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Influence of Hydro-Distillation Time on the Yield and Quality of Dill Volatile Constituents

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ABSTRACT

The effects of hydro-distillation time (1, 2, 3, 4, 5 and 6 h) on the essential oil (EO) extracted from dill (*Anethum graveolens* L.) fruits were investigated by GC and GC/MS. EO content (ml g^{-2}) increased with several treatments of hydro-distillation time compared with the first hour of hydro-distillation. After 5 h of hydro-distillation the yield of EO remained 2.5 ml g^{-2}, then it stabilized. The main constituents of dill EO were apiol (34.6-48.7%), carvone (19.2-27.1%), (*R*)-limonene (7.7-15.0%) and α-pinene (7.4-16.3%). Apiol and carvone gradually increased with an increase in hydro-distillation time but (*R*)-limonene and α-pinene decreased. The monoterpene hydrocarbons presented a gradual decrease (39.3% in the first hour to 19.9% after 6 h), the total amount of oxygenated monoterpenes increased from 60.0% in the first hour to 79.5% after 6 h, the sesquiterpene hydrocarbons appeared to increase in the 3rd hour (1.1%) compared with other distillation times while oxygenated sesquiterpene appeared to increase after 4 h (0.4%).

Keywords: *Anethum graveolens* L., apiol, carvone, (*R*)-limonene, oxygenated monoterpene, α-pinene
Abbreviations: EO, essential oil; **MH**, monoterpene hydrocarbon; **OM**, oxygenated monoterpene; **OS**, oxygenated sesquiterpene; **SH**, sesquiterpene hydrocarbon

INTRODUCTION

Changes in the composition of an essential oil (EO) can be caused by environmental factors such as the soil or climate in which a plant is grown, and by different harvesting methods or distillation techniques (Chatzopoulou and Katsiotis 1995). The isolation and concentration techniques normally used may well alter the quantitative as well as the qualitative composition of the obtained EO relative to the composition of the compound percent in the plant material (Chatzopoulou and Katsiotis 1995). The techniques employed in the extraction of an EO from a plant have a significant effect on the final composition of the product obtained (Charlwood and Charlwood 1991). Preliminary studies, carried out on different plant materials, confirmed these effects on EO yield and composition, and especially on *Eucalyptus globulus*, which was changed by the different distillation durations (Lassak 1992). Hydro-distillation, in which water from a still flask is separated by filtration and used together with fresh water for immersing the plant material in a subsequent distillation, was used for obtaining EO from aromatic plants (Stanković *et al*. 2001, 2004, 2005). Hydro-distillation is the most widely used and economical method to obtain dill EO (Baydar *et al*. 2008). Distillation time had a significant effect on the yield and composition of the EO extracted from coriander (*Coriandrum sativum* var. microcarpum) fruits and could be manipulated to alter the linalool content of the EO (Smallfield *et al*. 2001). Distillation time influences the quality of key lime (*Citrus aurantifolia*) EO with 1.10% of carbonylic compounds obtained within 10 h. Aldehyde content increased significantly during distillation time, and after 10 h, the EO has contained more than 3% aldehydes due to oxidative reactions (Gamarra *et al*. 2006). Baydar *et al*. (2008) investigated the effect of hydro-distillation time (30, 60, 90, 120, 150 and 180 min) on EO composition of damask rose (*Rosa damascena* Mill.); distillation up to 150 min increased the EO yield by 225% compared with the treatment of 30 min, although a longer distillation time gave a higher methyl eugenol concentration whose content increased steadily (0.69% after 30 min to 1.65% after 150 min). According to Chatzopoulou and Katsiotis (1995), the investigation on the influence of the distillation time on integral and comminuted *Juniper communis* L. berries performed with classic hydro-distillation and the simultaneous distillation extraction method showed that: (a) it is not possible to completely obtain the EO from integral berries (0.3% yield) and that the yield from the comminuted plant material was increased in the first three hours of distillation (1.75-2.2%); (b) the ratio of the three main constitutes groups liberated, i.e. monoterpene hydrocarbons, oxygennated monoterpenes and sesquiterpene depend on the isolation method as well as the distillation time applied on the *Juniper communis* L. berries. Distillation time up to 150 min increased the EO yield and methyl eugenol concentration extracted from Damask rose (*Rosa damascena* Mill.) (Baydar *et al*. 2008). The EO isolated from sage (*Salvia officinalis* L.) dried aerial parts obtained at different hydro-distillation times (30 min, 1, 2 and 3 h) ranged from a yield of 2.0-2.1% (v/w), showed no major difference with the different periods of distillation (Miguel *et al*. 2011). The effects of distillation time on EO yield and the concentration of artemisinin in the plant residue from distillation (PRD) were investigated by Valtcho *et al.* (2011); they indicated that the duration of the distillation time had a significant effect on EO yield. The EO yield varied from 0.05 to 0.35% in the fresh *A. annua* samples, with the lowest oil yield from the shortest distillation time and the highest yield from the longest distillation times (160 and 240 min). Artemisinin was apparently degraded during the distillation, showed a decrease (84%) in plant samples that have been subjected to distillation for 1.25 min compared with un-distilled control. Artemisinin in plant samples continued to decrease up to 20 minutes and was undetected in HPLC system in samples subjected to 40-240 min distillation time. Obtained results demonstrated that PRD consti-

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tutes a byproduct that is devoid or artemisinin. The highest EO yields were obtainable at distillation time of 160-240 min. Cannon *et al*. (2011) determined the effect of distillation time (1.25, 2.5, 5, 10, 20, 40, 80 and 160 min) on peppermint EO yield, and the concentration and yield of eucalyptol, menthofuran, menthol, menthone, menthylacetate and *t*-caryophyllene. EO yield increased from 2.5 to 5 min. Numerically, the highest EO yield was achieved after 80 min distillation time; however, this was not significantly different from yields at 20, 40, or 160 min. Obtained results suggested that there was no significant increase in EO yield after 20 min distillation time. The concentrations of menthol, menthone, and menthylacetate were not significantly affected by the length of the distillation time. The concentration of eucalyptol was the highest at the shortest distillation time, lower in 5, 10, or 20 min distillation time, and the lowest at the longest distillation time (160 min), while the concentrations of caryophyllene and menthofuran followed exactly the opposite trend. With the exception of eucalyptol, the yield of the other constituents increased up to 20 or 40 min, and there was no significant increase after that time. Obtained results demonstrated that 20 to 40 min distillation time would be sufficient to obtain the highest EO yield with desirable EO composition. The EOs isolated from sage (*Salvia officinalis* L.) dried aerial parts obtained at different hydro-distillation times (30 min, 1, 2 and 3 h) ranged from a yield of 2.0-2.1% (v/w), showed no major difference with the different periods of distillation (Miguel *et al*. 2011).

Anethum graveolens L. or dill, belonging to *Apiaceae* (*Umbelliferae*) family, is an annual aromatic herb known for culinary and medicinal use since ancient times. It is cultivated in the most parts of Europe,the United States of America, India, Egypt and Japan (RaduLescu 2010). In recent years the scientific literature reports pharmacological effects of dill such as antibacterial (Singh *et al*. 2001; Lopez *et al*. 2005), antimycobacterial (Stavri and Gibbons 2005), antioxidant (Satyanarayana *et al*. 2004; Singh *et al.* 2006; Taher *et al*. 2007), cancer chemopreventive (Zheng *et al*. 1992). The well-known properties of dill from the traditional medicine such as carminative, stomachic, diuretic have been reported (Hosseinzadeh 2002; Amin and Sleem 2007). The dill EO has hypolipidemic activity and could be a cardioprotective agent (Hajhashemi and Abbasi 2008). The chemical composition of dill EO varies depending on the plant parts. In the leaves oil monoterpenic hydrocarbons are predominant, amounting to 79.14% (62.71% α-phellandrene and 13.28% limonene). In the flowers EO oil the content of α-phellandrene and limonene is 32.26 and 33.22%, respectively. Anethofuran (dill ether) is present in leaves and flowers with 16.42 and 22%, respectively, but is missing in the fruit EO oil. The main compound in fruits EO is carvone (75.21%), while the content of α-phellandrene is only 0.12% and limonene is 21.56% (Valtcho *et al.* 2011).

This paper presents the results from the analysis of the volatile composition of dill or *Anethum graveolens* L. fruits by using the classic method of distillation, considering the influence of the isolation time parameter on the yield and quality of the obtained volatile constituents.

MATERIALS AND METHODS

Plant material

Anethum graveolens L. or dill fruits were obtained from the Institute of Medicinal and Aromatic Plants, Egyptian Ministry of Agriculture.

EO isolation

The EO was isolated from the dry ripening fruits by hydro-distillation for 1, 2, 3, 4, 5 and 6 h using a Clevenger-type apparatus according to the European Pharmacopoeia method (Council of Europe 2008). The EO was stored at 20°C in the dark until analysis.

GC/MS

The EO was analyzed on a VG analytical 70- 250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m × 30 m, 0.25 µm coating thickness, fused silica capillary column, injector 222°C, detector 240°C, linear temperature 80-270°C at 10°C/min. Diluted samples (1/100, v/v, in *n*-pentane) of 1 µl were injected, at 250°C, manually and in the splitless mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program.

Qualitative and quantitative analyses

Identifications were made by library searches (Adams 2007) combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature (Adams 2007) or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of *n*-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature (Adams 2007). Component relative concentrations were calculated based on GC peak areas without using correction factors.

Statistical analysis

In this experiment, 1 factor was considered: distillation time (1, 2,3,4,5 and 6 h). For each treatment there were 4 replicates. The experimental design followed a complete random design. According to Snedecor and Cochran (1990), the averages of data were statistically analyzed using 1-way analysis of variance (ANOVA-1). Significant values determined according to *P* values ($P \leq 0.05$ = significant, $P < 0.01$ = more significant and $P < 0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart T 1982).

RESULTS AND DISCUSSION

According to **Table 1**, EO content (ml g^{-2}) increased with several treatments of hydro-distillation time compared with the first hour of hydro-distillation. After 5h of hydro-distillation the yield of EO remained 2.5 ml g^{-2}. Then it stabilized (2.5 ml g^{-2}). The lowest EO yield (1.1 ml g^{-2}) was recorded after 1 h of hydro-distillation. ANOVA indicated that EO yield were highly significant in hydro-distillation time treatments. These results agree with those obtained by previous literature on EO-bearing plants; distillation time had significant effects on the yield and composition of the EO extracted from coriander (*Coriandrum sativum* var. microcarpum) fruits (Baydar *et al*. 2008); distillation time up to 150 min increased the EO extracted from *Juniper communis* L. berries. (Chatzopoulou and katsiotis 1995); the duration of the distillation time had a significant effect on *Artemisia annua* EO yield (Valtcho *et al.* 2011).

Twenty-nine constituents were identified in EO extracted from dill fruits, accounting for 99.3 to 99.8% of total constituents, and belong to four chemical main classes. Oxygenated monoterpene (OM) class was the major one,

Table 1 Effect of hydro-distillation time on essential oil content extracted from dill fruits.

Hydro-distillation time	Essential oil (ml g^{-2})
1 h	1.1
2 h	1.2
3 h	1.8
4 h	2.4
5 h	2.5
6 h	2.5
F-ratio	65.98 ***

* $P \leq 0.05$ according to *F*-values of one-way analysis of variance (ANOVA-1).
** $P < 0.01$ according to *F*-values of one-way analysis of variance (ANOVA-1).
*** $P < 0.001$ according to *F*-values of one-way analysis of variance (ANOVA-1).

Table 2 Effect of hydro-distillation time on EO constituents extracted from dill fruits.

No.	Components (%)	RI*	Hydro-distillation time (hours)						*F*-ratio
			1	2	3	4	5	6	
1	α-Pinene	929	16.3	15.1	12.5	11.7	11.3	7.4	276.8***
2	α-Thujene	939	0.3	0.2	0.2	0.1	0.2	0.2	2.4
3	Camphene	954	0.1	0.1	0.1	0.1	0.1	0.1	2.3
4	Sabinene	973	1.3	1.1	0.8	2.5	1.1	0.5	20.8***
5	*β*-Pinene	980	0.6	0.5	0.4	0.6	0.6	0.7	2.4
6	Myrcene	991	1.2	1.1	0.9	0.3	0.1	0.4	47.4***
7	α-Phellandrene	1005	3.2	1.2	2.3	0.6	1.2	1.4	41.6***
8	γ-Terpinene	1018	0.1	1.1	0.9	1.3	3.0	1.1	14.6***
9	*p*-Cymene	1026	0.1	0.2	0.2	0.5	0.1	0.3	10.2**
10	*β*-Phellandrene	1031	1.1	0.1	0.1	0.2	0.1	0.1	145.5***
11	Eucalyptol	1033	0.2	0.1	2.2	1.1	1.2	0.1	127.7***
12	(*R*)-Limonene	1054	15.0	13.6	11.7	11.5	11.4	7.7	66.7***
13	(*E*)-Limonene oxide	1133	0.1	1.6	0.3	1.9	0.3	0.1	296.0***
14	*cis*-Verbenol	1140	0.1	0.3	0.1	0.3	0.1	0.1	9.6**
15	1,6-Dihydrocarveol	1192	0.1	0.1	1.2	1.1	0.1	0.1	106.2***
16	Dihydrocarvone	1193	0.1	1.4	0.1	0.1	0.1	0.1	31.7***
17	Estragole	1195	0.1	0.5	1.4	0.1	0.1	0.7	22.8***
18	Verbenone	1204	1.7	0.1	0.3	1.4	1.1	0.1	40.7***
19	1,8-cineole	1219	0.3	0.1	0.2	0.1	0.4	0.5	12.0***
20	Citral	1240	0.1	0.1	0.1	0.1	0.1	0.1	2.5
21	Carvone	1242	19.2	19.4	22.1	22.6	23.1	27.1	635.2***
22	Eucarvone	1245	1.4	1.6	1.4	2.2	1.1	0.9	24.3***
23	Carvacrol	1298	0.1	0.2	1.3	0.1	0.2	0.1	37.1***
24	Germacrene-D	1480	0.1	0.1	1.1	0.3	0.1	0.1	144.0***
25	Myristicin	1520	0.1	0.1	0.2	0.2	0.1	0.1	2.4
26	Carotol	1594	0.1	0.2	0.3	0.4	0.2	0.1	6.2**
27	Apiol	1680	34.6	39.0	37.1	37.6	41.1	48.7	382.7***
28	Carveol	1846	1.7	0.4	0.2	0.3	0.1	0.3	80.1***
29	Dill ether	2000	0.1	0.2	0.1	0.4	0.6	0.4	5.8**
Monoterpene hydrocarbons			39.3	34.3	30.1	29.4	29.2	19.9	1912.6***
Sesquiterpene hydrocarbons			0.1	0.1	1.1	0.3	0.1	0.1	144.0***
Oxygenated monoterpenes			60.0	65.2	68.3	69.6	69.8	79.5	341.4***
Oxygenated sesquiterpenes			0.1	0.2	0.3	0.4	0.2	0.1	8.2**
Total identified			99.5	99.8	99.8	99.7	99.3	99.6	

RI*= retention index on SPB-5 column

* $P \leq 0.05$ according to *F*-values of the one-way analysis of variance (ANOVA-1).

** $P < 0.01$ according to *F*-values of the one-way analysis of variance (ANOVA-1).

*** $P < 0.001$ according to *F*-values of the one-way analysis of variance (ANOVA-1).

followed by monoterpene hydrocarbons (MH). The remaining fractions as sesquiterpene hydrocarbons (SH) and oxygenated sesquiterpene (OS) formed the minor classes (**Table 2**). The main constituents of dill EO as detected by GC-MS were apiol, carvone, (*R*)-limonene and α-pinene. Apiol and carvone were gradually increased with an increase in hydro-distillation time but (*R*)-limonene and α-pinene decreased. The highest amount of apiol (48.7%), carvone (27.1%) resulted after six hours while the highest amount (*R*)-limonene (15.0%) and α-pinene (16.3%) resulted after 1 h of hydro-distillation. ANOVA indicated that the changes in all constituents were highly significant for hydro-distillation time treatments except the constituents of *p*-cymene, *cis*-verbenol, carotol and dill ether were more significant while α-thujene, camphene, citral and myristicin were insignificant. Similar constituents were obtained by Jozef *et al*. (2002) as well as Khalid and Shafei (2005) from dill fruits. These results agree with those obtained by previous literature on EO - bearing plants; distillation time had significant effects on the yield and composition of the EO extracted from coriander (*Coriandrum sativum* var. microcarpum) fruits as well as could be manipulated to alter the linalool content of the EO (Smallfield *et al*. 2001). Distillation time process influences the quality of key lime (*Citrus aurantifolia*) (Gamarra *et al*. 2006); distillation time up to 180 min increased the methyl eugenol concentration extracted from Damask rose (*Rosa damascena* Mill.) (Baydar *et al*. 2008); artemisinin was apparently degraded during the distillation, showed an decrease (84%) in plant samples that have been subjected to distillation for 1.25 min, compared with undistilled control (Valtcho *et al*. 2011); Artemisinin in plant samples continued to decrease up to 20 min and was undetected in HPLC system in samples subjected to 40-240 min distillation time (Valtcho *et al*. 2011).

In **Table 2** the percentages of the chemical constituents of EO obtained after 1-6 h hydro-distillation are shown. The MH presented a gradual decrease (39.3% in the first hour to 19.9% after 6 h), the total amount of OM increased from 60.0% in the first hour to 79.5% after 6 h, the SH appeared to increase in the 3rd hour (1.1%) compared with other distillation times while OS appeared to increase in the 4th hour (0.4%).

The changes in all EO classes were highly significant except for OS, which was more significant (**Table 2**). OM appeared to increase throughout the hydro-distillation period from 1-6 h. This increase is rather negligible through losses had occurred, probably, because of their solubility. These compounds are considerably water-dissolved and probably were partially re-dissolved in the separation part of the hydro-distillation apparatus returning back to the flask, and moreover, presumably because of the extended period of hydro-distillation (Koedam *et al*. 1988). These results agree with those obtained by Chatzopoulou and Katsiotis (1995), who they indicted that the three main constitutes groups liberated, i.e. MH, OM and sesquiterpene, depend on the isolation method as well as the distillation time applied to *Juniper communis* L. berries.

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