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Original Research

HPTLC Finger Printing Analysis of Aqueous Root Extract of *Rotula aquatica* Lour. for Steroid and Terpenoid Contents

Vijayakumari B[1]., Sasikala V[1]., Radha S.R[1] and Hiranmai Yadav R[2]*

[1]Department of Botany, Avinashilingam University for Women, Coimbatore-6410043, Tamil Nadu, India

[2]School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar-382 030, Gujarat, India.

Abstract

The medicinal plants are considered to be the most important in the healing system due to their qualities. There is a high demand for the medicinal plants in pharmaceutical and cosmetic industry. The medicinal plants acquire these qualities due to the phyotchemicals present in them. The plant products are friendly to the user and poses minimum or no side effects hence they are preferred for treatment. The plants have many secondary metabolites that give them protection from different biotic and abiotic stresses. The objective of present work was to study the HPTLC fingerprinting of aqueous root extract of *Rotula aquatica*. A CAMAG HPTLC system equipped with LINOMAT 5 applicator, a TLC scanner3, REPROSTAR with 12 bit CCD camera and WIN CATS-4 software were used. In the present study aqueous extract of *Rotula aquatica* plant roots contains steroids and terpenoids in addition to different other components. The HPTLC fingerprinting profile developed for aqueous extract of *Rotula aquatic* will help in proper identification of compounds. By isolating and identifying marker compounds, new drugs can be formulated to treat various ailments.

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***Corresponding Author:**
Hiranmai Yadav R
E-mail:
mayahiranbt@gmail.com

INTRODUCTION

Steroids are a large group of substances that mediate a very wide variety of biological responses. These are phytochemicals that occur in plants and act as a natural defense system for the host plants and provide colour, aroma and flavor. The plants form the basis for synthetic drug discovery. Plant derived medicines have made large contributions to human health and well being. Natural products and secondary metabolites formed by living systems have great potential in human therapy (Aiyegoro *et al.,* 2008; Chew et al., 2011). The natural products have inspired many developments leading to development of synthetic methodologies in developing several analogues of lead compounds with therapeutic potential. They have the potential to treat various ailments (Mukherjee *et al*., 2010).

Plant based products are used for medicinal, therapeutic and other purposes and contain significant amount of biologically active components that impart health benefits (Parimala *et al*., 2012). Standardization and characterization of herbal drug is of concern in every drug industry. The advent of more advanced techniques has lead to the identification, characterization and standardization of the raw materials necessary for drugs formulation more accurately. HPTLC is one of those methods that help to assess the quality of plant extracts and its derived compound or formulation (Selvamani *et al*., 2009; Jain *et al*., 2011). Typical product of HPTLC is a fingerprint individual chromatographic track representing a mixture of organic compounds. It is adopted in the modern validation of herbal drugs. (Reich and Widmer, 2009; Gallo *et al*., 2011; Piccin *et al*., 2012).

Rotula aquatica is a plant widely used in traditional system of Indian medicine for anti-inflammatory, diuretic, laxative and in hemorrhoids treatments. It is found mostly in aquatic systems (Mengi and bakshi, 2009; Mamta et al., 2010). The medicinal value of the plant lies in their component phytochemicals like alkaloid, flavanoid, phenolic compounds and other nutrients. This plant is an important traditional medicine in Ayurveda for kidney and bladder stones (Priya *et al*., 2013) and are proved to be effective in urolithiasis study by Umesh and Christiana (2011). The present study was an attempt to elucidate the active components steroids and terpenoids found in the plant *Rotula aquatica* by HPTLC method.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh plant of *Rotula aquatica* Lour. was collected from Kuttiyadi (Malapuram district) in Kerala State, India. The collected plant material was identified and their authenticity was confirmed by comparing the voucher specimen at the herbarium of Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. The herbarium registered number was BSI/SRC/5/23/2012-13/Tech/415.

Processing of the Plant Material

Freshly collected plant material was cleaned to remove adhering dust, divided into different parts (Leaf, stem, root, flower and fruit) and then dried under shaded. The dried samples were powdered and used for further studies.

Successive Solvent Extraction

The air dried, powdered plant material was extracted in soxhlet extractor successively with petroleum ether, chloroform, methanol and aqueous. Each time before extracting with the next solvent, the material was dried in hot air oven. Finally, the material was macerated using hot water with occasional stirring for 24 hrs and the aqueous extract was filtered. The different solvent extracts were concentrated by rotary vacuum evaporator and then air dried. The dried extract obtained with each solvent was weighed.

High Performance Thin Layer Chromatography (HPTLC) Analysis

A Camag HPTLC system consisting of a Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used for the analysis of aqueous root extract. All the solvents used for HPTLC analysis were obtained from MERCK. The 100mg sample was dissolved in 1ml of HPTLC Grade water and centrifuged at 3000rpm for 5 min and used for HPTLC analysis as test solution. The samples (2µL) were spotted in the form of bands of length 6mm with a Camag microlitre syringe on precoated silica gel glass plate 60F-254 (20X10 cm) with 250µm thickness (E-Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland). The sample loaded plate was kept in thin-layer chromatography (TLC) twin through developing chamber after saturated with solvent vapour with respective mobile phase and the plate was developed in the respective mobile phase up to 90 mm. Linear ascending development was carried out in (20X10 cm) twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate was developed twice with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25±2 °C). The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under white light, UV light at 254 and 366 nm. The plate was photo-documented at UV 366 nm and daylight using photodocumentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The Rf values and finger print data were recorded by WIN CATS software (Version 1.3.4 Camag).

Steroid

The Toluene-Acetone (9:1) was employed as mobile phase. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried at 100 ºC in hot air oven for 3 min.

Terpenoid

The n-Hexane-Ethyl acetate (7.2:2.8) was employed as mobile phase. The plate was sprayed with anisaldehyde sulphuric acid reagent and dried at 100 ºC in hot air oven for 3 min.

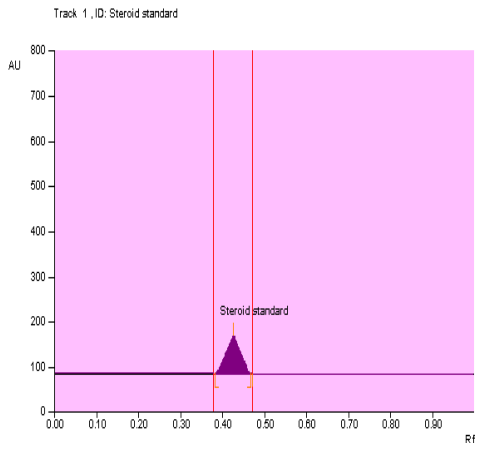
RESULTS AND DISCUSSION

Steroid Profile

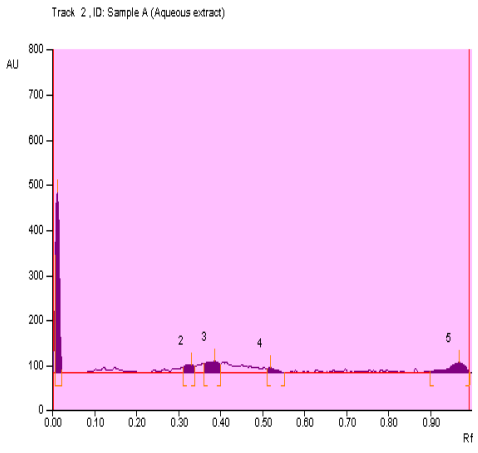
HPTLC analyses were tested with different compositions of the mobile phase in order to obtain high resolution and reproducible peaks. Toluene-Acetone (9:1) showed the presence of five different types of steroids and five different R_f values that ranged from 0.01 to 0.97 (Fig 1, 2 and Table 1). The steroid profile of *R. aquatica* is compared with HPTLC chromatogram of standard stigmasterol, which indicated the presence of stigmasterol on the aqueous root extract.

Table 1: Steroid profile of the aqueous root extract of *R. Aquatic*

Track	Peak	Rf	Height	Area	Assigned Substance
STD	1	0.43	93.1	3480.8	Steroid standard
Sample A	1	0.01	344.7	2812.3	Unknown
Sample A	2	0.33	18.1	372.4	Unknown
Sample A	3	0.39	25.9	722.8	Steroid 1
Sample A	4	0.52	13.0	201.6	Unknown
Sample A	5	0.97	21.9	828.8	Unknown



(a) HPTLC chromatogram of standard stigmasterol – *peak densitogram display (scanned at 366nm)*



(b) HPTLC chromatogram of aqueous root extract of *R. aquatica* –peak densitogram display (scanned at 366nm)

Figure 1*:* Densitogram display of aqueous root extract of *R. aquatic* for detection of steroid compounds

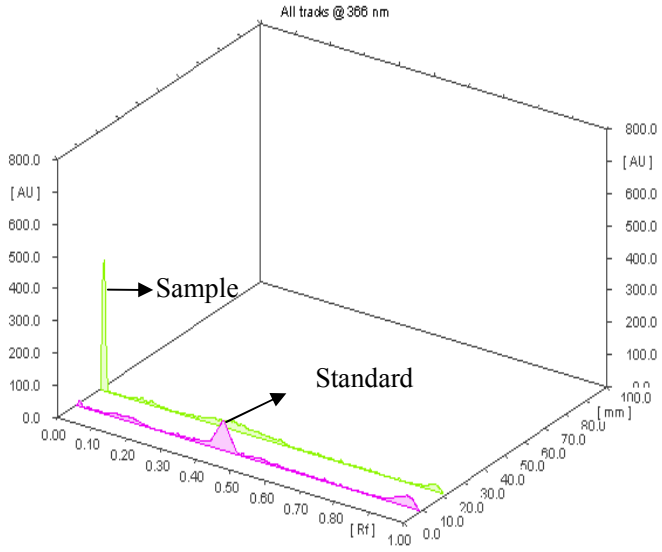


Figure 2: 3D display of HPTLC chromatogram of aqueous root extract of *R. aquatica*

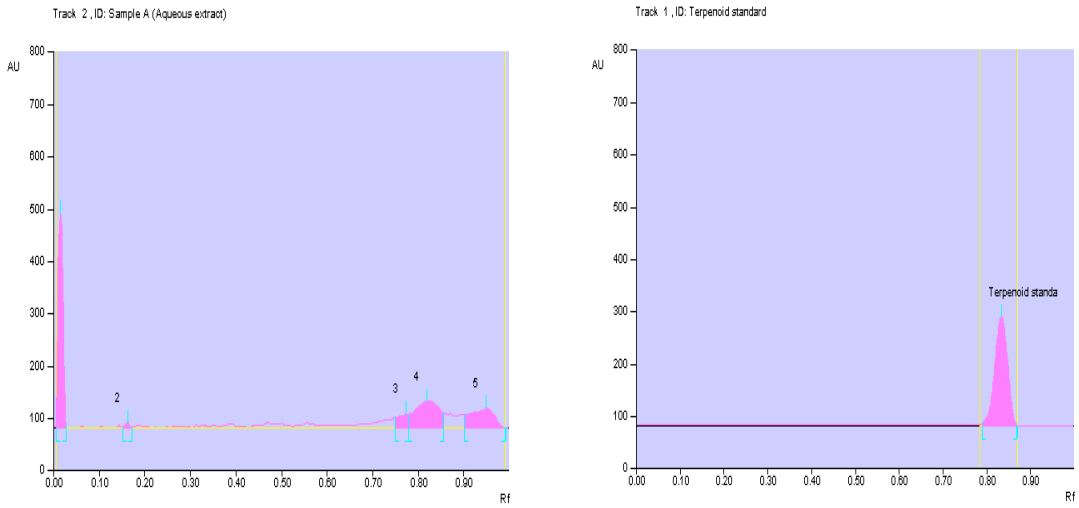
Mariswamy *et al.,* (2011) also have revealed the presence of thirty different types of steroids with thirty different R_f values ranging from 0.04 to 0.97 in the methanolic extract of stem, leaves, root, flower and seeds of *Aerva lanata.* Maximum numbers (eleven) of steroids were observed in the leaves followed by root. The methanol root extract of *Pseudarthria viscida* showed the presence of ten different types of steroids with ten different R_f values with a range of 0.01 to 0.94 (Hemlal and Ravi, 2012). According to Tresina *et al.,* (2012) the ethanol leaf extract of *Eugenia floccosa* contained fourteen different types of steroids with fourteen different R_f values with a range of 0.02 to 0.96.

Terpenoid profile

HPTLC analysis with different mobile phases was tested in order to obtain high resolution and reproducible peaks. n-Hexane-Ethyl acetate (7.2:2.8) was found to be suitable in which aqueous root extract of *R. aquatica* showed the presence of five different types of terpenoids and five different R_f values with ranges from 0.01 to 0.95 (Table 2). The terpenoid profile of *R. aquatica* is compared with HPTLC chromatogram of standard gallic acid (Figure 3 and 4).

Table 2: Terpenoid profile of the aqueous root extract of *R. aquatic*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.83	239.7	8765.3	Terpenoid standard
Sample A	1	0.01	526.9	5864.0	Unknown
Sample A	2	0.16	10.3	92.2	Unknown
Sample A	3	0.77	28.1	543.1	Unknown
Sample A	4	0.82	52.1	2529.7	Terpenoid
Sample A	5	0.95	37.9	1824.3	Unknown



(a) HPTLC chromatogram of standard solanesol; peak densitogram display (scanned at 366nm)

(b) HPTLC chromatogram of aqueous root extract of *R. aquatica* -peak densitogram display (scanned at 366nm).

Figure 3: Densitogram display of aqueous root extract of *R. aquatica* for detection of terpenoid compounds

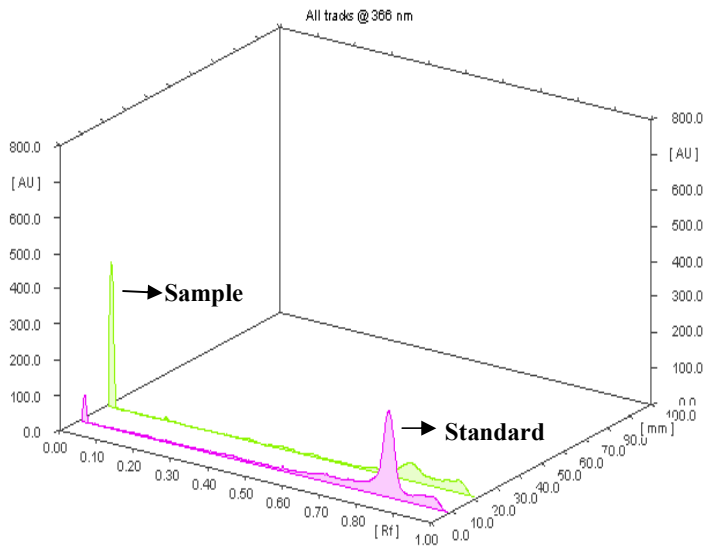


Figure 4*:* 3D display of HPTLC chromatogram of aqueous root extract of *R. aquatica*

Sophia *et al.,* (2011) reported that the n-hexane extract of the whole plant of *Emilia sonchifolia* showed the presence of seven different types of terpenoids with seven different R_f values in the range of 0.01 to 0.96. The present result coincides with the findings of Yamuna Devi *et al*. (2012) where methanolic extract of stem, leaves, root, flower and seeds of *Aerva lanata* showed the presence of twenty seven different types of terpenoids with twenty seven different R_f values in the range of 0.06 to 0.97. Tripathi *et al*. (2012) has documented the utility of HPLC to detect phenolic compounds in crude leaf extracts of *Psidium guajava* and *Syzygium cumini* and *Eucalyptus.*

In present era the HPTLC has become one of the best methods for quality assessment of botanical products**.** The botanicals can be easily compared with this fingerprint approach and adulteration can be recognized. The validation of the novel products from herbal extracts needs the assessment using powerful analytical instruments (Nicoletti and Toniolo, 2012).

CONCLUSIONS

Plant resources form the precursors and products of a variety of industries like pharmaceuticals, food and cosmetics**.** Essentially the drug research is more important in the present era and most researches are being carried out in this field. The use of advanced analytical methods leads to the discovery of new compounds that may help to treat the diseases. Such a method is HPTLC that is used in the present study to assess the presence of steroids and terpenoids in the plant root extract of *Rotula aquatic*. Different peaks formed with different Rf values demonstrated the presence of these compounds of medicinal importance in them. The study results may conclude that presence of different phytochemical compounds in the plant could be attributed to its high medicinal properties.

Conflicts of Interest

None declared.

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