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Research Article

Antioxidant activity of polyphenol compounds extracted from *Nypa fruticans* Wurmb. (Nipa palm) fruit husk with different ethanol concentration

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Abstract: Oxidative stress is a condition characterized by a higher content of free radicals than the potential antioxidants in the body. Exogenous antioxidants are needed to resolve this condition. The *Nypa fruticans* (Nipa palm) fruit husk is a source of polyphenol potential and can be used as a natural antioxidant agent. Therefore, this study aimed to determine the effect of ethanol concentration on polyphenol and tannin contents and their antioxidant activities. The polyphenol substances were extracted using several ethanol concentrations, whereas the antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl method. The results show that the ethanol concentration has no effect on the yield of extraction. However, it affects the total polyphenol and tannin contents with high levels in the 50% and 70% ethanol concentrations. Fifty percent ethanol exhibits more effective antioxidant activity when compared to other ethanol concentrations. Therefore, a 50% ethanol concentration is a suitable solvent to extract polyphenol and tannin substances from nipa palm fruit husk and can be used as an alternative natural antioxidant.

1. INTRODUCTION

Oxidative stress is a condition caused by an imbalance between free radicals and antioxidant potential in the body (Rad *et al*., 2020). A free radical is a substance with an unpaired electron in the outer orbital that is highly reactive with other molecules such as lipids and proteins, including deoxyribonucleic acid (Lobo *et al*., 2010). When the free radical level is higher than the potential endogenous antioxidant, the body needs an external source of antioxidant (exogenous antioxidant), which can be obtained from natural sources through functional foods or dietary supplements (Xu *et al*., 2017).This antioxidant is generally composed of some

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bioactive compounds, such as polyphenols, vitamins, pigments, and polysaccharides (Lourenço *et al*., 2019).

A polyphenol compound is a type of secondary metabolite that is generally found in plants. Polyphenol substances include phenolic acids, flavonoids, stilbenes, and lignans. Tannins are a class of polyphenol compounds that belong to the flavonoid subgroup. Polyphenol groups are known as antioxidant agents because they inhibit free radical formation. It can act as a radical scavenger by donating hydrogen atoms or electron transfer mechanisms. Polyphenols can be extracted from terrestrial and aquatic plants, such as seaweeds (Ismail *et al*., 2023), water lettuce (*Pistia stratiotes*) (Herpandi *et al*., 2021), and yellow velvetleaf (*Limnocharis flava*) (Serang & Laili, 2021). A previous study reported that tannin compounds were successfully extracted from coconut (Family Arecaceae) husk using an ethanol solvent (Buamard & Benjakul, 2017). The family Arecaceae, also known as the Palmae family, contains species of tropical shrubs, climbers, and trees commonly known as palm trees. *Nypa fruticans* Wurmb. is also a family of Arecaceae widely found in tropical countries. The empirical observation indicated that coconut husks show morphological similarity with the nipa palm fruit husk. Additionally, nipa palm endosperm and leaves have been reported to contain polyphenol compounds (Gazali *et al*., 2019; Prasad *et al*., 2013). Therefore, we hypothesized that the nipa palm fruit husk is also composed of polyphenols, including tannins.

In general, polyphenolic compounds can be extracted from plants using an ethanol solvent or its mixture with water (Mojzer *et al*., 2016). Ethanol was chosen as a solvent due to its relatively low toxicity and classification as a safe food additive (Plaskova & Mlcek, 2023). A previous study reported that different ethanol concentrations have a significant effect on the total phenolic and tannin contents of *Centella asiatica* (Chew *et al*., 2011). Also, different ethanol concentrations show different effects on the total polyphenol and tannin contents of *Andrographis paniculata* (Thoo *et al*., 2013). These conditions indicated that different ethanol concentrations have different effects on the total polyphenol and tannin content. Therefore, we hypothesized that different ethanol concentrations would also have different effects on the polyphenol and tannin contents of the nipa palm fruit husk extract. Thus, this study aimed to investigate the effects of the ethanol concentration on the total polyphenol and tannin contents of the nipa palm fruit husk extract as well as its antioxidant activity.

2. MATERIAL and METHODS

2.1. Materials

The *Nypa fruticans* fruit husk was collected from Sung Sang Village, South Sumatra. The ethanol absolute, Folin-Ciocalteu'sphenol reagent, natrium carbonate (Na_2CO_3), tannic acid, gallic acid, and *2,2*-diphenyl-*1*-picrylhydrazyl (DPPH) powder were purchased from Sigma Aldrich.

2.2. Preparation and Extraction

The small pieces of fruit husk were oven-dried (Memmert Universal Oven UN55) at 45°C for 24 hours and ground to obtain dried fruit husk powder using a grinding machine (Microphyte disintegrator B-One DM-120M). It was extracted using several concentrations of ethanol solvent with the maceration method according to previous methods (Chew *et al*., 2011; Złotek *et al*., 2016). Briefly, 20 g of extract was mixed with 200 mL of each ethanol concentration (50%, 60%, 70%, and 80%; ethanol mixed with distilled water) in the Erlenmeyer flask. The extraction was performed at 30°C for 3 hours on the hot plate and stirred with a magnetic stirrer (IKA-C MAG HS 7) at 120 rpm. After the extraction time, the filtrate and residue were separated using filter paper (Whatman No. 42). The filtrate was collected in a collection tube, whereas the residue was reextracted in the same condition as the first extraction, and five extractions were performed in total. After that, all the filtrates were collected in a new

collection. The filtrate was evaporated at 45°C using a rotary vacuum evaporator (Biobase RE-301) to remove the solvent and was completely dried using a freeze dryer (Biobase BK-FD10S) to obtain a dried extract. The percentage yield of extraction (%) was calculated as dried fruit husk powder (g) divided by dried extract (g) and multiplied by 100%.

2.3. Total Polyphenol and Tannin Contents Analysis

The polyphenol content of the nipa palm fruit husk ethanol extract was analyzed according to the previous method (Chandra *et al*., 2014). Briefly, 100 mg of the extract was dissolved in 10 mL of distilled water in an Erlenmeyer flask. Then, 0.2 mL of the extract solution was mixed with Folin-Ciocalteu's reagent (1:1, v/v) in the reaction tube and allowed to react at room temperature for 5 minutes. After the reaction time, 1 mL of 8% natrium carbonate solution was pipetted into the reaction tube, and the volume was increased to 3 mL with distilled water. The mixture was allowed to react at room temperature for 30 minutes. After that, it was centrifuged (Oregon LC-04S Centrifuge) at 3,000 rpm for 30 minutes. The supernatant was collected and absorbance was measured using a UV-Vis spectrophotometer at 765 nm immediately. Gallic acid was used as a standard; therefore, the total polyphenol content was calculated as mg gallic acid equivalent per g of dried sample (mg GAE/g).

The total tannin of the nipa palm fruit husk ethanol extract was measured according to the previous study (Rajkumar *et al*., 2022). Briefly, 2 mg of extract was dissolved in 2 mL of ethanol solvent in a tube. Then, 0.1 mL of the extract solution was pipetted into a reaction tube and added to 7.5 mL of distilled water. The mixture was then added to 0.5 mL of Folin-Ciocalteu's reagent and 1 mL of 35% natrium carbonate, bringing the volume up to 10 mL with distilled water. After 30 minutes of reaction at room temperature, the absorbance was immediately measured using a UV-Vis spectrophotometer at 700 nm. Tannic acid was used as a standard; therefore, the total tannin content was calculated as mg tannic acid equivalent per g of dried sample (mg TAE/g).

2.4. Antioxidant Activity Assay

The antioxidant activity of nipa palm fruit husk ethanol extract was analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Sudirman *et al*., 2022). Briefly, the extract was dissolved in ethanol to make a serial concentration (0 – 1,000 µg/mL). Then, 1 mL of each sample concentration was mixed with 0.2 mM DPPH solution (1:1, v/v) and incubated at 37°C for 30 minutes. The absorbance was immediately measured using a UV-Vis spectrophotometer (Genesys 150 ThermoScientific) at 517 nm. The antioxidant activity was calculated as the inhibition of the extract on the DPPH radical according to this formula:

$$\text{Percentage (\%) of inhibition} = \frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{blank}}} \times 100\%$$

Whereas: Abs_{blank}, the absorbance at 517 nm without sample; Abs_{sample}, the absorbance at 517 nm with sample.

2.5. Data Analysis

All data were expressed as the mean ± standard deviation (SD). The total polyphenol, tannin, and antioxidant activity were analyzed by one-way analysis of variance and Duncan's post-hoc test at $p<0.05$. All graphics were produced using GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, United States).

3. RESULTS

3.1. Yields of Extraction

The yield of crude extract from *N. fruticans* fruit husk is shown in Table 1. The different ethanol concentrations also have different effects on the extraction yield. The higher-yield extract is at the 50% ethanol concentration (17.74%±5.85) and lower at the 70% ethanol concentration (13.66±4.78%).

Table1. Yield of crude extract from *N. fruticans* husk with different ethanol concentrations.

Parameter	Ethanol concentrations (%)			
	50	60	70	80
Yield* (%)	17.74±5.85	14.25±0.91	13.66±4.78	14.83±2.34

*The data are shown as the mean ± SD (*n*=3).

3.2. Total Polyphenol and Tannin Contents

The total polyphenol content of the *N. fruticans* fruit husk ethanol extract is shown in Figure 1. The ethanol concentrations of 70% (16.63±2.25 mg GAE/g), 50% (15.48±1.48 mg GAE/g), and 80% (14.71±1.62 mg GAE/g) are significantly higher on the total polyphenol contents when compared to 60% (8.08±1.27 mg GAE/g). Whereas, the tannin content of *N. fruticans* fruit husk ethanol extract is shown in Figure 2. The ethanol concentrations of 70% (257.34±0.66 mg TAE/g) and 50% (255.54±11.45 mg TAE/g) are significantly higher on the total tannin contents when compared to 80% (232.87±2.86 mg TAE/g) and 60% (228.73±2.90 mg TAE/g).

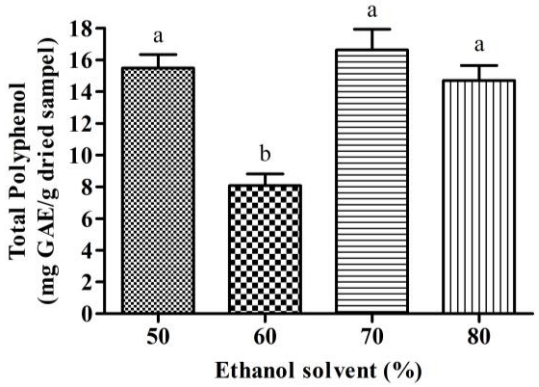

Figure 1. Total polyphenol content of the extract from *N. fruticans* fruit husk with different ethanol concentrations. The data are shown as the mean ± SD (*n*=3). Different letters above the bars indicate a statistically significant difference at $p<0.05$.

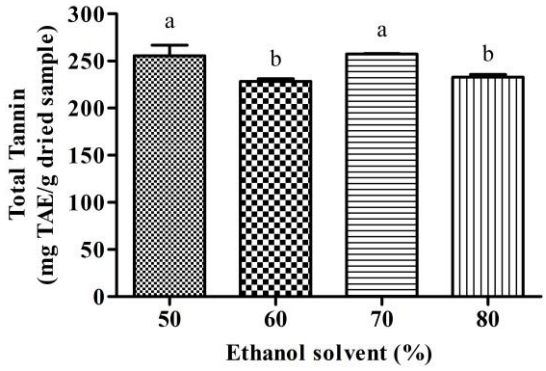

Figure 2. Total tannin content of the extract from *N. fruticans* fruit husk with different ethanol concentrations. The data are shown as the mean ± SD (*n*=3). Different letters above the bars indicate statistically a significant difference at $p<0.05$.

3.3. Antioxidant Activity

The antioxidant activity of the *N. fruticans* fruit husk ethanol extract is shown in Figure 3. Different ethanol concentrations significantly affected the antioxidant activities of the extract. The ethanol concentration of 50% showed the highest antioxidant activity, with the half-maximum inhibitory concentration (IC_{50}) of about 208.96±1.58 µg/mL and the lower in the 80% (429.98±50.11 µg/mL).

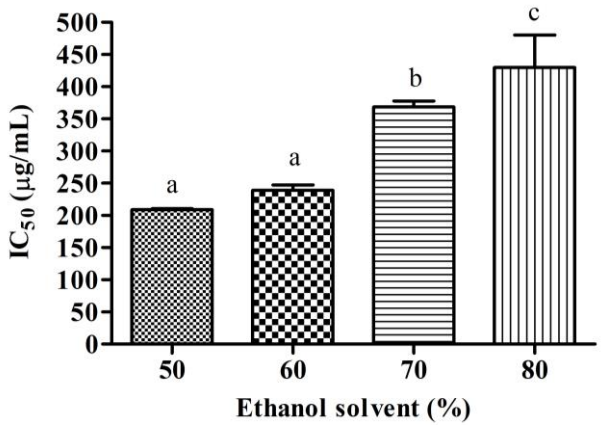

Figure 3. Antioxidant activity of the polyphenol extract from *N. fruticans* husk with different ethanol concentrations. The data are shown as the mean ± SD (n=3). Different letters above the bars indicate a statistically significant difference at p<0.05.

4. DISCUSSION and CONCLUSION

In this study, we successfully extracted polyphenol and tannin compounds from *Nypa fruticans* fruit husk. The different ethanol concentrations also have different effects on the extraction yield (Table 1). A previous study reported that the highest yield of ethanol extracts from *Sauropus androgynus* leaf is 50% ethanol (37.77±0,93%) and the lowest is 96% (33.55±2.77%) (Hikmawanti *et al*., 2021). A previous study reported that the yield of 70% ethanol crude extract from *Pistia stratiotes* leaf was about 16.80% (Sudirman *et al*., 2022). Additionally, the yield of extraction from peanuts using 80% ethanol is 13.01% and that using 20% ethanol is 8.67% (Jitrangsri *et al*., 2020). Different extraction yields are due to different ethanol concentrations caused by the different solvent polarities of each ethanol solvent. Solvents with high polarity also had the ability to extract a wide range of compounds (Do *et al*., 2014). Several factors can affect the extraction process, including the solvent type (Sulaiman *et al*., 2017). A previous study reported that different ethanol concentrations have been used for bioactive compound extraction from plants (Sun *et al*., 2015). Ethanol was chosen as a solvent due to its relatively low toxicity and classification as a safe food additive (Plaskova & Mlcek, 2023); therefore, the extract can be used as a functional food ingredient (Chemat *et al*., 2019).

Different ethanol concentrations show a different effect on the total polyphenol content of nipa palm fruit husk (Figure 1). The highest total phenolic content of grape stem was also extracted with 50% ethanol (Moreno *et al*., 2019).Additionally, 70% ethanol also shows the highest concentration of polyphenol content in some medical plants (Haq *et al*., 2019; Lezoul *et al*., 2020). The different ethanol concentrations used in the extraction also indicate different effects on the tannin contents (Figure 2). A previous study also reported that 50% ethanol solvent is the optimum concentration for tannin extraction from *Areca catechu* nut (Jakfar & Azwar, 2023). These results indicated that different ethanol concentrations also have different effects on the total polyphenol and tannin content. A previous study also reported that different ethanol concentrations have a significant effect on the total phenolic and tannin contents of *Centella asiatica* (Chew *et al*., 2011). Additionally, different ethanol concentrations also show

different effects on the total polyphenol and tannin contents of *Andrographis paniculata*(Thoo *et al*., 2013). In the solvent extraction method, the solvent only extracts those phytochemical or bioactive substances that a have similar polarity to the solvent, according to the "like dissolves like" principle. The ethanol concentration is the factor that has a significant effect on the bioactive compound extraction (Zhang *et al*., 2007).

The ethanol extracts from nipa palm fruit husk exhibit antioxidant activity (Figure 3). The low IC_{50} value indicated that the extract exhibits high potential antioxidant activity and *vice versa* (Goutzourelas *et al*., 2023). This condition is due to its high level of polyphenol and tannin content. A previous study reported that the ethanol extract of *Piper crocatum* exhibits antioxidant activity (Safithri *et al*., 2022). Polyphenol compounds are known as a source of natural antioxidant agents by single electron transfer (SET) or hydrogen atom transfer (HAT) mechanisms that inhibit free radicals and result in the reduction of the adverse effects of free radicals (Lee *et al*., 2015). In a previous study, tannin also exhibited antioxidant and antiradical properties (Maisetta *et al*., 2019).

Overall, in this present study, the bioactive compounds were successfully extracted from *N. fruticans* fruit husks using an ethanol solvent. Different ethanol solvents have different effects on the yield of extraction. However, it shows different effects on the total polyphenol and tannin contents, with a high content at 50% and 70% ethanol concentrations. Filthy percent ethanol also shows the highest antioxidant activity. Therefore, 50% ethanol solvent is a suitable solvent to extract polyphenol and tannin substances from nipa palm fruit husk and can be used as an alternative natural antioxidant.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Sabri Sudirman: Supervision and Writing – editing and final approval. **Aprilia Kusuma Wardana**: Methodology and Formal Analysis. **Herpandi:** Supervision. **Indah Widiastuti**: Writing -original draft. **Dwi Indah Sari**: Formal Analysis and Writing -original draft. **Miftahul Janna**: Methodology and Formal Analysis.

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