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FULL PAPER

Comparative study of the chemical compositions and antibacterial activity of the essential oils of two lagochilus species (*L. Archery*, *L. Cabulicus*) collected from the North Khorasan province of Iran

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The present research study reports on the extraction by hydrodistillation and the chemical composition of two Lagochilus species (*L. Archery*, *L. Cabulicus*) essential oils and antimicrobial properties from Iran. The chemical composition of the essential oils was determined by gas chromatography coupled to a mass spectrometer. An agar well diffusion method evaluated the antimicrobial effect of the essential oils of the tested species against 3 *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus atrophaeus*. In the essential oils of *L. Cabulicus*, 30 compounds which constituted 86.65% of the essential oils have been identified, the main constituents of α-pinene (36.01%), β-myrcene (10.21%), Germacrene D (6.03%), Limonene (4.75%) and cis-verbenone (4.73%) were the main components of the essential oils. 21 compounds were identified in the essential oils of *L. Aucheri* which made up 84.28% of the essential oils. α-pinene (24.74%), β-myrcene (12.85%), *p*-cymene (12.10%), Germacrene D (8.85%), Limonene (6.15%) and *p*-cymene-4-oil (5.23%) constituted the main components of this essential oil. Also, the results of microbial tests against 3 pathogenic bacteria showed significant effects on *Staphylococcus aureus* and *Bacillus atrophaeus,* and no effect was observed on *Escherichia coli*.

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Introduction

Natural remedies, especially herbal remedies, and even in some cases have been the only way to cure diseases [1]. One of the problems facing human societies in the biomedical and medical sciences is the bacterial resistance to chemical drugs and their side effects [2]. In cases of drug resistance, the drug may be modified, which may exacerbate the side effects [3]. On the other hand, environmental problems and the high cost of synthetic drugs have limited synthetic antibiotics to some extent [4]. Therefore, considering the increasing demand for essential oils and herbal extracts in the pharmaceutical, food, cosmetic, perfumery, and the substitution of herbal products instead of synthetic antibiotics, it is crucial to study the medicinal properties of native plants in each region. The Lamiaceae family is one of the largest globally dispersed plant families (except for the Arctic and South Pole) and has 200 species and two to five thousand species of aromatic shrubs and short shrubs. Most Lamiaceae produces terpenes and other compounds that are mainly stored in the epidermal glands of leaves and reproductive organs [5-7]. *Lagochilus* belongs to the Lamiaceae family, comprising 44 species worldwide, 33 growing in Central Asia. 5 species of this genus have been identified in the flora of Iran, two species of which are *Lagochilus cabulicus* and *Lagochilus aucheri* [8].

Studies have shown that the most essential compounds identified and isolated from the essential oils of the *lagochilus cabulicus* are β-springene, geranyllinalool, and (R) -(+) -α-pinene [9]. In another study, after examining the Antimicrobial effects have also been reported on *lagochilus cabulicus* collected from the Pamir Mountains in Afghanistan [12]. Other studies indicate that the main compounds of *L. cabulicus* are biologically and pharmacologically active flavonol glucosides, sterol acetates, and pentacyclic triterpene. There has not found any diterpene (lagochilin), as the main compound, in the aerial parts of *L. cabulicus* [13].

Due to the beneficial effects of this genus of plants, the present study investigates the antimicrobial properties and chemical composition of the essential oils of these plants.

Materials and methods

Plant collection: The aerial parts of plants were collected during the flowering stage from Baba Aman and Jozak regions located in North Khorasan province in the spring of 2021 and were identified and registered by the herbarium of the Medicinal Plants Processing Research Center of Mashhad University of Medical Sciences (Table 1).

TABLE 1 Name, place, date and voucher number of collection of study plants

plant	Plant collection place	Plant collection time	Voucher number
Lagochilus cabulicus (Benth.)	Babaaman	May 11,2021	FUMH-8380
Lagochilus aucheri (Boiss.)	Jozak	June 11,2021	FUMH-8381

Chemical compounds of *lagochilus aucheri* essential oils, Germacrene D, α-pinene and β-bourbonene were identified as the most important compounds [10].

Research has shown that *Lagochilus kotschyanus* Boiss also has antimicrobial activities that were determined by measuring the inhibitory growth zones (well diffusion assay) [11].

Experimental

Extraction of essential oils

250 g of plant aerial parts was extracted by hydrodistillation by Clevenger for 3 h. The essential oils were dried using anhydrous sodium sulfate and stored at +4 °C to prevent chemical decomposition.

GC-MS analysis

The chemical composition analysis and identification of essential oils were performed using Agilent 6890 series gas chromatography device equipped with a selective detector (Agilent Technologies-Little Falls- DE- USA). Helium was used as the carrier gas at a 1 mL/min flow rate and the capillary column TRB-5MS. The oven temperature was kept at 35 °C for 5 min and then gradually reached 150 °C at a rate of 15 °C/min and stopped at this temperature for 10 min and then at a rate of 20 °C/min. The temperature rose to 280 °C and was stopped for 10 min. The injection temperature was set at 270 °C. The compounds were identified by comparing their mass spectra with the NIST 08 and Wiley 275 libraries. Relative indices were also calculated from the inhibition time of normal C_3-C_{19} alkanes under similar conditions [14].

Antibacterial Test

The microorganisms studied in this study, including microbial strains of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (25923 ATCC) and *Bacillus atrophaeus* (DBIM 675) in lyophilized forms, were prepared from the Iranian Research Organization for Science and Technology (IROST).

In this study, the Institute of Clinical and Laboratory Standards (CLSI) guidelines were used with minor modifications. To evaluate the antibacterial activity of essential oils, the method of diffusion in agar well was selected. For this purpose, the first half of the McFarland microbial suspension was prepared. Wells were then made on the surface of the plate containing Müller-Hinton agar culture medium using a sterile tube (diameter 7 mm). Then the stock solution of essential oils in 10% dimethyl sulfoxide was prepared. Then serial dilutions of essential oils in the concentration range of 0.05-0.88 mg/mL were made, positive control (containing 10 μg gentamicin) and negative control (containing 10% DMSO). The wells were filled with 50 μL of essential oils, and then the Microplates were incubated at 37 °C for 24 h and incubated for *Bacillus atrophyus* at 30 °C for 24-48 h. Therefore, antibacterial activity was compared with measuring the growth inhibition zone diameter for indicator microorganisms. It was evaluated with gentamicin and 10% DMSO. The experiments were repeated three times.

Results and discussion

GC-MS analysis

The chemical compositions of *Lagochilus aucheri* and *Lagochilus cabulicus* essential oils are summarized in Table 2. Analysis of *L. aucheri and L. cabulicus* essential oils led to the identification of 21 and 30 compounds with a weight percentage of 84.28% and 86.65% of the total essential oil compounds respectively. Numerous Monoterpenes (M), Tetraterpenoids (T), Sesquiterpenes (S), Diterpenes (D) and Other compounds isolated from *L. aucheri and L. cabulicus* have been identified. (Table 3) Monoterpenes are classified into four categories: Monoterpene Hydrocarbons (M.H), Monoterpene Alcohols (M. Alc), Monoterpene Ketones (M. K), and Monoterpen Aldehydes (M. A). The main component of the essential oils of *L. aucheri* includes α-pinene (27.74%), β-myrcene (12.85%), *p - cymenene* (12.10%), Germacrene D (8.85%), limonene (6.15%), and *p*-cymen-4-ol (5.23%). Also, α-pinene (36.01%), β-myrcene (10.21%), Germacrene D (6.03%), limonene (4.75%), cis-verbenol (4.73%) were the main components of essential oils of *L. cabulicus* (Figure 1 and 2).

GC-MS chromatogram of *L.cabulicus* and *L.aucheri* are demonstrated in Figure 3.

TABLE 2 chemical compounds of *Lagochilus aucheri* and *Lagochilus cabulicus* essential oils

No	Compound	KI [a]	*Lagochilus cabulicus*	*Lagochilus aucheri*
1	*α- thujene*	931	1.76	0.4
2	*α-pinene*	939	36.01	27.74
3	*Camphene*	953	0.31	-
4	*Verbenene*	967	0.62	-
5	*Sabinene*	976	0.85	0.17
6	*β-pinene*	980	1.16	0.22
7	*β - myrcene*	991	10.21	12.85
8	*O- Cymene*	1022	-	0.62

9	*p - cymene*	1026	0.59	-
10	*Limonene*	1031	4.75	6.15
11	*β-ocimene*	1050	0.55	1.55
12	*Fenchone*	1087	1.13	0.26
13	*p - cymenene*	1089	0.96	12.10
14	*Linalool*	1098	1	-
15	*p-1,3,8-Mentatriene*	1111	-	0.42
16	*Endofenchol*	1112	0.48	
17	*Cyclooctanone*	1115	-	2.82
18	*α-campholenal*	1125	2.37	-
19	*Trans pinocarveol*	1139	1	-
20	*Sis verbena*	1140	4.73	-
21	*Pinocarvone*	1162	0.58	-
22	*p-menta 1,5 dien-8 -ol*	1166	0.69	-
23	*Terpinen- 4 -ol*	1177	0.57	0.26
24	*p-cymen -4- ol*	1183	0.62	5.23
25	*Myrtenol*	1194	1.09	0.3
26	*Verbenone*	1204	0.67	0.31
27	*Trans careful*	1217	0.69	0.64
28	*Month-1-en-4,8 dial*	1290	-	1.12
29	*β- damascenone*	1380	0.72	-
30	*β-bourbonene*	1384	1.6	-
31	*2,3,5 trimethyl forum*	1460	3.71	-
32	*Germacrene D*	1480	6.03	8.85
33	*Caryophyllene oxide*	1581	-	0.8
34	*Phytol*	1949	0.7	1.47
35	*Phytol (E)*	2221	0.5	-
	Total		86.65	84.28

TABLE 3 Classification of main compounds of *L. aucheri* and *L. Cabulicus* essential oils

Compounds / **plant**	**M**				**T**	**S**	**D**	**Other compounds**
	M.H	**M. Alc**	**M.K**	**M.A**				
L. Aurchery	49.7	6.97	0.57	-	-	9.65	1.47	2.82
L. Cabulicus	57.77	10.96	2.38	2.37	0.72	7.63	1.2	3.71

M: Monoterpenes, **M.H**: Monoterpene Hydrocarbons, **M. alco**: Monoterpene alcohols, **M.K**: Monoterpene Ketones, **M.A**: Monoterpene Aldehydes, **T**: Tetraterpenoids, **S**: Sesquiterpens, **D**: Diterpenses

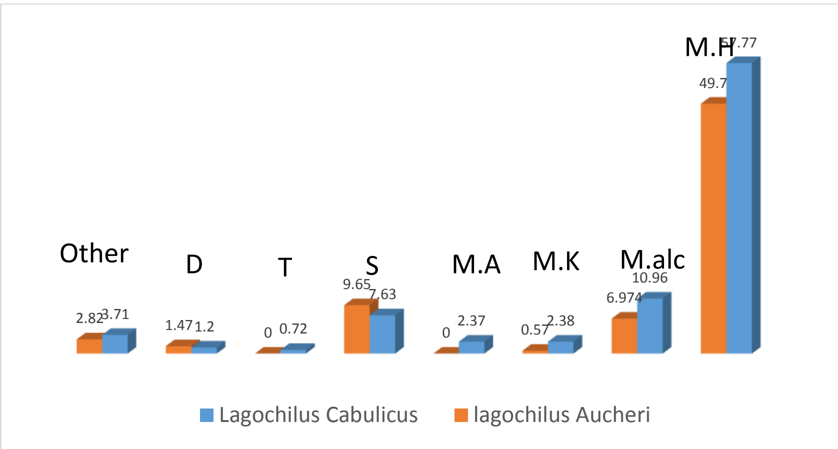


FIGURE 1 the main classified compounds

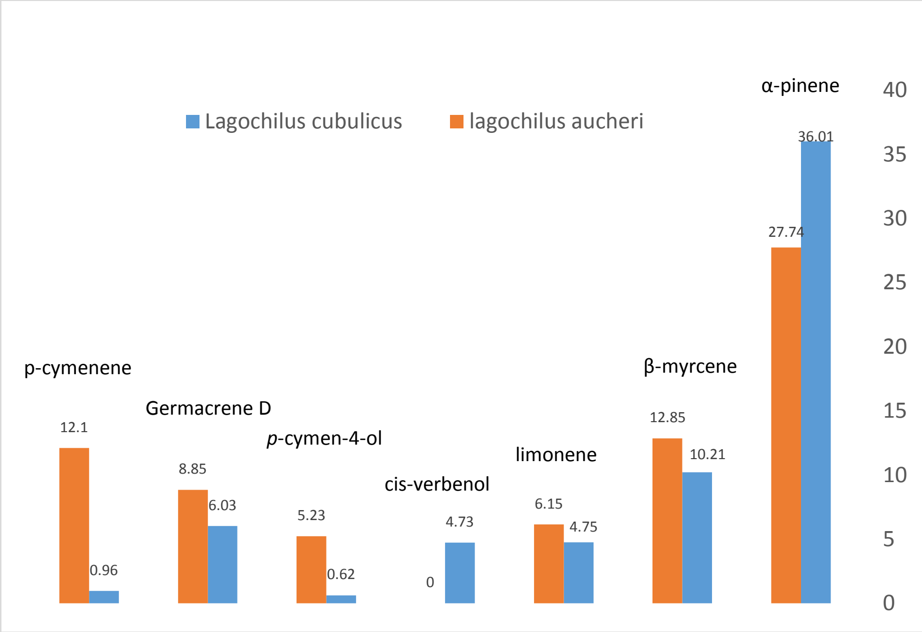
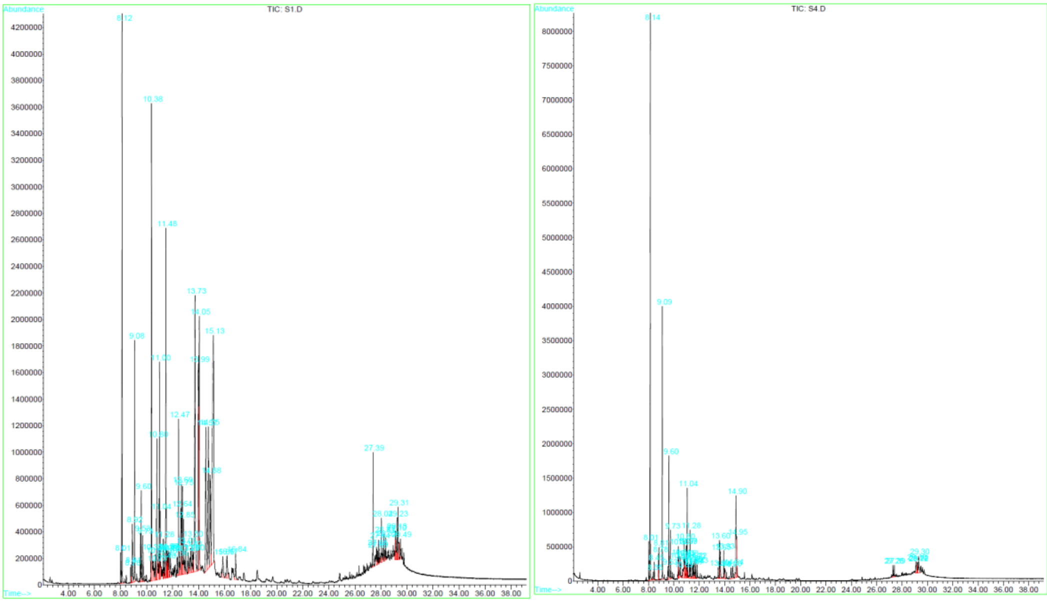


FIGURE 2 the main component of *L. aucheri* and *L. cabulicus* essential oils

Antimicrobial activity

The results of antimicrobial activity measurement of *L. aucheri and L. Cabulicus* essential oils are summarized in Table 4. The results show that *Escherichia coli* is resistant to plant essential oils and has no growth inhibition zones. However, the essential oils may affect two other bacteria, *Staphylococcus aureus,* and *Bacillus atrophies*. With decreasing the concentration of essential oils, the diameter of the growth inhibition zone has also decreased (Figure 4).



(a) (b)

FIGURE 3 GC-MS chromatogram of *L. aucheri* (a) and *L. cabulicus* (b)

Comparison of the results of this study showed that the compounds of α- pinene, β-myrcene, limonene and germacrene D are common in both essential oils with high weight percentages. The highest amount of α-pinene in *L. cabulicus* essential oil is 36.01% and in *L. aucheri* essential oils is 27.74%. β-myrcene with 12.85% in *L. aucheri* essential oils and 10.21% in *L. cabulicus* essential oils were another known ingredient. The amount of *p*-cymene and *p*-cymen-4-ol in *L. aucheri* essential oils is 12.10% and 5.23%, respectively, and *L. cabulicus* essential oils contained small amounts of these two compounds. Also, *L. cabulicus* essential oils has 4.73% of cis-verbenol which was very small in *L. aucheri* essential oils. Comparing the results of previous studies with is study, it can be concluded that the composition of α-pinene is the main component found in essential oils, which is common in both essential oils, but its amount is different. In addition, compounds such as β-bourbonene were present in *L.aucheri* essential oils collected from the Kandovan region [10], but the essential oils of the *L.aucheri* in the present study lacks this compound. This indicates that changing ecological conditions has a significant role in changing the type and amount of significant compounds of essential oils and as a result, can affect the chemical properties of the species.

TABLE 4 Evaluation of antibacterial activity of *L. aucheri and L. cabulicus ess*ential oils

	L.aucheri (mg/mL)					***L. Carbolic's (mg/mL)***					**Ge**
C / **Mic**	0.88	0.44	0.22	0.11	0.05	0.88	0.44	0.22	0.11	0.05	10 μg/mL
Escherichia coli (mm)	-	-	-	-	-	-	-	-	-	-	14.7
Staphylococcus aureus (mm)	17	15	14	10	-	20	20	19	17	15	18.9
Bacillus atrophies (mm)	13	12	11	-	-	15	14	10	-	-	19.7

C: Concentration, **Mic**: Microorganism, **Ge**: Gentamicin

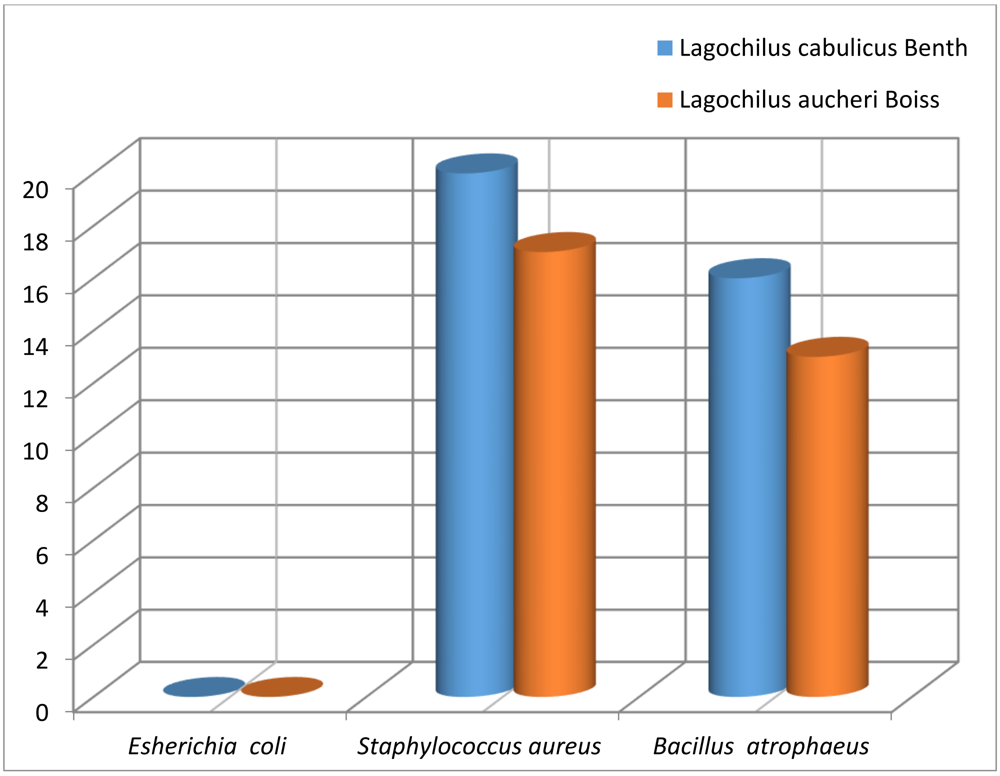


FIGURE 4 Comparison the diameter of the growth inhibition zone between antibacterial effects of the *L.aucheri* and *L.cabulicus* essential oils

The antimicrobial properties of essential oils were evaluated using the agar diffusion method. The results showed that both essential oils were ineffective against *Escherichia coli*, a gram-negative bacterium, but against two gram-positive bacteria, *Staphylococcus aureus* and *Bacillus atrophies* (respectively), with a growth inhibition zone diameter in the range of 10-20 mm and 10 -15 mm. According to research, gram-positive bacteria are more sensitive to essential oils than gram-negative bacteria. Due to the presence of outer membranes surrounding the cell wall in gram-negative bacteria, it seems logical that these bacteria are less sensitive to the antibacterial effect of essential oils. This outer membrane limits the release of hydrophobic material through this layer covering the lipopolysaccharide[15] Essential oils are more susceptible to *Staphylococcus aureus* than *Bacillus atrophies,* and the essential oils of *L.cabulicus* have more inhibitory power than *L.aucheri* essential oils. The high antibacterial activity of *L.aucheri* and *L.cabulicus* essential oils can be associated with the presence of high concentrations of α-pinene, β-myrcene, limonene, and Germacrine D [16,17] The antimicrobial effect of essential oils is not only due to the presence of their significant compounds, but also compounds with small amounts, such as terpinen-4-ol and linalool can also contribute to the antimicrobial activity of essential oils. The antibacterial properties of terpinen-4-ol against several microorganisms have been reported[18] The solid inhibitory properties of linalool against 12 bacteria and 10 fungi have also been demonstrated [19]. It is also possible that compounds with a lower percentage are likely to have a synergistic effect with other effects and active compounds [20].

Conclusion

The results of this study suggest that the essential oils of the two plant species studied may be useful alone or in combination with other antibacterial agents for the treatment of some bacterial infections

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