

## Phytochemical Studies and Antibacterial Activities of *Ruellia tuberosa* L.

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### Abstract

The medicinal plant *Ruellia tuberosa* L. of Acanthaceae family is a tropical plant widely distributed in South East Asia. In phytochemical investigation, 10 compounds were studied. Among them, glycoside, alkaloid, reducing sugar, terpene, phenolic compound, polyphenol, saponin and tannin were present but flavonoid and steroid were absent in the powder of study plant. Moreover, the antibacterial activity were carried out by using 95% ethanol, ethyl-acetate and n-hexane. Ethanol extract, n-hexane extract and ethyl-acetate extract were obtained the best inhibition zone diameter (0.7 cm) on *Salmonella typhi*. The inhibition zone of ethanol extract showed the most effective on *Staphylococcus aureus* (0.8 cm) and less inhibition zone showed on *Pseudomonas aeruginosa* (0.3 cm). All of the bacteria strains except in *E. coli* were showed sensitive to different plant extracts. *E. coli* strain was resistant to all of the plant extracts (n-hexane, ethyl-acetate and ethanol). These are not showed inhibition zone on plant extracts.

**Keywords:** *Ruellia tuberosa* L., Phytochemical, Antibacterial activity

### 1. Introduction

*Ruellia tuberosa* L. belonging to the family Acanthaceae is growing abundantly in shade places along the roadside. It is well known plant as Byauk and as well as Na-ga-hmaing in traditional medicine of Myanmar society. In other country, its also known as minnie root, fever root, snapdragon root and sheep potato plant is a species of flowering plant in Acanthaceae family. These plant names are also called popping pod, duppy gun and cracker plant come from the fact that children like to play with the dry pods that pop when rubbed with spit or water [10].

The *Ruellia* species are used medicinally to cure gonorrhea, syphilis, eye sores and in renal infections. In traditional medicine, *Ruellia tuberosa* L. are used to cure diuretic, anti-diabetic, antipyretic, antihypertensive and antidote [1]. The whole parts of genus *Ruellia tuberosa* L. are used in bladder stones and in bronchitis. Paste of the leaves are also used for skin diseases and boil. Roots are used as anthelmintic. In Bangladesh, *Ruellia tuberosa* is used for treatment of gonorrhea [9].

In Myanmar, the leaves of *Ruellia tuberosa* L. are used to cure ulcers, boil, blain. The liquid of the drained leaves used to cure for impetigo in children skin disease. The whole plant parts of the *Ruellia tuberosa* L. have

antidote activity. These plants are used to cure antidote for snake-bite, centipede and gecko-bite according to [2].

The preliminary phytochemical investigation was carried out by ethanol extracts for study of plants containing chemical constituents (alkaloid, steroid, flavonoid, glycoside, tannin, reducing sugar, phenolic compound, saponin, polyphenol and terpenoid).

Antibacterial activities were also made with various extract (ethanol, ethyl- acetate and n-hexane) of the whole plant of *R. tuberosa* L. by using agar well diffusion method against six different types of test organisms. The present research aimed to investigate the chemical composition of *Ruellia tuberosa* L. as well as to determine the antibacterial activity.

### 2. Materials and Methods

#### 2.1. Morphological Studies

The specimens of *Ruellia tuberosa* L. were collected from Amarapura Township in Mandalay Region. The specimens were collected during flowering and fruiting periods. The collected specimens were identified by the help of available literature [4], [6] and [8]. The morphological characters were made from the fresh specimens of both the vegetative and reproductive parts.

#### 2.2. Phytochemical Studies

The plant samples were extracted with ethanol (EtOH) and distilled water. Before phytochemical investigation, the powdered of *Ruellia tuberosa* L. were stored in air tight container to prevent moisture changes and contamination. Ethanol and distilled water extracts of *Ruellia tuberosa* L. were tested by using reagents to the solution. After the tests were detected by visual observation of color change or by the precipitate formation according to [7]. General reactions in these analyses revealed the presence or absence of these compounds in the crude extracts tested at Department of Chemistry, University of Mandalay.

#### 2.3. Antibacterial Studies

##### 2.3.1. Preparation of Crude Extracts

The plant materials were washed with water, air dried at room temperature and then grinded to get fine powder and stored in air-tight containers. The whole plants of *Ruellia tuberosa* L. were extracted by using hot continuous extraction technique in a soxhlet extraction method by using 95% ethanol and water. Then, the extracts were evaporated by placing them on

hot water bath at 50°C. All obtained extracts were weight before packed in water proof plastic flacks and stored at 4°C until.

### 2.3.2. Determination of Antibacterial Activity Microorganisms

The test organism (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella boydii*, *Salmonella typhi*, *Pseudomonas aeruginosa*) used in this study. All the test strains were obtained from Microbiology Laboratory, Biotechnology Department, Mandalay Technological University. Before testing the bacteria were grown in Nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

### Culture Media

Muller-Hinton agar for antibacterial susceptibility were used. All culture media will be prepared and treated according to the manufacture guide lines (Hi-Media) [3].

### Agar well diffusion method

The antimicrobial activity of *Ruellia tuberosa* extract was studies against *Staphylococcus aureus*, *Shigella boydii*, *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus* and *Pseudomonas* sp.. Bacteria pathogens were inoculated in Nutrient broth and incubated for 6-8 hours at 37°C. These pathogen cultures were swabbed throughout on nutrient medium with the help of sterile cotton wool. Swab in three directions to ensure complete plate coverage. Sterilize a cork bore by autoclaving. Then, the agar well (0.9 cm in diameter) were prepared by scooping out the media with sterile borer. The extract solution (n-hexane, ethyl-acetate and ethanol) was filled into each well (30 mg per well) incubated at 37°C for 24 hours and one well filled with solvent for control.

### Antibacterial tested

The plates inoculated with different bacterial strains were incubated at 37°C for 24 hours. Antibacterial activities were evaluated by measuring inhibition zone of complete growth inhibition surrounding bacterial growth.

## 3. Results

### 3.1. Morphological Studies

Family : Acanthaceae  
 Scientific name : *Ruellia tuberosa* L.  
 Local name : Byauk; Na-ga-hmaing  
 Common name : Minnine root, fever root

Perennial erect herbs with tuberous roots, up to 20-35 cm high; stems and branches quadrangular, tumid at the node. Leaves obovate, 1.9-8.6 µm in length and 0.7-3.6 cm in breadth, cuneate at the base, entire along the margins, slightly acute at the apex, sparsely pubescent

on both surfaces; petioles 0.3-1.1 cm long. Inflorescences axillary or terminal dichasial cymes. Flowers bisexual, zygomorphic, pentamerous, pale blue or violet, 3.5-4.0 cm in diameter at anthesis, bracts linear. Calyx 5 partite, linear-lanceolate, persistent in fruit. Corolla 5-lobed, infundibuliform, subequal. Stamens 4, didynamous, included, attached to the base of corolla tube; filaments filiform, white; anthers ditheous, basifixed. Ovary oblongoid, glabrous, bilocular with many ovules in each locule on the axile placentae; style filiform; stigma bifid. Fruits capsule, 1.0-2.9 cm long, oblongoid, mucronate at the apex, green, brown colour in dry. Seeds many, brown colour, ovoid, compress.



Figure 1. Habit of *Ruellia tuberosa* L.



Figure 2. A branch with inflorescences and seeds of *Ruellia tuberosa* L.

### 3.2. Phytochemical Studies

#### 3.2.1. Phytochemical investigation of powder of the whole plant *Ruellia tuberosa* L.

Preliminary phytochemical investigation on the whole plant of *Ruellia tuberosa* L. was done on the ethanol and distilled water extracts and the presence or absence of active constituents in this plant was shown in Table 1.

**Table 1. Preliminary phytochemical examination of the whole plant of *Ruellia tuberosa* L.**

Test	Solvent Extract	Reagent	Result	
			Observation	Remark
Alkaloid	1% HCl	Wagner's reagent	Reddish brown ppt	+
		Dragendroff's reagent	Orange ppt	+
Flavonoid	95% EtOH	EtOH, Mg ribbon conc: HCl	Green colour	-
Polyphenol	95% EtOH	1% FeCl <sub>3</sub> and 1% K <sub>3</sub> [Fe(CN) <sub>6</sub> ]	Greenish blue colour	+
Tannin	95% EtOH	Dil H <sub>2</sub> SO <sub>4</sub> , 1% FeCl <sub>3</sub>	Yellowish brown colour ppt	+
Phenolic Compound	Distilled Water	10% FeCl <sub>3</sub> Solution	Purplish blue colour	+
Glycoside	Distilled Water	Distilled water, 10% lead acetate	Yellow colour ppt	+
Reducing sugar	Distilled water	Benedict's solution	Orange red ppt	+
Saponin	Distilled Water	NaHCO <sub>3</sub>	Frothing	+
Terpene	Pep-ether	CHCl <sub>3</sub> , acetic anhydride, conc: H <sub>2</sub> SO <sub>4</sub>	Yellow ppt	+
Steroid	95% EtOH	CHCl <sub>3</sub> , acetic anhydride, conc: H <sub>2</sub> SO <sub>4</sub>	Yellow colour	-

+ = Presence - = Absence ppt = precipitate

### 3.3. Antibacterial Studies

#### 3.3.1. Antibacterial activity of ethanol, ethyl-acetate and n-hexane extract of the whole plant of *Ruellia tuberosa* L.

The study of antibacterial activity was carried out by using 95% ethanol, ethyl, acetate, n-hexane. The results were shown in Table 2. According to this result, ethanol extract, n-hexane extract and ethyl-acetate extract were obtained the best inhibition zone diameter (0.7 cm) on *Salmonella typhi*. The inhibition zone of ethanol extract showed the best on *Staphylococcus aureus* (0.8 cm) and less inhibition zone on *Pseudomonas aeruginosa* (0.3 cm). All of the bacteria strain except in *E. coli* was showed sensitive to different plant extract. *E. coli* strain was resistant to all of the plant extract (n-hexane, ethyl-acetate and ethanol). These are not showed inhibition zone.

**Table 2. Antibacterial activity of different solvent extracts from the whole plant of *Ruellia tuberosa* L.**

Organisms	Control (Ethanol)	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>
<i>Bacillus cereus</i>	0.2 cm	0.5 cm	0.4 cm	0.6 cm
<i>Escherichia coli</i>	-	-	-	-
<i>Staphylococcus aureus</i>	0.2 cm	0.4 cm	0.5 cm	0.8 cm
<i>Shigella boydii</i>	0.2 cm	0.6 cm	0.6 cm	0.5 cm
<i>Salmonella typhi</i>	0.3 cm	0.7 cm	0.7 cm	0.7 cm
<i>Pseudomonas aeruginosa</i>	0.3 cm	0.5 cm	0.7 cm	0.3 cm

- = no inhibition

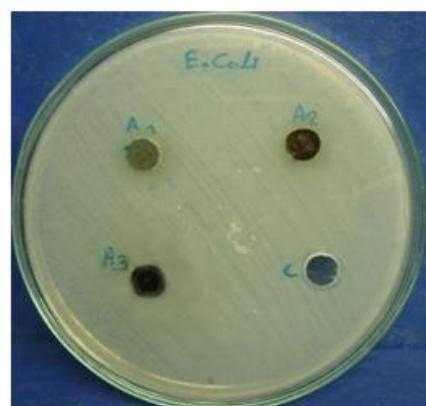
A<sub>1</sub> = *Ruellia tuberosa* L. with n-hexane extract

A<sub>2</sub> = *Ruellia tuberosa* L. with ethyl acetate extract

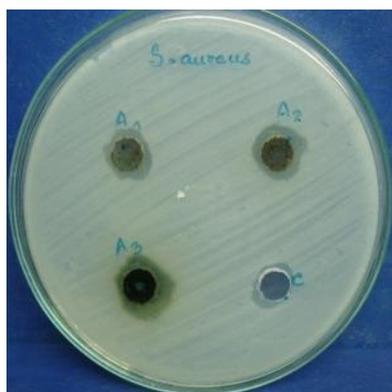
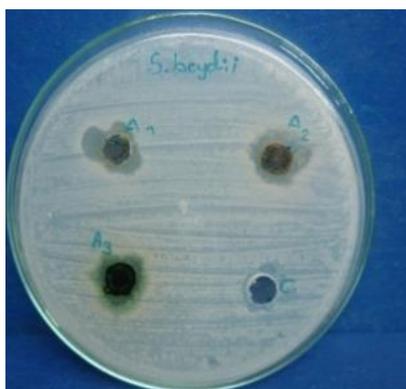
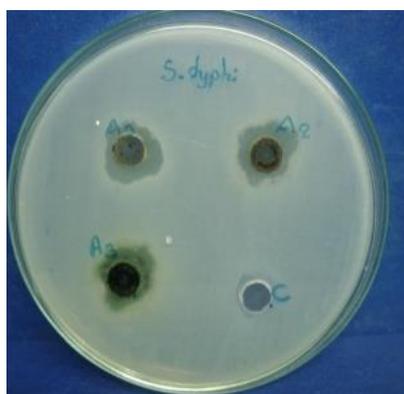
A<sub>3</sub> = *Ruellia tuberosa* L. with ethanol extract



A. *Bacillus cereus*



B. *Escherichia coli*

C. *Staphylococcus aureus*D. *Shigella boydii*E. *Salmonella typhi*F. *Pseudomonas aeruginosa*

**Figure 3. Antibacterial activity of the whole plant of *Ruellia tuberosa* L.**

#### 4. Discussion and Conclusion

In the present research work, the whole plants *Ruellia tuberosa* L. family Acanthaceae with the various extracts was carried out for phytochemical and activity against some of the bacterial strains, This plant was erect herbs with tuberous roots; leaves simple, obovate; inflorescence dichasial cymes; flowers bisexual, zygomorphic, pentamerous, pale blue or violet; fruits capsule, oblongoid, mucronate at the apex. These characters were in accordance with those described by [4], [6] and [8].

The presence of the secondary metabolites in the crude extracts of this plant may be responsible for some of the biological activity. The preliminary phytochemical investigation on the whole plants of *Ruellia tuberosa* L. contains alkaloid, terpene, polyphenol, glycoside, phenolic compound, reducing sugar, tannin and sponin but flavonoid and steroid were absent.

According to antibacterial activity investigation, ethanol extract more effective than those of the other extract. The ethanol extract most effective on the *Staphylococcus aureus* bacterial strain (0.8 cm). The ethanol extract, n-hexane extract and ethyl-acetate extract effective on *Salmonella typhi* (0.7 cm). *E.coli* strain not effective on all plant extract.

*Escherichia coli* to urinary tract infections, neonatal meningitis, septicemia, diarrhoea and dysentery, *Staphylococcus aureus* to skin infections and food poisoning, *Shigella boydii* to dysentery, *Salmonella typhi* to cause systemic infections, typhoid fever, *Pseudomonas aeruginosa* to pneumonia, septic shock, urinary tract infection, gastrointestinal infection, skin and soft tissue infections, *Bacillus cereus* to eye infection, soft tissue and cutaneous infections, according to [5].

In conclusion, *Ruellia tuberosa* L. contains potential antibacterial and phytochemical components that may be used for against various pathogens. To do this, the three extracts were tested to the 6 bacterial species. This plant crude extracts could serve as potential sources of new antibacterial agents. However, further investigations are required to identify the active constituents and to verify the medicinal properties of the active constituents.

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