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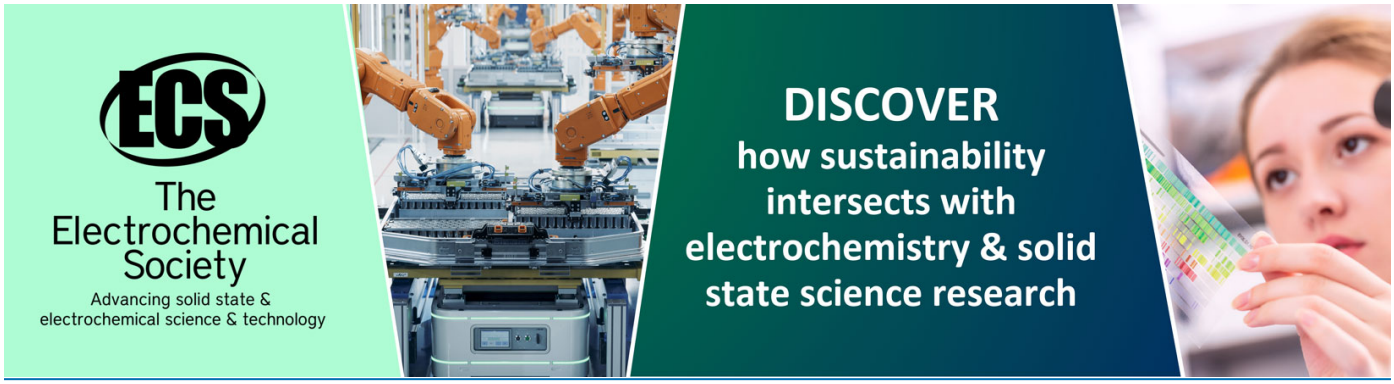

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Effect of extraction solvent on total polyphenol content, total flavonoid content, and antioxidant activity of soursop seeds (*Annona muricata* L.)

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Abstract: Soursop (*Annona muricata* L.), a plant native to South America, is widely distributed in tropical and subtropical regions of the world. In this study, extract of *A. Muricata* was obtained using various solvents including distilled water, ethanol, ethyl acetate, and chloroform. Total polyphenol content, total flavonoid content and the antioxidant activity of *A. muricata* seed extracts were investigated using various in vitro assays. The highest extraction yield (23.60%) was obtained by using chloroform. The extract obtained by ethanol showed the highest total polyphenol content (282.71 ± 8.64 mg GAE/100g DW) and the highest flavonoid content (86.57 ± 3.20 mg QE/100g DW). The same extract also exhibited the highest DPPH (341.57 ± 6.90 AAE/100g DW), ABTS (382.20 ± 9.71 mg AAE/100g DW) radical scavenging activity and FRAP (369.84 ± 7.96 mg AAE/100g DW). These results indicate that *A. muricata* can be used in dietary applications with the potential to reduce oxidative stress.

1. Introduction

In recent years, using medicinal plants has been receiving a great deal of public attention in clinical and therapeutic potential [1-8]. Soursop (*Annona muricata* L.) belong to the Annonaceae family which cultivated mainly in tropical and subtropical countries [9]. *A. muricata* has been extensively described in the scientific literature as possessing biological properties such as antioxidant, anti-inflammatory, antidiabetic, anti-bacterial, anti-convulsivant, antiviral and diuretic properties [10]. The main group of secondary metabolites in *A. muricata* is acetonegins, terpenoids, coumarins, alkaloids [11].

The antioxidant activity of multiple plants plays an essential role in different fields due to potential health benefits. The previous study demonstrated that consumption of natural antioxidant, like phenolics, may help decrease the risk of degenerative diseases such as cancer and cardiovascular

diseases [12]. Moreover, antioxidants play an essential role in the human protection body against free radical disorders acting as radical scavengers. Polyphenols can reduce and prevent damage to the human body due to free radicals promote. Flavonoids can produce mechanisms that may inhibit invasion and kill tumor cells. This research has chosen the seeds for extractions due to harvested easily without killing the plants. Plant extracts can be applied in different fields such as pharmaceuticals, cosmetic products, and dietary supplements [13].

The extraction process is widely known as a process of extracting substances with plant biological activity from raw materials. There are different techniques to obtain antioxidants from plants including microwave-assisted extraction, soxhlet extraction, maceration. However, extraction yield depends not only the extraction method but also the many other extract conditions [14]. Many different solvent systems were used to extract polyphenols from plant materials. Both extraction efficiency and extraction activity depend significantly on solvents [15]. The antioxidant capacity of phenolic compounds is strongly influenced by the polarity of the solvent used for extraction. Therefore, the selection of solvents is essential to plant material samples. The extraction solvent system is normally selected according to the purpose of extraction, polarity capacity of target components, polarity of unwanted components, total cost, safety and environmental issues [16]. Many previous studies have been carried out to evaluate the antioxidant activity from leaves of *A. muricata*, but the researches on seeds of this plant are very limited. For these reasons, in this study, the distilled water, ethanol, ethyl acetate and chloroform are the solvents with different polarities selected to carry out the experiments. The extracts obtained from *A. muricata* seeds shall be used to determine the total phenolic and flavonoid contents and evaluate the antioxidant activity in three models of DPPH, ABTS and FRAP to select suitable solvents for the extraction process and propose nutritional products made from soursop seeds which are applicable in the processing industry.

2. Materials and methods

2.1. Plant Material

Annona muricata L. seeds were collected from Tan Phu Dong district, Tra Vinh province, Vietnam in January 2019. The seeds were washed with water and dried to moisture contents of 10%. Then, the seeds were ground to powder and keep at -20°C.

2.2. Extraction procedure

The classic extraction method is chosen to find the best extraction solvent, four different experiments were set. In each experiment, 5.0 g of sample powder was extracted with 100mL of the desired solvent, included distilled water, ethanol, ethyl acetate and chloroform. Extraction was performed at room temperature (RT) for 24 h.

2.3. Total polyphenol content (TPC)

Based on the method of Spiridon et al., total polyphenol content was determined [17]. First, the 0.5 mL extract was pipetted into a test tube containing 2.5 mL Folin-Ciocalteu reagent 10% (v/v). After 5 minutes, 2 mL Na_2CO_3 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 30 minutes in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in mg gallic acid equivalents per 100 g of dried weight (mg GAE/100g DW).

2.4. Total flavonoid content (TFC)

Based on the aluminum chloride colorimetric method, the total flavonoid content was determined [18]. Mixing 0.5 mL the extract with 0.1 mL 10% $AlCl_3$. Then, 0.1mL 1M CH_3COOK and 4.3 mL distilled water was added and vigorously shaken. The absorbance was spectrophotometrically measured at 415 nm and the results were shown in mg quercetin equivalents per 100 g of dried weight (mg QE/100g DW).

2.5. DPPH Scavenging Activity

Based on Vuong et al., the antioxidant activity of the *A. muricata* seed extracts was tested using DPPH assay (Analytical chemistry laboratory - University Nguyen Tat Thanh) [17]. 1.5mL DPPH ($OD_{517\ nm}$ = 1.1 ± 0.02) into 0.5 mL solution sample. The sample solution with pre-concentration and the mixed the stable at room temperature in the dark within 37 min. The optical measurement of the mixture by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. Blank sample, but 0.5 mL solution replaced EtOH 99.7%. Standard sample: Vitamin C (0.01g ± 0.01) was dissolved EtOH 99.7% into volume flask 100mL, in the dark (C = 100 µg/mL). The DPPH scavenging activity was expressed as mg ascorbic acid equivalents (AAE) per 100 g of dried weight (mg AAE/100g DW).

2.5.1. ABTS Scavenging Activity

Based on Thaipong et al., ABTS scavenging activity was used [19]. First, adding 10 mL of 2.6 mM $K_2S_2O_8$ in 10 mL of 7.4 mM ABTS solution in 24 hours. Next, preparing the working solutions by putting 1ml of stock solution into 60 mL of methanol ($OD_{734\ nm}$ = 1.1 ± 0.02). Then, 0.5 mL of sample added with 1.5 mL of the working solution for 30 minutes RT. Using UV-VIS spectrophotometer measured the mixture at 734 nm. The ABTS scavenging activity was expressed as mg ascorbic acid equivalents per 100 g of dried weight (mg AAE/100g DW).

2.6. Ferric Reducing Antioxidant Power (FRAP)

FRAP was estimated as described by Vuong et al. [17]. First, FRAP solution is made by mixing 20 mM $FeCl_3$, 10 mM TPTZ in 40 mM HCl and 300 mM acetate buffer. Second, add 0.5ml extraction sample into a 1.5 mL FRAP solution at 30 min at RT. Then, using UV-spectrophotometer was estimated at 593 nm. The results were shown as mg ascorbic acid equivalents per 100 g of dry weight (mg AAE/100g DW).

2.7. Statistical Analyses

The data were analyzed by one-way ANOVA followed by using Fisher's Least Significant Difference (LSD) test using Statgraphics Centurion XV Version 15.0. Differences were considered statistically significant at $P < 0.05$ for all tests.

3. Results and discussion

3.1. *Effects of solvent type on extraction yield*

There are different factors which affected to extraction efficiency including sample particle size, extraction method used and so on. The yield of extraction reliant on the solvent with varying polarity, extraction time, pH and so on [20]. In this studied, *A. muricata* seeds extracts were obtained by using distilled water, ethanol, ethyl acetate and chloroform. Table 1 shows the percentage yield of various extractions. The highest extraction yield was obtained by using chloroform (23.60%). The percentage oils of extraction declined in the following order: chloroform (20.85%) > ethyl acetate (20.01%) > ethanol (16.7%) > distilled water (<0.5%). This reawsult confirms that oils rises relevant to the decreasing polarity of the solvent extraction. Therefore, *A. muricata* seed is rich in less polar compounds which are mainly oils.

Table 1. Percentage yields and oils of different extracts from the seed of A. muricate

Solvent of extraction	Percentage yield of extraction	Percentage oils of extraction
Distilled water	21.60	< 0.5
Ethanol	21.90	16.70
Ethyl acetate	23.27	20.01
Chloroform	23.60	20.85

3.2. Effects of solvent type on TPC and TFC

The TPC and TFC values of the *A. muricata* seed extracts are shown in figure 1. The TPC values of the extracts range from 61.15 ± 4.65 mg GAE/100g DW to 282.71 ± 8.64 mg GAE/100g DW. The TFC values of the extracts range from 13.08 ± 1.45 mg QE/100g DW to 86.57 ± 3.20 mg QE/100g DW. As can be seen from figure 1, ethanol extraction achieved the highest of TPC and TFC. TPC and TFC was declined in the following order: ethanol, chloroform, ethyl acetat and distilled water. Therefore, the majority of phenolic compounds in *A. muricata* seeds have a moderate to low polarity. Moreover, the content of nonphenol compounds in water extracts higher than other extracts.

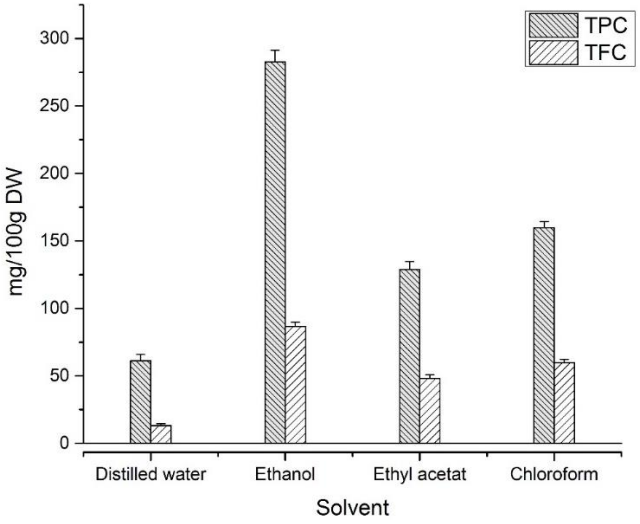

Figure 1. Effect of the solvent nature on TPC and TFC of *A. muricata* seeds.

3.3. *The effects of different solvent sort on antioxidant activity*

Phenolics are the main antioxidant component in plants, and their total content is often equivalent to their antioxidant activity [21]. In this studied, the effect of the extraction solvent on antioxidant capacity from *A. muricata* seed also tends to be related to the TPC and TFC. Table 2 the effect of different extraction on the antioxidant activity. The values of DPPH, ABTS scavenging, FRAP range from 86.14 ± 8.83 mg AAE/100g DW to 341.57 ± 6.90 AAE/100g DW, 114.05 ± 8.41 mg AAE/100g DW to 382.20 ± 9.71 mg AAE/100g DW and 93.91 ± 9.71 mg AAE/100g DW to 369.84 ± 7.96 mg AAE/100g DW respectively. Highest and lowest activity was exerted, respectively, in ethanol and distilled water extract of *A. muricata*. Many studies have demonstrated polyphenol solubility in extraction solvent depending on the polarity of the solution, in which ethanol is one of the most suitable solution and widely used to extract polyphenols from plants [22].

Table 2. The effects of different solvent sort on antioxidant activity

	Antioxidant capacity		
Solvent of extraction	DPPH (mg AAE/100g DW)	ABTS (mg AAE/100g DW)	FRAP (mg AAE/100g DW)
Distilled water	86.14 ± 8.83^{a}	114.05 ± 8.41^{a}	93.91 ± 9.71^{a}
Ethanol	341.57 ± 6.90^{d}	382.20 ± 9.71^{c}	369.84 ± 7.96^{d}
Ethyl acetate	102.39 ± 7.42^{b}	135.60 ± 5.44^{b}	100.27 ± 9.71^{b}
Chloroform	126.76 ± 8.48^{c}	147.26 ± 10.3^{b}	129.94 ± 14.02^{c}

Values are means of three replications ± standard deviations (SD).
In the same column, means followed by different letters are significant different ($p < 0.05$)

3.4. Correlation of TPC and TFC with the antioxidant capacity

Figure 2 illustrates the correlation between TPC and TFC with DPPH, FRAP, and ABTS. The correlation coefficient values (R^2) was highest between TPC and DPPH activity ($R^2 = 0.8945$) than that of TPC and ABTS activity ($R^2 = 0.8816$) followed by TPC and FRAP activity ($R^2 = 0.8723$). The correlation coefficient values of TFC with total antioxidant capacity was highest ($R^2 = 0.7089$) between TPC and DPPH activity than that of TPC and ABTS activity ($R^2 = 0.6920$) followed by TPC and FRAP activity ($R^2 = 0.6768$).

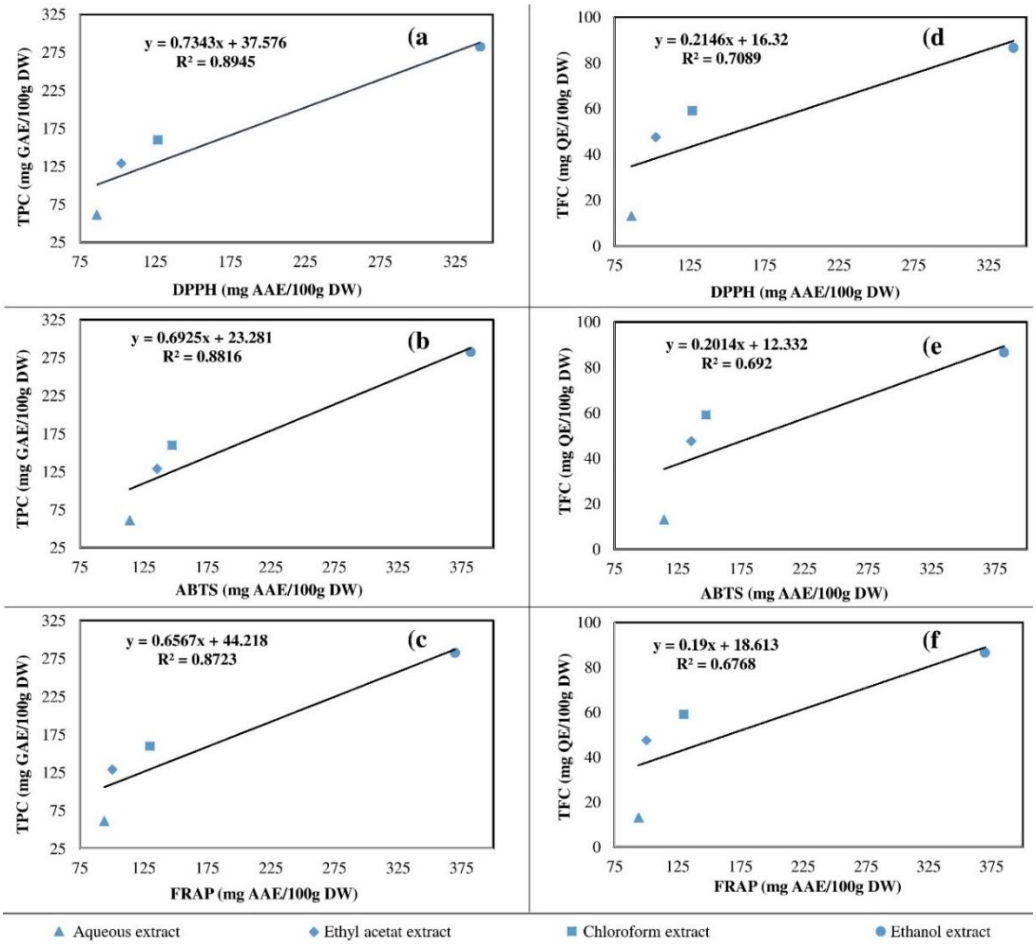

Figure 2. Linear correlations for the extracts between: (a) TPC and DPPH, (b) TPC and ABTS, (c) TPC and FRAP, (d) TFC and DPPH, (e) TFC and ABTS, and (f) TFC and FRAP.

Reports have shown that there is a positive correlation between total phenolic contents and antioxidant activity of plant extracts [23, 24]. Natural phenolic and flavonoid compounds are secondary plant metabolites that hold an aromatic ring bearing at least one hydroxyl group. Phenolic compounds are excellent electron donors because their hydroxyl groups can directly contribute to antioxidant action. Furthermore, some of them stimulate the synthesis of endogenous antioxidant molecules in the cell. According to multiple reports in the literature, phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems [25]. Linear regression analysis results demonstrate that antioxidant activity was positively correlated with TPC and TFC in *A. muricata* seeds extracts. TPC was highly correlated with TFC. The high correlations illustrate the function of phenolic compounds as the principal contributor to the antioxidant activities of the *A. muricata* seed extracts.

4. Conclusions

This study aimed to determine the TPC, TFC, and antioxidant activity of *A. muricata*. Antioxidant activity was performed via ABTS and DPPH radical scavenging assays. The phytochemical was extracted separately with distilled water, ethanol, ethyl acetate, and chloroform. The results show that the highest extraction yield was obtained by using chloroform (23.60%). This present study was also aimed at determining TPC and TFC. The TPC of ethanol extract of *A. muricata* measured by Folin-Ciocalteau reagent in terms of gallic acid equivalent was 282.71 ± 8.64 mgGAE/g. TFC of the plant sample as quercetin equivalent was 86.57 ± 3.20 mg QE/g). The same extract also exhibited the highest DPPH (341.57 ± 6.90 AAE/100g DW), ABTS (382.20 ± 9.71 mg AAE/100g DW) radical scavenging activity and FRAP (369.84 ± 7.96 mg AAE/100g DW).

Acknowledgments

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