



Research Article

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ISOLATION AND PURIFICATION OF BIOACTIVE COMPOUNDS FROM THE MEDICINAL FERN *ADIANTUM LATIFOLIUM* LAM.

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ABSTRACT

The Pteridophytic plant *Adiantum latifolium* Lam. is an important medicinal fern belongs to the family Adiantaceae. The plant is popularly known as “broad leaf maidenhair fern” and they possess shiny black rachis, which is employed in folk medicine Worldwide. The plant is called “Avenca” in Brazil and famous for anti-inflammatory, anti-infectious and diuretic Properties. *Adiantum latifolium* Lam. has been used in Latin American traditional medicine as anxiolytic and analgesic. In the current investigation, Ethyl acetate extract of *Adiantum latifolium* Lam. was subjected to column chromatographic separation, which was successively eluted with Ethyl acetate, hexane, chloroform, methanol and their mixtures of increasing polarity. Elution with Chloroform: Methanol afforded a yellow color and this fraction was separated. The presence of Secondary metabolite Flavonoid was detected by Thin Layer chromatography, by recording R_f values. The isolated Phytoconstituent flavonoid was studied by Ultraviolet Visible Spectrophotometer and Fourier transform infrared spectroscopy. Ultraviolet Visible spectroscopy profile showed the peaks at 340 and 304 with the absorption of 0.072 and 10.061 respectively; which was taken at the wavelength of 100-1100 nm. The infrared spectrum confirmed the presence of Amines, Alkenes, Alkyl halide in the plant extract. Thus, the present experimental plant *Adiantum latifolium* Lam. possesses compounds with different curative properties and proved the beneficial role of bioactive compounds in human health is well documented. Further analysis for phytochemicals as well as therapeutic molecule present in this plant is continued.

Keyword: *Adiantum latifolium* Lam, Chromatography, Spectroscopy studies, secondary metabolites, phytoconstituent

INTRODUCTION

The Pteridophytes are considered to be the primitive vascular plant group which are scattered all over the World. These plants are considered as an essential group and a great deal of phytochemical work has recently been aimed at resolving relationship among the desperate groups¹. Among the Pteridophytic plants, *Adiantum* is a genus of about 150 species belonging to the family Adiantaceae²⁻⁵. The present experimental plant *Adiantum latifolium* Lam is distributed in Tropical America from Mexico to South America, as well as the Greater Antilles, Virgin Islands and Trinidad. In India it is distributed throughout Kerala, whereas in Tamil Nadu it is reported in Kanyakumari District. The experimental plant is a Terrestrial species with widespread creeping rhizome, often branched, up to 0.4 cm thick, densely scaly all over, apex is acuminate, margin sparsely fimbriate, pale brown hairs and scales densely distributed all over the costa and rachis.

Various Species of *Adiantum* was traditionally used for Respiratory problems such as cough, cold, fever, pneumonia and mucous formation⁶⁻⁸. *Adiantum latifolium* Lam. has been used in traditional medicine as anxiolytic and analgesic for many years⁵. Based on the references, medicinal and phytochemical trials^{9,10} the present experimental plant is explored for its bioactive compound through isolation and purification techniques like Column and Thin layer chromatography, and evaluation through UV- Vis and FTIR spectroscopic studies.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Sample

Experimental plant *A. latifolium* Lam. was collected from Kanyakumari District (Tamil Nadu) and were authenticated by Dr. Raju Antony, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram, Kerala. A voucher specimen was deposited in the Department of Botany, (Voucher No. 001).

The collected plants were washed by using running tap water and then shade dried until the whole plant was ready to grind. The dried sample were ground and stored for further process. To prepare the sample for Column and TLC, the powdered plant sample was first defatted with hexane for two times, then extracted with ethyl acetate by soaking for 24 hours and this extract was used for further separation and purification process.

Column Chromatography

The slurry was prepared by using silica gel. A well-stirred suspension of silica gel (120 gram) in petroleum ether was poured into a column. The plant sample extract was loaded on the silica gel column. The column was successively eluted with ethyl acetate, hexane, chloroform, methanol and their mixtures of increasing polarity¹¹.

Thin Layer chromatography

The fraction obtained from the column chromatography were drawn with capillary tubes and applied as spots on a Stationary Phase i.e.) silica-gel coated plate, about 1 cm from the base. The Plate was dipped into a suitable solvent system such as n-Butanol, Acetic acid and water in the ratio of 4:1:5. The plate was placed in a container with enough solvent in a well-covered tank. The solvent migrates up the Plate. As the solvent rising through, thin layer separates different components of the mixture at different rates which appear as spots in the thin layer. After the solvent has reached almost the top edge of the plate, nearly 3/4th of the plate, the plate is removed from the tank and dried briefly at moderate temperatures 60-120°C. The presence of Secondary metabolites in the extracts was detected through thin layer by spraying suitable reagent like Ammonia¹². Also, the R_f value could be calculated to identify the compound.

R_f Value

It is a ratio of distance travelled by the sample and distance travelled by the solvent.

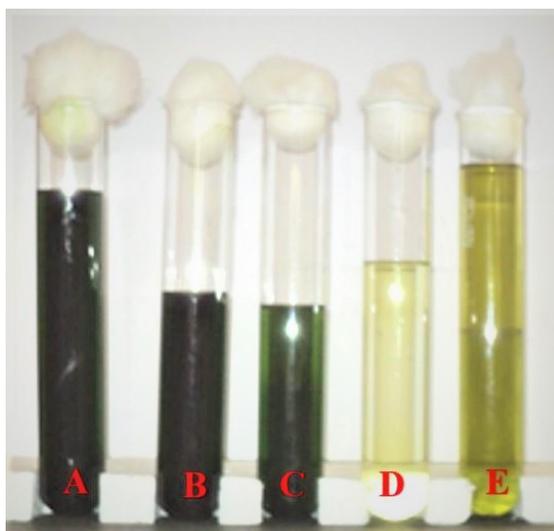
$$R_f = \frac{\text{Distance of the sample (solute) from the origin}}{\text{Distance of the solvent from origin}}$$

Ultraviolet Visible Spectrophotometer- UV-Vis

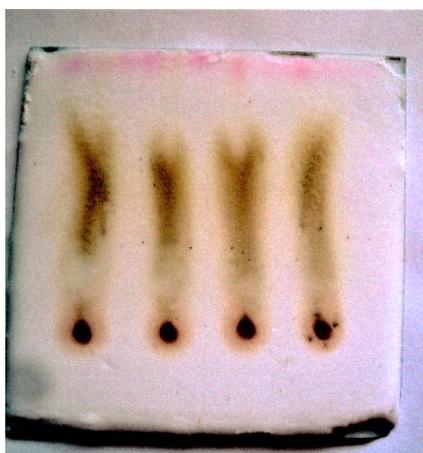
UV-Vis Spectroscopy is used to find out the Absorption Maxima of compounds with a wide range of wavelength. To detect the UV-VIS spectrum profile, the extract eluted from TLC was scanned with the wavelength ranging from 100 to 1100 nm by using lamda 35 model spectrums. The absorption values for wavelength of UV-Vis spectrum was tabulated¹³.

Fourier transform infrared spectroscopy - FTIR

The isolated extract eluted through the TLC were used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample disc and loaded in FTIR Spectroscope. Analysis was performed to detect the characteristic peaks and their functional groups using Perkin Elmer spectrophotometer system at range of 400 to 4000/cm. with a resolution of 4cm⁻¹. Peak values were recorded, and functional groups were analyzed¹⁴.



A). EtOAc (B). Hexane, (C). Chloroform, (D). Methanol (E). Chloroform : Methanol (7:1)
Plate 1: Column Chromatographic fractions of *Adiantum latifolium* Lam.



A



B

Plate 2: Thin layer chromatography of *Adiantum latifolium* Lam

Table 1: Analysis of Bioactive compound in *A. latifolium* Lam. by Thin layer chromatography

Phytoconstituents	Rf Value	Results	Literature (Gordana, 2003)
Flavonoid	3.3/4.6	0. 71	(Rf = 0.72)

Table 2: UV-Vis Spectrum of *A. latifolium* Lam.

Wavelength (nm)	Absorption
304	10.061
340	0.072

UV-VIS: Ultraviolet visible

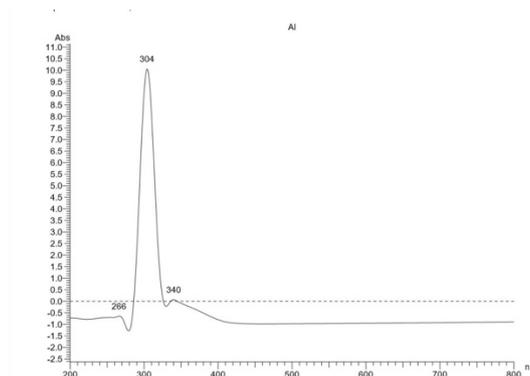


Figure 1: UV-Vis Spectrum of *A. latifolium* Lam.

Table 3: FTIR spectral peak values and functional groups of *A. latifolium* Lam.

S. No.	Nm	Functional Group	Molecular motion
1	3325	Amines	N-H
2	1637	Alkenes	C=C
3	655	Alkyl halide	C-Cl
4	597	Alkyl halide	C-Br
5	557	Alkyl halide	C-Br

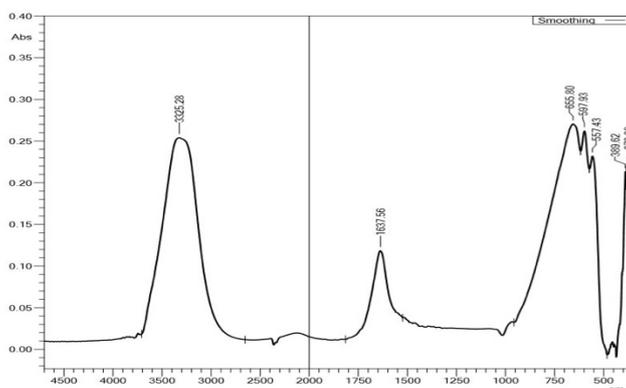


Figure 2: FTIR spectrum of *A. latifolium* Lam.

RESULTS AND DISCUSSION

Chromatographic Separation of compounds from *A. latifolium* Lam.

The column with sample extract was successively eluted with Ethyl Acetate, Hexane, Chloroform, Methanol and Combination of Chloroform and Methanol 7:1 ratio on increasing polarity. When eluted with chloroform and methanol in 1:7 proportions the elution showed yellow color (Plate - 1). The selection of solvent in systematic order proves the effect of polarity on the extraction and the extracted phytochemicals¹⁵. The resulted column selected fraction of *A. latifolium* Lam. sample showed yellow color and it indicates the presence of flavonoid. The presence of flavonoids

was already proved by qualitative phytochemical test as per standard procedure¹⁶.

Thin Layer Chromatography expresses the following results from the powder sample extract of *A. latifolium* Lam. Solvent Phase of n-Butanol, Acetic acid, and Aqueous (4:1:5) was used for eluting the experimental sample. Similarly, the selected solvent was used in the methanolic leaf extract of *Vitex negundo* in TLC technique¹⁷. The extract of *A. latifolium* Lam. applied as a spot, reached Rf value of 0.72 in the present thin layer chromatography plate¹⁸ (Plate - 2). This Rf value 0.72 when compared with literature data was identified as flavonoid compound in the ethyl acetate extract of *A. latifolium* Lam. (Table 1)¹⁹. The precise

position and relative intensities of these maxima give valuable information on the nature of the flavonoids²⁰

Similarly, the plants of *Calendula officinalis* contain flavonoid-glycosides in the ethyl acetate, n-butanol and water extracts²¹. Studies also proved that Flavonoid compounds are used as Antioxidative Activity, Hepatoprotective, Anti-Inflammatory, Anticancer and Antiviral activity which is obtained from *Vitex negundo* L plant extracts¹⁷.

The flavonoids in significant quantities are incorporated into the human systems through the regular diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens and so may be biological "response modifiers". *In-vitro* studies show that flavonoids also have anti-microbial activity²². The flavonoids are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in fruit, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey"²³.

UV - Visible and FT-IR spectral analysis of sample isolated from Chromatography

UV-Vis spectrum of *A. latifolium* was taken at the wavelength of 100-1100 nm which resulted peaks at 340 and 304 with the absorption of 0.072 and 10.061 respectively (Figure 1 and Table 2). The present study of UV-VIS spectrophotometer also clearly revealed the presence of flavonoid compound. The UV-Vis result of the present experimental plant coincides with literature data obtained for the presence of flavonoids in *Vitex negundo* plant extract²⁴.

The FTIR spectrum as per studies provides the functional constituents present in the plant extract. Based on the peak value in the region of infrared radiation, the results of FT-IR Spectrum are tabulated in Table 3. The results of FTIR analysis confirmed the presence of the Amines (N-H), Alkenes (C=C), Alkyl halide (Cl and C-Br) as shown in Figure 2 and Table 3. The present functional groups are also reported in various medicinal plants such as *Aerva lanata* (L.)Juss.ex Schult., ethanolic extracts of *Ichnocarpus frutescens* (L.), *Eclipta alba* (L.) and *Eclipta prostrate* (L.) and these bioactive compounds are responsible for various medicinal properties.^{25,26}

CONCLUSION

The Chromatographic and spectroscopic results on the Pteridophytic experimental plant *A. latifolium* Lam. has reported the presence of flavonoid compound such as flavonoid glycoside in the fraction of ethyl acetate extract. The presence of medicinally important phytochemical such as flavonoid was strengthened by the RF value of the compound. The analytical method UV-Vis FTIR analysis also further confirmed the presence of total flavonoid in the extract of experimental plant *A. latifolium*. The results indicate the importance of the bio actives present in the Pteridophytic plants as strong therapeutic agents against dreaded diseases.

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