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Phytochemical, Antimicrobial, Anthelminthic and Antidiarroheal Activity of Traditional Plant *Randia uliginosa* Retz

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Abstract

The present study was designed to evaluate the phytochemical, antimicrobial, antidiarroheal, anthelminthic activity of methanol, chloroform and petroleum ether extract of whole plant of *Randia uliginosa* Retz. (Family- Rubiaceae) by *in vivo* and *in vitro* test. Different crude extracts of *Randia uliginosa* Retz. have been shown to possess phyto-constituents including carbohydrates, alkaloid, glycosides, steroids, tannins and saponin. Concentration 50 mg/mL of methanol extract showed maximum anthelminthic activity which is comparable to the standard (Piperazine Citrate, 10 mg/mL). Methanol extract of *R. uliginosa* at a dose of 30 µL/disc showed maximum antimicrobial activity. Significant ($p<0.01$) level of reduction in fecal dropping was found in 250 mg/kg methanol extract and highly significant at 500 mg/kg methanol extract. The maximum inhibition of defecation 56.98% is observed in 500 mg/kg extract which is comparable to standard Loperamide. These data demonstrate that the plant may contain bioactive compounds possessing antimicrobial, antidiarroheal and anthelminthic activities.

Keywords: *Randia uliginosa* Retz; Antidiarroheal; Anthelminthic; Antimicrobial

Introduction

Traditional medicine is an important source of potential drugs for contemporary applications in various infectious diseases. Natural products have played an important role as new chemical entities. Between 1981 and 2002 approximately 28% of new chemical entities of medicine were natural product-derived [1]. Koehn and Carter in their research paper found that natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereo centers that can be challenging to derive synthetically [2].

An important change in resistance prevalence rates has occurred with the shift from Gram-positive to multi-resistant Gram-negative bacteria, for which treatment options are limited or entirely lacking which excelled the search for new antimicrobial principles in traditional medicinal plants [3]. The alkaloids present in the plants may be responsible for the antibacterial activity [4]. The antimicrobial activity may also be due to the presence of flavonoids [5], saponins [6], steroids [7]. The plant extracts are specially tested in drug-resistant organisms to get a more effective and valuable antimicrobial agent [8].

Many synthetic chemicals like diphenoxylate, loperamide and antibiotics are available for the treatment of diarrhoea but they have some side effects. The natural drugs are used as antidiarroheal drugs, which are not always free from adverse effects. Therefore, the search for safe and more effective agents has continued to be an important area of active research [9]. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on traditional medicine. Different plants such as roots of *Jatropha curcus*, roots and unripe fruit of *Aegle marmelos*, *Jussiaea suffruticosa*, *Mangifea indica*, *Musa paradisiaca*, *Ocimum sanctum*, *Xanthium indicum* are traditionally used [10]. The present study was undertaken to evaluate the antidiarroheal potential of *Randia uliginosa* Retz.

Yadav and Singh in their research paper found that about half of the world's population suffers from helminthiasis and the number is increasing day by day [11]. It is not only limited to tropical and subtropical countries but is also to endemic in many regions because of poor sanitation, poor family hygiene, malnutrition and crowded living condition. Potent anthelminthic are available today and treatment is frequent done by using different type of drugs. However the high costs of modern anthelminthic have limited effective control of the parasites. In some cases, wide spread use of low quality anthelminthic are used for the development of resistance and hence causes reduction in use of anthelminthic. It was found that plants like *Benincasa hispida*, *Caesalpinia bonducella*, *Allium sativum, Zingiber officinale*, *Curcurbita mexicana and Ficus religiosa* have anthelminthic effect [12-14].

So, on the above information demonstrate that drugs derived from plant origin have a promising future in helminthiasis, microbial and diarrheal treatment. Hence in the present study, methanol, chloroform and petroleum ether extracts of whole plant of *R. uliginosa* Retz. were examined for its antimicrobial, antidiarroheal, anthelminthic and phytochemical properties.

Materials and Methods

Collection, identification and preparation of *R. uliginosa* Retz. extracts

The whole plant of *R. uliginosa* Retz. was collected from Jahangirnagar University campus, savar, Dhaka and was identified by experts in Bangladesh National Herbarium, mirpur, Dhaka (Accession number of *Randia uliginosa* Retz.- 37959). The collected plant parts were thoroughly washed with water and dried in hot air oven at 60°C for 3 days. The dried parts were ground to coarse powder with a mechanical grinder. Plant powder sample was extracted by methanol, petroleum ether and chloroform. Extraction was performed at room temperature and preserved in petridish in refrigerator [10].

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Source of chemicals

All the chemicals/drugs and solvent used in this study were of analytical grade and purchased from Merck, Sigma- Aldrich, Well's Health Care Spain and Incepta Pharmaceuticals Ltd., Bangladesh.

Animals and treatment

Due to anthelminthic activity earthworms *Pheretima posthuma* (Annelida) were collected from moist soil at Jahangirnagar University area. Earthworms were washed with normal saline to remove soil and fecal matter and identified by Zoology Department, Jahangirnagar University. For the purpose of antidiarroheal activity *Swiss albino* mice of either sex were collected from Pharmacology lab, Jahangirnagar University. Animals were maintained under standard environmental conditions temperature (24.0 ± 1.0°C), relative humidity 55-65% and 12 h light/12 h dark cycle. Pellets of mice foods from ICDDRB were given to the mice with fresh water *ad libitum*. All protocols for animal experiment were approved by the institutional animal ethical committee.

Phytochemical screening

Qualitative phytochemical tests including Dragendroff reagent for alkaloids, Molisch's test for carbohydrates, Frothin test for saponin, HCl test for flavonoids, Salkowski's test for steroids, Ferric chloride test for tannins, General test for glycosides were performed for the determination of presence of different class of constituents in the extract using the methods described by Ghani [10].

Authentication for anthelminthic activity

Anthelminthic activities of the plant extracts were proposed by Ajayieoba et al. [15]. This study was evaluated in adult earthworm (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being. The groups of equal sized earthworms consisting of 3 earthworms in each group were released in 50 mL of sample with desired concentrations 10, 25 and 50 mg/mL. Group of earthworms in 1% Tween 80 was used as control group and group of earthworms in Piperazine citrate (10 mg/mL) used as reference. Observations were made for the time taken for paralysis and death of individual worms. Paralysis as said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Death was concluded when the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C.

Tests for antimicrobial activity

The antimicrobial activity of the plant extract was performed by the well accepted Bauer-Kirby method [16]. The microorganisms used in the antimicrobial activity assay of the plant extracts were carried out on both Gram-positive (*Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Serratia spp.*, *Salmonella typhi* and *Pseudomonas spp.*) bacteria. In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in laminar air hood and all types of precautions were highly maintained. The test microorganisms were seeded into respective medium by spread plate method. Sterilized metrical (Cocksville, USA) filter paper discs were taken and soaked with 30 µL, 20 µL and 10 µL of solutions of test samples. After then the filter paper discs were placed on test organism-seeded plates. Amoxicillin (10 µL/disc) used as standard antimicrobial disc and methanol, Chloroform and Pet. ether extracts (10, 20, 30 µL/disc) were used as sample discs. The antibacterial assay plates were incubated at 37°C for 24 h. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter.

Tests for antidiarroheal activity

The antidiarroheal activity of the plant extract was studied in castor oil-induced diarrhea in mice according to the method described by Shoba and Thomas [17]. Screening of the mice for the experiment was done observing the diarrhoea induced after giving 0.5 mL of castor oil. Mice fasted for 24 h were divided into control, positive control (Loperamide) and test samples (RUME, RUCL and RUPE) containing five mice in each group. Control group received 1% Tween 80 in water at the dose of 10 mL/kg per oral. Positive control group was given Loperamide in suspension at the dose of 3 mg/kg per oral. Test groups were given the methanol, pet- ether and chloroform extracts at the doses of 250 and 500 mg/kg. After 1 hour, each group was given 0.5 mL of castor oil orally. Then each animal was placed in a separate cage with blotting paper lined floor. The blotting papers were changed every hour. The animals were observed for the next 4 hours to record the characteristic of diarrhoea. The percent (%) inhibition of defecation was calculated using the formula.

$$\% \text{ Inhibition of defecation} = [(A-B)/A] \times 100$$

Where, A=Mean number of defecation caused by castor oil; B=Mean number of defecation caused by drug or extract.

Statistical analysis

Statistical analysis was carried out using Independent- Sample T test and one way ANOVA using SPSS 16.5 for windows. The results obtained were compared with the control group. The difference were considered significant when $P<0.05$.

Results and Discussion

Phytochemical screening

Preliminary phytochemical screening of the crude extracts of different parts of *R. uliginosa* Retz. revealed the presence of different kind of chemical groups that are summarized in Table 1.

Different extracts of *R. uliginosa* Retz. have been shown to possess phytoconstituents including carbohydrates, alkaloid, glycosides, steroids, tannins and saponin. No flavonoid was detected. These phytoconstituents present in the extracts may account for their various pharmacological activities shown in other investigations.

Anthelminthic activity of *R. uliginosa* Retz.

Earthworm used in anthelminthic activity determination of the plant extracts. Among the three different extract of *R. uliginosa* Retz. methanol extract have shown significant anthelminthic activity. Concentration 50 mg/mL of methanol extract showed maximum activity which is comparable to the standard (Piperazine citrate, 10 mg/mL) summarize in Table 2.

Piperazine citrate produces hyper polarization and reduced excitability by increasing chloride ion conductance of worm muscle membrane that leads to muscle relaxation and flaccid paralysis [18]. The methanol extracts not only demonstrated paralysis, but also caused death of worms especially at higher concentration (50 mg/mL). Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents. Tannins were shown to produce anthelminthic activities [19] and it is possible that tannin can bind to free proteins in the gastrointestinal tract of host animal [20] or glycoprotein on the cuticle of the parasite and may cause death [21].

Antimicrobial activity determination

The result of antimicrobial screening of different extracts of *R. uliginosa* has been presented in Table 3. Methanol extract of *R. uliginosa* at a dose of 30 µL/disc showed maximum efficacy against all the microorganisms. Other than the methanol extract of *R. uliginosa* showed moderate activity against gram positive and gram negative bacteria. The standard, Amoxicillin, exhibited significant zone of inhibition against all the test organisms.

The methanolic extract of leaf exhibited significant antimicrobial activity and it's probably attributed to the presence of steroids [7] and saponin [6] which has been detected earlier in phytochemical screening in Table 1. On the other hand, each of the extract showed moderate antimicrobial activity against all the microorganism except *Bacillus cereus*. So in this context it can be concluded that *R. uliginosa* Retz. will be a greater source of antimicrobial agent.

Tests for antidiarroheal activity

In castor oil-induced diarrhea all the extracts showed dose dependent reduction in fecal dropping Table 4. Significant ($p<0.01$) level of reduction in fecal dropping was found in 250 mg/Kg methanol extract and highly significant at 500 mg/Kg methanol extract. The maximum inhibition of defecation 56.98% is observed in 500 mg/Kg extract which is comparable to standard loperamide.

Conclusion

On the basis of the findings of the present study it can be assumed that the extract of *R. uliginosa* Retz. has strong antimicrobial, antidiarroheal and anthelminthic properties which are similar to the positive controls. These results indicated that this plant could be a potential source for discovery of newer antimicrobial, antidiarroheal and anthelminthic "leads" for drug development. Present study finding supports the traditional claims and provides a scientific basis for antimicrobial, antidiarroheal and anthelminthic effect of *R. uliginosa* Retz.

Future Directions

Based upon the results of the current investigations and previous reports more specific, defined and advanced studies can be carried out. Isolation of the active constituents from the crude extracts and subsequent tests in both *in vitro* and *in vivo* studies with evaluation of their exact mode of action and chronic toxicity profile may help to reach in a concrete conclusion about the current findings.

Extracts	Alkaloid Test	Steroid Test	Glycoside Test	Saponin Test	Carbohydrate Test	Tanin Test	Flavonoid Test
RUME	+	+	+	+	+	+	-
RUPE	+	+	+	+	+	+	-
RUCF	+	+	+	+	+	+	-

+: Present; -: Absence; RUME: Methanol extract; RUPE: Pet. ether extract; RUCF: Chloroform extract

Table 1: Result of chemical group test of different extracts of *R. uliginosa* Retz.

Treatment group	Doses (mg/ml)	Time for paralysis (minutes)	Time for death (minutes)
Control	-	No paralysis	No death observed
Piperazine citrate	10	26 ± 0.83**	37 ± 0.62**
RUME	10	>90	>90
	25	44 ± 2.5	55 ± 3.45
	50	35 ± 2.6**	46 ± 2.5**
RUCL	10	>90	>90
	25	68 ± 4.5	83 ± 0.2
	50	51 ± 4.7	68 ± 2.9
RUPE	10	>90	>90
	25	51 ± 3.05	66 ± 2.1
	50	47 ± 0.6	54 ± 2.2

The values are mean ± SEM. **P<0.01, significantly different from control; Done by independent sample t-test (n=3); RUME: Methanol extract of *R. uliginosa* Retz; RUPE: Pet. ether extract of *R. uliginosa* Retz.; RUCF: Chloroform extract of *R. uliginosa* Retz.

Table 2: Anthelminthic activity of *R. uliginosa* Retz.

Test microorganism	Zone of inhibition in mm									
	Doses µl/disc (RUME)			Doses µL/disc (RUPE)			Doses µL/disc (RUCL)			Standard Amoxicillin
	10	20	30	10	20	30	10	20	30	10
Bacillus subtilis	7.5	13	19	6	9	12	6.5	7	9	28.5
Bacillus cereus	ND	ND	5.5	ND	7.5	9.5	ND	ND	7	9.5
Staphylococcus aureus	8.5	10.5	13	6.5	8	11	7	9.5	10.5	12.5
Pseudomonas mirabilis	11.5	11	12.5	8.5	9	9.5	9.5	11	11.5	9.5
Escherichia coli	12.5	13.5	14.5	7	8.5	9	8	9	11	18
Serratia spp.	9	13	11	7	8.5	9	7.5	8.5	9	10.5
Salmonella typhi	8.5	10	11.5	6.5	9.5	11	6.5	7	9	12
Pseudomonas spp.	9	9.5	12.5	7	9.5	9.5	6	6.5	7.5	20.5

*ND: Not Defined, Zone of inhibition in mm are shown by mean values; RUME: Methanol extract of *R. uliginosa* Retz.; RUPE: Pet. ether extract of *R. uliginosa* Retz.; RUCF: Chloroform extract of *R. uliginosa* Retz.

Table 3: The zone of inhibition produced by the different extracts of *R. uliginosa* Retz. against some gram positive and gram negative bacteria.

Group	Dose (Per oral)	No. of fecal droppings in 4 hours	% Inhibition of defecation
Control	-	18.6 ± 0.850	-
Loperamide	3 mg/kg	6.2 ± 1.284**	66.6667
RUME	250 mg/kg	10 ± 0.42*	46.236
	500 mg/kg	8 ± 0.59**	56.989
RUCL	250 mg/kg	17 ± 0.72	8.602
	500 mg/kg	13 ± 0.69	30.107
RUPE	250 mg/kg	18.2 ± 0.81	2.150
	500 mg/kg	15.4 ± 0.64	17.204

Values are mean ± SEM, (n=6); Done by Dunnet t-test using SPSS 16.6 for windows; *p<0.05, **p<0.01, significantly different from control; RUME: Methanol extract of *R. uliginosa* Retz.; RUPE: Pet. ether extract of *R. uliginosa* Retz.; RUCF: Chloroform extract of *R. uliginosa* Retz.

Table 4: Effect of different extracts of *R. uliginosa* Retz. on castor oil-induced diarrhea in mice.

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