The Journal of Medicinal Plants and By-products

Original Article

Antimicrobial Properties and Essential Oil Profile of *Ziziphora clinopodioides* from Three Regions of Lorestan Province

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Article History: Received 12 May 2025/Accepted in revised form 24 December 2025

ABSTRACT
This study was conducted to identify one of the endemic medicinal plants and its use in medical science. With this aim, the quantity and quality of essential compounds in the *Ziziphora clinopodioides* plant under various environmental conditions and its antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method were investigated in three regions of Lorestan Province. These regions including region 1 (Borujerd), region 2 (Khorramabad) and region 3 (Aleshtar). 25, 27 and 21 compounds were identified in regions 1, 2, 3 respectively. The main compounds in regions 2 (Khorramabad) and 3 (Aleshtar) were Pulegone, Isomenthone and P-mentha- 3,8 -diene. The main constituents of the essential oil extracted from samples collected in region 1 (Borujerd) differed significantly from those obtained from the other two regions. Thymol, E- Caryophyllene, Carvacrol and γ-Terpinene were the main components of region 1. Significant negative correlation between soil organic carbon percentage and β-myrcene composition. The findings indicated that the essential oil of *Z. clinopodioides* collected from region 1 exhibited notable antimicrobial properties against both *S. aureus* and *E. coli* compared to regions 2 (Khorramabad) and 3 (Aleshtar). The different compositions of essential oil of plant collected from region 1 from regions 2 and 3 can be attributed to the distinct climatic and geographical conditions of region 1. the results of this research showed that the difference in the geographical conditions of the growing region of the *Z. clinopodioides* plants studied in this research has caused a significant difference in the components of the essential oil compounds, their amount and their antimicrobial effects, which can be used to improve the essential oil compounds in the pharmaceutical, health and food industries. It is suggested to investigate the effect of the main components of the essential oil separately for a better understanding of the mechanism of antimicrobial activity.
Keywords: Photochemistry, Medicinal plants, Antibacterial activity, *Ziziphora clinopodioides*, Lorestan Province, Environmental change

INTRODUCTION
The genus Ziziphora, commonly known as Kakuti in Iran, includes four species and several subspecies, including Mountain Kakuti (*Ziziphora clinopodioides*), Annual Kakuti (*Ziziphora tenuior*), Iranian Kakuti (*Ziziphora persica*), and Sarsan Kakuti (*Ziziphora capitata*), which are widely distributed in different regions of Iran [1-4]. Among these, *Z. clinopodioides* is particularly renowned for its aromatic properties and has significant value in traditional Iranian medicine, where it is utilized to treat digestive disorders [5]. Various species of Ziziphora are utilized for their analgesic properties, as well as in the treatment of digestive problem, heart diseases, depression, diarrhea, coughs, migraines, and fevers [6, 7]. Research indicates that the *Z. clinopodioides* plant is particularly rich in polyphenolic compounds, flavonoids, and free amino acids; notably, its flavonoid components have demonstrated significant antioxidant, anti-inflammatory, and anti-cancer activities [8].

A significant collection of effective antifungal and antibacterial agents is currently used to destroy infectious agents. However, the genetic variability observed in microbial pathogens, alongside the emergence of resistant strains and the potential side effects associated with conventional drugs, underscores the urgent need to find alternatives. This has prompted increasing interest in replacing synthetic antimicrobials with plant-derived compounds, which are believed to have fewer side effects [9]. Numerous studies have investigated the antimicrobial and antifungal properties of essential oils derived from various plants against a wide range of pathogenic microorganisms affecting both plants and animals [10]. Notably, Gram-positive bacteria such as *Staphylococcus aureus* remain a leading cause of hospital-acquired infections [11] and are highly problematic due to their ability to develop resistance to all clinically available antibiotics through mutation in chromosomal genes [12]. Essential oils have been of interest due to their antimicrobial and antifungal properties against various pathogenic micro-organisms and fungi [10]. Among these pathogens, the Gram-positive Bacterium *Staphylococcus aureus* is a considerable threat, which is one of the main causes of hospital-acquired infections [11] The proliferation of antibiotic-resistant *S. aureus* significantly reduces effective treatment options and affects public health on a global scale [13]. Alarmingly, the World Health Organization estimates that antibiotic resistance contributes to approximately 70,000 deaths per year [14], highlighting microbial resistance as one of the most critical challenges to modern healthcare [15]. Additionally, *Escherichia coli* has a profound effect on public health in terms of morbidity and mortality, with associated economic costs reaching several billion dollars worldwide each year [16]. The review of research conducted on *Z. clinopodioides* essential oil in different regions reveals significant variation in its main essential oil compositions, highlighting the substantial effect of habitat conditions on both the quantity and quality of the essential oil produced by this species [17].

Furthermore, analysis of variance has demonstrated that growth location significantly affects the concentration of Pulegone at the 5% level, while also resulting in notable differences in levels of α-Pinene and Sabinene at a probability level of 1% [18]. A multi-year study of *Z. clinopodioides*, it was observed that increasing altitude in the Sarayan and Birjand regions correlated with a higher percentage of Pulegone, particularly in areas with the highest altitude above sea level [18]. Pulegone not only acts as a flavoring agent in foods, beverages, and toothpaste but also plays an important role in the synthesis of antibacterial compounds in the pharmaceutical industry [19]. Experiments evaluating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic extracts from *Z. clinopodioides* showed that the extract effectively inhibited the growth of all investigated urinary tract bacteria in an experimental rat model, except for *Pseudomonas aeruginosa*. Notably, the MBC for most strains aligned closely with their respective MIC values, indicating a potent bactericidal effect [20].

The effective utilization of medicinal plants depends on a comprehensive understanding of the endemic species and the environmental factors that influence the quantity and quality of essential oils for enhancing secondary metabolites. Also, the development of resistance in bacterial agents, fungi, viruses, and parasites indicates significant challenges to modern medicine, highlighting an urgent need to discover new therapeutic sources. Therefore, this study was conducted to characterize the essential oil composition and assess the antimicrobial efficacy of the valuable medicinal plant *Z. clinopodioides* collected from three distinct regions of Lorestan Province.

MATERIALS AND METHODS

In July 2023, a study was conducted to identify the native populations of *Ziziphora clinopodioides* and to investigate the profile of essential oil compounds as well as their antimicrobial properties in different regions. Three distinct habitats were selected from Lorestan Province, Khorramabad, Aleshatar, and Borujerd (Figure 1)-and sampling was conducted during the full flowering period. Subsequently, samples were identified by an expert at the Agricultural and Natural Resource Research Center in Lorestan Province. The geographical characteristics, climatic conditions, and soil properties of the studied areas are detailed in Table 1. To quantify the essential oil content aerial parts of the plants were transported to the laboratory immediately following harvest and dried at room temperature for 10 days. After drying these samples were finely crushed and ground. The ground material was weighed and placed in to a distillation flask for essential oil extraction via steam distillation using Clevenger apparatus. The resulting essential oil was subsequently measured in both weight and volume. The percentage of essential oil content was calculated using the following equation:

Percentage of Essential Oil= Weight of Essential Oil/ Weight of Plant Material [21]

To identify essential oil compounds, it was carried out in the central laboratory of Lorestan University using a gas chromatography (GC) and a gas chromatography coupled to a mass spectrometer (GC/MS) manufactured by Shimadzu, Japan. For GC-MS analysis, a SCION SQ W/436, SSL-T21 model, gas chromatograph coupled to a SCION-MS, single Quadrupole-MS mass spectrometer, EI source, was used. Compounds were separated on a 15 m × 0.25 mm i.d. fused-silica capillary column coated with 0.25 µm film of DB-5ms (agilent) and a split/splitless injector with a 1 mm internal diameter glass liner. Ultra-pure helium was used as carrier gas with ionization voltage of 70 eV. Injector and interface temperatures were 280◦C and 200ºC, respectively. Mass ranged from 40 to 600 amu. The program of oven temperature was the same as mentioned above for the GC. After the essential oil was injected into the above devices by comparing the components with the standard compounds using the inhibition time of the compounds and the inhibition index and comparing with the internal reference mass spectra library (NIST11), the essential oil compounds were identified.

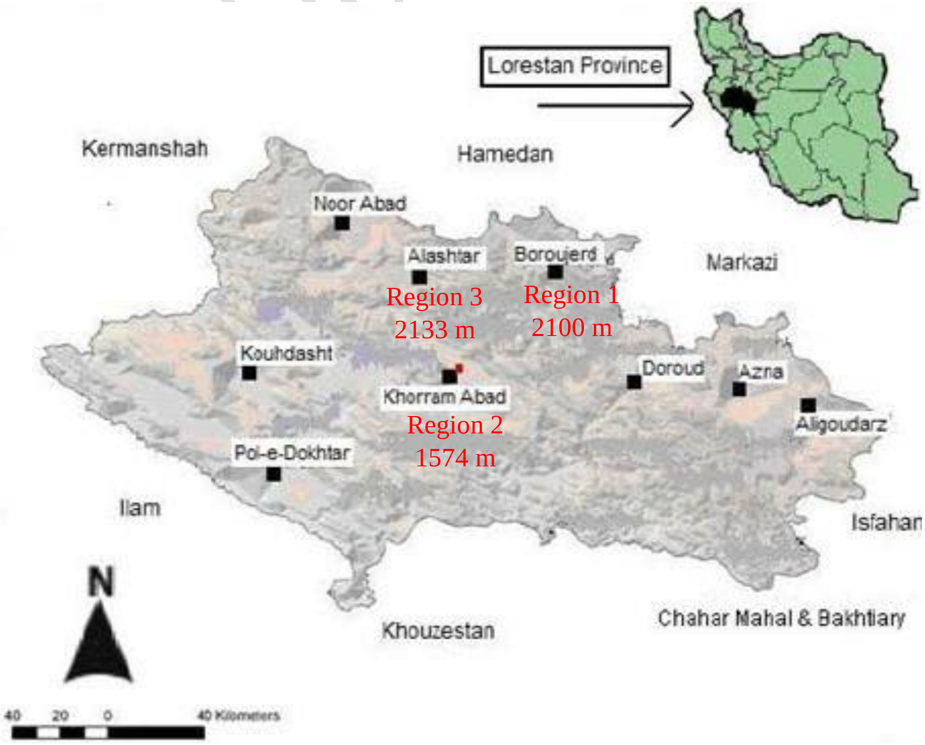

Fig. 1 Geographical distribution of studied *Ziziphora clinopodioides f*rom different regions of Lorestan province

Table 1 Geographic and soil characters of *Ziziphora clinopodioides* sample collection regions

Regions of sample collection	Code	The longitude and latitude of the region	altitude above sea level (m)	organic carbon percentage Co%	P (mg/kg)	K (mg/kg)
Razan, Borujerd	Region 1	48º 50′ 33″ 33º 33′ 3″	2100	1.13	356	8.3
Lands near Evander village, Khorramabad	Region 2	48º 11′ 16″ 33º 44′ 34″	1574	1.98	470	7.7
Garrin Mountain, Bardbal village, Aleshtar	Region 3	48º 16′ 55″ 33º 45′ 28″	2133	2.1	560	7.2

Investigating the Antimicrobial Effect of Essential Oil by Disk Diffusion Method

Colonies of standard strains of *Escherichia coli* and *Staphylococcus aureus* according to the 1/2 McFarland standard were prepared on Mueller-Hinton agar medium and cultivated by the lawn culture method. Then, 30 µl of essential oil inoculated into a blank disk. 30 µl DMSO was also inoculated into a blank disk and used as a control. After one night of incubation at 37 ºC, the effects of essential oils were examined to observe the formation or absence of growth inhibition [22].

Statical Analysis

All the experiments were performed in triplicate. Duncan's tests for antimicrobial activity at 5% using MINITAB Version 17 tool and SPSS program. Means ±se and $P < 0.05$ show a statistically significant difference. Past software was used to generate correlation and clustering heat maps.

RESULTS

The essential oil composition of *Z. clinopodioides* from region 2 in Lorestan province was characterized by the identification of 27 compounds. Notably Pulegone (36.45%), Isomenthone (11.06%), P-mentha 3, 8-diene (8.68%), 1,8- Cineole (7.39%), Menthol (5.93), Piperitone (5.18%), γ-Terpineol (3.84), β-Pinene (3.27) and Menthone (3.09) were the dominant compounds which together constituted 95.41% of the total essential oil. Among the 21 compounds identified in the essential oil of *Z. clinopodioides* collected from region 3, the main constituents were Pulegone (32.91%), Menthone (11.45%), Isomenthone (10.26%), DL-menthol (9.96%), Thymol (8.01%), 1, 8-Cineole (4.45%), P-Mentha-3,8-diene (3.75%), Menthol (3.35%), β-Pinene (3.37%), and Piperitone (3.06%). Together, these 21 compounds constituted 96.78% of the total essential oil composition (Table 2).

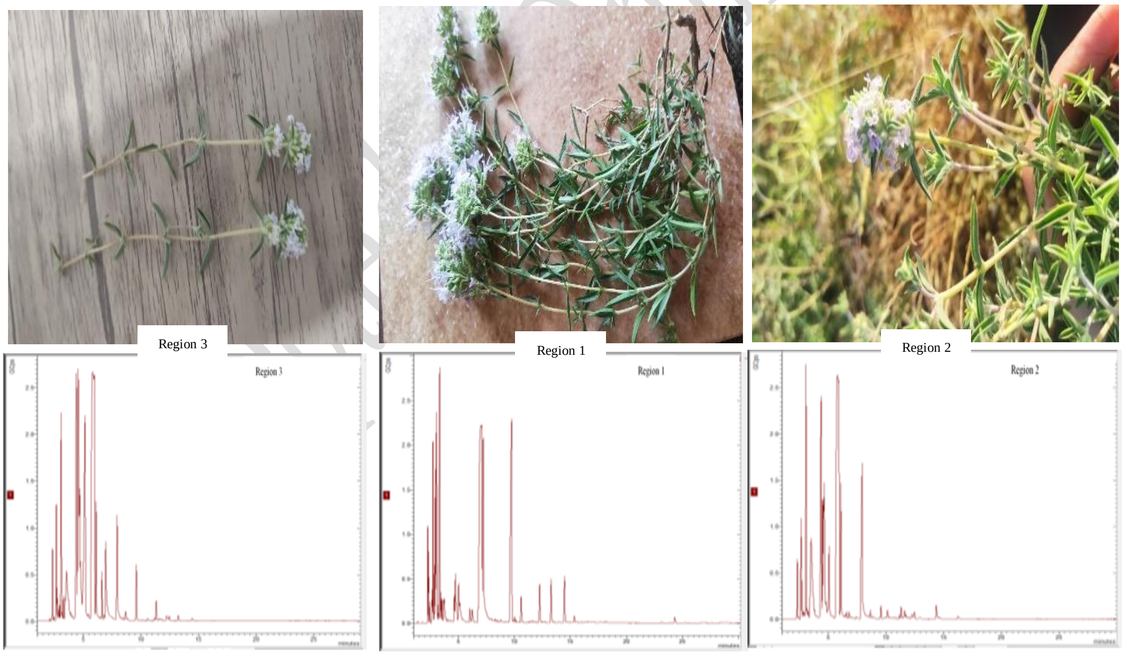

Fig. 2 GC/MS chromatogram of *Z. clinopodioides* from different regions of Lorestan Province

Ninety common essential oil constituents were identified in the populations from both region 2 and region 3. Notably, the concentrations of Pulegone, Isomenthone, P-mentha-3,8-diene, 1,8- Cineole, α-Pinene, β- Myrcene, Menthol, (Z)-β-Ocimene, Geranyl formate and Piperitone were higher in the *Z. clinopodioides* samples collected from region 2 than in those obtained from *Z. clinopodioides* samples in region 3. In contrast, the DL-menthol, E-Caryophyllene, Menthone and γ-Terpinene compound exhibited a different pattern with significantly higher concentration observed in the essential oil extracted from region 3 population. A total of 25 compounds were identified in the *Z. clinopodioides* samples collected from region 1, among which the main constituents included Thymol (27.52), E-Caryophyllene (15.38), Carvacrol (10.12), γ-Terpinene (9.25), p-Cymene (8.45), α-Terpinene (3.46), and β-myrcene (4.12). Collectively, 25 compounds constituted 97.79% of the total essential oil composition. Comparative analysis revealed that this population shared only seven common essential oil compounds with the other two populations.

Region 1 (Rozen, Borujerd) was characterized by high altitude (2100 m) and relatively lower organic carbon compared to the other two regions. It's levels phosphorus and potassium were low to moderate. While the soil in this region appears to be relatively exploitable but requires more organic carbon management, and the essential oils of plants collected in this region had the lowest amounts. Region 2 (lands near Evander village, Khorramabad) has medium altitude and relatively high organic carbon (1.98%); plants collected in this region had the highest percentage of essential oil (0.86%) and phosphorus (P) were also higher than in region 1, perhaps indicating more fertile soil. Soil from region 3 (Garrin Mountain, Bardbal village, Aleshtar) had the highest altitude, organic carbon and phosphorus content. The lowest potassium content was detected in this region. Plants collected from this region had relatively low essential oil content (0.5). The percentage of organic carbon (%Co) was higher in region 2 and 3 than in region 1, suggesting greater soil organic stability and potentially improved water and nutrient retention capacities in the former two regions. Phosphorus levels were relatively high in all regions; however, the highest phosphorus content is observed in region 3. The amount of potassium is relatively low in all three regions (between 7 and 8 mg/kg). Essential oil percentage is significantly higher in region 2.

Heat map analysis of essential oil components (Fig. 3) indicated that *Z. clinopodioides* collected from region 2 and 3 were clustered together, this result was expected given the closer distance between the two areas and the commonality of 19 compounds. The analysis revealed a significant negative correlation between soil organic carbon percentage and β-myrcene content ($r = -0.999^{*}$), while correlations between altitude, soil phosphorus, potassium, organic carbon percentages, and essential oil content-as well as other common essential oil compounds-across the three regions were found to be non-significant. Significant negative correlation was observed between Pulegone with γ-Terpinene ($r = -0.997^{*}$) and E-Caryophyllene ($r = -0.998^{*}$) but 1, 8- Cineole had positive and significant correlation with essential oil percentage ($r = 0.998^{*}$) (Fig. 5). The antimicrobial activity of essential oil was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli* using the disk diffusion method, with the diameter of the inhibition zone was measured to determine antibacterial efficacy. The findings indicated that the essential oil derived from *Z. clinopodioides* collected in region 1 exhibited the most significant anti-microbial activity, with an inhibition zone diameter 23 mm against both *S. aureus* and *E. coli*. This result showed that the pure essential oil from *Z. clinopodioides* in region 1 demonstrated superior performance in inhibiting these two bacterial strains compared to oils from regions 2 and 3, which showed a reduced inhibition zone diameter only 10 mm.

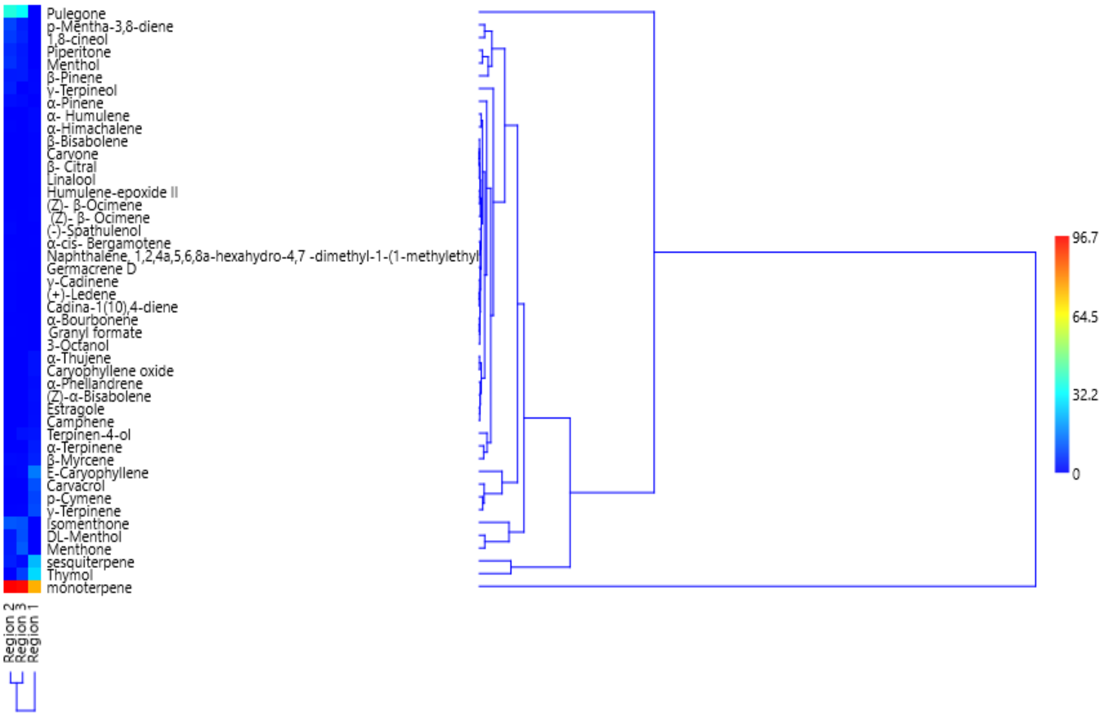

Fig. 3 The heat map analysis dendrogram of hierarchical cluster analysis for essential oil constituent's percentage of *Z. clinopodioides* from different regions of Lorestan Province

Table 2 Comparison of percentage of essential oil components of *Ziziphora clinopodioides* sample collection of three regions

Essential oil components	RI	Region 2 (%)	Region 3 (%)	Essential oil components	RI	Region 1(%)
α-Pinene	932	1/62	1/13	α-Thujene	924	2/11
Camphene	946	0/03		α-Pinene	932	1/58
β-Pinene	974	3/27	3/37	Camphene	946	0/50
β-Myrcene	988	1/03	0/80	β-Pinene	974	0/73
3-Octanol	998	0/23	0/23	β-Myrcene	988	4/12
α-Terpinene	1014	0/2	0/23	α-Phellandrene	1002	1/32
1,8- Cineole	1026	7/39	4/45	α-Terpinene	1014	3/46
(Z)- β- Ocimene	1032	0/26	0/11	p-Cymene	1020	8/45
γ-Terpinene	1054	0/28	0/4	(Z)- β-Ocimene	1034	0/39
p-Mentha-3,8-diene	1068	8/68	3/75	γ-Terpinene	1054	9/25
Menthone	1148	3/09	11/45	Linalool	1095	0/47
Isomenthone	1158	11/06	10/26	Terpinen-4-ol	1174	2/49
Menthol	1167	5/ 93	3/35	L-α-Terpineol	1192	0/87
Terpinen-4-ol	1174	-	1/92	Estragole	1196	1/48
DL-Menthol	1185	3/02	9/96	β- Citral	1238	0/54
γ-Terpineol	1199	3/84	-	Carvone	1239	0/54
Pulegone	1233	36/45	32/91	Thymol	1289	27/52
Piperitone	1249	5/18	3/06	Carvacrol	1298	10/12
Thymol	1289	-	8/01	α-cis- Bergamotene	1411	0/14
Geranyl formate	1298	0/31	0/13	(E)-Caryophyllene	1415	15/38
α-Bourbonene	1376	0/24	0/14	α- Humulene	1451	0/94
(E)-Caryophyllene	1417	0/47	0/87	β-Bisabolene	1505	1/51
α-Himachalene	1449	0/47	-	(Z)-α-Bisabolene	1511	1/73
1,2,4a,5,6,8a-hexahydro-4,7 -dimethyl-1-(1-methylethyl)- Naphthalene	1465	0/14	-	Caryophyllene oxide	1582	2/04
Germacrene D	1484	0/49	0/25	Humulene-epoxide II	1608	0/28
Cadina-1(10),4-diene1, 4-cadinadien-8a-ol	1486	0/38	-			
(+)-Ledene	1489	0/37	-			
γ-Cadinene	1513	0/29	-			
(-)-Spathulenol	1577	0/67	-			
Total		95/41	96/78			97/79
Essential oil percentage	0/86	0/5			0/091	

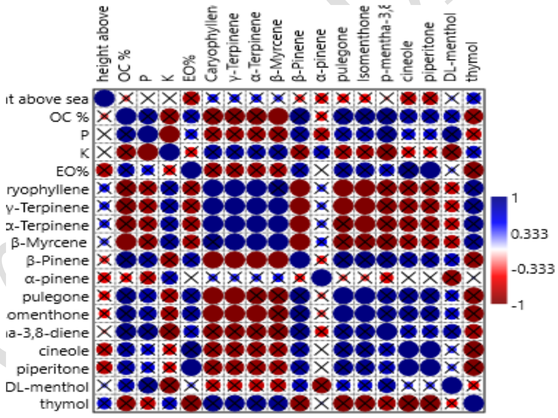

Fig. 4 The heat map analysis of correlation between main essential oils components and geographic and soil characters of *Z. clinopodioides* from different regions of Lorestan Province (p≥0.05 crossed)

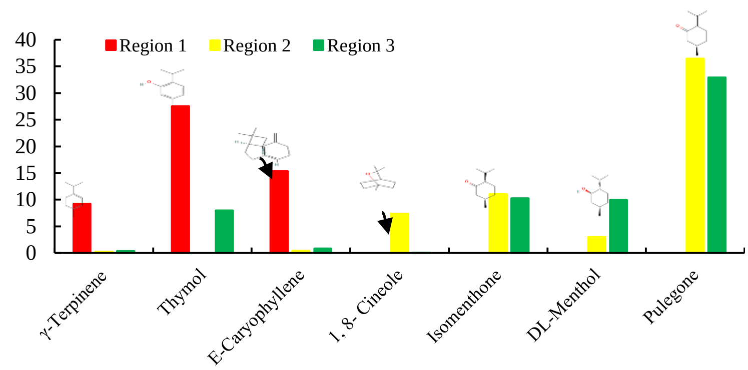

Fig. 5 Comparison of the amount of main chemical compounds of various *Z. clinopodioides* from different regions of Lorestan Province

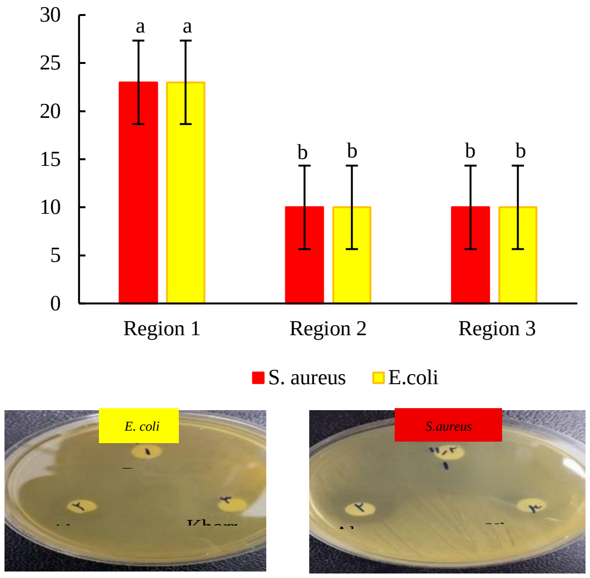

Fig. 6 Comparison of zone inhibition diameter of essential oils of *Z. clinopodioides* from different regions of Lorestan Province tested against two human pathogenic bacteria strains

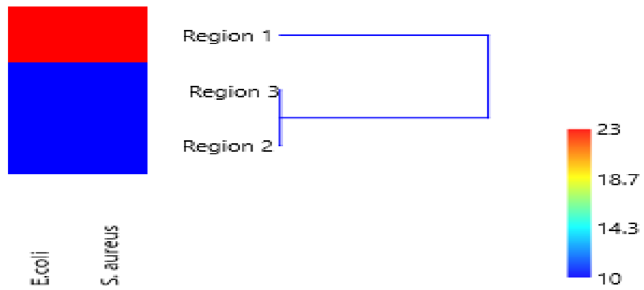

Fig. 7 The heat map analysis of the zone inhibition diameter of essential oils of *Z. clinopodioides* from different regions of Lorestan Province tested against two human pathogenic bacteria strains

DISCUSSION

This variation of essential oil components can be attributed to the distinct climatic and geographical conditions characteristic of each region. Specifically, the essential oil yield for *Z. clinopodioides* from region 2 was recorded at 0.86%, whereas the yield from region 2 was 0.5%. In contrast, samples collected from region 1 yielded a significantly lower percentage of essential oil at 0.091%. Notably, the main essential oil constituents of plants from region 1 differed considerably from those identified in the other two samples. Cluster analysis of the essential oil components placed plants from regions 2 and 3 in the same group, a result that was expected given the closer geographical proximity and the fact that they shared 19 common compounds. Plants collected from region 2, which were characterized by moderate levels of phosphorus, potassium, and organic carbon, as well as lower altitude, exhibited the highest essential oil yield compared to the other two regions. The essential oil of *Ziziphora clinopodioides* located in five natural habitats of Yazd province showed significant and negative relationship with EC and pH [23]. In the previous studies, Pulegone has been identified as the major component of *Z. clinopodioides* and *Ziziphora tenuior* essential oil, with reported concentration as high as 33.1%, 51.4% and 77.25% respectively [24, 25, 26, 27] whereas the major component of essential oil of *Z. clinopodioides* collected from mountain of Shirkooh on Yazd province was carvacrol [28]. This contrast with the profile of plants collected from Kermanshah region in 2018 where the main compounds were Geraniol (20.62%), Crocorol (18.17%), α-Terpineol (7.49%), γ-Terpineol (6.83%), Borneol (3.67%), and γ-Terpinene (3.53%). Further highlighting this phytochemical diversity, the essential oil of *Z. clinopodioides*, collected from Gilan-e Gharb in the western region of Kermanshah in 2015, was mainly characterized by high concentrations of Carvacrol (65.22%), Thymol (19.51%), P-cymene (4.86%), and γ-Terpinene (4.63%) [29]. This diversity in phytochemical profiles underscores the significant influence of geographical and environmental factors across different cultivation areas.

The increase in the concentrations of Carvacrol and Thymol in the essential oil from region 1 is consistent with previous research identifying these compounds as the main constituents of Z. *clinopodioides* essential oil [29-31]. This consistency underscores their biochemical significance within the species. Contrasting with the findings of Alipour *et al*. (2024), which reported that Pulegone levels increased with altitude in the perennial *Z. clinopodioides*, our study observed a non-significant decrease in Pulegone concentration with increasing altitude. Altitude also exhibited a positive, though non-significant effect on the concentrations of Thymol, Menthol, β-Myrcene, E- Caryophyllene, α-Terpinene, and γ-Terpinene compounds. Conversely, the levels of Isomenthone, 1,8-Cineole, and Piperitone showed a non-significant decrease with altitude. Similarly, the essential oil yield demonstrated a negative and non-significant correlation with altitude ($r = -0.818^{ns}$). This pattern aligns with a negative and significant correlation reported between essential oil percentage of and altitude in the *Artemisia aucheri* [32]. This discrepancy may suggest further investigation into the environmental factors that influence secondary metabolites profile at different altitudes. The variations in the chemical compositions of *Z. clinopodioides* observed in different studies may be attributed to several factors including plant age and growth phase, land slope, altitude, geographical and climatic conditions, harvesting time, and the extraction solvent used [33]. The environmental conditions of medicinal plant's habitat fundamentally affect its development in three key aspects: 1) the total quality of bioactive compounds, 2) the specific chemical constituents synthesized, and 3) the total dry biomass produced [34]. Notably, the observed variations among *Z. clinopodioides* populations emphasize the need for further research for plant breeding. The species has demonstrated considerable adaptability to agricultural conditions and exhibit remarkable yield in cultivation.

Furthermore, research has shown that the Pulegone compound has significant pesticide properties, which enhance its global market value [35, 36]. Due to the high concentrations of this compound in the essential oils from regions 2 and 3, more investigation is necessary to discover its potential applications in the developing biological and organic pesticides, as well as in health and medicinal products derived from these essential oils. The changes in the main essential oil constituents of *Z. clinopodioides* plant in regions 2 and 3 compared to region 1 may cause significant differences in their antimicrobial properties. Soltani Nejad *et al*. (2010) used the disk diffusion method to investigate the antibacterial effects of *Z. clinopodioides* essential oil, and indicted that this essential oil exhibited no inhibitory activity against *Pseudomonas aeruginosa* at the studied concentrations, while it showed significant antibacterial effect on *Listeria monocytogenes*, with an inhibition zone diameter 32 mm [37]. In line with these findings, also it is reported significant inhibitory and bactericidal effects for both the ethanolic extract and essential oil of *Z. clinopodioides* and against Gram-negative bacterial strains, as well as notable antibacterial activity against Gram-positive strains. However, it is important to note that neither preparation exhibited any growth inhibition or bactericidal activity against *Pseudomonas aeruginosa* [20].

In research on the biological activities of *Z. clinopodioides* collected from Karabagh and Armenia, it was determined that the essential oil of this plant has significant antimicrobial effects. These effects can be attributed to its complex chemical composition, which disrupts bacterial cell membranes by interfering with metabolic processes; additionally, its hydrophobic nature and environmental factors influence both quality and quantity of its bioactive compounds [38]. Collectively, the essential oil of *Z. clinopodioides* has demonstrated acceptable anti-microbial effect against important foodborne and human pathogenic bacteria [39, 40]. In summary, Ziziphora species exhibit anti-microbial, antioxidant, and immunomodulatory activities. respect their diverse biological activities and rich phytochemical profiles, they represent promising candidates for further pharmacological research [41].

Mazarei (2023) reported that the essential oil composition of *Z. clinopodioides* is predominantly composed of oxygenated monoterpenoids, such as Pulegone and Menthane-type structures, as well as aromatic monoterpenoids like Carvacrol and Thymol [42]. This high concentration of monoterpenoid confers significant anti-bacterial, anti-fungal, and insecticidal properties to the essential oil. Several studies have demonstrated that different bacterial strains exhibit varying sensitivities to the essential oil of *Ziziphora tanivivier* with *S. aureus* being particularly susceptible. One of the main components, Pulegone, has been shown to penetrate bacterial membranes, disrupting cellular integrity and ultimately leading to cell death. This disruption changed membrane permeability, resulting in the release of intra cellular substances and disrupted the vital functions of the bacterial [43]. Additionally, Carvacrol and its isomer Thymol-both present in certain Ziziphora extracts -exhibit a wide range of biological activities including anti-malarial, anti-cancer, antifungal, and antibacterial properties [44]. Research indicated that these compounds effectively inhibit the growth of both Gram-positive and Gram-negative bacteria while also demonstrating antifungal properties and antibiofilm activity. Furthermore, Thymol and Carvacrol can be a suitable alternative as antimicrobial agents against antibiotic-resistant pathogens [45].

Collectively, these findings emphasize the important role of Pulegone, Carvacrol, and Thymol in mediating the antimicrobial activity of the medicinal plant *Z. clinopodioides*. Antimicrobial compounds such as Thymol and Carvacrol are known to cause structural and functional damage to the cytoplasmic membrane [46]. Thymol disrupts critical energy production processes, thereby impairing a cell's ability to recover following exposure [47]. In contrast, Carvacrol alters cytoplasmic membrane fluidity by modifying the composition of membrane fatty acids [48, 49]. Essential oils can be categorized as either slow-acting and fast-acting based on their duration of action, with Carvacrol classified as a fast-acting anti-microbial compound [50]. While, the anti-microbial activity of essential oils is often attributed to one or two major components, it is increasingly recognized that the ratio of these components alone does not fully explain their effectiveness. Observations indicate that synergistic or antagonistic interaction between major and minor constituents in essential oils also play a significant role in determining the overall anti-microbial activity [51]. In this study, the notable anti-bacterial efficacy observed in the essential oil of *Z. clinopodioides* from region 1 can be attributed to several factors: (1) its distinct chemical composition compared to essential oil from other regions, and (2) synergistic interactions between the major and minor components; (3) a notably high concentration of sesquiterpenes; and. (4) Carvacrol exhibits a rapid anti-microbial effect. With regard to no research has been conducted to investigate the essential oil compounds of *Z. clinopodioides* from the study regions; while simultaneously investigating soil characteristics and their anti-microbial effects, the results of this research will help to better identify the native plants of Lorestan Province.

CONCLUSION

This study highlights that the geographical conditions significantly influence the anti-microbial properties of essential oil from *Z. clinopodioides*, particularly in region 1. The essential oils of the *Z. clinopodioides* plant collected from regions 2 and 3, having 19 common compounds, had the same anti-microbial effects against the studied bacteria. The *Z. clinopodioides* plant collected from region 1, with completely different compounds from the other two regions, showed the highest anti-microbial effects against the two bacteria studied.

These findings suggest potential applications in pharmaceuticals and the food industry, emphasizing the need for further research on its active compounds.

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