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Analytical Detection of Triterpenoids Present in the Hydroalcoholic Extract of *Ipomoea Aquatica* Forssk. in South India

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Abstract : Introduction: Qualitative analysis will help in the detection of phytoconstituents present in the herbal source accurately. The preliminary phytochemical screening and the thin layer chromatographic analysis are found more simple and sensitive and selective techniques in this way.**Aim and Objective:**The research was aimed to reveal the secondary metabolites like triterpenoids by using chemical tests and TLC methods. **Methods:** The tests for detecting triterpenoids were using Salkowski' sreagent and Sulphur powder tests. The TLC parameters were set silica gel G as adsorbent, Toluene: Ethyl acetate in the ratio of 9.3:0.7v/v as mobile phase, UV light of longer wavelength at 365nm as detection wavelength and R_f value as qualitative respect. **Results and Discussion:**The phytochemical tests were shown positive results for triterpenoids. The TLC analysis stated that the presence of nearly five different fluorescence spots with R_f values of 0.06, 0.11, 0.23, 0.36 and 0.59 respectively. **Conclusion:**Hence, revealing new class of lipophilliccomponents will assist to improve herbal drug products in the global market. This study could be used in research laboratories for detecting similar type of compounds using TLC analysis. Definitely this will give the good for opportunity for isolating out many therapeutically acting compounds.
Key words : Salkowski, lipophillic, triterpenoids, R_f value, water spinach,TLC.

Introduction

Triterpenoids, which are widely distributed natural compounds, are usually classified into three groups: acyclic, tetracyclic and pentacyclic. Triterpenoids have been shown with anti-cancer, anti-inflammatory, anti-proliferative, and anti-Alzheimer activities. A large number of bioactive pentacyclictriterpenoids, such as oleanolic acid, glycyrrhizin, glycyrrhetinic acid, ursolic acid, betulin, betulinic acid and lupeol have shown multiple biological activities with apparent effects on glucose absorption, glucose uptake, insulin secretion, diabetic vascular dysfunction, retinopathy and nephropathy. The versatility of the pentacyclictriterpenes provides a promising approach for diabetes management [1-2].

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Terpenoids are chemically lipid-soluble compounds and they can be extracted with petroleum ether generally. Sesquiterpene lactones, diterpenes, sterols and less polar triterpenoids extraction can be also performed by using benzene, ether and chloroform. Ethyl acetate and acetone extracts contain oxygenated diterpenoids, sterols and triterpenoids. Ethanol, methanol and water led to the extraction of highly oxygenated namely polar triterpenes as well as triterpenoid and sterol glycosides. Total extraction of the material carried out by any polar solvents such as acetone, aqueous methanol (%80) and aqueous ethanol and then re-extraction with hexane, chloroform and ethyl acetate is also leads to successive extraction of terpenoids and sterols [3-4].

TLC is a primary, easy to use and solvent used are unhazardus with no requirement of sophisticated instruments. Usually it is composed of stationary phase and mobile phase, which are performed on a sheet of solid surface such as glass, plastic, aluminum foil that is coated with absorbent material such as silica powder, aluminum oxide and cellulose, which is called as stationary. Mobile phase may consist of single or mixture solvents depending on extracts to separate. This mobile phase is drawn up through the stationary phase by capillary action allowing separation of various compounds on the basis of their solubility and retardation in stationary phase and mobile phase. Separation is achieved by competition of the solute molecules and the mobile phase for binding places on the stationary phase. The most common stationary phase used is a silica gel which is polar in nature, in case if two compounds in extracts have different polarity, highly polar compound will have strong interaction with silica and separated out initially in no time. Fewer polar compounds will separate in second position that has little interaction with stationary phase. On contrary, non polar compound will separate last, which would have non interaction with the stationary runs a longer distance on the plate. The complete understanding of handling TLC is handy for designing research to analysis, separate medicinal significant compounds. Optimization of the solvent system for TLC profiling for identification of amino acid, amines, alkaloids and secondary metabolites of curable plants is highly useful for production of medicinally prominent medicines and novel pharmaceutical products [5-6].

Experimental Procedure

Materials

The planned plant contents are tabulated in the table 01. The instruments, chemicals/reagents and glass wares/apparatus practical for the research are represented in the table 02, table 03 and table 04 individually.

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 4.0^0C 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 menstrum. The extract was kept at 40.0^0C for concentration and evaporation at the same temperature. Then the completely dried extract was cooled to room temperature and weighed [7-8].

Preliminary Phytochemicals Evaluation

Tests for Triterpenoids

Salkowski's test

1-2mg of the sample dissolved in 1.0ml of chloroform and 1.0ml of concentrated sulphuric acid. Formation of red colour at lower layer indicated the presence of steroiods and formation of yellow colour at lower layer indicated the presence of triterpenoids.

Sulfur powder test

Small amount of sulphur powder to the test solution was added and it had sink at the bottom[7-15].

Thin Layer Chromatographic Analysis

The existing constituents were separated by using proper mobile phase which was opteddepend on trial and error method [16-33].

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 105.0^0C for one hour

Mobile phase selection

Chamber: Twin trough mobile phase chamber
Chamber dimension: 20.0×10.0cm
Mobile phase selected for separation:
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 tests specific for triterpenoids were shown positive results and the details are denoted in the figure 01 and in the table 05.The chromatographic parameters were optimized through trial and error method and it is notified in the figure 02. The profile of TLC was done with the set specifications and those are designated in the figure 03 and table 06.The appearance of lipophillic five numbers of triterpenoids were confirmed by the various qualitative studies commonly meant for herbal analysis [34-38].

FIGURES AND TABLES:

Figure 01: Preliminary Phytochemical Tests for Triterpenoids

Figure 02: TLC for mobile phase selection (Trial and error method)

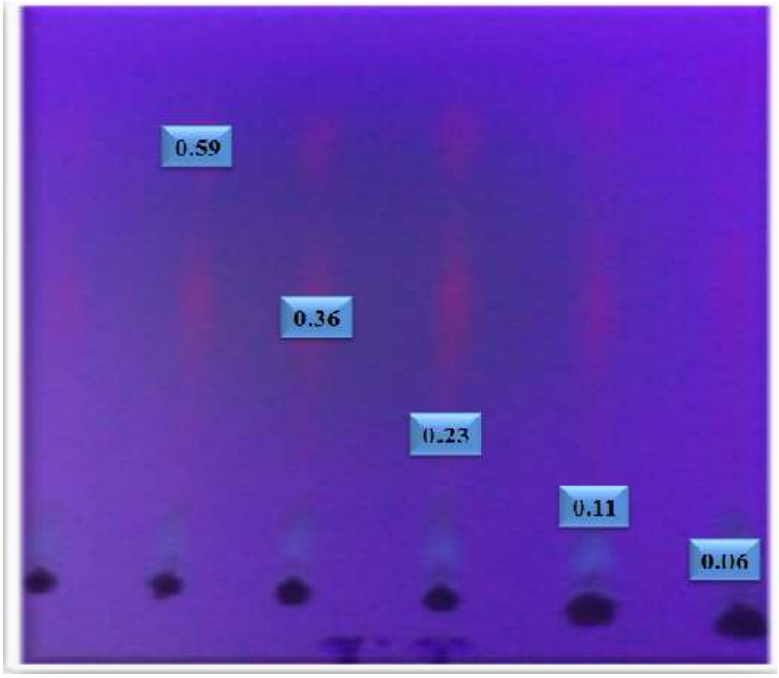

Figure 03: TLC Profile for Triterpenoids in Hydroalcoholic extract of *Ipomoea aquatica*

Table 01: Plant details

S. No.	Parameters	Subject
1.	Plant Name	Water Spinach, River 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 02: Instruments used

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

Table 03: 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.	Ethanol AR 99.9%	Jiangsu Huaxi International Trade Co., Ltd.	China
3.	Distilled water		
4.	Toluene (Sulphur free)	Reachem Laboratory Chemicals Private Limited	Chennai – 600 098
5.	Ethyl acetate LR	S d Fine chemicals Private Limited	Mumbai – 400 030
6.	Silica gel G for TCL	LobaChemie Private Limited	Mumbai – 400 005

Table 04: 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

Table 05: Report for Preliminary Phytochemical Tests forTriterpenoids

S. No.	Phyto-constituents	Chemical test	Observation	Inference
1.	Triterpenoids test	Salkowski's test	Yellow colour at lower layer	+
2.		Sulphur powder	Sulphur sinks at the bottom of the solution	+

Table 06: Thin Layer Chromatographic Analysis for Triterpenoids in Hydroalcoholic extract of *Ipomoea aquatica*

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.	Steroids and Tri-terpenoids	Toluene: Ethyl acetate (9.3: 0.7 v/v)	25	Blue Green Pink Pink Pink	0.7 1.4 2.9 4.6 7.5	12.8	0.06 0.11 0.23 0.36 0.59

Conclusion:

Mostly polar compounds exhibit pharmacological actions. In addition to that, these lipophilic triterpenoids also play a great role in giving therapeutic activities. Hence, this research will have support for the isolation, characterization and activity studies on the mentioned herbal for researchers in herbal industries, botanist, institutions, agricultural and pharmaceutical fields.

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