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Using coconut water for polyphenol extraction from *Artocarpus altilis*

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Abstract

This study investigates the potential of coconut water for the extraction of polyphenolic compounds from the leaves and branches of *Artocarpus altilis*. The objective is to identify a green and clean solvent as an alternative to environmentally harmful organic solvents. Two extraction methods were used: hot extraction (reflux) and cold extraction (maceration), with three types of coconut water (Malaysian dwarf, green Siamese, and strawberry coconuts) employed as solvents. The results indicated that the total polyphenol content in the extracts obtained with coconut water and an acetate buffer (pH 3.6) was higher than that of aqueous extracts (18.8–48.0 and 15–28.0 mg GAE/g, respectively). Furthermore, 6/24 extracts exhibited significant antioxidant activity, with IC₅₀ values below 50 µg/mL in the DPPH free radical scavenging assay. Based on MPI (mobile phase performance index) values, the optimal solvent system identified was a combination of Malaysian dwarf or strawberry coconut water with a 0.5 M acetate buffer (pH 3.6) at a 1:1 ratio, which yielded high polyphenolic content and antioxidant activity from *A. altilis* leaves and branches.

Keywords: *Artocarpus altilis*, antioxidant activity, coconut water, DPPH free radical scavenging assay, total polyphenol content

1. Introduction

Polyphenols are a diverse group of natural compounds found in plant species. The most prominent activity of polyphenols is their antioxidant capacity through a free-radical scavenging mechanism. Therefore, polyphenols can help prevent diseases related to free-radical damage, such as cancer, cardiovascular diseases, or neurological disorders [1]. Polyphenol compounds inhibit cell proliferation, suppress inflammatory pathways in fibroblast cell lines, and are involved in the expression of tumor

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suppressor genes [2]. Due to such significant potential in medical applications, the demand for extracting polyphenol compounds from natural sources is very high.

Cocos nucifera L. belongs to the Arecaceae family. It is widely distributed in countries around the world, particularly in tropical regions such as Vietnam, Indonesia, Malaysia, and the Philippines [3]. It is known as a low-cost raw material with numerous applications. Specifically, coconut oil is used as a foaming agent, stabilizer, and cleansing agent in cosmetics [4], while coconut flour, a gluten-free food, is suitable for patients with celiac disease [5]. Moreover, coconut water is considered a natural refreshing beverage rich in minerals such as Mg, K, Na, P, and vitamins like C, B3, and B5. Additionally, coconut water helps regulate blood pressure in hypertensive patients and may help combat myocardial infarction [6]. Furthermore, Makerri et al. discovered that coconut water could extract polyphenols from olive leaves more effectively than water, with results of 86.1 and 64.8 mg of naringin/g of extract, respectively [7].

Artocarpus altilis (Parkinson) Fosberg is primarily distributed in tropical climate zones. In folk medicine, leaves are used to treat gout, hepatitis, high blood pressure, and diabetes [8]. Furthermore, previous studies indicate that this plant possesses various biological activities, such as antibacterial [9], antioxidant [10], and cytotoxic potential against the PANC-1 pancreatic cancer cell line [8]. Most studies on polyphenol content and antioxidant activity have been conducted on the leaves, using organic solvents for extraction. From a methanol extract of *A. altilis* leaves, Leng et al. reported a polyphenol content of 144.16 ± 17.98 mg gallic acid/g and a DPPH free-radical scavenging ability of $63.88 \pm 1.87\%$ [11]. On the other hand, an ethanol extract of the leaves showed a polyphenol content of 26.2 mg GAE/g with a DPPH[•] radical scavenging IC₅₀ value of 140.54 μ g/mL [12].

In general, studies have focused on the polyphenol content in the leaves of *A. altilis*, using organic solvents for extraction, while the branches remain uninvestigated. Therefore, this study aims to evaluate the potential of coconut water as a clean, natural alternative for extracting polyphenols from both the leaves and branches, replacing toxic and environmentally harmful solvents.

2. Experimental methods

2.1. Materials

Leaves and branches of *A. altilis* were collected in Tien Giang Province, Vietnam, in November 2022. Malaysian dwarf coconuts were harvested in Dong Thap Province, while green Siamese and strawberry coconuts were harvested in Ben Tre Province in December 2022. All coconuts were harvested at maturity (about 7–9 months old), which is the optimal stage for drinking water, to ensure consistency in chemical composition. After harvesting, the coconut water was collected and stored in a freezer at -5 °C. All samples were botanically identified by comparing their characteristics with taxonomic databases.

2.2. Sample extraction procedure

2.2.1. Using coconut water and water as solvents

5g of dried sample (leaves or branches) was subjected to reflux extraction using three types of coconut water. The solvents consisted of coconut water-to-water ratios of 1:0 (100% coconut water), 7:3, 1:1, and 0:1 (100% water), respectively. The extraction was performed three times, with each time lasting 3 h using 150 mL of solvent (150 mL, 3 h \times 3). Similarly, samples were macerated with the same coconut water-to-water ratios. The maceration was also repeated three times, with each time lasting 24 h using 150 mL of solvent (150 mL, 24 h \times 3). The resulting extracts were dried to obtain crude extracts.

2.2.2. Using coconut water and buffer as solvents

The buffer-based extraction procedure was carried out similarly to section 2.2.1, but water was replaced with a pH 3.6 buffer solution. To optimize the process, various buffers (0.5 and 1.0 M citrate; 0.5 and 1.0 M acetate) were tested at coconut water-to-buffer ratios of 7:3 and 1:1. The effect of neutralization was also evaluated by comparing extracts neutralized to pH 7 (with 10% NaHCO₃) to non-neutralized (acidic) extracts. Finally, the solvent was evaporated to yield crude extracts.

2.3. Total polyphenol qualification

The total polyphenol content was determined using the Folin-Ciocalteu method [13]. Sample and 2 mL of 10% Folin-Ciocalteu reagent were added to a 10 mL volumetric flask. The mixture was shaken and incubated at room temperature for 5 minutes. 2.5 mL of 10% Na₂CO₃ solution was added, and the volume was brought up to 10 mL with water. The solution was mixed thoroughly, incubated in the dark for 30 minutes at room temperature, and its absorbance was measured at a wavelength of 737 nm using a Shimadzu UV-1800 spectrophotometer. The total polyphenol content was calculated as gallic acid equivalents (GAE), and the results were expressed as mg GAE per g of extract (mg GAE g⁻¹) and as the total polyphenol content in the entire extract (mg GAE).

2.4. Antioxidant activity assay

The antioxidant activity was determined based on the DPPH• free-radical scavenging method [14]. Sample and 1500 μL of a 100 μM DPPH• solution were added into tubes, followed by ethanol (EtOH) to reach a final volume of 3000 μL. The solution was mixed well, incubated in the dark for 30 minutes at room temperature, and its absorbance was measured at 517 nm using a Shimadzu UV-1800 spectrophotometer. The result was expressed as the percentage of inhibition (I%).

$$I\% = 1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

The IC₅₀ value (μg mL⁻¹), defined as the sample concentration required to scavenge 50% of DPPH• free radicals, was determined. Trolox was used as the positive control.

2.5. Evaluation of the optimal extraction solvent

As this study focuses on evaluating the polyphenol extraction potential and antioxidant activity of coconut water, the MPI (mobile phase performance index) was used as a standard for the overall comparison of extraction methods [15]. In this study, the MPI simultaneously evaluates the contribution of two factors, each accounting for 50%. A 50:50 ratio was chosen to objectively evaluate the mobile phase. This approach balances the total amount of extracted polyphenols with their actual antioxidant activity, ensuring the solvent performs well in both areas. Specifically, 24 extracts with high polyphenol content, obtained using coconut water combined with 0.5 M acetate buffer (pH 3.6), were evaluated using the MPI to select the most effective extract. The total polyphenol content values (P_i) and the 50% inhibitory concentrations (IC_{50-i}) were ranked to identify the minimum (P_{min}, IC_{50-min}) and maximum (P_{max}, IC_{50-max}) values. These were used to calculate the relative total polyphenol content \bar{P}_i and the relative 50% inhibitory concentration \overline{IC}_{50-i} .

$$\bar{P}_i = \frac{P_i - P_{\min}}{P_{\max} - P_{\min}}$$

$$\overline{IC}_{50-i} = \frac{IC_{50-i} - IC_{50-\min}}{IC_{50-\max} - IC_{50-\min}}$$

These relative values, ranging from 0 to 1, were used to calculate the MPI_i index for each sample to evaluate the optimal solvent system.

$$\overline{MPI}_i = \bar{P}_i \times 0.5 + \overline{IC}_{50-i} \times 0.5$$

2.6. Statistical analysis

All the samples were analysed in triplicate. The results were expressed as a mean ± SD (standard deviation). Data were analysed using an independent sample t-test to compare the means of two groups with equal variance.

3. Results and Discussions

3.1. Polyphenol content in aqueous and coconut water extracts

Figure 1 shows that the polyphenol content in the extract of *A. altilis* leaves and branches, using three different types of coconut water at coconut water-to-water ratios of 100:0, 7:3, and 1:1, ranged from 13.4 to 24.8 mg GAE/g of extract. In both extraction methods, the polyphenol content of samples using 100% coconut water was lower than that of the extracts obtained using 100% water. However, in the maceration method for leaf extracts, the polyphenol content extracted by the three types of coconut water at ratios of 7:3 and 1:1 was observed to be higher than that of aqueous extracts (p<0.05). Furthermore, there was no statistically significant difference between the results at these two ratios p>0.05).

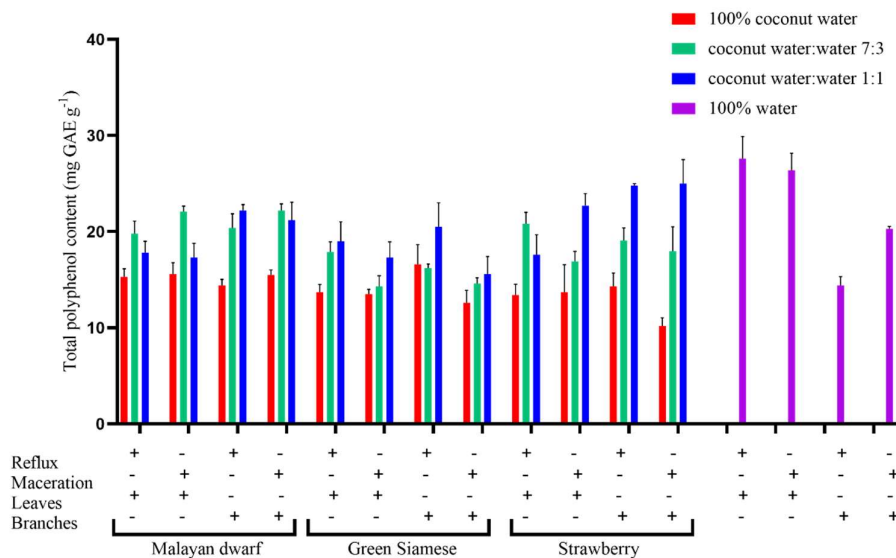


Figure 1. Polyphenol content per gram of extract (mg GAE/g) from the leaves and branches of *A. altilis* at different coconut water-to-water ratios (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

Figure 2 shows that the polyphenol content in the coconut water (without sample) is only about 60–70 mg GAE. This content only accounts for 15–20% of the total polyphenol content in the sample, which

indicates that the polyphenols naturally present in coconut water are insignificant compared to those in the sample extracts ($p < 0.05$). The total polyphenol content from the reflux method is higher than that of maceration ($p < 0.05$). This increase is attributed to the application of heat, which helps break down the bonds between lignin and phenolic acids, releasing otherwise insoluble compounds. Additionally, the degradation of lignin itself can produce more phenolic acids, which increases the total polyphenol content in the extracts [16]. Furthermore, the polyphenol content in the coconut water extracts of both leaves and branches was higher than that in the aqueous extracts. Specifically, the polyphenol content in the aqueous extracts was about 20–40 mg GAE using the reflux method and 15 mg GAE with the maceration method. In contrast, the results for the extracts with coconut water and water at various ratios ranged from 170–500 mg GAE ($p < 0.05$).

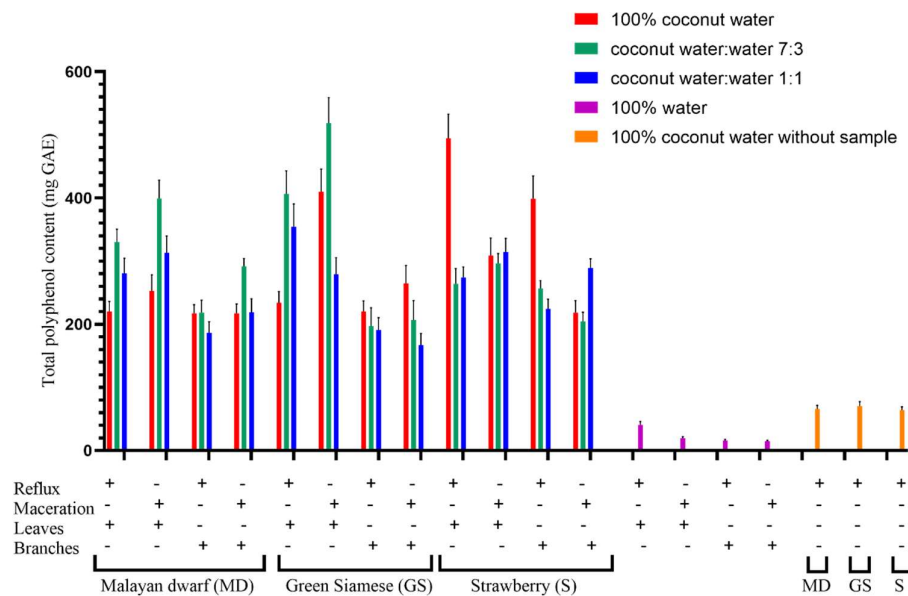


Figure 2. Total polyphenol content (mg GAE) from the leaves and branches of *A. altilis* at different coconut water-to-water ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

3.2. Polyphenol content in coconut water-buffer extracts

Figure 3 indicates that using a citrate buffer as the extraction solvent significantly decreased the total polyphenol content compared to the water-coconut water ($p < 0.05$). In contrast, combining coconut water with an acetate buffer (1:1) led to an increase in polyphenol content. This suggests that acidifying the solvent with an acetate buffer improved the polyphenol extraction efficiency. This can be explained that a low pH inhibits enzymatic oxidation and maintains the stability of phenolic compounds, particularly anthocyanins [17]. Besides, in an acidic solvent, polyphenols, which are weak acids, exist in their neutral form, thereby enhancing their solubility and extraction [18].

Furthermore, the results also revealed that extracts neutralized with NaHCO_3 after extraction with acetate buffer yielded a higher polyphenol content than their non-neutralized (acidic) counterparts ($p < 0.05$). This is because the Folin-Ciocalteu assay is conducted in an alkaline medium, where phenolic compounds must be converted to their phenolate form to reduce the reagent. In the acidic extracts, this conversion is limited, leading to an underestimation of the polyphenol content [19]. Based on these

findings, a 0.5 M acetate buffer (pH 3.6) and a post-extraction neutralization step with NaHCO₃ were selected for further experiments.

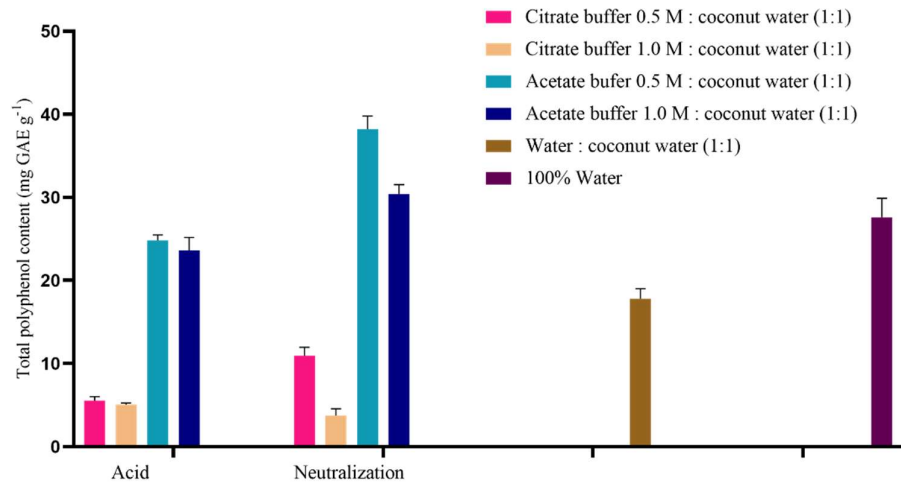


Figure 3. Polyphenol content per gram of extract (mg GAE/g) from *A. altilis* leaves at a 1:1 coconut water-to-buffer ratio (Malaysian dwarf coconuts).

The results from the polyphenol content in *A. altilis* leaves and branches, using different types of coconut water combined with an acetate buffer, showed a range of 18.8–48.0 mg GAE/g (Figure 4). The extracts were prepared with coconut water-to-buffer ratios of 7:3 and 1:1. Extracts yielded a higher polyphenol content than aqueous extracts ($p < 0.05$). Specifically, for the reflux method applied to the leaves, the 1:1 coconut water-to-buffer ratio had a higher polyphenol content than the 7:3 ratio. Among the different types of coconuts, Malaysian dwarf and strawberry coconuts yielded significantly better extraction of polyphenolic compounds than green Siamese coconuts ($p < 0.05$).

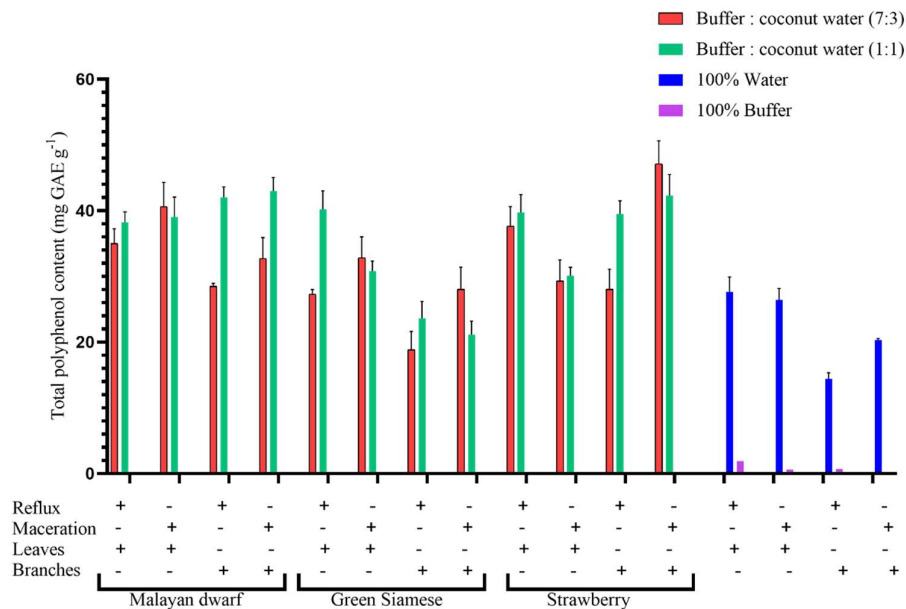


Figure 4. Polyphenol content per gram of extract (mg GAE/g) from the leaves and branches of *A. altilis* at different coconut water-to-buffer ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

Figure 5 shows that the total polyphenol content (mg GAE) in extracts obtained with coconut water and a buffer was from 500–2000 mg GAE. To verify the origin of the polyphenols, a control experiment was conducted on the coconut water solvent under identical extraction conditions. The results showed that the polyphenol content from the coconut water was minimal, accounting for only 12% of the total content measured in the *A. altilis* extracts. This confirms that the high polyphenol yield originates primarily from *A. altilis*, with a negligible contribution from the coconut water. The extract by the reflux method using a 7:3 ratio of Malaysian dwarf coconut water to buffer yielded the highest results for both the leaves and branches parts (1377 and 1875 mg GAE, respectively). Additionally, the reflux extraction method was found to be more effective than the maceration method ($p < 0.05$).

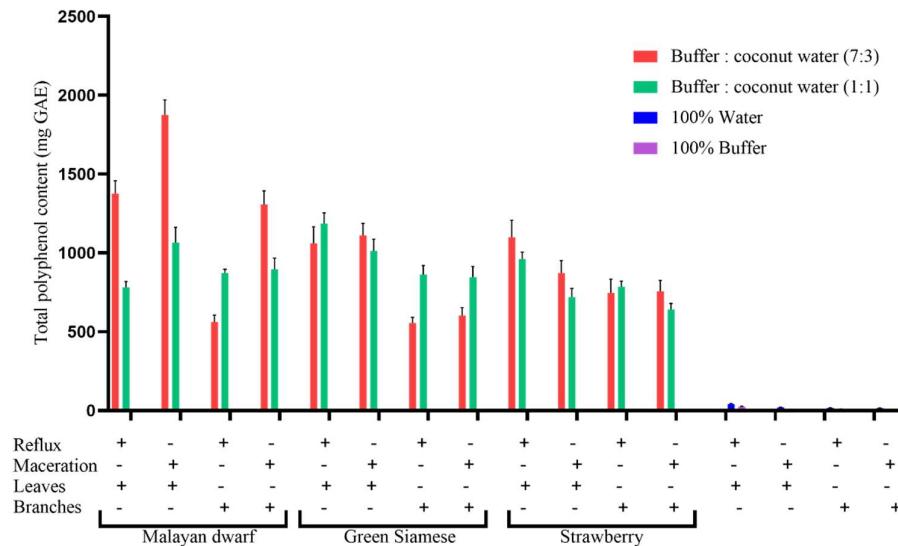


Figure 5. Total polyphenol content (mg GAE) from the leaves and branches of *A. altilis* at different coconut water-to-buffer ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

3.3. Antioxidant activity

The extracts were evaluated for their DPPH• free-radical scavenging activity at various concentrations (Figure 6). Overall, 22/24 extracts exhibited radical scavenging activity, whereas all coconut water extracts (without sample) had an $IC_{50} > 200 \mu\text{g/mL}$. Among the extracts of its leaves, 2/12 extracts showed good activity ($IC_{50} < 50 \mu\text{g/mL}$). Specifically, the refluxed extract from Malaysian dwarf coconut water: buffer (1:1) ($IC_{50} = 39.86 \mu\text{g/mL}$) and the macerated extract from Malaysian dwarf coconut water: buffer (7:3) ($IC_{50} = 46.67 \mu\text{g/mL}$). For the extracts of its branches, 4/12 extracts had IC_{50} values below $50 \mu\text{g/mL}$, specifically the macerated extracts from Malaysian dwarf and strawberry coconut water at a 1:1 ratio ($IC_{50} = 38.69$ and $39.09 \mu\text{g/mL}$, respectively), and the macerated extract from strawberry coconut water at a 7:3 ratio ($IC_{50} = 48.57 \mu\text{g/mL}$, respectively). Trolox was used as a reference standard to validate the experimental procedure, with the value of IC_{50} of $2.7 \mu\text{g/mL}$. Although the IC_{50} values of *A. altilis* extracts are higher than those of Trolox, they demonstrate significant antioxidant potential. The bioactivity of extracts is particularly promising, as they were obtained through a green extraction process rather than an organic solvent.

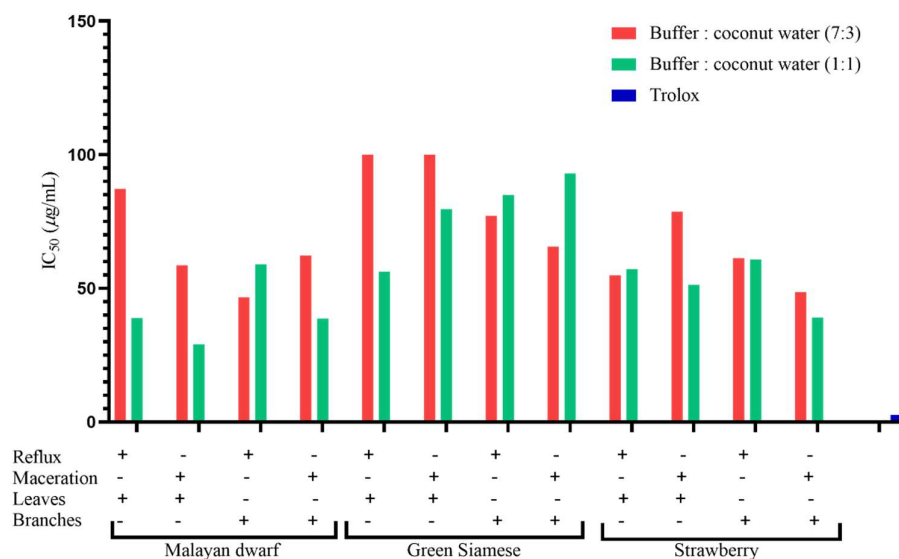


Figure 6. Antioxidant activity from the leaves and branches of *A. altilis* at different coconut water-to-buffer ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

3.4. Evaluation of optimal extraction solvent

Based on the results in Figure 7, the leaf extracts from the reflux method exhibited higher MPI values than those from the maceration method. This difference can be attributed to the effects of temperature during the heating process. High temperatures increase the solubility of compounds within the sample. Moreover, high temperatures provide greater kinetic energy. This causes molecules to move faster, which promotes the diffusion of compounds from the sample matrix into the solvent, thereby increasing the extraction rate. Furthermore, heat contributes to the degradation of the plant's cell wall, particularly lignin. Some intracellular compounds, such as polyphenols, are tightly bound to lignin. At high temperatures, these bonds can be cleaved, and the lignin structure can be broken down through the cleavage of its internal linkages. This breakdown of the cell wall makes it easier for the compounds to diffuse into the extraction solvent [16]. For both the reflux and maceration methods, extracts using a 1:1 ratio of Malaysian dwarf coconut water to buffer were the most effective, with MPI values of 0.92 and 0.89, respectively. Moreover, both extracts possessed high polyphenol content and potent antioxidant activity. These findings suggest that a 1:1 ratio of Malaysian dwarf coconut water to buffer is a promising solvent mixture for the extraction of polyphenols from *A. altilis* leaves

For *A. altilis* branches, the Malaysian dwarf coconut water-to-buffer ratio 1:1 (maceration), and strawberry coconut water-to-buffer ratios 1:1 and 7:3 (maceration) extracts showed the best MPI values of 0.88, 0.91, and 0.86, respectively (Figure 8). Furthermore, all three of these extracts demonstrated a strong ability to extract polyphenolic compounds as well as other compounds with antioxidant properties. These results suggest that Malaysian dwarf coconut water at a 1:1 ratio and strawberry coconut water at 7:3 and 1:1 ratios are promising solvents for the extraction of polyphenols from its branches. However, figures 7 and 8 show that some extracts with low polyphenol content exhibited good antioxidant activity. This can be explained by the antioxidant mechanisms. Specifically, the first mechanism is the abstraction of a hydrogen atom from the antioxidant (ArOH), which turns it into a free radical. The second mechanism involves the transfer of an electron from the antioxidant [20]. Based on the experimental results (Figures

7 and 8), a 1:1 ratio of Malaysian dwarf coconut water to acetate buffer is suitable for extracting polyphenol compounds from both the leaves and twigs of *A. altilis*.

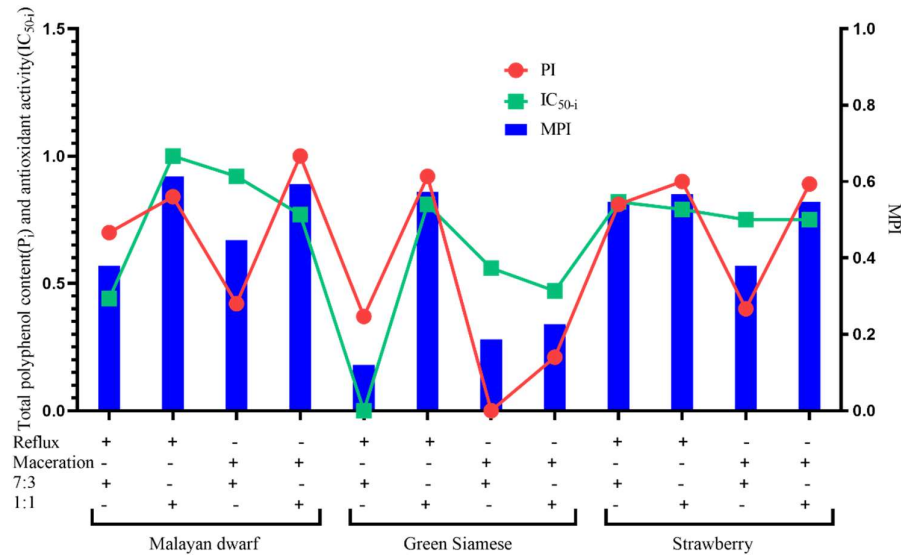


Figure 7. MPI values from the leaves of *A. altilis* at different coconut water-to-buffer ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

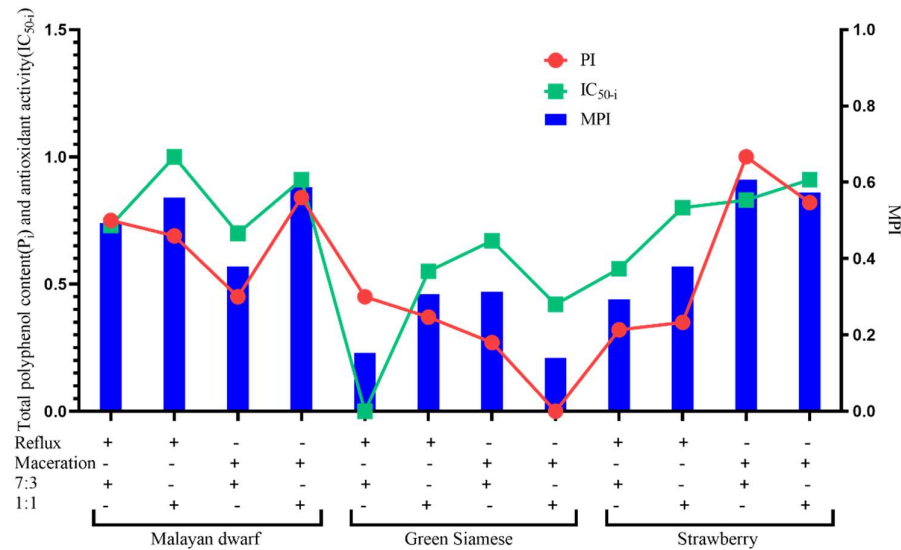


Figure 8. MPI values from the branches of *A. altilis* at different coconut water-to-buffer ratios. (Data columns are marked with +/- to indicate the use/non-use of the corresponding sample/method).

4. Conclusion

In conclusion, this study demonstrates that coconut water, particularly from Malaysian dwarf and strawberry coconuts, serves as a highly effective and sustainable green solvent for extracting polyphenols from *A. altilis*. Quantitatively, the coconut water-buffer mixture outperformed pure water, with 100% of the extracts exhibiting strong antioxidant activity ($IC_{50} < 100 \mu\text{g/mL}$) and 25% reaching high potency at

IC₅₀ < 50 µg/mL. While the extraction yield of the coconut water-buffer mixture is lower than that of ethanol (69.1 ± 4.2 mg GAE/g), it remains the superior choice for environmental sustainability. As a non-toxic, biodegradable, and renewable resource, it significantly reduces the ecological footprint and eliminates the risks associated with volatile organic solvents. These findings highlight the potential of coconut water to replace hazardous chemicals in green extraction processes, providing a cost-effective and eco-friendly solution for the industry.

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