Evaluation of polyphenol content and antioxidant activities of Dill leaves extract *Anethum graveolens* L.

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**Abstract:** Natural plants are an excellent source of materials for the extraction and application of bioactive compounds. Two extracts from the Dill leaves (*Anethum graveolens* L.), including the ethanol leaves extract (ELE) and the extract of the aqueous leaves (ALE), were applied to determine the polyphenol, flavonoid content and antioxidant activity of this plant. This plant preliminary phytochemical screening was found to have positive reactions for alkaloids, flavonoids, triterpenoids, saponins, tannins, coumarins and reducing assays. The findings of the quantitative determination revealed that the TPC of ELE and ALE was 69.76 ± 1.57 and 47.71 ± 1.44 mgGAE/g dry extract, respectively. In contrast, TFC was 49.10 ± 1.30 and 19.39 ± 0.61 mgQE/g dry extract, respectively. ELE exhibited the highest DPPH (IC₅₀ = 149.39 ± 5.74 µg/ml) and ABTS (IC₅₀ = 87.43 ± 3.07 µg/ml) radical scavenging activity. These results show that Dill can be used in culinary applications with the ability to minimize oxidative stress.

1. **Introduction**
Throughout recent years, the usage of medicinal plants has gained considerable media interest in terms of their scientific and therapeutic potential [1-4]. Various medicinal plants were investigated for their antioxidant abilities [5-8]. Given the tremendous advancements witnessed in recent decades of medical medicine, plants nevertheless offer a major difference to healthcare. Dill (*Anethum graveolens* L.) is a seasonal herb from the celiac Apiaceae family, belonging to the genus Anethum. Dill is a crucial aromatic herb used to savor various foods, including salads, sauces, soups, and seafood [9]. Numerous specific compounds were derived from plant's roots, leaves and flowers, which includes phenolic acids, α-phellandrene, triterpenes, flavonoids, coumarins, and so on [10]. In traditional medicine, Dill is applied as a diuretic and also solves gastrointestinal problems, including indigestion, stomach ache and colic to tract intestinal gas.

*Anethum sepulchre* L. (A. graveolens) has been thoroughly defined in the scientific literature as having biological properties including antimicrobials, antispasmodic, antisecretory, mucosal and anti-hyperlipidemic effects [11]. Natural antioxidants have now attracted significant interest due to their ability to scavenge free radicals [12]. Phenolic compounds of plants and their secondary metabolites-flavonoids and proanthocyanidins have also been identified as active bioactive components correlated with antioxidant properties and health benefits [13]. In addition to antioxidant function, it was reported that phenolic compounds from different plants have antibacterial capacities against various pathogenic
microorganisms [14]. The fact that exploring natural plant compounds for antioxidant and tolerance to contamination has now become an important part of its usage in the treatment of difference diseases. This study aims to evaluate the phenolic content, flavonoids content and antioxidant activities of Dill leaves extract as a new potential source antioxidant. Therefore, water extract and dill leaf extract were then packed, and all of their free radical scavenging activity was decided.

2. Materials and methods

2.1. Chemicals and reagents
Potassium acetate, potassium persulfate, aluminium chloride, sodium carbonate, ethanol, methanol, folin-ciocalteu phenol reagent, DPPH, ABTS, gallic acid, L-ascorbic acid, and quercetin were bought from Sigma-Aldrich.

2.2. Sample preparation
In January 2020, Dill (Anethum graveolens L.) leaves were collected from Tien Giang, Vietnam. To remove the water, the fresh leaves were dried in a 60°C drying oven for 8h. The dried leaves were milled to fine powder (1.0 mm) after drying, and processed for usage at room temperature.

2.3. Solvent extraction
With 300 mL of ethanol 70% and distilled water in a conical flask, approximately 10 g of dried sample was collected. The mixture was stirred 300 revolutions per minute using a hot plate stirrer at temperature of 70°C and time of 60 min. After the extraction. The dill leaves extract was then filtered through a filter paper of Whatman No. 1 and concentrated on a 40°C rotary Heidolph evaporator. Two extracts collected successively from Dill, namely the extract of ethanolic leaves (ELE) and the extract of aqueous leaves (ALE) used to assess TPC, TFC and antioxidant activity.

2.4. Phytochemical screening
Phytochemical screening of Dill leaves was determined by using standard methods to identify secondary plant metabolites, including alkaloids, tannins, saponins, flavonoids, steroids, phenolic compounds, and so on. There was different standard methods including Wagner’s test, Borntrager’s test, Fehling’s, Borntrager, Libermann-Burchard, Froth, Ferric chloride, and Shinoda test [15].

2.5. TPC
TPC is determined by using Folin-Ciocalteu method based on the description of Thuy et al [16]. It says as follows: 0.5ml diluted sample was added into a tube. Then, add 2.5 ml of 10% Folin-Ciocalteu solution and homogenize with a Vortex machine. After that, leave the solution in 5 minutes for it to react. After, adding 2.0 ml of 7.5% Na2CO3 solution and mixing together. Lastly, incubate the mixture at a RT in the dark for one hour and then measure the optical absorption at a wavelength of 765nm using the UV-Vis spectrometer. The result was shown in mg equivalent gallic acid per 1 gram of dry extract (mgGAE / g).

2.6. TFC
TFC is measured using the colourimetric aluminum chloride method, based on Mahboub et al. description [17]. 0.5 mL extract was added with 0.1 mL 10% AlCl3. Then, 1mL 1 M CH3COOK was mixed with 4.3 mL of distilled water, and shake vigorously. As a standard Quercetin was used. Reports were seen in mg quercetin equivalents per 1 gram of dry extract (mg QE / g) and spectrophotometrically calculated at 415 nm.

2.7. DPPH Scavenging Activity
DPPH is performed using the method Thuy et al describes [16]. The samples were packed and stored at -20°C in the dark before its use. The mixture diluted with methanol and adjust the absorbance of the
solution at a wavelength of 517 nm to 1.1 ± 0.02 at the same time. After the aforementioned steps are done, aspirate 0.5 ml of diluted sample into a test tube. The control sample will be replaced by methanol. Then, add to the test tube 1.5 ml DPPH solution (OD nm = 1.1 ± 0.02) and incubate in the dark for 30 minutes. The DPPH scavenging activity is determined according to the formula

\[
\text{DPPH scavenging activity (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100}
\]

IC50 is the sample concentration required to scavenge 50% of DPPH-free radicals.

2.8. ABTS Scavenging Activity

ABTS is conducted using the method described by Thuy et al [16]. ABTS free radical solution is prepared by adding 10 ml ABTS solution of 7.4 mM to 10 ml of 2.6 mM \( \text{K}_2\text{S}_2\text{O}_8 \) solution and incubated in the dark for 24 hours, then have the mixture diluted with methanol and adjust the absorbance of the solution at a wavelength of 734 nm to 1.1 ± 0.02 at the same time. After the aforementioned steps are done, aspirate 0.5 ml of diluted sample into tube. The control sample will be replaced by methanol. Then, add to the test tube 1.5 ml ABTS solution (OD nm = 1.1 ± 0.02) and incubate in the dark for 30 minutes. ABTS scavenging activity will be determined based on the formula:

\[
\text{ABTS scavenging activity (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100}
\]

2.9. Statistical Analyses

Both determinations in triplicate were selected and the outcomes were represented as mean and standard deviation (SD) values. The finding were analyzed using one-way ANOVA, accompanied by using the 15.0 version of Statgraphics Centurion XV using Fisher’s Least Significant Difference test. The variations were found statistically significant at P<0.05 for both measures.

3. Results and discussion

3.1. Phytochemical analysis

The extraction from A. graveolens leaves illustrated that the presence of various bioactive components which includes alkaloids, saponins, coumarins, flavonoids, triterpenoids, tannins, and reducing sugars (Table 1).

Table 1: Phytochemical screening of ethanolic, aqueous extracts of Anethum graveolens

| Chemical components | Ethanol | Aqueous |
|---------------------|---------|---------|
| Alkaloids           | +       | +       |
| Tannins             | -       | +       |
| Anthraquinons       | -       | -       |
| Flavonoids          | +       | +       |
| Terpenoids          | +       | +       |
| Coumarins           | +       | -       |
| Saponins            | -       | +       |
| Reducing sugars     | +       | +       |

(+ ) indicates present, (- ) indicates absence

As we known, previous studies have shown that plant secondary metabolites have been shown to have biological activity such as anti-inflammatory, anticancer, antibacterial, anti-diabetic, antioxidant, and disease prevention, and so on. Today, natural compounds are increasingly being noticed by scientists because they are used not only for food and cosmetics, but also for direct use as medicines to treat
diseases. The phenolic compounds showed different activities like such as antibacterial, antioxidant, anti-cancer, anti-inflammatory, xanthine oxidase inhibitors and anti-allergic activities [18]. The alkaloids from plants showed central nervous system activities, antibiotic activity, and anti-malarial activity [19]. Saponins are other phytoconstituents that are thought to protect living bodies from hypercholesterolemia and antibiotic properties [20]. Therefore, the diverse presence of natural compounds in plants has contributed to explain a number of therapeutic uses and potential sources of raw materials for the food and health care industries.

![Figure 1.](image)

Figure 1. Phytochemical analysis of Anethum graveolens leaves extracts. Ethanolic extract: Wagner’s test (a), Shinoda test (b), Libermann-Burchard test (c); Aqueous extract: Froth test (d), Fehling’s test (e), Ferric chloride test (f).

3.2. TPC and TFC in different fractions
The total polyphenol content ranged from 47.71 ± 1.30 to 69.76 ± 1.57 mg gallic acid equivalents per gram of dry extract (Table 2). Ethanolic extracts produce more TPC than aqueous extracts. The above results show that the TPC of the extracts reliance on the type of solvent. Many prior experiments have also shown that the solubility of phenolic substances in solvent extraction depend on the polarity of the solvent. Therefore, ethanol is the most effective solution for the removal of polyphenols from plants [21].

The TFC in the ethanolic extract was also higher than in the water with 47.71 ± 1.44 and 19.39 ± 0.61 mg quercetin equivalents per gram of dry extract, respectively. Water is a strong solvent for substances like saponins, tannins, and reduces sugars for the preliminary evaluation of chemical components of plants. Additionally, water is an inexpensive, readily accessible, and environmentally sustainable solvent, ideal for the application of extracts to foods. Ethanol solvents are more effective than distilled water in the extraction of flavonoid compounds from the dill leaves. This is also consistent with previous studies regarding the effect of extraction solvent on the TPC of plants [22]. Thus, ethanol 70% is a best solvent for polyphenol and flavonoid compounds extraction from Dill leaves.

Table 2. TPC, TFC, and IC₅₀ values of Anethum graveolens leaves extracts.

| Sample   | TPC (mg GAE/g) | TFC (mg QE/g) | IC₅₀ value (µg/ml) |
|----------|---------------|---------------|--------------------|
|          |               |               | DPPH               | ABTS               |
| ELE      | 69.76 ± 1.57ᵇ | 49.10 ± 1.30ᵇ | 149.39 ± 5.74ᵇ     | 51.17 ± 0.68ᵇ     |
| ALE      | 47.71 ± 1.44ᵃ | 19.39 ± 0.61ᵃ | 180.29 ± 3.92ᶜ     | 87.43 ± 3.07ᶜ     |
| Ascorbic acid | –              | –             | 3.05 ± 0.28ᵃ       | 2.51 ± 0.74ᵃ      |

The data is interpreted as mean ± SD for triplicate studies, and the ones in the same column accompanied by separate letters (a – d) are substantially different at p<0.05.
3.3. Antioxidant activity

DPPH and ABTS were the determinants of the antioxidant activity of dill leaves extracts. The antioxidant capacity of the extracts is expressed by the IC\textsubscript{50} value, at which the sample concentration inhibits 50\% of free radicals. The lower the amount of IC\textsubscript{50}, the higher the antioxidant function would be.

Figure 3. DPPH of different extracts from the leaves extracts of \textit{Anethum graveolens}. Ethanolic (A) and Water (B).

The DPPH ability of ethanolic extract and aqueous extract on the leaves of \textit{A. graveolens} is shown in Figure 3. The study surveyed the extract concentration range from 0 to 1000 μg/ml, then used Excel 2013 software to select the appropriate concentration range for linear paths. The scavenging ability of ELE and ALE was compared with the ascorbic acid (Table 2). The finding show that the ELE showed a better scavenging effect on DPPH radicals than ALE and the antioxidant activity depends on the concentration of the sample, the greater the concentration, the greater the antioxidant activity. The ELE showed antioxidant activity with IC\textsubscript{50} value of 149.39 ± 5.74 μg/ml while ALE exhibited an IC\textsubscript{50} value of 180.29 ± 3.92 μg/ml. The above results are consistent with the study of Selen et al. (2009) in which aqueous, and ethanolic Dill leaves extract displayed radical scavenging activity with IC\textsubscript{50} value of 193.39 ± 5,3 μg/ml and 475 ± 13.5 μg/mL, respectively [23].

Figure 4. ABTS of different extracts from the leaves extracts of \textit{Anethum graveolens}. Ethanolic (A) and Aqueous (B).

The ABTS scavenging ability of ELE and ALE is shown in Figure 4 and compared with ascorbic acid. The results showed that ELE and ALE were effective in scavenging ABTS radical in a concentration dependant manner. The ELE showed antioxidant activity with IC\textsubscript{50} value of 51.17 ± 0.68 μg/ml while AE illustrated an IC\textsubscript{50} value of 87.43 ± 3.07 μg/ml. The results shown that the antioxidant activity of dill leaves varies depending on the extraction solvent, and there is a link between the TPC of plant extracts and the antioxidant activity. This is consistent with previous studies on the Dill leaves...
that the extraction of strong antioxidant compounds in polar solvents [23-25]. 70% ethanol/water solvent was used for phenolic compounds derived from the Dill plants.

4. Conclusions
The ethanolic and water extracts from Dill leaves have antioxidant activity in which ELE gives better antioxidant effect than ALE. The results have identified dill leaves is a potential source of natural antioxidants with many natural compounds, including alkaloids, triterpenoids, flavonoids, tannins, saponins and coumarins. In particular, ELE has high phenolic and flavonoid content when extracted using 70% ethanol through quantitative results. The quantitative assessment revealed that the total ELE and ALE polyphenol content are 69.76 ± 1.57 and 47.71 ± 1.44 mgGAE/g dry extract, respectively. ELE had the strongest radical scavenging activity in DPPH (IC50 = 149.39 ± 5.74 μg/ml) and ABTS (IC50 = 87.43 ± 3.07 μg/ml). These results illustrated the potential for the use of dill as a source of natural antioxidant compounds to enhance cosmetic and biopharmaceutical applicability.

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