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Influence of extraction parameters on total phenolic contents, flavonoids and antioxidant capacity of extract from *Eryngium foetidum* leaves

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Eryngiumfoetidum leaf has unique spicy aroma and flavor popularly used in culinary of soup noodle. It's a great source of minerals, vitamins and essential oils. In this research, we investigated the influence of different extraction variables such as ethanol concentration (30, 40, 50, 60, 70%), temperature (40, 45, 50, 55, 60^{o}C), shaking duration (15, 20, 25, 30, 35 minutes), particle size (0.2, 0.4, 0.6, 0.8, 1.0 cm), solid-liquid ratio (1:10, 1:15, 1:20, 1:25, 1:30 g/mL) to the total phenolic, flavonoid, and antioxidant capacity of *Eryngiumfoetidum*extract. Results clearly revealed that 60% ethanol extraction, temperature 50^{o}C, shaking duration 30 minutes, particle size 0.4 cm, solid-liquid ratio 1:25 g/mL gave the highest total phenolic, flavonoid, and antioxidant capacity. These data gave very important finding to expoit the best phytochemical constituents inside this valuable herb.

Keywords: ***Eryngiumfoetidum, total phenolic, flavonoid, antioxidant, ethanol, shaking duration, particle size, solid-liquid ratio***

INTRODUCTION

*Eryngiumfoetidum*L.is a tropical perennial and annual herb. Its leaf has unique flavor and aroma with toothed margins grown in a basal rosette pattern. It has been used as ornamental, vegetable, culinary, medicine with various health benefits such as flu, pneumonia, diabete, constipation, fever, vomiting, diarrhoea, arthritic, anti-convulsant, anthelmintic, anti-inflammatory, analgesic, antimalarial, antibacterial, hypertension rheumatism, asthma, eye disease, poisoning, venereal disease (García et al., 1999; Zhang et al., 2008; Ekpong et al. 2006; Khoshbakht et al., 2006; Paula et al., 2011; Mohammad et al., 2012). It could be considered as a great source of calcium, iron, carotene, riboflavin, proteins and vitamins A, B, and C and essential oils in the aerial (Wong et al., 1994; Cardozo et al., 2004; Chowdhury et al., 2007; Banout et al., 2010; Aly et al., 2010).

Extraction of antioxidant components from spices and herbs was strongly influenced by major parameters such as kind of solvent, temperature, solid-liquid ratio, shaking duration, particle size (Ana et al., 2009). Solubility of phytochemical constituents and their diffusion to solvent was based on their chemical structure differing from simple to complex compounds; The suitable solvent for extracting target components should be selected carefully because the extracted constituents will be relied on kind of solvents introduced (Zarnowski and Suzuki, 2004). The most available solvents with different efficiency in extraction of phenolics from spices and herbs are methanol, ethanol, propanol, acetone, ethyl acetate, dimethylformamide (Ana et al., 2009). Ethanol is commonly selected for different implementations compared to other solvents due to its safety and affordability (Hemwimon et al., 2007; Guo et al., 2001).

Solvent concentration gradient within the plant matrix creates a driving force for the extraction process whereby individual component develops equilibrium between solvent and the plant tissues (Dahmoune et al. 2015). Kaushal et al. (2014) investigated several solvents such as petroleum ether, ethyl acetate, aqueous for extraction of antimicrobial activity, antioxidant activity and the total phenolic content of *Eryngiumfoetidum* L. The aqueous extract was found to exhibit the highest phytochemical constituents extracted. Objective of our study focused on different extraction variables such as ethanol concentration, temperature, shaking duration, particle size, solid-liquid ratio affecting to the total phenolic, flavonoid, and antioxidant capacity of *Eryngiumfoetidum*extract.

MATERIALS AND METHODS

Material

Eryngiumfoetidum leaveswere collected in local farm of SocTrang province, Vietnam. After collecting, they must be quickly conveyed to laboratory for experiments. They were washed in running tap water to remove foreign matters. Chemicals and reagents such as ethanol, FolinCiocalteu reagent, sodium carbonate, gallic acid, rutin were all analytical grade and purchased from Merk or Sigma-Aldrich.

Researching method

Eryngiumfoetidum leaveswere blanched in hot water in 5 seconds, dried on tray drier at 45^{o}C for 14 hours. The dried leaves were cut into different particle size (0.2, 0.4, 0.6, 0.8, 1.0 cm). 5 gram of these pieces was put into Erlenmeyer flask. Then ethanol solvent with different concentration (30, 40, 50, 60, 70%) was added, mixed in a shaking incubator under different temperature (40, 45, 50, 55, 60^{o}C) in different shaking duration (15, 20, 25, 30, 35 minutes) at 250 rpm with different solid-liquid ratio (1:10, 1:15, 1:20, 1:25, 1:30 g/mL). The mixture was centrifuged at 4000 rpm for 10 minutes to collect the supernatant. Replicate more times to completely extract the phytochemical components. The supernatant extract was then analyzed to determine total phenolic (mg GAE/g) , flavonoid (mg QE/g) and DPPH (%) radical scavenging assay.

Phytochemical determination

Total phenolic content (mg GAE/g) was evaluated using Folin–Ciocalteu assay (NizarSirag et al., 2014). Total flavonoid content (mg QE/g) was avaluated by the aluminium calorimetric method (Formagio et al., 2015).The antioxidant activity was evaluated using DPPH (%) radical scavenging assay which was described by Huang et al. (2005).

2.4 Statistical analysis

The experiments were run in triplicate with three different lots of samples. The data were presented as mean±standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTSAND DISCUSSION

Effect of ethanol concentration to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngiumfoetidum*leaf

Solvent extraction by using ethanol is a usual way to achieve bioactive components from spices and herbs. Solvent polarity plays a key role in intensity of phenol solubility. Selecting a suitable solvent is very necessary in extraction of functional components. Methanol and ethanol have different polarities to extract different phytochemical compounds due to their chemical characteristics in various matrices (Boeing et al., 2014). Methanol is more efficient in extraction of lower molecular weight phenolics, while acetone is suitable for extraction of higher molecular weight ones (Dai and Mumper, 2010). Methanol is much more efficiency to ethanol. However, ethanol is highly prefered owing to its bioavailability, low toxicity and high extraction yield (Franco et al., 2008) In our research, we examined the effect of different ethanol-to-water ratio (30, 40, 50, 60, 70%) to total phenolic, flavonoid, DPPH extracted from *Eryngiumfoetidum*leaf. Results revealed that there was no significant difference at 60% and 70% ethanol-to-water in respect of phytochemical constituents extracted (table 1). Therefore we selected 60% ethanol for next experiment.For flavonoid extraction from tea, ethanol showed higher efficiency than methanol and acetone (Wang and Helliwell, 2001). The extraction by water as solvent helped to extract hydrophilic antioxidants (Aziz et al., 2003). In one report, total phenolic efficiency increased by ethanol from 10% to 30% and remained constant from 30% to 60% (Giorgiaet al., 2007).

Table 1: Effect of ethanol concentration (%) to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngium foetidum* leaf

Ethanol (%)	30	40	50	60	70
Total phenolic (mg GAE/100 g)	41.32±0.01^{b}	42.59±0.03^{ab}	43.75±0.01^{ab}	45.01±0.02^{a}	45.09±0.03^{a}
Flavonoid (mg QE/100 g)	9.83±0.00^{c}	10.58±0.02^{bc}	10.96±0.00^{b}	12.07±0.01^{ab}	12.52±0.00^{a}
DPPH (%)	31.09±0.01^{c}	31.52±0.00^{bc}	31.91±0.02^{b}	32.54±0.00^{ab}	32.79±0.02^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Chew et al. (2011) examined parameters for phenolic extraction of *Orthosiphonstamineus* at 40% ethanol in 120 min at 65^{o}C. Methanol, ethanol, and acetone in various concentrations (50%, 75%, and 100%) were applied in the extraction of L. *aromatica* (Quy et al., 2014). Butsat and Siriamornpun (2016) proved that high amount of phenolics and antioxidant activity would be achieved by 80% methanol for 12h in extraction. Thom et al. (2018) demonstrated significant differences among total phenolic and total flavonoid obtaining by 80% methanol compared to other solvents. Thamizhiniyan et al. (2019) showed that the extracts obtained from *Pinusdensiflora* bark by 40% ethanol had signifcant antioxidant potential and total phenolic content. Nguyen et al. (2019) proved that methanol 40% was suitable for extraction of the antioxidant compounds from tamarind seeds. Nhu et al. (2019) showed the highest total phenolic content and antioxidant capacity of the extract from Thai basil was achieved by using 75% methanol as solvent. Alara et al. (2020) examined the extraction of total phenolic content, total flavonoid content, and antioxidants from *Vernoniaamygdalina* leaf with ethanol concentration (40–80%). These functional components were significant influenced by ethanol concentration.

Effect of extraction temperature to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngiumfoetidum*leaf

Result clearly shown in table 2 revealed that extraction temperature of 50^{o}C gave the best total phenolic, flavonoid, and antioxidant capacity which significantly different from other extraction temperatures. The phytochemical constituents increased with increasing temperature from 40^{o}C to 50^{o}C. However at higher temperature, these thermal sensitive elements could be degraded significantly. Higher temperature caused the rupture of phenolics and affected the membrane structure of the plant matrix making them less active (Chew et al., 2011). Ana et al. (2009) examined the influence of solvent and extraction temperature on phenolic extraction from grape seed. The best results were noticed at 50% ethanol at 80^{o}C. Elena et al. (2019) showed that 50% ethanol at 35◦C were appropriated for extraction of phenolics from tiger nuts by-products. Intan et al. (2017) proposed an extraction at 60 °C in 120 min by a water:ethanol solvent ratio of 90:10 v/v% on the extraction of phenolics and the anti-radical activity of *Clinacanthusnutans* Lindau leaves. Jahangiri et al. (2011) showed ethanol 80% at 80◦C to extract the total phenolics from *Ficuscarica*leaf. Applying high temperature above 65^{o}C might decompose the phenolics in the extract (Sólym et al., 2014).Nguyen et al. (2019) proved the highest total phenolic value was achieved by extraction in methanol 40% at 40^{o}C. DPPH were maximum by methanol 40% at 30^{o}C. Nhu et al. (2019) showed the highest total phenolic content and antioxidant capacity of the extract from Thai basil was achieved at the temperature of 55^{o}C. Alara et al. (2020) examined the extraction of total phenolic content, total flavonoid content, and antioxidants from *Vernoniaamygdalina* leaf with temperature (60–80^{o}C). These functional components were insignificant influenced by extraction temperature.

Effect of shaking duration to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngiumfoetidum*leaf

Result clearly shown in table 3 revealed that shaking duration of 30 minutes gave the best total phenolic, flavonoid, and antioxidant capacity which significantly different from other shaking conditions. It can be explained that the prolonged shaking duration over 30 minutes may cause bioactive degradation (Hithamani and Ramalakshmi, 2013).

Table 2: Effect of extraction temperature (ºC) to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngium foetidum* leaf

Extraction temperature (ºC)	40	45	50	55	60
Total phenolic (mg GAE/100 g)	45.01±0.02^{c}	46.23±0.02^{bc}	47.89±0.00^{a}	47.15±0.03^{ab}	46.89±0.01^{b}
Flavonoid (mg QE/100 g)	12.07±0.01^{c}	12.75±0.01^{bc}	14.51±0.02^{a}	14.08±0.02^{ab}	13.62±0.03^{b}
DPPH (%)	32.54±0.00^{c}	33.01±0.03^{bc}	34.62±0.01^{a}	34.03±0.00^{ab}	33.49±0.01^{b}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 3: Effect of shaking duration (minutes) to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngium foetidum* leaf

Shaking duration (minutes)	15	20	25	30	35
Total phenolic (mg GAE/100 g)	47.15±0.03^{b}	47.29±0.00^{ab}	47.53±0.02^{ab}	47.86±0.00^{a}	45.91±0.02^{c}
Flavonoid (mg QE/100 g)	14.08±0.02^{b}	14.23±0.02^{ab}	14.45±0.01^{ab}	14.78±0.01^{a}	12.93±0.01^{c}
DPPH (%)	34.03±0.00^{b}	34.35±0.01^{ab}	34.69±0.03^{ab}	34.95±0.02^{a}	33.16±0.00^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Table 4: Effect of particle size (cm) to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngium foetidum* leaf

Particle size (cm)	0.2	0.4	0.6	0.8	1.0
Total phenolic (mg GAE/100 g)	47.86±0.00^{a}	47.80±0.02^{a}	47.31±0.00^{ab}	47.04±0.02^{b}	43.62±0.03^{c}
Flavonoid (mg QE/100 g)	14.78±0.01^{a}	14.69±0.03^{a}	14.23±0.03^{ab}	14.05±0.00^{ab}	13.87±0.02^{b}
DPPH (%)	34.95±0.02^{a}	34.68±0.00^{ab}	34.31±0.01^{b}	34.05±0.00^{bc}	33.77±0.01^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 5: Effect of solid: liquid ratio to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngium foetidum* leaf

Solid: liquid ratio (g/mL)	1:10	1:15	1:20	1:25	1:30
Total phenolic (mg GAE/100 g)	47.80±0.02^{b}	47.98±0.00^{ab}	48.15±0.03^{ab}	48.57±0.01^{a}	48.60±0.00^{a}
Flavonoid (mg QE/100 g)	14.69±0.03^{c}	14.91±0.01^{bc}	15.35±0.02^{b}	15.76±0.01^{ab}	15.97±0.02^{a}
DPPH (%)	34.68±0.00^{c}	35.03±0.02^{bc}	35.77±0.00^{b}	36.01±0.03^{ab}	36.64±0.03^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Nhu et al. (2019) showed the highest total phenolic content and antioxidant capacity of the extract from Thai basil was achieved by shaking duration of 35 min.

Effect of particle size to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngiumfoetidum*leaf

Result clearly shown in table 4 revealed that particle size of 0.4 cm gave the best total phenolic, flavonoid, and antioxidant capacity which significantly different from other particle sizes. The smaller the particle size of *Eryngiumfoetidum*dried leaf, the higher the extraction efficiency and the higher the phenolics, flavonoids and antioxidant. The phytochemical constituents decreased with increasing particle size. It could be explained from an increase in the surface area available for molecular transport contributing to a more extensive mass transfer of solutes between phases (Maria et al., 2009). When the particle size was reduced, the accessible surface was increased, resulting in the observed enhancement of the extraction efficiency (Norra et al., 2016). In one report, the effect of particle size on the antioxidant characteristics of brown seaweed was examined. Dried samples with particle size of 0.25 mm in hot water extraction showed the highest total phenolic content and DPPH radical scavenging activity (Norra et al., 2016).

Effect of particle size to total phenolic, flavonoid and antioxidant capacity of extract from *Eryngiumfoetidum*leaf

Result clearly shown in table 4 revealed that particle size of 0.4 cm gave the best total phenolic, flavonoid, and antioxidant capacity which significantly different from other particle sizes. The smaller the particle size of *Eryngiumfoetidum*dried leaf, the higher the extraction efficiency and the higher the phenolics, flavonoids and antioxidant. The phytochemical constituents decreased with increasing particle size. It could be explained from an increase in the surface area available for molecular transport contributing to a more extensive mass transfer of solutes between phases (Maria et al., 2009). When the particle size was reduced, the accessible surface was increased, resulting in the observed enhancement of the extraction efficiency (Norra et al., 2016). In one report, the effect of particle size on the antioxidant characteristics of brown seaweed was examined. Dried samples with particle size of 0.25 mm in hot water extraction showed the highest total phenolic content and DPPH radical scavenging activity (Norra et al., 2016).

Effect of solid-liquid ratio to total phenolic, flavonoid and antioxidant capacity of extract from

Result clearly shown in table 5 revealed that solid-liquid ratio of 1:25 gave the best total phenolic, flavonoid, and antioxidant capacity from extract of *Eryngiumfoetidum*leaf which significantly different from other ones. Higher solid-liquid ratio required more energy and time (Pan et al., 2003). It could be explained by the mass transfer mechanism. The concentration gradient between the solid and liquid created the concentration variation between the interior cell tissue and the exterior solvent (Nguyen and Dang, 2017). Nhu et al. (2019) showed the highest total phenolic content and antioxidant capacity of the extract from Thai basil was achieved by using 1/100 (w/w) sample-solvent-ratio. Alara et al. (2020) examined the extraction of total phenolic content, total flavonoid content, and antioxidants from *Vernoniaamygdalina* leaf with feed-to-solvent ratio (1:8–1:12 g/ml). These functional components were significant influenced by feed-to-solvent ratio.

CONCLUSION

*Eryngiumfoetidum*L.was a very important herb in daily consumption. It has numerous pharmacological activities beneficial for human health. This study investigated the effect of different extraction parameters such as ethanol concentration, temperature, shaking duration, particle size, solid-liquid ratio to the total phenolic, flavonoid, and antioxidant capacity of *Eryngiumfoetidum*extract. It's concluded that these variables had significant influence to stability of phytochemical constituents during solvent extraction.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Minh Phuoc Nguyen arranged the experiments and also wrote the manuscript.

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