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In vitro Antibacterial Activity of the Essential oil from *Artemisia wallichiana* Besser

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ABSTRACT

Artemisia wallichiana Bess. (Family Asteraceae) grow wild in the central Himalayan region of Uttarakhand, India at an altitude range of 2500m. The emergence of new infections and increase of bacterial drug resistance has prompted interest for the development of new antibacterial agents from natural sources. This study is an attempt to assess the therapeutic potential of plant constituents as new antimicrobial drugs. Antibacterial activity of the oil sample was conducted against 11 bacterial strains using disc-diffusion method. The essential oil showed a broad spectrum of antibacterial activity against both the human and plant pathogenic bacteria. The oil showed the maximum activity against human bacterial strain P. aeruginosa (8.33 mm, MIC >100 µL mL^{-1}) followed by K. pneumonia (7.33 mm, MIC >100 µL mL^{-1}) and S. typhimurium (7.33 mm, MIC >100 µL mL^{-1}) while modest activity was observed against B. subtilis (6.33 mm MIC>100 µL mL^{-1}). Thus, it indicated the importance of this plant as natural agents for the treatment of infectious diseases caused by respective bacteria.

Graphical Abstract

Keywords: *Artemisia wallichiana*, Essential oil, Antibacterial activity, Disc-diffusion method.

INTRODUCTION

There is an increasing attention in the medicinal plants as a natural substitute to synthetic drugs [1]. Production of essential oils by plants is believed to be predominantly a defence mechanism against pathogens [2]. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use [3].In recent years, there has been increasing interest in healthy lifestyles and

healthy aging, as a result, many people are involved in searches for natural compounds that can improve health, especially those of plant origins [4].

The genus *Artemisia* known as "wormwoods" is one of the largest of herbs in the family Asteraceae, consisting of more than 800 species widely distributed throughout the world, especially, in South-West of Asia and Central Europe [5, 6].Several *Artemisia* species have been found to grow above 8000 ft., used for various purposes such as flavourings, fragrances, rodents, mite repellents and as folk medicine for antispasmodic, anti-pyretic, anti-inflammatory, abortifacient, antimicrobial and antifungicidal [7-10]. The essential oils of various species of the genus are used in soaps, detergents, cosmetics, perfumes, as aromatherapy claims and also as a purgative, to treat ear ache and fever, thus the genus *Artemisia* has always been of great interest to botanical, pharmaceutical and food industries [11, 12]. However, the volatile constituents of the essential oil of *A. wallichiana* have been previously investigated [13]. The aim of present study was to evaluate antibacterial activity of essential oil extracted from *A. wallichiana* collected from Chamoli region of Uttarakhand, which has not previously investigated.

MATERIALS AND METHODS

Plant materials: The plant material was collected in the month of August (flowering stage) from Chamoli District of Gharwal, Uttarakhand, India, at an altitude of 2500 meters. A voucher (specimen No. 87554) has been deposited at the Botanical Survey of India (BSI), Dehradun, India and Phytochemistry laboratory, Department of Chemistry, Almora, Kumaun University.

Extraction of the essential oil: Fresh aerial parts (~4 kg) were subjected to steam distillation. The distillate obtained after steam distillation of fresh plant material was treated with *n*-hexane for the extraction of organic constituents. The distillate was further shaken with dichloromethane to ensure complete extraction of constituents. The *n*-hexane and dichloromethane extracts were combined and dried over anhydrous Na_2SO_4. Solvent was distilled off in a rotary vacuum evaporator (Perfit-RV 1240, Buchi type) to get residual oil which was stored at ~4ºC.

Antimicrobial screening of essential oil

Pathogenic bacterial strains: The *in-vitro* antibacterial activity was evaluated against five human pathogenic bacterial strains *Bacillus subtilis* (MTCC No. 441), *Escherichia coli* (MTCC No. 443), *Klebsiella pneumoniae* (MTCC No. 3384), *Pseudomonas aeruginosa* (MTCC No. 424), *Salmonella typhimurium* (MTCC No. 3224) and six plant pathogenic bacterial strains *Agrobacterium tumefaciens* (MTCC No. 609), *Erwinia crysanthemi* (KUMSCC 328), *Ralstonia solanacearum* (BI0012), *Xanthomonas campestris* (BB0006), *X. oryzae* (BH0007) and *X. phaseoli* (KUMSCC 327).Some of the test strains were purchased from Indian Type Culture Collection (ITCC), ICAR, New Delhi and some provided by the Department of Biotechnology, Bhimtal, Kumaun University, which were procured from the Institute of Microbial Technology, Chandigarh. Indian Type Culture Collection (ITCC) and Microbial Technology Culture Collection (MTCC) numbers represent the standard strain numbers assigned to these microorganisms. The cultures of bacteria were maintained throughout the experiment at 4°C on their appropriate nutrient agar and used as stock cultures.

Antimicrobial activity by disc-diffusion method: Evaluation of antimicrobial activity of essential oil samples was done by disc-diffusion method described by Clinical and Laboratory Standards Institute [14]. The samples were dissolved in dimethyl sulphoxide (DMSO) to prepare desired concentrations. Inoculums of the microbial strains (1×10^6 CFU mL^{-1}) were plated using sterile swabs into petri dishes (90 mm) with 20 mL of Nutrient Agar, and then discs of Whatman paper-42 were soaked in sample solution (15 µL mL^{-1}) and placed onto inoculated petri dishes. Standard antibiotic streptomycin (15 mg mL^{-1}) was used as a positive control and DMSO as negative control. The petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples and then, incubated at 37±1ºC for 24 h [15]. Finally the zones of inhibition were measured.

Antimicrobial activity by broth dilution method: The evaluation of MICs was done using the agar dilution method with slight modifications described by the National Committee for Clinical Laboratory Standards [16]. Equal volumes of each microbial strain culture, containing approximately 1×10^6 CFU mL^{-1}, were applied onto MHB supplemented with the essential oil at concentration ranging from 25-250 µL mL^{-1} in tubes. These cultures were then incubated at 37°C for 24 h and then the cultures were finally inoculated on nutrient agar media to determine the growth of bacteria. Controls of bacteria without the oil were also applied. The concentration at which no visible growth was observed is considered as MICs.

Statistical analysis: Mean value ± SD was determined by using XLSTAT 14 statistical computer software package.

RESULTS AND DISCUSSION

Essential oil of *A. wallichiana* was tested against five human and six plant pathogenic bacteria. Results presented in table 1 showed that oil exhibited different antibacterial activity against both the tested bacterial strains. The oil showed the maximum activity against human bacterial strain *P. aeruginosa (*8.33 mm, MIC>100*)* followed by *K. pneumonia* (7.33 mm, MIC >100), *S. typhimurium* (7.33 mm, MIC >100) while modest activity was observed against *B. subtilis (6.33mm, >100)*.The

Table 1. Antibacterial activity of the *A. wallichiana* essential oil by disc-diffusion method

Bacterial strains	*A. wallichiana* oil		Reference antibiotic	
	ZOI (mean ± SD)[a]	MIC (µL mL^{-1})	ZOI (mean ± SD)[b]	MIC (mg mL^{-1})
Human Pathogenic				
B. subtilis	6.33±0.57	>100	26.33±0.57	100
E. coli	7.00±1.00	>100	31.66±1.15	50
K. pneumoniae	7.33±1.00	>100	30.33±0.57	50
P. aeruginosa	8.33±2.00	>100	27.33±1.15	75
S. typhimurium	7.33±1.00	>100	24.66±0.33	100
Plant pathogenic				
A. tumefaciens	10.00±1.00	100	33.00±1.00	50
E. crysanthemi	7.66±2.08	>100	25.66±0.57	50
R. solanacearum	8.00±1.24	>100	25.66±0.57	100
X. campestris	8.00±3.05	>100	23.66±0.52	100
X. oryzae	6.33± 0.57	>100	24.33±0.52	100
X. phaseoli	7.66±2.08	>100	32.66±1.00	50

[a]Inhibition zone diameter includes Whatman paper-42 (3 mm) at15 µL mL^{-1}
[b]Inhibition zone diameter includes Whatman paper-42 (3 mm) at15 mg mL^{-1}

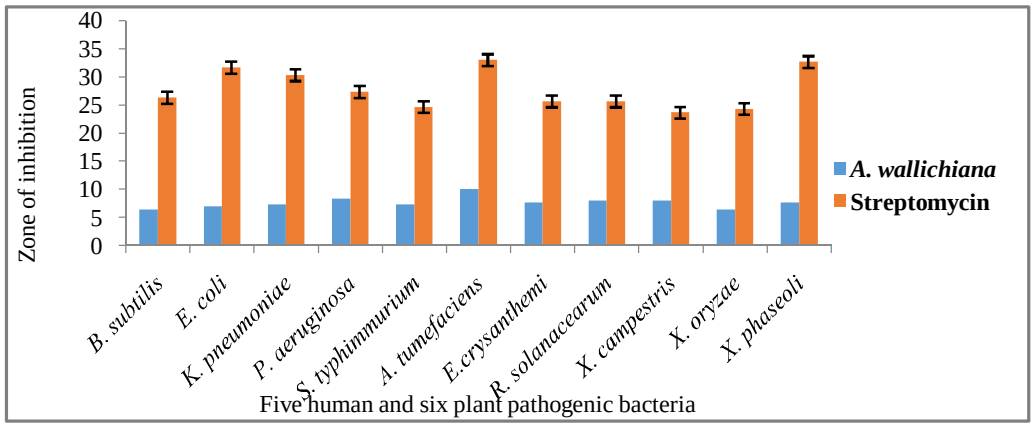

Figure 1. Antibacterial activity of the essential oil of aerial parts of *A. wallichiana* oil against eleven human pathogenic and plant pathogenic bacteria

order of resistivity of human pathogenic bacteria against oil was found *P. aeruginosa*> *K. pneumonia*> *S. typhimurium*> *E.coli*> *B. subtilis*. The oil demonstrated significant activity against plant pathogenic bacteria. The result showed highest antibacterial activity against *A. tumefaciens* (10 mm, MIC 100). As evident from the data in (Table 1), the oil demonstrated a higher level and broader spectrum of antibacterial activity, which was comparable to standard antibiotic (Streptomycin), used as positive control (Figure 1).

APPLICATION

To overcome the increasing resistance of pathogenic microbes, more effective, alternative biodegradable antimicrobial agents with novel modes of action and safer biomolecules need to be developed. We found that the essential oil from the aerial parts of *A. wallichiana* has significant antibacterial activity and would be important natural source for curing infections caused by microorganisms. It has been carried out against five human pathogenic bacterial strains *Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhimurium* and six plant pathogenic bacterial strains *Agrobacterium tumefaciens, Erwinia crysanthemi, Ralstonia solanacearum, Xanthomonas campestris*, *Xanthomona oryzae* and *Xanthomona phaseoli* by disc diffusion method.

CONCLUSION

The essential oil from the aerial parts of *A. wallichiana* showed varying degrees of antibacterial activity against tested bacterial strains. From the above experiment it can be inferred that extract suggest significant growth inhibiting effects on both human and plant pathogenic bacteria. The efficacy of oil of *A. wallichiana* against these microorganisms may provide a scientific ground for the application of the herb in the prevention and treatment of bacterial infections caused by various pathogenic bacteria, which have developed resistance to antibiotics. The results of this study present the herb as a good candidate to explore new alternative antibacterial agents to combat pathogenic microorganisms.

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