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ORIGINAL ARTICLE

Phytochemical components and antibacterial activity of two populations of *Senecio vulgaris* L. essential oils as traditional medicine plant

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ABSTRACT

Background and Objectives: *Senecio vulgaris* L., a member of the Asteraceae family, has been widely employed in traditional Iranian herbal practices for centuries. This research seeks to analyze and compare the essential oil compositions and antibacterial characteristics of two distinct populations of *S. vulgaris.*

Materials and Methods: Essential oils were obtained from the above-ground parts of these populations through hydrodistillation, and their chemical constituents were examined using gas chromatography-mass spectrometry. The antibacterial effectiveness of the essential oils against both Gram-positive and Gram-negative bacteria was evaluated employing the agar well diffusion technique.

Results: Monoterpene hydrocarbons were found to be dominated in both populations, with Humulene epoxide II being the primary constituent, constituting 17.87% in the first population and 21.55% in the second one. The agar-well diffusion method revealed significant antibacterial effects of the *S. vulgaris* essential oils. The findings indicated that the essential oil displayed heightened activity against *Escherichia coli* in both populations. Furthermore, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests indicated that *Pseudomonas aeruginosa* with concentrations of 400 µg/mL for both tests, was the most susceptible bacteria, while *Streptococcus pyogenes* with MIC = 800 and MBC>800 µg/mL was the most resistant in both populations of *S. vulgaris.*

Conclusion: This research highlights the significance of *S. vulgaris* as a valuable reservoir of monoterpene-rich oil exhibiting robust antibacterial characteristics, suggesting its potential use in the development of novel and naturally derived therapeutics for bacterial diseases.

Keywords: Gas chromatography-mass spectrometry; Herbal medicine; Humulene epoxide; Microbial sensitivity tests; *Senecio vulgaris*

INTRODUCTION

Contemporary research has increasingly focused on exploring the phytochemical properties of medicinal plants as a key reservoir of bioactive and therapeutic agents. Essential oils (EOs) obtained from various plant parts, particularly leaves and flowers, are abundant in aromatic and volatile compounds (1, 2). These compounds have been used for centuries to treat a variety of ailments due to their antioxidant,

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antimicrobial, and antifungal properties (3, 4).

EOs find extensive application across various sectors such as food, cosmetics, and healthcare (5). They offer a promising alternative to chemical drugs with fewer side effects (6). Presently, the resistance of pathogenic bacteria to chemical drugs has increased, highlighting the need for effective antimicrobial treatments. Studies have shown that antimicrobial compounds from plants can effectively eliminate bacteria and treat infections (7, 8). The medicinal properties exhibited by numerous plants strongly suggest the presence of antimicrobial compounds within them. Consequently, the demand for utilizing plants as an alternative to synthetic antimicrobial drugs has grown (9, 10).

The genus *Senecio* L., which is part of the tribe Senecioneae, ranks as one of the largest and most intricate genera within the Asteraceae family, comprising over 1,500 species widely distributed across various regions (11). Various species within this genus have been analyzed for their secondary metabolites (12-14). Among these species, *Senecio vulgaris* L. holds significant medicinal value, demonstrated by its antiscorbutic, anthelmintic, diaphoretic, purgative, and diuretic properties (15).

This investigation aims to examine, for the inaugural time, the chemical constituents of EOs derived from two distinct populations of *S. vulgaris* located in Iran. Specifically, the chemical profiles of the oils from these populations were compared and analyzed. Furthermore, the inhibitory and bactericidal potential of the oils were evaluated by measuring the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration.

MATERIALS AND METHODS

Plant material. The newly emergent aerial components, encompassing the stems, foliage, and inflorescences, of *S. vulgaris* populations, were procured during the anthesis phase in May 2021. The botanical specimens were gathered from two geographically distinct sites within the province of Mazandaran, Iran. The initial site was situated approximately 7 kilometers from Sari en route to Kiasar and Semnan, specifically in Tangelateh-Parchi Kola village, at geographical coordinates 36°29'11.3" N and 53°06'41.8" E (designated as S1 in the text). The subsequent site was located 5 kilometers from Sari towards Qaemshahr, in Kordkheil-Geleh Kola Sofla village, at geographical coordinates 36°29'24.1"N and 53°00'33.8"E (designated as S2 in the text). The taxonomic identification of the procured specimens was conducted utilizing the Flora Iranica reference (16). Voucher specimens (No. S1: 1179; No. S2: 1180) are archived within the Herbarium of Mazandaran University of Medical Sciences, Sari, Iran. Following this, the botanical materials were subjected to air-drying in a controlled dark and arid environment prior to their application.

Preparation of EOs. The dried *S. vulgaris* plants were grounded to a fine powder with the help of an electric grinder. For every 100 g sample of the dried plant material, a 5-hour water distillation process was performed utilizing a Clevenger device. The resulting EOs was then gradually evaporated at 40°C using a rotary apparatus until it dried. Following filtration, the oil was kept in dark containers at 4°C for subsequent research.

Gas chromatography/mass spectrometry (GC–MS) analysis. The EOs were analyzed with an Agilent 6890 gas chromatograph (Waldbronn, Germany) connected to an N-5973 mass spectrometer. A BPX5 capillary column measuring 30 m × 0.25 mm × 0.25 µm, with an ionization energy of 70 eV, was employed in the analysis. The analysis commenced at 50°C, with a gradual temperature increase of 3°C/min, ultimately reaching 240°C. Next, the temperature was incrementally raised to 300°C at a pace of 15°C per minute. Both the injector and detector temperatures were held constant at 290°C, with a 1-microliter volume of sample injected. The helium carrier gas flow rate was kept constant at 0.5 milliliters per minute. The chemical constituents were ascertained by analyzing the chromatograms for every oil sample, comparing retention indices with standards, using a computer database, and consulting the pertinent literature.

Microorganisms and media. The efficacy of *S. vulgaris* EOs against eight bacterial strains acquired from the Iranian Research Organization for Science and Technology (IROST) was investigated in terms of their potential antibacterial effects. The strains under study comprised four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 29737), *S. pyogenes* (ATCC 8668), *Bacillus subtilis* (ATCC 6633), and *B. cereus* (ATCC 11778). Additionally,

four Gram-negative bacteria were included: *E. coli* (ATCC 10536), *P. aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 10031), and *Acinetobacter baumanii* (ATCC 19660). The bacterial cultures were maintained on nutrient-rich agar media and preserved at a low temperature of 4°C for subsequent examinations.

Determination of MIC. Needed MIC to inhibit bacterial growth was established using the microtiter plate assay, following the CLSI 2012 guidelines. EOs of S1, S2 and S1+S2, were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 2000 µg/mL and then diluted with sterile Mueller Hinton broth (MHB) to create various concentrations (800, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL). Sterile 96-well microplates were utilized, with each well containing 95 µL of culture medium, 5 µL of bacterial suspension (at a 0.5 McFarland dilution), and 100 µL of the EO at the desired concentration. A 1:1 (v/v) ratio of combined EOs was also tested in the experiment. Subsequently, the microplates were incubated for 24 hours at a constant temperature of 37°C.

Determination of the MBC. The MBC of the samples were determined using the MIC assay. The MBC was determined as the minimum concentration required to eradicate all bacterial cells. To establish the minimum concentration necessary for complete bacterial death, the previously described microdilution technique was used. Following 24 hours of incubation, 5 µL from each well was transferred to Mueller Hinton Agar (MHA) and incubated at 37°C for 18-24 hours. *S. pyogenes* was cultured on MHA enriched with 5% Sheep Blood, under ambient air conditions. Following incubation, the colony-forming units were counted.

Agar well diffusion method. The antibacterial properties of the EOs S1, S2, and S1+S2 were evaluated through the agar well diffusion technique, adhering to the standards set forth by the Clinical and Laboratory Standards Institute (CLSI). Semi-solid Mueller Hinton agar plates were prepared and cultured with 0.5 McFarland suspensions of the test bacteria. 100 µL of the oils were poured into cavities measuring 6 mm in diameter. Additionally, a combination of EOs in a 1:1 (v/v) ratio was used. Following this, the agar plates were subjected to a 24-hour incubation period at a controlled temperature of 37°C. The assessment of antimicrobial efficacy was conducted by measuring the inhibition zone's diameter in millimeters. Dimethyl sulfoxide (DMSO) served as a negative control, whereas gentamicin (10 µg/disk) functioned as a standard positive control in the same experimental setup.

Statistical analysis. The statistical analyses were conducted utilizing SPSS version 16.0. The dataset was subjected to a one-way analysis of variance (ANOVA), with Duncan's multiple range test (DMRT) subsequently employed for comparative assessments. A significance level of $p \leq 0.05$ was established as the threshold for determining statistical significance. Means and standard deviations were computed using Microsoft Excel.

RESULTS

Chemical composition of EOs. The above-ground portions of both *S. vulgaris* populations were diluted, resulting in approximately 2 ml of pale yellow EO with a slight odor. The examination of the EOs via GC-MS revealed a total of 38 distinct compounds in the first population (S1) and 34 distinct compounds in the second population (S2). These compounds accounted for 75.79% and 80.12% of the total compounds, in that order (Table 1).

In the first population (S1), the dominant compounds identified were Humulene epoxide II (17.87%), alpha-Humulene (10%), Limonene (7.64%), para-Cymene (6.29%), Germacrene D (6.26%), and Caryophyllene oxide (4.97%). In the second population (S2), the dominant compounds found were Humulene epoxide II (21.55%), alpha-Humulene (12.31%), para-Cymene (8.82%), alpha-Phellandrene (5.95%), Limonene (5.40%), and Caryophyllene oxide (4.47%). The primary components in the EOs from both populations were monoterpene hydrocarbons (24.56% in S1 and 29.53% in S2), followed by Sesquiterpene Hydrocarbons (24.11%), oxygenated sesquiterpenes (24.11%) and Oxygenated Monoterpenes (0.99%) in the first population (S1). In the second population (S2), the most representative constituents were oxygenated sesquiterpenes (27.67%), Sesquiterpene Hydrocarbons (21.22%), and Oxygenated Monoterpenes (1.2%) (Table 1 and Figs. 1-3).

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Table 1. The chemical composition of the EOs in two *S. vulgaris* populations.

Components	Content %		RT	RI
	S1	S2		
1-Octene	0.15	0.11	5.34	791
1-Nonene alpha-	4.09	2.04	9.28	891
Pinene Sabinene	4.18	4.10	11.37	930
beta,pinene	0.22	0.20	13.44	975
Octen-3-ol< 1->	0.19	0.16	13.70	979
Beta-Myrcene	0.32	-	14.01	979
Alpha-Phellandrene	0.28	0.22	14.27	991
Para-Cymene	0.13	5.95	15.22	1003
Limonene	6.29	8.82	16.26	1025
beta Ocimene	7.64	5.40	16.42	1029
alpha-Terpinolene	0.20	0.69	16.75	1037
Undecane <n>	0.56	1.53	19.32	1089
para-Cymenene	-	0.16	19.45	1100
Dimethyl styrene <2,5>	0.12	-	19.45	1091
Cymene-8-ol<meta>	0.19	0.15	19.80	1099
Cymene-8-ol<para>	0.81	-	24.82	1180
Carvotanacetone	-	0.86	24.83	1183
1-Tridecene	0.18	0.13	27.59	1247
Carvacrol	-	0.26	19.19	1292
Cyclosativene	-	0.21	30.15	1299
alpha-Copaene	0.11	-	32.83	1371
beta-Elemene	1.87	1.31	33.11	1377
Caryophyllene	0.56	0.40	33.70	1391
beta-Farnesene	1.27	1.02	35.07	1409
beta-farnesene<Z>	0.09	0.80	35.35	1415
alpha-Humulene	1.11	-	36.31	1443
Aromadendrene <allo->	10.00	12.31	36.64	1455
4,11-Selinadiene	0.14	-	36.81	1460
gamma-curcumene	0.35	-	37.32	1477
Germacrene D	0.26	-	37.43	1483
Beta-Selinene	6.26	3.84	37.70	1485
alpha-Muurolene	1.01	0.41	38.08	1490
delta-Cadinene	0.40	0.41	38.40	1500
1,5-epoxysalvial-4(14)-ene	0.72	0.46	39.18	1523
Caryophyllene oxide	0.25	0.35	41.35	1578
salvial-4(14)-en-1-one	4.97	4.47	41.92	1583
Vulgarone B	-	0.27	42.36	1595
1,4,7, -Cycloundecatriene, 1,5,9,9-tetramethyl-,z,z,z-	0.67	-	42.18	1651
Humulene epoxide II	1.05	-	42.59	1610
Naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	17.87	21.55	43.06	1622
Eudesm-7(11)-en-4-ol	0.29	-	43.64	1638
Cedren-13-ol<8->	-	0.82	43.5	1612
Cubenol <1,10-di-epi->	0.35	-	47.78	1750
Apiol	-	0.21	43.64	1619
2-pentadecanone, 6,10,14-trimethyl	-	0.21	45.55	1678
Total Identified	0.64	0.40	50.99	1842
	75.79	80.12		

S1: first population of S. *vulgaris*.
S2: second population of S. *vulgaris*.
RT: Retention time.
RI: determined based on retention times in relation to n-alkanes (C6-C32) on the non-polar HP-5 chromatographic column.
-: Compound not detected.

eter of the inhibition zone of the oils and the combined oils against the bacterial strains is presented in Table 2. The *E. coli* sample exhibited the largest inhibition zone, with maximum diameters of 21.1 mm, 21.4 mm, and 21.5 mm for S1, S2, and the combined oil, respectively. In contrast, the *A. baumanii* sample had the smallest inhibition zone, with minimum diameters of 5.7 mm, 7.6 mm, and 8.9 mm for S1, S2, and the combined oil, respectively.

Table 2 further illustrates the findings regarding the MIC and MBC of the EOs, both individually and in combination. *S. pyogenes* exhibited the highest MIC and MBC values, whereas *P. aeruginosa* presented the lowest MIC and MBC values.

DISCUSSION

The examination of *S. vulgaris* EO led to the identification of 38 distinct components in S1 and 34 distinct components in S2, which collectively accounted for 75.79% and 80.12% of the total constituents, correspondingly. In S1, monoterpene hydrocarbons constituted a significant percentage, followed by sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and monoterpenoid hydrocarbons. In S2, oxygenated sesquiterpenes, and monoterpenoid hydrocarbons were the predominant components.

Previous studies on the genus *Senecio* have also reported the prevalence of monoterpenes, with some

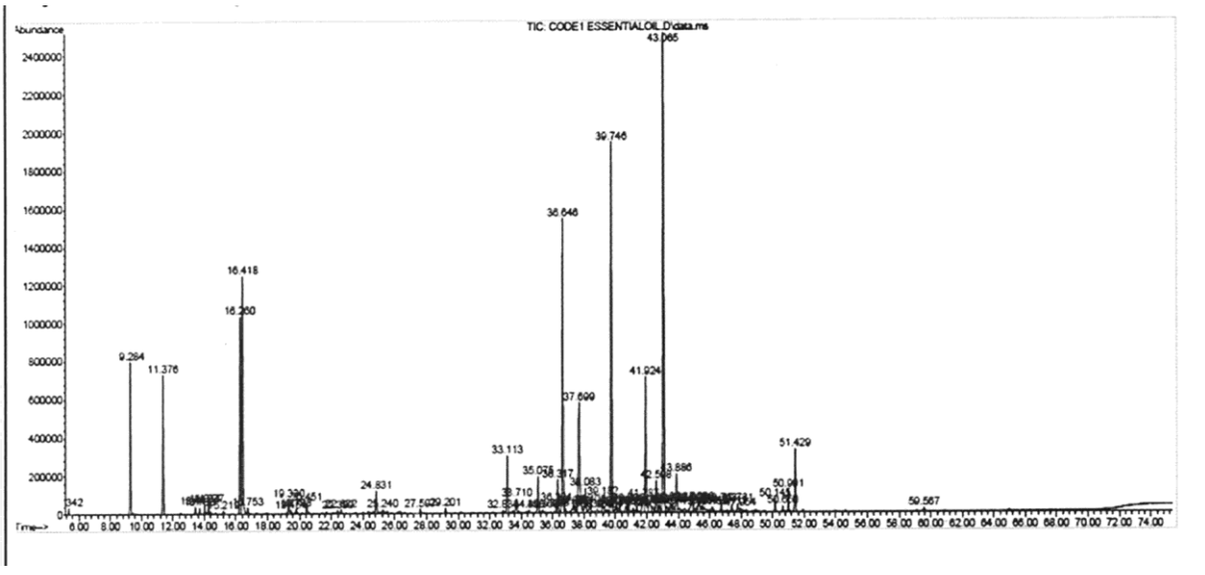

Fig. 1. GC–MS chromatogram of EO of *S. vulgaris* (first population)

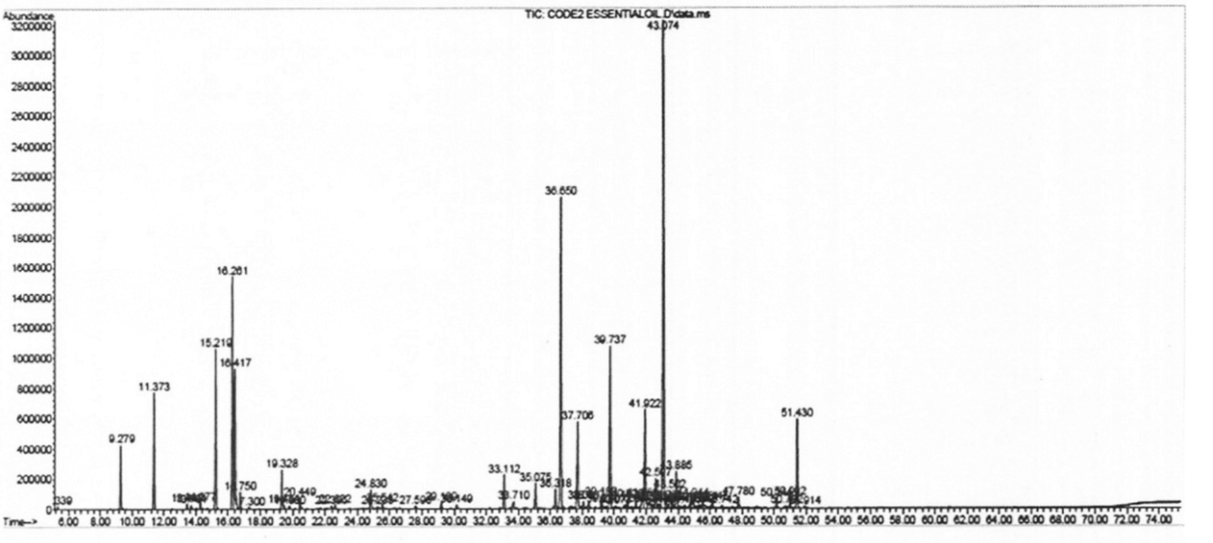

Fig. 2. GC–MS chromatogram of EO of *S. vulgaris* (second population)

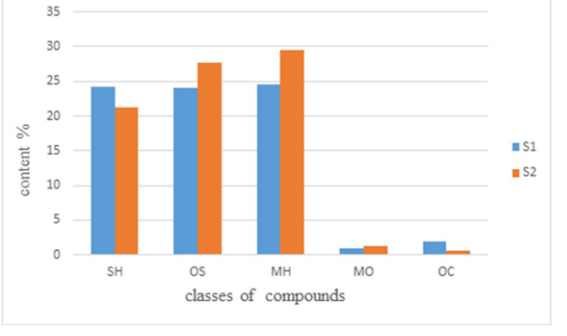

Fig. 3. Categories of natural compounds found in the EO of the first population of *S. vulgaris* (S1) and the second population of *S. vulgaris* (S2). MH: Monoterpene hydrocarbons; OM: Oxygenated Monoterpenes; SH: Sesquiterpene Hydrocarbons; SO: Oxygenated Sesquiterpenes. OC: Other Compounds.

species exhibiting dominance of oxygenated compounds (17, 18). In the present study, Humulene along with Humulene epoxide II were identified as the dominant component in both Iranian populations of *S. vulgaris.* Shareef and Hamid (19) introduced alpha-humulene as the major component in Corsican population of *S. vulgaris* in their study. In another study, the above-ground parts of *S. palmensis* were found to contain humulene-type sesquiterpene as the dominant component (20). Legault et al. showed that α-humulene leads to a reduction of intracellular glutathione levels and enhances reactive oxygen species production, which may play a role in its cytotoxic properties in *Abies balsamea* EO (21). The research investigates the essential oil composition of *S. gra-*

Table 2. Antibacterial activity of EOs of two *S. vulgaris* populations.

Microorganisms	S1			S2			Combined S1 and S2			Gentamicin
	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)	MIC (µg/mL)	MBC (µg/mL)	IZ (mm)
Staphylococcus aureus	14.3 ± 0.2^{b}	400	400	16.5 ± 0.5^{a}	400	400	16.7 ± 1.3^{a}	400	400	27 ± 1.1^{a}
Streptococcus pyogenes	17.2 ± 0.8^{a}	800	> 800	15.9 ± 1.3^{a}	800	> 800	17.4 ± 2.5^{a}	800	> 800	39 ± 0.7^{a}
Bacillus subtilis	10.8 ± 1.2^{b}	200	400	9.4 ± 2.6^{c}	400	400	10.9 ± 0.3^{b}	200	400	30 ± 0.5^{a}
Bacillus cereus	13.9 ± 0.6^{b}	50	50	17.2 ± 0.7^{a}	50	50	17.8 ± 0.8^{a}	25	50	21 ± 1.4^{b}
Escherichia coli	21.4 ± 0.2^{a}	200	200	21.1 ± 2.5^{a}	200	400	21.5 ± 1.2^{a}	200	200	23 ± 2.1^{b}
Pseudomonas aeruginosa	9.8 ± 2.3^{c}	25	25	11.9 ± 0.2^{b}	12.5	25	15.4 ± 1.6^{a}	12.5	12.5	20 ± 0.4^{b}
Klebsiella pneumoniae	18.7 ± 1.4^{a}	100	200	14.3 ± 3.2^{b}	200	200	19.1 ± 05^{a}	100	100	17 ± 0.8^{c}
Acinetobacter baumanii	5.7 ± 0.9^{c}	50	100	7.6 ± 0.4^{c}	100	100	8.9 ± 0.9^{b}	50	50	18 ± 0.4^{c}

IZ: Inhibition zone (mean ± SD).
MIC: diameter, the minimum inhibitory concentration
MBC: the minimum bactericidal concentration
S1: the EO of the first population of *S. vulgaris*
S2: the EO of the second population of *S. vulgaris*

ciliflorus, identifying 17 to 20 constituents across its flower, leaf, stem, and root parts, with monoterpene hydrocarbons being predominant (22).

Oladipupo and colleagues introduced alpha-pinene along with other compounds such as beta-pinene, p-cymene, beta-selinene, and others as the major compounds of *S. polyanthemoides* (23). In another study, p-cymene along with other Sesquiterpenes were identified as a dominant compound in Egyptian population of *S. glaucus* (24). According to Singh et al. (17), the volatile oil derived from the roots of *S. amplexicaulis* exhibited α-phellandrene as a predominant component (17). γ-selinene was recognized as a predominant compound in the EO derived from *S. anteuphorbium*, as documented by Elhidar et al. (25). Singh and colleagues (17), in their study demonstrated that monoterpene hydrocarbons were the primary constituents in *S. amplexicaulis.* Germacrene D, a sesquiterpenoid compound, was also found in the EOs of both studied populations of *S. vulgaris* in the current study. The predominant constituent identified within the EO of *S. rufinervis* by Mishra et al., was germacrene D, serving as the principal compound, succeeded by β-pinene, β-caryophyllene, and β-longipinene (26). In the present study, caryophyllene oxide was found to be a predominant component in the aerial parts of both populations of *S. vulgaris* analyzed. In the study conducted by Sánchez-Muñoz et al., the n-hexane extract derived from the aerial components of *S. salignus* specimens resulted in the isolation of the compounds β-caryophyllene and caryophyllene oxide (27). Joshi reported that the EO of *S. belgaumensis* contains 10.4% of the compound caryophyllene oxide (28). It has been established that caryophyllene oxide manifests cytotoxic properties in a manner that is both time- and dose-dependent (29). Furthermore, non-cytotoxic levels of β-caryophyllene have been demonstrated to amplify the growth inhibition effects of α-humulene, isocaryophyllene, and paclitaxel on tumor cell lines (30).

Variations in the chemical composition of EOs among the two *S. vulgaris* populations can be ascribed to a range of factors. A plethora of studies has suggested that this variability can be linked to diverse factors, encompassing the extraction technique, intrinsic and extrinsic variables, species and provenance, duration of drying, utilized plant parts, age of the plant, and genetic determinants. Furthermore, the variability in chemical composition is modulated by environmental conditions, the nutritional profile of the plants, geographic location, and the season of collection. Additionally, the method of EO extraction from distinct plant parts can further amplify the chemical diversity inherent within the species. These variations are essential in influencing the therapeutic properties and uses of EOs across several sectors, including pharmaceuticals, cosmetics, and food (31-33).

The antibacterial efficacy of EOs obtained from *S.*

vulgaris was evaluated against a spectrum of bacterial strains. The results indicated pronounced antibacterial activity against all bacterial strains subjected to testing. In terms of inhibition zones, the EO from *S. vulgaris* exhibited greater activity against *E. coli* in both populations, followed by *K. pneumoniae, S. pyogenes, S. aureus, B. cereus, B. subtilis, P. aeruginosa*, and *A. baumanii* in the first population, and *B. cereus, S. aureus, S. pyogenes, K. pneumoniae, P. aeruginosa, B. subtilis*, and *A. baumanii* in the second population. The combination of EOs from the two populations of *S. vulgaris* resulted in an increase in the diameter of the inhibition zone against various bacteria, indicating enhanced antibacterial properties in this mixture. This suggests that the chemical compounds present in the EOs can work synergistically to provide greater efficacy than using a single EO alone. The MIC and MBC tests revealed that in both populations of *S. vulgaris*, *P. aeruginosa* was the most sensitive while *S. pyogenes* was the most resistant. The gram-negative bacterium *P. aeruginosa* is a common and highly drug-resistant human pathogen (34).

However, it has been identified that this bacterium is susceptible to the EOs derived from *S. vulgaris*, thereby indicating a potential therapeutic avenue for infections instigated by this pathogen. Researchers have scrutinized the antibacterial characteristics of various taxa within the *Senecio* genus. For instance, the oil derived from *S. nutans* demonstrated a significant antibacterial efficacy, evidenced by an inhibition zone diameter of 22 mm and the MIC value of 0.4 mg/mL against the pathogenic bacterium *Vibrio cholerae* (35). In a distinct investigation, Loizzo et al. assessed the antibacterial and antifungal properties of *S. inaequidens* and *S. vulgaris.* The *S. vulgaris* extract, derived via a methanol extraction process, exhibited effectiveness in inhibiting the proliferation of the Gram-positive bacteria *B. subtilis* and *S. aureus*, whereas the extract from *S. inaequidens* did not display antimicrobial properties against these species. It is noteworthy that the extracts from both *Senecio* species had no discernible effect on Gram-negative bacteria (11).

El-Hamd et al. (36) reported that the aerial components of *S. aegyptius* var. *discoideus* yielded six novel eremophilane derivatives, specifically identified as 244 and 246–250. The antibacterial efficacy of these novel compounds was assessed against *B. cereus* and *Serratia* sp. Most of the synthesized compounds exhibited inhibitory effects on the growth of both microorganisms. Specifically, the compounds 1b-Hydroxy-8aH-eremophil-7(11), 9-dien-12, 8-olide and 1b,10b-Epoxy-8a-methoxyeremophil-7(11)-en-12,8-olide inhibited the growth of *B. cereus*, while having a negligible impact on the proliferation of *Serratia* sp. (36). In an additional study, *S. cannabifolius* demonstrated antibacterial activity against Gram-positive bacteria *B. subtilis* and *S. aureus*, but was ineffective against *E. coli* (12). Uçüncü et al. illustrated that the EOs extracted from *S. othonnae* and *S. nemorensis* exhibited antimicrobial properties against *B. cereus*, *S. aureus*, *Enterococcus faecalis*, and *Candida tropicalis*; however, the oil sourced from *S. racemosus* displayed activity exclusively against *Candida tropicalis* (37). In their investigation of *S. glaucus*, Zaher and his research team uncovered that the isolated benzofuran glucoside and flavonoid glycosides manifested moderate antimicrobial effects and minimal cytotoxicity against Panc-1 cancer cells (14).

As mentioned earlier, the antibacterial properties of EOs derived from two *S. vulgaris* population are primarily linked to their chemical makeup, particularly the presence of, monoterpene hydrocarbons in significant quantities. Determining the antibacterial properties of a specific compound can be challenging, as EOs from various populations may have distinct mixtures of active components. Additionally, the intricate composition of these EOs complicates the explanation of the action mechanisms of these mixtures. Considering the high levels of the two compounds, Humulene and Humulene epoxide II, found in the plants studied and the reported antimicrobial properties of these compounds (38, 39), it is likely that these two compounds play the most significant role in the antibacterial results obtained in this study.

CONCLUSION

The chemical profiling of the EO from the aboveground portions of two populations of *S. vulgaris* has revealed the presence of numerous biologically significant phytochemicals. Additionally, the major marker compound in the EO of both populations of *S. vulgaris* is Humulene epoxide II.

The EOs derived from the two *S. vulgaris* populations demonstrate considerable antibacterial efficacy and have shown potency against a range of bacterial species. Specifically, studies have shown that these

EOs are highly effective against *E. coli*. Furthermore, the effectiveness of the EOs increases when it is combined with each other. Consequently, this study indicates that *S. vulgaris* may be considered a natural source of monoterpene-rich oil with strong antibacterial properties. The findings from this study have the potential to motivate researchers to explore and discover affordable, natural sources of antimicrobial agents.

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