Chemical composition, antimicrobial and antioxidant activities of *Dittrichia graveolens* (L.) Greuter essential oil

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S u m m a r y

The aim of the study was to survey the chemical composition, antimicrobial and antioxidant activities of *Dittrichia graveolens* essential oil in *in vitro* conditions. GC-FID and GC-MS analyses were performed to evaluate the chemical composition of essential oil. The antimicrobial activity against different kinds of microorganisms was determined by microbroth dilution assay. The antioxidant activity was evaluated by DPPH free radical scavenging system. Studies of chemical composition of essential oil revealed the presence of borneol (38.2%) and bornyl acetate (14.9%) as major constituents. Essential oil showed strong antimicrobial activity against *Enterococcus faecalis* isolates and *Bacillus cereus*. *Staphylococcus aureus* and *Escherichia coli* are less sensitive to the oil. The strong antimicrobial activity of oil against clinical isolates of bacteria was demonstrated. In addition, *D. graveolens* oil notably reduced the concentration of DPPH free radical with higher efficacy than that of trolox at 70 min.

***Key words**: antioxidant and antimicrobial activity, borneol, bornyl acetate, Dittrichia graveolens, essential oil, stinkwort*

Abbreviations: DPPH, 2,20-diphenylpicrylhydrazyl; RI, Retention Indices; MOPS, morpholinepropanesulfonic acid; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; MIC, minimal inhibitory concentration; MLC, minimal lethal concentration

INTRODUCTION

The aromatic plants have been traditionally used in order to improve the shelf life, quality and nutritional value of food. Most of these properties are due to the essential oils that were produced by their secondary metabolisms [1]. Essential oils and their components are gaining interest because of multi purposes functional uses. Antimicrobial and antioxidant activities of plant essential oils are being increasingly reported from different areas of the world.

Many different microorganisms can spoil the food and pharmaceutical products during their shelf life. In recent years, the aromatic plants have been used as flavoring agents in foods and beverages due to their antimicrobial and antioxidant properties.

Dittrichia is a genus of Asteraceae family that is used as a food source in domestic and in traditional medicine for treatment of ischemic heart disease, angina pectoris, hypoglycemia, liver disorders, gastroduodenal diseases and ulcers, bronchia, rheumatic complaints, migraine and skin infections.

This genus of this annual herbaceous species is known as *Dittrichia graveolens* (*Inula graveolens*, *Cupularia graveolens*, and *Erigeron graveolens*) in Iran. It grows in Iran, Afghanistan, Pakistan, Mediterranean regions and Southwest of Asia [2]. It occurs along roadsides, on waste ground, in humid and degraded soils, and near subsalt lands. *D. graveolens* is commonly known as a stinkwort. It germinates in late spring or summer. It flowers in autumn, producing seeds with a pappus of numerous bristles which help in dispersal [3]. *D. graveolens* is the only green plant that grows after periods of dry weather.

Stinkwort is traditionally used for treatment of urinary tract infections [4], hemorrhoids, cold, wound infection, louse [5] and some other disorders [6]. It has been reported that the extract of stinkwort possesses conspicuous biological activities, such as high bactericidal effect against *Mycobacterium aurum* A^+, *Staphylococcus aureus*, *Escherichia coli* [7], cytotoxic [8], ichtyotoxic [9] and antiproliferative [10] effects.

The chemical composition of *D. graveolens* oil has been subjected to the number of studies. It has been found that stinkwort oil from Lebanese contains bornyl acetate, τ-cadinol, borneol [11]; from Greece bornyl acetate, epi-α-cadinole [12]; from Iran borneol, β-caryophyllene, bornyl acetate [13] as major components. Other report revealed the presence of bornyl acetate and camphene as the main components [14]. In this study, the chemical composition of stinkwort essential oil was analyzed, its antimicrobial and free radical scavenging activities in *in vitro* condition was surveyed.

MATERIALS AND METHODS

Plant material

The flowering aerial parts of *Dittrichia graveolens* (L.) Greuter (whole aerial part at flowering stage) were collected from Shoshtar suburb (Khozestan Province,

Iran) in October 2008. The voucher specimen was identified and deposited at the Herbarium of Agriculture Department, Research Center of Barij Essence, Kashan, Iran (No. 172-1).

Extraction, isolation and identification of the oil

The aerial parts of plant (100 g) were dried in shade and crushed. Materials were hydrodistilled for 6 h using a Clevenger type apparatus. The essential oil was dried by anhydrous sulfate and was kept into closed bottle until the analysis. The oil analysis was carried out using GC-FID and GC/MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230°C at a rate of 3°C/min., and maintained for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. The GC/MS analysis was conducted on a HP 6890 GC system coupled with a 5973 network mass selective detector with a the same as above capillary column, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature were programmed identically to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, NIST (National Institute of Standards and Technology) and data published in the literature [15].

Microbial strains and growth media

Tested bacteria include seven strains of *Staphylococcus. aureus* cultured from patients at Tehran University hospitals as well as *S. aureus* ATCC 25923 and eight isolates of *Enterococcus faecalis*, *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027. Fungi included seven clinical isolates of *Candida albicans*, one ATCC type strain (10231), *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* ATCC 15517. Bacteria and fungi were cultured on Brain Heart Infusion (BHI) agar and Sabouraud dextrose agar, respectively.

Determination of minimum inhibitory (MIC) and lethal (MLC) concentrations

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of oil were determined by microbroth dilution assay. The oil was serially diluted twofold with 10% DMSO containing 16–0.0125 μl/ml of oil. Vancomycin, gentamycin and amphotericin B from Sigma at concentration 16–0.0125 μg/ml were used as positive controls. These dilutions were

prepared in a 96-well microtiter plate. The RPMI 1640 buffered with 0.165 M morpholinepropanesulfonic acid (MOPS) (Merck KGaA, Darmstadt, Germany) (for fungi, yeast) [16], cation adjusted Muller Hinton broth (non fastidious bacteria) [17] and Todd Hewitt broth (*E. faecalis*) [18] were used as broth media. After shaking, 100 μl of oil was added to each well. The cultured bacteria were suspended in normal saline but spore of fungi and yeast were inserted in RPMI 1640 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). The turbidity of microorganisms was adjusted to 0.5 McFarland. The inoculations were 1×10^8, 1×10^6 CFU/ml for bacteria and fungi, respectively. The above mentioned microbial suspensions were diluted (1×10^6 CFU/ml for bacteria; 10^4 for fungi) and then 100 μl was added to each well and incubated at 35°C. The MIC was defined as the lowest concentration of compound that inhibits bacteria after 24 and fungi after 48 h. The MLC value was the first well that showing no growth on solid media.

Radical scavenging capacity of the oil by DPPH assay

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of ethanol solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [19].

1 mM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a stable antioxidant were used as positive controls (purchased from Sigma). Briefly, fifty microlitres of 1:5 concentrations of the oil in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 0, 30, 70 min incubation period at a room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated as follows:

$$I\% = [A_{blank} - A_{sample}/A_{blank}] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the tested compound. Tests were carried out in triplicate.

RESULTS

Chemical composition

The oil analysis showed 63 peaks of which 60 compounds were identified (tab. 1). These accounted for over 97.1% of total oil. The major components were borneol (38.2%), bornyl acetate (14.86%) followed by 3,4-diethyl phenol (7.5%), β-cubebene (7.1%) and caryophyllene oxide (4.0%).

Table 1

Chemical composition of *Dittrichia graveolens* (L.) essential oil

Compound	RI[a]	%
α-Pinene	928	0.09
Camphene	944	0.12
2-Heptenal	947	0.02
Benzaldehyde	958	0.04
2β-Pinene	971	0.05
6-Methyl-5-hepten-2-one	982	0.03
2,4-Heptadienal (cis,cis)	998	0.06
4-Ethyl-1,2-dimethylbenzene	1016	0.04
Limonene	1024	0.09
1,8-Cineole	1028	0.16
2-Methylene cyclopentanol	1048	0.02
o-Tolualdehyde	1066	0.03
β,β-Dimethylstyrene	1073	0.08
α-Terpinolene	1081	0.26
Nonanal	1089	0.14
6-Methyl-3,5-heptadiene-2-one	1098	0.04
β-Thujone	1102	0.03
Trans-ρ-mentha-2,8-dienol	1115	0.09
2-Ethyl hexanoic acid	1123	0.04
Trans-ρ-menthadien-1-ol	1131	0.07
Alcanfor	1144	0.61
Neroloxide	1149	0.44
Borneol	1165	38.2
ρ-Cymen-8-ol	1170	0.53
Trans-ρ-menth-2-en-1,8-diol	1189	2.19
Decanal	1195	0.18
β-Methyl-benzenepropanal	1211	0.5
Trans-carveol	1217	0.1
Nerol	1228	0.4
Carvol	1231	0.09
Vetiverol	1238	0.08
Piperitone	1247	0.25
Nonanoic acid	1257	0.14
4-Hydroxy-3-methylacetophenone	1269	0.11
Carvacrol	1286	0.06

Compound	RI[a]	%
Bornyl acetate	1289	14.86
1-(5(Methyl-2-furanyl)-1-buten-3-one	1293	0.13
ρ-Mentha-1(7),2-dien-8-ol	1299	0.39
2,4-Decadienal	1317	0.32
1-Methylnaphtalene	1319	0.04
Hydragine	1356	0.2
β-Damascenone	1379	0.4
Caryophyllene	1417	1.98
6,10-Dimethyl-5,9-undecadien-2-one	1436	0.34
3,4-Diethylphenol	1452	7.5
β-Lonone	1457	0.28
β-Selinene	1463	0.26
α-Selinene	1471	0.16
α-Amorphene	1488	0.4
6,6-Dimethyl-2-[2'-(trimethylsilyl)ethyl)cyclohex-2-enone	1493	1.7
Geranyl propionate	1540	1.8
Geranyl butyrate	1565	2.0
Caryophyllene oxide	1578	4.0
Cyercene	1581	1.1
β-Cubebene	1623	7.1
α-Cadinol	1659	0.38
5-Epi-paradisiol	1680	0.89
Geranyl hexanoate	1755	0.85
Hexafarnesyl acetone	1800	2.5
Isovaleric acid 3-phenylpropyl ester	1980	2.1

[a] retention indices

Antimicrobial activity

Nine different ATCC type strains of microbial species, including Gram-positive bacteria (*S. aureus*, *E. faecalis*, *B. subtilis*), Gram-negative bacteria (*E. coli*, *P. aeruginosa*), yeast (*C. albicans*, *C. glabrata*) and fungi (*A. niger*, *A. parasiticus*) were used to evaluate the possible antimicrobial activity of stinkwort oil. The results are given in table 2. The MICs and MLCs of oil against all of the tested microorganisms were in the range of 0.25–4 and 1–8 μl/ml, respectively. *E. faecalis* and *C. glabrata* were the most sensitive microorganisms with the lowest MIC and MLC values (0.5 and 1 μl/ml), whereas the least susceptible microorganisms were *S. aureus* and *E. coli* (4 and

8 μl/ml). The type culture collection strains employed in this study could be categorized from the most susceptible as *E. faecalis*, *C. glabrata* > *B. subtilis*> *C. albicans*, *P. aeruginosa* > *A. niger*, *A. parasiticus* >*S. aureus* and *E. coli*. Differences in antimicrobial activity of stinkwort oil were seen with respect to test bacterial strain. There were differences in results of antimicrobial effect of stinkwort on clinical and type culture collections of microorganisms (tab. 2, 3). The MIC values of clinical isolates of *S. aureus* were lower than that of ATCC *S. aureus*. The *E. faecalis* isolates were more sensitive than the other microorganisms. The essential oil of stinkwort exhibited the activity against 21 bacteria, 2 fungi and 10 yeast isolates. *S. aureus*, *E. faecalis* and *C. albicans* exert the MIC values ranging from 1–2, 0.125–1 and 0.5–2 μl/ml, respectively.

Table 2

Determination of MIC, MLC of the essential oil against ATCC type strains of microorganisms

Microorganisms	minimal concentrations							
	essential oil [μl/ml]		vancomycin [μg/ml]		gentamycin [μg/ml]		amphotericin B [μg/ml]	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
Staphylococcus aureus	4	8	2	2	-	-	-	-
Enterococcus faecalis	0.5	1	4	8	-	-	-	-
Bacillus subtilis	1	2	0.5	0.5	-	-	-	-
Escherichia coli	4	8	-	-	4	4	-	-
Pseudomonas aeruginosa	2	4	-	-	8	8	-	-
Aspergilus niger	0.25	4	-	-	-	-	0.25	0.5
Aspergilus parasiticus	0.25	4	-	-	-	-	1	1
Candida glabrata	0.5	1	-	-	-	-	0.5	1
Candida albicans	2	4	-	-	-	-	0.5	1

MIC – minimal inhibitory concentration, MLC= minimal lethal concentration

Table 3.

Determination of MIC, MLC of the essential oils against clinical strains of microorganisms

Microorganisms	minimal concentrations					
	essential oil [μl/ml]		vancomycin [μg/ml]		amphotericin B [μg/ml]	
	MIC	MLC	MIC	MLC	MIC	MLC
Staphylococcus aureus 26	2	4	4	8	-	-
Staphylococcus aureus Animal	2	4	1	1	-	-
Staphylococcus aureus 6	1	2	0.5	1	-	-
Staphylococcus aureus 4	2	4	0.25	0.5	-	-
Staphylococcus aureus 34	2	4	1	1	-	-
Staphylococcus aureus 8	2	4	0.25	0.5	-	-

Microorganisms	minimal concentrations					
	essential oil [μl/ml]		vancomycin [μg/ml]		amphotericin B [μg/ml]	
	MIC	MLC	MIC	MLC	MIC	MLC
Staphylococcus aureus 31	2	4	0.5	1	-	-
Staphylococcus aureus 34	2	4	1	1	-	-
Enterococcus faecalis	0.125	0.25	1	2	-	-
Enterococcus faecalis Tx0016	1	2	2	4	-	-
Enterococcus faecalis van A250	1	2	4	8	-	-
Enterococcus faecalis 43186	0.5	1	1	2	-	-
Enterococcus faecalis 11696	0.5	1	>16	>16	-	-
Enterococcus faecalis 25788	0.5	1	4	8	-	-
Enterococcus faecalis 6056	0.5	0.5	8	16	-	-
Enterococcus faecalisvan A256	1	1	4	8	-	-
Candida albicans 20	1	2	-	-	0.25	0.5
Candida albicans 35	0.5	1	-	-	0.5	1
Candida albicans 33	1	2	-	-	0.5	1
Candida albicans 27	2	4	-	-	0.5	1
Candida albicans purple	2	4	-	-	0.5	1
Candida albicans 25	0.5	1	-	-	1	2
Candida albicans green	2	4	-	-	0.5	1
Candida albicans 22	0.5	1	-	-	0.5	1

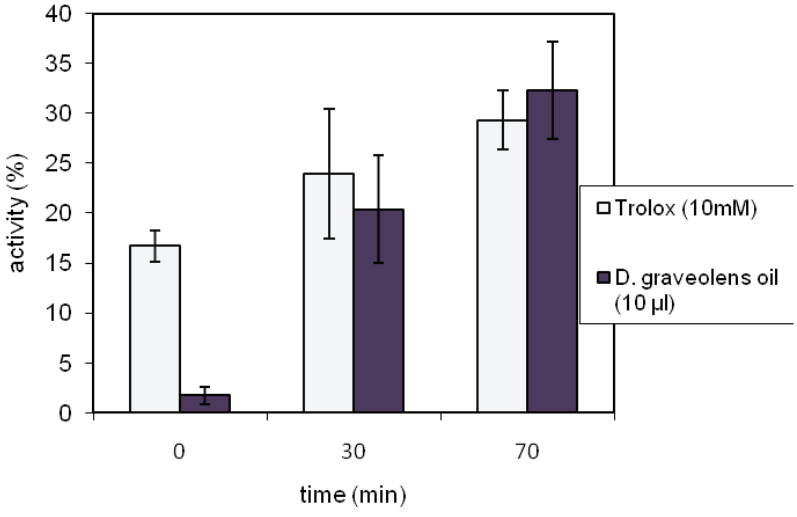

Figure 1.
Free radical scavenging of *D. graveolens* essential oil in comparison with Trolox

Free radical scavenging capacity of the oil

The literature search reveals that the oil of *D. graveolens* has not been the subject of antioxidant activity. The DPPH radical scavenging activity of stinkwort essential oil is shown in Fig. 1. 10 μl of essential oil notably reduced the concentration of DPPH free radical with an efficacy higher than that of 1mM Trolox (t=70 min). Stinkwort oil reduced the DPPH to 32.3%.

DISCUSSION

Ghosen *et al*. reported that stinkwort oil contained large quantities of bornyl acetate, τ-cadinole and borneol. Bornyl acetate and τ-cadinole were main components [11]. Petropoulou *et al*. reported epi-α-cadinol and bornyl acetate to be the major components. Epi-α-cadinol was the first one of them [12]. In our study, τ-cadinole was not detected in the oil and the amount of α-cadinol was trace. Harzallah-Skhiri *et al*. reported that bornyl acetate (33.4%) and borneol (21.4%) as the major components of aerial parts essential oil [20].

Our results are in agreement with the study that confirmed bornyl acetate and borneol as major components of stinkwort essential oil and we have shown that stinkwort essential oil has the same composition as of that study [20] although our results showed some quantitative differences from previous reports.

Borneol is a fragrance ingredient widely used in decorative cosmetics, foods as well as household cleaners and detergents. It is suggested that stinkwort could be a new borneol resource for food and cosmetic applications.

Major chemical components of essential oil play the prime role in antibacterial activities of stinkwort oil. There is a relationship between the chemical composition of essential oil and antimicrobial activities. Although they usually occur as complex mixtures, their activity may generally account for in terms of the major components. Probably, similar components such as borneol and bornyl acetate detected in our experiment could be responsible for this property.

The pronounced antimicrobial property of borneol as well as bornyl acetate was described previously [21-23]. Borneol exhibited strong antimicrobial activity against *S. aureus*, *C. albicans*, *E. coli*, *B. subtilis*, *P. aeruginosa* and *A. niger*, respectively [24]. While our results showed that stinkwort oil had less activity against *S. aureus* but strong effect against *E. faecalis*. Stinkwort essential oil is a complex mixture of major and minor compounds that can affect the antimicrobial activity of each component alone. Therefore, our result might be different from those of other investigators.

Although caryophyllene oxide, 1,8-cineole, carvacrol, caryophyllene are minor constituents of essential oil, they seem to be responsible for antibacterial activity in stinkwort. Carvacrol acts as antifungal and antibacterial agent [25]. Caryophyllene and caryophyllene oxide have been also claimed to indicate antibacterial and antifungal activity [26, 27]. Minor and major components, as well as possible

interaction between the substances could contribute to antimicrobial properties of oil. No studies have been performed concerning the antimicrobial activity of stinkwort essential oil, whereas several studies have been performed concerning the antimicrobial extracts of stinkwort [7]. Our results showed that stinkwort has the highest effect as antimicrobial agent.

Among the identified compounds in essential oil, borneol and bornyl acetate may be considered as main contributors of antioxidant activity [28]. Antioxidants play an important role in the protection of human against infections and degenerative diseases as well as in the reduction to reduce the risk of chronic diseases, including cancer and heart diseases. As it was mentioned above, investigation revealed that the stinkwort essential oil (t=70 min) is an antioxidant and its activity was higher than that of synthetic antioxidant. So, stinkwort can be used to protect human from oxidative stress damage. Further studies can lead us to identify the antioxidant components of essential oil for better use of this plant.

CONCLUSION

Our results revealed that borneol, bornyl acetate, 3,4-diethyl phenol, β-cubebene and caryophyllene oxide are the major components of stinkwort essential oil from flowering aerial parts (stem, leaves and flower) possesses antimicrobial and free radical scavenging activity. It can also explain why the stinkwort has been used as antimicrobial agents against different kinds of microbial spoilage in traditional medicines.

We exhibited the strong antimicrobial activity of oil against clinical isolates of microorganisms, especially *E. faecalis* that were established as antibiotic resistant bacteria and responsible for a wide variety of infectious diseases [29] but need further investigation to evaluate the suitability of remarkable antimicrobial properties against *E. faecalis* in practical applications. Regarding the antioxidant activity of stinkwort essential oil, the essential oil may be an alternative to more toxic synthetic antioxidants and preservatives in food products.

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ANALIZA SKŁADU CHEMICZNEGO ORAZ OZNACZENIE AKTYWNOŚCI PRZECIW- DROBNOUSTROJOWEJ I PRZECIWUTLENIAJĄCEJ OLEJKU Z DITTRICHIA GRAVEOLENS (L.) GREUTER.

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S t r e s z c z e n i e

Celem niniejszej pracy była analiza składu chemicznego oraz oznaczenie w warunkach *in vitro* aktywności przeciwbakteryjnej i przeciwutleniającej olejku z *Dittrichia graveolens*. Analizę składu chemicznego przeprowadzono za pomocą chromatografii gazowej z detektorem płomieniowo-jonizacyjnym (GC-FID) oraz chromatografii gazowej sprzężonej z detektorem spektroskopii masowej (GC-MS). Właściwości przeciwdrobnoustrojowe oznaczono, wykorzystując test wielokrotnych rozcieńczeń w stosunku do różnych rodzajów drobnoustrojów. Właściwości antyoksydacyjne olejku określono przy użyciu metody zmiatania wolnych rodników DPPH. Analiza składu chemicznego olejku wykazała obecność, jako głównych składników, borneolu (38,2%) oraz octanu bornylu (14,9%). Olejek z *Dittrichia graveolens* wykazywał silne właściwości przeciwdrobnoustrojowe w stosunku do szczepów *Enterococcus faecalis* oraz *Bacillus cereus*, natomiast *Staphylococcus aureus* i *Escherichia coli* były mniej wrażliwe na jego działanie. W badaniach wykazano silne działanie przeciwdrobnostrojowe w stosunku do różnych szczepów bakterii wyizolowanych od pacjentów. Ponadto olejek z *D. graveolans* wykazywał wyraźnie wyższy stopień zmiatania wolnych rodników w obecności DPPH w czasie 70 min. w porównaniu z troloksem.

Słowa kluczowe: *aktywność przeciwutleniająca oraz przeciwdrobnoustrojowa, borneol, octan bornylu, Dittrichia graveolens, olejek eteryczny*