Chemical Composition and Antimicrobial Activity of Essential Oils Obtained from Dill (*Anethum graveolens* L.) Grown in Western Romania

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In this study essential oils from inflorescences, stems, immature and mature seeds of dill (Anethum graveolens L.) grown in western Romania (Bucovăţ, Timiş County) were isolated by steam distillation. The isolated essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) and their antimicrobial activity was evaluated on an array of seven microorganisms: Shigella flexneri (ATCC 12021), Klebsiella pneumoniae (ATCC 13882), Salmonella typhimurium (ATTC 14028), Staphylococcus aureus (ATCC 25923), E. coli (ATCC 25922), Streptococcus pyogenes (ATTC 19615), Clostridium perfringens (ATCC13124). The main component of the oil from mature seeds is carvone 52.37% and limonene 39.20%, the concentration of the latter being slightly lower than in the oil obtained from immature seeds 40.69%, which also contains 34.62% carvone. The content of phellandrene varies in the oil samples analyzed: 12.61% in inflorescences, 3.67% in immature seeds, 30.45% in stems and 2.29% in mature seeds. Significant antimicrobial activity (tested by the Kirby-Bauer method) was recorded against Shigella flexneri, Klebsiella pneumoniae, Salmonella and E. Coli, while no inhibitory effects were observed against Streptococcus pyogenes and Staphylococcus aureus.

Keywords: dill, essential oil, steam distillation, GC-MS analysis, antimicrobial activity

Dill (*Anethum graveolens* L.) is a herbaceous annual plant in the *Umbelliferae* family, originated from Asia and the Mediterranean area, grown as a condimentary plant throughout the whole Romania. Dill has been known from ancient times, its condimentary value being given by its essential oil (EO), present in all the plant parts. The mature seeds contain 2.5-4% EO [1], its main components being carvone 41-67% and limonene 23-44% [2,3]. In the composition of the EO have been identified, along with the two main constituents, compounds such as: phellandrene, pinene, diterpenes, dihydrocarvone, cineol, myrcene, p-myrcene, isomyristicin, myristicin, myristin, apiol and dillapiol.

In addition to its aromatic properties, dill also exhibits a significant antimicrobial activity through its bioactive components isolated as EO. The presence of furocoumarin, oxypeucedanin, oxypeucedanin hydrate, falcarindiol, 5-(4"-hydroxy-3"-methyl-2"-butenyloxy)-6,7-furocoumarin, provides various degrees of antimicrobial activity against *Mycobacterium fortuitum*, *smegmatis*, *phlei*, *aurum* and *abscessus* [4]. D-Carvone and D-limonene generate strong antifungal activity against *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* [4,5], *Penicillium islandicum* and *Aspergillus flavus* [6]. D-Carvone also exerts antibacterial activity against both Gram-negative (*Escherichia coli*, *Yersinia enterocolitica*, *Salmonella choleraesuis* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, and *Listeria monocytogenes*) [5-7].

Elucidation of the relationship between the chemical composition of dill EO and its antimicrobial activity will allow the discovery of new natural sources of antiseptics with wide applications in the food and pharmaceutical industries.

The scope of this work is to establish the chemical composition and antimicrobial properties of the EO's isolated from inflorescences, immature seeds, stems and mature seeds of dill grown in western Romania.

Experimental part

Materials and methods

Raw material

The plant material utilized was harvested in three stages: inflorescences, immature seeds, and later stems and mature seeds (harvested when the plants reached maturity), in Bucovăţ, Timiş County (45°45'18"N, 21°22'52"E) in September 2010. After harvesting, the material was dried under natural conditions (sun-sheltered and naturally ventilated areas) and stored in double layer paper bags at 3-5°C.

Isolation of essential oils

The EO's were obtained through steam distillation for 4h, then dried on anhydrous sodium sulfate and stored for

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the GC-MS and antimicrobial activity analyses in hermetically sealed vials at 4°C.

Gas chromatography-mass spectrometry

Oil samples were analyzed by gas chromatography with a gas chromatograph HP6890, coupled with mass spectrometry HP 5973. The gas chromatograph has a split-splitless injector and a capillary column Factor Four™ VF-35ms, 35% phenylmethyl phase, 30m × 0.25 mm, 0.25 μm film thickness. The gas chromatography conditions include a temperature range of 50 to 250°C with 4°C/min, with a solvent delay of 5 min. The temperature of the injector was maintained at a temperature of 250°C. The inert gas was helium at a flow of 1.0 mL/min, and the volume of injected sample in the splitless mode was 2 μL. The MS conditions were the following: ionization energy, 70 eV; quadrupole temperature, 100°C; scanning velocity, 1.6 scan/s; weight range, 40-500 amu.

The percent composition of the essential oils was calculated. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the spectra library NIST 98 (USA National Institute of Science and Technology software).

Determination of antimicrobial activity

The essential oils were tested on the following strains: *Shigella flexneri* (ATCC 12021), *Klebsiella pneumoniae* (ATCC 13882), *Salmonella typhimurium* (ATTC 14028), *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922), *Streptococcus pyogenes* (ATTC 19615) *and Clostridium perfringens* (ATCC13124).

The antimicrobial activity was determined by the disc diffusion method using the Kirby-Bauer method [8]. The 6 mm diameter discs were prepared with Whatman No 1 filter paper. For the test was applied on discs 20μL of dill essential oil. Inoculum was prepared with fresh cultures of bacterial strains, which were grown in tryptic-soy agar for 18h at 37±1°C with physiological saline solution, 3 x 10^6 cells mL^{-1}. The inoculum density was compared with a McFarland standard solution of $BaSO_4$ (0.1mL of 1% $BaCl_2$ + 9.9 mL of 1% H_2SO_4). The cultures were cultivated on Mueller-Hington agar. The agar was then inoculated with the culture and incubated at room temperature for 25 minutes. The discs were arranged on the surface of the inoculated agar plates and pressed gently to adhere to the surface of the agar. The plates were incubated for 24-48h at 35-37°C. After incubation, the diameter of the zone of inhibition was measured. The EO isolated from stems was not analyzed because the obtained quantity was insufficient.

Statistical analysis

All data are displayed as average from at least three independent experiments. Statistical analysis was performed using SPSS v.17.0.

Results and discussions

The yield of EO (% v/w) was de 2.91% for mature seeds, 0.92% for immature seeds, 0.11% for stems and 0.67% for inflorescences; the chemical components identified are listed in table 1. In figure 1 are shown the chromatograms of essential oil from inflorescences, immature seeds, stems and mature seeds.

In the EO obtained from mature seeds 10 components were identified, representing 99.03 % of total, the main constituents being carvone 52.70% and limonene 39.45%. The content of carvone is lower in the EO from immature seeds, 34.17%, in this case the major constituent being limonene, 40.19%. Other components identified were dihydrocarvone 11.09%, phellandrene 3.62%, phellandrene 1.12%, p-cymene 2.02%. In this case 11 constituents were identified, representing 93.87% of total.

No.	Compounds	R.T. (MIN)	% of total			
			Mature seeds	Immature seeds	Inflorescences	Stems
1.	Thujene	5.46	-	0.04	0.11	0.27
2.	Pinene	5.69	0.13	0.32	0.61	1.58
3.	Sabinen	6.79	-	-	-	0.18
4.	Pinene	7.03	0.19	0.26	0.69	1.07
5.	Phellandrene	7.59	2.30	3.62	12.03	24.94
6.	1,3,8-p-Menthatriene	7.79	0.79	0.87	1.19	-
7.	Limonene	8.14	39.45	40.19	61.32	12.95
8.	p-Cymene	8.33	0.77	1.12	3.60	12.59
9.	Terpinene	8.51	0.83	2.02	7.64	31.66
10.	4-Carene	9.01	-	-	-	1.52
11.	3,9-epoxy-1-p-menthene	9.74	-	-	-	0.31

Table 1
COMPOSITION OF THE ESSENTIAL OILS (EO) OBTAINED FROM DILL GROWN IN WESTERN ROMANIA

12.	Pinocarveol	13.07	0.06	0.17	0.46	2.32
13.	Dihydrocarvone	13.66	-	-	-	0.18
14.	Ionene	14.02	1.81	11.09	0.40	-
15.	Ethanone, 1-(2,4-dimethylphenyl)-	14.01	-	-	-	0.19
16.	Carvone	14.47	-	-	-	0.21
17.	Phellandral	15.15	52.70	34.17	11.24	0.28
18.	Thymol	15.67	-	-	-	0.10
19.	Caryophyllene	16.22	-	-	-	0.17
20.	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	17.38	-	-	-	0.21
21.	Bisabolene	18.90	-	-	-	0.24
22.	Ionone	19.08	-	-	-	0.12
23.	Phenol, 2,4-bis(1,1-dimethylethyl)	19.82	-	-	-	0.06
24.	2-Pentadecanone, 6,10,14-trimethyl	20.16	-	-	-	0.21
25.	Phytol	25.06	-	-	-	2.37
26.	Thujene	29.50	-	-	-	3.71
	Identified from total area		**99.03**	**93.87**	**99.29**	**97.44**

No.	Test microorganism	Diameter (mm) of zone of inhibition			
		Mature seeds	Green seeds	Flowers	Stems
1.	*Shigella flexneri* **ATCC 12021**	13.13 ± 0.2	10.9 ± 0.26	9.0 ± 0.3	n.t.
2.	*Klebsiella pneumoniae* **ATCC 13882**	10.9 ± 0.4	24.93 ± 0.2	16.93 ± 0.25	n.t.
3.	*Salmonella typhimurium* **ATTC 14028**	21.03 ± 0.4	22.8 ± 0.45	n.d.	n.t.
4.	*Staphylococcus aureus* **ATCC 25923**	n.d.[a]	n.d.	n.d.	n.t.
5.	*E. coli* **ATCC 25922**	13.1 ± 0.3	25.13 ± 0.15	14.96 ± 0.4	n.t.
6.	*Streptococcus pyogenes* **ATTC 19615**	n.d.	n.d.	n.d.	n.t.
7.	*Clostridium perfringens* **ATCC13124**	25.06 ± 0.2	23.83 ± 0.35	n.d.	n.t.

[a]n.d. - not detected

[b]n.t. - not tested

Table 2
ANTIMICROBIAL ACTIVITY OF THE TESTED DILL ESSENTIAL OILS. INHIBITION IS EXPRESSED IN mm AND INCLUDE THE DIAMETER OF PAPER DISC (6 mm). RESULTS ARE PRESENTED AS MEAN ± STANDARD DEVIATION (SD) OF THE INHIBITION ZONE (n = 3)

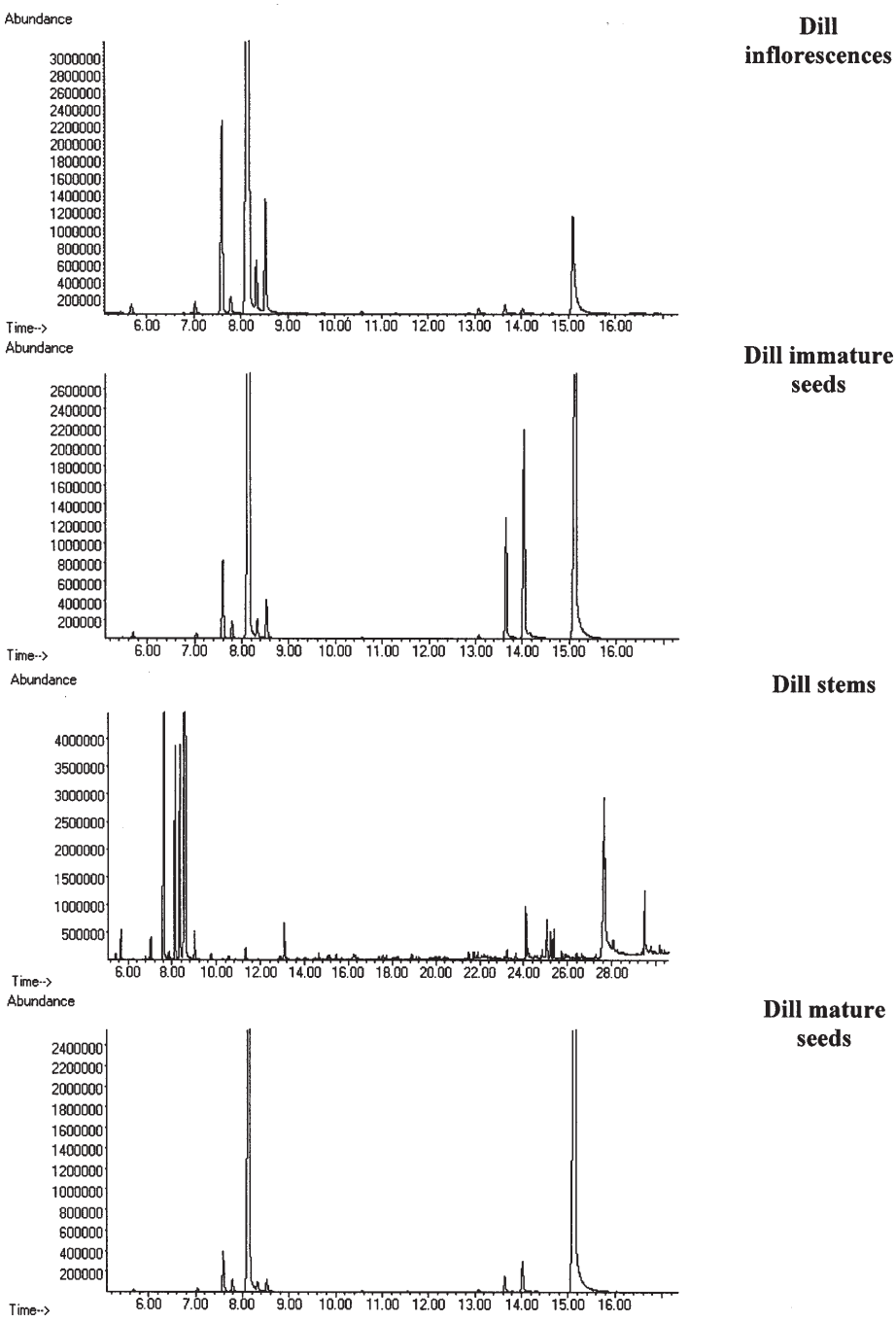

Fig. 1. Chromatograms of essential oils from *Anethum graveolens* flowers, immature seeds, stems and mature seeds

The EO from inflorescences has the highest content of limonene, 61.32%; 11 components were identified, representing 99.29 % of total. In the EO isolated from stems, 24 components were identified, representing 97.44% of total, the main constituent being p-cymene 31.66%, together with 24.94% phellandrene, 12.95% limonene, 12.59% phellandrene. In this case the content of carvone was 0.28%.

The experimental data obtained upon the evaluation of the antimicrobial activity (table 2) show significant bactericidal effects against *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *E. coli*.

Analyzing the diameters of the zones of inhibition, it can be concluded that the EO from dill immature seeds possesses the most efficient antimicrobial activity. It inhibits strongly the development of *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Clostridium perfringens* and moderately the one of *Shigella flexneri*. The EO isolated from mature seeds shows high efficiency against *Clostridium perfringens* and *Salmonella typhimurium*, while the EO isolated from inflorescences displays a moderate antimicrobial activity compared with the two above-mentioned EO's. Similar studies reported the efficiency of dill EO against *E. coli* and *Salmonella typhimurium* [9-11], and *Clostridium perfringens*, respectively [12].

The EO extracted from dill mature, immature seeds and inflorescences show no inhibitory effect on the growth of *Streptococcus pyogenes* and *Staphylococcus aureus*. The inefficiency of dill EO against *Staphylococcus aureus* has been reported occasionally in the past [13], these results being in contrast with other studies that report strong antibacterial activity [5-7,9,10,12,14].

These contradictory results can be reasoned on differences in pedoclimatic conditions, variety, different agricultural practices, as well as the loss of some active components with antibacterial properties caused by the high volatility of the EO or insufficient separation of EO from the plant material.

The experimental data obtained in the present study reconfirm the antibacterial properties of the dill EO, but the contradictory results demonstrate the necessity to continue these investigations.

Conclusions

The antimicrobial activity of *Anethum graveolens* EO's shown against *E. coli*, *S. typhimurium*, *Klebsiella pneumoniae*, *Clostridium perfringens* and *Shigella flexneri* recommend these oils or their constituents as possible alternative solutions of natural antiseptics with applicability in the food and pharmaceutical industries.

References

1.BAILER, J., AICHINGER, T., HACKL, G., HEUBER, G., HEUBER, K., DACHLER, M., Ind. Crop. Prod., **14**, no. 3, 2001, p. 229

2.SANTOS, P.A.G., FIGUEIREDO, A.C., LOURENCO, P.M.L., BARROSO, J.G., PEDRO, L.G., OLIVEIRA, M.M., SCHRIPSEMA, J., DEANS, S.G., SCHEFFER, J.J.C., Biotechnol. Lett., **24**, no. 12, 2002, p. 1031.

3.YAZDANI, D., JAMSHIDI, A.H., REZAZADEH, S., MOJAB, F., SHAHNAZI, S., J. Med. Plant., **3**, no. 11, 2004, p. 38.

4.STAVRI, M., GIBBONS, S., Phytother. Res., **19**, no. 11, 2005, p. 938.

5.DELAQUIS, P.J., STANICH, K., GIRARD, B., MAZZA, G., Int. J. Food Microbiol., **74**, no. 1-2, 2002, p. 101.

6.LOPEZ, P., SANCHEZ, C., BATTLE, R., NERIN, C., J. Agric. Food Chem., **53**, no. 17, 2005, p. 6939.

7.SINGH, G., MAURYA, S., DE LAMPASONA, M.P., CATALAN, C., J. Food Sci., **70**, no. 4, 2005, p. 208.

8.BAUER, A.W., KIRBY. M., Am. J. Clin. Pathol., **45**, no. 4, 1966, p. 493.

9.BADAR, N., ARSHAD, M., FAROOQ, U., Int. J. Agr. Biol., **10**, no. 3, 2008, p.329.

10.ELGAYYAR, M., DRAUGHON, F.A., GOLDEN, D.A., MOUNT, J.R., J. Food Protect., **64**, no. 7, 2001, p. 1019.

11.SOYLU, S., SOYLU, E.M., EVRENDILEK, G.A., Ital. J. Food Sci., **21**, no. 3, 2009, p. 347.

12.NEVAS, M., KORHONEN, A.R., LINDSTRÖM, M., TURKKI, P., KORKEALA, H., J. Food Protect., **67**, no. 1, 2004, p. 199.

13.ABED, K.F., Saudi J. Biol. Sci., **14**, no. 1, 2007, p.53.

14.JIROVETZ, L., BUCHBAUER, G., STOYANOVA, A. S., GEORGIEV, E. V., DAMIANOVA, S. T., J. Agric. Food Chem., **51**, no. 13, 2003, p. 3854

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