



Comparison of the Antifungal and Antibacterial Activity of *Ballota aucheri* Essential Oil and Fluconazole



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ABSTRACT

Aims Lamiaceae family are compromised the majority of species of the family have essence that was used for the nutritional, cosmetic, and pharmaceutical industry. This study aimed to investigate the chemical composition of essential oil from aerial parts of *Bollota aucheri* with its antibacterial and antifungal activity of it compared with fluconazole.

Materials & Methods This clinical laboratory research was conducted on the flowering aerial parts of *B. aucheri* that were collected during 2020-2021 from Zagros mountains in Kohgiluyeh and Boyer-Ahmad province, Iran. The essential oil of this plant was tested for antibacterial and antifungal properties and compared with fluconazole. The chemical composition of the essential oil was analyzed by GC/MS (Gas Chromatography Mass Spectrometry) and the antifungal activity of the plant essential oil was compared with fluconazole.

Findings Generally, β -Caryophyllene was the main compound of *B. aucheri* essential oil. The minimum inhibitory concentration (MIC) of the essential oil of the fruit of *B. aucheri* and fluconazole were 0.078 μ l/ml and 1 μ l/ml for *Candida albicans*, 0.078 μ l/ml and 0.5 μ l/ml for *C. glabrata*, and 0.078 μ l/ml and 0.178 μ l/ml for *C. parapsilosis*, respectively.

Conclusion The essential oil of *B. aucheri* could be used as a bactericidal and fungal alternative to antibiotics against microorganisms. Antifungal activity of *B. aucheri* essential oil is stronger than fluconazole.

Keywords *Ballota aucheri*; Essential Oils; *Candida albicans*; *Candida glabrata*; *Candida parapsilosis*; Lamiaceae Family; Fungal Infections

CITATION LINKS

[1] Invasive Candidiasis in critical care: Challenges and future directions [2] Risk factors for invasive candida infection in critically ill patients: A systematic review and meta-analysis [3] Antifungal drugs TDM: Trends and update [4] Chelerythrine reverses the drug resistance of resistant *Candida albicans* and the biofilm to fluconazole [5] A review on traditional uses, phytochemistry and pharmacological activities of the genus *Ballota* [6] A review of the phytochemistry, traditional uses, and biological activities of the Genus *Ballota* and *Otostegia* [7] Chemical composition of *Ballota macedonica* Vandas and *Ballota nigra* L. ssp. *foetida* (Vis.) Hayek essential oils- the chemotaxonomic approach [8] Volatile constituents analysis and antimicrobial activity of two subspecies *Ballota nigra* L. From Iran [9] Natural product isolation [10] Antifungal activity of essential oil and hydrosol extract of *Ballota nigra* L. and their protective effects against the black rot of tomatoes [11] Phytochemical analysis of *Ballota aucheri* Boiss of Iran [12] Essential oil composition and antifungal activity of aerial parts of *Ballota nigra* ssp *foetida* collected at flowering and fruiting times [13] Molecular identification, and antifungal susceptibility patterns of candida species isolated from candidemia patients in Yasuj, Southwestern Iran [14] Antifungal susceptibility profile and molecular epidemiology of recurrent vulvovaginal Candidiasis in Yasuj, southwestern Iran [15] Chemical composition and antimicrobial properties of *Teucrium polium* essential oil collected from dena mountain [16] Anti-planktonic and anti-biofilm formation activities of the essential oil and hydroalcoholic extract of *Myrtus communis* L. leaves and fruits against clinical *Candida* species [17] Susceptibility of candida spp. isolated from blood cultures as evaluated using the M27-A3 and new M27-S4 approved breakpoints [18] Clinical implications of revised piperacillin-tazobactam breakpoints in CLSI M-100 S32 [19] Breakthrough invasive fungal infections: Who is at risk? [20] Chromatin structure and drug resistance in candida spp [21] *Quercus brantii* Lindl. vaginal douche versus clotrimazole on vaginal Candidiasis: A randomized clinical trial [22] Gas chromatography-mass spectrometry profile and antimicrobial activities of *Ballota bullata* Pomel and *B. nigra* L. subsp. *uncinata* (Fiori and Bég.) A comparative analysis [23] Antifungal activity of *Ballota acetabulosa* against yeast candida and *Cryptococcus* species [24] Antifungal diterpenoids and flavonoids from *Ballota inaequidens*

Introduction

Candidiasis is an opportunistic infection that causes by different species of *Candida*. This infection has a variety of illnesses forms including superficial, cutaneous, and life-threatening systemic forms [1].

Some of the factors involved in this process such as a change in the balance of normal microbiota, immunosuppression, antibiotic therapy, and diabetes [2]. Generally, four classes of antifungal drugs are introduced for the treatment of different forms of mycotic infection [3].

Among these drugs, the azole class especially fluconazole is the most common drug used in different forms of candidiasis. However, resistance to fluconazole and its side effect is a challenging issue in recent years [4].

Significant research has been done on antifungal properties in herbal plants. The genus *Ballota* belongs to Lamiaceae family and consists of a variety of species with different habitats and therapeutic effects [5]. This species included *B. aucheri* Boiss., *B. nigra* L. and *B. platyloma* Rech. f., of which are endemic in different regions of Iran.

Ballota species uses in folk medicine for the treatment of gastrointestinal disorders, wounds, and upper respiratory inflammation and also as antibacterial, antiulcer, antispasmodic, diuretic, choleric, sedative agents, vermifuge, and anti-hemorrhoid [6]. *Ballota nigra* has been known in studies as an antioxidant, antispasmodic, anti-aging, anti-cough, and anti-vomiting. On the other hand, *B. hirsuta* Benth. as a wild Mediterranean shrub, is used in traditional medicine as a treatment for many diseases [7]. Recent studies on two species, *B. bullata*, and *B. nigra*, showed strong antibacterial and anti-candida effects. Some studies reported that herbal plants can inhibit or delay oxidative stress and in this way affect oxidative-related disease [8, 9].

Previous studies on the antioxidant properties of polyphenol-rich extracts of *B. nigra* showed the effectiveness of several glycosyl phenylethanoids in inhibiting LDL peroxidation [10]. Also, *B. aucheri* Boiss. widely uses in the biological control against plant pests. Various chemical compounds have been identified in *Ballota* species such as volatile oils, flavonoids, phenylpropanoids, terpenoids, saponins, and tannins [11]. Review studies have shown the antimicrobial activity of extracts, essential oils, and compounds isolated from *Ballota* species [6]. For instance, antifungal properties of essential oil of *B. nigra* ssp. *foetida* were investigated against saprophytic fungi and MIC value was reported 600 - 800 µg/ml [12]. However, no study has been conducted on the antifungal and antibacterial properties of *B. aucheri* in Iran as a native species in the south of the country.

This study aimed to compare the antibacterial and antifungal activity of the essential oil of aerial parts of *Ballota aucheri* and fluconazole.

Materials and Methods

This clinical laboratory research was conducted on the flowering aerial parts of *B. aucheri* that were collected during 2020-2021 from Zagros mountains in Kohgiluyeh and Boyer-Ahmad province, Iran.

Specimen

The collected plant was dried at room temperature and was identified and approved in Esfahan Agriculture and Natural Resources Research and Education Center (herbarium number: 17691). For essential oil preparation, 200gr of the specimen was used for the hydro distillation process by Clevenger-type apparatus for 24h. After this process, an essential oil sample was collected and stored at 4°C until further tests.

Gas chromatography/Mass spectrometry

The GC/MS method was used to analyze the components of the essential oil of the *B. aucheri* fruit at Kashan University of Medical Sciences. Chromatography (Brand Agilent, model 6890; USA) and mass spectrometry were used for qualitative and quantitative measurements of compounds. At first, the temperature was set at 60°C, and then it was raised to 246°C at a rate of 3°C. The combination of gas chromatography and mass spectrometry characteristics is used to identify different substances present in a sample.

Antifungal activities

The essential oil of *B. aucheri* was tested against clinical *Candida* isolates including 69 *C. albicans* (26 recurrent vulvovaginitis and 26 vaginitis, 1 otomycosis, 12 blood culture isolates), 14 *C. glabrata* (5 vaginitis, 1 recurrent vulvovaginitis, 8 urine isolates), and 12 *C. parapsilosis* (9 otomycosis, 3 blood isolates). These species were collected and identified based on the molecular method, and preserved in distilled water [13-16]. For this purpose, candidal suspensions were cultured on Sabouraud Dextrose Agar (Biolife; India) and incubated at 37°C. The antifungal susceptibility test was done according to CLSI-M27-A3 guidelines [17]. Based on this instruction suspension of *Candida* isolates was prepared in distilled water and standardized using a spectrophotometer (Brand JENWAY, Model 6320D; China) in 530nm and 75-77 transmittance.

Minimum inhibitory concentration (MIC)

Based on the broth microdilution method, serial dilution of essential oil and fluconazole were prepared in RPMI 1640 medium. For fungal isolates, standard suspensions were diluted 1:1000 in RPMI 1640 medium and 100 µl of them were added to each 96-well microtiter plate. At the end, all microplates were incubated at 35°C for 24h, and MIC was defined as the lowest concentration that causes 50% inhibition of fungal growth, compared to the positive control.

Antibacterial susceptibility

The essential oil was tested against different bacteria including Methicillin-Resistant *Staphylococcus*

aureus (MRSA) ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella* (UTI isolates). These microorganisms were obtained from the stock collection of the Microbiology Laboratory of Yasuj. The susceptibility test was carried out by microdilution method according to CLSI-M100 guidelines [18].

Statistical methods

Data were entered to Excel 2016 and were analyzed in SPSS 16 software by Wilcoxon test.

Findings

Chemical constituents

The main components of the essential oil of the *B. aucheri* plant are β -caryophyllene (22.16%), (Z)-beta-farnesene (9.57%), bicycloger macrene (7.95%), β -eudesmol (7.23%), β -bisabolene (5.57%), 2-Pentadecanone (3.79%), α -selinene (3.49%), germacrene D-4-ol (3.48%), α -cadinol (3.12%), and α -caryophyllene (3.09%; Table 1).

Table 1. Chemical composition of *B. aucheri* essential oil

PK	Name	RT (min)	Standard Retention Index	Conc. (%)
1	(E)-2-Hexenal	9.088	854	0.22
2	1-Octen-3-ol	13.323	978	1.46
3	Linalool	17.827	1098	2.09
4	Nonanal	17.953	1098	1.21
5	Camphor	18.244	1103	0.29
6	Geijerene	19.547	1139	1.27
7	Camphor	26.314	1143	0.60
8	Eugenol	26.903	1356	0.52
9	α -Copaene	27.640	1374	0.95
10	β -Elemene	28.246	1375	0.99
11	Dodecanal	28.755	1407	1.03
12	Isocaryophyllene	28.869	1413	0.55
13	β -Caryophyllene	29.549	1418	22.16
14	(Z)-beta-farnesene	30.161	1443	9.57
15	α -Humulene	30.298	1452	0.40
16	α -Caryophyllene	30.578	1454	3.09
17	Alloaromadendrene	31.052	1461	1.42
18	Germacrene D	31.344	1480	2.33
19	β -selinene	31.635	1485	2.88
20	Bicyclogermacrene	31.841	1494	7.95
21	α -Selinene	32.378	1502	3.49
22	Isodaucene	32.515	1502	1.85
23	(E,E)-a-Farnesene	32.664	1505	0.92
24	β -Bisabolene	33.544	1508	5.57
25	Germacrene D-4-ol	34.298	1511	3.48
26	Caryophyllene oxide	34.521	1581	2.24
27	Viridiflorol	34.870	1590	0.23
28	β -Oplophenone	35.024	1608	0.26
29	Junenol	35.716	1622	0.85
30	T-cadinol	36.156	1630	2.02
31	β -Eudesmol	36.425	1650	7.23
32	α -Cadinol	36.556	1653	3.12
33	Mintsulfide	37.356	1742	1.03
34	2-Pentadecanone	37.848	1779	3.79
35	Benzyl benzoate	38.031	1791	0.46
36	Phytone	41.208	1845	0.46
37	Palmitic Acid	44.277	1984	2.03

Antifungal activity

The essential oil of *B. aucheri* had antifungal activity against *C. albicans* isolates (MIC ranges: 0.039-1.25 μ l/ml) *C. parapsilosis* (0.039-0.15 μ l/ml) and *C. glabrata* (0.039-0.15 μ l/ml). There was a significant

difference between the mean of fluconazole and essential oil in total of *Candida* isolates ($p=0.001$). The difference between MIC values of *Candida* species was significant in essential oil ($p=0.0001$) and Fluconazole ($p=0.002$; Table 2).

Table 2. Minimum inhibitory concentration range, MIC₅₀, MIC₉₀, and MIC_{GeoMean} (MIC_{GM}) of *B. aucheri* compared with fluconazole against *Candida* species

Organisms	Range	MIC ₅₀	MIC ₉₀	MIC _{GM}	p-Value
Essential oil (μl/ml)					
<i>C. albicans</i>	0.039-1.25	0.078	0.625	0.076	0.0001
<i>C. parapsilosis</i>	0.039-0.15	0.078	0.15	0.1	
<i>C. glabrata</i>	0.039-0.15	0.078	0.078	0.055	
Fluconazole (μg/ml)					
<i>C. albicans</i>	0.03125-2	1	2	0.35	0.002
<i>C. parapsilosis</i>	0.0312-0.5	0.178	0.5	0.88	
<i>C. glabrata</i>	0.0625-32	0.5	7.6	0.7	

Antimicrobial activity

The essential oil of *B. aucheri* showed no inhibitory effect on *Klebsiella*. However, the results of MIC and Minimum Bactericidal Concentration (MBC) showed that the MIC value was lower for *E. coli* (3.5 μ l/ml) compared to MRSA (7 μ l/ml).

Discussion

We aimed to compare the antibacterial and antifungal activity of the essential oil of aerial parts of *Ballota aucheri* and fluconazole. Current treatments for malignancies and HIV and long stays in an intensive care unit (ICU), intervention therapy, and organ transplantation have made many fungal opportunistic diseases a high threat to humans [19]. Fungi acquire and maintain drug resistance through an evolutionary process through genetic mutation [20]. Also, the unavailability and high costs of drugs in low-income communities, as well as their potential drug side effects and resistance, have raised concerns about industrial chemical compounds. In recent years, patients became interested in herbal medicines due to fewer side effects, low costs, and availability [21]. In this study, the essential oil of *B. aucheri* fruit was analyzed by GC/MS method, and its constituent compounds were determined. The most compound present in this essential oil was β -Caryophyllene (22.16%), followed by (Z)-beta-farnesene (9.57%) and Bicyclogermacrene (7.95%), and other compounds had a concentration of less than 7%. In the study conducted by Fraternali *et al.*, β -Caryophyllene (22.6%, 21.8%), caryophyllene oxide (18%, 20.5%), Germacrene-D (16.5%, 13.1%) were the main compounds of plants and flowers of *Ballota nigra* L. *spp foetida* [12]. Compared to our study, the concentration of beta-caryophyllene is similar, but the concentration of caryophyllene oxide (2.24%) and germacrene (2.33%) is lower in the plant studied. Valerinol (18.3%), α -muurolol (7.9%), and spathulenol (7.1%) were identified as the main aromatic substances in *B. bullata* in Elmokni *et al.* study [22]. Besides who reported that, the essential oil of *Ballota nigra* L. subsp. *uncinata* is mainly

composed of hexadecanoic acid (31.8%) and linoleic acid (17.9%). Valerinol and Spatolenol compounds were not found in our studied species, and the concentration of hexanal and linalool was 0.22% and 2.09%, respectively.

Antimicrobial susceptibility profiles were assessed in the present study and showed that the essential oil of *B. aucheri* did not affect *Klebsiella*. Agree with our result, Bidgoli *et al.*, reported that *B. nigra* ssp. foetida and *B. nigra* ssp. anatolia was not able to inhibit the growth of *K. pneumonia* [18].

Besides, according to our results, the essential oil of *B. aucheri* had strong antibacterial activity against MRSA and *E. coli* with MIC values of 7 µg/ml and 3.5 µg/ml, respectively. Consistent with our results, Dulger *et al.* showed that the methanolic extract of the leaf of *B. acetabulosa* had an inhibitory effect on *E. coli* and *S. aeruginosa*, while it is not consistent with Bidgoli [23]. The reason for the difference in the results of different studies can be the difference in the type of *Ballota* species and the difference in its antimicrobial properties.

Previous studies have examined other species of the genus *Ballota*, and this study is the only study of the *B. aucheri* species. Our results indicated that the essential oil of *B. aucheri* had a better inhibitory effect on non-albicans species with MIC range 0.039-0.15 µg/ml. Also, based on MIC value the antifungal effect of *B. aucheri* on *Candida* species was more effective than fluconazole. However, there is a difference between our results and El Mokni *et al.*, who reported a MIC value of 625 µg/ml for *B. nigra* and *B. bullata* against *C. albicans*. Also, this author illustrated that gentamycin has less than the MIC value against *C. albicans* isolate [22]. The reason for the difference in these two studies seems to be the difference in the type of plant species under investigation. Consistent with our results in another study, Citoglu *et al.*, compared the antifungal properties of diterpenoid and flavonoids component of *B. inaequidens* against *C. albicans* and *C. krusei* isolates. Among these components, ballonigrine has the better antifungal activity against *C. albicans* isolate (MIC=3.1 µg/ml) and this inhibitory effect was better compared to fluconazole (MIC=12.5 µg/ml). Whereas, ballonigrine did not have a significant effect on *C. krusei* isolate and other constituent had lower MIC values (3.1 µg/ml) against this species such as 5-hydroxy-7,30,40-trimethoxy-, pachypodol, and 5-hydroxy-3,7,40-trimethoxyflavone [24].

Generally, one of the limitations of this study is the limited effect of essential oil on clinical isolates and the lack of simultaneous comparison in vivo conditions.

But based on the results of this study, it can be hoped that recurrent candidal infections will be treated in the future with different formulations of *B. aucheri* along with antifungal drugs. Moreover, if the results are confirmed in future studies, *Ballota aucheri* essential oil can be suggested as a supplement with

industrial drugs in patients with fungal and bacterial infections. This oil should be analyzed further, as an antifungal agent against different forms of candidiasis. It is suggested to evaluate and compare the effect of fluconazole with different formulations of *B. aucheri* for the treatment of recurrent *Candida* infection. Besides, it is recommended to conduct a similar study with a clinical trial design on patients with fungal and bacterial diseases.

Conclusion

The essential oil of *B. aucheri* could be used as a bactericidal and fungal alternative to antibiotics against microorganisms. Antifungal activity of *B. aucheri* essential oil is stronger than fluconazole.

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Conflicts of Interests: The authors declare that there is no conflict of interest.

Authors' Contribution: Gharaghani M (First author), Methodologist/Discussion author (25%); Ashrafzade Z (Second author), Introduction author (10%); Razmjoue D (Third author), Original researcher (10%); Sadeghi Mansourkhani H (Fourth author), Introduction author (10%); Salahi M (Fifth author), Original researcher (10%); Yousefi Mehryan SH (Sixth author), Introduction author (5%); Talaie T (Seventh author), Introduction author (5%); Nouripour-Sisakht S (Eighth author), Assistant/Discussion author (25%)

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