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In vitro Antibacterial Activity of the Essential oil from *Erigeron multiradiatus* (Lindl.) Benth

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ABSTRACT

Erigeron multiradiatus (Lindl.) Benth family Asteraceae grow wild in the alpine and subalpine meadow of Qinghai-Tibet plateau, at an altitude range of 2600–4300 m. The species has been reported to treat various diseases related with inflammation such as rheumatism, hemiparalysis, hyperpiesia, hepatitis, adenolymphitis and enteronitis. Antibacterial activity of the oil sample was conducted against 11 bacterial strains using disc-diffusion method. The *E. multiradiatus* oil showed maximum zone of inhibition against *Ralstonia solanacearum* (18.00±0.57 mm), *Bacillus subtilis* (15.33±1.52 mm) and *Pseudomonas aeruginosa* (13.00±1.00 mm) with minimum inhibitory concentration (MIC) of 50, 75 and 75 µL mL^{-1} respectively. It is finally concluded that the oil might be used as safer natural antibiotics.

Keywords: *Erigeron multiradiatus*, 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**]. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases [**2**]. Over the years, essential oils and plant extracts have evoked interest as sources of natural products. Essential oils produced by plant are believed to be predominately a defence mechanism against pathogens and pests [**3**]. Essential and their compositions are gaining increasing importance because of their relatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional use [**4**].

Erigeron multiradiatus (Lindl.) Benth , a perennial herb, is mainly distributed in the alpine and subalpine meadow of Qinghai-Tibet plateau, at an altitude range of 2600–4300 m [**5-6**]. The decoctions and infusions of this plant have been used under "Meiduoluomi" (vernacular name) in traditional Tibetan medicine for years to treat various diseases related with inflammation such as rheumatism, hemiparalysis, hyperpiesia, hepatitis, adenolymphitis and enteronitis [**7**]. *E. multiradiatus* contained a notable amount of flavonoids, and scutellarein-7-*O*-*β*-D-glucuronide (SG) and apigenin-7-*O*-*β*-D-glucuronide (AG) with significant anti-inflammatory activities [**8**]. Various biological and pharmacological activities have also been attributed to these two components, such as dilating blood vessel, improving microcirculation, increasing cerebral blood flow, and inhibiting platelet aggregation activity [**9-12**]. However, the volatile constituents of the essential oil of *E.multiradiatus* have been

previously investigated [**13**]. The aim of present study was to evaluate antibacterial activity of essential oil extracted from *E.multiradiatus* collected from Kumaun region of Uttarakhand, which has not previously investigated.

MATERIALS AND METHODS

Plant materials: The plant material was collected in the month of September(flowering stage) from Chipla kedar forest (Pithoragarh District), Uttarakhand state, India, at an altitude of 2700 meters. A voucher (specimen No. 116125) has been deposited at the Herberium of 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 µLl 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 hrs 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 37ºC 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 *E. multiradiatus* 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 evaluation of antibacterial activity of the essential oil from *E. multiradiatus* indicated significant activity against all bacterial strains and showed maximum inhibitory effect against plant pathogenic bacteria *X. phaseoli* (18.22±1.52), *R. solanacearum* (18.22±1.52 mm), *A. tumefaciens* (13.00 ± 1.00 mm) followed by *E. crysanthemi* (13.00±1.73 mm) and *X. oryzae* (10.66±0.57mm). Moreover, the oil showed significant antibacterial activity against human pathogenic bacteria, *B. subtilis* (15.33±1.52 mm), *P. aeruginosa* (13.00±1.00 mm), *E. coli* (12.66±2.08 mm) and *K. pneumonia* (10.00±1.00 mm). Based on the zone of inhibition and minimum inhibitory concentrations (MIC) values , *X. phaseoli* and *B. subtilis* were the most sensitive strains tested to the oil while *K. pneumoniae* and *S. typhimurium* were the most resistant towards the oil.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).

Table 1. Antibacterial activity of the *Erigeron multiradiatus* essential oil by disc-diffusion method.

Bacterial strains	*Erigeron multiradiatus*		Reference antibiotic	
	ZOI (mean ± SD)[a]	MIC (µL mL^{-1})	ZOI (mean ± SD)[b]	MIC (mg mL^{-1})
Human Pathogenic				
B. subtilis	15.33±1.52	75	26.33±0.57	100
E. coli	12.66±2.08	75	31.66±1.15	50
K. pneumoniae	10.00±1.00	100	30.33±0.57	50
P. aeruginosa	13.00±1.00	75	27.33±1.15	75
S. typhimurium	10.00±1.00	100	24.66±0.33	100
Plant pathogenic				
A. tumefaciens	13.00±1.00	75	33.00±1.00	50
E. crysanthemi	13.00±1.73	75	25.66±0.57	50
R. solanacearum	18.00±0.57	50	25.66±0.57	100
X. campestris	10.33±0.57	100	23.66±0.52	100
X. oryzae	10.66±0.57	100	24.33±0.52	100
X. phaseoli	18.22±1.52	50	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}

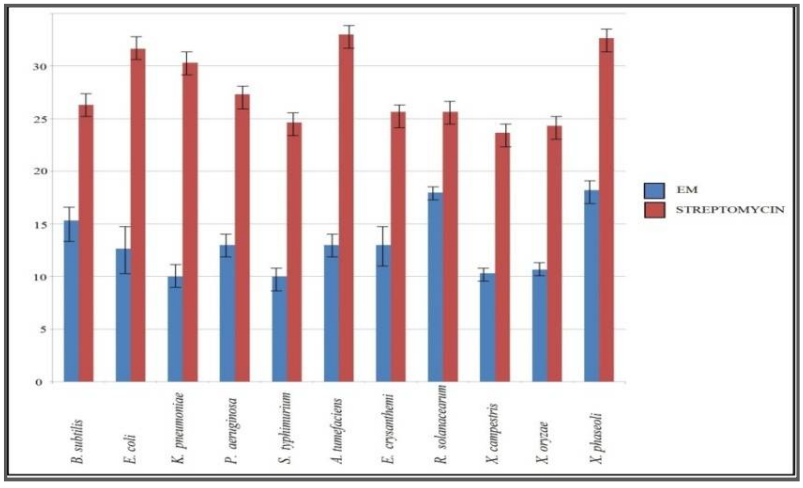

Figure 1. Antibacterial activity of the essential oil of aerial parts of *Erigeron multiradiatus* against eleven human pathogenic and plant pathogenic bacteria.

APPLICATION

Antibacterial activity of essential oil from the aerial parts of *Erigeron multiradiatus* (Lindl.) Benth 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.We found that the volatile extracts from the medicinal plants (aerial part) had significant antibacterial activity and would be important natural source for curing infections caused by microorganisms.

CONCLUSION

The essential oil from the aerial parts of *E. multiradiatus* 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 *E. multiradiatus* 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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