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GC-Mass and Phytochemical Investigation of Iraqi *Anethum graveolens* L. Seeds

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Abstract

Anethum graveolens L. is an annual aromatic plant belongs to Apiaceae (Umbelliferae) family. It has many medical uses since ancient times. Aim of this study is to analyze and study the chemical composition of Iraqi dill seeds hexane and ethanol crude extracts. The non-polar extracts were gained by using two different methods the first one was by extracting dill oil by Clevenger using distilled water as a solvent; the second method was by extracting crude dill extract using non polar solvent (hexane) by soxhlet apparatus for about four hours, and the ethanol as polar solvent was used to get crude polar extract from the seeds using soxhlet too. Then phytochemical tests were performed for ethanolic extract and revealed positive results for alkaloids, tannins, flavonoids and cardio active glycosides; while terpenoids and saponins showed negative results. The hexane extract showed positive result for terpenoids and flavonoids only. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis were done for the samples of hexane extract and oil extract as well and the results showed that apiol was the predominant compound with percentage area of 71.27% and thymol with percentage area 11.32% in the hexane extract while oil sample revealed the presence of D-carvone and D-limonine as main compounds with percentage area of 32.78% and 20.02%, respectively.

Introduction.

Anethum graveolens is an annual aromatic plant belongs to Apiaceae (Umbelliferae) family. It has many medical uses since previous times. The total height of this herb is 150 cm with pinnately divided leaves. Flowers are yellow in color and the seeds turn to brown when they ripe and have aromatic odor [1-2]. Aromatic odor will emit from the seeds after their ripening and getting brown color. This plant is cultivated and grown in Netherlands, USA, Pakistan, Germany, and Hungary [3]. It is also grown in India specifically in Punjab, Rajasthan, and Orissa cities [4]. Both of ground and whole seeds are used as seasoning in many manufactured foods such as sausages, bacon, pickles, and many manufactured meat. Powdered dill can be used as table spice for salads and soups [1, 5]. Anethum has many uses such as additives to gripe water to relief colic pain and flatulence in infants. It improves milk flow in lactating mothers. Its essential oil removes spasms and griping. It is also used in manufacturing of perfumes, chewing gum, beverages, and cosmetics for its pleasant aroma [6]. While the volatile increase appetite and used to remove intestine gases. Additionally, chewing the seeds remove bad breath [7]. The aqueous extract of dill had broad range antibacterial activity, antihyperlipidemic, antioxidant, diuretic, hypotensive, antihyperlipidemic activities and this is due to the structures of different active compounds like anethol and dillapiole [7-11]. To the best of our knowledge, there is no previous estimation of the main compositions of dill extracts cultivated in Iraq. Therefore, in the present study, crude extracts of Iraqi Anethum graveolens L. were extracted from seeds of the plant using two different solvents by two different extraction methods; then GC-Mass analysis was performed to determine which active compounds are present in the cultivated Iraqi dill seeds.

Material and Methods

2.1 Seeds Sample Collection:

Dry seeds of *Anethum graveolens* were collected from Iraqi herbs markets, grinded by mechanical grinder and used directly to prepare the studied samples.

2.2 Preparation of Extracts:

The studied seeds were grounded into powder and the non-polar extracts were gained by using two different methods the first one was by extracting dill oil by Clevenger using distilled water as a solvent; the second method was by extracting crude dill extract using non polar solvent (hexane) by soxhlet apparatus. On the other hand, ethanol as polar solvent was used to get crude polar extract from the seeds using soxhlet too. The three extracts were collected separately into clean glass bottles at room temperature until use. Then, the ethanolic and hexane extracts were examined by using different phytochemical tests.

2.3 Phytochemical study:

2.3.1 Saponins:

Saponins were identified using foam index method as follows: studied samples (2.5 mL) were added to sterile distilled water (10 mL) in a test tube. Then, it was covered and shacked vigorously for about 40 seconds. After that, they were allowed to stand for about 30 minutes. The honey comb froth indicates the presence of saponins.

2.3.2 Tannins:

Gallic tannins and catecholic tannins were detected by adding few drops of 10% ferric chloride to a few milliliters of extract that diluted with water. If blue color observed, then gallic tannins indicated and if it was green color, then it means the presence of catecholic tannins.

2.3.3 Cardio active Glycosides:

A mixture of 5 mL of the studied extracts and 25 mL of dil. H_2SO_4 was boiled in a test tube for about 15 min. After cooling to the room temperature, it neutralized with 10% sodium hydroxide. Cardio active glycosides were detected by formation of brick red precipitate after adding 5 mL of Fehling solution.

2.3.4 Alkaloids:

Both extracts were dissolved in dil. HCl and the precipitates were filtered. The following reagents were used to test the presence of alkaloids:

2.3.4.1 Wagner's Test:

Wagner reagent (which is a mixture of iodine solution together with potassium iodide) was added to the collected filtrate. Formation of reddish brown precipitate indicates the existence of alkaloids compounds in the sample.

2.3.4.2 Dragendroff's Test:

The studied samples were tested with Dragendroff's reagent (which is a solution of potassium bismuth iodide), the formation of orange precipitate indicates the presence of alkaloids.

2.3.5 Flavonoids:

1.5mL of 50% methanol was added to 4mL of each sample extracts. After mixing, it was warmed with magnesium metal and acidified with 5-6 drops of conc. HCl until red color is formed. Red color means the presence of flavonoids. If orange color is formed, means the presence of flavones.

2.3.6 Terpenoids:

Into a test tube, 0.5 mL of acetic anhydride and 0.5 mL of chloroform were added to 4 mL of both extracts. Red violet color was observed after slowly adding of conc. H_2SO_4 which indicated the presence of terpenoids.

2.4 Gas Chromatography-Mass Spectroscopy (GC-MS) Study:

The Gas chromatography and Mass Spectroscopy analysis was done at Ibn Al-bitar institution, Ministry of Industry and Minerals, Baghdad to determine the active compounds of the dill crude hexane extract and the dill oil sample.

Results and Discussion.

3.1. Phytochemical Study:

The qualitative phytochemical study was performed to determine the secondary metabolite in the studied dill extract. As shown in Table 1, the ethanolic extract revealed the presence of Flavonoids, Cardio active Glycoside, Alkaloids and Tannins; while Saponins and terpenoids were absent. In the hexane extract the results were opposite according to the polarity of the compounds under test so only saponin and terpenoids were present as long as they're non polar compounds

Table 1: Qualitative phytochemical study of *Anethum graveolens* Ethanol and hexane extracts.

Compounds	Ethanol extract	Hexane extract
Saponin	-	+
Flavonoids	+	-
Cardio active Glycosides	+	-
Alkaloids	+	-
Tannins	+	-
Terpenoids	-	+

(+): detected; (-): not detected.

3.2 GC-Mass Chromatography analysis:

The gas chromatography and mass spectroscopy analysis of hexane extract of *Anethum graveolens* L. shows the presence of two major compounds; Apiol and thymol at retention time of 19.45, 13.86 min. respectively with percentage of 71.27% and 11.27% respectively while other compounds such as 1,3-Benzodioxole, D-carvone and Tetracosane exist in trace amounts as shown in (Table 2 and Figure 1).

Table 2: GC-Mass results of the dill seeds Hexane extract.

Compounds	Retention time (min.)	Area %
Apiol	19.45	71.27
Thymol	13.86	11.27
Hexacosane	26.00	3.28
1,3-Benzodioxole	17.56	3.11
D-carvone	12.79	2.01
Tetracosane	23.07	1.53

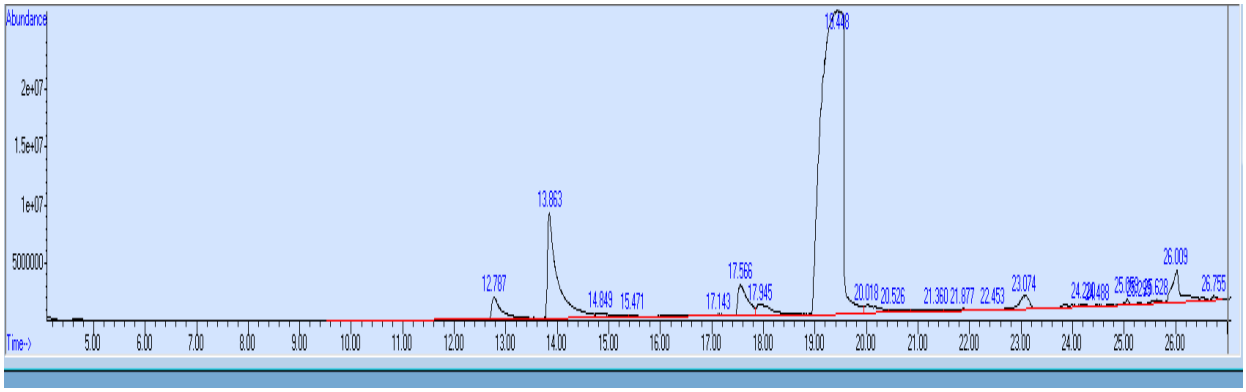

Figure 1: GC-MS analysis of Hexane extract of dill.

The gas chromatography of *Anethum graveolens* L. seeds oil shows the presence of two major compounds which are D-carvone and D-limonine at RT 12.92 and 8.77 min. respectively with percentage of 32.78% and 20.02%. Other compounds such as Hexadecanoic acid, Octadecanoic acid, Cyclohexanone, Apiol and Ethyl oleate present in different retention times with small amounts in percentage of 9.26%, 5.92%, 5.57%, 4.98% and 4.57% respectively.

Table 3: GC-Mass Results of the dill seeds oil.

Compounds	Retention time/min.	Area %
D-carvone	12.92	32.78
D-limonine	8.77	20.02
Hexadecanoic acid	23.73	9.26
Octadecanoic acid	26.08	5.92
Cyclohexanone	11.91	5.57
Apiol	19.02	4.98
Ethyl Oleate	25.81	4.57

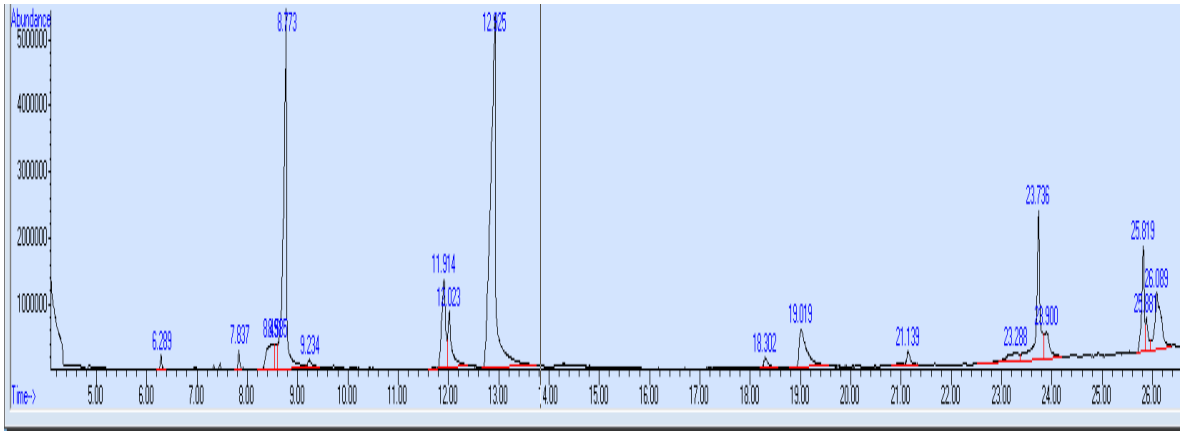

Figure 2: GC-MS analysis of dill oil sample.

The study results showed that both extraction methods used to analyze non polar compounds of the dill seeds revealed the presence of the most major compounds found in dill seeds which are apiol and carvone but indifferent percentages. So apiol was existing in high percentage (71.27%) in the hexane dill extract; while its percentage in oil extract was (4.89%). However, the carvone was present in high percentage (32.78%) in dill oil extract while it was present in hexane extract in low percentage (2.01%) which means that the method of extraction effects on these two important compounds. as previous studies showed that the main component of dill seeds oil were carvon (38.9%- 67.0%), limonene (23.0%-46.3%) [3, 5], camphor (11.44%) [6, 10] myristicin (11.7%), trans-dihydrocarvone (10.99%), and dihydrocarvone (3.1%), [13, 14, 15]; which supports our results. Apiole presents in dill oil samples with percentage of (14.4%-30.1%) but in our study it appeared in the hexane extract with more percentage. The amounts of these main components are varied depending on many causes like type of cultivation, geographic origin, and state of maturity, harvest time, plant part, extraction methods, and storage conditions [8]. In general, the aroma and biological activities

of dill essential oils were attributed to R-phellandrene, D-limonine, D-carvone, and the monoterpenes.

The current study showed that *Anethum graveolens* L. has alkaloids, tannins, cardio active glycoside and flavonoids in its ethanolic extract. While hexane extract consist only saponin and terpenoids as long as they're non polar compounds; so as a result we conclude that the dill extract as a whole has the most important active compounds which we can get by using different solvents polarity. The GC-Mass analysis revealed that active secondary metabolites such as carvone, limonine, apiol and thymol could be gained from different extraction methods which give a wide range of facilities by not being assigned to specific methods; especially apiol and carvone which presents in differential percentages according to the method of extraction. This Iraqi cultivated plant needs further studies to isolate these important active compounds and studied its biological activities of these identified components which have the functional rule for its future biological and pharmacological effectiveness.

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