International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 3; May 2017; Page No. 07-10

Preliminary photochemical assessment and HPTLC characterization of leaves of medicinal mangrove *Acanthus ilicifolius* L.

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Abstract
Acanthus ilicifolius is a medicinal plant used for treatment of skin diseases, small pox, ulcers, snake bite and rheumatism. The present study investigated preliminary phytochemical estimation and high performance thin layer chromatography (HPTLC) characterization of leaves. Phytochemical compounds like alkaloids, flavonoids, tannin, 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 photochemical of *A. ilicifolius.* HPTLC finger print of methanol extract showed eight peaks and the R_f values ranged in between 0.18 to 0.80 at 254nm and in 366 nm it showed seven peaks, R_f values ranged between 0.07 to 0.81. HPTLC analysis helps for the identification of plant species. Further research is necessary to extract the compounds from *A. ilicifolius* and it will give programmed for further phytochemical and pharmacuetical applications.

Keywords: *acanthus ilicifolius*, phytochemicals, tannin, saponin, extract

1. Introduction
Mangroves are part of a marginal ecosystem, able to tolerate extreme environmental factors such as high salinity and constant inundation (Vannucci, 2001) [8]. Mangroves and mangrove associates possess novel agrochemical products, compounds of medicinal value, and biologically active compounds (Bandaranayake, 2002) [1]. Leaves of *Acanthus ilicifolius* contained Protein, Alkaloids, Resin, Steroids, Tannins, Glycosides, Reducing sugar, Carbohydrates, Saponins, Steroids, Sterols, Terpenoids, Phenol, Cardioglycosides, and Catachol (Kokpol, 1984; Madhu and Madhu, 1997; Islam *et al*., 2012; Poompozhil and Kumarasamy, 2014) [4, 5, 2, 6]
A. ilicifolius has traditionally been used for treatment of skin diseases, small pox, ulcers, snake bite and rheumatism. Its antiviral, antioxidant and anticarcinogenic activity has been demonstrated recently. *A. ilicifolius* shows significant analgesic activity. (Sachin *et al.*, 2014) [7]
In Konkan, a decoction of the plant with sugercandy and cumin is given in dyspepsia with acid eructations. In Goa, the leaves are used as an emollient fomentation in rheumatism and neuralgia. The tender shoots and leaves ground small and soaked in water are applied to snake bite. (Rheede). In Siam and Cochin China, the plant is considered a cordial and attenuant, useful in paralysis and asthma. The tender shoots and leaves are useless in the treatment of snake-bite. (Kirtiker and Basu, 1933) [3].

2. Materials and methods
2.1 Collection of plant materials
A. ilicifolius (leaves) were collected from Ayiramthengu (9^0 7' N: 76^0 29' E.) of Kollam district in Kerala state (Figure 1). The plant materials were authenticated from Botanical Survey of India, Coimbatore.

Legenda
Kollam district

Fig 1: Aerial view of Kollam district and Kerala.

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. Ten grams of material was stirred overnight in 70% methonal (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 phyochemical 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 by (Harborne, 1973; Sofowora, 1993; Trease and Evans 1989).

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 was added. The appearance of brownish green or blue-black colour indicates the presence of tannins.
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.
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.
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.
5) **Test for Alkaloids:** Two ml of the extract was mixed with 0.2 ml of 1% HCl. Then 1 ml of Mayers' reagent was added. Any precipitate or turbidity indicates the presence of alkaloids.
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.
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.
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.
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.

2.3 Preparation of Extract

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

2.4 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.

2.5 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 winCATS Planar Chromatography Manager. The fingerprint so developed was used for the detection of phytocomponents present in the samples and the chromatograms and Rf 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 (sulfuric acid reagent and plate was heated at 110°C for 1minutes).

Result and discussion

3. Photochemical Screening

Various phyto components like alkaloids, saponins, flavonoids, phenols and tannins were present in the methanol extract of *A. ilicifolius* leaves (Table 3).

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

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

"+" present, "-" absent

3.1 HPTLC Profile

The results were shown using Toluene: Ethyl Acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system. TLC plate of *A. ilicifolius* methanol (leaf) extract scanned at 254 nm wavelength signified the existence of eight phytoconstituents whose Rf values ranged from 0.18 to 0.80. Peak one showing with an R_f value of 0.18 with area 1.77%. Peak two with an R_f value of 0.29, area of 0.55 %. Peak three with an R_f value of0.39 and area 1.93%. Peak four showing R_f value of 0.46 with area 3.62%. Peak five showing an R_f value of 0.51 with an area of 4.91%. Peak six showing R_f value of 0.58 with 3.27% area. Peak seven showing R_f value of 0.64 with area 2.77%. Peak eight showing R_f value of 0.80 with area 81.17%. The total peaks present in HPTLC profile of *Acanthuus ilicifolius* is eight with an area of 18778.2(AU). (Table 1, Figure 2 Plate 1).

Table 1: Phytochemical analysis of methanol extract of *A. ilicifolius* leaves at 254 nm.

Peak No	R_f Value	Area (AU)	% Area (AU)
1	0.18	333.1	1.77
2	0.29	103.9	0.55
3	0.39	362.4	1.93
4	0.46	679.7	3.62
5	0.51	922.5	4.91
6	0.58	613.4	3.27
7	0.64	520.4	2.77
8	0.80	15242.8	81.17

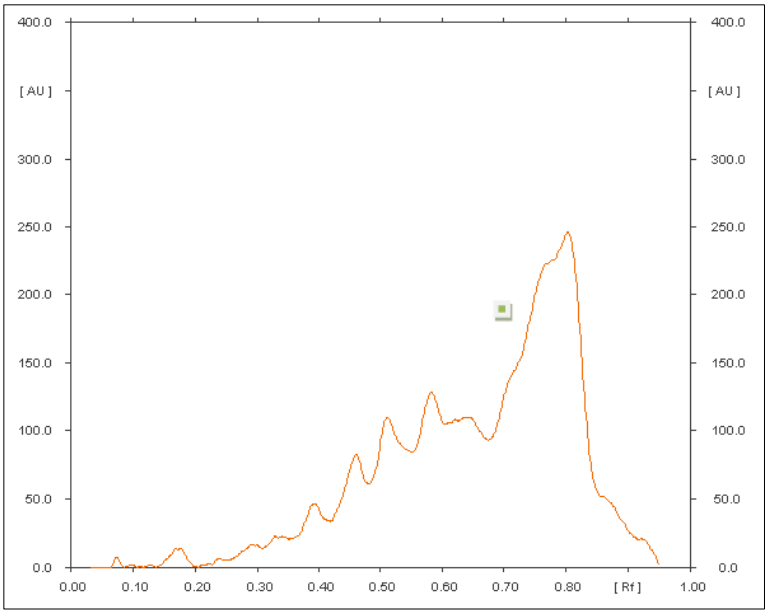
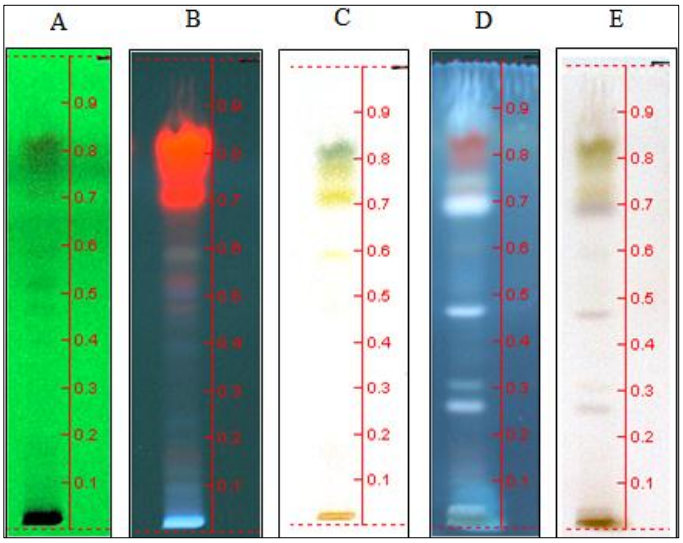


Fig 2: An overview of *Acanthuus ilicifolius* L., sample at 254



Plates 1: Phytochemical Profile of *Acanthuus ilicifolius* L., before (A, B & C) and after (D & E) derivatization

The methanol (leaf) extract scanned at 366 nm wavelength signified the existence of seven 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 1.11%. Peak two with an R_f value of 0.39with area of 1.21 %. Peak three with an R_f value of0.46 and area of 3.02%. Peak four showing R_f value of 0.51 with area 1.50%. Peak five showing an R_f value of 0.58 with an area of 10.03%. Peak six showing R_f value of 0.71 with area of 8.82% Peak seven showing R_f value of 0.81 with area 74.31%. The total peaks present in HPTLC profile of *A. ilicifolius* is seven with an area of 19671.6(AU). (Table 2, Figure 3 and Plate 1).

This is the first study to account the HPTLC fingerprint of methanol extracts of *A. ilicifolius* leaves showing topmost number of components using Toluene: Ethyl acetate: Formic acid: Methanol: 7:5:1:0.5 as solvent system. at a wavelength of 254nm and 366 nm.. The present study gives enough information regarding various phytochemicals present in the methanol extract of *A. ilicifolius.*

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

Peak No	R_f value	Area(AU)	% Area (AU)
1	0.07	218.9	1.11
2	0.39	237.8	1.21
3	0.46	593.8	3.02
4	0.51	295.9	1.50
5	0.58	1972.9	10.03
6	0.71	1734.8	8.82
7	0.81	14617.5	74.31

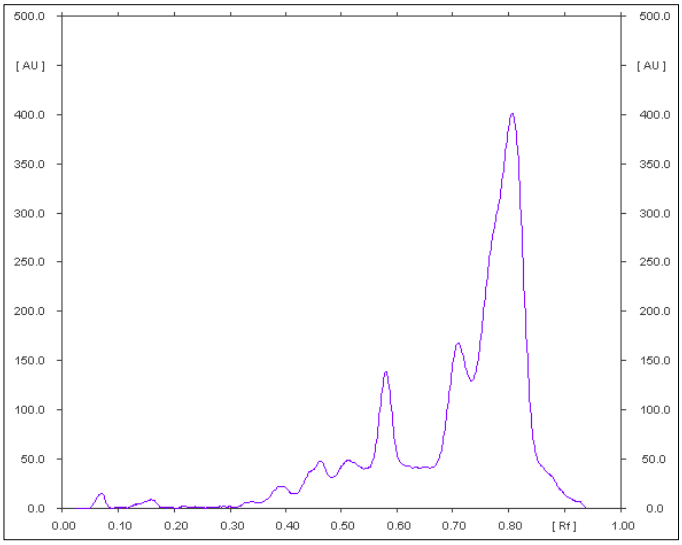


Fig 3: An overview of *Acanthuus ilicifolius* L., sample at 366 nm.

4. Conclusion

HPTLC fingerprint is a superior technique to check the genetic variability present in plant species. It is extreme tensible for selection of stationary and mobile phase, developing techniques, detection and derivatization simple. Further research is necessary to extract the compounds from *A. ilicifolius* and it will give a programme for further phytochemical and pharmacuetical applications.

5. Acknowledgment
The authors thank to CMS College Kottayam, Kerala for providing required facilities to carry out this research work

6. References

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