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Phytochemical Characterization and Antibacterial assessment of Essential oils Derived from *Teucrium stocksianum* Boiss. and Selected *Salvia* Species

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ABSTRACT

Species belonging to the genera *Teucrium* and *Salvia* (Lamiaceae) are widely recognized in ethnomedicine, particularly in the Baluchestan region, as important medicinal plants used in the treatment of ailments such as colds and gastrointestinal disorders, largely attributed to their terpene-rich essential oils (EOs). The aim of this study was to characterize the chemical composition and evaluate the antimicrobial activity of EOs obtained from selected *Salvia* species and *Teucrium stocksianum*. In the present study, EOs obtained from *Teucrium stocksianum* Boiss, *Salvia artemisioides* Boiss, *Salvia abrotanoides* Karel., and *Salvia yangii* Benth., collected from the Baluchestan region of Iran, were chemically characterized and evaluated for their antimicrobial activity. The oils were isolated by hydrodistillation and analyzed using gas chromatography–mass spectrometry (GC–MS). Antimicrobial activity was assessed by the microbroth dilution method against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, and expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The EO yield was 0.45% for *T. stocksianum* and ranged from 0.8% to 1.35% for the *Salvia* species. GC–MS analysis revealed that α-pinene (36.83%) and β-pinene (13.39%) were the major constituents of *T. stocksianum* oil, while 1,8-cineole (31.13%) was the predominant compound in *S. yangii*. Among the tested samples, the EOs of *T. stocksianum* and *S. yangii* exhibited notable antibacterial activity, particularly against *E. coli*, *S. aureus*, and *B. subtilis*, with inhibitory effects observed at concentrations below 20 mg/ml. Importantly, the EO of *T. stocksianum*, rich in monoterpenes such as α- and β-pinene, demonstrated strong antibacterial potential, with low MIC values *against B. subtilis* (0.5 ± 0.3 mg/ml) and *S. aureus* (2 ± 0.8 mg/ml). In contrast, the EOs of *S. abrotanoides* and *S. artemisioides* showed limited antimicrobial activity. These findings suggest that the EO of *T. stocksianum* is a promising natural antimicrobial agent, supporting its ethnomedicinal use and highlighting its potential applications in the development of plant-based therapeutic and preservative agents; however, further in vivo studies are required to confirm its efficacy and safety.

Keywords: Lamiaceae, Ssential oils, Secondary metabolites, Antimicrobial activity

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INTRODUCTION

The genera *Salvia* and *Teucrium*, including *Salvia artemisioides* Boiss, *Salvia abrotanoides* Karel., *Salvia yangii* Benth., and *Teucrium stocksianum* Boiss, belong to the Lamiaceae family, commonly known as the mint family. Lamiaceae is one of the most diverse and widely distributed families of flowering plants, comprising over 236 genera and approximately 7000 species [1]. Within this family, *Salvia* represents the largest genus, with more than 900 species worldwide; approximately 60 species occur in Iran, 17 of which are endemic. In contrast, *Teucrium* comprises around 340 species globally, including 12 species in Iran, three of which are endemic [2-3]. The genus *Salvia* includes numerous medicinal and aromatic species distributed mainly in Central Asia. Among these, *S. yangii* and *S. abrotanoides* are particularly widespread [4]. *Salvia s*pecies exhibit a broad spectrum of pharmacological properties, primarily attributed to their terpenoid constituents. These include antioxidant, anti-inflammatory, antimicrobial, antibiofilm, anxiolytic, and antidepressant effects, as well as the inhibition of amyloid-β aggregation and modulation of cholinergic activity [5-7]. *T. stocksianum* is a perennial aromatic herb native to northern Oman, the United Arab Emirates, Pakistan, and the mountainous areas of Iran. It typically grows to a height of 10–30 cm and is characterized

by grayish-white foliage and sessile flowers. *T. stocksianum* has been widely used in traditional medicine for the treatment of various ailments, including gastrointestinal disorders, inflammation, diabetes, and rheumatism [8-9]. In addition, it has been traditionally employed for the management of conditions such as ulcers, hypertension, epilepsy, throat pain, diarrhea, cough, jaundice, and abdominal pain. These diverse ethnomedicinal applications, together with reported cytoprotective, anti-ulcerogenic, and other pharmacological properties, highlight the therapeutic potential of *T. stocksianum* and suggest the presence of biologically active phytochemical constituents [8-9]. In addition to these traditional uses, EOs derived from *Teucrium* species have been shown to reduce microbial contamination in food systems, supporting their application as natural preservatives. Furthermore, *T. polium* EOs, particularly when combined with probiotic fermentation, have been reported to significantly reduce *Escherichia coli* populations during storage [10-11].

Pharmacological studies have provided scientific support for several of these applications. For example, the anti-angiogenic potential of *T. stocksianum* extract has been demonstrated using the chorioallantoic membrane assay, in which the extract significantly reduced blood vessel diameter at a dilution of 0.5% [12]. Moreover, methanolic extracts of *T. stocksianum* have exhibited significant antinociceptive activity in various animal models, along with strong antioxidant capacity and high total phenolic content. These effects are likely associated with the presence of diverse phytochemical constituents, including flavonoids, tannins, saponins, anthraquinones, steroids, phlobatannins, terpenoids, glycosides, and reducing sugars [13]. The ethyl acetate fraction of *T. stocksianum* has been reported to exert notable anti-inflammatory activity through the inhibition of both cyclooxygenase and lipoxygenase pathways involved in arachidonic acid metabolism, thereby supporting its traditional use in the treatment of inflammatory conditions [14]. Toxicological evaluations indicate that *T. stocksianum* is relatively safe. In contrast to *Teucrium* chamaedrys, which is known for its hepatotoxicity, no significant hepatotoxic effects have been observed following either acute or chronic administration of *T. stocksianum* [15-17]. In addition, ethanolic extracts of the plant have demonstrated hepatoprotective properties, with no lethality or histopathological abnormalities detected in the liver tissues of treated animals [16].

Overall, EOs are increasingly recognized as effective natural antimicrobial agents, offering promising alternatives to synthetic drugs that may be associated with adverse effects. Their biological activity is mainly attributed to the presence of bioactive compounds such as alcohols, phenols, and aldehydes [18]. In the Baluchestan region, selected *Salvia* species and *T. stocksianum* hold considerable importance in traditional medicine; however, there are limited reports on their chemical composition and biological properties. Therefore, the present study aimed to characterize the chemical composition of EOs obtained from these species and to evaluate their antimicrobial potential.

MATERIALS AND METHODS

Chemicals Used

The solvents were obtained from Cambridge, United Kingdom. *Escherichia coli* ATCC 11775, *Staphylococcus aureus* ATCC 12600, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* were used as tested microorganisms.

Plant Material

The branches and leaves of three *Salvia* species (*S. yangii, S. abrotanoides, and S. artemisioides*), as well as the aerial parts of *T. stocksianum*, were collected from Taftan Mountain (DarehGol region; 28°36′28.94″ N, 61°4′40.40″ E) and the Lashar area (the Tangeh Sarheh region; 26°33′26″ N, 59°57′52″ E) in Sistan and Baluchestan Province, Iran, during their flowering stage in early May 2023. The plant material was collected and taxonomically identified by Dr. Hadi Darroudi. Voucher specimens (HUS 239, HUS 241, HUS 242, and HUS 230) were deposited in the Herbarium of the Department of Production and Utilization of Medicinal Plants at the University of Saravan.

Analysis of Essential Oils by GC-MS and GC-FID

The EOs obtained by hydrodistillation were analyzed using a Thermo Quest GC–FID system and a Thermo Quest Finnigan GC–MS instrument. Separation of the volatile constituents was carried out on a DB-5 fused-silica capillary column. Helium was used as the carrier gas, and electron impact ionization was performed at 70 eV. The temperatures of the ion source and interface were maintained at 250 °C and 200 °C, respectively. The mass spectra were recorded over a range of 45–456 amu. The oven temperature program applied was the same as that used for GC analysis.

Identification of the volatile compounds was based on the calculation of Kovats retention indices using a homologous series of n-alkanes (C9–C21) under temperature-programmed conditions, and by comparison of both retention indices and mass spectral fragmentation patterns with literature data and the NIST library database. Quantitative analysis was performed using GC–FID, and the relative percentage of each component was calculated from its peak area relative to the total chromatographic area at a detector temperature of 250 °C.

Bioassays

To screen antimicrobial activity, the microbroth dilution assay was performed in accordance with the guidelines of the National Committee for Clinical Laboratory Standards [19]. The MIC values were determined using the microbroth dilution method [20]. A twofold serial dilution of EOs was prepared in 10% dimethyl sulfoxide (DMSO), ranging from 32 to 0.125 mg/ml. Mueller-Hinton broth was used as the bacterial growth medium. The microbial suspension was adjusted to approximately 1×10^6 colony-forming units per milliliter (CFU/ml) for bacteria and $0.5–1 \times 10^5$ CFU/ml for fungi [19]. Plates were incubated under appropriate conditions. MICs were defined as the lowest concentration at which no visible microbial growth was observed. The minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was determined as the lowest concentration at which no microbial growth was observed on solid media after 24 hours (for bacteria).

For the viability assay of *Staphylococcus aureus* in response to EOs, five flasks containing 5 ml of bacterial suspension in cation-adjusted Mueller-Hinton broth were prepared. EOs were added at their respective MIC values to each flask, except for the control. Immediately after EO addition, 100 µL from the control flask was sampled, serially diluted, and plated on nutrient agar to determine the initial cell density. At 30, 60, and 120 minutes at room temperature,

100 µL aliquots were withdrawn, transferred into a neutralizing buffer (0.075 M phosphate buffer, pH 7.9), serially diluted, and plated to determine the viable bacterial count (CFU/ml) for each oil. Experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Identification of Volatile Compounds in *Salvia* species and *T. stocksianum*

The chemical composition of EOs from three *Salvia* species is reported in Table 1. The EO of *S. abrotanoides* was found to be rich in geranyl acetate (60.81 %) and 1,8-cineole (5.77%). *S. artemisioides* contained δ-3-carene (44.84%) and 1,8-cineole (12.75%), while *S. yangii* was characterized by the presence of 1,8-cineole (31.13%) and δ-3-carene (23.77%).

A total of 33 compounds were identified in *T. stocksianum*, accounting for 98.10% of the total EO composition. As shown in Table 2, the predominant constituents in this species were α-pinene (36.83%), β-pinene (13.39%), trans- α-bergamotene (7.92%), and α-phellandrene (7.33%).

The results of our study support previous findings indicating that α-pinene and β-pinene are major constituents in the EO of *T. stocksianum* collected from southern and southeastern regions of Iran, including Bandar Abbas and Sistan and Baluchestan Province [21]. This observation is in agreement with reports on *Teucrium* species, where monoterpenes such as α-pinene and β-pinene are commonly identified as dominant components, suggesting a conserved phytochemical pattern within the genus [22]. However, the chromatographic profile of *T. stocksianum* EO in our study exhibited both qualitative and quantitative differences compared to those reported from other geographical regions. For instance, in samples collected from Pakistan, the major components were identified as δ-cadinene (12.92%), α-pinene (10.3%), myrcene (8.64%), β-caryophyllene (8.23%), germacrene D (5.18%), and limonene (2.36%) [23]. These variations may be attributed to environmental factors such as climate, altitude, and soil composition, as well as genetic differences among plant populations [24].

Antimicrobial Activity of Essential Oil

There are limited studies on the antibacterial properties of EOs from the studied plants, especially *Teucrium*. However, several investigations have reported the antimicrobial activity of *T. stocksianum* extracts. Antibacterial investigations of *Salvia* and *Teucrium* species from 2022 to 2025 were summarized in Table 3. According to the table, several *Salvia* and *Teucrium* species demonstrated strong activity against bacteria.

Table 1 Chemical composition of essential oils from three *Salvia* species.

No	Main components	RI	LRI	Rt	Amount (%)		
					S. artemisoides	*S. abrotanoides*	*S. yangii*
1	α-pinene	939	932	5.86	5.40	4.77	4.56
2	Camphene	957	946	6.28	3.64	2.10	3.78
3	β-Pinene	1020	974	6.90	1.58	0.83	2.20
4	Myrcene	992	988	7.01	0.66	0.78	0.65
5	δ-3-Carene	1019	1008	7.62	44.84	4.55	23.77
6	α-Terpinen	1012	1014	7.80	0.43	-	0.4031
7	p-Cymene	977	1020	7.97	0.37	0.12	1.3877
8	dl-Limonene	1026	1024	8.11	2.23	0.74	1.0417
9	1,8-Cineole	1048	1026	8.34	12.75	5.77	31.13
10	β-Ocimene	1036	1044	8.84	1.5	0.22	07
11	γ-Terpinen	1056	1054	9.44	0.68	-0.07	0.38
12	α-Terpinolene	1081	1086	9.565	1.27	0.20	1.06
13	Linalool	1115	1095	11.72	2.45	-	5.91
14	Camphor	1164	1141	13.69	-	-	0.69
15	Borneol	1191	1165	13.89	0.17	0.3	0.42
16	Linalyl acetate	1252	1254	12.08	3.57	-	-
17	Bornyl acetate	1294	1284	15.05	2.60	2.90	0.96
18	δ -Terpinyl acetate	1310	1316	13.8	0.56	-	-
19	α-Terpinenyl acetate	1353	1346	16.65	1.28	-034	1.07
20	Neryl acetate	1362	1359	17.34	0.29	-	0.5
21	Geranyl acetate	1393	1379	17.39	-	60.81	-
22	β-Caryophyllene	1418	1417	18.42	2.79	3.60	4.92
23	α-Humulene	1466	1452	19.35	2.86	3.34	5.06
24	α-Selinene	1494	1498	20.17	0.16	0.44	-
25	γ-Cadinene	1518	1513	20.71	0.10	0.3	0.60
26	Caryophyllene oxide	1589	1582	23.39	8.39	-	-
27	Ledene	1592	1496	23.82	-	6.51	4.35
28	Veridiflorol	1608	1592	24.83	-	-	1.06
29	γ-Cadinol	1653	1652	24.92	-	-	1.44
30	β-costol	1786	1766	25. 71	-	-	0.48
	Yield of EOs (%)				0.8	1.11	1.35
	Total identified				96.22	99.31	98.88

Rt: Retention time; RI: Retention indices; LRI: Literature retention indices

Our results show that the EO of *T. stocksianum*, which contains α-pinene, β-pinene, α-bergamotene, and α-phellandrene as major constituents, exhibited a bactericidal effect against *Pseudomonas aeruginosa*, with both MIC and MBC values of 32 mg/ml. This species also demonstrated significant antimicrobial activity against *Bacillus subtilis*, as indicated by its MIC value (Table 4). This antibacterial activity may be attributed to the high abundance of monoterpene constituents, particularly α-pinene and β-pinene, which are known to interact with bacterial cell membranes due to their lipophilic nature, resulting in increased membrane permeability and disruption of cellular integrity [34].

Table 2 Chemical composition of essential oil from *T. stocksianum*.

No	Main components	Rt	LRI	RI	Amount (%)
1	α-Phellandrene	4.779	920	919	7.33
2	α-Pinene	4.935	932	925	36.83
3	Camphene	5.224	946	940	0.44
4	Verbenene	5.322	953	951	0.16
5	Sabinene	5.697	963	965	2.37
6	β-Pinene	974	974	969	13.39
7	β-Myrcene	6.009	988	984	6.24
8	α-Terpinene	6.621	1014	1014	0.15
9	o-Cymene	6.800	1020	1023	0.89
10	d-Limonene	6.899	1024	1025	4.33
11	Trans-β-Ocimene	7.060	1033	1029	0.26
12	β-Ocimene	7.314	1044	1041	1.08
13	γ-Terpinene	7.609	1054	1055	0.30
14	α-Terpinene	8.354	1067	1068	0.20
15	α-Campholenal	9.313	1078	1080	0.64
16	(E)-3(10)-Caren-4-ol	9.671	1089	1086	0.40
17	Trans-Verbeno	9.792	1096	1099	0.27
18	Pinocarvone	10.295	1120	1117	0.39
19	Terpinen-4-ol	10.658	1189	1125	0.17
20	Bicyclo [3.1.1] hept-2-ene-2carboxaldehyde	11.178	1253	1265	0.59
21	Bornyl acetate	13.523	1284	1294	1.69
22	Trans-Caryophyllene	17.011	1417	1419	1.07
23	Trans-α-Bergamotene	17.358	1435	1439	7.92
24	Humulene	17.849	1452	1455	0.52
25	Selina-4,11-diene	18.576	1489	1479	1.25
26	Valencene	18.813	1498	1502	0.74
27	Cis,cis-4,6-Octadienol	19.108	1509	1511	-
28	(E,Z)-α-Farnesene	19.113	1522	1525	0.31
29	(-)-α-Panasinsen	19.414	1534	1539	4.40
30	δ-Cadinene	19.500	1555	1561	0.20
31	α-agarofuran	20.089	1568	1565	-
32	Spathulenol	20.811	1577	1579	-
33	Caryophyllene oxide	20.956	1593	1585	1.48
34	10-epi-γ-eudesmol	21.793	1620	1618	-
35	Veridiflorol	22.342	1645	1649	-
36	β-Eudesmol	22.457	1680	1678	1.50
37	α-Maaliene	22.50	1730	1734	-
38	α-Eudesmol	22.521	1791	1795	0.34
39	7-epi-α-selinene	22.636	1823	1821	-
40	γ-Gurjunene	22.659	1854	1859	0.25
41	1,8-Cyclopentadecadiyne	24.44	1893	1891	-
Yield of EO (%)			0.67		
Total identified			98.10		

Rt: Retention time; RI: Retention indices; LRI: Literature retention indices

Such effects are especially relevant in Gram-negative bacteria, where EOs can destabilize the outer membrane and promote leakage of intracellular components. In addition, terpenoid compounds may exert antimicrobial effects through multiple complementary mechanisms, including disruption of membrane potential, interference with enzyme systems, and induction of oxidative stress within microbial cells [34-35]. The observed activity is therefore likely the result of synergistic interactions among the major constituents rather than the action of a single compound. These findings support previous reports highlighting the antimicrobial potential of monoterpene-rich EOs and further emphasize the therapeutic relevance of *T. stocksianum* EO as a natural antimicrobial agent.

A new triol-based compound, teucriol, was identified in the extract of *T. stocksianum* and shown to exhibit notable antibacterial potential against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli* [9].

The antimicrobial potential of different leaf extracts obtained from *T. stocksianum* has been evaluated against a panel of eight clinically relevant human pathogens, including bacterial strains (*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) as well as fungal species (*Aspergillus niger* and *Aspergillus fumigatus*). These assessments were conducted using the agar well diffusion technique in combination with MIC determination. The findings indicated that all tested microorganisms were sensitive to the extracts, irrespective of the solvent employed. Notably, methanolic and ethanolic leaf extracts displayed the highest antimicrobial efficacy, suppressing microbial growth at levels comparable to those of reference agents such as chloramphenicol for bacterial strains and fluconazole for fungal species [36].

According to Table 1, the EO of *S. yangii*, which contains 1,8-cineole (31.13%) and δ-3-carene (23.77%) as dominant constituents, exhibited strong antimicrobial activity against *B. subtilis*, with a MIC value of 4 mg/ml. In contrast, the EOs of *S. abrotanoides* and *S. artemisioides*, whose main components are geranyl acetate (60.81%) and δ-3-carene (44.84%), respectively, showed weak antimicrobial effects, with MIC and MBC values exceeding 64 mg/ml. The pronounced activity of *S. yangii* EO may be attributed to its high content of 1,8-cineole, which has been reported to reduce bacterial viability by disrupting membrane integrity and increasing cellular permeability, leading to leakage of intracellular components [34]. In addition, 1,8-cineole has been associated with anti-virulence effects, including inhibition of quorum sensing and biofilm formation, and may enhance the efficacy of conventional antibiotics when used in combination [35]. The EO of *S. abrotanoides* was found to contain α-pinene (12%), camphor (23%), 1,8-cineole (22%), β-pinene (3.1%), and limonene (1.5%) as its major constituents, exhibiting antimicrobial activity against *Candida albicans* and gram-positive bacteria, particularly *Staphylococcus aureus* [37]. The inhibition zones ranged from 7.6 to 29 mm, with MIC values between 2 and 8 µL/ml. In contrast, *Aspergillus niger* and gram-negative bacteria were less susceptible. Notably, the MIC values of the whole EO were generally lower than those of its individual components, indicating the possibility of synergistic interactions among the components [37]. The antibacterial activity of *S. abrotanoides* EO may therefore be attributed not only to its major compounds but also to minor components and potential synergistic or antagonistic interactions within the oil.

Table 3 Antimicrobial studies on *Salvia* and *Teucrium* species during 2022-2025.

Species	Major compound(s)	Biological activity	Tested microorganisms
Salvia chamaedryoides [25]	Essential oils	Antibacterial	*Listeria monocytogenes*
Salvia lavandulifolia & Salvia officinalis [26]	Camphor-rich essential oils	Antimicrobial	*Proteus mirabilis, Bacillus subtilis, Candida albicans*
Salvia officinalis [27,28]	Phenolic-rich, polar fractions, camphor, thujone, cineole	Antibacterial, insecticidal, antibacterial	*Enterococcus faecalis, Citrobacter freundii Klebsiella pneumoniae, Staphylococcus aureus*
Salvia mirzayanii [29]	Essential oils	Antimicrobial	*Staphylococcus aureus, Escherichia coli, Giardia lamblia*
Salvia dominica [30]	Essential oil rich in linalyl acetate and cineole	Antioxidant, anticancer, antimicrobial	*Staphylococcus aureus, Escherichia coli, Candida albicans* (*in vitro*)
Teucrium luteum subsp. Flavovirens [31]	t-Cadinol, caryophyllene oxide	Antibacterial	*Enterococcus faecalis, Pseudomonas aeruginosa*
Teucrium polium [32-33]	α-pinene and limonene in essential oil	Antibacterial antifungal activity	*Staphylococcus aureus, Escherichia coli, Candida albicans*

In a viability test, it was reported that the antimicrobial activity of 1,8-cineole was initially greater than that of α-pinene and camphor; however, after 60 minutes, its effect gradually diminished, and camphor eventually exhibited higher antibacterial activity than α-pinene [37].

Furthermore, the therapeutic potential of 1,8-cineole against pathogenic bacterial species involved in chronic rhinosinusitis was evaluated by Schürmann et al., who found that it significantly inhibited bacterial proliferation and biofilm formation [38].

Similarly, *S. artemisioides* EO was reported to contain 1,8-cineole (29.9%), camphor (29.5%), and α-pinene (7.8%) as major constituents, along with δ-3-carene (5.1%), camphene (3.3%), and β-pinene (2.7%) [39]. Antimicrobial studies showed that the extracted oil exhibited strong inhibitory activity against five microbial strains, with inhibition zones reaching up to 18 mm. Moreover, MIC and MBC results demonstrated that *S. artemisioides* EO effectively inhibited *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* [40].

Table 4 Antimicrobial activity of essential oil by microbroth dilution

Strains	*Escherichia coli*		*Staphylococcus aureus*		*Pseudomonas aeruginosa*		*Bacillus subtilis*	
Sample	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Salvia yangii	8 ± 0.4	17 ± 0.7	9 ± 2	>64	>64	>64	4 ± 0.6	>64
Salvia artemisioides	>64	>64	>64	>64	-	-	-	-
Salvia abrotanoides	>64	>64	>64	>64	-	-	-	-
Teucrium stocksianum	3 ± 0.9	8 ± 0.3	2 ± 0.8	15 ± 2	32 ± 3	32 ± 2.7	0.5 ± 0.3	>64

α- and β-pinene are widely recognized for their antibacterial properties, primarily attributed to their disruptive effects on microbial cell membranes. Notably, α-pinene has been identified as an antibiotic resistance modulator in *Campylobacter jejuni*, where it influences resistance mechanisms and inhibits antimicrobial efflux, as demonstrated using insertion mutagenesis. These effects were assessed through broth microdilution and ethidium bromide accumulation assays. Furthermore, DNA microarray analysis revealed that α-pinene significantly modulated antibiotic resistance in *C. jejuni*, reducing the MIC of ciprofloxacin, erythromycin, and triclosan by up to 512-fold [40].

The antimicrobial activity of α- and β-pinene enantiomers has also been evaluated against a range of pathogens, including *Candida albicans*, *Cryptococcus neoformans*, *Rhizopus oryzae*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Reported MIC values for these enantiomers ranged from 117 to 6250 µg/ml. Interestingly, the negative enantiomers exhibited no significant antimicrobial activity at concentrations up to 20 µg/ml. In contrast, the positive enantiomers demonstrated higher efficacy, particularly against *C. albicans*. The positive forms were capable of completely eliminating *C. albicans w*ithin 60 minutes, whereas eradication of MRSA required approximately six hours [41].

Phytochemical screening confirmed the presence of α-pinene and β-pinene as the most active monoterpenes in *T. stocksianum*, and 1,8-cineole in *S. yangii*, which are likely responsible for the observed antimicrobial activities against *E. coli*, *S. aureus*, and *B. subtilis*. These findings highlight the significant antibacterial potential of both species, with *T. stocksianum* showing notable activity attributed to its monoterpene richness, while *S. yangii* demonstrates efficacy linked to its high 1,8-cineole content.

Taken together, the findings suggest that these EOs may represent promising natural sources of antimicrobial agents with potential therapeutic relevance. Nevertheless, further studies are warranted to more clearly elucidate the isolation and structural characterization of the bioactive compounds contributing to these effects. In addition, more comprehensive evaluations of their toxicological properties, pharmacokinetic behavior, and bioavailability would be valuable to better assess their safety and efficacy profiles. Such investigations may ultimately help to clarify their potential applicability in pharmaceutical formulations and clinical contexts, and contribute to bridging the gap between preliminary antimicrobial observations and practical therapeutic use.

CONCLUSION

The EOs of *T. stocksianum* and *S. yangii* demonstrated significant antibacterial activity against *E. coli*, *S. aureus*, and *B. subtilis*, with *T. stocksianum* oil showing particularly potent effects, as evidenced

by its low MIC and MBC values. Based on the present findings, it can be suggested that the antibacterial activity observed for *T. stocksianum* EO may be partially explained by the presence of α- and β-pinene, which are reported to affect microbial membrane stability. Similarly, the EO of *S. yangii*, in which 1,8-cineole was identified as the principal constituent, exhibited strong inhibitory effects against *E. coli* and *S. aureus*. These findings provide scientific evidence consistent with the ethnomedicinal application of these species for managing infectious and inflammatory disorders. However, as this study was conducted in vitro, further research, particularly in vivo studies, is required to confirm the therapeutic efficacy and safety of these EOs. In addition, assessing their effects on drug-resistant microbial strains would be a valuable direction for future investigations. Overall, the results highlight the potential of these EOs as promising natural antimicrobial agents and support their possible applications in pharmaceutical and food preservation systems.

Author Contributions

1) Concept, design, data collection, analysis and writing: Z.S.; 2) Data collection: H.D.; 3) Concept and design: S.H.H.; 4) Design, literature search and writing: H.K.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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