Phytochemical Content and Antioxidant activity in aqueous and ethanolic extracts of *Eryngium foetidum* L.

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**Abstract** *Eryngium foetidum* L. has been used as a spice for food and treatment for diabetes, rheumatism, anti-inflammatories, respiratory and stomach disorders. In Vietnam, due to high consumption of *Eryngium foetidum* L. in popular dishes and its significant benefits, the preliminary screening of the plant phytochemicals is required to extend its application and improve efficacy. The present research aimed at *Eryngium foetidum* L. extraction using aqueous and ethanolic as solvents to explore their phytochemical composition, total contents of flavonoids and phenolics and antioxidant activity. Phytochemical screening has detected the presence of flavonoids, tannins, alkaloids and terpenoids in both plant extracts. In addition, the total content of phenolics in both extract was equally significant while the total content of flavonoids was higher in ethanolic extract, as compared to the aqueous extract. The antioxidant actions of aqueous and ethanolic extracts were estimated using DPPH and ABTS scavenging assays. Results have shown that the former exhibited stronger DPPH scavenging activity than the latter. Findings from the present study suggested that ethanol and water can be useful for *Eryngium foetidum* L. extraction with high content of phytochemicals, phenolics and antioxidant activity.

1. **Introduction**

In the world, *Eryngium* genus consists of over 250 flowering plant species, which can be used as medicinal and edible plants [3]. *Eryngium foetidum* L. (*E. foetidum*) belongs to *Apiaceae* family which is widely planted in Vietnam. *E. foetidum* has long been used as a healthy food ingredient in Vietnam, West Indies and tropical regions, including Malaysia and Thailand. Beside its food application purposes, *E. foetidum* has been employed in traditional ethnomedicine to treat diabetes, rheumatism, several respiratory, inflammation and stomach disorders [1]. García et al [4] performed preliminary analysis of *E. foetidum* hexane extract and revealed the presence of various terpenes such as α-cholesterol,
phytochemicals such as carotenoids, ascorbates, tocopherols and phenols are strong antioxidants that play an important role in healthcare system [5] and pharmacology [6, 7]. The presence of flavonoids, saponins, tannins and some triterpenoids; yet no alkaloids in E. foetidum have been reported [2]. Therefore, E. foetidum can be considered as a major source of biologically active components with therapeutic activities such as antimutagenic, anticarcinogenic, anti-inflammatory and antioxidant properties [8-11].

According to Dalukdeniya et al (2017), methanol and chloroform extracts of E. foetidum have been shown to effectively inhibit proliferation of Listeria monocytogenes, Staphylococcus aureus and Streptococcus pneumoniae [12]. Meanwhile, the aqueous extract with a significant content of (E)-2-dodecenal (45.5%) and 2-dodecenoic acid (15.5%) showed a high inhibitory activity against Gram-negative bacteria such as Salmonella and Proteus mirabilis [12, 13] [14]. These major constituents were the subject of U.S patent applications as an effective treatments for burns, constipation, hypertension, asthma, stomach ache, infertility complications, diarrhea, worms, fungal and bacterial infections in human and other mammals [2]. Due to the significant role in pharmaceutical and food industries, in-depth studies on E. foetidum phytochemical profiles and antioxidant activity are on demand. The present study aimed to determine the phytochemical constituents, antioxidant actions and the total polyphenol and flavonoid contents of aqueous and ethanolic extracts of E. foetidum.

2. Materials and methods

2.1. Chemicals and equipment
Ethanol, methanol, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diaminonmonium salt (ABTS), 2,2’-diphenyl-1-picrylhydrayl (DPPH), Folin-Ciocalteu’s reagent and other general purpose laboratory chemicals were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). All the chemicals and reagents used in the study were of analytical grade.

2.2. Plant collection
E. foetidum was collected from a local market in Ho Chi Minh, Vietnam. After washing with distilled water, the plant was allowed to dry in an oven at 40°C and then grounded into fine powder that was passable through a 100 mm sieve.

2.3. Plant extraction
10g of plant powder was extracted using 300 mL ethanol and distilled water at 60°C for 1 h. Whatman No.1 filter paper was used to filter the obtained extract, which was then allowed to evaporate and dry at 40°C under reduced pressure. The obtained crude extracts were used for various analyses.

2.4. Phytochemical test

2.4.1. Detection of alkaloids. Crude extracts were dissolved in 1% HCl and filtered. Several tests for alkaloids presence such as Bouchardat’s test, Meyer’s test, Dragendorff’s test were then employed as previously described [1].

2.4.2. Detection of tannins. Crude extracts were dissolved in distilled water and placed in a bain-marie, then filtered and added with 5% FeCl3. Formation of dark blue or greenish black color indicated the presence of tannins.

2.4.3. Detection of anthraquinones. The ethanolic crude extracts were dissolved in chloroform, filtered and added with 1 ml of 10% NaOH. Appearance of pink precipitates indicated anthraquinones presence.

2.4.4. Detection of flavonoids. The content of flavonoids was measured using Willstatter’s test [15, 16] Crude extracts were dissolved in ethanol and filtered. Divide equally into 2 test tubes, added 0.05 g of magnesium powder and 1 ml HCl then heated for 5 min. the presence of flavanone, flavone, flavonol, and xanthone was indicated by appearance of orange, red or purple coloured precipitates while the presence of isoalloxazines, isoflavonoid, and auron was indicated by discoloration.
The calibration curve was constructed using a Vortex machine. Crude extracts were dissolved in acetic anhydride and added with 1 ml of chloroform. After filtering, 1 ml of H₂SO₄ was added. A reddish-brown ring separator was taken as positive result for terpenoids.

2.4.6. Detection of coumarins. Crude extracts were dissolved in ethanol and filtered. The obtained solution was divided equally into 2 test tubes, the first tube was added with 10% KOH and distilled water. These tubes were heated for 2 min, then cooled and observed under UV lamp at 365 nm of wavelength. The presence of coumarins was indicated by the first tube which fluoresced more strongly than the second one.

2.4.7. Detection of saponin. Crude extracts were dissolved in distilled water, heated and filtered. After being cooled down, the filtrate was filled with distilled water to 10 ml and vigorously shaken. Establishment of a stable persistent froth indicated a positive result for saponin presence.

2.4.8. Detection of reducing sugar. Crude extracts were dissolved in distilled water, boiled at 100°C for 5 min and filtered, then continuously added with Fehling’s solution A and B. The presence of reducing sugar was indicated by red precipitates formation.

2.5. Estimation of Total Phenolic Content (TPC)
The TPC in two different M. aquatica extracts was measured, following previous study by Pham et al [17]. The crude extracts were diluted to an appropriate concentration in methanol. Then, diluted sample was added with 10% Folin-Ciocalteu solution, homogenized using a Vortex machine and placed at room temperature for 5 min. Na₂CO₃ solution 7.5%, was added to the mixture, shaken well and further incubated in darkness for 1 h at room temperature. Absorbance measurement was at 765 nm using UV-Vis spectrophotometer (Cary 60, Agilent Technologies, Palo Alto, CA, USA) against a control which contained the reaction mixture with methanol instead of plant extract. Constructing a calibration curve involved the use of gallic acid solution prepared in methanol. TPC was expressed in milligrams of gallic acid equivalent per gram of extracted compounds (mgGAE / g DW).

2.6. Estimation of Total Flavonoid Content (TFC)
The TFC was conducted by the method of Mahboubi et al [18]. The crude extracts were diluted to an appropriate concentration in methanol and added with 10% AlCl₃, 1M CH₃COOK and distilled water. The mixture was shaken well and subjected to 30-min incubation at room temperature. Absorbance measurement was at 415 nm using UV-Vis spectrophotometer against a control sample, which contained the reaction mixture with methanol instead of plant extract. The calibration curve was constructed by using quercetin solution prepared in methanol. TFC was expressed in milligrams of quercetin equivalent per gram of extracted compounds (mgQE/ g DW).

2.7. Antioxidant Activity Assay

2.7.1. DPPH radicals scavenging activity. Estimating DPPH scavenging activity of E. foetidum extracts followed the modified method of Pham et al [17]. The prepared stock solution was added with methanol to give a working solution with absorbance value of 1.1 ± 0.02 at 517 nm. The working solution was mixed with plant ethanolic (0-4890 µg/ml) and aqueous (0-1360 µg/ml) extracts and the mixture was subjected to incubation without light at room temperature for 30 min. Absorbance measurement of the mixture was at 517 nm using UV-Vis spectrophotometer and methanol was used instead of plant extracts as a blank. Ascorbic acid (0-12.5 µg/ml) was used as a control. IC₅₀ values denoted the sample concentration required to remove DPPH free radicals by 50%. The percentage of DPPH inhibited by the samples was measured using the formula below:

\[ \text{% inhibition} = \frac{A_C - A_A}{A_C} \times 100 \]

Where \( A_A \) and \( A_C \) are the absorbance of samples (containing DPPH and plant extracts) and control (containing DPPH and methanol), respectively.
ABTS radicals scavenging activity

ABTS scavenging activity of E. foetidum aqueous and ethanolic extracts was evaluated following the modified method of Pham et al [17]. Stock solution which contained ABTS and K$_2$S$_2$O$_8$ was diluted with methanol to prepare working solution with absorbance value of 1.1 ± 0.02 at 734 nm. Different concentrations of the plant ethanolic (0-4890 µg/ml) and aqueous (0-3360 µg/ml) extracts were added to the working solution and subjected to incubation at room temperature for 30 min. The mixture absorbance was measured at 734 nm using UV-Vis spectrophotometer and methanol was used instead of plant extracts as a blank. Ascorbic acid (0-6.25µg/ml) was used as a control. IC$_{50}$ values denoted the sample concentration required to remove ABTS activity by 50%. The percentage of ABTS inhibited by the samples was measured using the formula below:

\[
\% \text{ inhibition} = \frac{A_C - A_A}{A_C} \times 100
\]

Where $A_C$ and $A_A$ are the absorbance of the control (containing ABTS and methanol) and samples (containing ABTS and plant extracts), respectively.

2.7.3. Statistical analysis. The experiments were performed in triplicates. All the data were analyzed by two-way ANOVA and represented as mean ± standard error (SE) using GraphPad Prism version 7.4 (GraphPad Software Inc., San Diego, CA, USA). $p < 0.05$ was considered as significant difference.

3. Results and discussion

3.1. Phytochemical screening

Previous studies have identified, isolated and analyzed the phytochemical profile and pharmacological activities of over 120 compounds from 23 Eryngium species, a majority of which are non-essential oil compounds such as triterpenoid, terpenoids, flavonoids, saponin, polyacetylenes, coumarins and steroids [19]. Phytochemical preliminary screening conducted on two E. foetidum extracts indicated that the consistent presence of these substances has been reported by previous studies. Table 1 showed similar positive result for tannins, flavonoids, terpenoids, reducing sugar and a minor quantity of alkaloids. In contrast, anthraquinones, saponins and coumarin were absent in both extracts. The extracts were subjected to examination of the total phenolic and flavonoid content.
3.2. Estimation of TPC and TFC
Phenolics are widely known as the largest phytochemical molecules in plants that exhibit antioxidant, antibacterial, anticancer, anti-inflammatory, and cardioprotective effects [20]. Determination of TPC was expressed in the form of GAE (the standard curve equation: \( y = 0.09206x + 0.03255, R^2 = 0.9981 \); whereas the TFC was expressed in the form of QE (the standard curve equation \( y = 0.00735x - 0.01790, R^2 = 0.9998 \)). The TPC and TFC of *E. foetidum* extracts were measured (Table 2). Results have shown that no significant difference was observed in the TPC of *E. foetidum* ethanolic and aqueous extracts (\( p > 0.05 \)). However, the TFC contained in ethanolic extract of *E. foetidum* was significantly higher than aqueous extract (\( p < 0.05 \)). Similar observation was reported by Do et al. (2014), possibly due to formation of nonphenolic and complex phenolic compounds which are more soluble in ethanol, as compared to water [30]. As the classes of phenolics and flavonoids are known for important bioactive activities [21], further studies are on high demand to exploit the potential content of TPC and TFC of *E. foetidum* extracts as well as other plant sources.

![Table 1: Phytochemical analysis of *E. foetidum* ethanolic and aqueous extracts. ‘+’ indicated presence and ‘−’ indicated absence of the compounds in the plant extracts.](image)

| Name of compound | Ethanol extract | Aqueous extract |
|------------------|----------------|----------------|
| Alkaloid         | +              | +              |
| Tannin           | +              | +              |
| Anthraquinone    | −              | −              |
| Flavonoid        | +              | +              |
| Terpenoid        | +              | +              |
| Coumarin         | −              | −              |
| Saponin          | −              | −              |
| Reducing sugar   | +              | +              |

3.3. Antioxidant activities
The antioxidant activity of the crude *E. foetidum* extracts was quantified by the percentage of scavenged ABTS and DPPH free radicals. Overall, as shown in Figure 2 and Figure 3, DPPH and ABTS scavenging activities of both aqueous and ethanolic extracts were improved as the sample concentration increased. Likewise, the result of IC\(_{50}\) values presented in Table 3 was also in close agreement. Since the antioxidant activity is inversely rational to IC\(_{50}\), a lower IC\(_{50}\) value represented a higher antioxidant potential and *vice versa*. In DPPH and ABTS assays, ascorbic acid showed strong antioxidant activities with IC\(_{50} = 5.33 \pm 0.28 \mu g/ml\) and IC\(_{50} = 2.33 \pm 0.10\), respectively. The antioxidant activities of ascorbic acid showed a clear difference from both extracts. In DPPH assay, the aqueous extract had lower IC\(_{50}\)


In contrast, both *E. foetidum* extracts showed no clear difference in ABTS scavenging activity, even at high concentrations that was mentioned above [23, 24]. In comparison with DPPH and ABTS scavenging activities of other plants such as *Eryngium palmatum* (IC$_{50}$ = 0.6 ± 0.0 µg/mL and IC$_{50}$ = 0.7 ± 0.0 µg/mL, respectively) and *Eucalyptus camaldulensis* (IC$_{50}$ = 10.52 ± 0.14 µg/mL and IC$_{50}$ = 9.86 ± 0.17 µg/mL, respectively), the antioxidant activity of *E. foetidum* was relatively low [25,29]. Therefore, further studies in optimizing the extraction process are required to obtain the extracts with improved antioxidant activity.

Table 3: IC$_{50}$ values of DPPH and ABTS scavenging activities of *E. foetidum* extracts. The experiment was repeated three times and data was represent as mean ± SE. The superscripted values (i.e. a and b) indicated significant difference at $p<0.05$ as compared to the control.

| Plant extract   | DPPH (µg/ml)         | ABTS (µg/ml)        |
|-----------------|-----------------------|---------------------|
| Ethanol extract | 6222.83 ± 95.33$^a$   | 6761.35 ± 141.64$^a$|
| Aqueous extract | 646.58 ± 20.58$^b$    | 5906.82 ± 286.61$^a$|
| Ascorbic acid   | 5.33 ± 0.28$^c$       | 2.33 ± 0.10$^b$     |

Figure 2: DPPH scavenging activities of (A) ethanolic and (B) aqueous extracts of *E. foetidum* as compared to (C) control.
Figure 3: ABTS scavenging activities of (A) ethanolic and (B) aqueous extracts of E. foetidum as compared to (C) control.

4. Conclusion
In the present study, E. foetidum leaf extracts have been demonstrated to have high flavonoid content and potential antioxidant activity. Particularly, the aqueous extract of E. foetidum exhibited a higher level of DPPH scavenging activity. The application of aqueous extract of E. foetidum for traditional medicine is rational because of its antioxidant properties standpoint. These findings provide a useful insight to extend the applications of E. foetidum for developing nutritious food products and additives or medication with improved antioxidant properties. In a long term, optimization work on extraction and tracking methods for absorption, circulation, as well as in vivo efficacy upon consuming the plant phytochemicals can be further investigated [27].

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