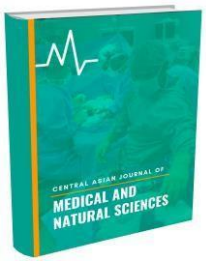

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Phytochemical Analysis and Antioxidant Activity of Flavonoids Extracted from Anethum graveolens using GC-Mass and DPPH Assay

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Abstract: The growing interest in natural compounds with medicinal potential has prompted researchers to explore the bioactive components of A. graveolens (Greater Celandine). The aim of this study is to evaluate the antioxidant activity of flavonoid extracts and the alcoholic extract of A. graveolens, in addition to determining the chemical composition of the alcoholic extract using GC-MS analysis. The results of GC-MS analysis showed the presence of a variety of chemical compounds in the alcoholic extract of A. graveolens, including alkanes, alcohols, organic acids, and silicon compounds. This chemical diversity indicates the possibility of biologically active compounds in the extract. The anti-oxidant activity study showed that both extracts (flavonoid and ethyl alcohol) have significant antioxidant activity, as their activity is similar to that of vitamin C, which is a well-known antioxidant. The A. graveolens alcoholic extract showed the highest percentage at the lowest concentration (200 µg/ml), which indicates that the antioxidant activity is higher at low concentrations. These results indicate that Nabat al-Shabat contains biologically active compounds with antioxidant properties. These compounds play a role in protecting cells from oxidative damage caused by free radicals, which are associated with the development of many chronic diseases. These results encourage further research to determine the specific chemical compounds responsible for the antioxidant activity in the nightshade, and to evaluate its activity and safety in various applications.

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1. Introduction

The search for natural compounds with potent biological activities has gained significant momentum in recent years, driven by the need for safer and more sustainable alternatives to synthetic drugs [1]. Among the countless medicinal plants, A. graveolens (commonly known as dill) has garnered attention due to its rich phytochemical profile and traditional use in herbal medicine. This wild plant, distributed across various regions of Iraq, is commonly found in fields, gardens, and along canals as a weed [2], [3]. It contains numerous bioactive compounds, including coumarins, flavonoids, and essential oils [4], [5], with flavonoids being of particular medical and economic importance [6], [7]. Despite its potential, A. graveolens has not been extensively studied, especially in terms of its medicinal properties and therapeutic potential. This study focuses on the chemical composition A. graveolens and the biological activity of its ethanolic extract and flavonoids.

The ethanolic extract of A. graveolens is particularly significant due to its ability to yield high concentrations of flavonoids and other bioactive components. Qualitative detection of these compounds is a crucial first step in understanding the plant's chemical

composition and its potential therapeutic applications. Techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and spectrophotometric assays are commonly used to identify and quantify these active biomolecules [8]. [9].

Antioxidants play a vital role in neutralizing free radicals, which are atoms or ions with one or more unpaired electrons. These free radicals are highly reactive and tend to interact with other molecules to gain the electrons needed to achieve stability [10], [11]. In addition to their antioxidant properties, flavonoids and other components of A. graveolens may also influence biological processes such as blood clotting. Coagulation factors play a critical role in maintaining blood clotting, and dysregulation of these factors can lead to clotting disorders or bleeding. Emerging evidence suggests that certain plant-derived compounds can modulate coagulation pathways [12], either by inhibiting or enhancing specific factors. This dual role highlights the potential of A. graveolens extracts not only as antioxidants but also as regulators of coagulation, offering a multifaceted approach to managing oxidative stress and coagulation-related disorders [13].

The ethanolic extracts of A. graveolens contain various bioactive compounds, including flavonoids, and exhibit significant antioxidant activity [14]. The extraction process typically involves the use of ethanol to obtain the plant extract, which is then analyzed to determine its phytochemical composition and biological activities [15], [16]. Total phenolic content can be determined using the Folin-Ciocalteu method, while flavonoid content is often quantified using spectrophotometric methods [17]. The extracts of A. graveolens demonstrate strong potential as antioxidants, with the ethanolic extract showing significant free radical scavenging activity [18]. When measured using the phosphomolybdenum method, the extract yields promising results compared to standard antioxidants [19], [20]. Additionally, the extract exhibits cytotoxic effects on leukemia cell lines, suggesting potential anticancer properties and also display peripheral and central analgesic effects [21], [22], further indicating the therapeutic potential of these extracts.

This study aims to qualitatively detect the ethanolic extract and flavonoids of A. graveolens and evaluate their antioxidant activity. Furthermore, by elucidating the chemical composition and biological activities of A. graveolens, this research seeks to contribute to the growing body of knowledge on natural products and their applications in medicine, paving the way for the development of new plant-based therapies.

2. Materials and Methods

The plant was collected during its flowering period from various regions in Al-Anbar Governorate between April and mid-June 2024. Standard collection protocols were followed, with plants typically gathered in the afternoon to ensure they were saturated with sunlight [23]. Healthy, disease-free, and undamaged parts of the plant were carefully selected. The aerial parts were collected meticulously to ensure clean samples free from insects and weeds. A sample of the plant was then taken to the Herbarium at the University of Anbar, College of Education for Women, for identification.

Drying and Extraction of The Plant

After cleaning, the plant was spread in a well-ventilated area away from direct sunlight and turned regularly. The drying process began immediately after collection to halt hydrolytic and enzymatic degradation and prevent microbial activity [24]. To prepare the ethanolic extract, 20 grams of powdered plant material were mixed with 200 mL of 80% ethanol and extracted using a Soxhlet apparatus at 40°C for 5 hours. The extract was then concentrated using a rotary evaporator, dried in an electric oven at 40°C, and stored in dark bottles in a refrigerator.

For flavonoid extraction, 86 grams of powdered plant material were mixed with 600 mL of distilled water and 10% HCl, followed by reflux extraction for 8 hours. After filtration and cooling, the non-sugar fraction was extracted using ethyl acetate, and the process was repeated three times. The extract was dried using a rotary evaporator at 45°C and stored for further analysis [25], [26].

Chemical Compound Identification using GC-MS

The chemical compounds in the A. graveolens extract were identified by dissolving 20 grams of plant powder in 200 mL of 80% ethanol. The analysis was performed using a GC-MS QP2010 Ultra (Shimadzu, Japan) [27].

Antioxidant activity using the DPPH Method

The antioxidant activity of the extracts was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely used method for evaluating the antioxidant potential of plant samples [28], [29]. The DPPH solution was prepared by dissolving 0.04 grams of DPPH in 100 mL of methanol to achieve a concentration of 400 µg/mL. Different concentrations of the ethanolic extract and flavonoids (20, 250.300, and 350 µg/mL) were prepared in triplicate. A standard solution of ascorbic acid (500 µg/mL) was also prepared. For the assay, 500 µL of each plant extract, the standard solution, and a methanol control were placed in separate tubes. Then, 500 µL of the DPPH solution was added to each tube, mixed thoroughly using a vortex, and incubated in the dark for 30 minutes. After incubation, the absorbance was measured at 517 nm using a spectrophotometer. The percentage inhibition of the DPPH radical (I%) was calculated using the following formula:

$$\text{Inhibition } \% = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100 \quad (1)$$

Where:

- A_{DPPH} = Absorbance of the control (methanol + DPPH)
- A_{Sample} = Absorbance of the sample

Statistical Analysis

The collected data were organized and analyzed using a simple experimental design based on a completely randomized design (CRD) with three replications per treatment. Statistical analysis was performed using Genstat (Tenth Edition, Version 10.3.0.0). Significant differences between means were tested using the least significant difference (L.S.D) method at a probability level of 0.05 [30].

3. Results and Discussion

Identification of A. graveolens extract Using GC-MS Technique

Figure (1) presents the results of the gas chromatography-mass spectrometry (GC-MS) analysis of the A. graveolens extract. A total of 29 compounds were identified, as detailed in Table (1). The table includes the molecular formula, percentage area, and retention time (in minutes) for each compound.

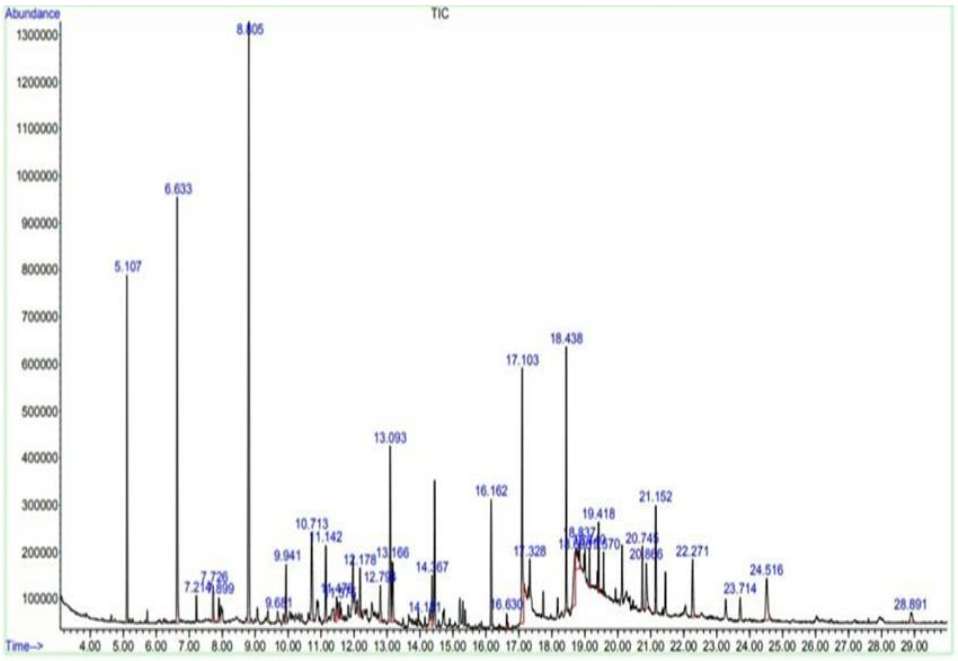

Figure 1. GC-MS Mass Spectrum Analysis of The Alcoholic Extract of A. Graveolens.

Table 1. Chemical Compounds Identified in The Ethanolic Extract of A. Graveolens using GC-MS.

Peak	Retention Time (min)	Percentage of Area (%)	Compound
1	5.339	0.88	Benzene
2	6.293	3.92	Ethane, 1,1-diethoxy-
3	7.333	8.96	Diglycerol
4	8.059	0.85	Glycerin
5	10.586	0.31	Oxirane, (ethoxymethyl)-
6	13.677	2.75	3-Hexanol
7	14.095	1.72	2-Pentene, 3-methyl-, (E)
8	17.387	0.84	Benzene, 1-methyl-4-(1-methylethyl)-
9	17.604	0.81	Cyclohexene, 1-methyl-4-(1-methylethenyl)-
10	18.981	1.04	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
11	20.867	0.99	Undecane
12	25.725	0.93	Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl)-
13	25.863	2.20	cis-Aconitic anhydride
14	29.212	3.27	Pyrimidine, 4,6-dimethoxy-5-nitro
15	29.886	0.59	Heneicosane, Heptacosane, Octadecane
16	30.223	0.71	Pentacosane, Octacosane, 10-Methylnonadecane
17	30.418	0.81	Heneicosane, Tridecane
18	31.349	2.53	Dodecane, Tritriacontane, Tetradecane, 5-methyl-
19	34.881	1.32	Tetradecane
20	38.396	0.99	Pentadecane
21	38.579	6.00	Pentasiloxane, dodecamethyl-
22	38.985	1.06	cis-Aconitic anhydride

23	39.734	1.35	2,2'-(1,4-Piperazinediyl)bis[N-(4-methoxyphenyl)succinimide]
24	40.282	1.06	cis-Aconitic anhydride
25	40.379	0.89	Pyrimidine, 4,6-dimethoxy-5-nitro-
26	40.454	4.07	Docosane, Heneicosane
27	40.854	0.92	Carbamic acid, (2-chloroethylidene)bis-, diethyl ester
28	41.260	0.99	Oxirane, (ethoxymethyl)-
29	42.117	0.89	1,2,3,4-Butanetetrol, [S-(R*,R*)]-
30	43.123	1.02	Diglycerol
31	46.872	4.02	Docosane, Heneicosane
32	47.203	0.83	Docosane, 1-iodo-
33	48.409	1.15	Heptacosane, Docosane, 1-iodo-
34	48.484	3.79	Eicosane, Docosane
35	48.821	0.97	Heptadecane, 9-octyl-, Eicosane, Docosane

The GC-MS analysis revealed that the ethanolic extract of A. graveolens contains a diverse array of chemical compounds. The GC-MS analysis of the alcoholic extract of the vegetable part of Nabat al-Shabat shows the presence of a variety of organic compounds, which indicates the complexity of the chemical composition of this extract, and 35 different compounds were identified, the difference in percentage ratios of the area (%) and retention time (Retention Time). The compounds with the highest percentage are Diglycerol (8.96% and 1.02%): It appears as one of the main compounds in the extract. L-diglycerol is a multi-hydroxyl organic compound, used in many industries, including food, cosmetics, and pharmaceuticals. Al-diglycerol is known for its moisturizing and soluble properties, and it can be used in the extraction of some other plant compounds.

Pentasiloxane, dodecamethyl-(6.00%): It is a silicone compound widely used in cosmetics and personal care products. It is known for its emollient and soluble properties, and it may be present in the extract as a result of the extraction process, or it may be a natural compound in the plant. Docosane, Heneicosane (4.07% and 4.02%): Docosane and Heneicosane are long-chain linear alkanes. These compounds are used in many industries, including candles and cosmetics. Ethane, 1,1-diethoxy-(3.92%):** Also known as diethyl acetal, it is a common solvent used in many industries. It may be present in the abstract as a result of the extraction process. Eicosane, Docosane (3.79%) are long-chain linear alkanes, and are used in many industries, including candles and cosmetics. The presences in the extract are the result of the extraction process or the formation of natural compounds in the plant. Pyrimidine, 4,6-dimethoxy-5-nitro (3.27% and 0.89%) is an organic compound containing a pyrimidine ring. It may be biologically active, but more research is needed to determine its effects. cis-Aconitic anhydride (2.20%, 1.06%, 1.06%) is a natural organic compound found in many plants. Hexanol is an aliphatic alcohol used in many industries, including the perfume and flavor industry. Dodecane, Tritriacontane, Tetradecane, 5-methyl- (2.53%) are long-chain linear alkanes. These compounds are used in many industries, including candles and cosmetics. The height of the beings in the extract is the result of the extraction process or the height of the formation of natural compounds in the plant. The results indicate that the alcoholic extract of the vegetable part of Nabat al-Shabat contains a variety of organic compounds, including alkanes, alcohols, organic acids, and silicon compounds. These compounds may have a variety of biological activities, including antioxidant, anti-inflammatory, and antimicrobial activity. GC-MS analysis is an important first step in determining the chemical composition of the alcoholic extract of the vegetable component of Nabat al-Shabat. There is a need for more research to determine the biological activities of the compounds in the extract and evaluate the safety of its use. The current study demonstrated that the extract of A.

graveolens contains coumarins, a large group of phenolic compounds characterized by a benzopyran core structure [31]. Coumarins are known for their role in preventing blood clotting, enhancing immune system activity, and reducing the risk of cardiovascular diseases [32].

Pentasiloxane, the most abundant compound in A. graveolens , belongs to the furocoumarins class. It is used medicinally to treat psoriasis and vitiligo and exhibits antibacterial activity against Gram-positive bacteria [33]. Another furocoumarin identified in the extract is Pimpinelline, which has been shown to help manage hypertension [34]. Our study found that the concentrations of furocoumarins such as cis-Aconitic anhydride in the ethanolic extract of A. graveolens were higher compared to previous studies. This discrepancy may be attributed to differences in extraction methods, as the quantity and type of bioactive compounds can be influenced by the plant's growth stage, solvent type, and environmental conditions [35].

The extract also contained several fatty acids, including Heneicosane and Hexadecanoic which has been shown to positively impact human health, particularly in lowering blood pressure and inhibiting platelet aggregation [36]. Linoleic acid, an essential fatty acid, plays a crucial role in cardiovascular health, acting as an anticoagulant and promoting wound healing [37], and Octadecadienoic acid (also known as stearic acid) is a saturated fatty acid commonly found in plant oils. It is primarily used in the production of cosmetics, detergents, and perfumes and exhibits antioxidant activity [38]. Docosane and palmitic acid) is another saturated fatty acid found in both animals and plants, widely used in the manufacture of soaps and cosmetics [38]. Our findings align with previous studies [39], confirming the presence of fatty acids in the aerial parts of A. graveolens. The presence of compounds not reported in previous studies may be due to differences in extraction techniques, plant growth conditions, or environmental factors. For instance, the stage of plant growth, the type of solvent used, and the extraction method can significantly influence the chemical profile of the extract [40]. This variability underscores the importance of standardizing extraction protocols to ensure consistency in the identification and quantification of bioactive compounds.

the GC-MS analysis of the ethanolic extract of A. graveolens revealed a rich chemical profile, including coumarins and fatty acids, which contribute to its potential therapeutic properties. The high concentration of Diglycero and other furocoumarins highlights their significance in the plant's biological activities. Additionally, the presence of essential fatty acids such as Linoleic acid and Oleic acid further supports the plant's traditional use in herbal medicine. These findings provide a foundation for further research into the pharmacological applications of A. graveolens and its bioactive compounds.

Antioxidant Activity of the Ethanolic Extract and Flavonoids.

The antioxidant capacity of the ethanolic extract and flavonoids extracted from A. graveolens was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This method is based on the reduction of DPPH to its reduced form, DPPH-H (1,1-diphenyl-2-picrylhydrazine), upon receiving a hydrogen atom from an antioxidant compound such as flavonoids or ascorbic acid. This reaction results in a color change, which can be measured spectrophotometrically to assess antioxidant activity.

To quantify the antioxidant activity, the percentage of DPPH radical scavenging was determined for both the ethanolic extract and flavonoid fraction and compared to that of ascorbic acid a known standard antioxidant, The results, illustrated in Figures 3 and 4, indicate a significant variation in antioxidant activity between the ethanolic extract and the flavonoid fraction, see Table 2. The highest DPPH radical inhibition was observed at a concentration of 150 μg/mL for both the ethanolic extract and the flavonoid fraction. However, the reference compound ascorbic acid exhibited a higher radical scavenging ability compared to both extracts.

Table 2. The Percentage of Antioxidant Activity in The Flavonoid Extract.

Concentration (Mg/Ml)	Antioxidant Activity (%)
200	90.67± 1.201
250	44.50± 2.322
300	65.50± 2.109
350	85.50± 1.443
Vitamin C	90.23± 0.44

Table 2 provides data on the antioxidant activity of flavonoid extract at different concentrations, in addition to the activity of vitamin C as a reference. The table shows the relationship between the concentration of the extract and the ratio of the extract, which is the measure of the ability of the extract to stabilize the free radicals. The percentage of absorption ranges from 44.50% at a concentration of 250 micrograms/ml to 90.67% at a concentration of 200 micrograms/ml. The theme of recruiting vitamin C as a reference, where the percentage of absorption reached 90.23%.

The extract shows a good antioxidant activity, where the percentage of oxidation ranges between 44.50% and 90.67%, it is noted that the highest percentage of oxidation (90.67%) was obtained at the lowest concentration (200 μg/ml), which indicates that the antioxidant activity is higher at low concentrations. Fluctuation of the ratio of alcohol with increasing concentration, where it decreases at 250 micrograms/ml and then increases at 300 and 350 micrograms/ml, but does not exceed the ratio obtained at 200 micrograms/ml. The convergence of the effectiveness of the extract at a concentration of 200 micrograms/ml of the effectiveness of vitamin C (90.23%), which indicates that the extract may be a good source of antioxidant compounds.

The Table 3 presents the data about the antioxidant activity of the ethanolic extract of alshabat at different concentrations, in addition to the effectiveness of vitamin C as a reference. The table shows the relationship between the concentration of the extract and the ratio of the extract, which is a measure of the ability of the extract to stabilize the free radicals.

Table 3. The Percentage of Antioxidant Activity in The Ethanolic Extract.

Concentration (μg/ml)	Antioxidant Activity (%)
200	91.87± 2.102
250	39.00± 4.837
300	60.83± 2.102
350	74.58± 0.102
Vitamin C	90.23± 1.10

The theme of the abstract test at four different concentrations: 200, 250, 300, and 350 micrograms/ml. The absorption rate ranges from 39.00% at a concentration of 250 micrograms/ml to 91.87% at a concentration of 200 micrograms/ml. Vitamin C was used as a reference, where the absorption rate reached 90.23%. The extract shows a good antioxidant activity, where the antioxidant percentage ranges between 39.00% and 91.87%. It should be noted that the highest percentage of the extract (91.87%) was obtained at the lowest concentration (200 μg/ml), which indicates that the antioxidant activity is higher at low concentrations. Fluctuation of the ratio of alcohol with increasing concentration, where it decreases at 250 micrograms/ml and then increases at 300 and 350 micrograms/ml, but does not exceed the ratio obtained at 200 micrograms/ml. The

convergence of the effectiveness of the extract at a concentration of 200 micrograms/ml of the effectiveness of vitamin C (90.23%), which indicates that the extract may be a good source of antioxidant compounds [41], [42], [43].

Antioxidants play a vital role in protecting cells from damage caused by free radicals, which are unstable molecules that can cause damage to cells and tissues. Free radicals can contribute to the development of many chronic diseases, such as heart disease, cancer, diabetes, and aging. The antioxidant compounds found in the extract of al-Shabat can help neutralize free radicals and reduce oxidative damage. The extract of the ethanolic shabbat shows good antioxidant activity, and it may be a promising source of natural antioxidant compounds. However, there is a need for more research to determine the chemical composition of the extract and evaluate its activity and safety in different applications.

It was observed that the ethanolic extract and flavonoids have the ability to inhibit the DPPH radical and there is a direct relationship between the concentration and the inhibition rate, which was confirmed by previous studies. All extracts prepared from A. graveolens showed a significant antioxidant activity, and this displacing effect is due to the plant's possession of active compounds such as phenols, which have antioxidant properties that are closely related to their chemical composition [44].

The antioxidant effectiveness of the ethanolic extract can be attributed to its content of phenols and flavonoids, which are characterized by their effective properties as antioxidants, as flavonoids are considered the most free radical displacing compounds due to their ability to donate a hydrogen atom through hydroxyl groups, which was confirmed in many studies [45], [46].

4. Conclusion

Certainly, here is the academic scientific conclusion based on the results of GC-MS analysis and the antioxidant activity of Al-Shabat extracts. Finding out the results of GC-MS analysis of the alcoholic extract of the aerial parts of Nabat al-Shabat for the presence of a variety of chemical compounds, including alkanes, alcohols, organic acids, and silicon compounds. These results indicate the complexity of the chemical composition of the extract, which makes it a probable source of biologically active compounds. In addition, the study of the antioxidant activity of flavonoid and ethyl alcohol extracts from Nabat al-Shabat showed promising antioxidant activity. All the extracts show a remarkable ability to inhibit free radicals, and the similarity of their activities is the effectiveness of vitamin C, which is a well-known antioxidant. These results indicate that Nabat al-Shabat contains biologically active compounds with antioxidant properties. These compounds may play a role in protecting cells from oxidative damage caused by free radicals, which are related to the development of many chronic diseases .The dominance of this study sheds light on the possible therapeutic potential of Al-Shabbat as a source of antioxidant compounds. These results encourage further research to determine the specific chemical compounds responsible for the antioxidant activity in Nabat al-Shabat. The contribution of this study to the development of new natural products with antioxidant properties.

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