Anticandida activities of essential oil of *Salvia macrosiphon*, in a single form and comparison with fluconazole

Abstract

Aims: Candidiasis is the most opportunistic infection with a high rate of recurrent infection. Salvia macrosiphon has antibacterial effect, however, its' antifungal effect was not studied. This study aimed to investigate the chemical composition of essential oil from leaves of Salvia macrosiphon with its antifungal activity of it compared with fluconazole.

Materials & Methods: Salvia macrosiphon leaves, a native plant of Kogiluyeh and boyerahmad states, were collected from Zagros heights, and used in this study. Then, 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: The results of GC-MS analysis proved the presence of at least 29 compounds in the essential oil of Salvia macrosphon. Amon these constitute, butyl benzoate (49.16%), n-hexyl benzoate (7%), and isopatolenol (4.83%) were the main compound. The minimum inhibitory concentration (MIC) of the essential oil (µl/ml) of Salvia macrosiphon and fluconazole (µg/ml) were 0.44 and 0.7 for Candida albicans, 0.056 and 0.7 for C. glabrata, and 0.1 and 0.088 for C.parapsilosis, respectively. Also, statistical analysis demonstrated that there was a significant difference between the mean of fluconazole and essential oil in total Candida isolates ($p=0.001$, $p<0.05$).

Conclusion: Based on the results and other studies, the essential oil of Salvia macrosiphon can be used as a new drug or a source of antifungal compounds alone or as a supplement with industrial drugs. Also, our findings in this study showed stronger antifungal activity of plant essential oil compared to fluconazole.

Keywords

Salvia Macrosiphon
Essential Oils
Candida Albicans
Candida Glabrata
Candida Parapsilosis

Introduction

Candidiasis is the most opportunistic infection and causes a variety of clinical forms. This infection may be superficial (e.g., oral, nail), mucocutaneous (e.g vaginal), or systemic (e.g candidemia, candiduria) [1]. Different species of *Candida* have been introduced as agents of candidiasis such as *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis* [2]. Although *C. albicans* is the most common cause of the disease, the prevalence of non-albicans species is also increasing [3]. Even though common *C. albicans* infections are easily treatable, systemic infections, frequently of a nosocomial nature, have a high mortality rate. Therefore, early diagnosis and management of infection are necessary [4]. There are different classes of antifungal drugs and uses for various forms of candidiasis. These drugs included azole classes (eg., imidazole and triazole), echinocandin (eg., caspofungin), and polyene (eg., amphotericin B) [5]. Fluconazole is one of the triazole drugs and is widely used in mucocutaneous and systemic forms of candidiasis. In the past two decades, several genes and mutations that increase resistance to fluconazole in clinical isolates, primarily in C. albicans, have been elucidated [6].

The use of herbal plants and their products has been considered for a long time. Some properties such as ease of access, lack of side effects, and drug resistance have caused these compounds to always be used in the treatment of various diseases. The genus of Saliva belongs to the Lamiaceae family and possesses more than 100 species. Many countries have been introduced as the inhabitants of this genus [7]. Iran is also an endemic region of this genus and there are 61 flora species in this region [8]. Generally, Saliva species are used in foods, cosmetics, perfumery, and pharmaceutical industries. Salvia macrosiphon) S. macrosiphon) known as Maryam Goli Lolei or Marvak in Persian has been used in traditional medicine. This species is the most valuable type of the Lamiaceae family and has therapeutic effects. The habitant of this species is in Iran and Afghanistan and due to its essential oil and tannin, its leaves have a strengthening and tonic effect, in addition, they facilitate digestion, are diuretic, anticonvulsant, antipyretic, and reduce blood sugar. It is used externally to heal and disinfect wounds [9]. Some fractions of *S. macrosiphon* have been introduced as antibacterial agents. For instance, the antibacterial effects of the plant's n-hexane, chloroform, and ethyl acetate fractions on Staphylococcus aureus and E. coli were tested in one study, and the results showed good activity with MIC values ranging from 0.61 to 2.5mg/mL [10]. Today, the problem of resistance of *Candida* species to existing chemical drugs, as well as complications and high cost of chemical drugs, is observed, and there is a tendency to use medicinal plants as medicinal and antifungal compounds [11]. Therefore, this study aimed to investigate the chemical composition of essential oil from leaves of *Salvia macrosiphon* with its antifungal activity of it compared with fluconazole.

Material and Methods

Design and samples

This study was designed based on the clinical laboratory research method from 2021 to 2022. 56 C.albicans were collected from the patient's samples including vulvovaginal swabs, ear swabs, blood and urine specimens, were studied.

Plant material

The aerial parts of *S. macrosiphon* were collected and the identification process was done and approved in the Kerman Agriculture and Natural Resources Research and Education Center and the herbarium number was obtained (herbarium number: 17714). The aerial parts were crushed and dried at room temperature. 200 g of dried powder mixed with 1 L of distilled water and the essential oil hydrodistilled in a Clevenger apparatus according to the British method for 3h. Finally, the essential oil was preserved at 4ºC in a dark condition until other steps.

Gas chromatography /Mass spectrometry

The GC/MS method was used to analyze the components of the essential oil of the *S. macrosiphon* 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 susceptibility test

1- Preparation of *Candida* isolate

56 *C.albicans* (including 15 blood cultures, 40 vulvovaginitis, recurrent vulvovaginitis, and 1 otomycosis isolates), 12 *C.glabrata* (6 vulvovaginitis, 6 urine culture isolates), 12 *C.parapsilosis* (3 blood cultures, 9 otomycosis isolates) were studied. Candidal isolates were collected from the patient's samples (vulvovaginal swabs, ear swabs, and blood and urine specimens). Vaginal samples were taken from suspected patients with VVC that had been referred to gynecology and (obstetrics) private midwifery clinics in Yasuj, Iran from 2021 to 2022. Other samples including blood culture, urine, and ear swabs were referred to the medical school of Yasuj University of Medical Science and identified based on molecular methods. All *Candida* isolates were subculture on Sabouraud Dextrose Agar (Biolife; India) and incubated at 37°C. Then fresh isolates were used for other steps.

2- Determination of minimum inhibitory concentration (MIC)

An antifungal susceptibility test was carried out based on CLSI-M27-A3 guidelines [12]. Fungal suspensions were prepared by fresh colonies and distilled water and standardized using a spectrophotometer (Brand JENWAY, Model 6320D; China) in 530nm and 75-77 transmittance. For broth microdilution processes, essential oil and fluconazole were diluted in RPMI 1640, and 100µL of each diluted solution was added to the 96-well microtiter plate. As well as the standard fungal suspensions were diluted 1:1000 in RPMI 1640 medium and added to each well. All microplates were incubated at 37ºC for 24-48h. Growth inhibition was determined after the incubation period and compared with positive control.

Statistical analysis

Data were entered into Excel 2016 and were analyzed in SPSS 16 software and descriptive rioncstatistics, and Wilcoxon test was calculated. Also, the data distribution was checked using Shapiro-Wilk and Kolmogorov-Smirnov tests, and the data distribution was not normal in any of the groups, and due to non-normality, non-parametric tests were used for data analysis.

Findings

Chemical component

The constituents of the essential oil of *S. macrosiphon* are presented in Table 1. Overall, 29 components were identified in the leaf essential oil. The main constituents of the oil were Butyl benzoate (49.16%), n-Hexyl benzoate (7%), Isospathulenol (4.8%), Cyperene (4.1%), Benzoic acid, 2-methyl-, butyl ester (3.88%), beta-caryophyllene (3.54%), and β-Elemene (3.02%).

Table 1. Chemical component of *Saliva macrosiphon*

PK	Name	RT (min)	Standard Ritention Index	Conc.
1	Methyl benzoate	17.707	1091	0.27
2	linalool	17.844	1098	3.31
3	Hexyl isobutyrate	19.461	1150	0.82
4	Ethyl benzoate	20.496	1170	0.31
5	Hexyl 2-methylbutanoate	22.690	1234	0.65
6	Hexyl 3-methylbutanoate	22.896	1243	0.34
7	Propyl Benzoate	24.09	1247	0.77
8	Butyl benzoate	26.416	1339	49.16
9	Octyl isobutyrate	27.669	1348	0.98
10	β-Elemene	28.286	1375	3.02
11	Cyperene	29.240	1398	4.10
12	Benzoic acid, 2-methyl-, butyl ester	29.440	1409	3.88
13	beta-caryophyllene	29.777	1418	3.54
14	(+)-Aromadendrene	30.555	1439	0.39
15	alpha-Humulene	31.166	1443	1.34
16	(E)-germacrene D	31.338	1480	1.62
17	Bicyclogermacrene	31.441	1494	0.71
18	δ-Selinene	31.784	1495	2.86
19	n-Hexyl benzoate	32.955	1576	7
20	Caryophyllene oxide	34.321	1581	1.19
21	Salvial-4(14)-en-1-one	34.504	1589	0.77
22	Ledene	35.556	1600	0.41
23	Caryophyllenol II	35.721	1614	0.93
24	Isospathulenol	36.379	1623	4.83
25	Isoaromadendrene epoxide	37.024	1682	2.29
26	Heptadecane	39.191	1700	0.45
27	Benzyl benzoate	39.545	1762	0.68
28	Octyl benzoate	39.905	1766	2.75
29	Palmitic acid	44.186	1984	0.63

Antifungal activities

As shown in Table 2, the MIC value showed that *S. microsiphon* has a better effect against different *Candida* species. Besides, the MIC value range was lower in non-albicans species (0.039-0.15µl/ml) than *C.albicans* (0.039-2.5µl/ml). Based on MIC_{90} fluconazole had poor activity against *C.glabrata* isolates (7.6µg/ml). However, a comparison of MICGM between *S. macrosiphon* and fluconazole revealed that only *C.parapsilosis* had this value lower in fluconazole (0.088µg/ml). Statistical analysis showed that there was a significant difference between the mean of fluconazole and essential oil in total *Candida* isolates (p=0.001, p<0.05).

Table2. Minimum inhibitory concentration range, MIC_{50}, MIC_{90}, and $MIC_{GeoMean}$ (MIC_{GM}) of *S. macrosiphon* compared with fluconazole against *Candida* species

Organisms	S. macrosiphon (µl/ml)				Fluconazole (µg/ml)			
	MIC range	MIC_{50}	MIC_{90}	MIC_{GM}	MIC_{range}	MIC_{50}	MIC_{90}	MIC_{GM}
Candida albicans (n=56)	0.039-2.5	0.078	0.46	0.44	0.0625-4	1	2	0.7
Candida parapsilosis (n=12)	0.039-0.15	0.078	0.141	0.1	0.0312-0.5	0.18	0.5	0.088
Candida glabrata (n=12)	0.039-0.15	0.078	0.078	0.056	0.0625-32	0.5	7.6	0.7

A comparison of mean and standard deviation showed that *Candida* isolates in *S. macrosiphon* essential oil were lower than fluconazole (Table 2). As shown, the difference in antifungal activity between *S. macrosiphon* essential oil and fluconazole was significant in all *Candida* species (p<0.05).

Table 3. Mean, standard deviation and p-value of different Candida species in two essential oil and fluconazole groups

Microorganism	Mean±Standard Deviation		p-value*
	S. macrosiphon	Fluconazole	
C. albicans	0.281±0.583	1.489±4.234	0.0001
C. parapsilosis	0.0867±0.0315	0.242±0.168	0.003
C. glabrata	0.677±0.0323	4.234±9.077	0.002

* Wilcoxon test

Discussion

Candidiasis is the most opportunistic infection and its treatment has always been a challenge [13]. The drug resistance of Candida isolates which are sometimes intrinsic and mostly acquired, has made it difficult to treat patients [14]. Therefore, choosing a suitable drug with the lowest cost and no recurrence of infection has always been desired. Herbal medicines with low side effects and availability have long been of interest [15]. In this study, the essential oil of S.macrosiphon was analyzed by the GC-MS method, and its constituent elements were determined. The most compounds in this essential oil are butyl benzoate (49.16%), n-hexyl benzoate (7%), isopatolenol (4.83%), cuprene (4.10%), benzoic acid 2-methyl-1-butyl ester (3.88%), linalool (3.31%), beta caryophyllene (3.54%), and beta elemen (3.02%). Various studies investigated the main compounds found in different species of Saliva. For example, in the study, Sajadi et al. proved the presence of at least forty-six compounds in the essential oil of S.macrosiphon. Among these compounds, beta-pinene (15.3%), germacrene-di (10.1%), spatholenol (7.7%), and 1 and 8-cineole (7.4%) were the main compounds in the essential oil of S.macrosiphon [16]. Also, in the study conducted by Taari et al., the most important components of the essential oil of this plant, 9- and 8-cineole, alpha-thujone, viridiflurol, and beta-thujone were introduced [17]. This is even though the above compounds were not detected in the present study. The difference in the composition of *Salvia* species essential oil can be attributed to reasons such as ecological differences. The compounds in the essential oils of plants are caused by ecological differences such as latitude and longitude, altitude, temperature, humidity, climate and soil, metabolic pathways, and biosynthesis of effective substances in these plants, which result in various secondary metabolites being biosynthesized under different environmental conditions. Various studies have confirmed this. Also, in similar weather conditions, secondary compounds in different plants have similarities. Of course, the conditions and types of studies are different, which can affect the results.

The present study was done to test the antifungal activity of S.macrosiphon based on the microdilution method. In the literature, the antimicrobial activity of some genera of Salvia essential oils against microbes has been reported [9, 18]. However, the use of broth microdilution method in the effect of antifungal activity of S.macrosiphon has been done for the first time in Iran in the present

study. The results of the present study showed that the essential oil of S.macrosiphon had great activity against Candida isolates. So, the essential oil of S.macrosiphon affected the growth of C. albicans and the MIC_{50} of this plant was equal to 0.78µl/ml. whereas, the MIC_{50} value was 1µg/ml for fluconazole against C. albicans isolates. Agree with results of the present study, the study conducted by Atai et al. reported MIC=1µg for Saliva species against C.albicans [19]. Also, in the study of Banaeian-Boroujeni et al., S.officinalis with a concentration of 1.25µg/ml was able to inhibit the standard strain of C. albicans [20]. However, in the study of Sookto et al., the effect of the extract and oil of several plants from the Lamiaceae family, including S.officinalis and Chamomile, on C. albicans was investigated. S. officinalis essential oil with a concentration of 78.2mg/l inhibited the growth of C.albicans [21]. Pozzatti et al reported that the essential oil of S.officinalis had no effect against fluconazole resistance and susceptible C.albicans [22]. This difference between our results and Pozzatti et al, may be due to the different species of Salvia tested. Besides, the present study revealed that S. officinalis essential oil had an inhibition effect against C.glabrata isolates (MIC_{GM}=0.056µL/ml). This antifungal activity has a better inhibitory effect compared to fluconazole (MIC_{GM}=0.7µg/ml) against C.glabrata isolates. However, Salari et al, reported the methanolic extract of S. rhytidea had anti-fungal activity against C.glabrata isolates (MIC50=100µg/ml) [23]. Resistance to fluconazole has been reported during the treatment of different forms of candidiasis [24, 25]. There is no study similar to the present study for the effect of the S.macrosiphon on Candida isolates and its comparison with fluconazole. However, results of the present study indicated that the MIC_{50} value of S.macrosiphon was lower than that of fluconazole against Candida isolates.

The mechanism of the inhibitory effect of S.macrosiphon is not clear. But in one study it was mentioned that probably due to the destruction of the cell wall by lipophilic compounds in this mechanism. Significant leakage of cell material indicates irreversible cell membrane damage [26]. On the other hand, the difference in the concentration of Saliva species in inhibiting the growth of Candida, which is observed in the results of various research, can be due to geophysical factors and different species of this plant, or the methods of essential oil extraction, oil preparation, and laboratory methods [23, 27, 28].

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

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 S.macrosiphon along with antifungal drugs. However, confirmation of the present results in similar studies is nessessary.

Conclusion

Saliva microsiphon has great anticandida activity than fluconazole. Therefore, it can be hoped that it can be used as an antifungal compound in the treatment of *Candida* infections in the future. However, obtaining definitive results requires specialized laboratory studies and identification of the effective components of plants and clinical studies. Therefore, in future studies, this issue can also be investigated in the patient with candidiasis.

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