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Essential Oils of *Anethum graveolens* L.: Chemical Composition and Their Antimicrobial Activities at Vegetative, Flowering and Fruiting Stages of Development

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Abstract

Antimicrobial activity of the essential oils obtained from vegetative herb, flowering herb and the seeds of *Anethum graveolens* was determined against eight microorganisms using the disk diffusion assay test as well as measuring minimum inhibitory concentrations (MIC) against diverse type of microorganisms including gram-positive bacteria, gram-negative bacteria and fungi. The results showed that the tested dill oils exhibited strong and a variable degree of antimicrobial activity against all of the tested microorganisms. The essential oils compositions were analyzed and determined by GC. The main components of the vegetative herb essential oils were α-phellandrene (46.33%), limonene (13.72%), β-phellandrene (11.01%) and p-cymene (17.88%). p-cymene (33.42%), carvone (13.10%) and dillether (19.63%)were the main components of the flowering herb, whereas, carvone (62.48%), dillapiole (19.51%) and limonene (14.61%) were identified as the major compounds in seed essential oil.

Keywords

Anethum graveoleus L., Antimicrobial Activity, Essential Oil Compositions

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1. Introduction

Essential oils are rich sources of biologicallyactive compounds. Recently, there has been a profound interest in the antimicrobial properties of the aromatic plants, particularly in their essential oils (Lis-Balchin and Deans, 1997; Pattnaik et al., 1997; Knobloch et al., 1989).Dill (*Anethum graveolens*), an important member of the family Apeaceae, and originates from Mediterranean and West Asia. It is cultivated for use as a vegetable and fruits and also as a source of essential oil. Its medicinal uses areas an antispasmodic, carminative, diuretic, stimulant and stomachic (Simon et al., 1984). Furthermore, the dill essential oil has hypolipidemic activity and could be used as a cardio-protective agent (Hajhashemi and Abbasi (2008). Dill has been reported topossess antibacterial (Rafii and Shahverdi, 2007), antihyperlipidemic, and antihypercholesterolemic (Yazdanparast and Alavi, 2001) properties, antifungal drugs and food preservatives (Tian et al., 2011). Majority of the essential oils are classified as generally recognized assafe and have low risk for resistance development inpathogenic microorganisms (Cardile et al., 2009). Some of the earlier studies had shown the antimicrobial activity of *A. graveolens* against *Saccharomyces cerevisia* and

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Listeria monocytogenes (Pascal et al., 2002). The quantity and chemical composition of dill essential oil varies depending on the plant parts and the developing stage of the plant at harvest time (Radelescu et al., 2010). Essential oil and extracts of dill have been reported to possess various degrees ofantimicrobial activity and this property may be probably due to the presence of furanocoumarin in dill (Stravri and Gibbons, 2005). Limonene and carvone, have exhibited strongantifungal activity against *Aspergillus niger*, *Saccharomyces cerevisiae* and*Candida albicans* (Stravri and Gibbons, 2005; Kaur and Arora, 2010). Gram-negative bacteria were shown to be generally more resistant than gram-positiveones to the antagonistic effects of essential oils because of the lipopolysaccharidepresents in the outer membrane. It restricts the diffusion of hydrophobic compounds through its lipo-polysaccharide covering, but this was not always true (Angienda et al., 2010; Sonali and Shekhawat, 2010).

Hence, there has been an increased interest in looking atantimicrobial properties of extracts from aromatic plantsparticularly essential oils.No information in the literature concerning variation of aroma composition, in dill volatile oil extracted from different parts and at various developing stages in Egypt. Our experiment showed the great differentiation in aroma composition on the basis of the three developing stages. It was possible to select the stage of the best aroma quality. The present study relates to determine theantibacterial activity against gram positive (*Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeroginosa* and *Staphylococcusaureus*); fungi (*Aspergillus niger*, *Trichoderma* sp.) and *Candida albicans*.However,to our knowledge and according to a literature survey, this is the first report on the antimicrobial activity of dill volatile oils at different developing stages (vegetative, flowering and fruiting)

2. Materials and Methods

2.1. Plant Material and Essential Oil Isolation

The different parts of *Anethum graveolens* plants (at vegetative, flowering and fruiting stages) were harvested from the Farm Station of National Research Centre, at Shalakan Kalubia Governorate, National Research Centre, Dokki, Giza, Egypt. The essential oil percentage was subjected to hydro-distillation for 3 hours using a modified Clevenger apparatus according to Gunther (1961). The resulted oil was dehydrated over anhydrous sodium sulphate and stored at freezer till used for gas liquid chromatographic (GLC) analysis.

2.2. Microorganisms and Growth Conditions

Microorganismes are three gram-positive bacteria such as *Bacillus cereus*,*Bacillus subtilis*, *Escherichia coli*; twogram-negative bacteria such as *Pseudomonas aeroginosa* and *Staphylococcus aureus* and two fungi such as *Aspergillus niger*, *Trichoderma sp*.and one yeast, *Candida albicans* were used to check the antimicrobial potential of essential oils of dill at different developing stages. These microorganisms were obtained from Microbial Chemistry Department, National Research Centre, and Egypt. The antimicrobial activity was determined by disc diffusion method (Ericsson and Sherris, 1971).

3. Results and Discussion

3.1. Essential Oil Constituents duringThree Development Stages

The main constituents of the essential oils distilled from fresh herb during vegetative stage were indicated in Table (1). The obtained data revealed that, α-phellandrene (46.33%), limonene (13.72%), β-phellandrene (11.01%) and p-cymene (17.88%) were the most abundant, but their quatitative contents varied to different extents. The results within our hands indicated that there are differences in volatile oils components according to variation of the development stages.

The quantities of the compounds identified by GLC in the volatile oil distilled from the fresh herb of dill during flowering stage are presented in Table (1). Altogether 17 compounds were identified. Three main compounds were identified, such as p-cymene (33.42%), carvone (13.10%) and dill ether (19.63%).

The relative percentages of identified main constituents of volatile oil distilled from air dried seeds of dill plants are indicated in Table (1). From these results, it is clear that carvone (62.48%), dillapiole (19.51%) and limonene (14.61%) were identified as the major compounds.

With regard to the major seven compounds in dill oil in three development stages, we find the following that α-phellandrene and β-phellandrene have the same behavior. The highest percentages for α-phellandrene (46.33%) and β-phellandrene (11.01%) in the vegetative stage and lowest percentages of them (0.59; 2.70%) in the flowering stage, respectively and both of them was disappeared in the fruiting stage. We also find that carvone and dillapiole have reverse behaviour, where carvone percentage increase with the development of the plant and this means that the highest of carvone (62.48%) and lowest of dillapiole (4.16%) percentages was in the fruiting stage, respectively and the highest of dillapiole (19.51%) and lowest of carvone (2.11%) percentages were in the vegetative stage. While the medium parentages of carvone (13.10%) and dillapiole (4.16%) were in the flowering stage. Similarly, limonene and p-cymene

have reverse behaviour, where the highest of limonene (14.61%) and lowest of p-cymene (0.30%) percentages was obtained from oil at fruiting and flowering stages, also the highest of p-cymene (33.42%) and lowest of limonene (0.48%) percentages was obtained from oil at flowering and fruiting flowering stages, and the medium parentages of limonene (13.72%) and p-cymene (17.88%) were in the flowering stage. The highest (19.63%) and lowest (0.45%) percentages of dill ether were obtained from dill plant at flowering and vegetative stages, respectively and the medium parentage (1.64%) was in the fruiting stage.

3.2. Antimicrobial Activity

The results recorded in Table (2) indicated that volatile oils of dill plants distilled individually from fresh herb during vegetative and flowering stages and from the air dried seeds (fruits) throughout fruiting stage had a considerable biologically activities against some microorganisms of bacteria such as *Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and *Staphylococcus aureus* and fungi such as *Aspergillus niger, Trichoderma* sp. and *Candida albicans.*

Basing the estimation on the values of growth inhibition zones (mm), it was clearly found that volatile oils from the fresh herb had a much stronger bacterial activity on *Bacillus cereus* and *Staphylococcus aureus* than that of volatile oil obtained from the seeds, because the two genus of bacteria were the most sensitive organisms to the action of volatile oil, while *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeroginosa* were the most resistant strains towards the volatile oils, because the tabulated values were absolutly more than less than 40 (mm) when inoculated with 12.5 and 25 ul/Disc. In this concern, the two genus of fungi and yeast were markedly sensitive organisms to the action of volatile oils distilled from herb and seeds with exception of *Candida albicans* that resistant strain towards the volatile oil from herb during flowering stage, thus susceptibility of microorganisms to volatile oils activity depended on strains of organisms, dill plant organs and stages.

The resulted given in Table (3) showed that the concentration of volatile oils (1 ul/ml) distilled from any dill organs was a strong inhibitor on the growth of *Bacillus cereus* (bacteria) and *Candida albicans* (fungi), while concentration of 2.5 ul/ml was a strong inhibitor on the growth of *Pseudomonas aerogenosa* (bacteria) and *Trichoderma* sp. (fungi), whereas, concentration of 3.5 ul/ml was a strong inhibitory on the growth of *Bacillus subtilis, Escherichiacoli,Staphylococcus aureus* (bacteria) and *Aspergillus niger* (fungi). Therefore, the responses of the strains (*Bacillus cereus* and *Candida albicans)* were markedly similar in that, low concentration of volatile oils of each dill organs at 1ul/ml were the most effective comparing to that of the strains under various concentrations of volatile oils. Jirovetz et al. (2003) found that, carvone (50.1%) and limonene (44.1%) were Antimicrobial testings showed high activity of the essential *A. graveolens* oil against the mold *Aspergillus niger* and the yeasts *Saccharomyces cerevisiae* and *Candida albicans*. Similar results have been reported by Saxena et al. (1990); Dwivedi and Dubey (1993); Lis-Balchin et al.(1996); Hassanein and Eldoksch (1997); Rasooli and Rezaei (2000); Singh et al. (2002); Leopold et al., (2003); Ozcan (2003); Sipailiene et al. (2003); Jirovetz et al.(2004) and Nevas et al. (2004)on volatile oil of *Anethum graveolens* plants.

Table 1. Principal constituents (%) of *Anethum graveolens* essential oil during three development stages

Compound	Vegetative Herb	Flowering Herb	Fruits
α-Pinene	1.63	-	-
β-pinene	0.27	-	-
α-phellandrene	46.33	0.59	-
Limonene	13.72	0.48	14.61
β-phellandrene	11.01	2.70	-
γ-terpinene	0.36	-	-
P-cymene	17.88	33.42	0.30
Linalool	-	0.35	0.01
Dillether	0.45	19.63	1.64
Dihydrocarvone	0.22	0.73	0.07
Sabinol	1.04	0.31	0.03
Carvone	2.11	13.10	62.48
Piperitone	0.23	4.60	0.21
Carveol	0.66	3.24	-
Nerolidol	0.71	1.48	0.01
Eugenol	0.79	1.55	-
Thymol	0.59	0.85	-
Carvacrol	0.24	1.62	0.02
Myristicin	1.07	0.64	0.05
Dillapiole	0.59	4.16	19.51

Table 2. Antimicrobial activity of different concentrations (ul/disc) of dill volatile oils expressed as (mm) inhibition zone.

Concentrations (ul/Disc) / Tested microorganisms	Diameter (mm) of zone of inhibition														
	Dill oil at vegetative stage					Dill oil at flowering stage					Dill oil at fruiting stage				
	1	2	5	12.5	25	1	2	5	12.5	25	1	2	5	12.5	25
Bacillus cereus	8	10	20	+	+	8	10	18	+	+	6	10	18	20	22
Bacillus subtilis	8	8	18	26	30	6	6	8	18	20	6	6	14	22	24
Staphylococcus aureus	8	10	23	+	+	6	12	28	+	+	6	12	18	32	+
Escherichia coli	10	12	14	16	26	6	10	10	10	14	-	10	18	20	20
Pseudomonas aerogenosa	6	10	18	25	30	6	10	20	25	30	6	8	18	24	32
Aspergillus niger	-	8	20	+	+	-	-	12	+	+	8	10	18	+	+
Trichoderma sp.	10	10	18	+	+	10	10	20	+	+	15	25	+	+	+
Candida albicans	6	8	18	+	+	6	6	10	20	28	6	10	26	+	+

(+) more than 40 (mm); (-) no inhibition

Table 3. Minimum inhibitory concentration of dill volatile oils at different developing stages (vegetative, flowering and fruiting).

Tested microorganisms	Concentration of dill volatile oils (uL/ml.)				
	0.5	1	2.5	3.5	5
Bacillus cereus	+	-	-	-	-
Bacillus subtilis	+	+	+	-	-
Staphylococcus aureus	+	+	+	-	-
Escherichia coli	+	+	+	-	-
Pseudomonas aerogenosa	+	+	-	-	-
Aspergillus niger	+	+	+	-	-
Trichoderma sp.	+	+	-	-	-
Candida albicans	+	-	-	-	-

The minimum inhibition concentration (MIC) is determined as the lowest concentration of dill volatile oils that totally inhibited microbial growth. (-) inhibition; (+) no inhibition

4. Conclusion

The antimicrobial activity of essential oil of dill isolation from different developing stages (vegetative, flowering and fruiting) was evaluated in vitro test against eight microorganisms. Essential oil of dill at different developing stages exhibited strong and a variable degree of antimicrobial activity against all the tested microorganisms.

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