STM JOURNALS

Research & Reviews: A Journal of Life Sciences
ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)
Volume 7, Issue 3
www.stmjournals.com

Preliminary Phytochemical Analysis and Haemolytic Activity Assay of Tuber Extract of *Ruellia tuberosa* L.

Rajanna L., Ramya Raju, G. S. Shailaja Sharma*
Department of Botany, Bangalore University, Jnana Bharathi Campus, Bangalore, Karnataka, India

Abstract

Presence of certain chemical compounds is responsible for medicinal properties of the plant. In the present study, the various biochemical tests were conducted to examine the presence of various phytochemicals in the methanolic, petroleum ether and chloroform tuber extracts of Ruellia tuberosa L. Also, the extracts were subjected to the in vitro haemolytic activity assay. The tests confirmed the presence of carbohydrates, alkaloids, tannins, terpenoids and flavanoids in all the three tested extracts. Saponins were absent in methanolic and petroleum ether extracts but were found to be present in chloroform extract of the tuber. The methanolic and the petroleum ether extracts have shown insignificant haemolysis (less than 20%) even at higher concentrations such as 100 and 200 µg/ml, making them safe and potential source for drug formulations and human consumption.

Keywords: *Phytochemicals, Ruellia tuberosa, tuber extracts*

****Author for Correspondence*** E-mail: shailaja.sharma.gs@gmail.com

INTRODUCTION

Ruellia tuberosa L. belongs to Acanthaceae. It is commonly called minnieroot, blue bell or cracker plant. It is a perennial herb native to Tropical America but introduced and naturalized in Africa, Pakistan, India, South East Asia and Australia. The plant is erect with hairy stem growing up to a height of 60–70 cm. Roots are slender, elongated and tuberous. The leaves are petiolate, oblong or obovate and show opposite decusate arrangement. Flowers are violet coloured and fruits are 2–4 cm long pods, which at maturity burst open, resulting in hurdling of seeds. It is believed to have medicinal properties and is used in the traditional system of medicine in parts of India, Taiwan, Suriname, America and Vietnam. The decoction of this herb is used to cure chronic bronchitis and is also used as diuretic for the treatment of stones in the bladder [1]. In Taiwanese traditional system of medicine, it is known to have antitoxic property and is used to cure abdominal pain, flu, hepatitis, high blood pressure and diabetes [2]. In Surinamese traditional system of medicine, it is used to cure joint pains and strained muscles. It is also known to have the anthelmentic, abortificient antipyretic, antidiabetic, antidotal, analgesic, antihypertensive and thirst-quenching properties [3]. Such immense medicinal properties of the plant are due to the phytochemicals present in it. However, certain phytochemicals may also cause adverse effects such as haemolysis when they enter the blood stream. Hence, the aim of the present study was to investigate the presence of various phytochemicals in the extracts of the tubers of the selected plant and to determine whether they are safe to the blood by conducting *in vitro* haemolytic analysis.

MATERIALS AND METHODS

Collection of the Plant Material

Fresh and healthy tubers of *Ruellia tuberosa* L. were collected from the Bangalore University Jnana Bharathi Campus. The collected tubers were washed thoroughly in the running tap water to remove dust, soil and other debris. The excess water content was removed with the help of clean cotton cloth and kept for drying under room conditions. These thoroughly dried tubers were ground to fine powder and kept in airtight container until it was used for extraction.

Preparation of the Crude Extract from Powdered Plant Material

10 gm of the tuber powder was weighed and boiled with 50 ml of the solvent in a beaker by keeping it in a water bath at 50°C for 4 h. The beaker was covered with an aluminum foil and

tightly wrapped with cling-wrap and gum-tape to prevent evaporation during boiling at 50°C for 4 h. It was then filtered with Wattman No. 1 filter paper, precipitate was discarded and the filtrate was transferred to an empty beaker of known volume. It was then concentrated by evaporating at 80°C in water bath. Thus, obtained crude extract was transferred to an Epindorf tube and stored in refrigerator (at 4°C) till used. Three such solvent extracts of the tuber powder were prepared separately using the solvents: methanol, chloroform and petroleum ether.

Preliminary Phytochemical Analysis

Preparation of Working Stock of Plant Extract for Priliminary Phytochemical Analysis

Working stock solutions were prepared separately by dissolving 120 mg of each of the three crude extracts in 1 ml of respective solvents. Thus prepared working stocks of plant extract (henceforth called as samples) were subjected to the preliminary phytochemical analysis.

Test for Alkaloids: **Dragendorff's Test**

400 µl of distilled water was added to 100 µl of sample and boiled with few drops of 1% HCl on flame. To this reaction mixture, 100 µl of Dragendorff's reagent was added. Formation of yellow precipitate indicated the test as positive.

Tests for Phenolic Compounds and Flavanoids

Ferric Chloride Test for Tannins

400 µl of distilled water was added to 100 µl of sample and treated with 500 µl of 5% $FeCl_3$ solution. Formation of dark green precipitate indicated the presence of tannins.

Sulfuric Acid Test for Terpenoids

500 µl of chloroform was added to 100 µl of sample, which resulted in separation of two layers. To this, 100 µl of concentrated H_2SO_4 was added drop wise. Formation of yellow coloured layer confirmed the presence of terpenoids.

Lead Acetate Test for Flavanoids

400 µl of distilled water was added to 100 µl of sample and treated with 500 µl of 10% lead acetate. Formation of yellow precipitate on shaking this reaction mixture indicated the presence of flavonoids.

Test for Carbohydrate: **Molish's Test**

200 µl of distilled water was added to 100 µl of sample and treated with 200 µl of Molish reagent. To this, 100 µl of concentrated H_2SO_4 was added drop wise. Formation of a violet ring indicated the test as positive for carbohydrates.

Test for Saponins: **Foam Test for Saponins**

400 µl of distilled water was added to 100 µl of sample and flame heated to achieve 50°C and was observed for the formation of froth, which is stable for 15 min for a positive result.

***In vitro* Haemolytic Activity Analysis**

Preparation of Working Stock of Plant Extract for in vitro Haemolytic Analysis

200 mg of each of the three crude extracts were reconstituted in 1 ml of 1X PBS separately to get 2 mg/ml or 200 µg/100 µl. These were considered as working stock solutions. They were further diluted with required amount of 1X PBS to get three test concentrations (viz., 50 µg/100 µl, 100 µg/100 µl and 200 µg/100 µl) of each solvent extract. These were used as test samples.

Isolation of Erythrocytes

5 ml of blood was collected from a healthy volunteer in a tube containing 5.4 mg of EDTA to prevent coagulation and centrifuged at 1000 rpm for 10 min at 4°C. Plasma was removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4. Washed erythrocytes were stored at 4°C and used within 6 h for the haemolysis assay. Just before the actual usage, it was further diluted to get 1/10th the original concentration by adding 900 µl of 1X PBS to 100 µl of stored erythrocyte suspension (E.S.).

In vitro Haemolysis Analysis

100 µl of test sample was added to 50 µl of the prepared diluted erythrocyte suspension and was incubated at 37ºC in a water bath for 60 min. Later the volume was made up to 1 ml by adding 850 µl of 1X PBS and was centrifuged at 300 rpm. Following the same procedure simultaneously, two other tests were

conducted where erythrocyte suspension was treated with either control 1X PBS or standard 1% SDS.

Spectrophotometric reading of the resulting haemoglobin in the supernatant of test samples, control and standard were taken at 540 nm to determine the concentration of the haemoglobin formed due to erythrocyte lyses or haemolysis. Percentage of haemolysis shown by the test samples were calculated by using the formula:

$$\% \text{ Haemolysis} = \frac{(\text{Absorbance of negative control} - \text{absorbance of sample})}{\text{Absorbance of negative control}} \times 100$$

Percentage of haemolysis of test samples, control and standard were tabulated and compared.

RESULTS AND DISCUSSION

In the present study, the three crude extracts of tubers prepared using the solvents methanol, chloroform and petroleum ether have shown positive response to all the phytochemical tests conducted except for that of saponins, inferring the selected plant possess carbohydrates, alkaloids, tannins, terpinoids and flavanoids. Saponins were found to be absent in methanolic and petroleum ether extracts but were present in chloroform extracts. The results are represented in the Table 1.

Table 1: *Responses Shown by Tuber Extract of Ruellia tuberosa L. to the Phytochemical Tests Conducted.*

Phytochemical Tests	Methanolic	Chloroform	Petroleum Ether
Test for Alkaloids			
Dragendorff's test:	+	+	+
Tests for Phenolic Compounds and Flavanoids			
Ferric chlorid test for tannins	+	+	+
Sulfuric acid test for Terpenoids	+	+	+
Lead acetate test for Flavonoids	+	+	+
Test for Carbohydrate			
Molish's test	+	+	+
Test for Saponins			
Foam test for Saponins	-	+	-

Therapeutic properties of a medicinal plant are due to presence of various groups of chemical compounds commonly called phytochemicals. The preliminary phytochemical analysis in the present study has revealed that the crude extracts of the tubers of *Ruellia tuberosa* L. posses various phytochemicals such alkaloids, tannins, terpenoids, flavanoids and carbohydrates. In the literature some of the alkaloids are proved to have some beneficial effects on human health such as antipyretic and analgesic activities [4]. Similarly, phenolic compounds are known to control high blood pressure and coronary heart diseases [5]. Hence the medicinal properties of this plant can be correlated with the presence of such phytochemical compounds.

Similar preliminary phytochemical analysis of this plant have been conducted by several authors [6–9]. Senthilkumar *et.al.* have tested only the methanolic extract of leaves of *Ruellia tuberosa* L. which was prepared using soxhlet apparatus [6]. Their results revealed that the phytochemicals such as alkaloids, glycosides, tripenoid, tannins and triterpenes were present in the methanolic leaf extract but flavanoids, and saponins were found to be absent. Luxmini *et al*. in 2015 tested methanolic extract of leaf, bark and roots for the presence of different phytocompounds and showed that alkaloids, flavanoids, saponins, steroidal glycosides and tannins were present in all the three extracts [7]. They had used soxhlet apparatus for the extraction. The results obtained in our present study are in agreement with that of the above mentioned studies. However both the previous studies have focused only on the phytochemicals present only in methanolic extracts of the plant parts and have not tested other solvent extracts.

Lakshmana and Gabriel in 2015 have tested three solvent-extracts of tuber for the presence of wide range of phytochemicals [8]. They have sequentially extracted the powdered plant material with hexane, chloroform and ethanol. Their results showed the mild presence of carbohydrates and flavanoids in all three extracts, complete absence of tannins and saponnins in all the three extracts and absence of alkaloids in only hexane and ethanolic extracts. The variation in the results showed by

them when compared to our present study may be due to the method of extraction followed and the solvents selected for the extraction.

Khachitpongpanit *et al.* have measured only the total phenols, flavanoids, tannins and alkaloid contents of chloroform extract of leaves, stem and roots [9]. They have not tested the presence of other phytocompounds in the extracts prepared.

Haemolytic activity assay conducted during our present investigation has revealed that the methanolic and petroleum ether extracts of tubers of *Ruellia tuberosa* L. show insignificant haemolysis even at higher concentrations. Methanolic and petroleum ether extracts exhibited 14.13 and 19.49% respectively at higher concentration (200 µg/ml) while chloroform extract showed a high haemolytic activity of 78.91% at the same concentration; proving that methanolic and petroleum ether extracts are safer for human consumption than chloroform extract. The high haemolytic activity of the chloroform extract may be due to the presence of saponins in it. The results are indicated in Table 2 and represented in Figure 1.

Literature on haemolytic activity assay of extracts of *Ruellia tuberosa* L. is not found, however another *in vitro* cytotoxicity test, brine shrimp assay has been carried out by Luxmini *et al*. [7]. They have demonstrated that presence of less cytotoxicity in methanolic extracts of roots (LC_{50} (ppm)=27.20) when compared to methanolic extracts of leaf (LC_{50} (ppm)=16.31) and bark (LC_{50} (ppm)=19.84). The results obtained in our present experiment also shows insignificant haemolytic activity of methanolic tuber extracts which is an evidence of safety of methanolic root extracts for drug formulation and thus is in agreement with the above mentioned brine shrimp assay carried out by Luxmini *et al*. [7].

Table 2: *Responses Shown Tuber Extracts of Three Solvents of Ruellia tuberosa L. to the Haemolyic Activity Assay.*

Concentration of the Samples Used	% of Haemolysis				
	Methanolic Extract	Chloroform Extract	Petroleum Ether Extract	Control (1X PBS)	Standard (1% SDS)
50 µg/ml	8.99	16.52	12.48	0.00	80.66
100 µg/ml	12.10	**73.65**	17.45		
200 µg/ml	14.13	**78.91**	19.49		

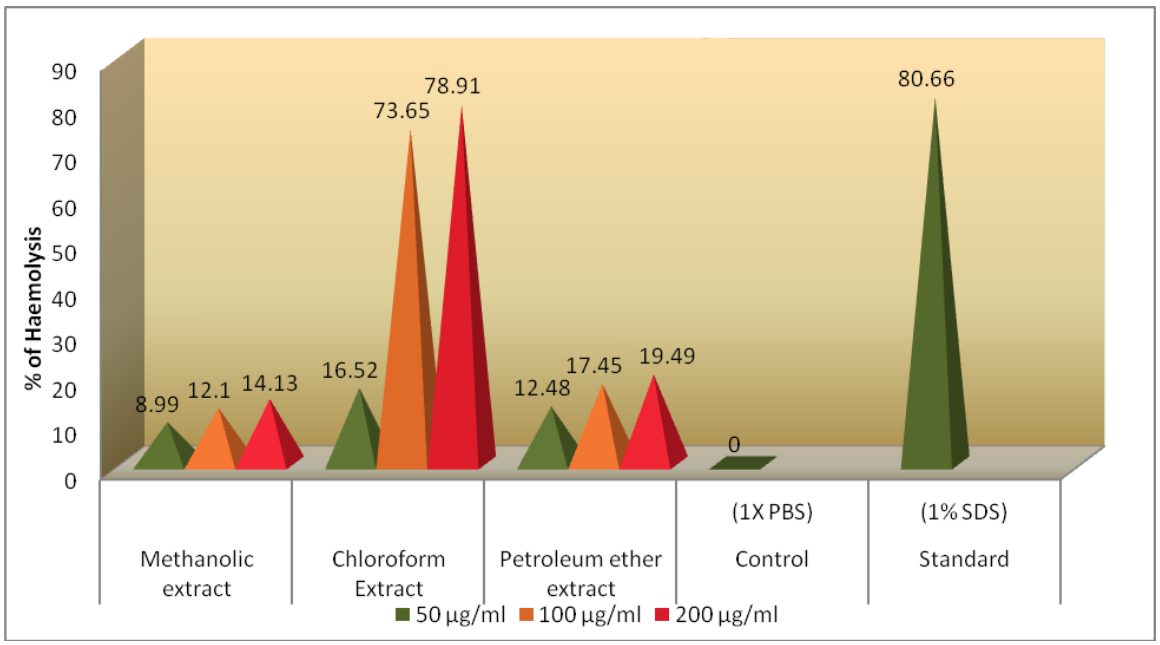

Fig. 1: *Graphical Representation of % of Haemolysis shown by Different Concentrations of the Plant Extract.*

CONCLUSION

The present study on preliminary phytochemical constitution of three solvent extracts of tubers of *Ruellia tuberosa* L. can serve as a basic knowledge about the various groups of compounds present in the plant which can further guide the studies on isolation and characterization of each specific compound present in this plant. The results shown by the plant extract in the haemolytic activity assay has proved that the methanolic and petroleum ether extracts of the tubers are not haemolytic and hence supports further testing of drug potentiality of this plant.

REFERENCES

1. Khare CP. *Indian Medicinal Plants an Illustrated Dictionary*. Verlag, Berlin: Springer; 2007; 561p.
2. Li TCS. *Taiwanese Native Medicinal Plants Phytopharmacology and Therapeutic Values.* New York, USA: Taylor and Francis Group CRC Press; 2006; 95p.
3. Kader MA, Parvin S, Chowduri MAU, *et al*. Antibacterial, Antifungal and Insecticidal Activities of *Ruellia tuberosa* (L.) Root Extract. *J Bio-Sci.* 2006; 20: 91–7p.
4. Crozier A, Clifford MN, Ashihara H. *Plant Secondary Metabolites Occurrence, Structure and Role in the Human Diet.* UK: Blackwell Publishing House Oxford; 2006; 103p.
5. Watson RR, Preedy VR, Zibadi S. *Phenols in Human Health and Diseases.* Vol. 1. London UK: Elsevier; 2014; 9–14p.
6. Senthilkumar P, Sambath R, Vasantharaj S. Antimicrobial Potential and Screening of Antimicrobial Compounds of *Ruellia tuberosa* using GC-MS. *Int J Pharm Sci Rev Res*. 2013; 20(1): 184–188p.
7. Luxmini KPAMK, Dharmadasa RM, Samarasinghe K, *et al*. Comparative Pharmacognostic Study of Different Parts of *Withania somnifera* and its Substitute *Ruellia tuberosa*. *WJAR*. 2015; 3(1): 28–33p.
8. Lakshmana P, Gabriel JJ. Comparative Phytochemical Analysis and Free Radical Scavenging Potentials of Tubers, Shoots of the Wild and *in vitro* Extracts of *Ruellia tuberosa* L. (Acanthaceae). *J Chem Pharma Res*. 2015; 7(5): 988–995p.
9. Khachitpongpanit S, Singhatong S, Sastraruji T, *et al*. Phytochemical Study of *Ruellia tuberosa* Chloroform Extract: Antioxidant and Anticholinestarase Activities. *Der Pharmacia Lettre*. 2016; 8(6): 238–244p.

Cite this Article
Rajanna L, Ramya Raju, Shailaja Sharma GS. Preliminary Phytochemical Analysis and Haemolytic Activity Assay of Tuber Extract of *Ruellia tuberosa* L. *Research & Reviews: A Journal of Life Sciences*. 2017; 7(3): 23–27p.