Journal of Medicinal Plants Research Vol. 5(5), pp. 751-757, 4 March, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil

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Accepted 8 November, 2010

The present study explores the chemical composition, antimicrobial and antioxidant activities of Omani basil (*Ocimum basilicum)*. Omani basil essential oil was extracted using a Clevenger type appartus. The oil yield of Omani basil was found to be 0.171%. A total of 75 compounds representing 99.8% of Omani basil oil were identified. Linalool (69.9%) was identified as the major component present in Omani basil oil, followed by geraniol (10.9%), 1,8-cineole (6.4%), α-bergamotene (1.6%) and geranyl acetate (1.4%). Omani basil essential oil exhibited strong antibacterial activity against all the bacteria tested except *Pseudomonas putida* and *Pseudomonas aeruginosa*. The strongest inhibition activity of Omani basil was observed against *Streptococcus pneumoniae 2* (60 mm), *Hemophilus influenzae* (45 mm), *Candida albicans* (45 mm), *S. pneumoniae* 1 (37 mm) and *Aspergillus niger* (35 mm). Total antioxidants content quantified in Omani basil essential oil using a commercial kit were found to be 50.32±1.8 mM. The essential oil extracted from Omani basil was found to have higher linalool content, antimicrobial and antioxidant activities than most of earlier reported values. In this regard, this variety can be very useful for medical purposes and in food and perfumery industries.

Key words: Omani basil, essential oil, GC-MS, antibacterial, antioxidant.

INTRODUCTION

Basil called Rehan in Arabic (*Ocimum basilicum* L.) belonging to the plant family Lamiaceae is an annual plant usually producing white-purple flowers (Daneshian et al., 2009). Omani basil can be distinguished from other basil varieties due to its height and different look. It is consumed as a seasoning in dry and fresh form. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. The economic value of basil essential oil is well known all-around the world due to its utilization for cookery, pharmaceutical and cosmetic purposes. Traditionally basil is used in folk medicine due to its stimulant, carminative and antispasmodic properties (Morris et al., 1979; Marotti et al., 1996). The medicinal value of any plant depends on bioactive phytochemical constituents that produce definite physiological action in the human body. Bioactive phytochemical constituents like alkaloids, phenolics, flavonoids, essential oils, tannins and saponins are usually responsible for medicinal importance of herbal plants (Krishnaiah et al., 2009; Kahkonen et al., 1999). Antioxidants obtained from natural plant sources are more potent and safe due to their harmless nature. Medicinal plant and wild herbs are always under investigation due to these facts (Kahkonen

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et al., 1999). Basil is one of the most important medicinal and aromatic plants because of the continuous and increase demand of its products from the local and international markets. Basil essential oil is extensively used for flavoring food stuffs such as souces, vinegars, pickles, ketchups, beverages, condiments and confectionery goods. Basil essential oil is also important part of toiletry products such as mouth washes and dental creams. In perfumery basil essential oil is used for compounding certain popular perfumes and jasmine blends. Basil is also recognized as a febrifuge and antimalarial plant. Thus, infusion of the plant is used for gouty joints, cephalalgia and gargle for foul breath. Relief in irrigation for throat, earache and ring worm is also well known properties of basil extracts (Husain et al., 1988).

The purpose of this study was to evaluate Omani basil as a new potential source of natural antioxidants and phenolic compounds. Our study also first time explores essential oil composition, antimicrobial and antioxidant activities of Omani basil.

MATERIALS AND METHODS

Plant material

Omani basil seeds (*O. basilicum* L.) were collected from small farmers, home gardens and wild areas in Oman and were grown to the flowering stage at Sultan Qaboos University, Muscat, Oman, Agriculture Experimental Station (AES), Oman. Seeds of Omani basil were grown in a sterilized soil mixture in 6 inch deep plastic trays. After two months these seedlings of Omani basil were transferred to shade house. Omani basil plants started flowering after three months

Chemical reagents

All chemicals used in the present study were of analytical grade and obtained from Sigma Co. (St. Louis, MO, USA).

Essential oil extraction

On flowering stage fifty basil plants were harvested and essential oil was extracted from them using Clevenger type distillation apparatus. Omani basil biomass was weighed (2.5 kg) and cut into small pieces and subjected to hydrodistillation for 5 h. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was yellow-greenish liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis.

Characterization of constituents in essential Oil

The oils obtained were immediately analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the chemical constituents present in the essential oils. The essential oils were analyzed with MS Clarus 600 Perkin Elmer equipped with FID detector. Column ULTRA-1 (0.20 mm x 25 m x 0.33 µm) packed with 100% dimethyl polysiloxane was used. The carrier gas used was helium with a flow rate 1.0 mL/min. Temperature was kept at 45°C for 6 min and programmed to reach 250°C for 10 min at the rate of 3°C per min with hold up time of 2min. Both injection and detection temperature of sample is adjusted at 250°C. Identification of individual compounds was based on the comparison of mass spectra of compound with mass spectra data base (NIST Mass Spectral Library v 2.0a 2002).

Antimicrobial testing

The antimicrobial activity of different essential oils was evaluated by the diffusion method. Briefly, the test was performed in sterile Petri dishes (100 mm diameter) in solid and sterile Muller–Hinton agar medium (Chocolate agar and Blood agar medium were used for *Hemophilus* species, and *S. pneumoniae*, respectively). The oils were adsorbed on sterile filter paper discs (10 µl per disc of 5 mm diameter) and placed on the surface of the media previously inoculated with a sterile microbial suspension (one microorganism per Petri dish). All Petri dishes were sealed with sterile laboratory films to avoid eventual evaporation of the test samples, then incubated at 37°C (in some experiment with 5% CO_2) for 24h, followed by the measurement of the zone diameter of the inhibition expressed in mm. The experiment was done in duplicate.

Total antioxidants content

Total antioxidants content was quantified for basil oil using a commercial kit (RANDOX, UK) and the measurements were of 10 samples (n = 10); mean ± SD. A summary of assay used for total antioxidant content determination is as follow: A colorimetric method using Randox assay kit (Randox Laboratories Ltd, UK) was used to measure TAC. The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzithazioline sulphonate) ABTS with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation $ABTS^+$ which has a relatively stable blue-green color, measured at 600 nm. Antioxidants in the assayed samples inhibit the oxidation of ABTS to $ABTS^+$ (cause suppression of the color production) to a degree that is proportional to their concentration. The capacity of the antioxidants was compared with that of standard Trolox, a water soluble tocopherol analogue, which is widely used as a traditional standard for TAC measurement assays.

RESULTS AND DISCUSSION

Yield of essential Oil

Omani basil essential oil was extracted using specially designed Clevenger type apparatus. Hydrodistillation is thought to be a suitable method to extract volatile components of essential oil. Besides, this is also one of the economical methods. The Omani basil plants were bushy type with height more than 40 inches (Figures 1 and 2). The biomass harvested per plant was 0.3222 ± 0.0112 kg. All soft parts of Omani basil plants were used for extraction of essential oil. The harvesting of biomass was done in month of May. The oil yield of Omani basil was found to be 0.171 ± 0.0112% (Table 1). A great variation in the essential oil content among the basil accessions, ranging from 0.07 to 1.92% was observed by Zheljazkov et al. (2008) in a previous study. The essential oil content and composition in medicinal and aromatic

Figure 1. Omani Basil (*Ocimum basilicum).*

Figure 2. Omani Basil (*Ocimum basilicum)* grown in a shade house

plants is affected primarily by plants genotypes and other conditions such as soil and climatic conditions, growing techniques, harvest time, irrigation, as well as fertilization (Merk, 1988; Muzika, 1989; Lee and Yang, 2005). The physical and chemical properties of Omani basil oil (Table 2) although not same but comparable to a previous study done by Hussain et al. (2008).

Determination of essential oil content and composition of Omani basil oil

The essential oil contents and components identified in the herbage of Omani basil are tabulated in Table 3, together with their relative percentages. A total of 75 compounds representing 99.8% of Omani basil oil were identified. Linalool was identified as the major component present in Omani basil oil (69.9%), followed by geraniol (10.9%), 1, 8-cineole (6.4%), α-bergamotene (1.638623%) and geranyl acetate (1.353949%). Omani basil mainly consisted of oxygenated monoterpenes followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Linalool was also found as a main component in basil oil in previous studies (Hussain et al., 2008; Telci et al., 2006; Mondello et al., 2002; Jirovetz and Buchbauer, 2001; Gurbuz et al., 2006). Jirovetz and Buchbauer (2001) found a high level of linalool (71.4%) in *O. basilicum* essential oil from Bulgaria. Bulgarian basil oil is considered as a high quality essential oil due to higher linalool contents. Omani basil essential oil was also found to contain linalool as a major constituent (69.9%). Linalool is a naturally-occurring terpene alcohol found in many spices and flowers. Linalool due to its floral pleasant scent has many commercial applications in perfumed hygiene products and cleaning agents including soaps, detergents, shampoos, and lotions. Linalool is also used as a chemical intermediate in synthesis of vitamin E. Additionally, linalool is used by pest professionals as a flea and cockroach insecticide (Klimánková et al., 2008; Ahmed et al., 2000). In a study it was found that inhaling linalool can reduce stress in lab rats. The findings could form the basis of new blood tests for identifying fragrances that can soothe stress (Nakamura et al., 2009).

Antimicrobial activity

The antimicrobial activity of Omani basil oil was tested against thirteen highly pathogenic bacteria is shown in Table 4. Omani basil essential exhibited strong antibacterial activity against all the bacteria tested except *P. putida* and *P. aeruginosa*, which were found highly resistant to Omani basil oil. The antimicrobial activity of basil was evaluated by measuring the zone of inhibition. The strongest inhibition activity of Omani basil was observed against *S. pneumoniae 2* (60 mm), *H. influenza* (45 mm), *C. albicans* (45 mm), *S. pneumoniae* 1 (37 mm) and *A. niger* (35 mm). *S. pneumoniae*, or pneumococcus, is a member of the genus *Streptococcus.* This gram positive bacteria is α-hemolytic and bile soluble aerotolerant anaerobe. In past it was found as a major cause of pneumonia and other diseases including otitis media, acute sinusits, meningitis, sepsis, bacteremia, osteomyelitis, septic artritis, peritonitis, pericarditis, cellulitis, brain abscess and endocarditis. *S. pneumoniae* is also a common cause of bacterial meningitis in adults and children and dogs and is one of the top two isolates found in ear infection, otitis media (Dagan, 2000). *H. influenzae*, formerly called Pfeiffer's bacillus or *Bacillus influenzae*, is a non-motile Gram-negative rod shaped

Table 1. Biomass and oil yield of Omani basil.

Harvested biomass per 50 plants	% oil yield	% moisture contents
16.11 kg	0.171 ± 0.0112	78.66 ± 1.33

Table 2. Physical and chemical properties of Omani basil oil

Appearance	Yellow-greenish liquid
Odor	Sweet vaguely anise-like minty pleasant odor.
Solubility	Water insoluble. Soluble in alcohol and other organic liquids
Boiling point	215 ± 2℃
Specific gravity	0.904± 0.043 @ 25℃
Optical rotation	--9.0 ± 0.043 @ 25℃
Refractive index	1.4532 ± 0.065 @ 25℃
Acid value	1.8 ± 0.30
Ester value	~ 35
Flash point	165 ℉

Table 3. GC-MS analysis of Omani essential oil.

Peak #	Compound name	Area %
1	3-hexen-1-ol	0.020
2	α-Pinene	0.128
3	Camphene	0.036
4	Sabinene	0.204
5	2- β -Pinene	0.363
6	1-Octen-3-ol	0.1000
7	β -Myrcene	0.524
8	3-octanol	0.011
9	cis-3-hexenyl acetate	0.001
10	α-Terpinene	0.023
11	Ortho-Cymene (cymol)	0.004
12	Limonene	0.322
13	1,8-Cineole	6.432
14	β-cis-Ocimene	0.025
15	β-trans-ocimene	0.328
16	γ-Terpinene	0.040
17	cis-Sabinene hydrate	0.044
18	1-octanol	0.176
19	Linalool	69.92
20	Octenyl acetate	0.007
21	fenchyl alcohol	0.015
22	2-Cyclohexen-1-ol	0.011
23	Camphor	0.673
24	Trans-chrysanthemal	0.007
25	Bicylogermacrene	0.013
26	Isoborneol	0.013
27	4-Terpineol	0.125
28	Limonene oxide	0.017
29	α-Terpineol	0.812
30	Myrtenol	0.071

Table 3. Contd.

31	p-Allylanisole	0.691
32	Acetic acid,decyl ester	0.044
33	Fenchyl acetate	0.061
34	Nerol (cis-geraniol)	0.269
35	Pulegone	0.015
36	z-citral	0.326
37	Geraniol	10.854
38	Carvone	0.050
39	E-citral	0.510
40	L-Bornyl acetate	0.089
41	Tridecane	0.016
42	Myrtenyl acetate	0.053
43	Acetic acid 1,3,3, trimethyl-2--oxa-bicyclo[2.2.2] oct-6-yl ester	0.057
44	Eugenol	0.090
45	Geranic acid	0.103
46	Geranyl acetate	1.354
47	β -Elemene	0.073
48	α-Cubebene	0.011
49	Methyleugenol	0.071
50	Trans-α-bergamotene	0.016
51	Trans-caryophyllene	0.037
52	α-Bergamotene	1.639
53	β -Sesquiphelandrine	0.024
54	α-Humulene	0.029
55	β -Farnesene	0.044
56	*epi*-Bicyclosesquiphellandrene	0.051
57	Germacrene-D	0.0658
58	trans- β -Farnesene	0.111
59	Bicylogermacrene	0.303
60	Germacrene A	0.374
61	Δ-Guaiene	0.375
62	β -Bisabolene	0.016
63	α-Amorpherene	0.271
64	β -Sesquiphellandrene	0.082
65	Δ-Cadinol	0.019
66	α-Cadinene	0.006
67	Sesquisabinene hydrate	0.021
68	.(+)-Aromadendrene	0.012
69	trans-Nerolidol	0.037
70	Spathulenol	0.003
71	Cubenol	0.114
72	tau.-Cadinol	0.858
73	β -Eudesmol	0.045
74	α-Cadinol	0.033
75	α-Bisabolol	0.031
	Total	99.81

bacterium. Naturally-acquired disease caused by *H. influenzae* seems to occur in humans only. *H. influenzae* cause dieases in human only when other factors such as a viral infection or reduced immune function held. Both *S. pneumoniae* and *H. influenzae* can be found in the human upper respiratory system (Fleischmann et al., 1995; Kennedy et al., 2007). *Candida albicans* is a diploid fungus (a form of yeast) specially found in

immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). It causes opportunistic oral and genital infections in humans (Rikkerrink et al., 1988; Sonneborn et al., 1999). High inhibitory activities against these microorganisms clearly suggest that Omani basil essential can be used as a natural agent in controlling human pathogens. Several researchers previously reported the antimicrobial activities of basil against various microbes (Wannissorn et al., 2005; Bozin et al., 2006; Lopez et al., 2005; Sokovic et al., 2006). Many scientists have linked basil antimicrobial effects to the presence of high content of linalool (Suppakul et al., 2003; Koutsoudaki et al., 2005; Sartoratotto et al., 2004; Juliani and Simon, 2002). Omani basil oil exhibited high antimicrobial activities which suggest its use as a potential natural food and medicines preservative.

Total antioxidants content

Total antioxidants content quantified in Omani basil essential oil using a commercial kit were found to be 50.32 ± 1.8 mM. Omani basil essential was found to have higher antioxidant content than previously reported values of many cultivars in literature (Gao et al., 2000; Wang and Lin, 2000). Given the high relative antioxidant activity of Omani basil suggest that this plant could constitute new sources of antioxidant phenolics in the daily diet and functional foods. Antioxidants are commonly found in medicinal plants are potent and safe due to their harmless nature and have been reported to have multiple biological effects. Among medicinal plants, the plants of genus *Ocimum* are known to very useful for their therapeutic potentials. In traditional medicine, basil has been used as an antiseptic, preservative, sedative, digestive regulator, diuretic, headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Kahkonen et al., 1999).

Conclusions

Following conclusions can be withdrawn from the present study:

- Omani basil was found to produce high biomass yield in less period of time.
- Essential oil extracted from Omani basil was found to have linalool as a major component.
- The basil essential was found highly active against human pathogens.
- The higher antioxidant concentration in Omani basil suggests it's importance for food and pharmaceutical industries.

ACKNOWLEDGEMENT

The authors would like to appreciate the corporation of Dr. Mustafa Walay in carrying out the present study.

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