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*Full Length Research Paper*

# Antibacterial and cytotoxic activities of the methanolic extracts of *Rhododendron arboreum*

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**The antibacterial activity of the methanolic extract of various parts of *Rhododendron arboreum* such as flowers, leaves, bark, stem and roots were investigated against medically important pathogens by determining the zone inhibition. The result showed that the extract had good antimicrobial activity against the tested bacterial strains such as *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilus* and *Salmonella typhi*. All parts showed low to significant antibacterial activity against the mentioned bacterial strains. The cytotoxicity of the crude methanolic extracts of various parts of *R. arboreum* was evaluated against *Artemia salina* at 1000, 100 and 10 µg/ml. All parts showed significant activity at concentration of 1000 µg/ml, good activity at 100 µg/ml and no activity at 10 µg/ml, against *A. salina.* This study shows a broad spectrum and great therapeutic potential of the plant with the ability to attract significant scientific attention.**

**Key words:** Antibacterial activity, *Rhododendron arboreum,* inhibition zones, cytotoxicity, *Artemia salina.*

## INTRODUCTION

The use of medicinal plants in the treatment and prevention of diseases is attracting attention by scientists worldwide. The use of plants whether herbs, shrubs or trees in parts or in whole in the treatment and management of disease and disorders date back to prehistoric days. Since the middle of the 19th century, numerous bioactive constituents have been isolated and characterized. Many of these are being used as the active ingredients of the modern medicine, or as the lead compounds for new drugs discovery.

Several plant derived medicines, rich in phenolic compounds (Scalbert, 1993), are used in protection against coronary heart diseases and carcinogenesis (Hertog et al., 1995). Pakistan possesses a vast array of medicinal flora; especially, the Khyber Pakhtoonhwa province is rich in medicinal plants. *Rhododendron arboreum* is a member of the family Ericaceae. The genus *Rhododendron* consists of 1000 species (Berg and Heft, 1991) which are distributed throughout the world, mostly concentrated in China, India, Malaysia and Nepal (Chamberlain, 1982; Rotherham, 1983). Phytochemically, *R. arboreum* was reported to contain a number of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, phlobatanins and steroids (Nisar et al., 2011). Most of the *Rhododendron* species are known as toxic plants and GTXs (Grayanotoxins I and III) are accounted for their toxicity. GTXs are secondary metabolites belonging to the diterpene class (Kan et al., 1994). Locally this plant is reported to be effective for the treatment of astringent, diuretic, choleretic, antispasmodic, chronic eczema, diarrhoea, dysentery and menstrual disorders (Hentschel et al., 1995).

Flavone glycoside and dimethyl ester of terphthalic acid isolated from the leaves of *R. arboreum* have potent anti-inflammatory property (Kamil et al., 1995). The leaves of *R. arboreum* showed potent antioxidant property (Dhan et al., 2007), while the ethanolic extract of the flowers showed potent anti-diabetic property (Bhandary and Kawabata, 2008).

The aim of this work was to investigate the cytotoxicity and antimicrobial activity of the methanolic extracts of different parts of *R. arboreum* such as flowers, leaves,

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bark, stem and roots.

## MATERIALS AND METHODS

### Collection of plant material

The plant *R. arboreum* was collected from Seran valley (Khyber Pakhtoonkhwa, Pakistan) in the month of February 2011. A voucher specimen for this collection has been deposited in the National Herbarium of Peshawar University, for future reference.

### Preparation of the extract

The shade dried plant material such as flowers (50 g), leaves (50 g), bark (50 g), stem (50 g) and roots (50 g) were crushed into small pieces and finally pulverized into fine powder. The plant materials were soaked in methanol with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature using rotary evaporator.

### Test organisms

The microorganisms used in the antibacterial activity are: *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilus* and *Salmonella typhi* were collected from stock culture in the Pathology Department, Agriculture University Peshawar, Pakistan. The organisms were stored on agar slants and kept in the refrigerator, prior to subculture.

### Antibacterial activity

The crude methanolic extracts of various parts of *R. arboreum* such as flowers, leaves, bark, stem and roots were screened against various human pathogens including *E. coli*, *S. aureus, B. subtilus* and *S. typhi* as per reported procedure (Alves et al., 2000; Stepanovic et al., 2003). In this method, 10 ml aliquots of nutrients broth (Sigma-Aldrich, USA) was inoculated with the test organism and incubated at 37°C for 24 h. Using a sterile pipette, 0.6 ml of the broth culture of the test organism was added to 60 ml of molten agar, which had been cooled to 45°C, mixed well and poured into a sterile Petri dish (for the 9 cm Petri dish, 0.2 ml of the culture was added to 20 ml of agar).

Duplicate plates of each organism were prepared. The agar was allowed to set and harden and the required numbers of wells were dug in the medium with the help of a sterile metallic cork borer ensuring proper distribution of the wells in the periphery and one in the center. Agar plugs were removed. Stock solutions of the test samples in the concentration of 1 mg/ml were prepared in the sterile dimethyl sulfoxide (DMSO), and 100 and 200 µl of each dilution was added in their respective wells. The control well received only 100 and 200 µl of DMSO. *Imipenem* was used as standard drug. The plates were left at room temperature for 2 h to allow diffusion of the samples then incubated face upwards at 37°C for 24 h. The diameter of the zones of inhibition was measured (mm) (Figure 2).

### Brine shrimp cytotoxicity test of crude extract

The materials and reagents used for cytotoxicity includes, test sample, *Artemia salina* (shrimp eggs), sea salt (38 g/L of D/W, pH 7.4), hatching tray with perforated partition, lamp to attract brine-shrimp larvae, micro pipette (5, 50 and 500 µl), vials tray, 9 vial samples, methanol, distilled water. The cytotoxic activity of the crude extract of the plants was carried out by following the method of Meyer et al. (1982).

### Hatching techniques

The hatching tray [a rectangular dish (22 × 32 cm)] was half-filled with filtered brine solution and (50 mg) eggs of brine shrimps were sprinkled in it and incubate at 37°C for 24 h. After the incubation period, brine shrimps were hatched and the plants extracts were applied to find out the cytotoxic effect of these extracts.

### Sample preparation

Test sample was dissolved (20 mg) in 2 ml of DMSO and from this solution 5, 50 and 500 µl was transferred to vials (3 vials/ concentration). The concentration was 10, 100, and 1000 µg/ml, respectively. After 2 days of hatching and maturation, 10 larvae/ vials were placed, using a Pastuer pipette. The volume was made 5 ml with seawater. It was incubated at 25 to 27°C for 24 h under illumination. Other vials were supplemented with DMSO and reference cytotoxic drug which served as negative and positive controls, respectively. The data was analyzed with Finney computer program to determine $LD_{50}$ values with 95% confidence intervals.

## RESULTS

Antibacterial assay of crude methanolic extract of various parts, that is, flowers, leaves, bark, stem and root of *R. arboreum* was done through the growth in the medium containing crude extracts by measuring the zone of inhibition (mm) with reference to the positive control. The results are summarized in Figures 1 to 5. Whereas, the cytotoxicity using brine shrimp lethality assay has shown that the methanolic extracts of various parts of *R. arboreum* is potent against *A. salina* having high $LD_{50}$ values of 20 µg/ml for leaves when compare to the reference drug (Table 1).

## DISCUSSION

### Antibacterial activity

Figures 1 to 5 shows the antibacterial activity of the methanolic extracts of flowers, leaves, bark, stem and roots of *R. arboreum* against the mention human pathogens. It was observed that the methanolic extract of flower showed potent antibacterial activity against *E. coli*, *S. aureus, B. subtilus* and *S. typhi* in the following order: *E. coli* (80%) > *B. substilus* (60%) > *S. typhae* (50%) > *S. aureus* (45%). Flower extract showed good to significant activity against the mentioned bacterial strains. Similarly, the methanolic extract of leaves showed antibacterial activity in the following increasing order: *B. substilus* (85%) > *S. typhae* (70%) > *S. aureus* (65%) > *E. coli* (10%). The antibacterial activity of leaves was from low to

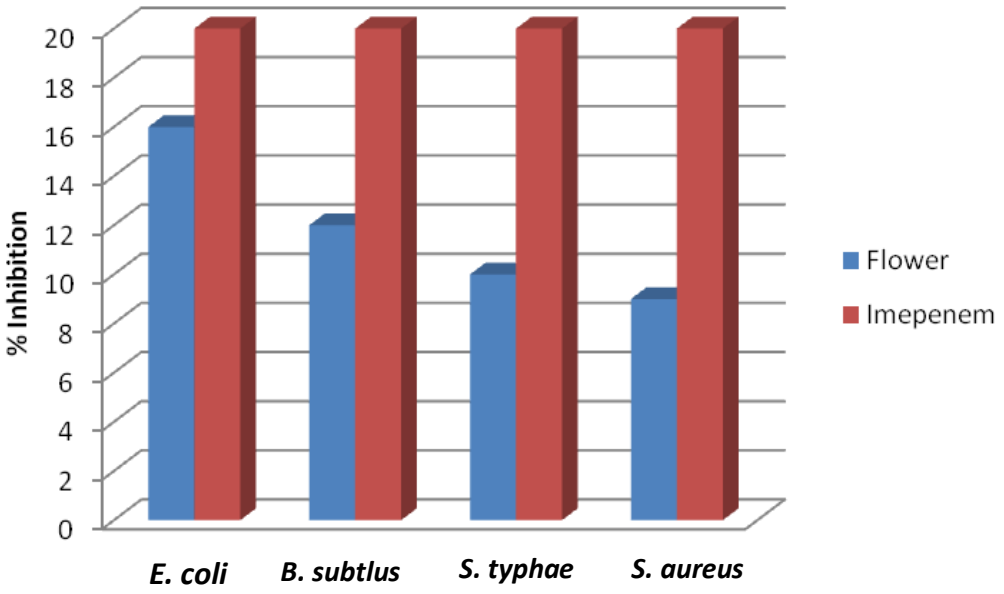


**Figure 1.** Antibacterial activity of flower extract of *R. arboreum* against various human pathogens.

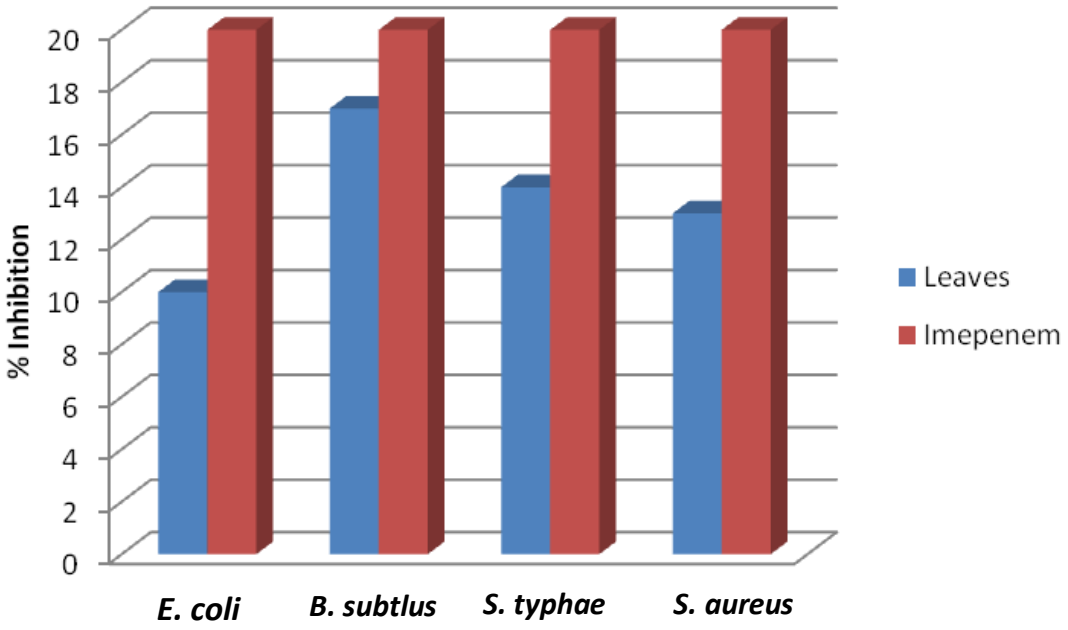


**Figure 2.** Antibacterial activity of leaves extract of *R. arboreum* against various human pathogens.

significant against the test bacteria. The bark extract was also active against the mentioned human pathogens. The antibacterial activity of the bark methanolic extract was in the following order: *S. typhae* (75%) > *E. coli* (70%) > *B. substilus* (55%) > and *S. aureus* (35%). Similarly, the methanolic extract of stem and roots showed the antibacterial in the following order: *S. aureus* (80%) > *E. coli* (75%) > *B. substilus* (65%) > *S. typhae* (50%) and *B. substilus* (80%) > *S. aureus* (65%) > *E. coli* (50%) > and *S. typhae* (25%) respectively. The high potency of the extracts of *R. arboreum* against these bacteria shows its scientific basis for its uses in traditional medicine in the treatment of different types of cough, diarrhea and dysentery. These antibacterial activities are likely due to the presence of the secondary metabolites present in the extract.

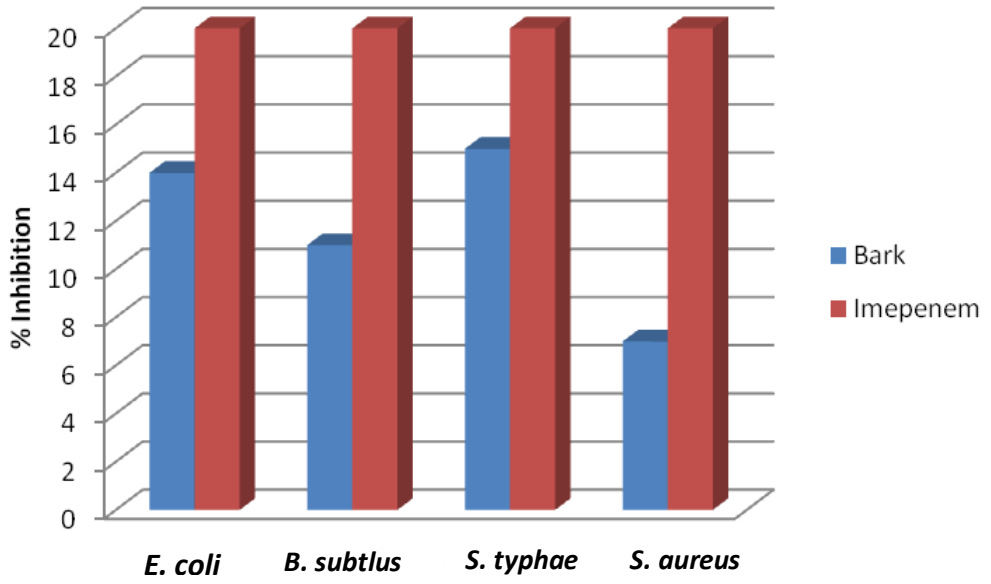


**Figure 3.** Antibacterial activity of bark extract of *R. arboreum* against various human pathogens.

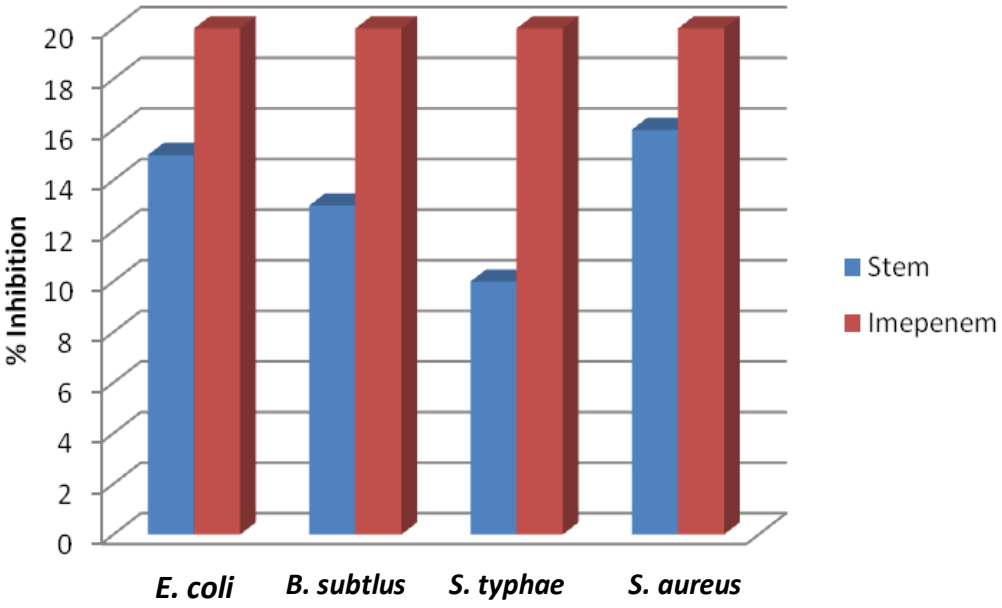


**Figure 4.** Antibacterial activity of stem extract of *R. arboreum* against various human pathogens.

## Brine shrimp cytotoxicity test

Brine shrimp lethality is a general bioassay which is indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions. It has earlier been reported that $LD_{50}$ values for general cytotoxicities are about one-tenth $LD_{50}$ values in the brine shrimp test (MacLaughin et al., 1991). The results of the cytotoxicity against *A. salina* of the crude methanolic extracts of various parts such as flowers, leaves, bark, stem and roots of *R. arboreum* of family Ericaceae are given in Table 1. Results showed that all parts of *R. arboreum* are toxic at 1000 µg/ml. The leaves extract showed significant cytotoxic activity against *A. salina*, where $LD_{50}$ was 20 µg/ml. The upper and lower limits were 186.513 and 9.890, respectively. The G value was 1.1530. The flowers extract showed good cytotoxic activity, the $LD_{50}$ was 64.23 µg/ml. The upper and lower limits were

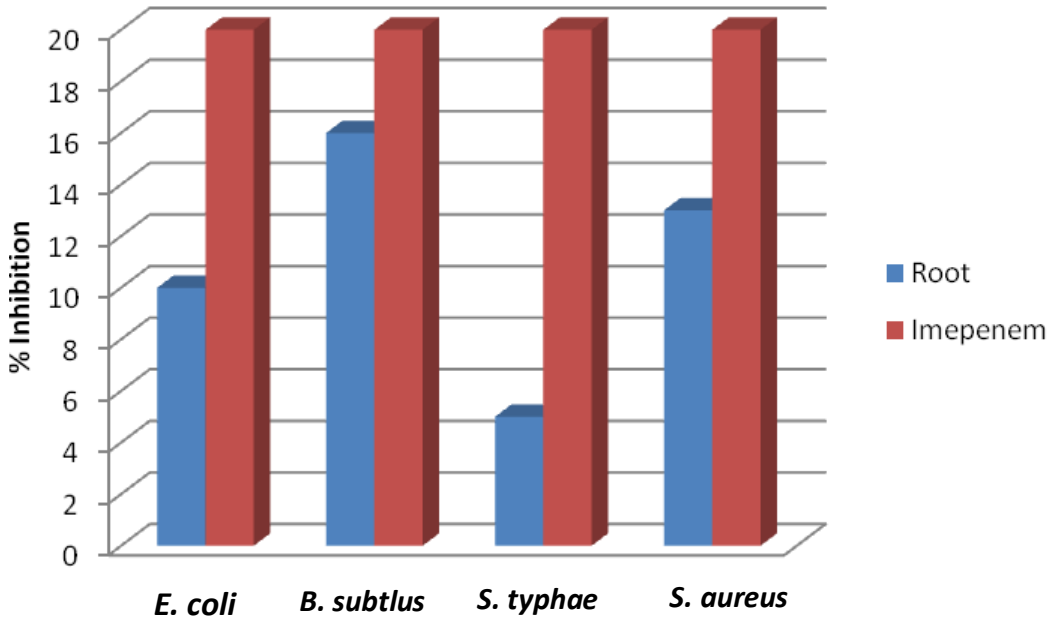


**Figure 5.** Antibacterial activity of root extract of *R. arboreum* against various human pathogens.

**Table 1.** Brine shrimp cytotoxicity test of methanolic extract of different parts of *R. arboreum* against *A. salina.*

| Part of plant | Dose (µg/ml) | No. of Survivors | $LD_{50}$ (µg /ml) | Upper limit | Lower limit | G value |
|---|---|---|---|---|---|---|
| Flowers | 1000<br>100<br>10 | 2<br>5<br>8 | 64.23 | 740.700 | 13.500 | 0.5554 |
| Leaves | 1000<br>100<br>10 | 2<br>3<br>6 | 20 | 186.513 | 9.890 | 1.1530 |
| Bark | 1000<br>100<br>10 | 3<br>7<br>10 | 545 | 1920.576 | 116.256 | 0.4011 |
| Stem | 1000<br>100<br>10 | 3<br>6<br>9 | 85 | 3507.41 | 54.03 | 0.5392 |
| Root | 1000<br>100<br>10 | 1<br>5<br>9 | 85 | 573.455 | 17.438 | 0.3652 |
| Standard drug | Etopoisde | | 7.4625 | | | |

No. of shrimps used in each experiment = 10; Values are mean ± SD of 3 replicates; G = Geometric mean.

740.700 and 13.500, respectively. The G value was 0.5554. Stem and roots had moderate cytotoxic activity against *A. salina,* where $LD_{50}$ values for stem and roots were 85 µg/ml respectively. The upper and lower limits

for stem were 3507.41 and 54.03 and for roots were 573.455 and 17.438, respectively. The G values for stem and roots were 0.5392 and 0.3652, respectively. Bark of *R. arboreum* showed poor cytotoxic activity against *A. salina*, where $LD_{50}$ was 545 µg/ml. The upper and lower limits were 1920.576 and 116.256, respectively. The G value for bark was 0.4011. The results show the ability of the extracts to exert a wide range of pharmacological effects. The presence of secondary metabolites such as alkaloids, flavonoids and glycoside may be responsible for the observed brine shrimps cytotoxicity activity of the extract. The brine shrimp cytotoxicity further supports the antibacterial activities of the extract of various parts of *R.arboreum* on some pathogenic organisms observed in this study.

## Conclusion

The results showed that *R. arboreum* can be used as a source of antimicrobial and cytotoxic agents at the concentration stated in Figure 1 and Table 1, respectively.

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