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Aromatic Profiles and Antimicrobial Activities of Two *Ocimum basilicum* Varieties

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Abstract

The volatile oils isolated from both *Ocimum basilicum* var. purpurascens and *Ocimum basilicum* var. basilicum, in Yemen, were analyzed by GC/FID and GC/MS. Twenty peaks were detected (100%) in *Ocimum basilicum* var. basilicum oil where *l*-Linalool was the highest one (39.0%) followed by *cis*-anethole (20.0%), guaiol (8.9%) and 1,8-cineole (7.2%). *Ocimum basilicum* var. purpurascens chromatogram revealed the presence of twenty peaks which represented 100% of the total peaks. *l*-Linalool (41.8%), *α*-copaene (22.3%) and 1,8-cineole (10.6%) were the most representative components. *Ocimum basilicum* var. basilicum, was more effective as antimicrobial agent than *Ocimum basilicum* var. purpurascens where it showed potent activity against *Bacillis subtilis* (MIC is 3.90 µL/mL) and moderate activities against both *Staphylococcus aureus* and *Escherichia coli* (MIC value for both 7.8 µL/Ml). *Ocimum basilicum* var. basilicum induced potent antifungal activities against *Saccharomyces cerevisiae* (MIC 3.90 µL/mL).

Keywords: Basil, Volatile oil, Antibacterial, Antifungal

Abbreviations: *AF: Aspergillus fumigatus* (RCMB 02568); *CA: Candida albicans* (RCMB 05036); *SC*: *Saccharomyces cerevisiae* (RCMB 05177); *SA*: *Staphylococcus aureus* (RCMB 010067); EF: *Enterococcus faecalis* (RCMB 010028); *BS*: *Bacillis subtilis* (RCMB 010063); *EC*: *Enterobacter coloacae* (RCMB 010072); *KP: Klebsiella pneumoniae* (RCMB 010052); *ECo*: *Escherichia coli* (RCMB 010093).

Introduction

Ocimum basilicum has been used in traditional medicine since long time all over the world as a remedy for a several ailments [1,2]. Lots of pharmacological activities have been traced for *O. basilicum* volatile oil, as antimicrobial, anti-inflammatory, analgesic, antioxidant and immunomodulatory activities [1,2].

Little variations in components concentration were observed in different locations as some major components present in one of the oils were in lesser concentrations in the other variety. For example, anethole in var. basilicum (20%), presents in low concentration in the second var. purpurascens (2.53%) and guaiol 8.8% is absent in the second variety. The major constituents *of Ocimum basilicum* var. basilicum from Brazil were eugenol (28.1%) and camphor (10.1%) [3]. However, in Colombia, the main

components were *l*-linalool (46.7%) and estragole (27.4%) [4]. Linalool was the major constituents in three oil samples collected from plants in Yemen, Tajikistan and Nepal [5]. In addition, *l*-linalool (29.7-39.3%) %) was the major compound in previous study of EO isolated from *Ocimum basilicum* var. purpurascens in Bangladesh and Brazil but estragole was the major constituent of EO collected in Russia [6-8].

This study was undertaken to chemically investigate the essential oils of *Ocimum basilicum* var. purpurascens and *Ocimum basilicum* var. basilicum from Yemen as well as evaluate their antimicrobial activities against selected bacteria and fungi.

Materials and Methods

Plant Material

Ocimum basilicum var. purpurascens and *Ocimum basilicum* var. basilicum were collected during the rainy season in 2-22/8/2015 from different location in Bani Matar District, Sanaa governorate, Yemen. The identification of the specimens was done by utilizing the available taxonomic and floristic literatures [9-12]. Voucher specimens have been deposited at the Herbarium of Faculty of Science, Ain Shams University and a duplicate of each herbarium specimen was kept at the Herbarium of Biology Department, Faculty of science Sanaa University.

Isolation of the Essential Oil

The fresh leaves and green branches of both taxa were chopped into small pieces. The essential oil was isolated from each part by hydrodistillation for 5 h using a Clevenger-type all glass apparatus. Each oil was transferred to a screw-capped glass vial, dried (Na_2SO_4) and stored at 4°C in the dark until analysis.

Analysis of Essential Oils by GC and GC-MS

GC analysis was carried out using a GC HP 5890 Hewlett Packard equipped with FID and HP-5 fused silica capillary column 30 m × 0.25 mm i.d., film thickness 0.25 µm, using a sample volume of 0.03 µL. Oven temperature was programmed from 60ºC to 240ºC at 3ºC/min; injector temperature 250ºC; detector temperature 280 ºC; carrier gas was helium, flow rate was 1.0 mL/min; automatic sample injection, 0.02 µL of the oil; split was 1/70. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization. GC–MS analysis was performed on a Perkin-Elmer quadrupole MS system (Model 5) coupled with the GC HP 5972, equipped with a HP-5 capillary column. Oven temperature was programmed from 45 ºC to 240ºC at 3ºC/min; injector temperature 250ºC; carrier gas was helium, flow rate was 0.5 mL/min; automatic sample injection, 0.02 µL of the oil; split: 1/70. The MS operating parameters were interface temperature: 300 ºC, ion source temperature: 200ºC, EI mode: 70 eV, scan range was 41–400 amu.

Identification of the Components

Mass spectra of the individual GC peaks were identified by a computer search of the commercial libraries (WILEY, NIST), as well as matching with published mass spectra [13]. The identification was further confirmed by the calculation of the retention indices (RI) relative to (C6–C22) *n*-alkanes [14].

Antibacterial Activity

This work has been done in Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasr City, Cairo, Egypt.

Antibacterial activity was investigated using agar-well diffusion method. The activity of the tested oils was studied against the *Staphylococcus aureus* (RCMB010027), *Enterococcus faecalis* (RCMB 010063), *Bacillis subtilis* (RCMB 010067) (as Gram-positive bacteria), and *Enterobacter coloacae* (RCMB 010072), *Klebsiella pneumoniae* (RCMB 010093), *Escherichia coli* (RCMB 010052) (as Gram-negative bacteria). The solution of 250 µ/mL of each oil and standard drug in DMSO was prepared for using against the tested bacteria. The centrifuged pellets of bacteria from a 24-hour-old culture containing approximately 104-106 CFU/ml were spread on the surface of nutrient agar (tryptone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 ml of H_2O, pH 7.0) which was autoclaved under 121°C for at least 20 min. The wells were created in a medium with the help of a sterile metallic bores and then cooled to 45°C. The activity was determined by measuring the diameter of the inhibition zone (in mm). Fifty microlitres of the oil (concentration: 250µL/mL) were loaded into the wells of the plates. All the samples were prepared in DMSO, and DMSO was loaded as control. The plates were kept for incubation at 37°C for 24 h and then the plates were examined for the formation of inhibition zone. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. Ampicillin was used as a positive control for Gram-positive bacteria and gentamicin for Gram-negative bacteria.

Antifungal Activity

The tested samples were screened separately *in vitro* for their antifungal activity against *Aspergillus fumigatus*

(RCMB 02568), *Candida albicans* (RCMB 05036), *Saccharomyces cerevisiae* (RCMB 05177) and Trichophyton mentagrophytes. These species were isolated from the infected organs of some patients on Sabouraud dextrose agar plates. The culture of fungi was purified by single spore isolation technique. The antifungal activity was done by agar-well diffusion method according to the following procedure: A homogeneous mixture of glucose–peptone–agar (40:10:15) was sterilized by autoclaving at 121 °C and 15 ×105 Pascal for 20 min. The sterilized solution (25 mL) was poured in each sterilized Petri dish in laminar flow and left for 20 min to form the solidified Sabouraud dextrose agar plate. These plates were inverted and kept at 30 °C in an incubator to remove the moisture and to check for any contamination.

Antifungal Assay

Fungal strain was grown in 5 mL Sabouraud dextrose broth (glucose: peptone, 40:10) for 3-4 days to achieve 105 CFU/ml cells. The fungal culture (0.1 mL) was spread out uniformly on the Sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5-10 min so that culture was properly adsorbed on the surface of Sabouraud dextrose agar plates. Small wells of size (4-2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. About 50 µL of the tested samples (250 µL/mL) were loaded into the wells of the plates. The samples were prepared as DMSO solutions, and DMSO was loaded as control. The plates were kept for incubation at 30°C for 3-4 days and then the plates were examined for the formation of inhibition zone. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Amphotericin was used as a standard for the antifungal activity.

Determination of Minimum Inhibitory Concentrations

Minimum inhibitory concentrations (MIC) were determined by using the microdilution broth method. This was done following the procedures recommended by the National Committee for Clinical Laboratory Standard [15,16]. Ampicillin, gentamycin, and amphotericin B were used as the reference compounds for bacteria and fungi. The reference compounds were dissolved in sterile distilled H_2O. The microtiter plates were incubated at 37°C for tested bacteria and 28 for tested fungi, and were read using microplate reader after 24 h for bacteria and 48 h for fungi.

Results

GC/MS Results

The essential oils composition seems to be similar in both varieties of *Ocimum basilicum*; basilicum and purpurascens where *l*-linalool is the dominant compound (39.0, 41.7%).

Ocimum basilicum var. basilicum oil was pale green in color. Twenty peaks were detected (100%). *l*-Linalool was the highest one (39.0%) followed by *cis*-anethole (20.0%), guaiol (8.9%) and 1,8-cineole (7.2%), (Table 1).

Ocimum basilicum var. purpurascens oil was pale green in color. Twenty peaks were detected which represented 100% of the total peaks. *l*-Linalool (41.8%), *α*-copaene (22.3%) and 1,8-cineole (10.6%) were the most representative components, (Table 2).

NO.	Formula	Compounds	KI	Oil Area [%]a)
1	$C_{10}H_{16}$	α-Pinene	936	0.58
2	$C_{10}H_{16}$	*β*-Pinene	981	0.75
3	$C_{10}H_{16}$	*β*-Myrcene	990	1.42
4	**$C_{10}H_{18}O$**	**1,8-Cineole**	**1035**	**7.16**
5	$C_{10}H_{16}$	*α*-Ocimene	1049	2.13
6	**$C_{10}H_{18}O_2$**	***l*-Linalool**	**1102**	**39.02**
7	$C_{10}H_{18}O$	*d*-Fenchyl alcohol	1121	0.48
8	$C_{10}H_{18}O$	Borneol	1175	1.25
9	$C_{10}H_{18}O$	Terpine-4-ol	1182	1.02
10	**$C_{10}H_{12}$**	***Z*-Anethole**	**1249**	**19.98**
11	$C_{12}H_{20}O_2$	Thujanol acetat	1277	1.31
12	$C_{12}H_{20}O_2$	Isobronyl acetat	1281	5.94
13	$C_{15}H_{24}$	α-Bergamotene	1409	1.87
14	$C_{15}H_{24}$	*allo*-Aromadendrene	1458	1.68
15	$C_{15}H_{24}$	α-Macrocarpene	1471	1.8

16	$C_{15}H_{24}$	Dauca-5,8diene	1472	0.48
17	$C_{15}H_{24}$	Germacrene D	1483	0.89
18	$C_{15}H_{24}$	*β*-Selinene	1487	2.45
19	$C_{15}H_{22}O$	Elemenone	1583	0.96
20	**$C_{15}H_{26}O$**	**Guaiol**	**1605**	**8.83**
Total all				100
Functional group		**Total peak (%, No. of identified compounds)**		
Monoterpene hydrocarbons		24.86		
Sesquiterpene hydrocarbons		9.17		
Monoterpene oxygenated		56.18		
Sesquiterpene oxygenated		9.79		
Total Hydrocarbons compounds		**34.03**		
Total oxygenated compounds		**65.97**		

Table 1: Essential oil composition of *Ocimum basilicum* var basilicum.
a Values are expressed as relative area percentage; bKovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).

NO.	Formula	Compounds	KI	Oil Area [%][a])
1	$C_{10}H_{16}$	α-Pinene	936	0.53
2	$C_{10}H_{16}$	*β*-Pinene	981	0.94
3	**$C_{10}H_{18}O$**	**1,8-Cineole**	**1035**	**10.6**
4	$C_{10}H_{16}$	*α*-Ocimene	1049	0.53
5	**$C_{10}H_{18}O$**	***l*-Linalool**	**1102**	**41.73**
6	$C_{10}H_{18}O$	*d*-Fenchyl alcohol	1121	2.16
7	$C_{10}H_{18}O$	Borneol	1175	1.88
8	$C_{10}H_{18}O$	Terpine-4-ol	1183	1.18
9	$C_{10}H_{18}O$	*α*-Terpineol	1186	0.77
10	$C_{10}H_{12}O$	*Z*-Anethole	1249	2.53
11	$C_{12}H_{20}O_2$	Thujanol acetat	1277	0.57
12	$C_9H_{10}O$	Cinnamyl alcohol	1306	1.6
13	**$C_{15}H_{24}$**	***α*-Copaene**	**1374**	**22.25**
14	$C_{15}H_{24}$	Bergamotene	1409	3.12
15	$C_{15}H_{24}$	*trans*-Muurola-3,5-diene	1457	1.78
16	$C_{15}H_{24}$	*α*-Macrocarpene	1471	2.04
17	$C_{15}H_{24}$	$_{\gamma}$-Gurjunene	1476	0.85
18	$C_{15}H_{24}$	Germacrene D	1483	1.3
19	$C_{15}H_{24}$	*β*-Selinene	1488	1.12
20	$C_{15}H_{26}O$	*α*-epi-Eudesmol	1608	2.52
Total all				100
Functional group			**Total peak (%, No. of identified compounds)**	
Monoterpene Hydrocarbons			2	
Sesquiterpene Hydrocarbons			32.46	
Monoterpene oxygenated			**63.02**	
Sesquiterpene oxygenated			2.52	
Total hydrocarbon compounds			**34.46**	
Total oxygenated compounds			**65.54**	

Table 2: Essential oil composition of *Ocimum basilicum* var. purpurascens.
a Values are expressed as relative area percentage; b Kovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).
a Values are expressed as relative area percentage; b Kovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).

Antimicrobial Activity

Tables 3 and 4 displayed zone of inhibition and minimum inhibitory concentration (MIC) of both oils.

The antimicrobial activity of these EOs was investigated against nine pathogenic microorganisms represented by six bacterial species, which were three gram-negative bacteria: *Enterobacter coloacae*, *Klebsiella pneumoniae*, *Escherichia coli*, three gram-positive bacteria: *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillis subtilis* and three fungal species: *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*

Ocimum basilicum var. basilicum, was more effective as antimicrobial agent than *Ocimum basilicum* var. purpurascens. Concerning *Ocimum basilicum* var. basilicum, it exhibited potent antibacterial activities against *Bacillis subtilis* with MIC values of 3.90 µL/mL and moderate activities with MIC value of 7.8 µL/mL against both *Staphylococcus aureus* and *Escherichia coli*. It also induced potent antifungal activities against *Saccharomyces cerevisiae* with MIC values of 3.90 µL/mL.

Linalool was found as a major component in *Ocimum basilicum* var. basilicum. It was reported that linalool exerted antifungal activity with anti-Trichophyton rubrum potential [17]. A Bulgarian study on basil oil, where the major components was linalool (54.95%), showed that it exhibited strong inhibitory effect against multidrug resistant clinical isolates from the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas* [18]. Anethole showed antimicrobial activities against coliforms [19].

The essential oil from the fruits of the Brazilian spice *Xylopia sericea* exhibited high bacteriostatic effect against *Staphylococcus aureus*, *Enterobacter cloacae*, *Bacillus cereus* and *Klebsiella pneumonia* where one of its major compounds is guaiol (13.93%) [20].

Eucalyptol itself possessed an activity against many microorganisms as *E. coli*, S. *aureus, Bacillus cereus* and *P. aeruginosa* [21] suggesting that eucalyptol may be responsible for the antimicrobial activity identified by this study [22].

Essential oil of *Ocimum basilicum* var. purpurascens did not show any antimicrobial effect. We can explain that Interactions between components may cause antagonistic, additive or synergistic effects. Some researchers have demonstrated that whole EOs usually induce higher antimicrobial; activity than the mixtures of their major compounds, suggesting that the minor compounds are critical to the synergistic activity, though antagonistic and additive effects have also been observed [23-25].

Conclusion

Ocimum basilicum var. basilicum induced potent to moderate activities against some Gram-positive, Gram-negative bacteria as well as some fungi.

This could courage us for using this oil as natural preservative in food, drugs and cosmetics preparations.

plant	Diameter of inhibition zone (mm)[a)b)c)d)]								
	Gram-negative bacteria			Gram-positive bacteria			Fungal species		
	EC	***KP***	***Eco***	***SA***	***EF***	***BS***	***AF***	***CA***	***SC***
OBB	NA	17.3±0.44	18.1±0.63	18.2±0.44	NA	19.2±0.37	17.3±0.58	NA	19.2±0.58
OBP	NA	NA	NA	NA	NA	NA	NA	NA	NA
Standard	**Gentamicin**			**Ampicillin**			**Amphotericin B**		
	23.8±0.63	20.2±0.12	27.3±0.44	28.9±0.14	25.3±0.58	26.4±0.34	23.7±0.10	21.9±0.12	27.8±0.58

Table 3: Antimicrobial activity of the volatile oils of *Ocimum basilicum* var. basilicum and *Ocimum basilicum* var. purpurascens expressed as inhibition zone diameter.

a) Mean zone of inhibition in mm _ standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. The concentration used for the standard antibiotic was 30 mg/mL.

b) The test was done using the diffusion agar technique. Well diameter: 6.0 mm. RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms.

c) Results are Mean±SD of triplicate values. The concentration of oil was 250 µL/mL.

d) 6–9 mm: no activity; 12–15 mm: low activity; 15–18 mm: good activity; above 18 mm: significant activity.

Abbreviation of plants names in Table 3.

AF: Aspergillus fumigatus (RCMB 02568), CA: Candida albicans (RCMB 05036), SC: Saccharomyces cerevisiae (RCMB 05177); SA: Staphylococcus aureus (RCMB 010067); EF: Enterococcus faecalis (RCMB 010028); BS: Bacillis subtilis (RCMB 010063); EC: Enterobacter coloacae (RCMB 010072); KP: Klebsiella pneumoniae (RCMB 010052); ECo: Escherichia coli (RCMB 010093)

Plant	Gram-negative bacteria			Gram-positive bacteria			NA : no activity.Fungal species		
	EC	*KP*	*ECo*	*SA*	*EF*	*BS*	*AF*	*CA*	*SC*
OBB	NA	15.63	7.81	7.8	NA	3.9	13.25	NA	3.9
OBP	NA	NA	NA	NA	NA	NA	NA	NA	1.95
Standard	**Gentamicin**			**Ampicillin**			**Amphotericin B**		
	0.98	3.9	0.98	0.49	0.98	0.49	0.49	0.98	0.49

Table 4: Antimicrobial activity (MIC) of volatile oils of *Ocimum basilicum* var. basilicum and *Ocimum basilicum* var. purpurascens plants.
Abbreviations are the same as Table 3.
NA: no activity, OBP: NA with all microorganisms

References

1. Othman AM, Awadh NA, Al Fadhli E, Salama M (2014) Topical herbal antimicrobial formulation containing Thymus laevigatus essential oil. WJPR 3(3): 3693-3703.

2. Chorianopoulos NG, Evergetis E, Mallouchos A, Kalpoutzakis E, Nychas GJ, et al. (2006) Seasonal variation in the chemical composition of the essential oils of Satureja species and their MIC assays against foodborne pathogens. J Agric Food Chem 54: 3139-3145.

3. Sartoratto A, Machado AL, Delarmelina C, Figueira GM, Duarte MC, et al. (2004) Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz J Microbiol 35(4): 275-280.

4. Roldán LP, Díaz GJ, Duringer JM (2010) Composition and antibacterial activity of essential oils obtained from plants of the Lamiaceae family against pathogenic and beneficial bacteria. Revista Colombiana De Ciencias Pecuarias 23(4): 451-461.

5. Sharopova FS, Satyal P, Ali NAA, Pokharel S, Zhang H, et al. (2016) The Essential oil compositions of *Ocimum basilicum* from three different regions: Nepal, Tajikistan, and Yemen. Chem Biodivers 13(2): 241-248.

6. Mondello L, Zappia G, Cotroneo A, Bonaccorsi I, Chowdhury JU, et al. (2002) Studies on the essential oil-bearing plants of Bangladesh. Part VIII, Composition of some Ocimum oils O. basilicum L. var. purpurascens; O. sanctum L. green; O. sanctum L. purple; O. americanum L., citral type; O. americanum L., camphor type. Flavour Fragr J 17(5): 335-340.

7. Maria Goretti Vasconelos De, Abreu Matos de, Lacerda Machado MI, Craveiro AA (2003) Essential oils of *Ocimum basilicum* L., *Ocimum basilicum* var. minimum L. and *Ocimum basilicum* var. purpurascens Benth. Grown in north-eastern Brazil. Flavour Fragr J 18(1): 13-14.

8. Carovic Stanko K, Orlic S, Politeo O, Strikic F, Kolak I, et al. (2010) Composition and antibacterial activities of essential oils of seven Ocimum taxa. Food Chem 119(1): 196-201.

9. Chaudhary SA (2001) In Labiate in flora of kingdom of Saudi Arabia Illustrated. Ministry of agriculture and water, national herbarium and national agriculture research, Riyadh KSA 2(2): 309-418.

10. Wood JRI (1997) Handbook of the Yemen Flora, Whitstable Litho Printers Ltd, Royal Botanic Gardens, Kew, UK, pp: 256.

11. Alfarhan AH, Al Turki, Basahy AY (2005) Flora of Jizan Region, King Abdulaziz City for Science and Technology (KACST), KSA 1: 348-360.

12. Boulos L (2002) Flora of Egypt. AL Hadara publishing, Cairo, Egypt 3: 6-34

13. Azab SS, Abdel Jaleel GA, Eldahshan OA (2017) Anti-inflammatory and gastroprotective potential of leaf essential oil of Cinnamomum glanduliferum (Wall) Meissn in ethanol-induced rat experimental gastritis. Pharmaceutical Biology 55(1): 1654-1661.

14. Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry, 4th (Edn.), Allured Publishing Corporation, Illinois, USA.

15. National Committee for Clinical Laboratory Standard (1997a) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard M7-A, Villanova, pp: 37(2).

16. National Committee for Clinical Laboratory Standard (1197b) Methods for dilution antimicrobial

susceptibility tests for yeast approved standard M27-A, Villanova 17(9).

17. De Oliveira Lima MI, Araújo de Medeiros AC, Souza Silva KV, Cardoso GN, de Oliveira Lima E, et al. (2017) Investigation of the antifungal potential of linalool against clinical isolates of fluconazole resistant Trichophyton rubrum. Mycol Med 27(2):195-202.

18. Opalchenova G, Obreshkova D (2003) Comparative studies on the activity of basil--an essential oil from *Ocimum basilicum* L.-against multidrug resistant clinical isolates of the genera Staphylococcus, Enterococcus and Pseudomonas by using different test methods. J Microbiol Methods 54(1): 105-110.

19. Wieczyńska J, Cavoski I (2018) Antimicrobial, antioxidant and sensory features of eugenol, carvacrol and trans-anethole in active packaging for organic ready-to-eat iceberg lettuce. Food Chem 259: 251-260.

20. Mendes RF, Pinto NC, da Silva JM, da Silva JB, Hermisdorf RC, et al. (2017) The essential oil from the fruits of the Brazilian spice Xylopia sericea A. St.-Hil. Presents expressive in-vitro antibacterial and antioxidant activity. J Pharm Pharmacol 69(3): 341-348.

21. Mahboubi M, Kazempour N (2009) The Antimicrobial Activity of Essential Oil from Perovskia abrotanoides Karel and its Main Components. Indian J Pharm Sci 71(3): 343-347.

22. Taha AM, Eldahshan OA (2017) Chemical Characteristics, Antimicrobial, and Cytotoxic Activities of the Essential Oil of Egyptian Cinnamomum glanduliferum Bark. Chem Biodiver 14(5).

23. Chorianopoulos NG, Lambert RJW, Skandamis PN, Evergetis, ET, Haroutounian SA, et al. (2006) A newly developed assay to study the minimum inhibitory concentration of Satureja spinosa essential oil. J Appl Microbiol 100(4): 778-786.

24. Gayathiri K, Sangeetha M, Sharanya VK, Prakash GS, Vimalavathini R, et al. (2016) Review: Potential pharmacological uses of natural products from Lamiaceae. Int J Pharm Res & Rev 5(5): 21-34.

25. Chorianopoulos NG, Nychas GJE, Serkos A, Haroutounian SA (2007) Essential oils of Lamiaceae family taxa as natural preservatives of food preparations. Food Chem 1(2): 202-215.