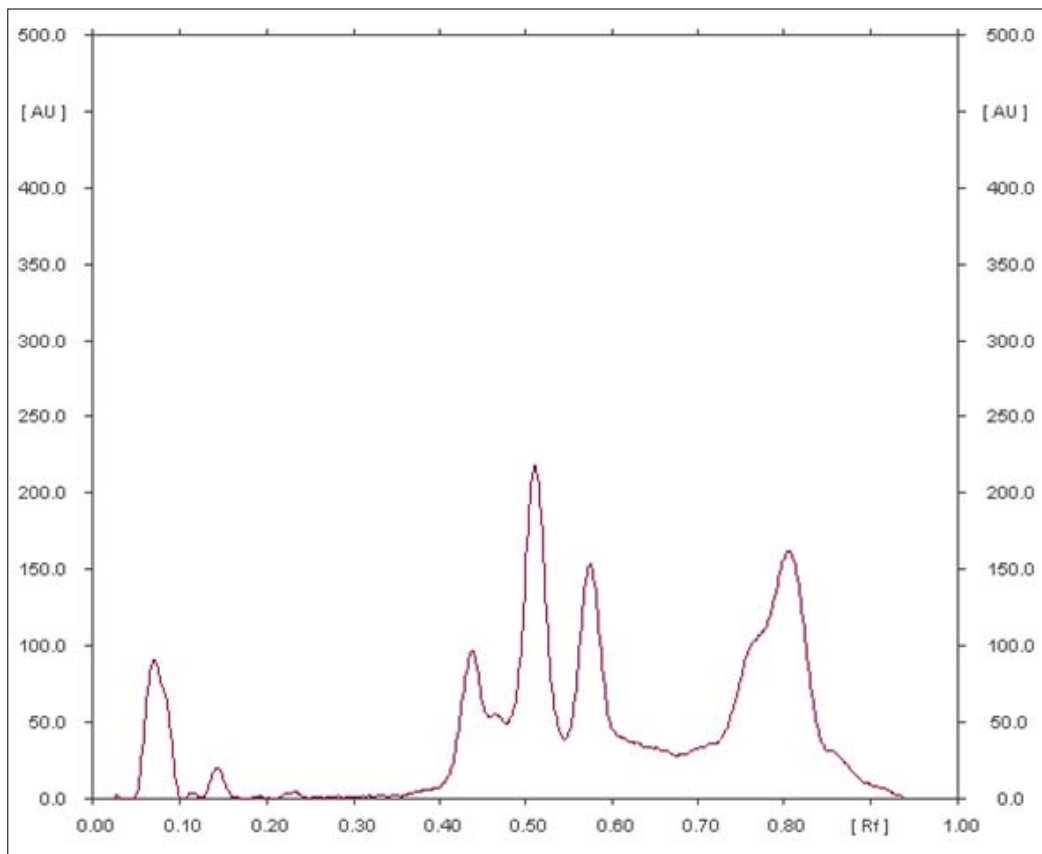
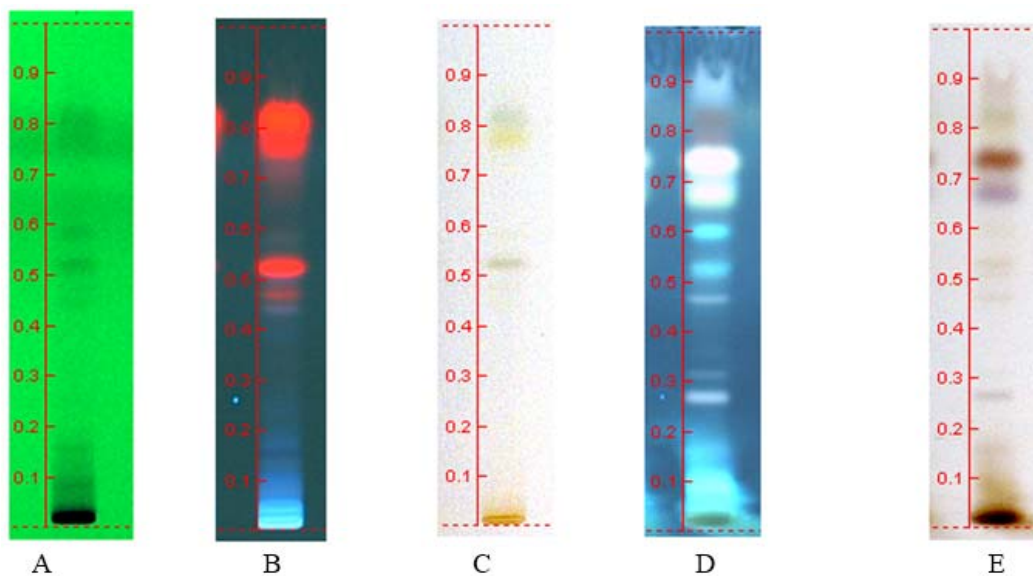


Fig B. An overview of *Avicennia marina* (Forssk.) Vierh. sample at 366 nm after derivatization



Plates: Phytochemical Profile of *Avicennia marina* (Forssk.) Vierh. before (A, B & C) and after (D & E) derivatization

4. Conclusion

The optimized HPTLC characterization and preliminary analysis method for *Avicennia marina* leaf is a quick, inexpensive, exact, error-free and reliable method for the identification. HPTLC analysis helps in distinguish the adulterant of species.

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