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Chemical Composition and Antibacterial Activity of the Essential Oil from *Cupressus arizonica* Greene Cones Against Clinical and Foodborne Pathogens

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ABSTRACT

Objective: The purpose of this study was to characterize the chemical composition of the essential oil (EO) derived from the cones of *Cupressus arizonica* Greene collected from Erzincan and to evaluate its antibacterial potential against a range of pathogenic microorganisms, including strains not previously investigated for this species.

Methods: The essential oil was obtained from fresh cones via hydrodistillation for 4 hours. Chemical profiling was performed using Gas Chromatography-Mass Spectrometry (GC-MS). The antibacterial activity was assessed against six pathogenic bacteria using the disc diffusion method, with imipenem serving as a positive control.

Results: GC-MS analysis identified 35 compounds, representing 93.83% of the total oil. The major components were *α* -pinene (53.02%), 3-carene (13.31%), *α* -myrcene (4.54%), sabinene (4.24%), and limonene (3.47%). The oil exhibited selective antibacterial activity, with inhibition zones recorded for *Salmonella enterica* (10.0 ± 0.3 mm), *Clostridium perfringens* (8.1 ± 0.5 mm), and *Bacillus cereus* (8.0 ± 0.6 mm). However, no inhibitory effect was observed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

Conclusion: This study identifies a distinct *α*-pinene/3-carene chemotype of *C. arizonica influenced by the regional flora of Erzincan. Notably, this research represents the first report of the inhibitory efficacy of C. arizonica cone essential oil against the anaerobic pathogen Clostridium perfringens. The findings suggest that this essential oil could be a promising natural candidate for targeted applications against certain foodborne and anaerobic pathogens.*

Keywords: *Cupressus arizonica, Essential oil, α-pinene, 3-carene, Clostridium perfringens*

INTRODUCTION

Medicinal and aromatic plants have been utilized for centuries as natural sources of therapeutic agents due to their diverse secondary metabolite profiles. Among these, the genus *Cupressus* L. (Cupressaceae), comprising evergreen trees and shrubs distributed across northern temperate regions, has gained significant attention for its pharmacological potential.[1,2] *Cupressus arizonica* Greene, commonly known as the Arizona cypress, is frequently cultivated for ornamental purposes.[3,4] *C. arizonica* is a perennial evergreen that typically reaches heights between 5 and 25 meters. Its short stems are quadrangular in shape, possessing a diameter of approximately 1–2 mm. The foliage consists of scale-like leaves ranging in color from a muted grey-green to a vibrant cyan-green, situated on rounded branchlets. The tree produces cylindrical or four-sided pollen cones (2–5 mm long and 2–2.5 mm wide), whereas its seed cones—measuring 10–35 mm—are globose to egg-shaped and dehisce upon reaching maturity.[5,6] *C. arizonica* cones-leaves are rich in volatile oils,[7,8] which showed insecticidal,[8] antibacterial,[3] antioxidant,[9] antifungal,[10] herbicidal[11] as well as larvicidal activities.[12,13]

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The increasing incidence of multidrug-resistant (MDR) microorganisms poses a significant danger to the global healthcare system, requiring the development of alternative antimicrobial drugs. Essential oils, complex combinations of volatile molecules generated from plants, have received interest for their broad-spectrum antibacterial characteristics, including action against both Gram-positive and Gram-negative bacteria.[13,14] Furthermore, certain essential oils function as chemosensitizers, increasing the efficiency of conventional medicines while restoring sensitivity in resistant bacteria.[15] Essential oils' antimicrobial properties occur by a variety of processes, including the disruption of bacterial cell membranes, increased permeability, and cell death.[16]

Previous investigations into the antibacterial properties of *C. arizonica* essential oils have primarily targeted a specific range of reference and clinical strains, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus,* and *Enterococcus faecalis*.[3] However, to the best of our knowledge, the inhibitory potential of its cone-derived essential oil against other critical pathogens—particularly *Clostridium perfringens* (ATCC 13124) and *Bacillus cereus* (ATCC 10876)—remains unexplored. Furthermore, this study evaluates the oil's efficacy against *Staphylococcus aureus* (ATCC 6538) and *Salmonella enterica* (ATCC 35664), providing a broader perspective compared to previously studied lineages. By expanding the antibacterial screening to include these previously untested strains alongside standard models like *E. coli* and *P. aeruginosa*, this research aims to offer a more comprehensive assessment of *C. arizonica* as a potential source for novel, broad-spectrum antimicrobial agents.

METHODS

Plant Material

Cupressus arizonica Greene cones were collected in October 2025 from Kavakyolu, Erzincan, Türkiye (39°46'04.15"N, 39°27'13.63"E; 1215 m altitude). Plant sample identified by Prof. Dr. Ali Kandemir, Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Pharmacognosy. The authentic sample was kept in the herbarium of EBYU (EBYU 000045). The plant material used in this study, [*Cupressus arizonica*], is not classified as an endangered or protected species in Türkiye. Therefore, no specific collection permit was required.

Extraction of Essential Oil

Essential oil extraction was performed by hydrodistillation using a Clevenger-type apparatus for 4 hours. Prior to distillation, the fresh cones were cut into small pieces to facilitate the release of volatile compounds. The obtained oil was subsequently dried over anhydrous sodium sulfate (Na_2SO_4) and preserved in sealed, dark-colored vials at +4°C to maintain its stability prior to analysis.[14]

GC-MS Analysis

Chemical analysis of the essential oil was done with a Thermo Scientific Trace 1310 GC-MS system and an HP-5MS capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness). Helium served as the transporter gas, with a constant flow rate of 1.2 mL/min and a split ratio of 50:1. The temperature gradient for the column oven was configured as follows: an initial hold at 60 °C for 3 minutes, then a 3 °C/min rise to 200 °C, and finally a 5 °C/min ramp to 240 °C, which was maintained for 5 minutes. The temperatures for the injection port, transfer line, and ion source were consistently maintained at 280 °C. Mass spectra were acquired in electron impact (EI) mode at 70 eV, utilizing a 3-minute solvent delay. A 1 µL sample (5% v/v in acetone) was injected into the system. The identification of volatile components was achieved by comparing their mass spectra with Wiley and NIST2004 library databases, while retention indices (RI) were calculated using the Van den Dool and Kratz equation relative to a series of *n*-alkanes. Quantitative data were expressed as relative area percentages based on the chromatogram peaks.[14,17-23] The compounds were validated by normalizing the peak area obtained from the FID data without applying any correction factor.[24] The peak area from GC-FID analysis was used to determine the relative percentages of the components. The type of column, carrier gas flow rate, oven temperature program, injection volume, split ratio, and other experimental parameters were all the same for both GC-MS and GC-FID studies.

Antibacterial activity

The antibacterial activity of the volatile oil was assessed against *Staphylococcus aureus* (ATCC 6538), *Clostridium perfringens* (ATCC 13124), *Bacillus cereus* (ATCC 10876), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 35664), and *Escherichia coli* (ATCC 25922) were assessed using the disc diffusion method following a standard protocol.[25-27]

Sterile blank discs with a diameter of 6 mm were put on Nutrient Agar medium after 100 µL of bacterial solution ($1x10^8$ CFU). A blank disc was injected with 5 µL of the test sample, and a positive control disc with the antibiotic imipenem was also put on the medium. The plates were then incubated at 37 °C for 24 hours. The inhibitory zones

were measured with a digital caliper, and the mean diameter ± standard deviation of triplicates was reported in millimeters.

RESULTS

The compositions of the volatile oil extracted from the cones of *C. arizonica* are presented in Table 1. The volatile oil was obtained as a yellowish liquid with a yield of 0.137% (v/w), calculated based on fresh weight. The volatile oil included 35 compounds (93.83% of the overall composition) according to GC/MS analysis (Table 1). The identified subtances were classified into monoterpene hydrocarbons (84.21%), oxygenated monoterpenes (4.49%), sesquiterpene hydrocarbons (2.50%), oxygenated sesquiterpenes (2.59%), and others (0.04%). The main substances (> 3%) were identified as α-pinene (53.02%), 3-Carene (13.31%), α-Myrcene (4.54%), sabinene (4.24%), and limonene (3.47%).

Table 1. Chemical compositions of *C. arizonica* cones essential oils

No	RT	RI*	RI**	Component	% Composition***
1	4.42	811	809	Acetonyldimethylcarbinol	0.04±0.1
2	5.84	910	908	Bornylene	0.05±0.1
3	6.28	925	926	Tricyclene	0.25±0.05
4	6.42	930	929	Thujene	0.43±0.05
5	6.66	938	939	α-Pinene	**53.02±0.1**
6	6.94	951	950	α-Fenchene	1.13±0.2
7	7.66	960	961	Sabinene	**4.24±0.09**
8	8.15	993	992	α-Myrcene	**4.54±0.08**
9	8.52	997	996	α-Fellandrene	0.18±0.02
10	8.70	1006	1005	3-Carene	**13.31±0.1**
11	8.86	1015	1017	α-Terpinene	0.21±0.07
12	9.12	1031	1030	Limonene	**3.47±0.02**
13	9.77	1040	1039	*trans*-β-Ocimene	0.03±0.07
14	10.08	1059	1060	γ-Terpinene	0.39±0.01
15	10.34	1063	1064	*cis*-Sabinene hydrate	0.08±0.2
16	10.94	1065	1065	α-Terpinolene	2.96±0.08
17	11.26	1099	1100	Linalool	0.05±0.01
18	12.02	1112	1111	α-Campholenic aldehyde	0.04±0.1
19	12.40	1139	1140	*trans*-Pinocarveol	0.11±0.02
20	12.55	1145	1144	Camphor	0.32±0.02
21	13.01	1146	1145	Verbenol	0.3±0.03
22	13.18	1164	1165	Borneol	0.14±0.02
23	13.37	1169	1168	3-Thujen-2-one	0.09±0.03
24	13.50	1173	1172	Terpinen-4-ol	0.72±0.05
25	13.88	1189	1190	α-Terpineol	0.71±0.02
26	14.91	1218	1217	Citronellol	0.43±0.05
27	16.50	1263	1264	Bornyl acetate	0.41±0.04
28	17.92	1338	1337	α-Terpinyl acetate	1.09±0.07
29	19.30	1401	1402	Junipene	0.12±0.01
30	20.03	1418	1417	*trans*-Caryophyllene	1.06±0.4
31	20.87	1453	1452	α-Humulene	0.91±0.03
32	21.54	1481	1480	Germacrene D	0.41±0.02
33	23.44	1532	1531	Nerolidol	0.06±0.01
34	24.42	1608	1607	α-Cedrol	2.27±0.03
35	25.04	1642	1643	Cubenol	0.26±0.02
				Monoterpene Hydrocarbons (2-14, 16)	84.21
				Oxygenated Monoterpenes (15, 17-28)	4.49
				Sesquiterpene Hydrocarbons (29-32)	2.50
				Oxygenated Sesquiterpenes (33-35)	2.59
				Others (1)	0.04
				Total identified	93.83

RI*: retention index as determined on an HP-5MS column using the homologous series of *n*-hydrocarbons, RI **: Retention indexes according to literature, % Composition***: Percent compositions were expressed as mean ± standard deviation of three independent measures

The results of the antimicrobial activity assay for *C. arizonica* volatile oil are summarized in Table 2. The disc diffusion analysis revealed that the essential oil possessed selective antibacterial properties. Moderate inhibitory activity was recorded against *Salmonella enterica* (inhibition zone: 10.0±0.3 mm), *Clostridium perfringens* (inhibition zone: 8.1±0.5 mm), *Bacillus cereus* (inhibition zone: 8.0±0.6 mm). However, the essential oil failed to suppress the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, indicating a lack of broad-spectrum activity at the tested concentration. Imipenem, utilized as a positive control, yielded significantly larger inhibition zones, ranging from 21.1±1.0 mm to 38.8±0.5 mm, confirming the comparative resistance or lower sensitivity of the pathogens to the essential oil.

Table 2. Disc diffusion (mm) results of *Cupressus arizonica* Greene cones essential oil for tested pathogenic bacteria

Bacterial strains	Essential oil (5 µL)	Imipenem* (10 mcg)
Staphylococcus aureus	ni	38.8±0.5 mm
Clostridium perfringens	8.1±0.5 mm **	21.9±0.6 mm
Bacillus cereus	8.0±0.6 mm**	21.1±1.0 mm
Pseudomonas aeruginosa	ni	24.9±1.2 mm
Salmonella enterica	10.0±0.3 mm**	32.2±1.4 mm
Escherichia coli	ni**	28.9±2.1 mm

ni: no inhibition, *: Positive control (Imipenem), **: inhibition zone diameters in mm (mean ± sd, n=3)

DISCUSSION

In this study, it was observed that 0.137% (v/w) volatile oil was obtained from *C. arizonica* cones by hydro distillation. This result differs from many previous studies that generally report higher yields ranging from 0.46% to 1.9%.[3,9,12,28]

The relatively lower essential oil yield obtained in the present study (0.137%, v/w) compared with previously reported values may be attributed to several factors. Essential oil yield in aromatic plants is strongly influenced by environmental and methodological parameters, including geographical origin, altitude, soil composition, climatic conditions, and seasonal variations during plant collection. In the present study, the plant material was collected from Erzincan at an altitude of 1215 m, and such pedoclimatic conditions may significantly affect the biosynthesis and accumulation of volatile metabolites. Additionally, the use of fresh cones, differences in plant maturity, and variations in hydrodistillation parameters may also contribute to the observed variation in oil yield. These factors are well known to cause substantial quantitative differences in essential oil production even within the same species.

A comparative assessment of the chemical profile reveals significant qualitative and quantitative discrepancies between the present study and previous reports on *C. arizonica* cone essential oils. Notably, acetonyldimethylcarbinol and bornylene were uniquely identified in our sample, whereas they were absent in the compared literature.[3,9,12,28] Furthermore, the concentration of 3-carene (%13.31) represents a major chemical marker for our study, contrasting sharply with the trace amounts (0.4% or less) reported in other studies. These findings suggest that our *C. arizonica* sample belongs to a distinct *α*-pinene/3-carene chemotype, likely influenced by the specific pedoclimatic conditions of the collection site. Additionally, while the compared literature showed a high diversity of sesquiterpene hydrocarbons such as zonarene and muurolenes, our oil exhibited a more dominant monoterpene profile, further distinguishing its phytochemical fingerprint.[3,9,11,12,28,29]

The chemical composition differences among *C. arizonica* cones can be attributed to genetic factors, environmental conditions of their growing regions (such as soil type and climate). This study also provides novel insights into the metabolic capacity of *C. arizonica*, shedding light on the ecological adaptation processes of this species.[14,20]

The antibacterial screening of *Cupressus arizonica* cone essential oil (EO) revealed a selective inhibitory profile, primarily targeting *Salmonella enterica*, *Clostridium perfringens*, and *Bacillus cereus*. In the present study, the highest susceptibility was observed in S. enterica with an inhibition zone of 10.0±0.3 mm. This finding is largely consistent with previous reports investigating the cones of *C. arizonica*, which documented inhibition zones of approximately 13 mm against *Salmonella typhimurium*. The slight quantitative differences in inhibition diameters may be attributed to variations in bacterial strain sensitivity or environmental factors influencing the secondary metabolite accumulation in the plant material.[3]

A notable deviation from the existing literature was observed regarding the activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. While our results indicated no inhibitory effect against these pathogens at the tested concentrations, several studies have reported contrasting findings. For instance, literature data indicates that *C. arizonica* cone EOs can produce significant inhibition zones for *E. coli* (10-19 mm), *S. aureus* (16 mm), and *P. aeruginosa* (15 mm).[3,30] Furthermore, some researchers have reported a broader spectrum of activity, with minimum inhibitory concentrations (MIC) reaching 1/250 v/v for all tested clinical and reference strains.[29]

In a recent study, the chemical composition and antioxidant activity of essential oils obtained from the female cones and leaves of *C. arizonica* were investigated. The essential oils were extracted by hydrodistillation, yielding 1.90% from the female cones and 0.65% from the leaves. GC–MS analysis identified 17 compounds in the cone oil and 45 compounds in the leaf oil. The essential oil from the female cones was mainly composed of monoterpene hydrocarbons, with *α*-pinene (72.20%) as the predominant compound, followed by *β*-myrcene, *β*-pinene, and *δ*-3-carene. In contrast, the leaf essential oil was rich in oxygenated monoterpenes, particularly umbellulone (17.33%).[31] In another study, the essential oils obtained from the leaves, cones, and stems of *C. arizonica* were investigated for their chemical composition and biological activities in a study. GC–MS analysis revealed that the oils were dominated by monoterpenes, particularly *α*-pinene, which was the major component in all parts and reached 72.97% in the stem oil, while the leaf oil was also rich in umbellulone and limonene. The antimicrobial activity of the oils was evaluated against several Gram-positive and Gram-negative bacteria, including *S. aureus*, *Listeria monocytogenes*, *P. aeruginosa*, *E. coli*, and *Acinetobacter baumannii*. The essential oils exhibited notable bactericidal and antibiofilm effects, showing stronger activity against *E. coli* and *P. aeruginosa*, whereas limited activity was observed against *A. baumannii* and *S. aureus*.[32]

This discrepancy in antibacterial efficacy is likely rooted in the unique phytochemical "fingerprint" of our EO sample. Our analysis identified a distinct *α*-pinene/3-carene chemotype, characterized by a high concentration of *α*-pinene (53.02%) and an uncommonly high level of 3-carene (13.31%). In contrast, literature reports that demonstrate high broad-spectrum activity often describe oils with higher sesquiterpene diversity (such as germacrene D or muurolene isomers) or different monoterpene ratios.[3,9,11,12,28,29] The selective inhibition observed in our study suggests that the high *α*-pinene and 3-carene content may be specifically effective against certain Gram-positive bacterials and *Salmonella* sp., but insufficient to penetrate the robust outer membrane of *P. aeruginosa* or overcome the specific resistance mechanisms of the tested *E. coli* and *S. aureus* strains. Furthermore, our study provides a significant contribution to the biological characterization of *C. arizonica* by reporting its inhibitory activity against *Clostridium perfringens* (8.1±0.5 mm). To the best of our knowledge, this study represents the first report on the antimicrobial activity of *C. arizonica* cone essential oil against *Clostridium perfringens*. This finding not only

enriches the biological profile of this species but also provides valuable data for the search for novel natural antimicrobial agents.

The selective antibacterial activity of EO can be primarily attributed to its dominant monoterpene profile, characterized by high levels of α-pinene (53.02%) and 3-carene (13.31%). The observed susceptibility of Gram-positive pathogens, such as *B. cereus* and *C. perfringens*, is linked to the lipophilic nature of these monoterpenes, which integrate into the bacterial lipid bilayer, causing membrane disruption and cellular leakage.[33,34] In contrast, the resistance of Gram-negative strains like *E. coli* and *P. aeruginosa* is largely explained by the presence of a hydrophobic lipopolysaccharide (LPS) layer in their outer membrane, which acts as a protective barrier against hydrophobic agents like α-pinene. However, the moderate inhibition recorded for *S. enterica* suggests that the high concentration of α -pinene in our sample may partially bypass this barrier. Furthermore, our findings provide a significant contribution by establishing the efficacy of EO against *Clostridium perfringens*, a pathogen often overlooked in the phytochemical screenings of this genus. This inhibitory effect is likely enhanced by the synergistic action of minor components such as limonene and *β*-caryophyllene, which are known to facilitate the penetration of major constituents into the bacterial cell.[35-37]

CONCLUSION

This study provided a detailed characterization of the chemical composition and antibacterial activity of *C. arizonica* cone essential oil collected from Erzincan, Türkiye. The oil exhibited a distinctive *α*-pinene/3-carene chemotype dominated by monoterpene hydrocarbons and demonstrated selective antibacterial activity against *S. enterica, B. cereus*, and notably *C. perfringens*. The inhibition of *C. perfringens* represents a novel finding that expands the known antimicrobial spectrum of *C. arizonica* essential oil.

Future studies should focus on determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values to better quantify its antimicrobial potency. In addition, investigations into the synergistic effects between the major constituents (such as *α*-pinene and 3-carene) and minor compounds could provide further insights into the mechanisms underlying the observed antibacterial activity. Studies evaluating the oil's cytotoxicity, safety profile, and potential applications in food preservation, pharmaceutical formulations, or natural antimicrobial products would also be valuable. Furthermore, comparative studies involving different plant parts, harvesting seasons, and ecological conditions may help to better understand the factors influencing the phytochemical variability and biological activity of *C. arizonica* essential oils.

Ethics Committee Approval: The research was conducted exclusively using plant material and in vitro microbiological assays. Therefore, in accordance with national and international research ethics guidelines, approval from an Ethics Committee was not required for this study. All experimental procedures were carried out in compliance with relevant institutional, national, and international guidelines.
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