IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY OF *RHODODENDRON ARBOREUM* PLANT EXTRACT ON SELECTED PATHOGENIC BACTERIAL ISOLATES

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Abstract: Technological inputs in healthcare system have although revolutionized the treatment practices but due to the side effects of synthetic drugs, there has been an abrupt global shift towards safe herbal healthcare options. Medicinal plants have been in use for curing several ailments for centuries together. Himalayan region is bestowed with immense medicinal plant wealth, can be efficiently used for safe drug formulations. *Rhododendron arboreum* is one of such plant resources of great economical and medicinal importance, found in entire Himalayan range. Plant has been in use for treatment of chronic eczema, diarrheal dysentery, irritable bowel syndrome and menstrual disorder. Antimicrobial potential of solvent extracts of dried *Rhododendron* flower (collected from Shimla, H.P.) was tested against various pathogenic bacteria *i.e. B. cereus, E. coli, S. typhi, Staphylococcus aureus, S. flexneri, P. aeruginosa* using agar well diffusion method. Of various solvents, petroleum ether showed no inhibitory effect while ethanolic, methanolic and acetone extracts were quite effective against all pathogens even in lower concentration. Results of MIC experiment revealed that 10–80 µg/100µl concentration of extracts was effective. Results obtained signify the relevance of traditional practices and suggest the application of biotechnological interventions for effective utilization of plants *i.e. Rhododendrom* in disease control.

Keywords: Extract, minimum inhibitory concentration, well diffusion methods

Introduction: Evolution of synthetic drugs is one of the major contributions of science and technological advancements in healthcare but consumption of these drugs for longer has resulted in adverse health impacts [3]. This has resulted in shifting of research communities towards exploring the safe and herbal healthcare products [10]. India is the global center for research and mining of natural and safer alternatives of medicine [5]. Even today more than 80% of the global population depends upon traditional medicine (WHO report) which also contribute a major part to the national economy each year [4] and have helped the rural India to sustain healthy life. The use of plants in healthcare finds reference in ancient Indian literature like Rig-veda, Atharva Veda, Charaka Samhita and Susruta Samhita. More than 700 herbal plants have been already described but exact number of medicinal plants is not available [1] [11] [14]. Indian Himalayan Region (IHR) is one of the mega hot spots of biological diversity hosting major part of medicinal plant species [8] [7] and Himachal is one of the bioresource rich and full of potential opportunities.

Besides many important plants like tulsi, neem etc. *Rhododendron arboreum* is one of very important plant resources with enormous medicinal importance. It is an evergreen plant with extremely variable in stature, hardness, flower color and leaf characteristics [2] [9]. *R. arboreum* was discovered in north central India is distributed in entire Himalayan range including Kashmir to Bhutan & in the hills of Assam & Manipur [9].Normally it grows at altitude of 4500 to 10,500 ft & can attain the height of 100 ft [13]. Various bioactive components *i.e.* pinitol, phenols and flavanoids, terpenoids and flavanoids and steroids are responsible for its medicinal, antimicrobial, anti oxidants and other biological activity. In present research work different extracts of *R. arboreum* flowers collected from Shimla, HP were studied for pharmacological properties.

Materials and Methods

Chemicals: Acetone, ethanol, methanol, petroleum ether, dimethyl sulfoxide (DMSO), and Muller-Hinton agar. All the chemicals used in present work were of high purity and analytical grade supplied by Himedia.

Plant material: *R. arboreum* flowers were collected from Kufri and summerhill forest around Shimla in Himachal Pradesh.

Microbial pathogen isolates: Pathogenic microbial isolates for pharmacological studies were procured from RL-V, Department of Biotechnology, H. P. University, Shimla Himachal Pradesh, India which were obtained from IGMC Shimla.

Preparation of extract: Flowers were washed properly and dried. Dried flower leaves were crushed with mortar and pastel to obtained fine powder. Powdered flowers were kept in dried containers. Extract of dried powder (P) were prepared in solvents (S): acetone, ethanol, petroleum ether and methanol via cold percolation method as mentioned in table I. Extracts were dried at room temperature to evaporate solvent from powder and stored at 4^{o}C till preparation of stock solution.

Table I: Preparation of extracts

Solvent	P:S	Parameters
Acetone	1:10	35°C, 120 rpm for 24 h
Ethanol		
Methanol		
Petroleum ether		

Stock solutions of different extracts were prepared in 80% dimethyl sulfoxide (DMSO). The final concentration of extract will be 200 mg/ml.

Pharmacological studies: Antimicrobial potential of plant extract was analyzed by using well diffusion method. Plant extracts were allowed to diffuse in agar Muller-Hinton agar medium already seeded with test organism and plates were incubated at 35°C for 24 h. Inhibitory zones, developed around the plant extract were measured in terms of diameter (mm). For comparative analysis different antibiotics were used as positive control as; ciprofloxacin for *S. aureus*, chloremphenicol for *P. aeruginosa*, *S. typhi* and tetracycline for *E. coli* and *B. cereus* while DMSO was used as negative control.

Minimum Inhibitory Concentration: Minimum inhibitory concentration (MIC) of all the extracts was determined against all five pathogens. MIC is the minimum amount of antimicrobial needed to inhibit or kill the pathogenic microbes. Out of selected pathogenic microbes extracts were most effective against *S. aureus,* hence selected for MIC determination. In order to determine the effectiveness of extract, different concentrations of extract from 0.1-0.8 mg/µl were tested against *S. aureus*.

Results : Antimicrobial property of *R. arboreum* was treated against 6 bacterial pathogens: *S. typhi, Shigella, E. coli, S. aureus, Pseudomonas, B. cereus*. Results of well diffusion methods has been shown in table II and further discussed in following sections:

Since petroleum ether was found ineffective against all pathogens and antimicrobial property was not exhibited hence it was not used for further studies. Acetone extract of *R. arboreum* was found effective against *S. typhi, E. coli and S. aureus.* Ethanol extract of *R. arboreum* flowers was effective against only *S. aureus* while methanolic extract was effective against all pathogens except *Shigella flexneri.* Hence extracts were tested against selected microbial pathogens in next experiments as per their past results. The inhibitory effect of different concentrations of extracts against respective pathogens was studies and the results have been shown in table: III

Table II: Effect of extract in different solvent on pathogenic bacterial isolates

Bacteria	Extracts of *R. arboreum* against pathogenic isolates			
	Petroleum ether	Acetone	Ethanol	Methanol
S. typhi	-	+	-	+
Shigella	-	-	-	-
E. coli	-	+	-	+
S. aureus	-	+	+	+
Pseudomonas	-	-	-	+
B. cereus	-	-	-	+

Table III: Antimicrobial effect of extract in different solvent against bacterial pathogens

	Acetone (µl)					Ethanol (µl)					Methanol (µl)				
	10	20	30	40	C	10	20	30	40	C	10	20	30	40	C
E coli	8	8	12	13	31	0	0	0	0	0	9	10	12	13	27
S typhi	9	12	13	14	30	0	0	0	0	0	8	9	10	11	27
S. aureus	17	18	20	21	31	9	13	14	16	33	12	16	17	18	26
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	7	8	9	11	30
B cereus	0	0	0	0	0	0	0	0	0	0	7	8	9	13	19

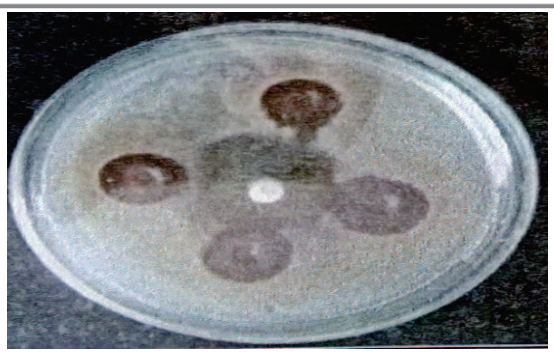

Fig. 1: Antimicrobial effect of acetone extract against *S. aureus*

All three extracts, acetone, ethanol and methanol extract were most effective against *S. aureus*. Fig. 1 shows the effect of acetone extract on *S. aureus*. **Minimum Inhibitory Concentration:** Since maximum inhibitory effect was shown by extracts against *S. aureus,* minimum inhibitory concentration (MIC) of ethanolic, methanolic and acetone extract was tested against *S. aureus* by serial dilution of plant extract with either agar or broth media. The results of MIC experiment have been shown in table V.

Table IV: MIC of extract of *Rhododendron arboretum* against *S. aureus*

Plant extracts			
Conc (mg/µl)	Methanol	Ethanol	Acetone
0.1	-	-	-
0.2	-	-	-
0.4	-	-	-
0..6	-	-	-
0.8	-	-	-

\- No growth, + growth

Discussion: Saklani and Chandra (2015) have also reported similar results while analyzing the antimicrobial potential of *R. arboreum* flowers. However methanolic extract was found most effective against *S. aureus*. In contrast to present work they found the minimum inhibitory concentration to be 60µg/ml against *Staphyloccus aureus* while in present work 0.1mg/µl is found to be the effective against pathogens. In another work by Lepcha *et. al.* (2015) regarding the medicinal importance of *Rhododendron* sp. of Sikkim, it has been found that out of 36 species within Sikkim only *R. arboreum*, displayed significant medicinal value. Ved Prakash et al (2016) have also reported that methanolic leaf extract leaf extracts of *R. arboreum* and *R. campanulatum* was effective against *Escherichia coli, Yersinia pestis, Bacillus cereus, Pseudomonas aeruginosa, Listeria monocytogenes* and *Staphylococcus aureus* in comparison to acetone leaf extract.

Conclusion: Among different extracts, petroleum didn't show any inhibitory effect against any of the six pathogenic isolates selected. Methanolic extract was found effective against all isolates except *Shigella* while ethanol extract was found effective against only *S. aureus*. Extracts were used in concentration of 10, 20, 30, 40µl respectively (0.2 mg/µl). 40 µl of extract have been found maximum effective against all pathogens. MIC results showed that even lower concentration of extract is sufficient to inhibit the growth of all pathogenic microbes. The results seem promising in inhibiting the growth of pathogenic strains. This suggests the possible use of extracts for treatment of different infections. These results also support the reliability of traditional treatment systems and traditional medicines.

Future Prospects: Further studies are needed to explore probability of purifying the bioactive compounds and using other technological inputs like green nanoparticles and evaluating the effectiveness of the purified plant extract/nanoparticles.

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