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ANTIBACTERIAL, ANTIFUNGAL AND INSECTICIDAL ACTIVITIES OF *RUELLIA TUBEROSA* (L.) ROOT EXTRACT

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Abstract

Context: Plants as therapeutics are popularized for thousands of years and people continue to rely on them for health care until now due to their effectiveness, easy availability, low cost and comparatively being devoid of serious toxic effects. Microorganisms such as bacteria, fungi and insects are developing resistance to the current therapies very easily and the currently available antibacterial, antifungal agents and pesticides are very much costly and toxic. So the current shift to the use of herbal antibacterial, antifungal agents and pesticides may be more effective, economic and advantageous.

Objectives: The present research was performed to investigate the antibacterial, antifungal and insecticidal activities of the methanolic extract of the dried root of the plant *Ruellia tuberosa* (L.).

Materials and Methods: Five Gram (+) ve bacteria namely *Staphylococcus aureus, Staphylococcus agalactiae, Bacillus cereus, Bacillus megaterium, Bacillus subtilis*; five Gram (-) ve bacteria namely *Pseudomonus aeruginosa, Escherichia coli, Shigella flexneri, Shigella dysenteriae, Shigella sonnei* were used as test bacteria for testing the antibacterial activity of the plant extract. Antifungal activity was observed against six fungi namely *Candida albicans, Aspergillus niger, Aspergillus ochreus, Aspergillus ustus, Rizopus oryzae* and *Trichophyton rubrum*. The disc diffusion assay method was used in both the cases and standard Kanamycin disc (30µg/disc) was used as the reference standard. The test for insecticidal activity was performed by using surface film activity testing method and *Tribolium castaneum* (Herbst) was used as the test insect.

Results: The methanol extract was active against all the bacteria and fungi tested and showed significant antibacterial and antifungal properties with the zone of inhibition 9 to 23 mm for antibacterial screening and 8 to 15 mm for antifungal screening. The insecticidal assay by surface film activity test also revealed strong insecticidal activity with 80% mortality rate of *Tribolium castaneum* (Herbst) at a dose of 50 mg/ml in 48 hours.

Conclusion: From our experiment it is informed that *Ruellia tuberosa* (L.) may be used to treat bacterial and fungal diseases and also as insect repellant and it is also possible to isolate antibacterial, antifungal and insecticidal drug from this plant.

Key words: *Ruellia tuberosa*, methanol extract, antibacterial, antifungal and insecticidal activity.

Introduction

Globalization interferes with infectious disease control at the national level while microbes move freely around the world, unhindered by borders, human responses to infectious diseases and are conditioned by jurisdictional boundaries (Stepanovic *et al.* 2003). According to WHO, important progress has been made in controlling major infectious diseases. About 43% of total deaths occured in developing countries due to infectious diseases in recent years (Carballo *et al.* 2002). Similarly freedom from insect infestation and contamination has become an important consideration in storage of grain and to maintain high quality food product by preventing them from attack of the most frequently invading organisms (Coolins 1998).

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Ruellia tuberosa (L.) is an erect, sub erect or diffuse perennial herb up to 60-70 cm tall and belongs to the family Acanthaceae, a native of Central America introduced into Indian gardens as ornament and widely distributed in South East Asia including Thailand and Laos. It is used medicinally in West Indies, Central America, Guiana and Peru. *Ruellia tuberosa* (L.) is commonly known as "Cracker plant" (Pandey 2005, Medicinal plants of the Guiana's and Chothani *et al.* 2010). In Siddha system of medicine, leaves are given with liquid copal as remedy for gonorrhea and ear diseases (Suseela and Prema 2007), used in stomach cancer (Reddy *et al.* 1991). Dried and ground roots in dose of two ounces cause abortion and also used in sore eyes (Kirtikar and Bashu 1935). The herb also exhibits emetic activity and employed as substitute of ipecac also used in bladder stones and decoction of leaves is used in the treatment of bronchitis (The wealth of India 1972). In Suriname's traditional medicine system, it is used as anthelmintic and also in the management of joint pain and strained muscles. In folk medicine, it has been used as diuretic, antipyretic, antidiabetic, antidotal, thirst- quenching agent and analgesic and antihypertensive agent (Chiu and Chang 1995, Chen *et al.* 2006). *Ruellia tuberosa* (L.) is used as cooling agent in urinary problems and uterine fibroids (Lans 2001 and 2006). It has recently been incorporated as a component in an herbal drink in Taiwan (Balick *et al.* 2000). It is reported that it contains flavonoids, steroids, triterpenoids and alkaloids (Lin *et al.* 2006, Subramanian and Nair 1974, Singh *et al.* 2002, Andhiwal and Varshney 1985). The aim of this study was to explore the antibacterial and antifungal activity of *Ruellia tuberosa* (L.) roots to determine the scientific basis for its use in folk medicine to treat microbial pathogen and other infectious diseases. Besides this, we made an attempt to investigate the importance of *Ruellia tuberosa* (L.) roots as strong natural insecticide.

Materials and Methods

Collection of plant Materials

The fresh roots of *Ruellia tuberosa* (L.) were collected during the month of July 2009, from the village Haibotpur, situated near Natore district of Bangladesh and identified by Md. Arshed Alom, Taxonomist, Department of Botany, University of Rajshahi, Bangladesh.

Extraction of plant Materials

The collected roots were sun dried for 10-12 days and then kept in an electric oven for 72 hrs at 40°C. Then the dried roots were pulverized into coarse powder with the help of a grinding machine. The ground powder (900 gm) was extracted with methanol (4.5 Liters) in an air tight clean flat bottomed container for 12 days at room temperature with occasional stirring and shaking (Trease and Evans 1997).The extract was then filtered first through a fresh cotton plug and finally with a whatman filter paper. The filtrate was then evaporated to dryness in vacuum by a rotary evaporator at 40 - 50°C to afford a brownish mass (35 gm) and kept for further analysis.

Collection of microorganisms

Antibacterial activity was determined against five Gram (+) ve bacteria (*Staphylococcus aureus, Streptococcus agalactiae, Bacillus cereus, Bacillus megaterium* and *Bacillus subtilis*) and five Gram (-) ve bacteria (*Pseudomonus aeruginosa, Escherichia coli, Shigella flexneri, Shigella dysenteriae* and *Shigella sonnei*). The antifungal screening was carried out against six fungi (*Candida albicans, Aspergillus niger, Aspergillus ochreus, Aspergillus ustus, Rizopus oryzae* and *Trichophyton rubrum*). All these organisms were collected from the Microbiology Research Laboratory of Pharmacy Department, University of Rajshahi, Rajshahi, Bangladesh.

Collection of test insect

For insecticidal screening the insect *Tribolium castaneum* (Herbst) used in the experiment was provided from the stock cultures of the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh.

Growth media and conditions

Nutrient agar media (Difco Laboratories) pH 7.2 and Sabouraud dextrose agar media (Biolife Vole Monza) pH 5.6 were used for antibacterial and antifungal screening respectively.

Antibacterial screening

The *invitro* antibacterial activity of the extract was determined by disc diffusion method (Bauer *et al.* 1996). The test sample was prepared by dissolving 50 mg of the methanol extract in 2 ml of respective solvent to give a concentration of 25 µg/µl. Sample discs were prepared by allowing each sterile disc (6 mm in diameter) of filter paper to absorb 20 µl of a test solution in aseptic condition. The discs were allowed to dry until complete evaporation of solvent. Dried and sterilized filter paper discs, each containing a test sample of 500 µg of the test agent was placed on nutrient agar medium uniformly seeded with the test microorganisms. Kanamycin disc (30 µg/disc) and blank disc were used as the positive and negative control respectively. The plates were incubated at 37°C for 24 hours for optimum growth of the organisms. The antibacterial activity of the extract was determined by measuring the diameter of the zone of inhibition expressed in millimeter.

Antifungal screening

The extract was screened for its antifungal activity by disc diffusion method at the concentration of 500µg/disc. Sabouraud dextrose (20 ml) plates were prepared and incubated by spread plate method under aseptic conditions. The sterile impregnated discs with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with agar surface. Control discs of Kanamycin were prepared and placed on the agar surface. All the plates were incubated at 37°C for 72 hours and the size of the inhibition zones were measured. The mean zone of inhibition of the three replicated tests (triplicate analysis) of the plant extract is expressed in millimeter.

Insecticidal screening

For the conduction of surface film activity test of the plant extract, 60 mm petridishes were taken. The plant extract (50 mg) was dissolved into 1 ml methanol. This was poured into the lower part of the petridish. A control experiment applying only the solvent into the petridish was also set at the same time under the same conditions (Bousquet 1990). After completing all the arrangements, treated petridishes were placed in a secured place at room temperature. The whole experiment was observed from time to time and mortality was observed first after 30 minutes and then after 12 hrs, 24 hrs, 36 hrs and finally after 48 hrs of exposure and data were recorded. A simple microscope was used to observe each and every beetle by tracing natural movements of each organism. In some cases hot needle was taken closer to the bodies (without movement) to confirm death. Attention was also paid to recover the insects if occurred.

Results and Discussion

The result representing antibacterial activity of methanol extract of the roots of *Ruellia tuberosa* (L.) is presented in Table 1. The highest activity of the plant extract was 23 mm diameter of zone of inhibition found against Gram (-) ve bacteria, *Shigella dysenteriae followed by Shigella sonnei* (22 mm), *Shigella flexneri* (21 mm), *Escherichia coli* (18 mm) and *Pseudomonus aeruginosa* (17 mm) at a concentration of 500 µg/disc. The plant extract was less effective against Gram (+) ve than Gram (-) ve bacteria. The lowest antibacterial

activity was observed against Bacillus *megaterium* with the least zone of inhibition, 9 mm diameter and then 10 mm against *Bacillus cereus*, 11 mm against *Bacillus subtilis* and 13 mm against *Staphylococcus aureus* and *Streptococcus agalactiae.* The reason for different sensitivity could be due to morphological difference between microorganisms.

Table 1. Antibacterial activities of the methanolic extract of *Ruellia tuberosa* (L.) roots

Test bacterial strains	Diameter of Zone of Inhibition(mm)	
	Methanol extract 500 µg/disc	Std. Kanamycin 30 µg/disc
	Gram (+ve) bacteria	
Bacillus subtilis	11	21
Bacillus megaterium	09	22
Bacillus cereus	10	23
Staphylococcus aureus	13	22
Streptococcus agalactiae	13	20
	Gram (- ve bacteria	
Escerichia coli	18	28
Shigella dysenteriae	23	29
Shigella sonnei	22	28
Shigella flexneri	21	26
Pseudomonus aeruginosa	17	27

The methanolic extract of roots of *Ruellia tuberosa* (L.) was found to be effective against various fungi as indicated by the zone of inhibition (Table 2). Maximum inhibition was obtained against *Candida albicans* (18 mm) followed by *Aspergillus niger* (16 mm), *Aspergillus ochreus* (15 mm), *Aspergillus ustus* (14 mm), *Rizopus oryzae* (10 mm) at a concentration of 500 µg/disc in comparison to reference standard kanamycin 30µg/disc. The least zone of inhibition was noted against *Trichophyton rubrum* (8 mm).

Table 2. Antifungal activities of the methanolic extract of *Ruellia tuberosa* (L.) roots

Test fungal strains	Diameter of Zone of Inhibition(mm)	
	Methanol extract 500 µg/disc	Standard Kanamycin 30 µg/disc
Aspergillus niger	16	23
Aspergillus ochreus	15	21
Aspergillus ustus	14	21
Candida albicans	18	26
Rizopus oryzae	10	19
Tricophyton rubrum	08	17

The insecticidal activity of methanol extract of *Ruellia tuberosa* (L.) roots has been studied by testing it against the insect, *Tribolium castaneum* (Herbst) and the results are represented in Table 3. The maximum mortality rate of *Tribolium castaneum* (Herbst) was 80% at a dose of 50 mg/ml in 48 hrs. The results have shown that the methanolic extract of *Ruellia tuberosa* (L.) root was highly toxic to insects.

Table 3. Insecticidal activities of the methanolic extract of *Ruellia tuberosa* (L.) roots.

Extract	Amount of extract (mg/ml)	Number of insect used	Number of insect killed					Mortality %
			30 min	12 hrs	24 hrs	36 hrs	48 hrs	
Methanolic extract of *Ruellia tuberosea* Linn. Roots.	50	15	-	1	5	10	12	80%

As an evolutionary process, plants on which insects, microorganisms and mammals are feeding, usually acquire self defending capabilities by producing a variety of secondary metabolites such as alkaloids, terpenoids, steroids and aromatic compounds which are presumably unpleasant or even toxic to the enemy. Inside the tissue of nearly all the healthy plants, there are a lot of microorganisms which are called endophytes. Endophytes are mutualistic to their host; at least some of them are thought to be making returns for the nutrition from the plant by producing special substances such as secondary metabolites to prevent the host from successful attack of fungi, pest and mammals. As a matter of fact, metabolites of endophytes were reported to inhibit a number of microorganisms (Fisher *et al.* 1984, Gurney and Mantle 1993).

Yang and Tang (1998) reviewed the plant used for insect control and found that there is strong connection between medicinal and pesticidal plants. Not only the world wide annual losses of food grains storage caused by insects have been estimated to be about 10% of the world's production, but loses of 25% or more may also occur in tropical countries through insect attack after harvest (Howe 1965).

Previous phytochemical invstigations on this plant have revealed the presence of flavonoids, steroids, triterpenoids and alkaloids. So, the insecticidal activity showed by the extract of *Ruellia tuberosa* (L.) roots may be due to the presence of such type of phyto-constituents.

Human pathogenic microorganisms, phyto-pathogens are prone to developing drug resistance to decrease substantially the effectiveness of those pesticides (Rosenberger and Meyer 1981). Therefore, there is an urgent need to work towards the development of safer antimicrobial agents and bio-pesticides that are expected to be renewable, non-petrochemical, naturally eco-friendly and easily obtainable.

Conclusion

From the results obtained, it is evident that *Ruellia tuberosa* (L.) root possesses potential inhibitory activity against human pathogens (bacteria and fungi) and insects. Hence, there is a need to isolate possibly by purification of the various phytochemical groups in the extracts. The further isolation of such bioactive components could perhaps clarify the pharmacological properties of *Ruellia tuberosa* (L.) root and be further exploited for pharmaceutical use.

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