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Original Article

ESSENTIAL OIL CONTENT AND CHEMICAL COMPOSITION OF EIGHT DILL (*ANETHUM GRAVEOLENS* L) CULTIVARS CULTIVATED UNDER EGYPTIAN CONDITIONS

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ABSTRACT

Objective: The objective of this research was to evaluate the essential oil content and composition of the eight dill cultivars under Egypt conditions.

Methods: A two years field experiment was conducted on eight dill cultivars in 2010/2011 and 2011/2012 seasons. The volatile oil content of the eight dill cultivars was studied at fruiting stage. Extracted volatile oil by water distillation was analyzed with GC-MS.

Results: Essential oil % of the eight dill cultivars under study was varied from 1.93-3.26%. *Anethum graveolens* cv. Common was the highest in essential oil followed by cv. Local, cv. Compatto, cv. Bouquet, cv. Elephant cv. Vierling, cv. Tetra and then cv. Dukat which gave the lowest in essential oil content. From the results of GC/MS obtained for the eight seeds dill cultivars, two chemotypes were found. The seven European cultivars that belong to type 1 contained limonene (9.60–18.0%), carvone (81.35–89.98%), whereas the Local cultivar belongs to type 2 (cv. Local) contains limonene (18.81%), carvone (56.61%), dillapiole (15.71%) and piperitone (7.41%). No dillapiole and piperitone were found in the essential oil of the first type cultivars (European cultivars).

Conclusion: The results obtained in this research work clearly indicated superiority of European cultivar (Common) to all cultivars on volatile oil content. Also all European cultivars were best in the volatile oil quality. European cultivars contain the highest percentage of carvone and were free from dillapiol and piperitone.

Keywords: Dill, Cultivar (cv.), Essential oil, Chemotype, Carvone, Limonene, Dillapiole, Piperitone

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INTRODUCTION

Anethum graveolens L. (dill, Apiaceae (umbelliferae) family) is an annual important aromatic plant originates from the Mediterranean and West Asia and grown worldwide as an essential-oil crop or as a culinary crop. So, it has been used as a spice and medicine [1]. The fruits used for many medicinal purposes as a diuretic, carminative, sedative, lactagogue, diuretic, antispasmodic, stimulant and to treat neuralgio, bronchial asthma, haemorrhoids, bronchial asthma, genital ulcers, dysurea, neuralgio, remal colic, and dysmenorrhoea [2, 3]. Also, essential oil of dill seed has been investigated in connection with their antimicrobial, antiseptic and exhibits anticarcinogenic activity and antioxidant [4].

The seeds essential oil of dill has been reported to range between 1.75–5.8% [1, 5-9]. The high quality of dill seed oil depending on the high content of carvone (>30%) and low content of dillapiole (0 to 5%) [10, 11].

The productivity and quality of essential oil depend on cultivar, climate, sowing and harvest dates, environmental stress, and management practices [6-8, 12-18]. The main components of dill seed oil was carvone (49.5%-67%) and limonene (23%p-46.3%) [1, 19, 20]. In another report, dill apiole and carvone as the main components [21]; furthermore, Wall and Friesen [11] found limonene, carvone and α-phellandrene as the main components of dill seed oil. In other studies the dill seed oil components differed to be limonene (43.7%), carvone (41.2%), dihydrocarvone (3.1%), and myristicin (11.7%) [6]. Carvone (38.89%), dill apiol (30.81%), limonene (15.93%) and trans-dihydrocarvone (10.99%) were major components [21]. Also, Singh *et al.* [22] found that carvone (55.2%), limonene (16.6%), dill apiole (14.4%) and camphor (11.44%), four major compounds in dill seed oil.

Due to their commercial interest, the essential oils from the fruits (seeds) of dill growers are looking for new cash crops. However, in our opinion, such goal may be achieved by introducing new cultivars of dill to cultivate in the first time under conditions of Egypt, there has been no evaluation of these imported European dill cultivars under Egypt Conditions and so clarifying to what extent the different cultivars of dill can superior and through selecting the superior cultivars under Egypt conditions. Therefore, to make this study more representative, seven European cultivars were chosen for this study compared with the Local cultivar under Egypt condition. To evaluate these cultivars of dill as potential new cash crops for essential oil production in Egypt based on having been developed for essential oil production and their productivity in Egypt and to assess the content and quality performance of dill cultivars oils and also to determine the chemotype of these cultivars cultivated in Egypt.

MATERIALS AND METHODS

A field experiment was conducted at the Experimental Farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt, during two successive seasons (2010/2011 and 2011/2012). The physical and chemical properties of the soil sample were determined according to Jackson [24] to indicate that the field soil is sandy loam, having a physical composition as follows: 51.1% sand, 25% silt, 23.9 % clay and 0.47% organic matter. Soil chemical analysis was as follows: E. C (ds/m) = 4.9; pH= 8.05 and available N, P and K =0.07, 0.53 and 2.8 mg/kg, respectively.

The seeds of eight cultivars of dill, (*Anethum graveolens*) viz., cv. Tetra, cv. Bouquet, cv. Compatto, cv. Dukat, cv. Vierling, cv. Elephant and cv. Common were introduced from the HEM ZADEN B. V-P. O. Box 4-1606 ZG Venhuizen, The Netherlands. Besides the seeds of the Local cultivar of dill in Egypt, the seeds of the eights cultivars were sown on 15th October in the two seasons into 3 x 3.5 m plots with 60 cm spaced rows, and 20 cm between the hills. The normal agricultural practices normally don for the dill were performed for all cultivars. The plants were harvested on 20th May and the seeds were collected for seeds essential oil. The experimental layout was a complete randomized block design with three replications.

The essential oil of all cultivars were extracted from seeds of each cultivar separately by water-distillation using Clevenger apparatus for 2 hr according to the method described in the British Pharmacopoeia [25] and expressed as (ml/100 g fruits). The resulted essential oils were dehydrated over anhydrous sodium sulfate and were kept in the refrigerator until GC-MS analyses.

GC/MS analyses conditions

The volatile oil of eight cultivars was analyzed with gas chromatography-mass spectrometry (GC-MS) instrument stands with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC/MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). The carrier gas was helium at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 40 ^{o}C for 1 min; rising at 4.0 ^{o}C/min to 160 ^{o}C and held for 6 min; rising at 6 ^{o}C/min to 210 ^{o}C and held for 1 min. The injector and detector temperatures were held at 210 ^{o}C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Statistical analysis

The essential oil content of eight cultivars was analyzed with the analysis of variance (ANOVA) using JMP 10 program (SAS Institute, NC, USA). The mean values of treatments were compared using Tukey's HSD test. Values accompanied by different letters are significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

Table I shows that essential oil % in eight cultivars of dill. In the two seasons, cv. Common showed significantly higher essential oil % than the rest of cultivars. Cultivar Local had the second higher essential oil %. On the meantime, cv. Compatto has the third higher essential oil %. On the other hand, cv. Boquet, cv. Elephant, cv. Veirling, cv. Tetra and cv. Dukat all had lower essential oil %. No significant differences were observed between cv. Boquet, cv. Elephant, cv. Veirling, cv. Tetra and cv. Dukat. Kruger and Hammer [6] reported that essential oil percentage of different dill cultivars (fruits) vary from 1.91% to 7.25%. Also, Badoca and Lamartib [7] observed that the oil content of European dill seeds varied from 1.75-5.8%.

Table 1: Essential oil % of the seeds of different dill (*Anethum graveolens*) cultivars cultivated under Egyptian conditions

Cultivar	Ist season	2nd season
Tetra	2.067 d*	2.10 c
Bouquet	2.267 cd	2.20 c
Compatto	2.467 c	2.70 b
Dukat	1.933 d	2.13 c
Vierling	2.033 d	2.20 c
Elephant	2.10 d	2.23 c
Common	3.267 a	3.03 a
Local	2.83 b	2.73 b

*Numbers accompanied by different letters are significantly different at $P \leq 0.05$ using one-way ANOVA.

Table (2) shows the relative percentages of main components of the essential oil extracted from the seeds of the studied dill cultivars during the season of 2011/2012 and analyzed with GC-MS are shown in table (2). The total identified components were more than 99.81 in the essential oils of all studied cultivars. The compounds that are defined have been distributed in three groups, i.e., major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace ones (less than 1%).

It is clarified that carvone and limonene only exhibited as majors of seven dill imported cultivars whereas, carvone, limonene, and dillapiol were majors in the local cultivar. Dill apiol was identified in the Local cultivar only, and piperitone was also represented as a minor compound in the local cultivar only. 1-pentene-3-ol, 1-propanol, n-h eptanal, α-thujene, β-thujene, sabinene, β-pinene, 1-methyl-2(methylene bi cyclopropyl), myrcene, cinnamyl alcohol, 1,3,8-para-menthatriene,(4E)-4-decen-6-yne, tetracyclo[4.4.0.0(2,4).0(3,5)] decane,3(iodomethyl)tricycle [3.2.1.0(2,4)]oct6ene, 3-phenylpropanal, α-phellanderene, o-cymene, 1,8cineole, sabinene, cis-limonene oxide, camphor, dill ether, dihydrocarvone, 2,8-dimethyl-1,8-nonadiene, 1,7octadiene, cyclopentane, isopropyl, 2,5-octadiene, cis-dihydrocarveol acetate, 10-undecyn-1-ol, decane, lupulone, E-3-hexenyl acetate, 3-ethyl2pentene, cyclooctene oxide, carvomenthene, piperitenone oxide and hexagermane were considered as traces.

Carvone and limonene were the major constituents of dill seeds volatile oil in Romania [26]. Similar results were reported regarding the seed oil composition in which limonene and carvone were the main constituents as mentioned by [1, 4, 19, 27]. In contrast, another report reviled that dillapiole and carvone were the main components in the dill seed oil [20]. Furthermore, carvone, limonene, and α-phellandrene were found as main components of dill seed oil [11]. Bulgarian cultivar contains limonene, carvone, dihydrocarvone and myristicin [6].

According to the major compounds, it was obviously clear that cv. Tetra showed the highest % of carvone (89.98%) followed by cv. Common (88.81%), cv. Elephant (87.23%), cv. Vierling (85.79%), cv. Compatto (85.22%), cv. Dukat (83.63%) and cv. Bouquet (81.35%), then Local cultivar (56.61%). On the contrary, the highest percentage of limonene in the Local cultivar (18.81%) followed by cv. Bouquet (18.00%), cv. Dukat (15.81%), cv. Compatto (13.80%), cv. Vierling (13.62%), cv. Elephant (11.40%) and cv. Common (10.75%), then cv. Tetra (9.60%). This is an indication that both carvone and limonene have behaved inversely. Dillapiol and piperitone were detected only in the essential oil of Local cultivar where all imported cultivars were free of these two compounds. In Egypt, Mahran *et al.* [2] defined four major components of dill were limonene, dillapiole, carvone and piperitone.

From our results, we can detect a correlated relation between the percentages of carvone and limonene in the seeds essential oils of the all studied cultivars, in which the carvone increased at the expense of limonine and vice versa. As it can be seen from fig. (1) [27], carvone formation depends on the relative activities of the enzymes in the monoterpenes biosynthetic pathway.

This proves results obtained by Sandermann and Bruns [28] reported that limonene is an intermediate in the biosynthesis of carvone. During fruit development, the content of carvone increases at the expense of limonene. Accumulation of limonene and carvone in dill fruits is a developmentally regulated process. Whereas limonene accumulation predominates in the early stages of development, carvone accumulation predominates in the later stages such that, when the fruits mature [29, 30]. Although it has been suggested that carvone and limonene accumulation continue until fruit maturity [31].

Table 2: Chemical composition (%) of the essential oils of the different dill cultivars cultivated under Egyptian condition (second season)

Compound	Cultivar							
	Tetra	Bouquet	Compatto	Dukat	Vierling	Elephant	Common	Local
	%							
1-pentene-3-ol	-	0.01	-	-	-	-	-	-
1-propanol	-	-	0.01	-	-	-	-	-
n-heptanal	-	-	-	-	-	0.01	-	-
α-thujene	-	-	0.01	-	-	0.04	-	0.01
β-thujene	-	-	-	-	-	-	-	0.09
sabinene	-	-	0.01	-	-	o. o4	-	0.04
β-pinene	-	-	-	-	-	-	-	0.02
1-methyl-2(methylenebicyclopropyl)	-	-	-	0.01	-	0.03	-	-
myrcene	0.01	0.01	0.02	0.02	0.01	0.06	0.01	0.09
cinnamyl alcohol	0.03	-	-	-	-	-	-	-
1,3,8-para-menthatriene	-	-	0.03	-	-	-	-	-
(4E)-4-decen-6-yne	-	-	-	0.09	-	-	-	-
tetracyclo[4.4.0.0(2,4).0(3,5)]decane	-	-	-		0.06		-	-
3(iodomethyl)tricyclo[3.2.1.0(2,4)]oct6ene	-	-	-	-	-	0.09	-	-
3-phenylpropanal	-	-	-	-	-	-	0.02	-
α-phellanderene	-	-	-	-	-	-	-	0.32
o-cymene	-	0.01	0.01	-	-	0.03	-	0.11
limonene	9.60	18.00	13.80	15.81	13.62	11.40	10.75	18.81
1,8Cineole	-	-	-	-	-	0.05	-	-
cis limonene oxide	-	0.02	0.01	0.02	0.02	0.03	-	-
camphor	-	-	-	-	-	-	-	0.19
dill ether	-	0.04	-	0.02	-	0.01	-	-
dihydrocarvone	0.30	0.35	0.05	-	0.32	0.51	0.19	0.34
2,8-dimethyl-1,8-nonadiene	-	-	-	0.04	-	-	-	-
1,7octadiene	-	-	-	-	-	-	0.03	-
cyclopentane, isopropyl	-	-	0.39	-	-	-	-	-
2,5-octadiene	-	-	-	0.24	-	-	-	-
cis dihydrocarveol acetate	-	0.02	-	0.01	-	-	-	-
10-undecyn-1-ol	-	-	-	0.02	-	-	-	-
decane	-	-	-	-	0.01	-	-	-
carvone	89.89	81.35	85.52	83.63	85.79	87.23	88.81	56.61
lupulone	-	-	-	-	-	-	0.01	-
E-3-hexenyl acetate	-	-	-	-	0.01	-	-	-
3-ethyl2pentene	-	-	-	0.01	-	-	-	-
cyclooctene oxide	-	-	-	-	-	0.08	-	-
piperitone	-	-	-	-	-	-	-	7.41
carvomenthene	-	-	-	-	-	-	-	0.05
piperitenone oxide	-	-	-	-	-	0.22	-	-
dillapiole	-	-	-	--	--	--	--	15.71
hexagermane	-	-	-	-	-	-	0.01	
Total Identified	99.83	99.81	99.86	99.92	99.84	99.83	99.83	99.80

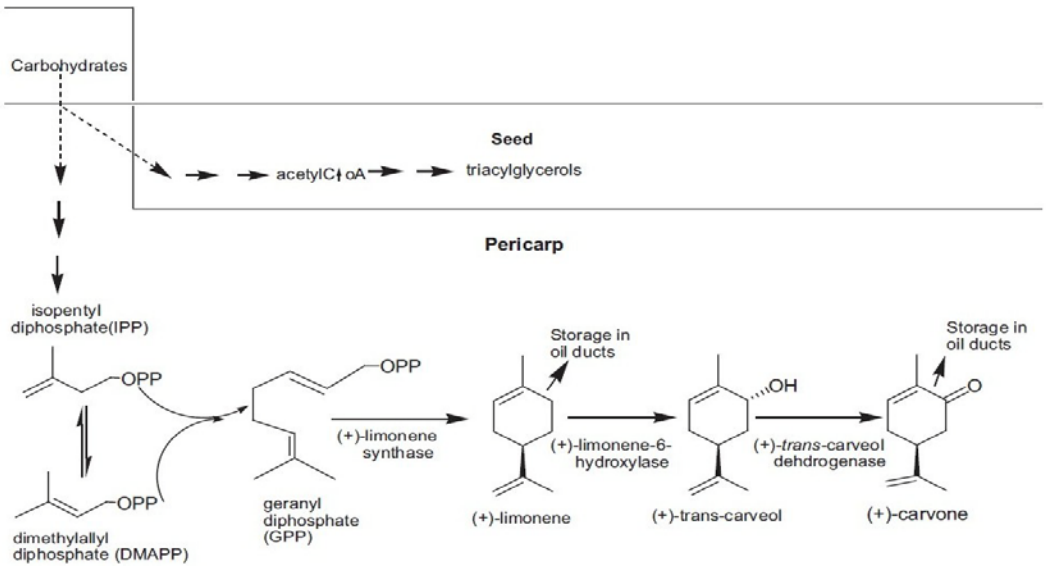

Fig. 1: Enzymatic pathway depicting synthesis of limonene and carvone in seeds of *Anethum graveolens*

Earlier studies by [6, 7] concluded that, there was four chemptypes of dill seeds essential oil: (type1) limonene (36.9-46.7%), carvone (17.8-45.6%), myristicin (0.2-20.3%), dillapiol (8.0-22.3%); (type2)limonene (31.0-40.9%), carvone (25.1-47.4%), dillapiol (6.3-31.8%); (type 3) limonene (39.5-50.7%), carvone (43.7-57.7%) and (type 4) limonene (43.7%), dihydrocarvone (3.1%), carvone (41.2%) and myristicin (11.7%).

In our study, we find the chemotypes of dill can be summarized as shown in table (3): Type (1) carvone and limonene (all European cultivars; cv. Tetra, cv. Bouquet, cv. Compatto, cv. Dukat, cv. Vierling, cv. Elephant and cv. Common). Type (2), carvone, limonene, dillapiol and piperitone (cv. Local).

1- Tetra cultivar, carvone (89.98%) and limonene (9.60%)

2- Bouquet cultivar, carvone (81.35) and limonene (18.00%)

3- Compatto cultivar, carvone (85.22%) and limonene (13.80%)

4- Dukat cultivar, carvone (83.63%), and limonene (15.81%)

5- Vierling cultivar, carvone (85.79%) and limonene (13.62%)

6- Elephant cultivar, carvone (87.23%) and limonene (11.40%)

7- Common cultivar, carvone (88.81%) and limonene (10.75%)

8- Local cultivar, carvone (56.61%), limonene (18.81%), dillapiol (15.71%) and piperitone (7.41%).

Table 3: The main differences in compounds of different studied dill cultivars essential oils

Compound	Cultivar							
	Tetra	Bouquet	Compatto	Dukat	Vierling	Elephant	Common	Local
	%							
Limonene	9.60	18.0	13.80	15.81	13.62	11.40	10.75	18.81
Carvone	89.98	81.35	85.22	83.63	85.79	87.23	88.81	56.61
Piperitone	-	-	-	-	-	-	-	7.41
Dillapiol	-	-	-	-	-	-	-	15.71

CONCLUSION

It may be concluded that the European cultivars contain the highest percentage of carvone and were free from dillapiol and piperitone, thus, we may recommend these cultivation in Egypt.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Santos PAG, Figueiredo AC, Lourenço PML, Barroso JG, Pedro LG, Oliveira MM, *et al.* Hairy root cultures of *Anethum graveolens* (dill): establishment, growth, time-course study of their essential oil and its comparison with parent plant oils. J Biotechnol Lett 2002;24:1031-6.
2. Mahran GH, Kadry HA, Thabet CK, El-Olemy MM, Al-Azizi MM, Schiff PL. GC/MS analysis of volatile oil of fruits of *Anethum graveolens*. Pharm Biol 1992;30:139-44.
3. Peirce A. The American pharmaceutical association practical guide to natural medicines. Stonesong Press: New York; 1999.
4. Weiss E. Spice Crops. CABI Publishing, 10E 40th street, Suite 3203, New York, NY 10016 U.S.A; 2002.
5. Bailer J, Aichinger T, Hackl G, de Hueber K, Dachler M. Essential oil content and composition in commercially available dill cultivars in comparison to caraway. Ind Crops Prod 2001;14:229-39.
6. Kruger H, Hammer K. A new chemotype of *Anethum graveolens* L. J Essent Oil Res 1996;8:205-6.
7. Badoca A, Lamartib A. A Chemotaxonomic evaluation of *Anethum graveolens L.* (dill) of various origins. J Essent Oil Res 1991;3:269-78.
8. Bowes KM, Zheljazkov KVD, Cadwell CD, Pinckock JA, Roberts JC. Influence of seeding date and harvest stage on yields and essential oil composition of three cultivars of dill (*Anethum graveolens* L.) grown in Nova Scotia. Can J Plant Sci 2004;84:1155-60.
9. Said-Al Ahl HAH. Physiological studies on growth, yield and volatile oil of dill (*Anethum graveolens*). Ph. D. Thesis, Fac. Agric., Cairo Univ., Cairo, Egypt; 2005.
10. Guenther E. The essential oils. Vol. III. D Van Nostrand Co. Inc., New York; 1949. p. 640-76.
11. Wall DA, Friesen GH. The effect of herbicides and weeds on the yields and composition of dill (*Anethum graveolens* L.) oil. Crop Prot 1986;5:137-42.
12. Frank JR, Baron JJ, Guest RT. Pesticides for use in herbs, spices, and medicinal plants. Herb Spice Med Plant Dig 1987;5:1-12.
13. Kothari SK, Singh JP, Singh K. Chemical weed control in Bulgarian coriander (*Coriandrum sativum* L.). Trop Pest Manage 1989;35:2-5.
14. Zheljazkov V, Zhalnov I. Effect of herbicides on yields and quality of *Coriandrum sativum* L. J Essent Oil Res 1995;7:633-9.
15. Rangappa M, Bhardwaj HL, Showhda M, Hamama AA. Cilantro response to nitrogen fertilizer rates. Herbs Spices Med Plants 1997;5:63-8.
16. Gil A, De La Fuente EB, Lenardis AE, Pereira ML, Suarez SA, Arnaldo B, *et al.* Coriander essential oil composition from two genotypes grown in different environmental conditions. J Agric Food Chem 2002;50:2870-7.
17. Faber B, Bangert K, Mosandl A. GC-IRMS and enantioselective analysis in biochemical studies in dill (*Anethum graveolens* L.). Flavour Fragrance J 1998;12:305-14.
18. Yili A, Yimamu H, Maksimov VV, Aisa HA, Veshkurova ON, Salikhov ShI. The chemical composition of essential oil from seeds of *Anethum graveolens* cultivated in China. Chem Nat Compd 2006;42:491-2.
19. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oil. Int J Food Microbiol 2002;74:101-9.
20. Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV, Damianova ST. Composition, quality control and antimicrobial activity of the essential oil of a long time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. J Agric Food Chem 2003;18:3854-7.
21. Kapoor R, Giri B, Mukerji KG. *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in dill (*Anethum graveolens* L.) and carum (*Trachyspermum ammi* (Linn.) Sprague). World J Microbiol Biotechnol 2002;18:459-63.
22. Babri RA, Khokhar I, Mahmood Z, Mahmud S. Chemical composition and insecticidal activity of the essential oil of *Anethum graveolens* L. Sci Int (Lahore) 2012;24:453-5.
23. Singh G, Maurya S, Lampasona MPD, Catlan C. Chemical constituents, antimicrobial investigations, and antioxidative potentials of *Anethum graveolens* L. Essential oil and acetone extract: Part 52. J Food Sci 2005;70:M208-M215.
24. Jackson ML. Soil Chemical Analysis Prentice-Hall of India; 1973.
25. British Pharmacopoeia, British Approved Names. A Dictionary of Drug Names for Regulatory Use in the UK. Stationary Office Press: London, UK; 2002.
26. Radulescu V, Popescu ML, Ilies D. Chemical composition of the volatile oil from different plant parts of *Anethum graveolens* L. (*Umbelliferae*) cultivated in Romania. Farmacia 2010;58:594-600.
27. Jana S, Shekhawat GS. *Anethum graveolens*: an Indian traditional medicinal herb and spice. Pharmacogn Rev 2010;4:179-84.
28. Sandermann W, Bruns K. Biogenese von carvon in *Anethum graveolens* L. Planta Med 1965;13:364-8.
29. Bouwmeester HJ, Kuijpers AM. Relationship between assimilate supply and essential oil accumulation in annual and biennial caraway (*Carum carvi* L.). J Essent Oil Res 1993;5:143-52.

30. Bouwmeester HJ, Davies JAR, Smid HG, Welten RSA. Physiological limitations to carvone yield in caraway (*Carum carvi* L.). Ind Crops Prod 1995;4:39-51.

31. Von Schantz M, Huhtikangas A. Über die Bildung von limonene und carvon in Kümmel. *Carum carvi*. Phytochemistry 1971;10:1787-93