

## Original Research Article

# Phenolic constituents of *Anethum graveolens* seed extracts: chemical profile and antioxidant effect studies

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### ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

**Background:** *Anethum graveolens* (dill) is a common herb used in traditional Romanian cuisine, as well as in phytotherapy. Dill seeds were reported to be rich in antioxidants. As part of the diet, this herb may play a role in prevention the damage caused by oxidative stress and reducing the risk of pathologies such as cancer, diabetes, neurodegenerative and cardiovascular diseases. Moreover, interest in food additives of natural origin has increased in recent years.

**Methodology:** In this study, the total phenolics contents, the phenolic profile and the antioxidant properties of the methanolic and hydromethanolic extracts of Romanian dill seeds were investigated. They were applied the Folin-Ciocalteu spectrophotometrically method, respectively reverse-phase high-performance liquid chromatography.

**Results:** The highest content of total phenolics was found in acidified methanol samples (46.5 - 46.8 mg GAE/g dry seeds). RP-HPLC analysis highlights important content of quercetin, kaempferol, caffeic acid, sinapic acid, gallic acid, vanillic acid, ( $\pm$ ) - catechin and umbelliferone. Antioxidant activity, measured via DPPH free radical scavenging ability, showed very high values (93.5 - 95.6% for the crude extracts and 67.5 - 93.2% for extracts at a concentration of 0.25 mg/mL). Methanolic extract exhibited the best IC<sub>50</sub> value (88.7  $\pm$  0.01  $\mu$ g/mL).

**Conclusion:** All experiments proved the antioxidant activity of dill seed extracts.

*Keywords: Dill seeds, maceration, ultrasound-assisted extraction, RP-HPLC, phenolic compounds, oxidative stress*

## 1. INTRODUCTION

Plants are an inexhaustible source of health promoting compounds. Medical studies have shown the relationship between a diet rich in phytonutrients and a lower risk for the development of age-related diseases, such as cancer, type 2 diabetes, cardiovascular diseases and neurodegenerative disorders [1-3]. Increased oxidative processes (oxidative stress) and overproduction of free radicals in the human body, such as reactive oxygen species and reactive nitrogen species, can adversely affect large biomolecules such as lipids, proteins, lipoproteins and DNA, resulting in changes in their structure and functions and the pathogenesis of such diseases [2,4,5]. Studies also suggest that flavonoids are potent anti-inflammatory agents [2,6]. While oxidative stress and inflammation are important

in triggering many disorders, antioxidant polyphenols could prevent some damage at the molecular level, playing an important role in preventing such pathologies [2,3].

Synthetic phenolic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are scarcely accepted as antioxidants in the food, cosmetic due to their ineffectiveness and toxicity [4,7]. In recent years, the search for antioxidants and natural food additives has become a growing concern [8].

*Anethum graveolens*, commonly known as dill, is an annual aromatic plant belonging to the family *Umbelliferae* (*Apiaceae*), originating in the South-West Asia or South-East Europe and cultivated since ancient times [9]. In Europe, dill is used as a culinary plant as well as for several medical purposes, including as an anti-asthmatic, antispasmodic analgesic, digestive, carminative and diuretic herb [3]. Dill seed oil is used to treat various conditions, including colic in infants, transit disorders (constipation, diarrhea), flatulence, nausea, abdominal discomfort and indigestion. In addition, *A. graveolens* has been reported to possess antimicrobial, antihyperlipidemic and antihypercholesterolemic activities [9,10,11], anticarcinogenic and antioxidant properties [10,12,13], increases the effectiveness of insecticides [9] and has vermifugal activity against intestinal worms [10]. Dill seed extract showed significant protective activities of the gastric mucosa, antisecretory and anti-ulcer effects in mice by oral administration of hydrochloric acid and absolute ethanol [14], antinociceptive properties in inflammatory pain [11], anti-stress, antioxidant and memory improvement activities [15], anticonvulsant effect against pentylenetetrazole-induced seizure [16] and broad antimicrobial spectrum against several bacteria or fungi [9,10,12].

Based on the above considerations, the main objective of this study was to investigate the phenolic composition of the alcoholic and hydroalcoholic extracts obtained from dill seeds found on the Romanian market. The total phenolic content as well as the profile of the individual polyphenols such as flavonoids, phenolic acids and coumarins were determined. A preliminary assessment of the antioxidant activity of dill seed extracts was performed.

## **2. MATERIALS AND METHODS**

### **2.1 Chemicals and Reagents**

All chemicals (methanol, acetic acid, sodium carbonate) used for assays were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), quercetin, kaempferol, caffeic acid, sinapic acid, gallic acid, vanillic acid, ( $\pm$ ) - catechin and umbelliferone were purchased from Sigma-Aldrich. 2N Folin-Ciocalteu reagent was purchased from Merck Co. Acetic acid, methanol and water of HPLC grade used for HPLC analyses were purchased from Merck Co.

### **2.2. Sample preparation**

The dill seeds were purchased from the market (Plafar, Stefmar Company). The dried seeds were ground into a fine powder using a mechanical blender and then subjected to phytochemical extraction. In order to study the influence of solvent concentration and extraction technique, four solvents systems (methanol:water 4:1, methanol:water 1:1, methanol: 6N HCl 9:1 and methanol) were used following two techniques – maceration and ultrasound-assisted extraction.

The dill seed powder (1 g) was macerated with 10 mL of solvent at room temperature for 48 h. The mixture was shaken from time to time. A mixture of dill seed powder (1 g) and 10 mL of solvent was ultrasonicated (ultrasonic bath Elma S300H) at room temperature for 30 min. The resulting extracts were filtered through a 0.45  $\mu$ m Millipore filter and made up to 10 mL

with the same solvent. The extraction solutions were stored at +4 °C in the refrigerator until further use.

## **2.3. Phytochemical characterization of dill seed extracts**

### **2.3.1 Determination of total phenolics content**

The total phenolics content of each extract was evaluated spectrophotometrically using the Folin-Ciocalteu assay, according to a procedure described in the literature [17] with slight modifications. The Folin-Ciocalteu reagent reacts with phenolic compounds changing the color from yellow to blue. To 8 mL of distilled water was added 1 mL of dill seed extract sample and 1 mL of 2N Folin-Ciocalteu reagent (diluted 1:10). The mixture was shaken and allowed to stand for 1 min before adding 2 mL of 20% aqueous Na<sub>2</sub>CO<sub>3</sub> solution and then it was kept at 40 °C for 1 h. In the standard sample, the dill seed extract was replaced with 1 mL of standard solution of gallic acid. The absorbance value of the resulting blue color was measured at 765 nm using a SPECORD 210 PLUS UV-VIS spectrophotometer Analytik-Jena. The total phenolics content was expressed as mg of gallic acid equivalents per gram of dry sample ± standard deviation (mg GAE/g dry dill seeds ± SD) through a calibration curve with gallic acid (gallic acid concentration in the range of 0.05 - 0.25 mg/mL; R<sup>2</sup> = 0.9975). Data were obtained from the average of the independent determination.

### **2.3.2 Analysis of phenolic compounds by reverse-phase high-performance liquid chromatography (RP-HPLC)**

The RP-HPLC analysis was performed using a High-Performance Liquid Chromatography System HPLC ACME 3000 Younglin Instrument (Young Lin Instrument Co. Ltd, Korea), equipped with SP 930D module and UV 730D detector module. The determination was made under isocratic elution, using as mobile phase the mixture of methanol:water:acetic acid 300:700:2 and the elution flow set at 1 mL/min. Chromatographic separation of the sample constituents was obtained using a YMC-Pack ODS AQ reverse-phase column (150 cm long, 4.6 mm internal diameter, YMC Co. Ltd Japan) with an injection volume of 20 µL from each sample at room temperature. The detection was performed at the analytical wavelength of 300 nm. The determination of the components in dill seed extracts was performed by the external standard method, using pure substances as standards [18]. The calibration curve showed the linearity of detector over the tested range. The correlation coefficient (R<sup>2</sup>) values were greater than 0.995, indicating an appropriate adjustment of the experimental data. The specificity of the method was assessed by comparing the consistency of the retention time between a sample and the corresponding reference standard. Retention time (R<sub>t</sub>) is shown in Table 2.

## **2.4. Antioxidant activity – DPPH scavenging assay**

The DPPH radical scavenging activity of each sample was carried out using a method described in literature with minor modifications [19]. Each sample stock was diluted to final concentrations of 0.063 - 2.5 mg/mL with 95% methanol. Various concentrations of each extract sample (0.2 mL) were mixed with freshly prepared DPPH methanolic solution (2.8 mL, 3 mM concentration) and the mixture was kept at room temperature in the dark for 60 min. The analysis was performed using a SPECORD 210 PLUS UV-VIS spectrophotometer Analytik-Jena. The absorbance values were recorded at 517 nm for each sample and gallic acid was used as a reference standard. The scavenging activity was measured as a decrease in the absorbance of the samples versus DPPH solution (control). The DPPH radical scavenging ability of each extract sample was calculated using the following equation:

$$\text{DPPH radical scavenging rate (\% inhibition)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/\text{Abs}_{\text{control}}] \times 100$$

where  $\text{Abs}_{\text{control}}$  is the absorbance of DPPH solution (without extract) and  $\text{Abs}_{\text{sample}}$  is the absorbance of extract sample. The antioxidant activity of standard and samples was expressed as  $\text{IC}_{50}$  value (mg GAE/mL required to cause a 50% inhibition of the DPPH radical). The  $\text{IC}_{50}$  values were graphically determined by plotting the percentage of inhibition against inhibitory concentration. All experiments were carried out in triplicate.

## 2.5. Statistical analysis

The experimental results were expressed as mean  $\pm$  standard deviation (SD). The correlation coefficient ( $R^2$ ) was established by regression analysis. Data results were analyzed for statistical comparisons using Student's t-test and Design Expert Application (trial version). Analysis of variance (ANOVA) was also carried out to process the experimental results. Values of  $P < .05$  were considered statistically significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical investigations

#### 3.1.1 Identification of phenolic compounds by RP-HPLC

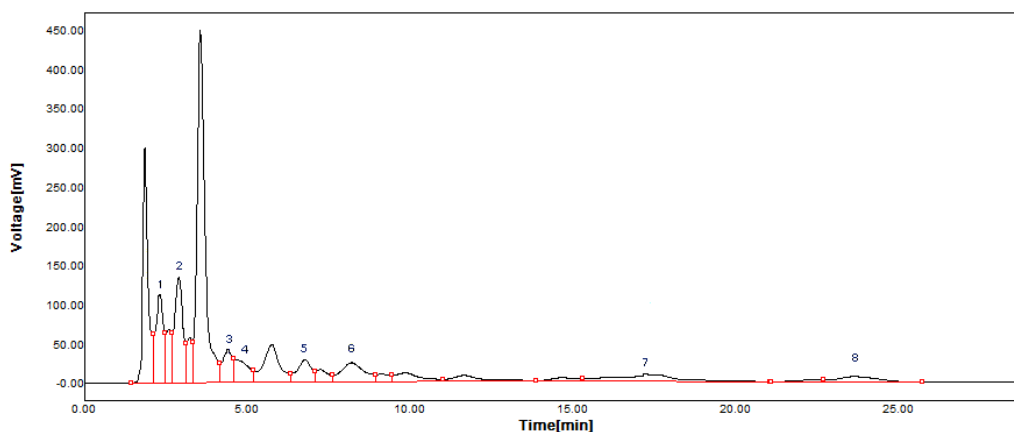
To determine the phenolic compounds, dill seed extracts were prepared using four solvents systems – methanol:water 4:1, methanol:water 1:1, methanol:6N HCl 9:1 and methanol (99.9%, concentrated methanol), respectively following two techniques – maceration at room temperature and ultrasound-assisted extraction (sonication extraction) (Table 1), and the extracts were analyzed by liquid chromatography (reversed-phase high-performance liquid chromatography RP-HPLC). Flavonols such as quercetin and kaempferol, phenolic acids including caffeic acid, sinapic acid, gallic acid and vanillic acid, flavanols such as catechin, as well as coumarins such as umbelliferone were screened in extracts.

**Table 1. The solvents systems used for extraction**

Solvents systems	Solvents	Ratio	Technique of extraction
A	methanol:water	4:1	maceration
B	methanol:water	4:1	sonication
C	methanol:water	1:1	maceration
D	methanol:water	1:1	sonication
E	methanol:HCl 6N	9:1	maceration
F	methanol:HCl 6N	9:1	sonication
G	methanol	conc	maceration
H	methanol	conc	sonication

*Methanol conc = methanol 99.9%*

Figure 1 shows a typical separation of the analyzed extracts under optimized chromatographic conditions. According to the RP-HPLC analysis, the phenolic compounds investigated – flavonols, phenolic acids, ( $\pm$ ) - catechin and umbelliferone were identified in the studied samples.



**Fig. 1. Chromatogram of *A. graveolens* seed extracts (sample G).**

1: Gallic acid, 2: ( $\pm$ )-Catechin, 3: Vanillic acid, 4: Caffeic acid, 5: Umbelliferone, 6: Sinapic acid, 7: Kaempferol, 8: Quercetin.

Quantitative analysis of phenolic components was performed on each dill seed extract. The quantitative determination of phenolic compounds in extracts A-H is summarized in Table 2. Quercetin and kaempferol are the flavonols identified in dill seed extracts. Although generally extracted in good amounts, quercetin is found in lower concentrations in methanolic samples G and H. The concentration was improved using hydromethanolic mixtures (samples A, B, C and D, concentration in the range of 0.75 - 1.05 mg/g dill seeds) and increased with increasing methanol content (samples A and B), which is in accordance with literature data on the solubility of quercetin in methanol [20]. When extraction was performed using solvents systems whose pH was adjusted in the acidic range with 6N hydrochloric acid (systems E and F, pH 1.5), quercetin could not be determined. The concentration of kaempferol was higher when the extraction was performed with more polar solvents (systems C and D – methanol:water 1:1, concentration of 0.77 and 0.58 mg/g seeds, respectively). Unlike quercetin, the addition of 6N hydrochloric acid to methanol increased the content of kaempferol in the extracts (systems E and F, concentration of 0.78 and 0.86 mg/g seeds, respectively). Although the literature has reported the presence of rutin in the seeds of *A. graveolens* [13,21], this was not reflected in the results of this study, since the rutin was not determined.

Phenolic acids were determined in all dill seed extracts. Hydroxycinnamic acids such as caffeic acid and sinapic acid are common ingredients of human diet and are often found in fruits, vegetables and grains, accounting for a high percentage of total phenolic compounds [14]. Due to its low solubility in water (0.6 mg/mL) [22] compared to its solubility in methanol or ethanol (approximately 50 mg/mL) [23], caffeic acid was not detected or detected in very small amounts in samples A, B, C and D. However, methanol and acidified methanol have been shown to be better solvents for caffeic acid, allowing its extraction (samples E, F, G and H, concentration in the range of 0.18 - 0.27 mg/g seeds). A similar situation occurred in the case of sinapic acid, which is more soluble in methanol than water [24]. Therefore, sinapic acid was identified in good amounts in samples with high rates of methanol (samples A, E and G, concentration in the range of 0.24 - 0.28 mg/g seeds). The influence of pH change on yield during the extraction procedure was not visible.

**Table 2. Phenolic content of *A. graveolens* seed extracts A - H by RP-HPLC**

Compound	Compound content (mg/g dill seeds $\pm$ SD)								$R_t$ (min)
	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Sample H	
Quercetin	1.05 $\pm 0.04$	0.91 $\pm 0.07$	0.83 $\pm 0.09$	0.75 $\pm 0.05$	ND	ND	0.67 $\pm 0.09$	0.47 $\pm 0.01$	23.63
Kaempferol	0.18 $\pm 0.01$	0.16 $\pm 0.01$	0.77 $\pm 0.06$	0.58 $\pm 0.07$	0.78 $\pm 0.06$	0.86 $\pm 0.07$	0.28 $\pm 0.01$	0.17 $\pm 0.01$	17.46
Caffeic acid	ND	ND	0.08 $\pm 0.01$	0.09 $\pm 0.01$	0.27 $\pm 0.01$	0.26 $\pm 0.01$	0.21 $\pm 0.01$	0.18 $\pm 0.01$	4.58
Sinapic acid	0.28 $\pm 0.02$	0.17 $\pm 0.02$	0.11 $\pm 0.02$	0.09 $\pm 0.02$	0.24 $\pm 0.03$	0.18 $\pm 0.02$	0.24 $\pm 0.02$	0.16 $\pm 0.01$	8.20
Gallic acid	0.68 $\pm 0.05$	0.29 $\pm 0.07$	0.38 $\pm 0.02$	0.12 $\pm 0.02$	1.64 $\pm 0.02$	0.97 $\pm 0.09$	0.93 $\pm$ 0.09	0.51 $\pm 0.04$	2.31
Vanillic acid	0.29 $\pm 0.03$	0.29 $\pm 0.03$	0.25 $\pm 0.03$	0.21 $\pm 0.03$	0.39 $\pm 0.03$	0.26 $\pm 0.02$	0.43 $\pm 0.03$	0.31 $\pm 0.03$	4.40
( $\pm$ )-Catechin	0.91 $\pm 0.07$	0.93 $\pm 0.05$	0.82 $\pm 0.06$	0.59 $\pm 0.06$	0.67 $\pm 0.05$	0.41 $\pm 0.04$	1.0 $\pm 0.10$	0.98 $\pm 0.10$	2.90
Umbelli ferone	0.45 $\pm 0.01$	0.41 $\pm 0.02$	0.55 $\pm 0.02$	0.45 $\pm 0.02$	0.48 $\pm 0.01$	0.38 $\pm 0.01$	0.34 $\pm 0.03$	0.31 $\pm 0.01$	6.78

SD: standard deviation; ND: not determined;  $R_t$ : retention time

Hydroxybenzoic acids such as gallic acid, syringic acid and vanillic acid are generally found in small amounts in edible plants [3] and some studies showed their presence in dill herb [25]. According to RP-HPLC analysis, the concentration of vanillic acid is quite similar in all samples regardless of the extraction solvent (concentration in the range of 0.25 - 0.39 mg/g seeds). A better result was obtained in the case of methanolic extract G (concentration of 0.43 mg/g). However, it was not supported by the sonication method, probably due to the short extraction time. Gallic acid was dominant among phenolic acids. The best results were obtained for methanol (sample G – 0.93 mg/g seeds) and acidified methanol as extraction systems (sample E – 1.64 mg/g seeds and sample F – 0.97 mg/g seeds), compared to experiments when hydromethanolic mixtures were used (sample A – 0.68 mg/g seeds and sample C – 0.38 mg/g seeds) which can be explained by the higher solubility of gallic acid in methanol or ethanol than water [26]. Considering the two extraction methods, the gallic acid concentrations are significantly higher in the extracts obtained by maceration (samples A, C, E, G) compared to those obtained by sonication for the same solvents systems (samples B, D, F, H). The concentrations of vanillic acid and sinapic acid also vary depending on the extraction process, but these differences are less significant.

( $\pm$ ) - Catechin is a flavanol soluble in ethanol, methanol and water [27]. A good ( $\pm$ ) - catechin content was found in all samples (concentration in the range of 0.59 - 1.0 mg/g seeds) with the highest concentrations in methanolic extracts (samples G and H, concentration of 1.0 mg/g and 0.98 mg/g seeds, respectively), as well as in less polar hydromethanolic solutions (samples A and B, concentration of 0.91 mg/g and 0.93 mg/g seeds, respectively). The addition of hydrochloric acid to methanol decreased the ( $\pm$ ) - catechin content in extracts (samples E and F, concentration of 0.67 mg/g and 0.41 mg/g seeds, respectively).

The most consistent results were obtained for umbelliferone, with concentrations in a narrow range (0.31 - 0.55 mg/g seeds) regardless of the solvents mixtures and extraction

procedures. However, hydromethanolic mixtures (samples A, B, C and D) seem to be better solvents than methanol and acidified methanol, when the umbelliferone content is slightly decreased. The concentration of umbelliferone determined in dill seed extracts is close to the concentration described in the literature [28].

The experimental results showed that the extraction efficiency is closely related to the polarity of the medium, the extraction yields showing sometimes significant differences due to the different polarity of the solvents.

As can be seen, an efficient extraction of active ingredients (flavonols, phenolic acids, ( $\pm$ ) - catechin and umbelliferone) with methanol or hydromethanolic solutions by maceration at room temperature is possible, and the maceration process accompanied by sonication can ensure an optimal extraction if the working time is well determined. Methanol and aqueous methanol have been reported to be very good solvents for catechin, flavones and polyphenols [25,29]. Changing the pH of methanolic solutions during the extraction procedure had significant results in particular cases. Some phenolic compounds are mainly found in the form of glycosylated derivatives in natural products, which may explain the increase in their amount during acidic extraction.

### **3.1.2 Determination of total phenolics content**

The bioavailability of plant phytochemicals is influenced by the microstructure of the plant tissue in which they are located, the storage conditions and by the thermal conditions to which they are subjected during processing [25]. The total phenolics content and phenolics content except umbelliferone respectively, expressed in mg gallic acid equivalent (GAE)/g dry dill seeds are shown in Table 3.

**Table 3. Total phenolics content and total phenolics content except umbelliferone of *A. graveolens* seed extracts**

<b>Sample</b>	<b>Total Phenolics Content (mg GAE/g dill seeds)</b>	<b>Total Phenolics Content except umbelliferone (mg GAE/g dill seeds)</b>
A	43.3 $\pm$ 0.17	38.8 $\pm$ 0.13
B	42.5 $\pm$ 0.20	38.1 $\pm$ 0.22
C	43.4 $\pm$ 0.26	37.9 $\pm$ 0.28
D	43.6 $\pm$ 0.33	39.1 $\pm$ 0.18
E	46.8 $\pm$ 0.21	42.3 $\pm$ 0.17
F	46.5 $\pm$ 0.16	42.7 $\pm$ 0.11
G	35.2 $\pm$ 0.12	31.8 $\pm$ 0.21
H	26.7 $\pm$ 0.14	23.3 $\pm$ 0.17

Regardless of the extraction technique followed, much more phenolic compounds were extracted by hydromethanolic mixtures (samples A - F), which may be referred to the polarity of the solvents (Table 3). Phenols comprise a wide variety of compounds such as polyphenols, flavonoids, condensed tannins. Phenolic compounds are mostly hydrophilic, while the flavonoids are mostly hydrophobic [30]. However, due to their structures, flavonoids such as quercetin, kaempferol and catechin were easily extracted by hydromethanolic solvents. Significantly close results were obtained for samples A-D where the total amount of phenols is in the range of 42.5 - 43.6 mg GAE/g dry seeds. It should also be noted that the acidification of the extraction solvents with 6N hydrochloric acid (systems E

and F) led to a slight increase in the level of total phenolics content (46.5 - 46.8 mg GAE/g dry seeds), which can be due to hydrolysis or other chemical cleavage processes of glycosylated phenolic derivatives found in the vegetal product [31]. The lowest values of total phenolics content were found in methanolic samples G and H (35.2 and 26.7 mg GAE/g dry seeds, respectively), because of the lower solubility of phenols in methanol than in hydroalcoholic mixtures.

Umbelliferone is a hydroxycoumarin and a monophenol compound which is also determined by the Folin-Ciocalteu method. The quantitative determination of umbelliferone by RP-HPLC-UV method showed contents of 0.45 - 0.55 mg/g dill seeds in the case of hydromethanolic solutions and 0.34 mg/g in the case of methanol extraction, respectively. To determine the actual amount of polyphenols in the extracts, we prepared umbelliferone solutions and determined the gallic acid equivalent corresponding to each concentration. The polyphenols content of dill extracts was determined by the difference (Table 3).

As expected, the total phenolics content of *A. graveolens* seed extracts exceeds the content of flavonols, phenolic acids, ( $\pm$ )-catechin and umbelliferone identified by RP-HPLC. The likely explanation might be the presence of other phenolic derivatives besides the mentioned compounds. However, Folin-Ciocalteu is a simple method and currently the most widely used technique for quantifying phenolics content [32].

There are several reports showing different phenolics content depending on the specific parts of *A. graveolens* [33]. Therefore 9.8 mg GAE/g of dry weight consisting of phenolic acids, flavonoids and essential oils were obtained from the leaves and branches [34]. Ethanolic extracts of *A. graveolens* (seeds and leaves) from Romania gave 26.4 mg GAE/g seeds compared to 7.6 mg GAE/g leaves [13]. Higher values for polyphenols were obtained from extracts of *A. graveolens* originating in Algeria, which showed 96.54 mg GAE/g of dry whole plant, compared to 167.7 mg GAE/g of dry seeds [33]. It is also reported that fresh dill extracts contain more total polyphenols (35.23 mg/g weight in 50% acetone) than samples obtained from frozen and dried samples [25,35]. Our results are comparable to the data described in the literature.

### 3.2 DPPH radical scavenging activity

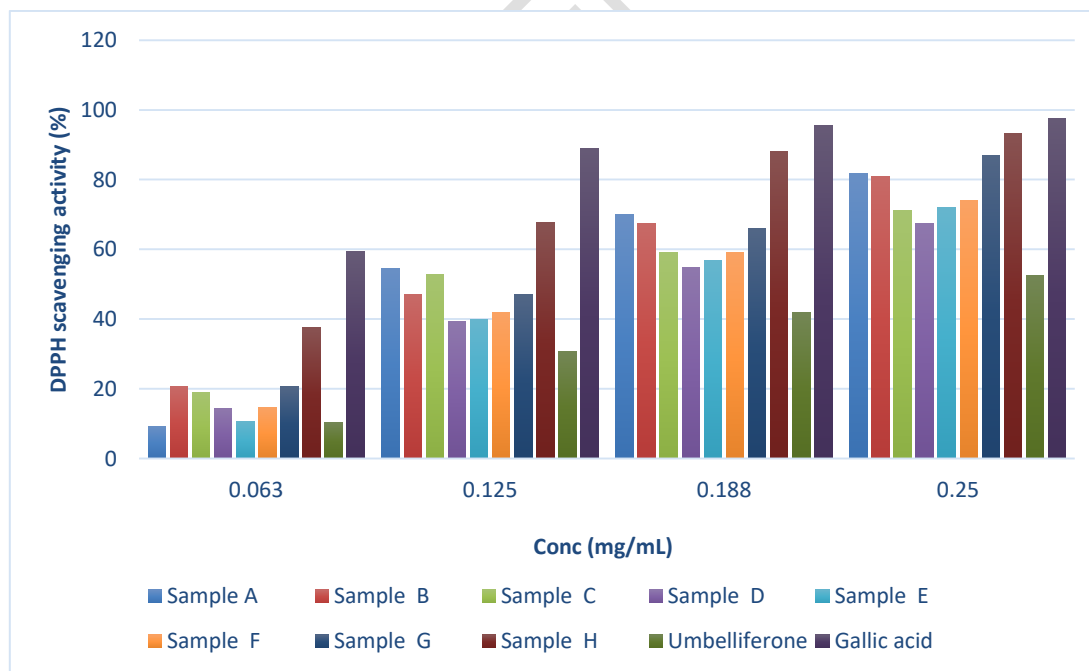
Phenolic compounds can act as hydrogen or electron donors, reducing agents and metal ion chelators depending on their chemical structures and the number of hydroxyl groups [36]. Therefore, the antioxidant potency of *A. graveolens* extracts was investigated by means of in vitro assay based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. This test is based on a combination of hydrogen atom transfer (HAT) and single electron transfer (SET) reactions between phenols and the DPPH radical in alcoholic solutions (proton-coupled electron transfer). Since phenolic compounds can undergo both HAT and SET processes, the reaction mechanism depends mainly on the chemical structure of the antioxidant [37].

The extent of the reaction depends on the capacity of antioxidant to participate in the proton-electron transfer. The results of the DPPH test for *A. graveolens* seed extracts compared to gallic acid as standard antioxidant are shown in Table 4 and Figure 2. Gallic acid was chosen as a representative of phenolic derivatives. It should be noted that all methanolic extracts of dill seeds significantly scavenged DPPH radicals, showing an inhibitory activity in the range of 67.5 - 93.2% at a concentration of 0.25 mg/mL, compared to the free radical scavenging activity of gallic acid ( $97.6 \pm 2.37\%$  at a concentration of 0.25 mg/mL). It is also noteworthy that the acidification of the methanolic extraction mixtures, as well as the increase in the polarity of the solvents caused the decrease of the antiradical abilities (e.g. samples E, F, C and D, DPPH inhibition in the range of 67.5 - 74.1%). The concentration of

extracts that inhibit 50% of DPPH free radicals (half maximal inhibitory concentration  $IC_{50}$ ) was also determined. The  $IC_{50}$  values of the extracts are ranged between 88.7 - 168.8  $\mu\text{g/mL}$ . A lower  $IC_{50}$  value shows a stronger DPPH radical scavenging activity. Compared to standard gallic acid ( $IC_{50} = 55.4 \pm 0.02 \mu\text{g/mL}$ ), the extracts showed good antiradical activity. The best value was obtained for the sample H ( $IC_{50} = 88.7 \pm 0.01 \mu\text{g/mL}$ ) with high antiradical activity.

**Table 4. DPPH radical - scavenging activity of *A. graveolens* seed extracts (crude extracts and 0.25 mg/mL extracts) and  $IC_{50}$  values**

Sample	DPPH scavenging activity (%) (crude extract)	DPPH scavenging activity (%) (0.25 mg/mL)	$IC_{50}$ ( $\mu\text{g/mL}$ )
A	94.6 $\pm$ 6.55	81.7 $\pm$ 1.55	107.7 $\pm$ 1.03
B	94.2 $\pm$ 3.60	81.0 $\pm$ 2.60	133.9 $\pm$ 1.55
C	94.1 $\pm$ 2.64	71.2 $\pm$ 2.55	119.6 $\pm$ 1.12
D	93.4 $\pm$ 4.72	67.5 $\pm$ 4.25	168.8 $\pm$ 2.11
E	95.6 $\pm$ 3.53	72.1 $\pm$ 2.73	162.1 $\pm$ 1.92
F	95.0 $\pm$ 2.54	74.1 $\pm$ 2.65	154.9 $\pm$ 1.69
G	93.5 $\pm$ 3.21	87.0 $\pm$ 1.51	134.9 $\pm$ 1.42
H	93.6 $\pm$ 5.51	93.2 $\pm$ 4.75	88.7 $\pm$ 0.01
Gallic acid	-	97.6 $\pm$ 2.37	55.4 $\pm$ 0.02
Umbelliferone	-	52.4 $\pm$ 0.65	236.1 $\pm$ 2.31



**Fig. 2. DPPH radical - scavenging activity of *A. graveolens* seed extracts**

The radical scavenging activity of the crude dill extracts was also tested by the DPPH assay. The extracts that contained the highest amount of total phenolics (samples E and F, 46.5 mg GAE/g dry seeds and 46.8 mg GAE/g dry seeds, respectively) showed the best effect in

inhibiting DPPH, reaching 95.6% (Table 4). Although we cannot make a linear correlation between the values of the total phenolics content and the antioxidant activity of dill seed extracts, it can be generally said that the antiradical abilities have increased with increasing amounts of phenolic derivatives. Therefore, phenolic compounds can be considered important components responsible for the radical scavenging activity of *A. graveolens* seed extracts. These findings support some previous reports showing that phenolic and polyphenolic derivatives can act as free radical scavengers. Through a proton-electron transfer process, they form stable antioxidant free radicals which interrupt the oxidation chain of free-radicals and further protect biomolecules [25]. The results also suggest that phenolic compounds are only partially responsible for the antiradical activity of the extracts and other phytochemical constituents may contribute to this activity. Relevant examples are samples G and H which showed much lower total phenolics content compared to samples A - D. However, their antioxidant activities are very close (Table 3, Table 4). In fact, samples G and H showed the best values of inhibitory activity at the concentration of 0.25 mg/mL (87% and 93.2%, respectively), compared to the activity of gallic acid (97.6%). Therefore, the antioxidant activity of the extracts should not be attributed exclusively to the total phenolics content.

As a plant-derived hydroxycoumarin, umbelliferone have been reported to exhibit pharmacological properties including antioxidant, antibacterial and antifungal, antitumor, anti-inflammatory and anti-hyperglycemic activities [38]. Our study showed that the DPPH radical scavenging ability of umbelliferone increased in a concentration dependent manner. Compared to the standard GA (97.6% DPPH inhibition), umbelliferone has a moderate radical inhibition (52.4%) at the concentration of 0.25 mg/mL (Table 4).

Several authors have investigated the antioxidant potential of *A. graveolens* seed extracts by evaluating their ability to scavenge DPPH or ABTS radicals in order to determine the relationship between the phenolic constituents and biological activity and have suggested that antioxidant activity might be related to the phenolics content and their chemical structures [7,13,39]. On the other hand, Swieca and Gawlik-Dziki [25] did not find any correlation between the phenolic content and the antioxidant activity of the dill herb extract. In addition, there are studies indicating that polar solvents such as water, methanol or ethanol provide dill seed extracts with higher antioxidant activity compared to other fractions [7,39]. Therefore, Ramadan et al [39] reported that aqueous extract of dry dill seeds showed 89.7% DPPH radical inhibition compared to the synthetic antioxidant tert-butyl hydroquinone (99.7% DPPH radical inhibition). Our findings are in agreement with these published data showing that *A. graveolens* seed extracts are effective DPPH radical scavengers.

#### 4. CONCLUSIONS

Dill seeds are one of the most commonly used spices in Romanian traditional cuisine and food industry. Moreover, due to their pharmacological properties, dill seeds are used in Romania as medicinal products for the treatment of certain diseases.

In the present work, the total phenolics contents, the phenolic profile and the antioxidant properties of the methanolic and hydromethanolic extracts of Romanian *Anethum graveolens* seeds are reported. Quercetin, kaempferol, gallic acid and ( $\pm$ ) - catechin were found in good amounts and the results suggest that dill seeds are a potential source of bioactive compounds as well as of natural antioxidants as an alternative to synthetic additives.

#### COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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