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Research Article

Fluorescence Detection of Phytoconstituents in Hydroalcoholic Extract of *Ipomoea aquatica* Forssk. by Thin Layer Chromatographic Analysis

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ABSTRACT

Introduction: The medicinal plant water spinach is applicable for many therapeutic activities like anti cancer, anti oxidant, etc. the plant is used for purification of metals and waters of different categories. The benefit of hydroalcoholic extract in this study is non toxic, preservative and high yield of extract from the herbal powder. Thin layer chromatographic analysis is one of the best analytical methods for the separation and identification of phytoconstituents from the crude extract. The spots separated on the stationary phase could be detected by using the non destructive fluorescence method. The detection will be done at shorter and longer wavelength in UV cabinet. Aim and Objective: The aim was to separate and identify the various active metabolites present in the crude extract of the herb of interest using R_f value by thin layer chromatographic analysis. Method: The thin layer chromatographic parameters were set by trial and error method. Ethyl acetate: Methanol: Water (10.0:1.35:1.0 v/v/v) for alkaloids, Ethyl acetate: Pyridine: Water (5.0:1.0:4.0 v/v/v) for glycosides, Toluene: Acetone: Formic acid (4.5:4.5:1.0 v/v/v) for tannins and Toluene: Ethyl acetate (9.3:0.7 v/v) for triterpenoids were the mobile phases for the individual constituents. The constituents were identified at 365nm. Results and Discussion: Nearly 24 various constituents of alkaloids, glycosides, flavonoids, triterpenoids and tannins were separated using respective mobile phases through R_f value. Conclusion: The mentioned TLC profile for the plant of interest will hopefully help the researchers in herbal field to develop more herbal medicines requiring the particular constituent or its extract.

Keywords: TLC**,** R_f, alkaloids, glycosides, flavonoids, tannins, triterpenoids and UV light.

INTRODUCTION

Extraction methods are widely used to get phytoconstituents from the plant. This is the basic method to detect the compounds present in each and every plant. The application of this process for an important medicinal plant *Ipomoea aquatica* helps in revealing its phytochemicals. These are formed in the primary and secondary metabolism of the herb. The metabolic products are extracted into suitable solvents in which those are soluble. The solvents for extraction are available with various polarities. Mostly therapeutically active secondary metabolites of the plant will come into polar solvents like alcohol, water, etc. Thereby extraction is found as the most important basic method to recognize the phytochemical potential of a natural source[1-6].

Phytochemical Analysis is devoted to the publication of original articles concerning the development, improvement, validation and/or extension of application of analytical methodology in the plant sciences. The spectrum of coverage is broad, encompassing methods and techniques relevant to the detection (including bio-screening), extraction, separation, purification, identification and quantification of compounds in plant biochemistry, plant cellular and molecular biology, plant biotechnology, the food sciences, agriculture and horticulture[7-17].

Phytoconstituents of the herbals are important source for herbal drug development. Hence, the study on detection about those is playing majorly in the pharmaceutical, botanical, agricultural and other life sciences fields. These chemicals are divided into two categories based on its metabolism such as primary and secondary metabolites. Among this, the later one acts as ailments in treating many diseases in human and are known as traditional herbal medicine[18-26].

Ipomoea aquatica belonging to the family Convolvulaceae is a semi-amphibious, equatorial plant ripened as a vegetable for its tender boughs and leaves. It is beginned all over the tropical and subtropical domains of the planet, although it is not acknowledged where it was emerged[27-38].

The medicinal plant water spinach is applicable for many therapeutic activities like anti cancer, anti oxidant, etc. the plant is used for purification of metals and waters of different categories. The benefit of hydroalcoholic extract in this study is non toxic, preservative and high yield of extract from the herbal powder. Thin layer chromatographic analysis is one of the best analytical methods for the separation and identification of phytoconstituents from the crude extract. The spots separated on the stationary phase could be detected by using the non destructive fluorescence method. The

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Table 1: Plant details.

S. No.	Parameters	Subject
1.	Plant Name	Water Spinach
2.	Botanical Name	*Ipomoea aquatica* FORSSK.
3.	Family	Convolvulaceae
4.	Location	Parambikulam – Aliyar Riverine, Pollachi
5.	Part of the plant	Whole plant
6.	Authentication No.	BSI/SRC/5/23/2017/Tech./3269
7.	Place of Authentication	BSI, Coimbatore-641003, Tamil Nadu, India

Table 2: Instruments used.

S. No.	Name of the Instrument	Model Name
1.	Precision Balance	Wensar
2.	Hot plate	Cintex
3.	Ultra Sonicator	Labman
4.	Electrical Water bath	Technico
5.	UV cabinet	CAMAG and Deep Vision

Table 3: Chemicals/Reagents used.

S.No.	Name of the Reagent	Company	Location
1.	Petroleum Benzine boiling range 60.0^{0}C-80.0^{0}C GR (Petroleum ether)	Merck Specialities Private Limited	Mumbai – 400 018
2.	Pyridine GR		
3.	Ethanol AR 99.9%	Jiangsu Huaxi International Trade Co., Ltd.	China
4.	Distilled water		
5.	Toluene (Sulphur free)	Reachem Laboratory Chemicals Private Limited	Chennai – 600 098
6.	Formic acid LR		
7.	Acetic acid Glacial LR		
8.	Acetone LR		
9.	Methanol LR	S d Fine Chemicals Limited	Mumbai – 400 030
10.	Ethyl acetate LR		
11.	Silica gel G for TCL	Loba Chemie Private Limited	Mumbai – 400 005

detection will be done at shorter and longer wavelength in UV cabinet[39-55].

MATERIALS AND METHODS

Materials

The intended plant contents are given in the table 01. The instruments, chemicals/reagents and glass wares/apparatus effective for the research are described in the table 02, table 03 and table 04 respectively.

Miscellaneous

Aluminium foil, Muslin cloth, Filter paper, Tripod stand, Test tube holders and test tube stands and butter paper.

Methods

Plant Collection, drying and powdering

The plant was collected from Parambikulam – Aliyar Riverine in Pollachi. The collected portions of the plant were washed with distilled water three times. They were allowed to dry under shade kept over the news paper. Then the half dried portions were cut into small pieces using stainless steel knife and kept under shade only for drying completely. It took 22 days for complete drying. The dried material was pulverized into coarse powder by means of manual blender. The powdered plant material was stored in air tight containers at 4.0^{0}C for further use. 350.0g of coarse powder of drug was weighed and was taken in a 5000.0ml Round bottomed flask. Petroleum ether was added to remove the fatty matters associated with the powder. The solvent retained was evaporated at room temperature after rinsing for few minutes. Then the dried defatted powder was immersed in 2000.0ml of solvents which comprises 1000.0ml of distilled water and 1000.0ml of ethanol (50:50 v/v). After 7 days, the content of extraction was strained through a muslin cloth. The marc was separated from the menstruum. The extract was kept at 40.0^{0}C for concentration and evaporation at the same temperature. Then the completely dried extract was cooled to room temperature and weighed[56-66].

Preliminary Phytochemicals Evaluation

The experimental procedures were followed for detecting metabolic products of the plant. From the analysis, primary metabolites such as carbohydrates, proteins & aminoacids and secondary metabolites such as “alkaloids, glycosides, flavonoids, tannins, steroids & triterpenoids” were found positive for the tests carried out[67-75].

Thin Layer Chromatographic Analysis

The existing constituents were separated by using proper mobile phase which was selected based on trial and error method[76-77].

Chromatographic parameters

Stationary phase selection

Principle: Adsorption

Support material: Glass plate

Dimension of the plate: 20.0×10.0cm

Adsorbent: Silica gel G

Method of thin layer preparation: Pouring method

Layer thickness: 1.0mm

Plate activation temperature: at 105.0^{0}C for one hour

Mobile phase selection

Chamber: Twin trough mobile phase chamber

Chamber dimension: 20.0×10.0cm

Mobile phase selected for separation:

Alkaloids

Ethyl acetate: Methanol: Water (10.0:1.35:1.0 v/v/v).

Glycosides

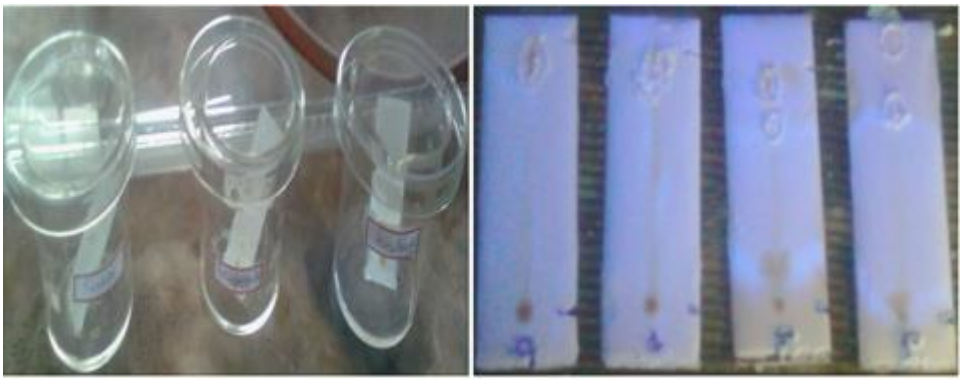

Figure 1: TLC for mobile phase selection (Trial and error method).

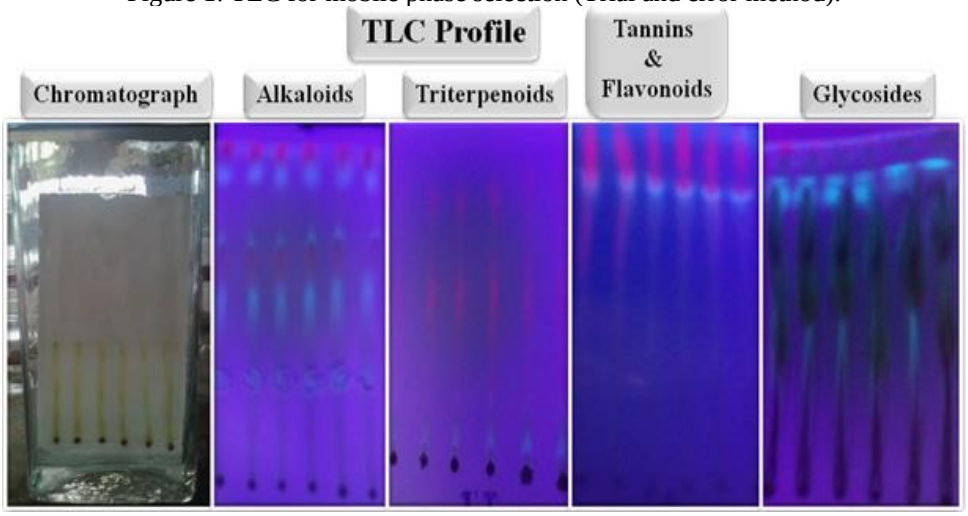

Figure 2: TLC Profile of Hydroalcoholic extract of *Ipomoea aquatic.*

Table 4: Glass wares/Apparatus used.

S. No.	Name of the Glassware	Capacity	Brand Name
1.	Round bottomed flask	1000.0ml	Riviera
2.	Funnel	Medium Size	Sh Borosilicate Glass
3.	Beaker	1000.0ml	Borosilicate Glass
4.	Measuring cylinder	10.0ml	Riviera
5.	Measuring cylinder	50.0ml	Sh Borosilicate Glass
6.	China dish	Big & Small size	Chinese Porcelain
7.	Stirrer	Small size	Sh Borosilicate Glass
8.	Conical flask	250.0ml	Borosilicate Glass
9.	Test tubes	10.0ml	Borosilicate Glass
10.	Pipettes	5.0ml	Borosilicate Glass
11.	Mobile phase chamber (Twin trough)	20×10cm	CAMAG
12.	Beaker	250.0ml	Borosilicate Glass
13.	Petridish lid	Medium size	Borosil S - Line

Ethyl acetate: Pyridine: Water (5.0:1.0:4.0 v/v/v).

Tannins and Flavonoids

Toluene: Acetone: Formic acid (4.5:4.5:1.0 v/v/v).

Steroids and Triterpenoids

Toluene: Ethyl acetate (9.3:0.7 v/v).

Chamber saturation time: 45.0 minutes

Fluorescence detection: at 365.0nm (Longer wavelength)

Calculation of R_f value:

$$R_f = \frac{Distance\ travelled\ by\ the\ solute\ from\ the\ sample\ application\ position}{Distance\ travelled\ by\ the\ solvent\ from\ the\ sample\ application\ position}$$

Ideal R_f value: 0.1-0.9

RESULTS AND DISCUSSION

The TLC mechanisms are interpreted in the figure 01, figure 02 and table 05.

The existence of separated compounds on the stationary phase adsorbent was noticed with different colour of fluorescence at the longer wave length. It was pointed out that the presence of more than ten variable metabolites. These could be quantified, isolated and characterized by using suitable pharmaceutical analytical techniques.

CONCLUSION

Table 5: Thin Layer Chromatographic Analysis.

S. No.	Sample of interest	Mobile phase	Develop-ment time (min)	Fluorescence spot colour at 365nm	Distance travelled by the solute (cm)	Solvent Front (cm)	R_f value
1.	Alkaloids	Ethyl acetate: Methanol: Water (10.0: 1.35: 1.0 v/v/v)	33	Green	0.9	13.6	0.07
				Blue	1.8		0.13
				Green	3.0		0.22
				Blue	4.7		0.35
				Green	6.5		0.48
				Blue	7.5		0.55
				Dark Blue	10.6		0.78
				Green	11.9		0.88
				Pink	12.8		0.94
2.	Glycosides	Ethyl acetate: Pyridine: Water (5.0: 1.0: 4.0 v/v/v)	107	Blue	5.2	12.3	0.42
				Green	6.9		0.56
				Green	8.4		0.68
				Blue	10.3		0.84
				Blue	10.8		0.88
				Pink	11.8		0.96
3.	Tannins and Flavonoids	Toluene: Acetone: Formic acid (4.5: 4.5: 1.0 v/v/v)	30	Brown	3.0	13.2	0.23
				Blue	10.3		0.78
				Pink	10.9		0.83
				Pink	12.5		0.95
4.	Steroids and Tri-terpenoids	Toluene: Ethyl acetate (9.3: 0.7 v/v)	25	Blue	0.7	12.8	0.06
				Green	1.4		0.11
				Pink	2.9		0.23
				Pink	4.6		0.36
				Pink	7.5		0.59

TLC is the best sensitive analytical method for the separation and identification of natural products. The non destructive method of detection supports the separated constituents for isolation by means of scrapping method. The various colour of fluorescence indicates that the presence of different type of similar compounds. After isolation, the particular compound could be characterized using spectroscopic analytical techniques. The mentioned TLC profile for the plant of interest will hopefully help the researchers in herbal field to develop more herbal medicines requiring the particular constituent or its extract.

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