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Essential oil composition and antimicrobial, antioxidant activities of *Oliveria decumbens* Vent

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Abstract

A scientific experiment was conducted by utilizing herbal essential oil plants of *Oliveria decumbens* Vent by subjecting to extraction of essential oil through the standard microwave assisted hydro distillation (MAHD) method. The chemical composition of the essential oil detected with GC/MS technique. The obtained results clearly quantified main components oil like, m-thymol (34.80%), thymol (34.36%), myristicin (20.88%), etc. Further, antimicrobial activity of *Oliveria decumbens* Vent oil was noticed, which clearly showed on gram-positive and gram-negative effects respectively and some of the fungal species too. However, antibacterial activity against gram positive bacteria, *Staphylococcus aureus* was respected MIC=8 and antifungal activity against alternaria fungus was MIC=16 clearly noticed. Moreover, antioxidant activity was determined with DPPH oil through which obtained results was antioxidant activity of oil ($IC_{50} = 2.73 \pm 0.172$) recorded. Apart, total phenol reagent with Folin-Ciocalteu and gallic acid was determined with the standard curve and obtained results of total phenol of $IC_{50} = 0.072 \pm 0.027$ value was clearly evident.

Keywords: *Oliveria decumbens* Vent, GC/MS, antimicrobial, antioxidant.

1. Introduction

The popular “umbelliferae” family is one of the biggest and popularly known as vegetable’s family that have been distributed in all over the world. In addition to that, 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 [1, 2]. *Oliveria decumbens* Vent belongs to Apiaceae family is an endemic plant of flora iranica that grows in high temperature areas of south and west of Iran [3]. This plant have been calling since back several years with the different common Persian names are “*Mooshkorok*”, “*Den*”, and “*Denak*” [4] etc. In the background of traditionally known medicines, it is used for several purposes like, Indigestion, Diarrhea, Abdominal pain, Fever [5], etc. But currently, shift to different dimension, in regard to much research performed on antimicrobial compounds from plant extracts and essential oils, the goal being to identify novel lead structures with significant biological activities [6]. Moreover, the most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [7]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reactions and pathogens. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential [8-10]. Hence, keeping the above concept in mind, the prime focus of this research experiment was to understand the antioxidant and antimicrobial properties of *Oliveria decumbens* Vent.

2. Materials and methods

2.1 Chemicals

Folin-Ciocalteu, sodium carbonate methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, gallic acid and over all the total chemicals were purchased from the Merck Germany.

2.2 Plant material

All parts of the plant of *O. decumbens* Vent were collected during May 2015 from Southern Iran (Bandar Abbas). The all parts of the plant were kept at room temperature (25 °C) and dried under the shade area of the standard laboratory condition and plant samples were identified in the medicinal herbs Research Center of Bandar Abbas.

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2.3 Essential oil extraction

Microwave assisted hydro distillation was conducted in a domestic microwave oven operating at 2450 MHz and 990 W (White-Westinghouse, Pittsburg, USA). The delivered power level of the microwave oven was measured using IMPI-2 L test [11]. As in the case of conventional HD, 350 g of distilled water and 70 g of dried *O. decumbens* Vent were placed in flat-bottom flask combined to a cleverger apparatus. Moreover, microwave assisted hydro distillation was performed until further no more essential oil was obtained. Experiments were performed starting from the beginning each time to collect the essential oil samples at different extraction times since taking continuous data was not possible. For each condition, experiments were replicated twice [12]. The extraction of essential oil from *O. decumbens* Vent carried out utilizing the standard technique of MAHD as shown in Figure-1. The essential oil obtained with the intake of sodium sulfate and the vials were stored at 4 °C. As well as, the tests for the antimicrobial and antioxidant were conducted and essential oil was analyzed by GC/MS technique.

2.4 Gas Chromatography Mass Spectrometry (GC-MS) Analysis

The MAHD oil of *Oliveria decumbens* Vent was analyzed by GC and GC-MS, then gas chromatographic analysis was carried out in a Perkin-Elmer 8500 gas chromatograph equipped with an FID detector and a BP-1 capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The carrier gas was helium with a flow rate of 2 mL/min and the oven temperature was maintained at 60 °C for the first 4 minutes and later increased at a rate of 4 °C/min until reaching a temperature of 280 °C. The injector and detector temperatures were set at 280 °C. However, the confirmation of peak identity was accomplished by GC-MS. The mass spectra were recorded in an Agilent 7890 MS detector coupled with an Agilent 7890 gas chromatograph equipped with an HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The mass spectrometer conditions were as follows: ionized potential, 70 eV; source temperature, 200 °C [13]. Moreover, the components of the essential oil were identified by comparing their mass spectra fragmentation patterns with those stored on a Wiley7n.l MS computer library. Kovats' retention indices of all the constituents were obtained by interpolating between bracketingn-alkenes [14, 15].

3. Antimicrobial activity

3.1 Microorganisms utilized for the experiment

Microorganisms such as, gram negative-bacteria *Kleb seilla oxytoca* (ATCC 1402), Gram positive bacteria-*Staphylococcus aureus* (ATCC 1337) and the fungus like *aspergilsniger* (ATCC 5154), *Fusarium solani* (ATCC 5284) and *alternaria* (ATCC 5224) were utilized for the present experiment. All microorganisms were prepared in Institute of Biological Research, Islamic Azad University, Firozabad, Iran.

3.2 Assessment of antimicrobial activity

The antimicrobial activity of essential oils of plants has been used to determine the microdilution method [16]. Briefly, 100µl of a suspension of the test microorganism (108cells/ml)

was spread on Mueller-Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for the fungi. The 6 mm sterile disks, each containing 100µl of essential oil were placed on the microbial lawns. The bacteria and fungi plates were incubated at 37 °C for 24 hrs and 30 °C for 48 hrs [17] respectively.

3.3 Minimal inhibitory concentration (MIC)

The extracted oil was tested for antibacterial activity using the macro broth dilution method in broth media Mueller-Hinton (Difco). In these experiments, 100 µl of a suspension containing 1x 10⁶ CFU/ml was added to 100 µl of susceptibility test broth containing two fold dilutions of the EO in glass test tubes (13 by 100 mm) fitted with loose plastic non-screw caps. All tubes were incubated in air at 37 °C for 24 hrs. The MIC was considered the lowest concentration of the sample that prevented visible growth [18].

3.4 Antioxidant activity

The DPPH radical scavenging assay was determined according to the method reported by Brand-Williams *et al.* [19]. Briefly, 1mM DPPH radical solution was prepared in methanol and then 200 µl of this solution was mixed with 20µl of the equal sample solution in ethanol. After incubation for 30 minutes in the dark condition, the absorbance was measured at 515 nm. Moreover, it is important to note that, the decreasing of the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values were an average of three replicates was followed by the standard method of Obame *et al.* [20].

3.5 Total phenolic content analysis

The total phenolic content was determined with the Folin-Ciocalteu reagent as described by Singleton and Rossi [21]. Essential oil solution (0.5 ml) containing 500 µg oil was taken in a volumetric flask [22] with 1 mL of Folin Ciocalteu reagent (10-fold dilution with distilled water) and allowed to stand at 22°C for 5 minutes. Then, 4 mL of sodium carbonate (10.59 g/L) solution was added to the mixture followed by 15 min incubation at 22°C and absorbance was measured at 765 nm using a UV-visible spectrophotometer (Prim, SECOMAM, France). The total phenolic content was determined using a standard curve of gallic acid at 0.02 - 0.1 mg/mL concentrations. Finally, the total phenolic content was calculated for each sample and expressed as milligrams of gallic acid equivalent per 100 mL of juice [23].

4. Results and discussion

The composition of essential oil extracted from *O. decumbens* Vent using Microwave-assisted hydro distillation technique was detected with gas chromatography mass spectrometry. The total 14 combinations of essential oil were presented in the table 1 and figure-2. The main components of essential oil observed were m-Thymol (34.80%), Thymol (34.36%), Myristicin (20.88%) recorded meticulously in the form of MAHD (%) percentage from the *O. decumbens* Vent. The details was presented in the figure-3.

Table 1: Chemical compounds of essential oil of *Oliveria decumbens* Vent through MAHD

No.	Compound	KI	MAHD %
1	p-Cymene	1026	2.04
2	Moslene	1060	0.81
3	verbenone	1216	0.43
4	Thymol	1287	34.36
5	Carvacrol	1288	34.80
6	Hydroxyl-p-cymen	1302	0.73
7	Myristicin	1526	20.88
8	β -Elemene	1553	0.74
9	spathulenol	1583	0.64
10	Caryophyllene oxide	1590	0.41
11	Torreyol	1647	0.55
12	α -Eudesmol	1657	0.83
13	γ -Cadinene	1660	1.62
14	zizanal	1697	0.98
Total		20092	99.82

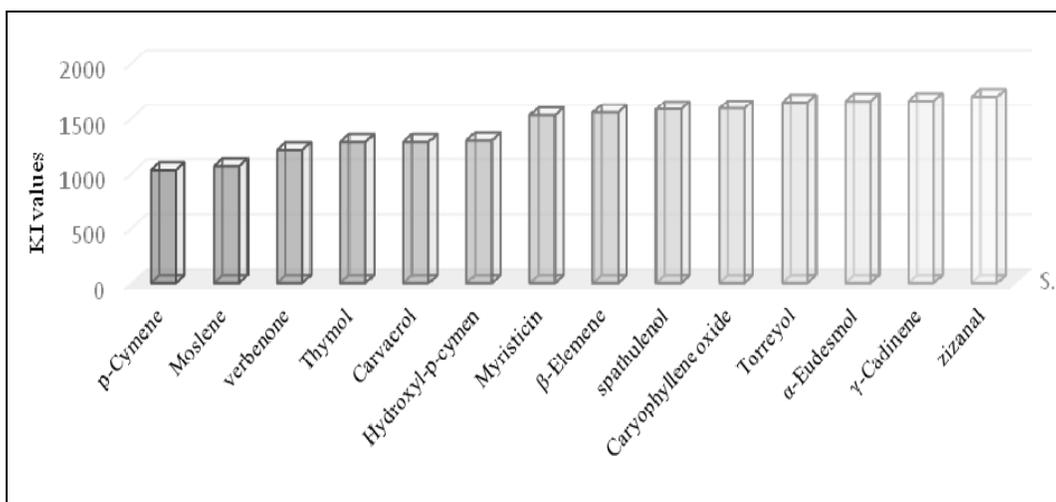


Fig 2: The KI values of *Oliveria decumbens* Vent

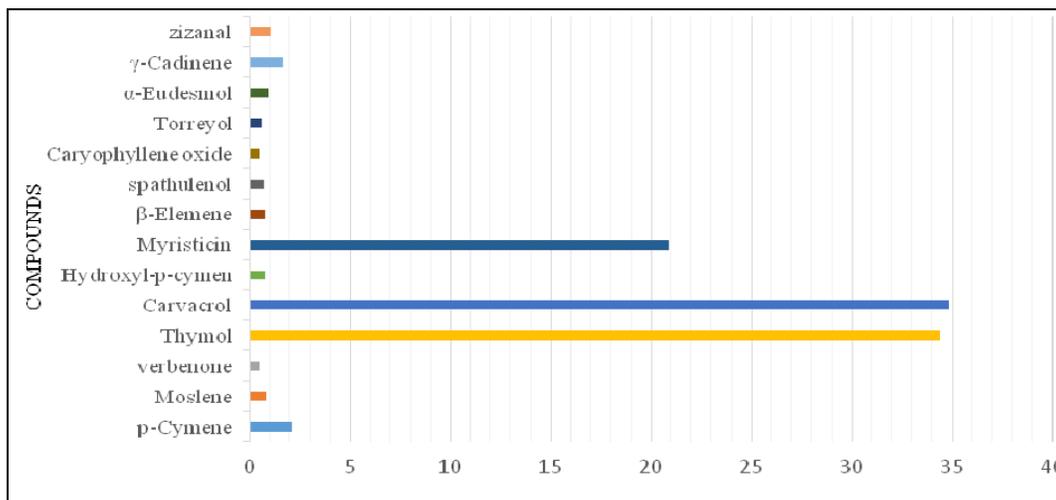


Fig 3: The percentage of different chemical compounds of *Oliveria decumbens* Vent

4.1 Antimicrobial activity

The antibacterial activity of essential oils of *Oliveria decumbens* Vent was calculated (mg/ml) and the total number of organism tested were *S. aureus*, *K. Oxytoca*, *A. Alternaria*, *F. Solani* and *A. Niger*. The results of bacterial against *Staphylococcus aureus* (MIC=8) and *Klebsiella Oxy Tuka* (MIC=16) were detected as bactericidal activity against *Staphylococcus Aureus*. Whereas, antifungal activity was also observed from fungus *Alternaria* and *K. Oxytoca* that showed

MIC=16 each. Further, fungus *fusarium Solani* and *Niger Aspergillus* were clearly observed of MIC=64. The antifungal activity against *Alternaria* fungus was also noteworthy in the present experiment carried out against *Fusarium* and *Aspergillus* that were both of them showed the MIC=64 same activity. Moreover, the activity of the antibacterial essential oils against *Staphylococcus aureus* bacteria and *Alternaria* fungus were due to the composition of the essential oil of thymol. The thymol is a combination of

non-polar organic compounds with a capacity of the showing high solubility and permeability of the outer membrane of the bacterial cell membrane, which has also damage and increases the synthesis and preventing activities of it. Apart, essential oil has also observed that the effect on gram-positive bacteria too, which overall have presented in table 2 and figure 4.

Table 2: Antibacterial activity of essential oils of *Oliveria decumbens* Vent (mg/ml)

Tested organism	<i>S. aureus</i>	<i>K. oxytoca</i>	<i>A. alternaria</i>	<i>F. solani</i>	<i>A. niger</i>
Essential oil	8	16	16	64	64

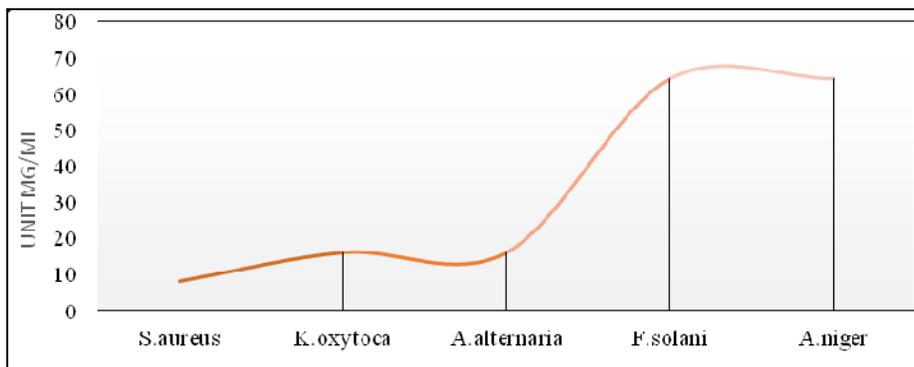


Fig 4: The antimicrobial activity (mg/ml) for different organism tested from the essential oil of *O. decumbens* Vent

4.2 Antioxidant activity and total phenol

The total phenolic content is responsible for antioxidant activity in medicinal plants [24]. It is well acknowledged that, the free radical may cause many disease conditions such as cancer and coronary heart disease in human [25, 26]. The total phenol reagent was used for the testing Folin–Ciocalteu and gallic acid was used as the standard curve and the antioxidant activity (Table 3) and same was represented as Figure 5.

Table 3: Antioxidant activity and total phenolic content of essential oil *O. decumbens* Vent

DPPH IC ₅₀ (mg/ml)	Total phenolic content (mg catechin equivalent/g oil)
2.73 ± 0.172	0.072 ± 0.027

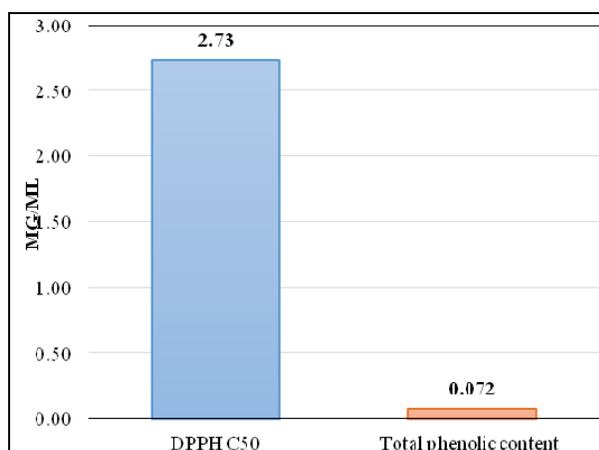


Fig 5: Antioxidant activity (mg/ml) and total phenolic content (catechin equivalent/goil) of the essential oil of *O. decumbens* Vent

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