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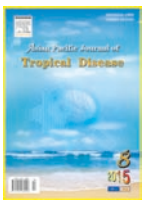

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Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.)

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ABSTRACT

Objective: To identify the major volatile constituents and evaluate the antibacterial activity of essential oil isolated from the aerial parts of *Ocimum basilicum* (*O. basilicum*) Linn. grown in Oman.

Methods: The fresh plant material was collected in the month of September from Seeb Nursery in Muscat Governorate, Sultanate of Oman. The aerial parts of *O. basilicum* Linn. were separated from fresh plant and essential oil was isolated by hydrodistillation method by using Clevenger apparatus. A greenish yellow oil was obtained in 0.6% v/w yield which was analyzed by gas chromatography–mass spectrometry for its chemical composition. The antibacterial activity of oil was also evaluated against three Gram–positive and four Gram–negative pathogenic bacterial strains by agar well diffusion method.

Results: A total of thirty six chemical constituents were identified and linalool (69.87%) was found to be the major constituent. Other main identified constituents included geraniol (9.75%), p-allylanisole (6.02%), 1,8–cineole (4.90%), trans–α–bergamotene (2.36%) and neryl acetate (1.24%). The essential oil of *O. basilicum* showed excellent antibacterial activity against Gram–positive bacteria and moderate activity against Gram–negative bacteria.

Conclusions: Omani basil is characterized by a high content of linalool which makes it useful in food, pharmaceutical and perfumery industries.

1. Introduction

Ocimum basilicum (*O. basilicum*) Linn., popularly known as sweet basil in English, “Tulsi” in Hindi and “Raihan” in Arabic, belongs to the family Lamiaceae. It is an annual herb that is widely grown as a commercial ornamental crop in tropical and warm temperate regions of the world including Asia, Africa, Central and South America[1,2]. Basil is an erect branching herb that grows 0.3 to 1.3 m high, with light green silky leaves. Its leaves are simple, opposite, 3 to 11 cm long, 1 to 6 cm wide, ovate, acute and usually toothed containing numerous oil glands which store essential oils[3]. The flowers of sweet basil are white to purple in color and arranged in a terminal spike[4]. Omani basil can be distinguished from other cultivars of basil based on its height and different look[5].

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The basil herb, especially its aromatic leaves, possesses excellent medicinal properties and therefore, it has been used in traditional system of medicine as a tonic, vermifuge, diuretic, antispasmodic and for the treatment of upper respiratory tract infections[6-8]. Due to their strong aroma, the leaves are also used as a fragrance and flavoring agent for food, beverages, condiments and oral care products.

Essential oil of *O. basilicum* is of economic significance due to its widespread utilization in food, cosmetics and pharmaceutical industries[9]. It is used as a folklore medicine to promote digestion, to stimulate respiratory circulation, and to alleviate mental fatigue and cold symptoms. It is also applied externally on the skin to treat acne[10]. Many scientific studies have been conducted worldwide to investigate the potential of basil essential oil in search of potent antimicrobial agents[11,12].

Ocimum oil has been reported to exhibit powerful antibacterial activities against both Gram–positive and Gram–negative bacteria[13]. However, the chemical composition of basil essential oil varies significantly depending upon the cultivar, season, chemotype and origin of the plants[1,14]. Various chemotypes of

O. basilicum oil have been reported to contain linalool, methyl chavicol, methyl cinnamate, methyleugenol, eugenol and geraniol as major components[15]. Hanif, *et al*. reported that linalool (69.9%) was the major component present in Omani basil oil while copaene (25.5%) and methyl chavicol (52.4%) were the major constituents of Ethiopian and Iranian *O. basilicum* species respectively[1,5,16]. Thus, it is very clear that the basil oil of different plant species and at different seasons may have different phytochemical composition so that different medicinal activities can be exhibited.

The present study was, therefore, undertaken to investigate the chemical composition, to identify the major volatile constituents and to evaluate the antibacterial activity of essential oil isolated from the aerial parts of *O. basilicum* L. grown in Oman.

2. Materials and methods

2.1. Chemicals and test microorganisms

All the chemicals and solvents used in this present study were of analytical grade procured locally. Clevenger apparatus used for the isolation of essential oils was from Borosil®, India. The Gram–negative bacterial strains such as *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium* (*S. typhimurium*) and *Klebsilla pneumoniae* (*K. pneumoniae*) and Gram–positive bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), and *Bacillus cereus* (*B. cereus*) were obtained from Department of Natural Sciences, Oman Medical College, Sultanate of Oman.

2.2. Collection of plant material

The fresh *O. basilicum* plant material was collected in the month of September from Seeb Nursery in the Muscat Governorate of Sultanate of Oman. The plant was identified by the Pharmacognosy Professor of Nizwa University and a voucher specimen was deposited in the herbarium of Department of Pharmacy, Oman Medical College, Oman, for future reference.

2.3. Isolation of essential oil

The aerial parts (stem and leaves) of *O. basilicum* Linn. were separated from fresh plant material, washed under tap water for clean, shaded dried and weighed. Two hundred gram of dried aerial parts were subjected to hydrodistillation for 6 h by using Clevenger apparatus to isolate volatile oil. The greenish yellow volatile oil (1.2 mL) was separated from aqueous layer, dried over anhydrous sodium sulphate, filtered and stored at 4 ℃ in a sealed dark brown bottle prior to further analysis.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

The isolated essential oil was analyzed on a Perkin Elmer Clarus 600 GC equipped with flame ionization detector and Rtx®-5MS capillary column (30.00 m × 0.25 mm inner diameter × 0.25 μm film thickness). The injection, mass transfer line and ion source were set at 290 ℃, 260 ℃ and 260 ℃ respectively. The oven temperature was programmed from 40 ℃ (held for 2 min) to 280 ℃ at a rate of 3 ℃/min. Helium was used as carrier gas with a constant flow rate of 1 mL/min. The injected volume of volatile oil was 1 μL with a split ratio of 100:1. The mass spectra were recorded at an ionization voltage of 70 ev in electron ionization mode and all data were obtained by collecting the full scan mass spectra within the range of 40-500 (Figure 1).

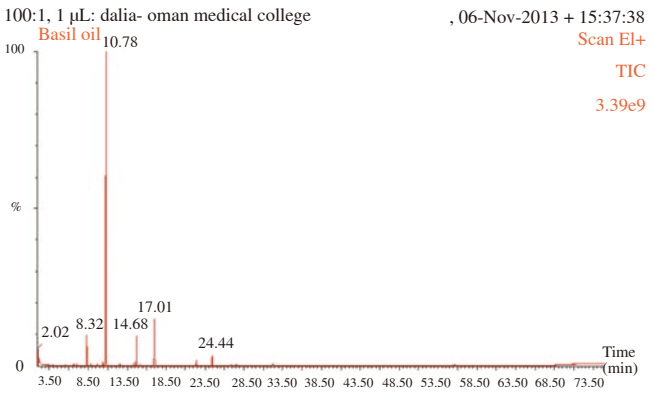

Figure 1. A chromatogram of essential oil isolated from Omani basil.

2.5. Identification of volatile compounds

The volatile components were identified on the basis of GC retention time on fused silica capillary column, by comparison of their mass fragmentation pattern with literature reports and by computer matching with standard spectra (Wiley Access Pak V7, May 2003 and NIST2005 version 2.1.0).

2.6. Evaluation of antibacterial activity

The antibacterial activity of the *O. basilicum* essential oil was evaluated against a range of Gram–positive (*S. aureus*, *S. epidermidis*, *B. cereus*) and Gram–negative pathogenic bacteria (*E. coli*, *P. aeruginosa*, *S. typhimurium* and *K. pneumoniae*). Antibacterial activity was determined by using standard agar well diffusion method and antimicrobial activity was compared with that of the commercially available basil oil. Three wells of 6 mm diameter were dug on each inoculated agar plate of a bacteria with sterile cork borer and 10 μL of extracted basil oil (test), commercial basil oil (standard for comparison) and paraffin oil (control) were added to the wells. The plates were then incubated at 37 ℃ for 24 h following which the diameter of zone of inhibition (clear zone) was measured in mm.

3. Results

3.1. Chemical composition of Omani basil oil

The yield of the hydrodistilled basil oil during cropping season of late summer was found to be 1.2 mL *i.e.* 0.6% v/w on fresh weight

basis which was greater than the previously reported value by Hanif *et al.*[5]. It has been reported that yield of basil oil may vary considerably from 0.07% to 1.92% in various basil accessions[17]. The content and composition of Omani basil oil identified by GC–MS analysis are presented in Table 1.

Table 1

Chemical composition of basil essential oil by GC–MS.

Compound name	QM (%)	MW	Formula	RT (min)	%
α–pinene	91.4	136	$C_{10}H_{16}$	5.51	0.075645
Sabinene	94.7	136	$C_{10}H_{16}$	6.54	0.138788
β–pinene	97.2	136	$C_{10}H_{16}$	6.66	0.363849
β–myrcene	99.0	136	$C_{10}H_{16}$	6.99	0.366589
dl–limonene	98.0	136	$C_{10}H_{16}$	8.23	0.244261
1,8–cineole	99.0	154	$C_{10}H_{18}O$	8.33	4.896275
β–ocimene	97.8	136	$C_{10}H_{16}$	8.85	0.267457
γ–terpinene	96.9	136	$C_{10}H_{16}$	9.25	0.031569
Octanol	96.4	130	$C_{10}H_{18}O$	9.64	0.257395
Fenchone	94.7	152	$C_{10}H_{16}O$	10.34	0.704858
Linalool	97.9	154	$C_{10}H_{18}O$	10.74	69.867420
Camphor	96.8	152	$C_{10}H_{16}O$	12.52	0.368525
Unidentified	–	–	–	13.40	0.073742
4–terpineol	94.3	154	$C_{10}H_{18}O$	13.82	0.088366
α–terpineol	97.2	154	$C_{10}H_{18}O$	14.39	0.585312
p–allylanisole	98.8	148	$C_{10}H_{12}O$	14.67	6.018883
n–octyl acetate	95.6	172	$C_{10}H_{20}O_2$	15.22	0.257536
Fenchyl acetate	98.7	196	$C_{12}H_{20}O_2$	15.58	0.10786
Nerol	90.2	154	$C_{10}H_{18}O$	15.91	0.042481
Z–citral	85.3	152	$C_{10}H_{16}O$	16.44	0.046011
Geraniol	97.4	154	$C_{10}H_{18}O$	17.01	9.754251
E–citral	94.9	152	$C_{10}H_{16}O$	17.68	0.089267
Endobornyl acetate	98.2	196	$C_{12}H_{20}O_2$	18.33	0.140703
Myrtenyl acetate	91.0	194	$C_{12}H_{18}O_2$	19.97	0.067036
Exo–2–hydroxycineole acetate	88.2	212	$C_{12}H_{20}O_3$	20.66	0.039701
Neryl acetate	96.5	198	$C_{12}H_{22}O_2$	22.36	1.237073
β–elemene	98.7	204	$C_{15}H_{24}$	22.69	0.107110
Methyleugenol	86.7	178	$C_{11}H_{14}O_2$	23.23	0.037340
Trans–α–bergamotene	99.3	204	$C_{15}H_{24}$	24.44	2.355691
Cis–β–farnesene	91.6	204	$C_{15}H_{24}$	25.28	0.057345
β–farnesene	94.6	204	$C_{15}H_{24}$	26.40	0.097899
Germacrene B	94.6	204	$C_{15}H_{24}$	26.88	0.190904
Germacrene A	88.3	204	$C_{15}H_{24}$	27.21	0.068046
Germacrene D	96.3	204	$C_{15}H_{24}$	27.55	0.190098
β–sesquiphellandrene	93.0	204	$C_{15}H_{24}$	27.92	0.069857
Unidentified	–		–	31.38	0.045462
Tau–cadinol	96.6	222	$C_{15}H_{26}O$	32.31	0.605034
Phytol	87.1	296	$C_{20}H_{40}O$	47.71	0.044364

MW: Molecular weight; QM: Quality match with the MS libraries; RT: Retention time.

A total of 38 phytochemicals were detected but only 36 chemical constituents were identified (99.87%). The number of identified components in this study was small as compared with the previous study in which a total of 75 compounds were identified representing 99.8% of Omani basil oil[5]. Linalool was identified as the major component (69.87%), followed by geraniol (9.75%), p–allylanisole (6.02%), 1,8–cineole (4.90%), trans–α–bergamotene (2.36%) and neryl acetate (1.24%).

Although there was a difference in number and concentration of the basil oil constituents in both studies, linalool (69.87%) was identified as the major component with almost similar concentration present in Omani basil oil in both studies. In our study, the concentration of p–allylanisole was found to be approximately ten times of the value observed in Omani basil oil by Hanif *et al*[5]. This difference could be due to seasonal variation and stage of plant growth. Two compounds with retention time of 13.40 min (0.074%) and 31.38 min (0.045%) could not be identified by GC–MS analysis.

The basil volatile oil primarily consisted of oxygenated monoterpenes, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and diterpene hydrocarbon.

3.2. Antibacterial activity

The antimicrobial activity of the basil essential oil was tested against three Gram–positive and four Gram–negative pathogenic bacterial strains. The results of the antibacterial activity reported as diameter of zone of inhibition (mm) are shown in Table 2.

Table 2

Antibacterial activity of basil oil against Gram–positive and Gram–negative bacteria reported as diameter of zone of inhibition. mm.

Pathogen		Extracted basil oil	Commercial basil oil
Gram–positive	*B. cereus*	25	–
	S. aureus	9	–
	S. epidermidis	–	–
Gram–negative	*E. coli*	11	–
	S. typhimurium	10	–
	K. pneumoniae	–	–
	P. aeruginosa	–	–

–: No inhibitory effect.

The basil oil inhibited the growth of two Gram–positive (*S. aureus* and *B. cereus*) and two Gram–negative (*E. coli* and *S. typhimurium*) bacteria. *B. cereus* showed the highest susceptibility to the extracted oil followed by *E. coli*.

The zone of inhibition for the four bacteria was observed in the following order: *B. cereus* (25 mm) > *E. coli* (11 mm) > *S. typhimurium* (10 mm) > *S. aureus* (9 mm). *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* were found to be highly resistant to *O. basilicum* essential oil. However, the commercially available basil oil showed no inhibitory effect on the panel of organisms used, which probably mean that there was a major difference in chemical composition of the isolated and marketed essential oil.

4. Discussion

Medicinal and aromatic plants have been used by mankind since ancient time to treat various ailments. Medicinal plants are rich source of bioactive compounds and have played an important role in drug discovery[18]. Recently, there has been a growing interest in the medicinal properties of essential oils which are concentrated essences of the plant materials such as fruits, buds, flowers, leaves, *etc*. Plant essential oils are primarily secondary plant metabolites which have been reported to possess various biological activities such as antimicrobial, anticancer, anti–inflammatory, analgesic,

as well as antifungal activities, *etc*.[2,19]. Over the years, many pathogenic bacterial strains have developed high antimicrobial resistance, this necessitates the discovery of new effective antibiotic treatment. Essential oils are considered as the important source of bioactive compounds especially antibacterial agents, as they have shown promising antimicrobial action against a wide range of microorganisms[20].

Basil is an aromatic, annual herb which has been known for centuries for its medicinal properties. There are more than 150 species of *Ocimum* known, but among all the species, sweet basil (*O. basilicum* Linn.) is the major essential crop in many countries, including Oman. Basil is known as "Raihan" in Arabic and is a very popular culinary herb in Arab world because of its rich spicy and peppery flavor. In Indian system of traditional medicine, it is known as the queen of herbs because of its useful medicinal actions[21]. Omani basil have up to eight different varieties which like other aromatic and medicinal plants, yield different content and composition of essential oil depending upon the plant genotypes, geographic distribution, environmental conditions, harvest time, irrigation, fertilization, *etc*.[4,22]. Therefore, the present study was conducted to analyze the composition of Omani basil oil isolated during the late summer (September) season and to evaluate its antimicrobial activity against seven pathogenic bacterial strains.

The essential oil yield from the Omani basil in the present study (0.6% v/w) was found to be comparable to previous reports[11,23]. However, it was more than the yield obtained from one of the eight Omani basil varieties by Al–Maskri *et al*. in winter, spring and summer seasons (0.1%, 0.3% and 0.1% respectively) and less than the yield reported by Telci *et al*. for Turkish *O. basilicum* landraces[9,24]. Such variations in the yield of basil essential oil might be due to the different geographical and environmental conditions of the regions[25,26].

The chemical composition of basil oil has been the subject of considerable studies due to its traditional use, economic value and demand in international market owing to utilization in various industries. Omani basil oil on GC–MS analysis showed the presence of 38 chemical compounds out of which only 36 could be identified (Table 1). Linalool was identified as the major component (69.87%) and thus this Omani basil variety can be classified as chemotype I based on its high linalool content. The other important components of this chemotype included geraniol (9.75%), p–allylanisole (6.02%), 1,8–cineole (4.90%), trans–α -bergamotene (2.36%) and neryl acetate (1.24%). Al–Maskari *et al*. also characterized the presence of linalool, geraniol, 1,8–cineole and p-allylanisole in Omani basil essential oil but the content of linalool (26.5%–56.3%) was lower while geraniol (12.1%–16.5%) content was higher than our study results[9]. They detected β–farnesene in the oil extracted only during winter and spring seasons while we detected it in our sample in summer season, though in a smaller amount. The number of chemical components detected in our basil oil is much lower than that of the seventy five earlier report for Omani basil of linalool chemotype[5].

The linalool content (69.90%) was almost similar to our result (69.87%) but they obtained higher concentration of geraniol (10.90%), 1,8–cineole (6.40%), and neryl acetate (1.40%). However, content of trans–α-bergamotene (1.600%) and p–allylanisole (0.691%) in their oil was much less than our study results. The linalool content in the essential oil extracted from Thai Siam (sweet basil) ranged from 24.60% to 48.65% of total oils and was less than Omani basil oil[26]. It is noteworthy that oxygenated monoterpenes constituted approximately 90% of the isolated basil oil followed by monoterpene hydrocarbons and sesquiterpenes. Due to the high content of linalool, the Omani sweet basil may find application as a scent in soaps, detergents, shampoos, cosmetics, food and perfume industries.

The antibacterial activity of basil oil was evaluated by agar well diffusion method against seven bacterial strains. Basil oil exhibited an excellent antimicrobial activity at a dose level of 10 μL against *B. cereus* and an moderate activity against *E. coli*, *S. typhimurium* and *S. aureus*. It has been reported that volatile oils are generally more active against Gram–positive than Gram–negative bacteria as Gram–negative bacteria possess an outer membrane surrounding its cell wall[27]. However, it failed to inhibit the growth of *S. epidermidis*, *K. pneumoniae* and *P. aeruginosa* bacterial strains. Several other antibacterial studies conducted on sweet basil elsewhere, reported that it was active against an array of microorganisms and linked the antimicrobial activity with high linalool content[13,23]. The commercially available basil oil was used for comparison purpose which did not show any inhibitory effect on the panel of microbes used. Thus, it could be concluded that chemical composition of marketed oil is significantly different from the isolated oil. Basil chemotypes which have eugenol or methyl chavicol as the major component exhibit a good antimicrobial activity against a wide range of microorganisms.

This study reveals the content, chemical composition and antibacterial activity of essential oil from basil grown in Oman. The results showed slight variation in the content and composition of extracted Omani basil oil with regard to the previously published data but identified linalool as the major component. It showed a mixed antibacterial activity against Gram–positive and Gram–negative bacteria. Further detailed investigations are needed to study the effect of plant growth stages and seasons on chemical composition and on antimicrobial activity of this miraculous medicinal herb in Oman.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Sajjadi SE. Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. *DARU* 2006; **14**(3): 128-30.

[2] Hossain MA, Kabir MJ, Salehuddin SM, Rahman SM, Das AK, Singha SK, et al. Antibacterial properties of essential oils and methanol extracts of sweet basil *Ocimum basilicum* occurring in Bangladesh. *Pharm Biol* 2010; **48**(5): 504-11.

[3] Kathirvel P, Ravi S. Chemical composition of the essential oil from basil (*Ocimum basilicum* Linn.) and its *in vitro* cytotoxicity against HeLa and HEp–2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts. *Nat Prod Res* 2012; **26**(12): 1112-8.

[4] Daneshian A, Gurbuz B, Cosge B, Ipek A. Chemical components of essential oils from basil (*Ocimum basilicum* L.) grown at different nitrogen levels. *Int J Nat Eng Sci* 2009; **3**(3): 8-12.

[5] Hanif MA, Al–Maskari MY, Al–Maskari A, Al-Shukaili A, Al-Maskari AY, Al–Sabahi JN. Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. *J Med Plants Res* 2011; **5**(5): 751-7.

[6] Srivastava S, Cahill DM, Conlan XA, Adholeya A. A novel *in vitro* whole plant system for analysis of polyphenolics and their antioxidant potential in cultivars of *Ocimum basilicum*. *J Agric Food Chem* 2014; **62**(41): 10064-75.

[7] Ramesh B, Satakopan VN. *In vitro* antioxidant activities of *Ocimum* species: *Ocimum basilicum* and *Ocimum sanctum*. *J Cell Tissue Res* 2010; **10**(1): 2145-50.

[8] Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. *J Med Plants Res* 2009; **3**(2): 67-72.

[9] Al–Maskri AY, Hanif MA, Al–Maskari MY, Abraham AS, Al–Sabahi JN, Al–Mantheri O. Essential oil from *Ocimum basilicum* (Omani Basil): a desert crop. *Nat Prod Commun* 2011; **6**(10): 1487-90.

[10] Matiz G, Osorio MR, Camacho F, Atencia M, Herazo J. [Effectiveness of antimicrobial formulations for acne based on orange (*Citrus sinensis*) and sweet basil (*Ocimum basilicum* L) essential oils]. *Biomedica* 2012; **32**(1): 125-33. Spanish.

[11] Shirazi MT, Gholami H, Kavoosi G, Rowshan V, Tafsiry A. Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetus minuta* and *Ocimum basilicum* essential oils. *Food Sci Nutr* 2014; **2**(2): 146-55.

[12] Vlase L, Benedec D, Hanganu D, Damian G, Csillag I, Sevastre B, et al. Evaluation of antioxidant and antimicrobial activities and phenolic profile for *Hyssopus officinalis*, *Ocimum basilicum and Teucrium chamaedrys*. *Molecules* 2014; **19**(5): 5490-507.

[13] Tankeo SB, Lacmata ST, Noumedem JA, Dzoyem JP, Kuiate JR, Kuete V. Antibacterial and antibiotic-potentiation activities of some Cameroonian food plants against multi-drug resistant Gram–negative bacteria. *Chin J Integr Med* 2014; **20**(7): 546-54.

[14] Bilal A, Jahan N, Ahmed A, Bilal SN, Habib S, Hajra S. Phytochemical and pharmacological studies on *Ocimum basilicum* Linn–a review. *Int J Curr Res Rev* 2012; **4**(23): 73-83.

[15] Padalia RC, Verma RS, Chauhan A, Goswami P, Chanotiya CS, Saroj A, et al. Compositional variability and antifungal potentials of *Ocimum basilicum*, *O. tenuiflorum*, *O. gratissimum* and *O. kilimandscharicum* essential oils against *Rhizoctonia solani* and *Choanephora cucurbitarum*. *Nat Prod Commun* 2014; **9**(10): 1507-10.

[16] Unnithan CR, Dagnaw W, Undrala S, Ravi S. Chemical composition and antibacterial activity of essential oil of *Ocimum basilicum* of Northern Ethiopia. *Int Res J Biol Sci* 2013; **2**(9): 1-4.

[17] Zheljazkov VD, Callahan A, Cantrell CL. Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *J Agric Food Chem* 2008; **56**(1): 241-5.

[18] Hossain MA, Harbi SRAL, Weli AM, Al–Riyami Q, Al–Sabahi JN. Comparison of chemical constituents and antimicrobial activities of three essential oils from three different brands' clove samples collected from Gulf region. *Asian Pac J Trop Dis* 2014; **4**(4): 262-8.

[19] Rana IS, Rana AS, Rajak RC. Evaluation of antifungal activity in essential oil of the *Syzygium aromaticum* (L.) by extraction, purification and analysis of its main component eugenol. *Braz J Microbiol* 2011; **42**(4): 1269-77.

[20] Sharma V, Gupta RC, Singh B, Dhaliwal HS, Srivastava DK. Volatile oil composition and antimicrobial activity of essential oil of two varieties of *Ocimum sanctum* (l.) from Dhameta (Kangra)–a North Indian region. *Int J Recent Sci Res* 2014; **5**(1): 142-7.

[21] Johnson M, Babu A, Irudayaraj V. Antibacterial studies on *in vitro* derived calli of *Ocimum basilicum* L. *J Chem Pharm Res* 2011; **3**(1): 715-20.

[22] Bakkour Y, El–Achi N, Tabcheh M, El-Nakat H, El Omar F. Chemical composition and antioxidant activities of the essential oils from green and ripe berries of *Juniperus excelsa* growing in Lebanon. *Int J Pharm Life Sci* 2013; **4**(2): 2362-7.

[23] Hussain AI, Anwar F, Sherazi STH, Przybylski R. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chem* 2008; **108**(3): 986-95.

[24] Telci I, Bayram E, Yilmaz G, Avci B. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). *Biochem Syst Ecol* 2006; **34**(6): 489-97.

[25] Chikkaswamy BK, Prasad MP, Chandrashekharaiah KS. Detection of genetic diversity in *Ocimum* species using RAPD markers. *Int J Res Pharm Sci* 2013; **4**(1): 58-64.

[26] Wesołowska A, Kosecka D, Jadczak D. Essential oil composition of three sweet basil (*Ocimum basilicum* L.) cultivars. *Herba Polonica* 2012; **58**(2): 5-16.

[27] Saha S, Dhar TN, Sengupta C, Ghosh P. Biological activities of essential oils and methanol extracts of five *Ocimum* species against pathogenic bacteria. *Czech J Food Sci* 2013; **31**(2): 194-202.