

*Original Research Article***PHYTOCHEMICAL STUDY OF AERIAL PARTS OF *RANUNCULUS MURICATUS* FOR THE PHARMACOLOGICAL ACTIVE COMPOUNDS****Muhammad Shahzad Aslam*, Bashir A. Choudhary, M Uzair, Abdul Subhan Ijaz.**

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ABSTRACT

Phytochemical study for the pharmacological activity is an important segment of drug designing. This initial work establishes a foundation for product development. The pharmacological active compounds are finally developed into a pharmaceutical preparation. This is used in actual clinical and therapeutical practice to cure miscellaneous pathological problems. Thus; we have aimed this study for the extraction of compounds using two solvents of different polarities, so that maximum number of compounds can be extracted. Analytical technique used for the isolation and identification of the various compounds is thin layer chromatography. Phytochemical analysis of aerial parts showed the presence of Tannins, saponin and cardiac glycosides. Cardioactive glycosides, Tannin and saponin were reported in aerial parts of *Ranunculus muricatus* for the first time. Therefore *Ranunculus muricatus* can be used for further isolation and structural determination of cardioactive compounds.

Keyword : *Ranunculus muricatus*, cardioactive glycosides, Tannin, Saponin, phytochemical study

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INTRODUCTION

Treatment of ailments with the help of plants having medicinal values is constantly a noticeable characteristic of Islamic training. Different Surahs in the Holy Quran such as in Al-Momeenoon, Al-Rehman, Al-Bakra and Al-Inaam narrating the significance of medicinal plants. Islamic medicine begins with Hazrat Adam (A.S.) and was ended at Hazrat Muhammad (SAW) but pharmaceutical search and gathering of these medicines is still continued throughout the world [1]. Natural source such as plants have always been a noteworthy role in discovery and production of new pharmaceuticals which are clinically useful in future. They may always been a source of making fully synthetic drugs [2].

Ranunculus muricatus (Figure 1) belong to genus *Ranunculus* that possesses more than 600 species. It is distributed throughout the northern hemisphere and southern temperate regions in the tropic where they usually limited to higher altitude. The most common use of *Ranunculus* species in traditional medicines are anti-rheumatism, intermittent fever and rubefacient. It has been found that the constituents that produce these affects are due to Protoanemonin, anemonin [3]. Traditionally it was used against plague, abscess and tumors of plague [4]. Whole plant is also used in periodic fever and asthma [5].

Ranunculus pseudo-muricatus Baltter & Hallb is the synonym of *Ranunculus muricatus* [6]. It is also known as spinyfruit buttercup and its vernacular name is Jal dhania. Commonly it is also known as Latokari, Kor gandal [5].

The aim of the present paper is phytochemical assessment of the aerial parts of *Ranunculus muricatus* for the determination of groups which are pharmacologically active.



Figure 1: *Ranunculus muricatus*: Sketch of aerial parts & flower

MATERIAL AND METHOD

Material:

Rotary Evaporator, Extraction bottle, Dichloromethane (DCM), Methanol, Ultrasonic bath, Dragendorff's reagent, dilute ammonia solution, separating funnel, chloroform, acetic acid, Mayer's reagent, carbontetrachloride (CCl₄), Five silica gel 60 F²⁵⁴ TLC plates (20x20cm) (Merck),

Collection of Plant material:

Ranunculus muricatus was collected from Jallo pind, Lahore as identified by Dr. Altaf Hussain Dasti, Professor, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan giving the Catalog number 271-stw. Total wet weight of plant collected was 15kg. It was then reduced to 5 kg of dried plant. The plant was then ground till it become powder. The total weight of powder drug was 1 kg and 600 grams.

Extraction:

Maceration is the technique for extraction for finely ground plant material. Measured quantity of plant material (200grams) was taken in a glass bottle. After that quantified volume of dichloromethane was added to it with constant sonication in ultrasonic bath. It takes 24 hours to be settle down and then filtration was performed. Repeat the process three times with dichloromethane and then methanol. The Dichloromethane used during

first, second and third soaking was 900 ml, 450ml and 250 ml respectively and 700 ml, 300ml and 250 ml for methanol respectively. Rotary evaporator was used for the concentration of both extracts under reduced pressure labeled with codes as RMAPD and RMAPM respectively.

Phytochemical Analysis

Test for Alkaloids:

Powdered drug (0.5-1g) was boiled with dilute hydrochloric acid (10 ml) in a test tube for one minute; it was allowed to cool and fragments to settle down. Filtered the supernatant liquid into other test tube. Pour three drops of Dragendorff's reagent. Clear precipitate or turbidity seemed, representing the occurrence of alkaloids. To confirm the existence of alkaloids, the remaining part of solution was made alkaline to litmus paper with dilute NH_3 solution. It was then extracted with CHCl_3 (5ml) by shaking it gradually and permit the layers to isolated. The lower CHCl_3 layer was detached and extracted with dilute CH_3COOH (10 ml). The CHCl_3 layer was cast-off. The extract was distributed into 4 parts and adds few drops of Wagner's reagent, Mayer's reagent and Dragendorff's reagent separately to each of 3 parts while the 4th parts worked as untreated control. An observation of turbidity or precipitate compared with untreated control with either or all reagents confirmed occurrence of alkaloids [7].

Test for Anthraquinone glycosides:

Borntrager's test:

Ground drug (0.1g) was extracted with hot H_2O (10 ml) for five minutes. Filter the solution when it was hot. Cool afterward and extracted with CCl_4 (10ml). The CCl_4 was detached, washed with water (5ml) and shaken with dilute NH_3 solution (5ml). Absence of pink to cherry red color showed the absence of free anthraquinone. Ground drug (0.1g) was dissolved with Iron (III) chloride (10 ml) and HCl (5ml). Δ the solution on heated bath for ten minutes. Filter the solution when it was hot. Cool afterward and extracted with carbon tetrachloride (10ml). The carbon tetrachloride was detached, washed with water (5ml) and shaken with dilute NH_3 solution (5ml). Absence of pink to cherry red color showed the absence of bound anthraquinone [7].

Test for Cardioactive glycosides:

Keller Kiliani test:

Drug used for analysis was crushed then taking 1g. 10 ml of alcohol (70%) with 1g of crushed drug was boiled on water bath for two minutes. Filter the extract and add distilled water (twice amount) to dilute the filtrate. After then lead sub acetate was added. Filter it. CHCl_3 or CCL_4 was used for extraction of filtrate by vigorous shaking. Transfer the organic portion in a crucible. Evaporate the organic portion and add 3ml of Iron (III) chloride (3.5 %) in a residue. Transfer the portion in a test tube and then H_2SO_4 was added cautiously along the wall of test tube [7].

Test for Tannins:

Lead acetate test:

Powder of plant was dissolved in distilled water and boiled. After boiling filter the solution and added lead acetate in the filtrate which gave precipitate, are indicating the presence of tannin.

Test for Saponin:

Grounded drug (0.5g) was shaken with H₂O. Consistent foam showed presence of saponin [7].

Thin Layer Chromatography

Requirements:

Test samples, organic solvents (chloroform, methanol, ethyl acetate, n-hexane and isopropyl alcohol), Spotting capillary, coated TLC plates, TLC tank, oven and UV illuminator.

Spotting and Development of TLC plates:

Ten mg of each methanolic and dichloromethane extracts of aerial parts were dissolved in 1ml of methanol and dichloromethane (HPLC grade), respectively. Five silica gel 60 F254 TLC plates (20x20cm) were marked at 1cm from each side and cut into smaller plates. 5-10ul of sample was applied by capillary on the line marked. Samples were applied at equal distance for simple TLC and spot was no more than 6mm in diameter.

Visualization of TLC plates:

TLC plates were first visualized in the UV light (254nm and 366nm) and visualized spots were marked for the determination of R_f value. Subsequently sprayed with the Godin's reagent.

Godin reagent:

This reagent was prepared by mixing equivalent volumes of 1% vanillin in ethanol and 3% perchloric acid in water. TLC plates were sprayed with this mixture and then with 10% sulphuric acid in ethanol. Sprayed TLC plates were then heated at 100C°. Different spots were observed [8].

Measurement of R_f value:

Both distances, covered by mobile phase and substances were measured and R_f value was calculated

as:

$R_f = \text{Distance traveled by the component} / \text{distance traveled by the solvent front}$

RESULTS

Extraction:

For extraction of Aerial part of *Ranunculus muricatus* maceration process was adopted. The solvent used for extraction were methanol and dichloromethane. The results are shown in the table 1.

Phytochemical analysis of crude extract :

Phytochemical studies were carried out for detection of secondary metabolites i.e. alkaloids, anthraquinone glycosides, cardiac glycosides, saponins and tannin; in plant material. The results of the study are shown in table 2.

Table 1: Results of extraction of plant material with different solvents.

Plant Name	Part Used	Solvent Used	Extract obtained (gm)	Sample codes
<i>Ranunculus muricatus</i>	Aerial part	Dichloromethane	2.7	RMAPD
		Methanol	5.3	RMAPM

Table 2: Results of identification of secondary metabolites in crude extract.

Plant Name	Part used	Alkaloids	Anthraquinones	Cardiac glycosides	Saponins	Tannins
<i>Ranunculus muricatus</i>	Aerial part	-	-	+++	+++	+++

+++ = Strongly positively results - = No results

Chromatographic method:

Result of TLC of dichloromethane extract of *Ranunculus muricatus*:

UV active components were observed at 254nm with Rf values as follows:

0.08, 0.18, 0.28, 0.39, 0.54, 0.67, 0.77, 0.82, 0.87.

UV active components were observed at 366nm with Rf values as follows:

0.08, 0.18, 0.28, 0.39, 0.54, 0.67, 0.87

Colored components became visible is Purple "P.U" following Godin reagent and 10% sulfuric acid spray having Rf values given below:

0.87

The photograph of developed TLC plate of dichloromethane extract of *Ranunculus muricatus*.

Result of TLC of methanol extract of *Ranunculus muricatus*:

UV active components were observed at 254nm with Rf values as follows:

0.36, 0.52, 0.94

Some colored components became visible i.e. P. U and P. I following Godin reagent and 10% sulfuric acid spray respectively having Rf values given below:

0.20, 0.30, 0.46, 0.69, 0.80

The photograph of developed TLC plate of methanol extract of *Ranunculus muricatus*.

DISCUSSION

Current investigation deals with the phytochemical evaluation of *Ranunculus muricatus* (Ranunculaceae). The end result of phytochemical analysis of secondary metabolites displayed the occurrence of cardiac glycosides, tannin and saponin in aerial part of crude extract of *Ranunculus muricatus*. There have been reports of the presence of alkaloids, anthocyanin, carbohydrates, coumarins, Jbenolie and phytosterols in ethanolic extract of *Ranunculus muricatus* [9]. In comparison with the crude extract of *Ranunculus muricatus* under current investigations it has been found that these constituents were reported for the very first time which can be explored for further phytochemical studies and isolation of cardioactive glycosides. Thin Layer Chromatography (TLC) has been a good analytical technique for isolation and identification of the various compounds. Number of UV visible components from dichloromethane and methanolic extract of aerial parts of *Ranunculus muricatus* has been identified through their R_f value. It has been found the difference in R_f value in comparison between methanolic and dichloromethane extract.

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