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Research Article

FINGER PRINTING ANALYSIS OF THE ALKALOIDS FROM *SPHAERANTHUS AMARANTHOIDES* LEAVES USING HPTLC ANALYSIS

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ABSTRACT

Objective: The present study is conducted to identify the alkaloids from leaf ethanolic extracts of *Sphaernathus amaranthoides*.

Methods: The leaves ethanolic extracts was subjected to column chromatography and elutes were collected. The collected elutes were analyzed using TLC and HPTLC.

Results: The collected elutes were showed the presence of alkaloids in the preliminary phytochemical analysis, further proved the presence of alkaloids with the TLC and the Rf value is 0.85 and from HPTLC there are nine different Rf values were identified which refers to nine alkaloids.

Conclusion: With the above Rf values we have concluded the presence of alkaloids.

Keywords: phytochemical ; *Sphaeranthus amaranthoides* leaf extracts; alkaloids;TLC;HPTLC.

INTRODUCTION

Plants are well known for the primary and secondary metabolites like carbohydrates, proteins and amino acids and flavonoids, phenolics, glucosides, saponins, tannins, terpenoids and coumarins etc. These secondary metabolites impart medicinal properties to the plants [1]. Therefore it is mandatory to resolve the type of secondary metabolites, nature and their pharmacological, antimicrobial and clinical research, to reveal their bioactivities, to identify the active components and their side effects and to enhance the purity of the pharmacologically important active compounds [2]. These active secondary metabolites are qualitatively and quantitatively estimated by various techniques like spectroscopy and chromatography. Chromatography techniques are the popular tools for the separation and identification of the bioactive compounds. Thin layer and High performance thin layer chromatography can be applied for this identification. HPTLC finger print analysis help in the identification of the biochemical constituents of the plant[3].

Sphaeranthus amathanthodies is widely distributed in the regions of Asia, Africa, it is weed in the paddy fields. Traditionally plant is used in the treatment of tumors in Tirunelveli district in Tamilnadu. The ethanolic extracts of the plant possess antidiarrhoel, antibacterial, hepatoprotective, and antioxidant, wound healing activities. With the above information the present study was focused towards the HPTLC finger printing analysis of the medicinally important weed *sphaeranthus amaranthoides.*

MATERIALS AND METHODS

Collection of plant material and preparation of crude extract

Sphaeranthus amaranthoides Burm. F (Asteraceae) plant leaf was collected from Tirunelveli district. This Plant was examined and botanically identified by a botanist V. Chelladurai Research Officer-Botany. The collected leaf was shade dried for three weeks to get consistent weight and made in to coarse powder and is used for further studies.

Preparation of ethanolic extract

The leaf powder was soaked in the petroleum ether for two days to dissolve the chlorophyll and then the leaf material was transferred in to the ethanol for five days. On fifth day leaf material was filtered. The filtered extract was subjected to rotaryevapour to remove the ethanol and to get the concentrated extract in powder form.

Purification of alkaloids from crude extract

The order of solubility for alkaloids Chloroform > acetone > ethanol > methanol > ethyl acetate>ether>n hexane Silica gel G60 emulsion was used as a stationary phase in glass column. (1.5×50cm) and chloroform and acetone (10:1, 9:2, 8:3, 7:4) were used as mobile phases.. A larger section of the alkaloids are easily soluble in chloroform and relatively less soluble in other organic solvents [4]. The Uv-vis spectrophotometer was used to measure the absorbance of the alkaloids on a Cary E-100 Varion type Spectrophotometer. The absorbance was measured between 200nm-800 nm.

Thin layer chromatogrphy

The elutes were spotted on TLC (20×20×2mm) and manifested by Dragendroff`s and Mayer`s reagents, then the compounds of similar Rf were collected in one group for further analysis [5-6]. The plates were visualized basing on the chemical structure of the compound at visible light, UV-254 nm and 365 nm and by using spray reagents [7].

High performancethin layere chromatography

HPTLC was used for further analysis of purity of the alkaloids. HPTLC studies were carried out following Harborne [8] and Wagner *et al* [7]. N-hexane: ethyl acetate: formic acid (100:13.5:10) were used as mobile phase for alkaloids. In the present study a densitometric HPTLC was performed for the characteristic development of alkaloid finger print profile. The sample was loaded in TLC silica gel plates 60F 254 5.0x10.0 cm. using hamilton syringe and CAMAG Automatic TLC Sampler 4 (ATS4). After loading the sample the TLC plates were placed in the in trough developing chamber. The mobile phase used was n-Hexane:Ethyl acetate: Formic acid(60:40:1.0). The developed plates were dried using hot air oven at 60^0c to evaporate the solvent and sprayed with the reagent to detect the alkaloids. The plate was kept at photo documentation chamber CAMAG TLC reprostar Scanner 3. The images were captured at white and UV366nm. Before derivatization, the plate was fixed in scanner stage and scanning was done at 254nm. The Peak table, Peak display and Peak densitogram was identified.

RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the enthanolic extracts confirms the presence of flavonoids, alkaloids, saponins, tannins,

phenolic compounds, steroids, terpenoids. The extract was subjected to column chromatography to separate alkaloids. Alkaloids were identified from these elutes when subjected to qualitative analysis (table 1).

Table: 1 Qualitative analysis for Alkaloids

Test	Observations	Results
Mayer Reagents	Cream precipitate was formed in significant amount	+
Wagner Reagent	Dark Brown precipitate was formed in significant amount	+
Dragendorff Reagent	Orange precipitate was formed	+
Hager's Reagent	Dark Yellow colour precipitate was formed	+

THIN LAYER CHROMATOGRAPHY

The TLC plate was developed by using n-Hexane:Ethylaceate:formic acid(60:30:10). The plate was sprayed with Dragandraffs and mayer's reagents. The sample was analysed and visualized using UV254nm. The sample showed in a light orange colour with 0.85 Rf(Figure:1)

Fig.1

High performance thin layer chromatography for alkaloids

The HPTLC profile was shown in table 2. The orange coloured bands showed in the chromatogram under visible light after derivetisation. After spraying the 10% H_2SO_4 orange coloured bands were changed into brown colour this confirms the presence of the alkaloids(figure 2, 3 and 4a&b). Ten alkaloids were identified with different Rf values, the Rf values are 0.15,0.24,0.29,0.32,0.38,0.61,0.75,0.84,0.90,0.97 of the peaks 1,2,3,4,5,6,7,8,9,10 respectively. The Rf value peak and area were given in the table 2.

Previous studies indicate that alkaloids from different plants have good cytotoxic and antimicrobial activities. The phytochemical analysis of ethanolic extract revealed the presence of the secondary metabolites [9]. The alkaloids present in the plant extract are used to treat psoriasis, chronic skin eruptions and chronic rheumatism and also may show a good antibacterial activity against *staphylococcus epidermidis and Klebsiella pneumonia, and Escherichia coli*[10].

CONCLUSION

Due the adverse effects of synthetic antibiotics and anticancer agents, in recent years scientists are on search for alternative medicine. There are some diseases which are chronic and needs a long duration medication, plant based drugs are less toxic and have no side effects. The alkaloids isolated from the *sphaeranthus amaranthoides* may have promising antibacterial and anticancer property which will offer a possibility to lead a molecule for drug development.

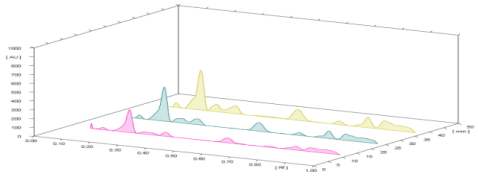

Fig.2: HPTLC

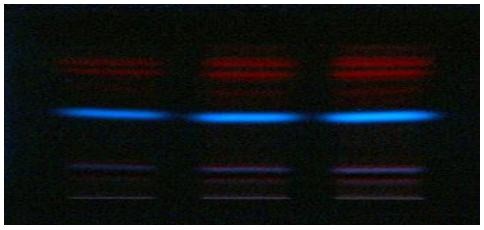

Fig 3

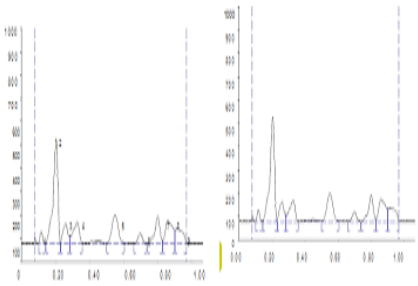

Fig. 4: a&b Graph showing the densitogram of the different alkaloids

Table: 2

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.10	0.3	0.12	52.0	4.66	0.14	0.3	693.7	2.29	unknown *
2	0.14	0.5	0.20	450.5	40.32	0.23	1.5	10751.1	35.52	unknown *
3	0.23	3.8	0.26	84.7	7.58	0.28	30.1	2147.1	7.09	unknown *
4	0.29	30.3	0.33	91.1	8.15	0.36	0.2	2826.7	9.34	unknown *
5	0.50	1.9	0.55	122.2	10.94	0.60	1.6	3886.5	12.84	unknown *
6	0.66	0.1	0.70	42.3	3.79	0.74	0.0	986.7	3.26	unknown *
7	0.74	0.0	0.80	116.0	10.38	0.83	12.0	2948.5	9.74	unknown *
8	0.83	12.0	0.86	97.6	8.73	0.90	58.1	3589.9	11.86	unknown *
9	0.90	58.2	0.92	61.1	5.46	0.97	3.4	2438.1	8.06	unknown *

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