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Antimicrobial potential of essential oil *artemisia sieversiana*: a medicinal plant from the high altitude nubra valley, Ladakh

RIGZIN CHUSKIT[1], RISHIKESH SINGH[1,2], SHALINDER KAUR[1*] AND DAIZY R. BATISH[1]

[1]Department of Botany, Panjab University, Chandigarh − 160014, India

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ABSTRACT

Artemisia sieversiana Ehrh. ex Willd. (common name: wormwood; family: Asteraceae) is a medicinal plant thriving at high altitudes. It is extensively utilized in traditional folk medicine by various ethnic communities to alleviate and treat a wide range of health issues. However, despite its widespread use, the chemical characterization and identification of bioactive compounds responsible for these benefits is still incomplete, especially in the context of Trans−Himalayan region. Prior research has demonstrated the substantial efficacy of this plant against various pathogenic bacteria, although these studies utilized essential oil (EO) extracted from different geographic regions. There is a need for further research to explore the antibacterial activity of A. sieversiana EO from the Trans−Himalayan, Ladakh region, as the unique environmental conditions of this region may influence the plant's chemical composition and biological activity. This research therefore focusses on characterizing the phytochemical composition and evaluating the antibacterial properties of EO extracted from A. sieversiana, specifically from the Nubra valley, Ladakh (India). The antimicrobial potential of A. sieversiana essential oil was tested against a diverse spectrum of gram-negative (Erwinia herbicola and Pseudomaonas syringae) and gram-positive bacteria (Bacillius cereus, B. pumilus, Rhodococcus fasciens, and Streptomyces scabiei) plant and food pathogenic bacteria. Gas Chromatography−Mass Spectrometry (GC−MS) analysis led to the identification of 28 components with camphor (18.8%), borneol (11.2%), and 1,8−cineole (9.7%) as the major constituents. Further, the EO was more active against gram−positive bacteria, with a greater inhibition percentage for all gram−positive bacteria (24.68%−38.90%) than for gram−negative bacteria (0%−50.19%) at 10 µg mL^{-1}. Moreover, the antibacterial activity showed a concentration dependent response, escalating from 24.68% to complete inhibition (100%) as the EO concentration increased from 10 to 160 µg mL^{-1}. Notably, the EO demonstrated strongest activity against B. pumilus, even more than the common antibiotic Rifampicin. Among gram−negative bacteria, Erwinia herbicola showed the highest susceptibility. This observation shows that the essential oil of A. sieversiana exhibits promising antimicrobial activity and highlighted their potential for further exploration and utilization.

Keywords: Biological properties, Trans−Himalayan, GC−MS, Secondary metabolites

INTRODUCTION

Agricultural production is the backbone of our civilization, sustaining global populations and ensuring food security (Rafael, 2023). However, it faces a persistent and formidable adversary in the form of microbial attacks on crops. These attacks, caused by plant pathogens, represent a significant challenge to agricultural sustainability, leading to substantial crop losses and consequential economic damage (Collinge *et al.* 2022). To combat these threats, agriculture heavily relied on chemical−based antimicrobial agents, which have proven effective in controlling plant pathogens. Yet, their extensive use has unveiled a complex web of unintended consequences, raising serious concerns for both the environment and human health. Residues of these compounds find their way into our food and water supply, posing potential health hazards (Xu *et al.* 2021). Pesticides and fungicides have been associated with a range of health issues, from acute toxicity in farmworkers to chronic exposure−related diseases in the general population. Such concerns emphasize the urgency for transitioning towards safer, eco−friendly alternatives in agricultural practices (Yan *et al.* 2022). This has led to a surge in research on safe and sustainable alternatives, with natural products at the forefront (Atanasov *et al.* 2015). Among the natural products, plant essential oils (EO), have gained a significant attention as antimicrobial agents for combating plant and food pathogens, owing to their perceived safety and efficacy compared to synthetic additives (Gupta *et al.* 2023). EO

[2]Department of Earth and Environmental Sciences, Amity University, Punjab 140306, India
Corresponding author: shalinderkaur@hotmail.com

possess the potential to serve as an ideal substitute for synthetic mycotoxin inhibitors (Wang *et al.* 2018), insecticides (Mahmoudvand *et al.* 2016), additives (Liu and Yang, 2012) and inhibitors of various plant pathogens (Soylu *et al.* 2006). Generally, EO are secondary metabolites within plants that play a crucial role in their defense mechanisms (Pavela and Benelli, 2016). Apart from protecting plants, EO play key role in several ecological processes, including various plant to plant interactions, and offer advantages to the community due to their application in the food, fragrance, and medicinal sectors (Kaur *et al.* 2011). Consequently, they possess diverse biological qualities, including their ability to exhibit antimicrobial activities (Bhavaniramya *et al.* 2019). This has prompted increased exploration of botanical alternatives, with an emphasis on reduced effects and a lower likelihood of resistance development.

Within the Asteraceae family, the *Artemisia* genus, stands as one of the largest one, encompassing nearly 500 species distributed worldwide (Jiang *et al.* 2022). Several species of *Artemisia* such as *A. annua* L., *A. arborescens* L., *A. campestris* L., and *A. cana* Pursh. possess antimicrobial, antirheumatic, antiseptic, anti−inflammatory, antimalarial, and antivenin properties (Abad *et al.* 2012). In India, *A. sieversiana* Ehrh. ex Willd. is a temperate annual species that can be found growing extensively in Trans−Himalayan region. In Ladakh, the plant can be seen flourishing in sandy and marshy soils at elevations ranging from 2500−3500 m above sea level and is valued for its anti−septic, discutient, and tonic properties (Gurmet *et al.* 2017). Decoction prepared from the flowers and leaves function as wormicide and considered as a natural source of 'sieversinin' and 'siersin', both possessing antimicrobial properties (Gurmet and Sharma, 2016). However, no prior studies have reported chemical constituents of *A. sieversiana* EO collected from high elevational ranges of Trans−Himalayan Ladakh.Various factors e.g., ecotypes, cultivars, plant species, growth conditions and environmental stresses highly affect the concentration and composition of EO (Al−Maqtari *et al.* 2021). The chemical composition of EO of *A. sieversiana* from China (Liu *et al.* 2010), Kazakhstan (Suleimenov *et al.* 2009), and Tibet (Li *et al.* 2017) has showed eucalyptol, geranyl butyrate, myrcene, α−bisabololand chamazulene as major constituents. The plants thriving at high altitudes may produce additional secondary metabolites to withstand harsh environmental conditions (Hashim *et al.* 2020; Kumar *et al.* 2021). Variations in both the quantity and quality of oil constituents may significantly influence the biological and physical properties of the oils (Bunse *et al.* 2022). Exploring the antibacterial attributes of EO against food and plant pathogens possesses significant value, as it holds the potential to pave the way for innovative and eco−friendly methods to manage these harmful micro−organisms. This study could thus yield several advantages, such as food safety, minimized food loss, and the promotion of environmentally sustainable agricultural practices. Considering its numerous applications in traditional medicine, it is crucial to delve deeper into the core constituents and the simultaneous antimicrobial effects unraveled in the EO of *A. sieversiana*. Therefore, the objectives of the present study were (i) to determine the chemical constituents of EO from *A. sieversiana* collected from high altitude region of Ladakh, India, and (ii) to evaluate the antimicrobial potential of *A. sieversiana* EO.

MATERIALS AND METHODS

Plant collection

Fresh aerial parts of *Artemisia sieversiana* were collected from the Nubra valley of Leh district, Ladakh during August, 2021 from an altitude of 4174 m above sea level (34°22'32.7" N' and 77°39'14.5" E) (Fig. 1). The plants were identified and cataloged (voucher no. 916) by the Botanical Survey of India, Dehradun, India. Following a thorough cleaning process, the aerial parts of the plant were shade−dried for 15 days at room temperature. Subsequently, the essential oil extraction was performed using Clevenger−type apparatus through hydro−distillation (Issa *et al.* 2020). The obtained oil was dehydrated using anhydrous sodium sulfate (Na_2SO_4) and then preserved in a glass container at 4 °C until subsequent applications and analysis. The percentage essential oil yield was calculated using the equation:

$$\text{EO Yield (\%)} = \frac{M}{B_m} \times 100$$

where M is the mass of the extracted oil (g) and B_m is the initial plant biomass (g) (Eid *et al.* 2021).

Fig. 1: *Artemisia sieversiana* in its natural habitat in Trans−Himalayan region of Ladakh, India

Gas Chromatography−Mass Spectrometry (GC−MS) analysis

Qualitative analysis of EO was performed employing Gas Chromatography−Mass Spectrometry (GC−MS). A ZB−5MS capillary column (Phenomenex, USA) with dimensions of 30 m × 0.25 mm × 0.25 µm film thickness was utilized. For the analytical process, the Shimadzu QP 2010 instruments (Shimadzu, Tokyo, Japan), equipped with an AOC−5000 auto−injector, was utilized. For analysis, helium (He) was employed as the carrier gas, flowing at a rate of 1.05 ml min^{-1}. The starting oven temperature was configured at 70°C and maintained for a period of 5 minutes. Afterward, a gradual temperature ascent occurred, reaching 220°C with an increment of 4°C min^{-1} and this temperature was maintained for 5 minutes. Mass spectrometry (MS) data were acquired at a level of 70 electronvolts, encompassing a mass spectrum within the range of 40 to 800 atomic mass units. The injector temperature was configured at 240°C and interface temperature at 250°C. 2 µg of EO, was prepared by diluting 5 mg of EO in 2 ml of dichloromethane. This sample was then introduced into the system with a split ratio of 10:1. Using the similar operational parameters as those applied for EO analysis, A standard mixture of n−alkane spanning from C_9 to C_{24} was introduced into the GC−MS system to ascertain the retention indices (RI). Compound identification relied on spectral comparisons between the mass spectra of samples and entries in the (NIST 11) and Wiley databases. Furthermore, validation of compound identity was achieved through comparisons of the retention indices with values derived from existing literature (R_{lit}).

Antibacterial activity

Bacterial strains

The bacterial strains were sourced commercially from the Microbial Type Culture collection and Gene Bank (MTCC) at the Institute of Microbial Technology (IMTECH), Chandigarh (India) on April, 2019. Two strains of gram−negative bacteria *Pseudomonas syringae* (MTCC 1604) and *Erwinia herbicola* (MTCC 6720) along with four strains of gram−positive bacteria, *Bacillus cereus* (MTCC430), *Rhodococcus fasciens* (MTCC 8495), *Streptomyces scabiei* (MTCC 3966), and *B. pumilus* (MTCC 2296) were utilized for the assessment of antibacterial activities of *A. sieversiana* EO. Among these strains, *B. cereus* is a soil and food borne pathogen, whereas the other five are associated with plants.

Each bacterium was inoculated overnight at 37 °C in nutrient culture medium. Further. the antibacterial activity of the essential oil was examined through disc diffusion method (CLSI, 2012). For the assay, Petri plates of nutrient agar media were prepared and each plate was inoculated by evenly spreading 100 µl of already prepared suspension containing $1{\times}10^7$ CFUs (colony forming units) of bacteria and allowed to settle down. Different concentrations of EO (10, 20, 40, 80, and 160 µg mL^{-1}) were prepared by dissolving oil in dimethyl sulphoxide (DMSO). Sterile discs (⌀ = 6 mm, Himedia) were impregnated with 10 μl of EO and placed on the inoculated agar plates. Four discs per Petri plate were kept equidistantly to determine the zone of inhibition (ZOI). Discs impregnated with DMSO were used as negative control, while commercially relevant antibiotic, Rifampicin (30 μg $disc^{-1}$) was used as positive control. The plates were then incubated for 24 hours at 25 ± 2°C. Following incubation, the antimicrobial activity was assessed by measuring the ZOI in mm using Vernier calliper. The ZOI of test concentrations of EO was compared to the positive control. Each treatment concentration was checked for its activity in triplicate and mean diameter of ZOI was calculated.

Statistical analysis

All assays were conducted in triplicates, and the outcomes presented as mean ± standard error (SE). Data analysis involved the use of one−way analysis of variance (ANOVA), followed by *post hoc* Tukey's test to discern differences in mean values of ZOI at a significance value of $P \leq 0.05$. Statistical analysis was conducted using SPSS Inc., Chicago, IL, 16.0 Version.

RESULTS AND DISCUSSION

Essential oil composition

The EO of *A. sieversiana* aerial parts was dark bluish in colour with a yield of ~0.37% (v/w). The GC−MS analysis, revealed the presence of 28 components, representing 90.16% of the overall oil composition. The extracted EO showed a remarkable diversity in its phytochemical constituents. The EO was comprised mainly of monoterpenes and sesquiterpenes; however, monoterpenes (79.4%) constituted a greater proportion of the mixture. Within the monoterpenes, the content of oxygenated ones (75.68%) was higher than the hydrocarbon ones (3.72%). Sesquiterpenes constituted 9.59%, while monoterpenes constituted a total of 79.40% of the EO. Overall, the oil was discovered to be monoterpenoid in nature. Oxygenated monoterpenes *viz.* camphor, borneol, 1,8−cineole, and neryl isovalerate with a composition of 18.8, 11.2, 9.7, and 7.1%, respectively, were identified as the most abundant constituents of the EO. Furthermore, significant levels of geranyl isovalerate (5.3%), *α*−terpineol (5.20%), and linalool (5.13%) were also present in the oil of *A. sieversiana*. The chemical components identified in the analysis are presented in Table (1) along with their respective relative percentages and retention indices.

The chemical constituents of *A. sieversiana* EO collected from Xinjiang, China revealed the presence of 17 compounds, collectively constituting 99.17% of the oil, where *α*−thujone (64.46%) emerged as the predominant component (Jiang *et al.* 2022). Contrastingly, *α*−thujone was conspicuously absent in our study. This clearly supports the fact that a number of factors including geographical location, climate, environmental stress like drought, and the timing of the harvest etc., affect the distinct chemical profiles of EO obtained from the same plant species (Walia *et al.* 2020; Jiang *et al.* 2022). Similarly, studies on the composition of *A. sieversiana* EO obtained from different regions including Beijing and inner Mongolia, also displayed a considerable variability. The EO from Beijing was represented by 9.12, 9.20, 7.90, and 7.90% of eucalyptol, geranyl butyrate, camphor, and borneol, respectively (Liu *et al.* 2010). On the other hand, EO from Mongolia was primarily composed of neryl propanoate (22.88%), *β*−nerol (11.01%), and *β*−cubebene (7.50%) (Zhang *et al.* 2022). In our study, *A. sieversiana* EO exhibited a good amount of camphor (18.8%) in contrast to the typically observed range ~2%−8%. However, borneol range was in line with the previously observed range as reported in other EO from different geographical regions (Liu *et al.* 2010; Jeppesen *et al.* 2012; Vasylievna *et al.* 2015). Moreover, our study identified lower/absence of several compounds present in other studies. These compounds encompassed geranyl butyrate, cis−verbenol, *β*−pinene, chamazulene, *α*−bisabolol, *α*−phellendrene, *α*−thujone, nerylproponate, and *β*−cubebene (Liu *et al.* 2010; Jeppesen *et al.* 2012; Vasylievna *et al.* 2015; Jiang *et al.* 2022). These observations indicate the intricate interdependence of environmental variables and geographical origin (which may affect edatope, temperature, and precipitation) in shaping the chemical profiles of *A. sieversiana* EO.

Table 1: Chemical profile of *Artemisia sieversiana* essential oil assessed by GC−MS analysis

Constituents	RT	RI_{cal}	Area (%)	MOI
Monoterpene hydrocarbons				
β−Myrcene	6.847	989	2.63	RI, MS
o−Cymene	7.962	1025	0.58	RI, MS
γ−Terpinene	9.044	1058	0.51	RI, MS
Oxygenated monoterpenes				
1,8−cineole	8.234	1034	9.7	RI, MS
Linalool	10.428	1100	5.13	RI, MS
Camphor	12.155	1148	18.8	RI, MS
Lavandulol	12.701	1164	2.34	RI, MS
Borneol	13.056	1174	11.2	RI, MS
Terpinen−4−ol	13.340	1181	3.8	RI, MS
α−Terpineol	13.855	1196	5.2	RI, MS
Nerol	14.881	1224	4.14	RI, MS
trans−Geraniol	15.785	1250	0.44	RI, MS
Bornyl acetate	17.007	1284	2	RI, MS
Geranyl propionate	24.324	1499	0.53	RI, MS
Geranyl isovalerate	26.481	1568	5.3	RI, MS
Neryl isovalerate	26.741	1577	7.1	RI, MS
Oxygenated Sesquiterpene				
Viridiflorol	26.958	1584	0.53	RI, MS
Sesquiterpene Hydrocarbons				
β−Elemene	20.69	1390	1.1	RI, MS
Caryophyllene	21.73	1421	1.7	RI, MS
β−*cis*−Farnesene	22.735	1451	0.58	RI, MS
Germacrene−D	23.743	1482	3.81	RI, MS
β−Selinene	24.008	1490	0.98	RI, MS
Bicyclogermacrene	24.218	1496	0.28	RI, MS
α−Calacorene	24.66	1510	0.33	RI, MS
β−Sesquiphellandrene	25.065	1523	0.28	RI, MS
Phenylpropanoid				
Methyleugenol	20.945	1397	0.23	RI, MS,
Aliphatic Alcohol				
7−Octen−4−ol	6.563	978	0.27	RI, MS
Acid Aliphatics				
Butanoic acid, 2−methyl−, 2−methylbutyl ester	10.517	1103	0.33	RI, MS
Ester Derivatives				
cis−3−Hexenyl−α−methylbutyrate	15.05	1229	0.34	RI, MS
Total identified Compounds−28			Percentage	
Monoterpene hydrocarbons−3			3.72%	
Oxygenated monoterpenes−12			75.68%	
Oxygenated sesquiterpene−1			0.53%	
Sesquiterpene hydrocarbons−8			9.06%	
Phenylpropanoid−1			0.23	
Aliphatic Alcohol−1			0.27%	
Acid Aliphatics−1			0.33%	
Ester Derivatives−1			0.34%	
Total oil percentage−			90.16%	

RT: Retention time; RI_{cal}: Retention index calculated relative to C_9–C_{24} n–alkane series on the ZB–5MS column; Area (%): Relative percentage of the chemical constituent; MOI: Methods of identification, MS: identified on the basis of computer matching of mass spectra of peaks with Wiley 7 and NIST 11 libraries, RI: identified by matching of retention index with published literatures

The EO derived from *A. sieversiana* EO exhibited varying degrees of growth inhibition against diverse plant and food pathogenic bacterial species. The concentration-dependent impact of the EO (10µg mL^{-1} to 160 µg mL^{-1}), on pathogen growth was a key observation. Among different strains of plant pathogens, *R. fasciens* and *E. herbicola* exhibited reduced susceptibility to the EO compared to *B.cereus*, the foodborne pathogen. This discrepancy was evident in significant variations in their zone of inhibition (ZOI) at both lower and higher concentrations i.e., at 160 µg mL^{-1} (*R. fasciens*: 10.61 mm; *E. herbicola*: 11.63 mm vs. *B. cereus*: 21.16 mm); and 10 µg mL^{-1} (*R. fasciens*: 6.72 mm; *E. herbicola*: no activity vs. *B. cereus*: 7.74 mm).

Similarly, upon comparing ZOI, the susceptibility of bacterial strains to the EO varied significantly, with gram−positive bacteria generally exhibiting greater susceptibility than gram−negative bacteria. Among the gram−positive bacteria, *B. pumilus* demonstrated the highest susceptibility, with the EO's ZOI (22.32 mm) surpassing that of the positive control, rifampicin (22.00 mm) at the highest concentration (160µg mL^{-1}), contrastingly, *R. fasciens* showed the lowest susceptibility with ZOI (10.61 mm). Similarly, among gram−negative bacteria, (*P. syringae*) ZOI increased from 7.59 to 11.71 mm when exposed to10µg mL^{-1}−160 µg mL^{-1}exhibiting higher susceptibility at both lower and higher concentrations compared to *E. herbicola*, that showed no activity at lower concentration, while at 160µg mL^{-1} an ZOI of 11.63 mm was observed.

Table 2: One−way ANOVA showing differences among antibacterial activity of different bacterial strains between varying concentrations of *Artemisia sieversina* essential oil

Type of strain	F value					
	Bacillus cereus	*Bacillus pumilus*	*Rhodococcus fasciens*	*Streptomyces scabiei*	*Erwinia herbicola*	*Pseudomonas syringae*
Gram (+)	59.841*	54.700*	552.940*	393.374*	−	−
Gram (−)	−	−	−	−	207.817*	548.457*

**Denotes significant variation at P ≤ 0.001*

In general, with the increase in the concentration of EO, the inhibitory effect also increased. A significant ($P \leq 0.001$) inhibitory effect on the growth of both gram−negative and gram−positive bacteria was observed (Table2). When compared with rifampicin, the highest percentage inhibition among the gram−negative bacteria was observed in *E. herbicola*, with ~80% reduction in growth at 160 µg mL^{-1} over control (Table 3). Conversely at lower concentrations 10 µg $mL^{-1}$and 20 µg mL^{-1}, there was no discernible activity observed. In case of *P. syringae*, percentage inhibition increased from 50.2% to 77.4% at a concentration (10µg $mL^{-1}$to 160 µg mL^{-1}) (Table 3). The size of the inhibition zone varied depending on both the concentrations of EO used and the specific bacterial strains tested. For instance, the inhibition percentages exhibited by *R. fasciens* increased from 32% to 50.4%, while those of *S.scabiei* increased from 26% to 53.7%, and of *B. cereus* increased from 25% to 68.1% when exposed to increasing concentrations of the EO (10µg mL^{-1}, to 160 µg mL^{-1}) over control. An increase in the ZOI with increase in concentration of EO was observed among all the tested strains of bacteria (Table 3 & 4). Similar findings regarding the efficacy of EOs in inhibiting bacterial growth have been documented in previous studies against *E. herbicola* and *Pseudomonas putida* (Pandey *et al.* 2014). Additionally, there are comparable reports for other species within the *Pseudomonas* genus, where EO derived from *Origanum majorana* L. demonstrated efficient inhibition of bacterial growth (Sabiha *et al.* 2023). A slight disparity in antimicrobial activity between various studies reported in the literature could be attributed to the chemical polymorphism of the EO and the bacterial strains tested (Shafaghat *et al.* 2009; Tariq *et al.* 2019; Houti *et al.* 2023).

Our findings indicate that the EO derived from *A. sieversiana* sourced from the high altitude Ladakh region, demonstrated a significant antimicrobial efficacy. This suggests its potential application in the development of novel−plant based antimicrobial pharmaceutics product. The pharmacological effects of EO can be attributed to its diverse array of constituents. Specifically, borneol, camphor, and eucalyptol has earlier been demonstrated to have bacteriostatic activity against a wide spectrum of microorganisms (Verma *et al.* 2011), which was in line with our study, with the presence of these component as a major constituent. Additionally, minor components may also contribute potentially to enhance the overall biological activity of the EO (Burt, 2004).

Table 3: Antibacterial activity of essential oil of *Artemisia sieversiana* against some gram−negative bacteria

Concentration (µg mL^{-1})	Zone of inhibition (mm)	
	Erwinia herbicola	*Pseudomonas syringae*
10	ND	7.59±0.15e
20	ND	8.71±0.15d
40	7.38±0.10c	8.89±0.11d
80	8.59±0.16c	10.14±0.21c
160	11.63±0.98b	11.71±0.13b
Rifampicin (+ control)	14.67±0.49a	15.12±0.41a
DMSO (− control)	ND	ND

ND− Not Detected; Data represented in mm as Mean ± S.E. Different alphabets in a column represent significant difference at P ≤ 0.05 applying post hoc Tukey's test

Artemisia grown in Iran, for instance, also exhibited strong antimicrobial activity against various microorganisms, including fungicidal and bactericidal potential (Behbahani *et al.* 2017). Further, in a comprehensive study involving 34 fungal strains and 64 bacterial strains, the EO demonstrated varying susceptibilities (Kordali *et al.* 2005). Although, the precise mechanisms of action of EO remain incompletely understood, it is hypothesized that these constituents exert toxicity by disrupting the integrity of bacterial and fungal cell membranes (Filipowicz *et al.* 2003). For instance, borneol has been proven to possess the ability to inhibit respiration and ion transport (Marinas *et al.* 2023), while camphor disrupts the cell membrane of bacteria, fungi, and viruses, leading to the leakage of cell contents and ultimately causing cell death (Tariq *et al.* 2019).

Table 4: Antibacterial activity of essential oil of *Artemisia sieversiana* against some gram−positive bacteri

Concentration (µg mL^{-1})	Zone of inhibition (mm)			
	Bacillus cereus	*Bacillus pumilus*	*Rhodococcus fasciens*	*Streptomyces scabiei*
10	7.74±0.11e	8.56±0.34c	6.72±0.07e	6.18±0.02c
20	8.44±0.05e	12.10±1.31bc	6.93±0.03e	6.73±0.05c
40	11.76±0.36d	13.40±0.48bc	8.28±0.15d	7.62±0.39c
80	15.32±0.35c	15.13±1.99b	9.37±0.18cd	12.54±0.63b
160	21.16±0.85b	22.32±0.94a	10.61±0.38b	12.81±0.48b
Rifampicin (+ control)	31.36±0.48a	22.00±0.86a	21.05±0.53a	23.84±0.45a
DMSO (− control)	ND	ND	ND	ND

ND− Not Detected; Data represented in mm as Mean ± S.E. Different alphabets in a column represent significant difference at P ≤ 0.05 by applying post hoc Tukey's test

The diverse array of chemical components found in *A. sieversiana* EO thus provide a valid explanation for its antimicrobial effectiveness against both gram−negative and gram−positive bacteria. This explains its role as a natural inhibitor of microbial growth, making it a potential tool in combating crop and food pathogenic bacteria. In conclusion, the present study highlighted camphor and borneol as the primary components within the *A. sieversiana* EO, showcasing variations in its composition due to ecological influences. The EO demonstrated notable inhibitory effects against a range of food and plant pathogenic bacteria, suggesting its potential as a natural antimicrobial application. However, further investigation into its toxicity across diverse microrganisms is essential to ensure its safety and effectiveness for practical use.

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