Chemical Composition and Antimicrobial Activity of *Oliveria decumbens* Volatile Oil from West of Iran

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Abstract

Background: ***Oliveria decumbens*** **Vent. is an endemic plant of Flora Iranica that grows in high temperature areas of south and west of Iran. It is used for indigestion, diarrhea, abdominal pain and fever in traditional medicine.**

Objective: In this investigation, chemical composition and antimicrobial effects of *Oliveria decumbens* has been studied.

Methods: The essential oil of aerial parts of *Oliveria decumbens* was obtained by hydrodistillation and analyzed by GC-MS. The antimicrobial activity of the essential oil was also investigated against three Gram positive bacteria, two Gram negative bacteria and two fungi using disc diffusion method.

Results: 10 components were identified in the essential oil of *Oliveria decumbens*. The main components were γ-terpinene, myristicin, thymol, ρ-cymene and carvacrol. The essential oil showed high antimicrobial activity against Gram-positive bacteria, *Escherichia coli*, *Aspergilus niger* and *Candida albicans* but low antibacterial effect against *Pseudomonas aeroginosa*.

Conclusion: High amount of thymol, carvacrol and ρ-cymene in *Oliveria decumbens* essential oil may be a reason of antimicrobial effects of the plant and the plant essential oil can be considered as a natural source of preservatives in food and cosmetic industries.

Keywords: ***Oliveria decumbens*****, Essential oil, Antimicrobial activity, GC-MS**

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Introduction

Essential oils are natural compounds which are obtained from aromatic plants. They are volatile liquid, insoluble in water, highly soluble in alcohol, ether, vegetable and mineral oils and are complex mixture of different chemical compounds. The chemical composition of essential oil differs in each species or subspecies and is characteristic for the species providing different toxic and medicinal properties to each one. The proportion of the essential oil components mainly depends on season but percentage of total essential oil is generally higher during summer [1]. Exact function of essential oils has not been known but several investigations have demonstrated antimicrobial activity of these compounds [2 - 4]. Therefore, recently, many of them are used in food industry to prolong shelf life of food products [1]. Identification of components in essential oils requires the use of several techniques. One of the most popular methods of studying essential oil composition is gas chromatography- mass spectrometry (GC-MS), which allows the identification of the specific natural compounds found in an essential oil by comparing their retention times indexes and their MS spectra [5].

Umbelliferae family is one of the biggest vegetable's families that has global distribution. Most of the plants belonging to this family produce terpenes and other types of volatile compounds. Many researches have been executed about antibacterial and preservative effects of herbal essential oils like oils that extract from plants of umbelliferae family [6, 7]. *Oliveria decumbens* Vent. belongs to Umbelliferae family and is an endemic plant of Flora Iranica that grows in high temperature areas of south and west of Iran [8]. In traditional medicine, it is used for indigestion, diarrhea, abdominal pain and fever [9]. According to our knowledge, there are no published reports on the chemical composition and antimicrobial activity of *Oliveria decumbens* essential oil from west of Iran. Thus the aim of this study is the identification of the volatile oil components of the plant aerial parts by GC-MS. In addition, antimicrobial activities against three Gram positive bacteria, two Gram negative bacteria and two fungi have also been evaluated.

Material and Methods

Plant Material

The aerial parts of the plant were collected in may 2006 from Charmahale va Bakhtiary: Lordegan, Sarkhon, Shalile to Duabe Bazoft and Karoon river, 1200 m and identified by Dr. V. Mozaffarian, Research Institute of Forests and Rangelands, Tehran, Iran (No. 54897, TARI).

Isolation of Essential Oil

The air dried and powdered aerial parts of the plant were subjected to hydrodistillation for 4h using a Clevenger type apparatus. The obtained essential oil was dried with anhydrous sodium sulphate and stored at +4°C before using.

Identification and Quantification of the Oil Components

Analytical gas chromatography was carried out using a HP-6890 GC with capillary column DB-1 (30 m × 0.25 mm i.d., 0.25 µm f.t.); carrier gas, He; flow rate 1.5 ml/min; split ratio, 1:25 and using a flame ionization detector. The column temperature was programmed at 50 °C for 1 min and then heated to 265 °C at a rate of 2.5 °C/min, then kept constant at 265 °C for 20 min; injector

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temperature, 265 °C; detector temperature, 300 °C; H_2 flow, 35 ml/min; air flow, 400 ml/min. GC-MS was performed on a Thermoquest 2000 with a quadrupole detector, on capillary column DB-1 (see GC). The MS operated at 70 eV ionization energy. Retention indexes were calculated using retention times of n-alkanes that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of retention indexes (RI, DB-1) with those reported in the literature [5] and by comparison of their mass spectra with the Wiley library.

Microorganisms

Three Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, Methicillin resistant *Staphylococcus aureus* and *Bacillus subtilis* ATCC 6633), two Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 8739) and two fungi (*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) were used in the experiments.

Antimicrobial Activity Assessment

Antimicrobial activity of the essential oil of *Oliveria decumbens* was determined using disc diffusion method. The bacteria inocula were prepared by suspending overnight colonies from Muller-Hinton (MH) agar media in 0.9% saline. The *C. albicans* and *A. niger* inocula were prepared by suspending colonies from 48h and 72h old Sabouraud dextrose (SD) agar cultures in 0.9% saline respectively. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml). MH and SD agar plates (100 mm diameter) were seeded individually with bacterial or fungal suspensions using a sterile cotton swab.

The essential oil was dissolved in dimethylsulfoxide (DMSO) 10% v/v at a concentration of 5 µg/ml and diluted in a two-fold manner to make the concentrations of 2.5, 1.25, 0.625, 0.312 and 0.156 µg/ml. Blank discs loaded with 20µl of the essential oil with different concentrations and put on the surface of plates. Blank discs containing 20µl DMSO 10% were used as negative controls. The plates containing bacteria were incubated at 30-35^{o}C for 24h and those containing fungi were incubated at 20-25^{o}C for 48h. After incubation, the mean inhibition zone diameter for each concentration was determined and the lowest one considered as the minimum inhibitory concentration (MIC).

Results

The essential oil of *Oliveria decumbens* aerial parts was light yellow with a sharp odor, in a yield of 2%.

10 components have been detected in the essential oil of *O. decumbens*, representing 99.73% of the total oil that have been shown in table 1.

The results of antimicrobial tests indicated that the essential oil has potent antimicrobial activities against Gram-positive bacteria (*Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis*), *Escherichia coli* (Gram-negative), *Aspergillus niger* and *Candida albicans* (fungi) but low antibacterial effect against *Pseudomonas aeroginosa* (Gram-negative). Moreover, the antifungal effect of the essential oil against *A. niger* is lower than *C. albicans* (table 2).

Discussion

The results obtained from *Oliveria decumbens* essential oil analysis showed that the oil contained 78.32% monoterpenes that all

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Table 1- Identified compounds in the essential oil of *Oliveria decumbens* Vent. Aerial parts

Compound	RI*	Amount (%)
Thujene	917	0.19
α-pinene	921	0.19
β-pinene	962	2.12
β-myrcene	975	0.29
ρ-cymene	1018	19.40
Limonene	1024	2.53
γ-terpinene	1053	23.33
Thymol	1271	20.46
Carvacrol	1289	9.54
myristicin	1501	21.68

Identified: 99.73%
*RI: Retention index in reference to C_8-C_{22} n-alkanes.

Table 2- Minimum inhibitory concentration of *Oliveria decumbense* Vent. essential oil against Gram-positive and Gram-negative bacteria and fungi by disc diffusion method

Microorganism	MIC (µg/ml)
Staphylococcus aureus ATCC 6538	0.312
Bacillus subtilis ATCC 6633	0.625
Methicillin-resistant *Staphylococcus aureus* (isolated)	0.625
Escherichia coli ATCC 8739	1.25
Pseudomonas aeroginosa ATCC 9027	>10
Candida albicans ATCC 10231	0.625
Aspergillus niger ATCC 16404	2.5

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of them except myrcene had cyclic structure and 30.0% of that was oxygenated compounds including thymol (20.46%) and carvacrol (9.54%). Among other monoterpenes, γ-terpinene and ρ-cymene were higher than others. Myristicin with phenylpropanoid structure has high concentration in the essential oil as well.

Another investigation which has been done on this plant from south of Iran (Shiraz) by Amin et al. [9] has shown the plant contains β-pinene (0.42%), β-myrcene (0.05%), ρ-cymene

(8.71%), limonene (0.76%), terpinene (18.94%), thymol (47.06%), carvacrol (23.31%) and myristicine (0.63%). Comparison between compounds of the plant essential oils from south and west of Iran shows thujene and α-pinene don't exist in the plant of south. In additioan, in our investigation myristicin is one of the major components of the essential oil (21.68%) along with ρ-cymene, γ-terpinene and thymol, whereas the amount of myristicin is very low in the plant of south (0.68%) and thymol is the major one. In both study, percentage of thymol is about twice of carvacrol. It is concluded that kind of components and proportion of each one in *Oliveria decumbens* essential oil are different in each growing area.

Comparison between results reported about antimicrobial properties of different essential oils is very difficult that varies in different methods of antimicrobial effects evaluation, source of essential oil and different genus of used microbes [10]. Several studies have been carried out on antimicrobial effects of plants and they have demonstrated that thymol and carvacrol are the most important components that are responsible for antimicrobial properties. In a study performed by Kim et al. [11], antibacterial effects of carvacrol on *Salmonella typhi* Murium and its rifampin-resistant genus was studied. In this research, carvacrol showed powerful bactericidal effect against rifampin-resistant genus in a sample of fish food. In another study, Karman et al. [12] showed powerful bacteriostatic effect of *Thymus revolatus* essential oil on *staphylococcus aureus*. They illustrated high amount of carvacrol in essential oil is possible reason of this effects. Similar study by Rasoli and Mirmostafa [13] about bactericidal effects of *Thymus pubescens* essential oil (with high amount of carvacrol) on *Staphylococcus aureus* and *Escherichia coli* was executed. Like previous study, high amount of carvacrol in the essential oil was mentioned as reason of powerful bactericidal effect of volatile oil. Similar results by Bagamboula et al. [14] and Horváth et al. [15] were obtained in studies about effects of Thyme and compounds of carvacrol and thymol on *Shigella sonnei*, *Shigella flexneri*, *Erwinia amylovora* and *Erwinia carotovora*. Delgado et al. [16] showed that when thymol and ρ-cymene were applied simultaneously, it resulted in a greater antibacterial effect than when the compounds were used separately. Therefore, the presence of high amount of thymol, carvacrol and ρ-cymene in *Oliveria decumbens* essential oil from west of Iran, could be considered as a reason of antimicrobial effects of the plant. According to the obtained results from this research and increasing usage limitations of synthetic antimicrobial agents because of side effects and microbial resistances, it is necessary to replace these substances with natural ones.

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