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Characterization of essential oils from fruits of Umbelliferous crop cultivated in Sudan I. *Pimpinella anisum* L (Anise) and *Anethum graveolens* L. (Dill)

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Abstract
The Umbelliferae (Apiaceae) are mostly temperate herbs almost always with umbellate inflorescences comprising more than 300 genera and more than 3000 species. Some plants of the family such as anise, dill, coriander, fennel, and cumin are famous for their medicinal and aromatic properties. The objective of this study is to give an overview about content and constituents of oils from two umbelliferous crops, *Pimpinella anisum* L (Anise) *Anethum graveolens* L (Dill), cultivated in Sudan. The essential oils from the samples were obtained by hydro-distillation, whereas their chemical composition were identified using GC-MS analysis. The essential oils content of *P. anisum* and *A. graveolens* fruits were 3.1 and 1.8% (v/w), respectively. Monoterpenes comprised the main constituents (80.36%) of *P. anisum* oil, all of which were oxygenated, whereas sesquiterpenes represented about the (19.65%), (14.86%) of which were oxygenated. The main identified constituent was anethole (78.21%), followed by longifolene (2.64%), Estragole (1.86%) and zingiberene (1.06%). Cis-anethole was not detected in the present investigated oil, although it was detected in anise essential oil from other countries.
The monoterpenes constitute about (82.77%) of the *A. graveolens* oil, of which oxygenated fraction comprised (58.26%) of the oil. Oxygenated sesquiterpenes fraction represented about (25. 28%) whereas the sesquiterpenes hydrocarbons were detected as trace compounds. The major constituents of the oil were D-carvone (40.09%), dill apiole (25.58%), D-limonene (22.98%), cis-dihydracarvone (5.42%) and trans-dihydracarvone (3.77%). Composition of essential *A. graveolens* oil of dill cultivated in Sudan was found to be different from some other oils, but it was very similar in the chemical composition to that obtained from fruits cultivated in Egypt.

Keywords: *P.* anisum, *A. graveolens*, Anethole, D-carvone, GC-MS

1. Introduction
The Umbelliferae (Apiaceae) are mostly temperate herbs almost always with umbellate inflorescences comprising more than 300 genera and more than 3000 species. The species are commonly further distinguished by the presence of hollow stems and sheathing petioles. The leaves are nearly always alternate and pinnately or palmately compound or more than once compound; stipules are generally absent. The flowers are typically small, mostly bisexual. The fruits form below where the petals and stamen originate. Fruits or seeds are in pairs, commonly conspicuously ribbed, and sometime winged. The family has a cosmopolitan distribution, but most of its members are confined to northern temperate regions and high altitudes in the tropics [1-3]. Plants belonging to this family are extensively used for food and medicinal purposes. Some plants of this family such as carrots, parsley, and celery are common vegetable crops, while other members like anise, dill, coriander, fennel, and cumin are famous for their medicinal and aromatic properties [3, 4].
Pimpinella anisum L. (Anise) is a slow growing annual herb which is cultivated throughout the world. For cultivating of anise plant a warm, sunny and dry autumn is ideal to meet economical yield and high quality of essential oil [5, 6].
Previous studies of *P. anisum* essential oil revealed detection of *trans*-anethole as the major constituent; e.g. a total of seventeen components were identified in the essential of *P. anisum* cultivated in Pakistan. The major constituent was *trans*-anethole (82.1%) followed by γ-himachalene (7.0%) [7].
The essential oil obtained by hydrodistillation from the mature fruits of *P. anisum* grown in Serbia characterized by higher amounts of *trans*-anethole (96.80%) [8].
Anise fruits and essential oils are extensively used for flavoring of foods and confectionaries as well as some medicines and toothpastes and mouthwashes. They also exhibited many biological activities [9-13].

Anethum graveolens L. (Dill) is a biennial or annual aromatic herb. It is native to Southern Europe, West*ern* Asia, Southern Russia and Mediterranean region but it is now widely cultivated in all different areas of the world [14-15]. *A. graveolens* essential oil composition has been investigated in many regions.

The main constituents of essential oil of dill fruits, cultivated in Thailand, were dill apiole (19.98-48.9%), D-carvone (18.05-28.02%) and D-limonene (26.96-44.61%) [16].

Carvone (62.48%), dill apiole (19.51%) and limonene (14.61%) were identified as the major compounds in essential oil of dill fruits cultivated in Egypt [17]. Carvone (75.9%) was the most characteristic compound of Estonian dill seed oil [18].

Anethum graveolens is used for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic. The fruit has an antispasmodic effect on the smooth muscle of the gastrointestinal tract [19].

This study was conducted to give an overview about content and constituents of oils from two umbelliferous crops, *Pimpinella anisum* L (Anise) *Anethum graveolens* L (Dill), cultivated in Sudan.

2. Materials and Methods

2.1 Materials

Pimpinella anisum and *Anethum graveolens* fruits were brought from Nile Province (Northern Sudan) schemes in Feb.-March/2016.

2.2 Essential oil isolation

One hundred grams of each provided herbal sample were subjected to hydrodistillation for 4 h using Clevenger's apparatus. The distilled essential oil was dried over anhydrous sodium sulphate, filtered and stored in a sealed vial at 4°C. The yield of the oil (v/w %) was calculated based on the plant dry matter.

2.3 GC-MS analysis of the oils

The essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS-QP-2010 Plus-SHIMADZU). The sample was dissolved in dichloromethane (1%) and injected at 250 °C (injector temperature) into a capillary column type HP-1(30 m X 0.25 mm i.d, 0.25 µm film thickness, stationary phase (95% diethyl-5% diphenylsiloxane), using helium as a carrier gas at a flow rate of 1.2 ml/min. The injected volume was 1 µl and the injection mode used was split (split ratio 300), the injection temperature was 250 ºC. The oven temperature was raised from 35 ºC (hold for 3 min) to 240 °C at the rate of 5 °C/min, then at the rate of 3 °C/min, raised to 280 ºC, hold for 3 min. Interface temperature was 250 ºC; the ion source temperature was 200 ºC. The start time was 4 min and the end time was 61.33 min. The mass and scan range was set at *m/z* 35-800.

2.4 Identification of components

The essential oils constituents were identified by their retention time and computer matching of their mass spectra with those found in NIST and Wiley libraries database.

3. Results and Discussion

The essential oil content, prepared by hydrodistillation from *P. anisum* fruits, was found to be 3.1% (v/w), which considered to be high yield when compared to the yield of *P. anisum* fruits essential oil from different regions world-wise (1.5–3.5%) [20, 21].

The oil obtained was pale yellow liquid, bearing the characteristic aromatic odor of anise. The refractive index and specific gravity of prepared anise oil were 1.55017 and 0.882 respectively, at 27 ºC.

Investigation of the chemical constituents of the oil was carried-out using GC-MS. The gas chromatogram showed that fourteen compounds were detected in anise oil. The constituents of the essential oil of *P. anisum*, as well as their percentage abundance, are shown in Table (1) according to their retention times.

Table 1: Essential oil composition of *P. anisum* fruit oil

Peak	Renton Time	Compound	Area%
1	19.435	Estragole	1.86
2	22.154	Trans-anethole	78.50
3	23.321	D-Elemene	0.16
4	24.802	β-Elemene	0.10
5	26.350	***α***-fimachalene	0.28
6	27.085	Longifolene	2.64
7	27.388	Zingiberene	1.06
8	27.630	β-himachalene	0.12
9	27.713	***β***-bisabolene	0.27
10	28.114	Unidentified	0.16
11	30.735	Spathulenol	0.31
12	35.244	Cadinol	0.71
13	36.369	Unidentified	4.49
14	37.604	Pentadecanoic acid	0.35

Monoterpenes comprised the main constituents (80.36%) of the oil, all of which were oxygenated, whereas sesquiterpenes represented about the (19.65%), (14.86%) of which were oxygenated (Fig. 1).

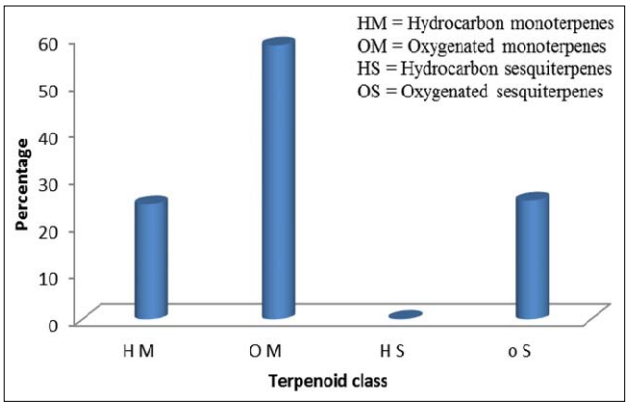

Fig 1: Terpenoid classes of *P. anisum* fruits essential **oil**

Within the identified constituents of the essential oil of *P. anisum* fruit, trans-anethole was the main constituent (78.21%), followed by longifolene (2.64%), Estragole (1.86%) and zingiberene (1.06%).

Interestingly, cis-anethole, which is toxic, was not detected in the present investigated oil, although it was detected in anise essential oil from other countries [7, 8].

On the other hand, the yield of aniseed may noticeably vary depending on ecological conditions such as temperature, precipitation and soil fertility. Previous studies showed the effects of row spacing, water supply, fertilization, sowing time, sowing density on anise seed yield and quality were studied under field and greenhouse conditions [7].

Dill essential oil content, prepared by hydrodistillation from seeds, was found to be 1.8% (v/w). The oil obtained was pale yellow liquid, bearing the characteristic aromatic odor of dill. The refractive index and specific gravity of prepared dill oil were 1.50038 and 0.895 respectively, at 27 ºC.
Sixteen compounds were detected in dill oil gas chromatogram; their percentages are presented in Table (2).

Table 2: Essential oil composition of *A. graveolens* fruits oil

Peak	Renton Time	Compound	Area%
1	10.827	α-Pinene	0.08
2	12.200	Sabinene	0.02
3	12.287	*β*-Pinene	0.05
4	12.808	*β*-myrcene	0.15
5	13.243	*α*-phellandrene	0.52
6	13.940	cymene	0.30
7	14.091	D-limonene	22.98
8	16.070	Benzene	0.41
9	17.559	Limonene oxide	0.12
10	19.032	3,9-epoxy-1-p-menthene	0.18
11	19.398	Trans-dihydrocarvone	3.77
12	1.618	Cis –dihydracarvone	5.42
13	20.889	D –carvone	40.09
14	28.147	Myristicin	0.18
15	28.868	*β*-Asarone	0.15
16	30.642	Dill apiole	25.58

The monoterpenes constitute about (82.77%) of the oil, of which oxygenated fraction comprised (58.26%) of the oil. Oxygenated sesquiterpenes fraction represented about (25. 28%) whereas the sesquiterpenes hydrocarbons were detected as trace compounds (Fig. 2).

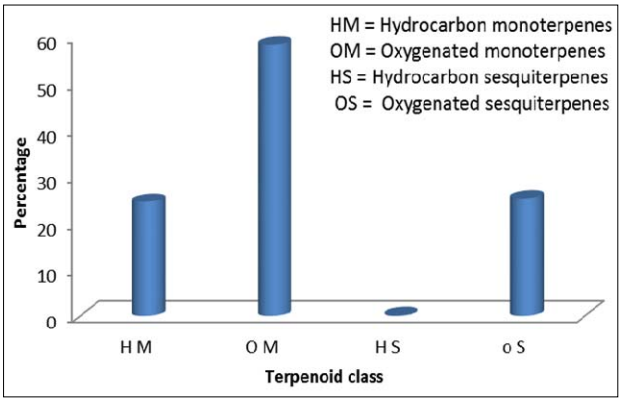

Fig 2: Terpenoidal composition of *A. graveolens* essential oil

The major constituents of the oil were D-carvone (40.09%), dill apiole (25.58%), D-limonene (22.98%), cis-dihydracarvone (5.42%) and trans-dihydracarvone (3.77%).
Composition of essential oil of dill cultivated in Sudan was found to be different from some other oils like that obtained from Thailand in which the main constituents was dill apiole (19.89-48.9%) [16], but it was very similar in the chemical composition to that obtained from fruits cultivated in Egypt, especially in the presence of the three main constituents which were carvone followed by dill apiole and limonene (Fig. 3).

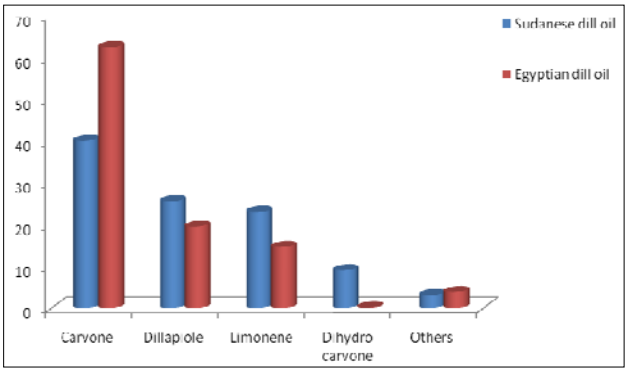

Fig 3: Major constituents in Sudanese and Egyptian dill oils

4. Conclusion

In Northern Sudan climatic conditions, cultivated *P. anisum* fruits essential oil was characterized by presence of *trans*-anethole as the main constituent (78.21%) and absence of *cis*-anethole, whereas the essential oil of *A. graveolens* fruits contained carvone as the main constituent (40.09%).

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