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

**MD Hossain S1, MD Al-Amin**, **MD Hossain A2** and **MD Rana S**

1Department of Pharmacy, Prime Asia University, Banani, Dhaka, Bangladesh
2Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh
3Department of Pharmacy, Daffodil International University, Dhaka, Bangladesh

**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/kg). 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 defeation 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 the shift from Gram-positive to multi-resistant Gram-negative bacteria, for which treatment options are limited or entirely lacking [2].

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 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 bonduc*, *Allium sativum*, *Zingiber officinalis*, *Curcuma 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].

*Corresponding author: MD Al-Amin, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh, Tel: 0088016871392, E-mail: shantopher2016@gmail.com*

Received October 18, 2016; Accepted November 01, 2016; Published November 04, 2016

Citation: Hossain MDS, Al-Amin MD, Hossain MDA, Rana MDS (2016) Phytochemical, Antimicrobial, Anthelminthic and Antidiarroheal Activity of Traditional Plant *Randia uliginosa* Retz. Nat Prod Chem Res 4: 245. doi: 10.4172/2329-6836.1000245

Copyright: © 2016 Hossain MDS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 anthelminthical 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 mices 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 Dragendorff 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. Parasitology 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 move 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 diarrhoea 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.

\[
\text{% Inhibition of defecation} = \left(\frac{A-B}{A}\right) \times 100
\]

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 defeation 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 ± 2.6²                  | 46 ± 2.5                 |
| RUCF            | 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: Antimicrobial activity of R. uliginosa Retz.

| Test microorganism | Zone of inhibition in mm | Standard Amoxicillin |
|--------------------|--------------------------|---------------------|
|                    | Doses µL/disc (RUME) | Doses µL/disc (RUPE) | Doses µL/disc (RUCF) | 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 | 7  | 9  | 9.5 |
| Staphylococcus aureus | 8.5                    | 10.5                | 13                  | 6.5 | 8  | 11 | 7  | 9  | 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.
Table 4: Effect of different extracts of *R. uliginos* Retz. on castor oil-induced diarrhea in mice.

| 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.

Acknowledgements

This study was supported both financially and technically by Phytochemical and Pharmacological Lab, Department of Pharmacy, Faculty of Life Science, Jahangirnagar University, Savar, Dhaka.

References

1. Newman DJ, Cragg GM, Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 66: 1022-1037.
2. Koehn FE, Carter GT (2005) The evolving role of natural products in drug discovery. Nat Rev Drug Discovery 4: 206-220.
3. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, et al. (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. The Lancet Infectious Disease 10: 597-602.
4. Zuo YG, Meng FY, Hao XY, Zhang YL, Wang GC, et al. (2008) Antibacterial alkaloids from chelidonium majus linn. (papaveraceae) against clinical isolates of methicillin-resistant staphylococcus aureus. J Pharm Pharm Sci 11: 90-94.
5. Cushnie TPT, Lamb AJ (2005) Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26: 343-356.
6. Avato P, Bucci R, Tava A, Vitali C, Rosato A, et al. (2006) Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. Phytother Res 20: 454-457.
7. Talebi-Contini SH, Salvador MJ, Watanabe E (2003) Antimicrobial activity of flavonoids and steroids isolated from two Chromolaena species. Braz J Pharma Sci 39: 403-408.
8. Abu-Shanab BG, Adwn D, Safiya A, Jarrar N, Adwan K (2004) Antimicrobial activities of some plant extracts utilized in popular medicine in Palestine. Turkish Journal of Biology 28: 99-102.
9. Maiti A, Dewanjee S, Mandal SC (2007) In Vivo Evaluation of Antidiarrhoegal Activity of the Seed of *Swietenia macrophylla* King (Meliaceae). Tropical Journal of Pharmaceutical Research 6: 711-716.
10. Ghani A (2003) Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edn. Asiatic Society of Bangladesh, 5 old Secretariat Road, Nimtali, Dhaka, Bangladesh.
11. Yadav P, Singh R (2011) A review on anthelmintic drugs and their future scope. International Journal of Pharmaceutical and Pharmaceutical Research 3: 17-21.
12. Bhattacharjee C, Debjit B, Tiwari P, Tripathi KK, Dutta AS (2010) In Vitro anthelmintic activity of *Benincasa hispida* (Petha) thumb leaves. International Journal of Pharma and Bio Sciences 1: 9.
13. Wadkar GH, Kane SR, Matapati SS, Hogade MG (2010) In vitro anthelmintic activity of *Caesalpinia bonducella* (Linn) Flem Leaves. Journal of Pharmacy Research 3: 926-927.
14. Iqbal Z, Nadeem QK, Khan MN, Akhtar MS, Waraich FN (2001) In Vitro Anthelmintic Activity of *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana* and *Ficus religiosa*. Int J Agri Biol 3: 454-457.
15. AjajeebbaEO, Onochara PA, OlarenwaajuOT (2001) In-vitro anthelmintic properties of *Buchholzia coioaceae* and *Gynandropis gynandra* extract. Pharm Biol 39: 217-220.
16. Bayer AW, Kirby WMM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 45: 493-496.
17. Shoba FG, Thomas M (2001) Study of antidiarrheal activity of four medicinal plants in castor-oil induced diarrhea. Journal of Ethnopharmacology 76: 73-76.
18. Martin RJ (1985) Y-Aminobutyric acid and Piperazine activated single channel currents from Ascaris suum body muscle. Br J Pharmacol 84: 445-461.
19. Niezen JH, Wagorn GC, Charleston (1995) Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa* or *Hedysarum coronarium*), which contains condensed tannins. J Agri Sci 125: 281-289.
20. Athnosiadou S, Kyriazakis I, Jackson F, Coop RL (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vivo studies. Vet Parasitol 99: 205-219.
21. Thompson DP, Geary TG (1995) The structure and function of helminthes surfaces. Biochemistry and Molecular Biology of Parasites 1: 203-232.