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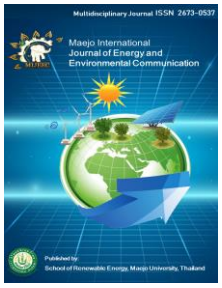

ARTICLE

Effect of agitation on the extraction of phenolic compounds from the ripe pericarp of *Wodyetia bifurcate*

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ABSTRACT

In this study, the ripe pericarp extracts of *Wodyetia bifurcata* were prepared by solvent extraction techniques with and without agitation. The influence of agitation was examined on the extraction yield, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of the ripe pericarp extracts of *Wodyetia bifurcata*. Antioxidant capacity of extracts was evaluated by measuring the scavenging effect on 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radicals. Compared to solvent extraction without agitation, solvent extraction with agitation significantly ($p < 0.05$) increased the extraction yield, TPC, TFC and antioxidant capacities (ABTS and DPPH) of the ripe pericarp extracts of *Wodyetia bifurcata*. In addition, higher agitation speed resulted in higher extraction yield, TPC, TFC and antioxidant activities (ABTS and DPPH) of the ripe pericarp extracts of *Wodyetia bifurcata*.

1. Introduction

Foxtail palm (*Wodyetia bifurcata*), a member of the Arecaceae family, has gained interest owing to its high economic value, adaptability and fast growth (Perez et al., 2009; Naderali et al., 2017). Foxtail palm is mainly used for ornamental purposes. However, a recent study revealed the presence of phenolic compounds in the aerial parts of *Wodyetia bifurcata* (Sengab et al., 2015). Phenolic compounds exhibit various physiological properties such as antioxidant (Hanula et al., 2020), anti-microbial (Alshwyeh, 2020), anti-inflammatory (Sengab et al., 2015; John and Shahidi, 2019) and anti-cancer (Kim et al., 2010; Palanisamy et al., 2022a) activities.

The pericarp of *Wodyetia bifurcata* consists of three layers: an epicarp, which turns to orange or red at maturity; a mesocarp; and an endocarp (Dowe, 2010). In the present study, the recovery of phenolic compounds from the ripe pericarp of *Wodyetia bifurcata* was investigated. Solvent extraction is an easy and efficient method commonly used in the preparation of plant extracts (Dai & Mumper, 2010; Palanisamy et al., 2022b). The efficiency of solvent extraction is affected by extraction conditions. It has been reported that agitation during extraction had an influence on the performance of extraction of phenolic compounds from Tamarindus indica seeds (Sarkar & Ghosh, 2017; Palanisamy et al., 2021) and from Averrhoa bilimbi (Muhamad et al., 2014).

Therefore, the purpose of this study was to investigate the influence of agitation during solvent extraction on the extraction

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yield, total phenolic content (TPC) and total flavonoid content (TFC) of the ripe pericarp extracts of *Wodyetia bifurcata*. In addition, the antioxidant capacity of the extracts was evaluated using two different methods, namely ABTS and DPPH assays.

2. Materials and Methods

2.1. Plant material

Ripe foxtail palm fruits were harvested from Samut Sakhon province, Thailand in August 2021. The fruits were washed with clean water, dried at ambient temperature by a blower and then kept at -18 °C in a freezer until use. After removing from the freezer, the foxtail palm fruits were allowed to thaw at room temperature for approximately 30 minutes. The outer skin (epicarp) and middle skin (mesocarp) of foxtail palm fruits were then separated and grinded using a blender. Extractions were performed on the same day or next day of sample preparation.

2.2. Solvent extraction

Ten grams of sample and 100 mL of solvent (60% ethanol) were mixed in a 250 mL Erlenmeyer flask. The flask was placed in a water bath shaker (Memmert, Germany) operating at 0, 50 and 90 spm (strokes per minute) for 30 minutes at room temperature. After extraction, the mixture was vacuum filtered through Whatman No. 1 filter paper. The extract was kept in an amber bottle at -18 °C until analysis. All samples were prepared and analyzed in triplicate.

2.3. Determination of extraction yield

The extraction yield was determined using a slightly modified method of Hegazy (2017). The extract (10 mL) was pipetted into an aluminum dish. The sample was dried in a hot air oven at 105 °C for about 8-10 hours until obtaining a constant weight. The extraction yield was expressed as grams of solid per gram of dry weight sample (g/ g DW).

2.4. Determination of total phenolic content (TPC)

The TPC was examined using a method based on Hanula et al. (2020) with slight modification. The extract (0.05 mL), Folin–Ciocalteu reagent (0.25 mL) and distilled water (1.95 mL) were mixed in a test tube and allowed to react for 6 minutes. Then, 7% sodium carbonate solution (0.75 mL) was added and mixed. After incubation in the dark for 2 hours at room temperature, the absorbance was measured at 765 nm against a blank solution. The blank solution was prepared by replacing the extract (0.05 mL) with distilled water (0.05 mL). The TPC was expressed as milligrams of gallic acid equivalent per gram of dry weight sample (mg GAE/ g DW).

2.5. Determination of total flavonoid content (TFC)

The procedure according to Wang et al. (2008) was slightly modified for determination of the TFC. The extract (1.5 mL) and 2% $AlCl_3$ ethanol solution (1.5 mL) were mixed in a test tube. After standing for 1 hour at room temperature, the absorbance was measured at 420 nm against a blank solution. The blank solution was prepared by replacing the extract (1.5 mL) with ethanol (1.5 mL). The TFC was calculated as milligrams of quercetin equivalent per gram of dry weight sample (mg QE / g DW).

2.6. Determination of antioxidant activity

2.6.1. ABTS radical scavenging capacity assay

The ABTS radical scavenging capacity was evaluated using the method of Thoo et al. (2010). 7 mM ABTS (10 mL) and 2.45 mM potassium persulfate (10 mL) were mixed to prepare the ABTS radical solution in an amber bottle and kept in the dark at room temperature for 12–16 hours. The absorbance of ABTS radical solution was adjusted to be 0.7±0.02 at 734 nm by diluting with ethanol before use. The extract (0.1 mL) and ABTS radical solution (3.9 mL) were mixed in a test tube. After standing in the dark for 6 minutes at room temperature, the absorbance was measured at 734 nm against ethanol as a blank. The ABTS radical scavenging capacity was calculated as micromoles of trolox equivalent per gram of dry weight sample (µmol TE/ g DW).

2.6.2. DPPH radical scavenging capacity assay

The DPPH radicals scavenging capacity was evaluated using the method of Donadone et al. (2020). The extract (0.1 mL) and 60 µM DPPH solution (3.9 mL) were mixed in a test tube. After incubation in the dark for 30 minutes at room temperature, the absorbance was measured at 517 nm against ethanol as a blank. The DPPH radical scavenging capacity was expressed as micromoles of trolox equivalent per gram of dry weight sample (µmol TE/ g DW).

2.7. Statistical analysis

The extraction processes were performed in triplicate and results were expressed as the mean ± standard deviation (SD). Data were analysed using analysis of variance (ANOVA) and significant differences between means were evaluated using Duncan's test.

3. Results and Discussion

3.1 Effect of agitation on extraction yield

In this study, the extraction of phenolic compounds from the ripe pericarp of *Wodyetia bifurcata* was carried out using solvent techniques with agitation (50 and 90 spm) and without agitation. All the extractions were performed at room temperature for 30 minutes using a sample-to-solvent ratio of 1:10 (g/mL). The extraction yield of the ripe pericarp extracts of *Wodyetia bifurcata* obtained by solvent extraction with and without agitation is presented in Table 1. The results showed that solvent extraction with agitation significantly ($p < 0.05$) increased the extraction yield of the ripe pericarp extracts of *Wodyetia bifurcata* compared to

solvent extraction without agitation. Additionally, the extraction yield of the ripe pericarp extracts of *Wodyetia bifurcata* significantly ($p < 0.05$) increased with increasing the agitation speed from 50 spm to 90 spm.

The extraction yield obtained by solvent extraction with agitation at 50 spm (0.20 g/ g DW) and at 90 spm (0.25 g/ g DW) showed 1.5-fold and 1.9-fold higher than that obtained by solvent extraction without agitation (0.13 g/ g DW), respectively. The results also indicated that the extraction yield of the ripe pericarp extracts of *Wodyetia bifurcata* significantly increased by 25%, when the agitation speed increased from 50 spm to 90 spm.

Table 1 The extraction yield of the ripe pericarp extracts of *Wodyetia bifurcata* obtained by solvent extraction with and without agitation

Agitation speed (spm)	Extraction yield (g/ g DW)
0	0.13 ± 0.05^{c}
50	0.20 ± 0.05^{b}
90	0.25 ± 0.03^{a}

Means ± standard deviations followed by different letters in the same column are significantly different based on Duncan's test at $p < 0.05$.

3.2. Effect of agitation on TPC and TFC

The effect of agitation on TPC and TFC of the ripe pericarp extracts of *Wodyetia bifurcata* is shown in Figure 1A-B. Compared to solvent extraction without agitation, solvent extraction with agitation significantly ($p < 0.05$) increased the TPC and TFC of the ripe pericarp extracts of *Wodyetia bifurcata*. In addition, higher agitation speed resulted in higher TPC and TFC of the ripe pericarp extracts of *Wodyetia bifurcata*.

The TPC obtained by solvent extraction with agitation at 50 spm (12.54 mg GAE / g DW) and at 90 spm (16.32 mg GAE / g DW) showed 1.9 fold and 2.4 fold higher than that obtained by solvent extraction without agitation (6.76 mg GAE / g DW), respectively, whereas the TFC obtained by solvent extraction with agitation at 50 spm (0.40 g QE/ g DW) and at 90 spm (0.58 g/ g QE/ g DW) showed 1.7 fold and 2.4 fold higher than that obtained by solvent extraction without agitation (0.24 g QE/ g DW), respectively. The results also suggested that the TPC and TFC of the ripe pericarp extracts of *Wodyetia bifurcata* significantly increased by 30% and 45%, respectively, when the agitation speed increased from 50 spm to 90 spm.

A similar observation was found by Das & Eun (2018) and Muhamad et al. (2014) who studied the extraction of bioactive metabolites from green tea and the extraction of polyphenol and antioxidant from *Averrhoa bilimbi*, respectively. In the solvent extraction, turbulence caused by agitation greatly contributes to the high rate of mass transfer between solvent and solute (Patil et al., 2019; Lanjekar & Rathod, 2021). Furthermore, a higher agitation speed resulted in a higher convective mass transfer rate (Muhamad et al., 2014).

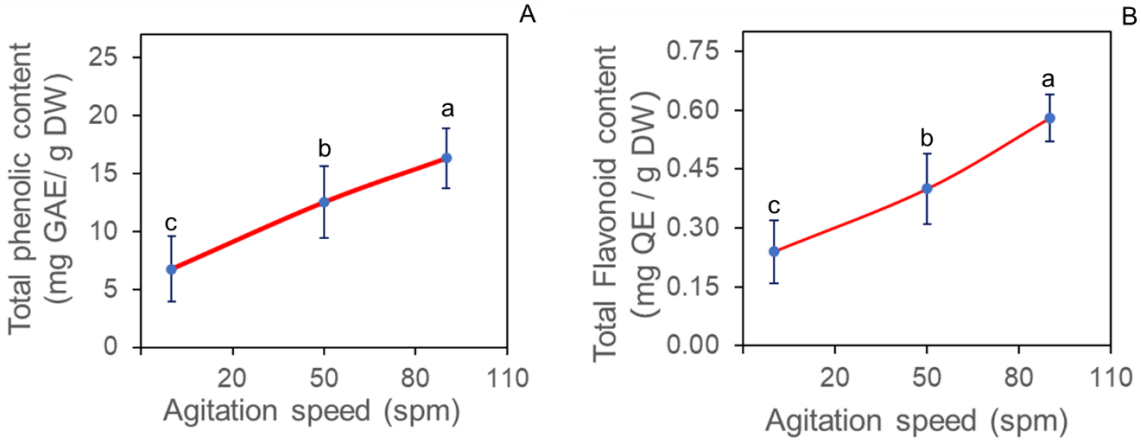

Figure 1 Effect of agitation on (A) TPC and (B) TFC of the ripe pericarp extracts of *Wodyetia bifurcata*. Each value is expressed as mean ± SD of triplicate determinations. Values denoted by different lower-case letters are significantly ($p < 0.05$) different.

3.3. Effect of agitation on antioxidant activity

Figure 2A-B demonstrates the impact of agitation on the antioxidant activities (ABTS and DPPH) of the ripe pericarp extracts of *Wodyetia bifurcata*. As evident in Figure 2A and B, solvent extraction with agitation significantly ($p < 0.05$) increased the antioxidant activities (ABTS and DPPH) of the ripe pericarp extracts of *Wodyetia bifurcata* compared to solvent extraction without agitation. As the agitation speed increased from 50 spm to 90 spm, the antioxidant activities (ABTS and DPPH) of the ripe pericarp extracts of *Wodyetia bifurcata* increased significantly ($p < 0.05$).

The ABTS radical scavenging capacity obtained by solvent extraction with agitation at 50 spm (1,068.56 µmol TE/ g DW) and at 90 spm (1,289.54 µmol TE/ g DW) showed 1.7 fold and 2.0 fold higher than that obtained by solvent extraction without agitation (629.43 µmol TE/ g DW), respectively, whereas the DPPH radical scavenging capacity obtained by solvent extraction with agitation at 50 spm (838.24 µmol TE/ g DW) and at 90 spm (1067.80 µmol TE/ g DW) showed 1.7 fold and 2.1 fold higher than that obtained by solvent extraction without agitation (498.33 µmol TE/ g DW), respectively. The results also suggested that the ABTS and DPPH radical scavenging activities of the ripe pericarp extracts of *Wodyetia bifurcata* significantly increased by 21% and 27%, respectively, when the agitation speed increased from 50 spm to 90 spm.

A similar result has been observed in the extraction of polyphenol and antioxidant from *Averrhoa bilimbi* (Muhamad et al., 2014).

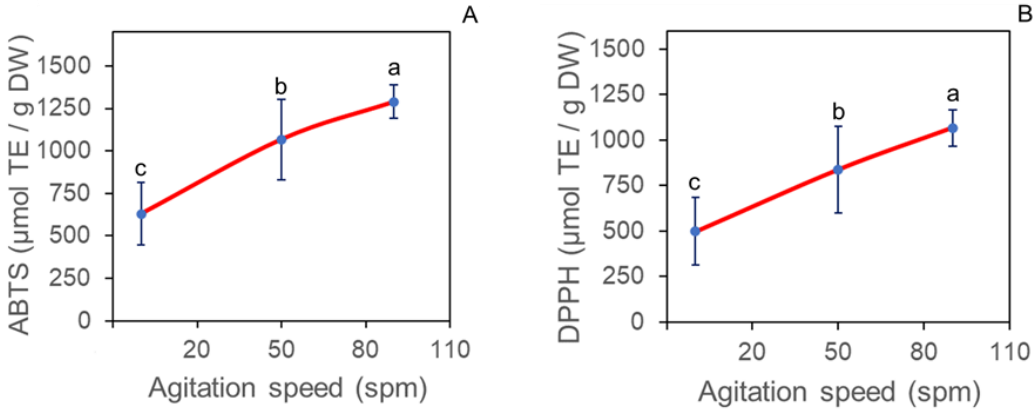

Figure 2 Effect of agitation on (A) ABTS and (B) DPPH radical scavenging activities of the ripe pericarp extracts of *Wodyetia bifurcata*. Each value is expressed as mean ± SD of triplicate determinations. Values denoted by different lower-case letters are significantly ($p < 0.05$) different.

4. Conclusion

The influence of agitation during solvent extraction was investigated for efficient extraction of phenolic compounds from the ripe pericarp of *Wodyetia bifurcata*. Compared to solvent extraction without agitation, solvent extraction with agitation exhibited significantly higher extraction yield, TPC, TFC and antioxidant activities (ABTS and DPPH). In addition, solvent extraction with higher agitation speed showed significantly higher extraction yield, TPC, TFC and antioxidant capacities (ABTS and DPPH) compared to solvent extraction with lower agitation speed. These results suggested that the solvent extraction efficiency was significantly improved by agitation. In addition, higher agitation speed resulted in higher extraction performance.

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