Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2017.0301.05**

CHEMICAL AND BIOLOGICAL ANALYSIS OF THE EXTRACT FROM THE PLANT *RUMEX HASTATUS* FOR ITS SECONDARY METABOLITES

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ABSTRACT: Secondary metabolites are important source of potential drugs that can be used as medicine. *Rumex hastatus* has been growing in Pakistani northern areas commonly in Muree, Gilgit/ Baltistan. Overall, the whole plant is used in medicines for example to release blood pressure, sexually transmitted diseases, blood purifier, aches in throat and skin disease. The plant collected from Muree was deride, chopped, grinded to coarse powder and soaked in different solvents (Methanol, Chloroform, water, ethanol) for different chemical tests. Filtrates were concentrated under reduced pressure to avoid thermal decomposition on rotary evaporator. Tests were performed for tannins, alkaloids, saponins, steroids, flavonoids, glycosides and anthraquinones. From all tests, it has been concluded that the plant *Rumex hastatus* consists of alkalloids, saponins, tanins, steroids, flavonoids, cardiac glycosides, anthraquonone, phenolic compounds. The crude extract contain biological potential that showed 74% inhibition of urease and found to be cytotoxic with LD_{50} of 200 µg/mL.

KEYWORDS: Alkalloids, Cytoxic Activity, *Rumex hastatus,* Secondary Metabolites.

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1. INTRODUCTION

Natural products especially secondary metabolites obtained from plants, fungus, and many other natural sources are most important source of potential drugs leading to pharmaceutical industry (Mishra & Tiwari,2011), (Rey-Ladino et al., 2011), (Cragg & Newman, 2005), (Haefner, 2003), (Butler, 2004). A natural product has been used long before in the form of traditional medicines and home remedies (Kinghorn et al., 2011). A time before about 20th century, crude and semi-pure extracts of plants, animals, microbes and mineral has been utilized to cure the human and domestic animal illnesses but with the 20th century it was concluded by scientist that the chemical compounds present in extract would be utilized for their healing power instead crude extract.Thus, this conclusion lead to the new idea in pharmacology that chemical compounds has been isolated from extracts, purify them and then characterized and then utilized to cure the diseases (Lahlou, 2013). *Rumex hastatus*is the plant of family Polygonaceae that has been growing in northern areas most commonly in Muree, Gilgit/ Baltistan. This plant locally known as "khatimal" and it is widely distributed in cultivatedareas, dry slopes, rocks at an altitude of 700-2500 meter. It is shrub likeplant with single layered roots. It flowers from May to June and flowersare greenish white and fruit is pinkish (Hameed et al., 2010). Leaves of *R. hastatus* substantially produce acids due to whichleaves have a pleasant acidic taste and used in chutneys and pickles.Overall, the whole plant is used in medicines e.g.; it is used against bilious complaints, piles, bleeding of lungs. The leaf extract of plant is used torelease blood pressure. *R. hastatus* also have activity against sexually transmitted diseases including AIDS (Sahreen et al., 2011). The juice ofthe plant acts as an astringent and used as blood purifier. Its fresh tubeis chewed to relieve the aches in throat. The root is used as laxative, alternative, tonic and moreover as anti-rheumatic and against skindisease (Manandhar, 2002). The leaves and shoots of *R. hastatus* are reported to be used as diuretic, refrigerant and as cooling agent (Ali et al., 2006), (Hussain et al., 2006).

2. MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant was collected from Muree hills in July 2008. The plant was identified by Mr. Farrukh Nisar, Department of Botany, University of Gujrat.

Preparation of sample: The collected plant material was deride under shade for one week. The deride plant was chopped, grinded to coarse powder.

Extraction of Plant: The deride plant material was soaked in different solvents (Methanol, Chloroform, water, ethanol) for 07 days to prepare different extracts for different chemical tests. After soaking, extracts were filtered and filtrates were concentrated under reduced pressure to avoid thermal decomposition on rotary evaporator.

Preparation of wagner's reagent: Wagner' s reagent was prepared by dissolving 5 g potassium

iodide and 1 g iodine in water (Yadav et al., 2014), (Vaghasiya et al., 2011).

Preparation of dragnedroff's reagent

A). 0.85 mg of bismith-nitrate in 10 ml of glacial acetic acid and 40 ml of water (Yadav et al., 2014), (Vaghasiya et al., 2011).

B). 8 g potassium iodide in 20 ml of water.

A + B ⟶ dragnedroff' s reagent.

Analysis of extract from *Rumex hastatus*: Following tests were performed to determine the secondary metabolites inextract of *R. hastatus*.

Test for tannins: In a test tube 2 ml of water extract was taken and 2 ml freshly prepared $FeCl_3$solution was added to it. The appearance of blue-black precipitates confirmed the presence of tannins (Yadav &Agarwala, 2011), (Wadood et al., 2013), (Sharma et al., 2014).

Tests for alkaloids: In a test tube 2 ml of methanolic extract was taken and 1 ml of HCl and 6 drops of wagner' s reagent were added to it. The brownish-red precipitates confirmed the alkaloids in extract of R. hastatus (Yadav &Agarwala, 2011), (Wadood et al., 2013).

In another test tube, 2 ml of MeOH extract was taken and 6 drops of dragnedrff's reagent was added to it. The appearance of yellow precipitates indicated the presence of alkaloids (Yadav &Agarwala, 2011), (Wadood et al., 2013).

Test for Saponins: 0.5 ml water extract was taken in a test tube and 1 ml glacial acetic acid + 3ml $FeCl_3$ soln. + 3 ml conc. H_2SO_4 were added to it. Green-blue color indicated the presence of Saponins (Yadav & Agarwala, 2011), (Wadood et al., 2013).

Test of steroids: In a test tube, 1 ml chloroform extract was taken. Then 2 ml acetic anhydrideand 1 ml conc. H_2SO_4 were added to it. Blue-green ring formation indicated the presence of steroids (Yadav & Agarwala, 2011), (Wadood et al., 2013), (Sharma et al., 2014).

Test of flavonoids: 2 ml methanolic extract was taken in a test tube. 5 ml conc. HCl + 0.5 mg Mg ribbon were added to it. Pink tomato red colour indicated the presence of flavonoids (Yadav &Agarwala, 2011), (Wadood et al., 2013).

Test for cardiac glycosides: In a test tube 2 ml ethanolic extract was taken. 1 ml glacial acetic acid, 2 ml $FeCl_3$ soln. And 1 ml conc. H_2SO_4 was added to it. Green-blue colour confirmed the presence of cardiac glycoside (Yadav & Agarwala, 2011), (Wadood et al., 2013), (Sharma et al., 2014).

Test of Anthraquinones: 1ml of methanolic extract of plant was taken in a test tube and 10 % HCl was added to it and boiled for few minutes on water bath. Then it was filtered and cooled. Then a small volume (approx. equal) chloroform and 10 % NH_3 were added to the filtrate and heated it. Appearance of rose color indicated the presence of anthraquinones (Yadav &Agarwala, 2011), (Wadood et al., 2013).

3. RESULTS AND DISCUSSION

From all tests which were performed for different classes of the natural products/ secondary metabolites, it has been concluded that the plant Rumex hastatus consists of alkalloids, saponins, tanins, steroids, flavonoids, cardiac glycosides, anthraquonone, phenolic compounds and due to presence of these constituents, plant R. hastatus has been utilized as a cure in a number of ailments in local community in form of plant extract, paste, juice.

Table: Phytochemical analysis of the plant Rumex hastatus

Sample	Test For	Results
Rumex hastatus	Alkalloids	+
	Saponins	+
	Tanins	+
	Steroids	+
	Flavonoids	+
	Cardiac glycosides	+
	Anthraquinones	+

Biological screening of the extract of R. hastatus for bioassays

The crude extract of R. hastatus was tested for its biological potential that showed 74% inhibition of urease as an anti-urease assay and was also found to be cytotoxic with LD_{50} of 200 µg/mL.

4. CONCLUSION

From all investigation related to secondary metabolites of *R. hastatus*, we have concluded that plant *R. hastatus* was a rich source of biologically active secondary metabolites. Moreover, it has potential as cytotoxic as well as anti-urease.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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