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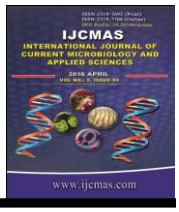

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Studies on Antibacterial activity of Leaf Extracts of *Rhododendron arboreum* and *Rhododendron campanulatum*

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A B S T R A C T

The antibacterial activity of the methanolic and acetone leaf extracts of *Rhododendron arboreum* and *Rhododendron campanulatum* was determined *in-vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis, Bacillus cereus, Pseudomonas aeruginosa, Listeria monocytogenes* and *Staphylococcus aureus* following agar-well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results showed low to significant antibacterial activity against the mentioned bacterial strains. Methanolic leaf extract was found to be more effective against selected pathogenic bacterial *spp*. as compared to acetone leaf extract. Further the leaf extract of both plants inhibited gram- positive bacteria more efficiently than gram- negative bacteria. Therefore, the leaf extracts of these plants can be selected for further investigation to determine their therapeutic potential.

Introduction

The use of plants for treating various diseases is as old as the human species. Plants are traditionally being used for medicinal treatment of numerous human disorders including infectious diseases caused by different microorganisms. Due to the increasing resistance of several microorganisms to commonly used antimicrobial agents, there is an urgent need for novel antimicrobial compounds. Because of available antimicrobials failure to treat infectious diseases, researchers have focused on the investigation of natural products as potential source of new bioactive compounds. According to the World Health Organization, approximately 65% of the World's populations integrate various medicinal plant products into their primary health care strategies (WHO, 2000; Farmsworth and Soejarto, 1991). Significantly, in developing countries about 80% of the population is used to prepare traditional medicine formulations from numerous plant sources (Kim, 2005).

Plants of the genus *Rhododendron* belong to the woody representatives of the family Ericaceae, which are typically used in a wide range of ethno-medical applications. *Rhododendron* genus consists of about 1025 species, which are mainly found at higher altitudes (Chamberlaim, 1996). In India, there are about 80 species and 14 sub-species of *Rhododendron*, which are distributed in the Himalayan region at the altitude ranging 1500-5500 meters (Bhattacharya, 2011). Extracts of different species of *Rhododendron* are generally used in traditional medicine in the countries of their indigenous habitats (Rehman *et al*., 2010).

Rhododendron arboreum, also known as burans or gurans, is an evergreen shrub or a small tree with a showy display of bright red flowers. Phytochemically, *R. arboreum* was reported to contain a large number of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids and phlobatanins (Nisar *et al*., 2011). The leaves of *Rhododendron arboreum* were reported for significant antioxidant property (Anpin *et al*., 2010) while the ethanolic extract of the flowers showed potent anti-diabetic activity (Bhandary and Kawabata, 2008). Further it was also revealed that the methanolic extract of the flowers had potent antiglycation potential in rats (Verma *et al*., 2011).

R.campanulatum is an evergreen gregarious shrub which is found in the outer and inner ranges of the Alpine Himalayas from Kashmir to Bhutan at altitudes of 9,000 to 14,000 ft. The leaves of this plant are said to possess several medicinal and poisonous properties (Chopra *et al*., 1949; Kirtikar and Basu, 1933). *R. campanulatum* is known for its traditional medicinal value for different ailments like body ache, sore throat, digestion, skin diseases, rheumatism, syphilis, cold and fever, etc. (Popescu and Kopp, 2013; Pushpandan *et al*., 1996; Prakash *et al*., 2007; Kunwar *et al*., 2010; Paudal *et al*., 2011; Tantry *et al*., 2011). The traditional method of using this plant is to mix the leaves and stem with tobacco and snuff to get relief from hemicranias and colds (DiPasuquale, 2005; WHO, 2001; Shakya and Bista, 2002).

Therefore, in the present work an attempt has been made to analyse the antibacterial potential of methanolic and acetonic extracts of *Rhododendron arboreum* and *R. campanulatum* against selected pathogenic bacterial strains.

Materials and Methods

Collection of Plant Materials

Leaves of *Rhododendron arboreum* and *Rhododendron campanulatum* were collected from Churdhar area of District Sirmaur, Himachal Pradesh, India. The collected plant materials were brought to the laboratory for further analysis.

Processing of Plant Material

Leaves of *R. arboreum* and *R. campanulatum* were plucked and collected from respective plants, washed thoroughly under tap water and then with 2% Mercuric chloride. After that the leaves were cut into smaller pieces for quick drying. Cleaned leaves were shade dried for 15-20 days. The dried plant materials were crushed into fine powder with the help of pestle mortar. Finally the fine powder was stored in air tight container at room temperature.

Preparation of Methanolic and Acetone Extracts

5gm dried leaves of *Rhododendron arboreum* and *R. campanulatum* were taken in separate Erlenmeyer flasks to which 50ml

of required solvents i.e., methanol and acetone were added. The flasks were covered with aluminium foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using rotary evaporator. The extracts were collected and weighed. Finally, stock solution of conc. 50 mg/ml was prepared.

Procurement of Bacteria

Bacterial strains used for antibacterial studies were procured from Department of Biotechnology, Himachal Pradesh University, Summer Hill Shimla, India. Pathogens used for the study were *Escherichia coli, Pseudomonas aeruginosa, Yersinia pestis, Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes.*

Revival of Pathogen

The collected pathogens were revived in nutrient broth and stored in nutrient agar slants at 4°C.

Screening the Antibacterial Activity of Methanolic and Acetone Extracts of *Rhododendron arboreum* and *Rhododendron campanulatum*

Screening of plant extracts (methanol & acetone) of *R. arboreum* and *R. campanulatum* was done using agar-well diffusion method. Nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium Chloride 1g, Peptone 5g, Agar 20g, Distilled Water 1000 ml) was used throughout the investigation. The medium was autoclaved at 121.6°C for 30 minutes and poured into petriplates. Bacteria were grown in nutrient broth for 24 hours. A 100µl of bacterial suspension was spread on each nutrient agar plate. Agar wells of 8 mm diameter were prepared with the help of sterilized stainless steel cork borer in each Petri plate. The wells in each plate were loaded with 25%, 50%, 75% and 100% concentration of prepared leaf extracts of *R. arboreum* and *R. campanulatum.* The petri plate kept as a control contained pure solvent only. The plates were incubated at 37 ± 2^0C for 24 hours in the incubation chamber. The zone of growth inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in perpendicular direction in all the three replicates and the average values were tabulated. Percentage inhibition of bacterial species was calculated after subtracting control from the values of inhibition diameter using control as standard (Hemashenpagam and Selvaraj, 2010).

Percentage of growth inhibition= (Control-Test/Control) x100

Control=average diameter of bacterial colony in control.

Test=average diameter of bacterial colony in treatment sets (Kannan *et al.*, 2009).

Results and Discussion

In the present study, two common plants namely, *Rhododendron arboreum* and *Rhododendron campanulatum* were tested for their antimicrobial properties against selected human pathogens. Results obtained revealed that the tested plant extracts possess considerable potential antibacterial activity against *Escherichia coli, Yersinia pestis, Pseudomonas aeruginosa, Bacillus cereus, Listeria monocytogenes and Staphylococcus aureus* (Table 1 and 2).

The screening revealed that the methanolic leaf extract of *R. arboreum* was quite effective against *Listeria monocytogenes*

(24.45±1.65mm at 100%, 22.00±0.00mm at 75%, 18.70±2.22mm at 50% and 16.00±1.05mm at 25%) and showed minimum inhibition was against *Pseudomonas aeruginosa* (16.10±1.87mm at 100%, 14.15±0.24mm at 75%, 12.40±0.59mm at 50% and 10.00±0.00mm at 25%) while the acetone leaf extract was found to be most effective against *Staphylococcus aureus* (22.50±2.54mm at 100%, 20.20±0.90mm at 75%, 17.65±1.94mm at 50% and 15.60±1.75mm at 25%), and showed minimum inhibition against *Pseudomonas aeruginosa* (15.10±0.89mm at 100%, 13.00±1.9mm at 75%, 11.85±1.87mm at 50% and 10.15±0.89mm at 25%) as given in table 1.

Table.1 Percent Inhibition of Growth of Pathogenic Bacterial *spp*. at Different Concentrations of Methanolic and Acetone Extracts of *R. Arboreum*

Extract	**Concen-tration in %**	**Inhibition zone diameter (In mm)**					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanolic Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	14.45±0.25	15.80±1.30	10.00±0.00	12.45±0.77	16.00±1.05	14.50±0.78
	50	16.20±1.27	17.95±1.56	12.40±0.59	13.85±0.98	18.70±2.22	17.20±0.44
	75	18.45±1.55	20.50±2.00	14.15±0.24	15.90±1.08	22.00±0.00	19.60±0.67
	100	20.43±0.47	23.00±0.00	16.10±1.87	18.30±06	24.45±1.65	21.35±1.33
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	14.00±0.89	13.50±1.44	10.15±0.89	12.15±1.55	11.00±0.54	15.60±1.75
	50	15.90±0.70	15.20±0.76	11.85±1.87	14.30±1.00	13.45±0.66	17.65±1.94
	75	18.10±1.08	17.60±0.70	13.00±1.91	17.30±0.87	16.10±0.79	20.20±0.90
	100	19.25±0.76	19.75±2.03	15.10±0.98	19.00±1.56	19.55±0.24	22.50±2.54

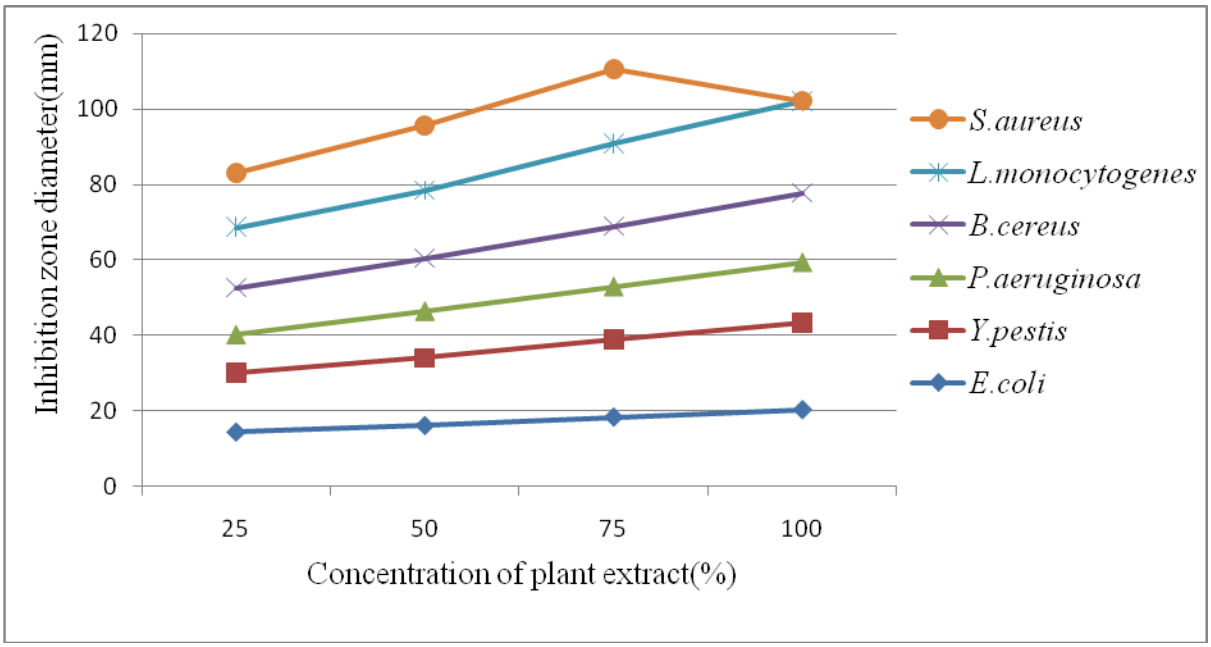
Each data represent mean of three replicates ± S.D

Table.2 Percent Inhibition of Growth of Pathogenic Bacterial *spp*. at Different Concentrations of Methanolic and Acetone Extracts of *R. campanulatum*

Extract	**Concen-tration in %**	**Inhibition zone diameter (In mm)**					
		E. coli	*Y. pestis*	*P. aeruginosa*	*B. cereus*	*L. monocytogenes*	*S. aureus*
Methanolic Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	16.22±0.50	12.42±0.74	14.10±1.01	17.00±0.37	18.24±2.08	15.16±0.86
	50	18.38±0.29	15.08±0.45	16.60±0.33	20.67±1.58	21.00±0.00	17.54±0.31
	75	21.00±0.60	17.50±1.22	19.76±0.58	23.45±1.42	24.80±0.75	20.35±1.70
	100	23.87±1.20	20.68±0.96	21.90±0.44	26.18±1.15	28.32±0.48	22.90±2.00
Acetone Extract	Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	25	12.40±0.76	16.00±0.19	14.05±0.16	17.90±0.00	15.85±0.90	14.00±1.09
	50	15.00±0.87	18.28±0.32	16.43±0.98	21.00±1.32	18.00±1.54	17.18±0.00
	75	17.20±1.21	21.76±0.45	19.28±0.55	24.92±1.47	22.14±1.42	21.66±0.53
	100	19.15±0.67	23.34±0.25	21.10±1.45	28.22±0.77	25.86±2.77	25.50±1.73

Each data represent mean of three replicates ± S.D

Figure.1 Antibacterial Activity of *R. arboreum* against Selected Bacterial Strains: (A) Methanolic Leaf Extract; (B) Acetone Leaf Extract



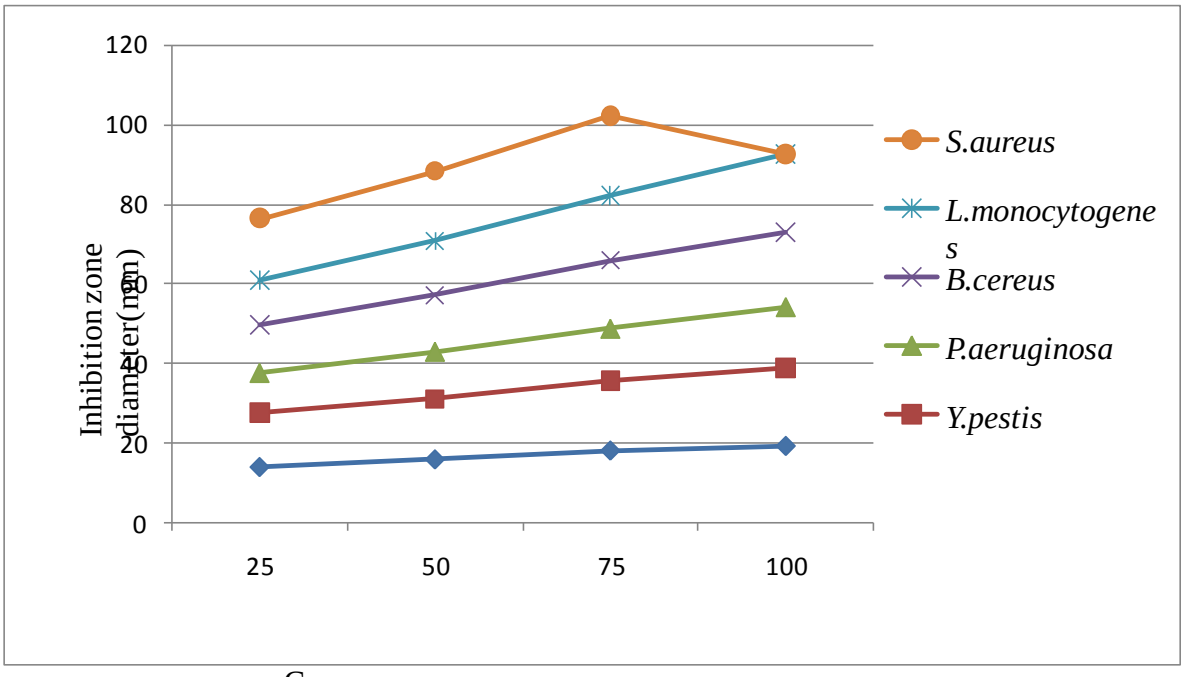
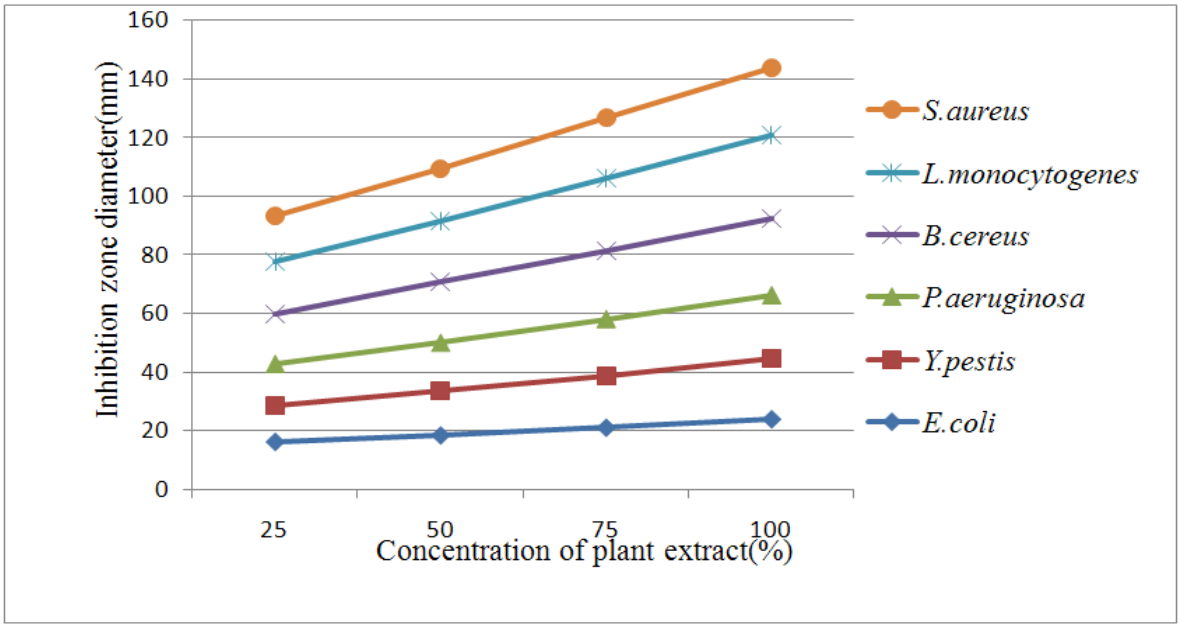
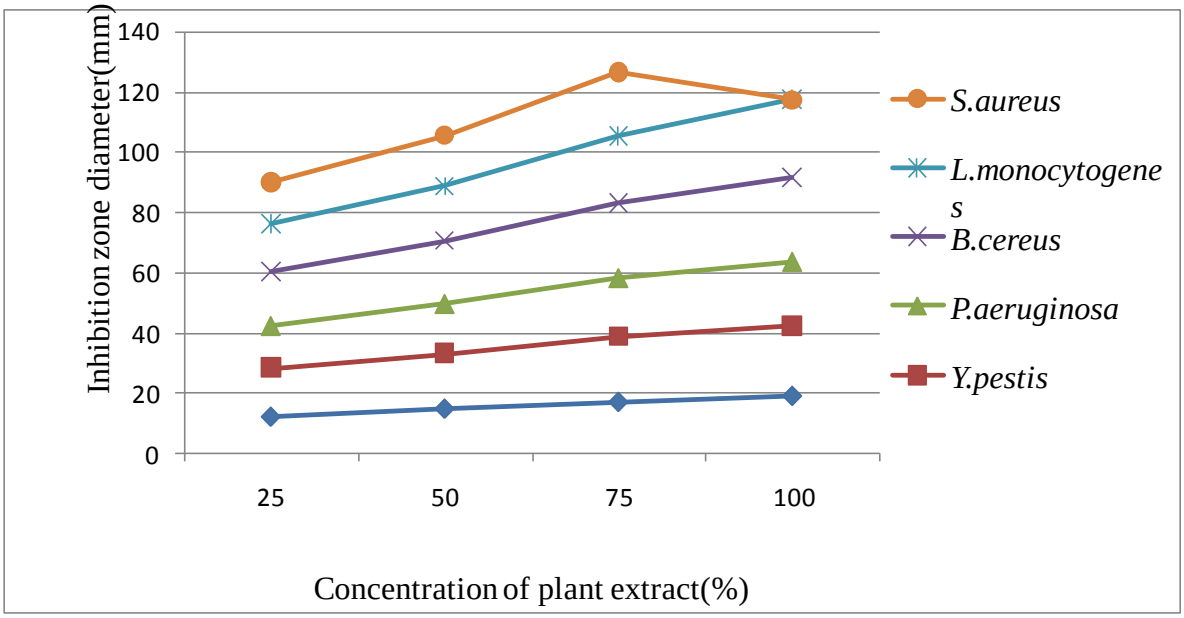


Figure.2 Antibacterial Activity of *R. campanulatum* against Selected Bacterial Strains: (a) Methanolic Leaf Extract; (b) Acetone Leaf Extract





In case of *R. campanulatum*, the methanolic leaf extract was found to be most effective against *Listeria monocytogenes* (28.32±0.48mm at 100%, 24.80±0.75mm at 75%, 21.00±0.00mm at 50% and 18.24±2.08mm at 25%), and it affected minimum inhibition to *Yersinia pestis* (20.68±0.96mm at 100%, 17.50±1.22mm at 75%, 15.08±0.45mm at 50% and 12.42±0.74mm at 25%). Acetone leaf extract showed considerable potential against *Bacillus cereus* (28.22±0.77mm at 100%, 24.92±1.47mm at 75%, 21.00±1.32mm at 50% and 17.90±0.00mm

at 25%), and minimum inhibition was found in *Escherichia coli* (19.15±0.67mm at 100%, 17.20±1.21mm at 75%, 15.00±0.87mm at 50% and 12.40±0.76mm at 25%) as given in table 2.

It was concluded from the above experimental observations that the plants *Rhododendron arboreum* and *Rhododendron campanulatum* showed potent antibacterial activity against different bacterial strains at all concentrations. Methanolic leaf extract was found to be more effective against pathogenic bacterial *spp*. as compared to acetone leaf extract. Further the leaf extract of both plants showed more inhibition effect in gram-positive bacteria than in gram- negative bacteria. Possible reasons for this antibacterial activity of *R. arboreum* and *R. campanulatum* are presence of alkaloids, tannins, saponins, terpenes and flavonoids in their leaves (Saklani and Chandra, 2015; Paudel *et al*., 2011). Findings of present study are preliminary and further investigations are required to determine the exact nature of the bioactive compounds which may be present in the leaves.

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Conflict of Interest

The authors hereby declare that there is no conflict of interest regarding the manuscript and experimentation done.

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