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RESEARCH ARTICLE

Headspace-SPME/GC-MS Analysis of the *Anethum graveolens* L. volatiles from Saudi Arabia with different fiber coatings

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Abstract

Anethum graveolens L. (Apiaceae) commonly known as dill is widely used as a spice crop and medicinal herb worldwide. The aim of the study was to extract and compare the volatile constituents present in the seeds of *Anethum graveolens* by headspace solid phase microextraction (HS-SPME) using three fiber coatings [polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane (PDMS), and divinylbenzene/carboxen on polydimethylsiloxane (DVB/CAR/PDMS)]. The volatile compounds were characterized by gas chromatography-mass spectrometry (GC-MS) on the DB-5 column. A total of 15 constituents representing around 94-98% of the total components were identified. Carvone (26-35%), limonene (14-42%), dillapiole (9-34%) and *cis*-dihydrocarvone (8-14%) were found as the predominant constituents. The results showed that the relative abundances of the extracted compounds from dill seeds varied depending on the nature of SPME fibers.

Keywords: *Anethum graveolens*, dill seeds, Headspace-SPME/GC-MS, carvone, dillapiole

Introduction

Genus *Anethum* L., belonging to the Apiaceae family, is represented by only one species as *Anethum graveolens* L. (dill), which is locally known as "Shabat" or "Ein Jaradeh" in the Arabian Peninsula (Bailer, 2001). Dill is an important food crop in the world and it originated in the Mediterranean and South-west Asia and South-east Europe regions, however, it is, now, cultivated all around the world (Singh, 2005).

The leaves are incorporated as a spice in salads, soups and many Asian and Middle East cuisines, while the seeds are widely used to prepare tea, in pickling and baking. The plant is globally appraised for its medicinal and aromatic uses. In Ayurvedic medicine, Anethum seed is a major constituent of over 60 ayurvedic preparations; the tea prepared from the seed is commonly used for colics and to ease digestion (Jana & Shekhawat, 2010). The native people in Saudi Arabia use the seeds as an appetizer, carminative, mouth wash, anthelmintic, antispasmodic and aphrodisiac (Youssef, 2013).

Other compounds isolated from dill seeds are coumarins, flavonoids, phenolic acids, and steroids. The essential oil can be obtained from different parts of the plant including the leaves, flowers, fruits, and seeds with different percentages and varying contents depending on the part investigated, the geographic region, seasonal variations and the method of extraction used (Hussein et al., 2015). Previous works carried out in different countries around the world revealed that carvone and limonene are the major components of the dill seed essential oil. *A. graveolens* have been described to have antibacterial, antifungal, insecticidal, anti-inflammatory, antioxidant, antidiabetic, anticancer, antispasmodic, adaptogenic, diuretic and antihypercholesterolemic (Dahiya and Purkayastha, 2012, Chahal et al., 2016; Babri et al., 2012; Naseri et al., 2012; Panda et al., 2008; Zheng et al., 1992). A clinical study on 40 pregnant women showed that drinking dill seeds infusion enhanced the release of oxytocin and shortened the duration of the first stage of labor (Monsefi et al., 2006). It was reported that limonene, one of the main constituents of the seed oil showed a contractive effect on uterine myometrium (Ma et al., 2015).

The present study was carried out to develop a quick and easy method to analysis of volatile composition of dill seeds using three different SPME headspace analysis and gas chromatography-mass spectrometry (GC-MS).

Materials and Methods

Sample

The seeds of *A. graveolens* were purchased from a local market in Riyadh city and, kindly, identified by a taxonomist in the Pharmacognosy Department, Faculty of Pharmacy, King Saud University. A voucher specimen was deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, King Saud University.

Headspace-solid phase microextraction (HS-SPME)

The headspace solid-phase microextraction was used to isolate the volatile compounds of *A. graveolens* seeds. Three types of fibers, 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 100 µm polydimethylsiloxane (PDMS), and 50/30 µm divinylbenzene/carboxen on polydimethylsiloxane (DVB/CAR/PDMS) (Supelco Inc., Bellefonte, PA, USA), were chosen for the determination of volatile components from *A. graveolens* seeds. About 2 grams of uncrushed seeds was transferred to 30 mL beaker that covered with aluminium foil for 30 min at 35 to 45 °C. The each fiber separately was exposed to the sample headspace for 30 min, prior to thermal desorption. After the collection process SPME fibres needle directly inserted into the splitless injection port of the GC-MS system for thermal desorption for 2 min.

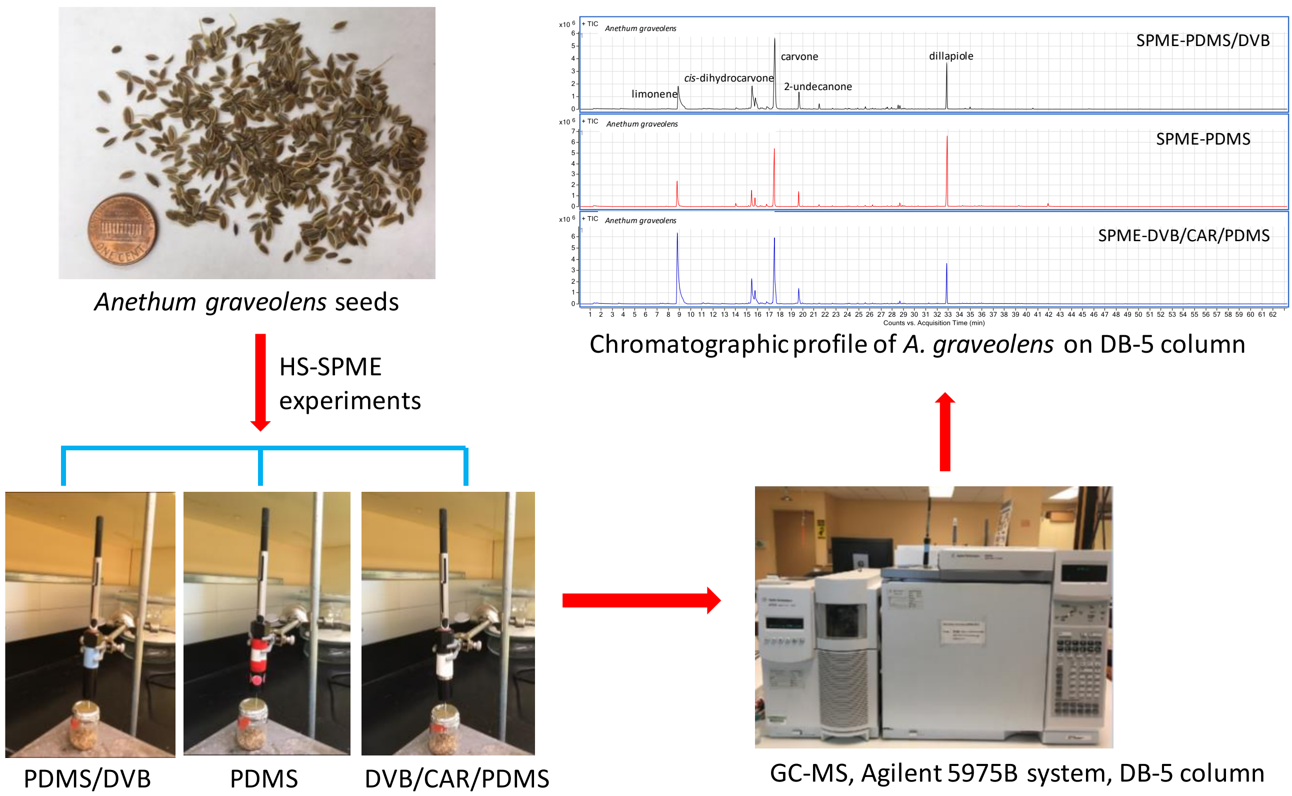
Gas chromatography-mass spectrometry (GC-MS)

The volatile compounds extracted from *A. graveolens* seeds by HS-SPME fibers were subsequently analysed by GC-MS using an Agilent 5975B (Agilent Technologies, Santa Clara, CA, USA) system (Figure 1). An apolar column DB-5 (30 m x 0.25 mm inner diameter with 0.25 µm film thickness, Agilent Technologies) was used with helium as carrier gas (1.3 mL $min^{-1)}$. GC oven temperature was 1.3 min at 60 °C and then 3 °C min^{-1} to 246 °C. The PTV injector temperature was 200 °C. Mass spectra were recorded at 70 eV. Mass range was *m/z* 35 to 450, ion source temperature was 230 °C and the scan rate was 2.8 sec^{-1}.

Compound identification

The volatile composition of the samples was identified by comparison of their arithmetic indices (Dool & Kratz, 1963) relative to a homologous series of C_5-C_{25} alkanes on DB-5 capillary column and mass spectra to published data and the mass spectra database (MassFinder (2004), Adams Library (2007), Flavours and Fragrances of Natural and Synthetic Compounds 3 (2015), NIST 2017 and Wiley 11/NIST 2017), and our own library "SHRS Essential Oil Constituents-DB-5" which was built up from authentic standards and components of known essential oils. Authentic standards used in this study were purchased from Sigma-Aldrich Ltd, St. Louis, MO, USA [limonene (Cas # 5989-27-5), α-terpineol (Cas #10482-56-1), carvone (Cas # 6485-40-1), 2-undecanone (Cas # 112-12-9), β-caryophyllene (Cas # 87-44-5)].

Figure 1. SPME/GC-MS in analysis of dill seeds of volatile compounds using three different SPME fibers



Results and Discussion

The present study shows the preliminary investigation of the volatile constituents in *A. graveolens* seeds using HS-SPME-GC/MS analysis with three fibers including polydimethylsiloxane/divinylbenzene (PDMS/DVB), polydimethylsiloxane (PDMS) and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). The chemical composition of the dill seeds is shown in Table 1, where the compounds are listed in the order of their elution. A total of fifteen compounds, representing 94-98% of the total composition of dill seeds, were identified. The relative percentages of the predominant constituents were found as carvone (26-35%), limonene (14-42%), dillapiole (9-34%) and *cis*-dihydrocarvone (8-14%).

Table 1. Volatile compounds of *Anethum graveolens* seeds using three different SPME fibers (PDMS/DVB, PDMS, DVB/CAR/PDMS) by GC-MS on DB-5 column

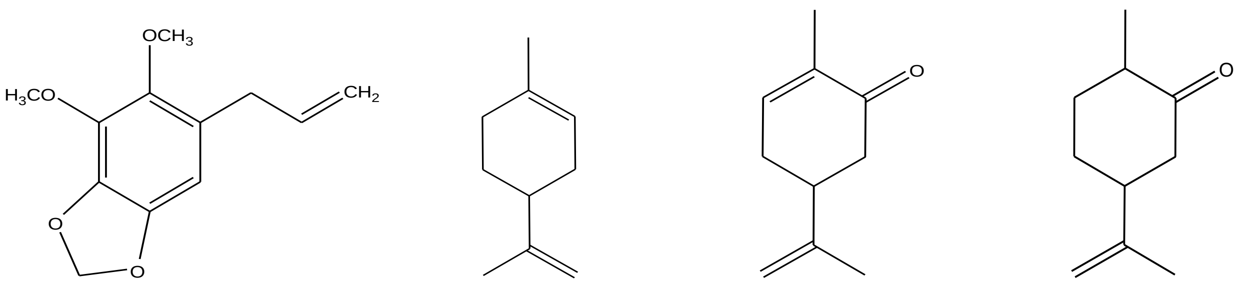
[a]AI $_{extperiment}$	[b]AI $_{literature}$	Compound name	PDMS/DVB	PDMS	DVB/CAR/PDMS
1030	1024	limonene	20.7	14.0	42.0
1187	1186	α-terpineol	0.5	0.4	<0.1
1193	1191	*cis*-dihydrocarvone	13.9	7.7	10.9
1200	1200	*trans*-dihydrocarvone	6.8	4.2	1.0
1239	1279	carvone	35.0	28.7	26.1
1288	1293	2-undecanone	5.9	5.6	4.7
1407	1417	β-caryophyllene	0.4	0.4	<0.1
1415	1432	*trans*-α-bergamotene	0.8	0.5	<0.1
1438	1453	geranyl acetone	<0.1	0.6	<0.1

1470	1489	β-selinene	0.5	<0.1	<0.1
1479	1498	α-selinene	0.5	0.4	<0.1
1507	1513	γ-cadinene	0.9	1.3	0.6
1502	1517	myristicin	<0.1	0.4	<0.1
1506	1522	δ-cadinene	<0.1	0.4	<0.1
1600	1620	dillapiole	11.4	33.8	8.9
		Total	<97.3	<98.4	<93.6

[a]Experimental arithmetic indices calculated against *n*-alkanes using DB-5 apolar column. [b]Arithmetic retention indices from Adams Library (2007)

The results showed that the relative abundances of extracted compounds from dill seeds varied based on the nature of SPME fibers. Among the fibers tested, PDMS fiber coating showed the highest total amount of volatile compounds detected in dill, followed by PDMS/DVB and lastly, DVB/CAR/PDMS. Dill seeds, as an aromatic plant, is qualified based on carvone content (Zawirska-Wojtasiak & Wasowicz). Limonene (fresh-citrus like) and carvone (spicy-caraway) are responsible for the dill aroma (Jirovetz et al., 2013) high content of carvone can be related to quality of dill seeds (Zawirska-Wojtasiak & Wasowicz). The PDMS/DVB fiber extracted the highest carvone among the three tested fibers. We found that the PDMS/DVB fiber might be the most suitable fiber coating for the quality control of dill seeds.

Figure 2. Structure of the main compounds identified in the sample



Dillapiole Limonene Carvone Dihydrocarvone

Comparing our results with earlier published data on the volatile composition of *A. graveolens* seeds revealed some similarities as well as some differences. In the study performed by Hussein et al., 2015, carvone (62.48%), dillapiole (19.51%) and limonene (14.61%) were identified as the major compounds in the seed essential oil. Limonene was the dominant compound in an Indian (83.0%) and Egyptian (30.3%) samples. On the other hand, carvone was the major constituent of the seed volatiles in samples analyzed from Pakistan (55.2%), India (41.5%), Tajikistan (51.7%), China (41.51%) and Thailand (45.16%); reaching 73.6% of the oil composition in a study from Uzbekistan (Babri et al., 2012, Khaldi et al., 2015, Chahal et al., 2016, Sharopov et al., 2013, Ma et al., 2015, Yilli et al., 2009).

Conclusions

The objective of the present investigation was to analyze the chemical profile of the volatile compounds responsible for the characteristic flavor of dill seeds by SPME using three different fibers. The highest amount of carvone was extracted with PDMS/DVB fiber which suggest that it can be used as a quality control tool for dill seeds.

Headspace solid-phase microextraction (HS-SPME) coupled with GC-MS technique can be a simple, fast and sensitive method for the analysis of volatile compounds from agriculturally important crops. Compared to traditional methods, SPME is fast, easy to use, inexpensive, and solvent-free technique that can be suitable for various applications samples from medicinal and aromatic crops. Sampling from headspace-SPME can be extended from volatile to non-volatile compounds depending on the affinity of the fiber coatings. This technique also successfully allows the detection of volatile collection from small amounts of samples in the headspace mode. The SPME technique can be, routinely, used in combination with wide range of analytical instruments such as gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS). The SPME technology is expected to be widely used in the future for chemical analysis and chemical profiling of plant extracts.

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