Evaluation of flavonoid compound in coconut waste and its antioxidant activity

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Abstract. The coconut tree, Cocos nucifera L., is planted for a variety of purposes. According to the Malaysian Agricultural Research and Development Institute (MARDI), rising coconut production has resulted in an increase in agricultural waste (coconut husk) in Malaysia (536,606 million coconuts in 2018 compared to 22,167 million coconuts in 2016). More than 60% of solid waste degradation in the environment is caused by the manufacture of coconut husks each year. Recycling coconut waste, particularly coconut husk, as a source of natural compounds offers environmental and economic benefits. The objectives of this study are to extract the flavonoid compounds from coconut husk from three different types of coconut fruit (Pandan Coconut, MAWA Coconut and Yellow Coconut) by using ethanolic solvent and compare them. Secondly, the antioxidant activity of coconut husk was determined by using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Thirdly, the alkaline reagent test was used to determine the presence of flavonoid compounds. The flavonoid compounds are existed in the coconut husk ethanolic extract and it have the antioxidant activity. IC50 of the standard compounds, ascorbic acid was 9.2 µg/ml. The most powerful radical scavenging effect was seen in Yellow Coconut husk extract, which had an IC50 value of 8.4 µg/ml. Flavonoids found in the husk of coconuts are known to have antioxidant properties. This shows that coconut husk extract has enormous potential as a natural preservative and a good bioresource of antioxidants.

1. Introduction

The exocarp, which is the outer layer, and the mesocarp, which is the fibrous husk, make up the coconut husk [1]. A sign of the economic significance of the coconut palm is the fact that it is grown in more than 90 nations throughout the world [2]. Coconuts are abundant along tropical countries' coasts, including Malaysia. The growing demand for coconut products like coconut water and coconut fruit pulp has led to an increase in the production of coconut shells and husks, which has a severe impact not only on the environment but also on the economy and society. Many people are unaware that the manufacturing of coconut products generates a significant amount of waste in the form of

coconut husks. Around 85% of the husks from the nearly 50 billion coconuts grown worldwide are tossed as waste, putting fuel to the flame that is global pollution. The husk of the coconut is removed during processing, and millions of tonnes of husk are generated each year. There are around 745,657.1 million tonnes of coconut consumed in Malaysia each year [3]. Malaysia is forced to import coconut from Indonesia and the Philippines to make up for a yearly deficit of 250,126 metric tonnes (mt) caused by overconsumption. Three different types of coconut were utilized in this research which are Yellow Coconut, Pandan Coconut and MAWA coconut. Malayan Yellow Dwarf Coconut plants, often known as Yellow Coconut trees, produce pale yellow fruits when mature. In Malaysia, the Aromatic Green Dwarf, also known as the Pandan coconut, is the preferred premium coconut species for the manufacture of tender drinking nuts. MAWA coconuts are the result of a hybrid between the Malayan Dwarf Variety (MRD or MYD) and the West African Tall. The three types of coconut were used as there were no research was done for those coconut. Nowadays, natural phenolic compounds have grown in popularity as food additives due to their safety and abundance [4]. The presence of such beneficial biological activities indicated that coconut husks are not wastes but rather valuable natural resources. The objectives of the research are to extract the bioactive compounds from coconut husk, to determine the presence of flavonoid compounds and compare them and evaluate the antioxidant activities from coconut husk extract of three different types of coconut fruits.

2. Methodology

Ethanolic extraction method was used to extract bioactive compounds by using 60% ethanol, ascorbic acid was used as a standard. The extraction was performed by soaking approximately 20 g of dried coconut husk in a 200 mL solution of 60% (v/v) ethanol for the 1: 10 ratios for 72 hours at room temperature (26 to 30 °C) in 250 mL conical flask. The aluminium foil paper was used to seal the conical flask and was labelled with a permanent marker. The sealed conical flask with three varieties of coconut husk extracts was placed in bio-shaker at room temperature for 72 hours or three days. Separately, the extracts were filtered through Whatman No.1 filter papers by using vacuum filtration process. The filtrates were concentrated under reduced pressure at 50 °C at 100 rpm using a rotary evaporator (Bibby Sterlin Ltd, UK) to obtain concentrated extracts of coconut husk, respectively. The extraction yield of three different types of coconut husk ethanolic extract was calculated to compare which extract give more percentage yield. The yield of coconut husk extract (%) was calculated as follows:

$$Y_{extract}(\%) = \frac{M_{extract}}{M_{feed}} \times 100 \quad (1)$$

where $Y_{extract}$ is the extraction yield, $M_{extract}$ is the mass of crude extract (g), and M_{feed} is the mass of feed (g).

Alkaline reagent test was conducted to determine the presence of flavonoid compounds. Approximately 2 mL of test solution was treated with a few drops of sodium hydroxide solution and observed for a vivid yellow colour that faded upon the addition of diluted HCl [5]. Figure 1 shows the flowchart representing steps in alkaline reagent test.

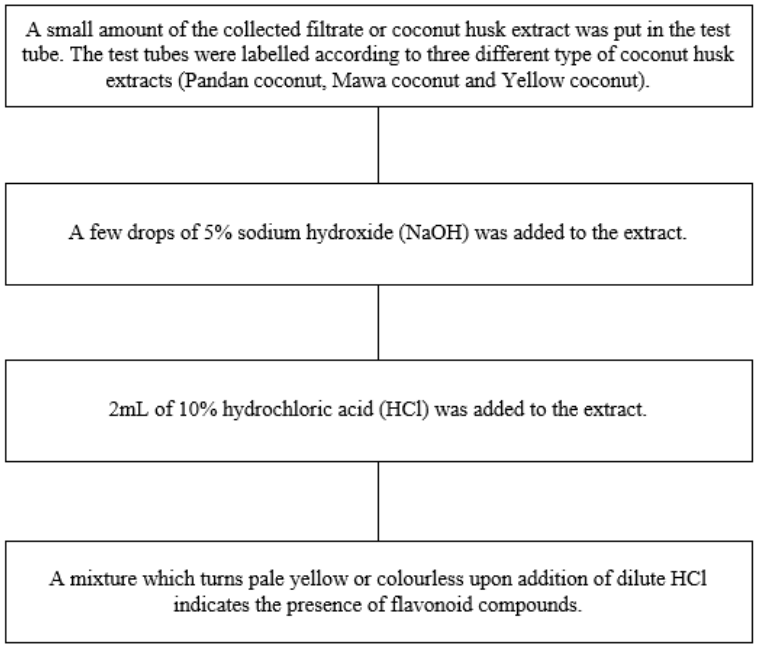

Figure 1. Flowchart representing steps in alkaline reagent test

The antioxidant activities of coconut husk from three varieties were determined by DPPH radical scavenging assay, ascorbic acid was used as positive control. Figure 2 shows the steps for the preparation of DPPH solution while Figure 3 shows the flowchart representing steps in DPPH analysis for the coconut husk extract.

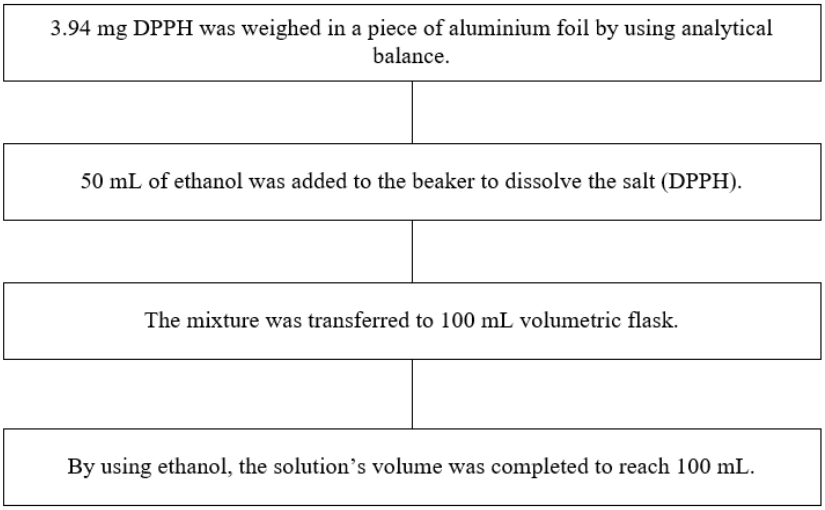

Figure 2. Preparation of DPPH solution

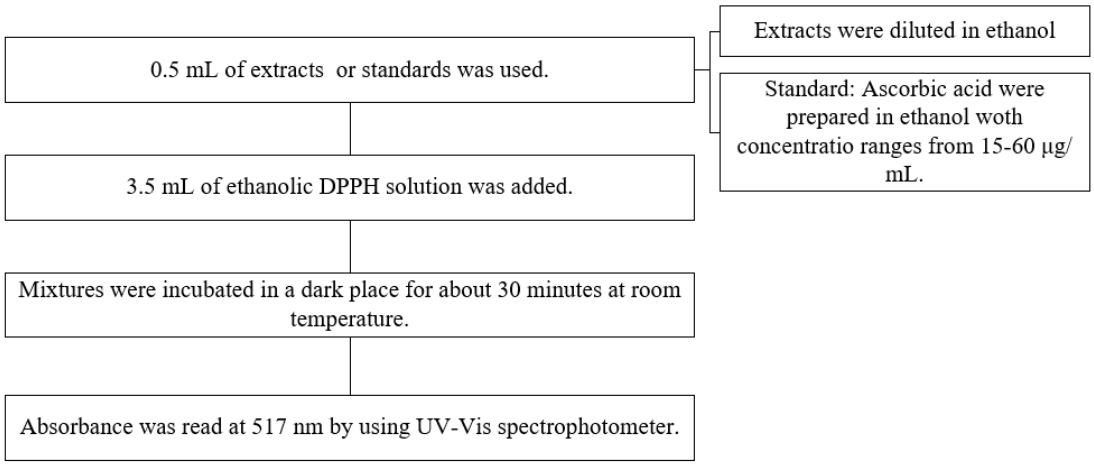

Figure 3. Flowchart representing steps in DPPH analysis

The absorbance of the mixtures was evaluated using UV-Vis Spectrophotometer at 517 nm and DPPH radical scavenging activity of coconut husk extracts was evaluated using equation below:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Abs control at 517 nm} - \text{Abs sample at 517 nm})}{\text{Abs control at 517 nm}} \times 100 \qquad (2)$$

3. Results and Discussion

The extraction yields of ethanolic coconut husk extract are shown in Table 1. Yellow Coconut husk ethanolic extract has highest extraction yield (16.85%) while MAWA coconut husk ethanolic extract has lowest extraction yield (9.65%). The presence of flavonoid compounds was detected using an alkaline reagent test. Ascorbic acid served as a positive control. Positive outcomes were indicated by a change in colour from concentrated yellow colour of mixture to pale yellow colour or colourless [6]. The presence of flavonoid compounds is indicated by the appearance of an intense yellow colour after the addition of sodium hydroxide and a transition to a colourless with the addition of hydrochloric acid. As shown in Figure 4, the positive colour change can be seen. Ascorbic acid (a), Yellow Coconut husk extract (b), Pandan Coconut husk extract (c), and MAWA Coconut husk extract (d) passed the alkaline reagent test. Referring to Figure 5, as the concentration of the extract increases, the absorbance of the DPPH radical decreases. Antioxidants in the extract decrease the absorbance of the DPPH radical at 517 nm, a measure of its radical-scavenging capacity. Maximum ethanol absorption of a stabilized DPPH radical occurs at 517 nm [7]. The radical scavenging interactions between antioxidant molecules and radicals, which result in hydrogen donation, are likely responsible for an antioxidant's ability to lower DPPH radical absorption.

Table 1. The extraction yields of the three different types of coconut husk extract

Varieties	Initial weight (g)	Final weight (g) (Dried extract)	Extraction yield (%)
Yellow Coconut husk (Malayan Yellow Dwarf)	20.00	3.37	16.85
Pandan Coconut husk (Aromatic Green Dwarf)	20.00	2.32	11.60
MAWA Coconut husk	20.00	1.93	9.65

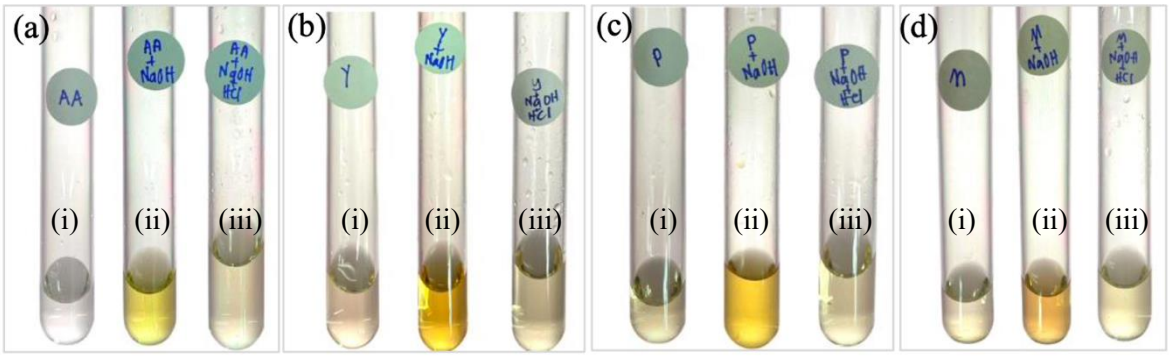

Figure 4. Alkaline reagent test (a) Ascorbic acid (i) Ascorbic acid/extract (ii) Ascorbic acid/extract + NaOH (iii) Ascorbic acid /extract + NaOH + HCl (b) Yellow Coconut husk extract (c) Pandan Coconut husk extract (d) MAWA Coconut husk extract

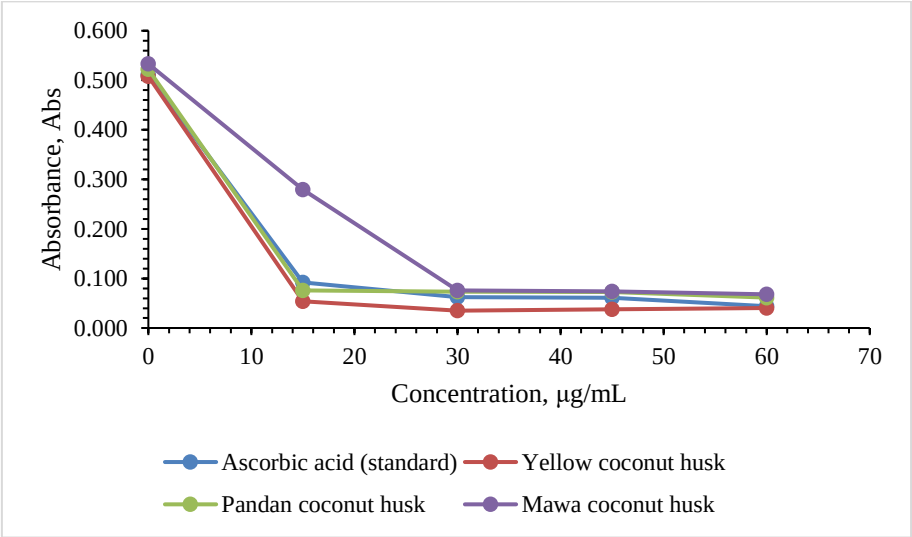

Figure 5. Free radical scavenging activity of the coconut husk extracts

Figure 6 shows the percentage inhibition exhibit by the three different types of ethanolic coconut husk extract and ascorbic acid (standard) used in the study together with amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} of the standard compounds, ascorbic acid was 9.2 µg/ml. The most powerful radical scavenging effect was seen in Yellow Coconut husk extract, which had an IC_{50} value of 8.4 µg/mL. The ability of Yellow Coconut husk extract to scavenge radicals was shown to be significantly more powerful than that of the synthetic antioxidant ascorbic acid. As demonstrated by Yellow Coconut husk extract, a higher flavonoid compound content is directly correlated with a more powerful radical scavenging effect. The computed IC_{50} value of Pandan coconut husk extract at four different concentrations (15 µg/ml, 30 µg/ml, 45 µg/ml, and 60 µg/ml) was 8.8 µg/ml, and MAWA coconut husk extract was 16 µg/ml. The radical scavenging activity in coconut husk extracts decreased in the following order: Yellow Coconut > Pandan Coconut > MAWA Coconut. Consequently, these husk extracts demonstrate moderate free radical scavenging activity. In a dose-dependent way, coconut husk extracts quenched the DPPH free radical. As the concentration of the extract rises, so will its DPPH quenching activity.

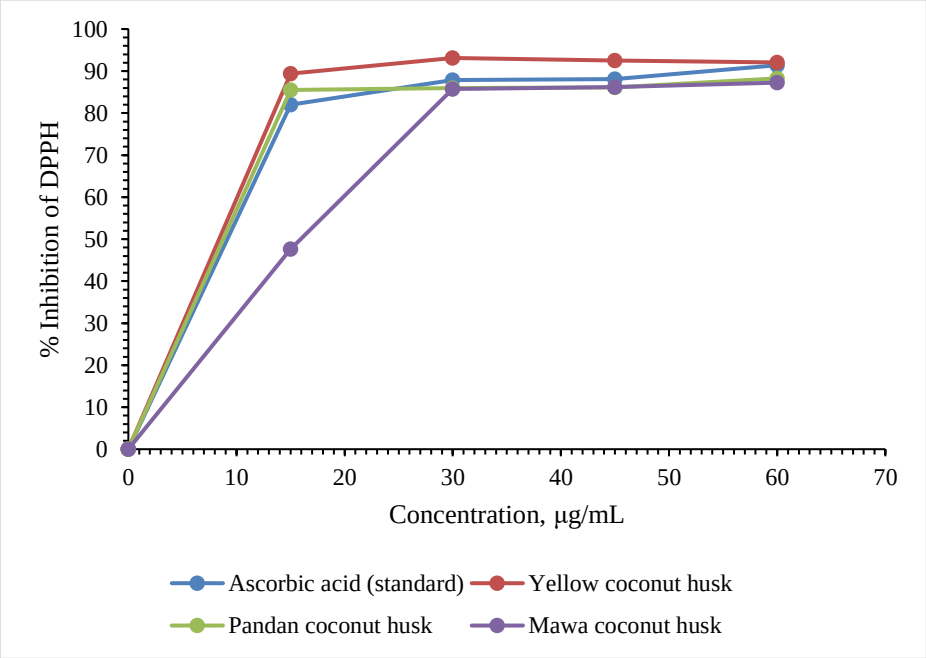

Figure 6. DPPH radical scavenging capacity of coconut husk extracts

4. Conclusion

The extraction of bioactive compounds from coconut husk using a solution containing 60 % ethanol was successful. The use of an alkaline reagent test allowed for the confirmation of the presence of flavonoid compounds in the husk extract of three different types of coconut (Pandan Coconut, Yellow Coconut and MAWA coconut). Because of the changes in colour that occurred in the sample upon the addition of sodium hydroxide (NaOH) and diluted hydrochloric acid (HCl), the presence of flavonoid has been verified. The extracts used in this study also include a high concentration of radical scavengers, often known as antioxidants, like flavonoids. The most powerful radical scavenging effect was seen in Yellow Coconut husk extract, which had an IC_{50} value of 8.4 µg/ml. The extract of Yellow Coconut husk has the maximum number of flavonoid components, exhibited the highest level of antioxidant activity. It is possible that the high radical scavenging property of Yellow Coconut husk extract is related to the presence of hydroxyl groups in the chemical structure of flavonoid compounds. These hydroxyl groups can offer the required component for acting as a radical scavenger. For future works, more research is needed to have more solid answer regarding the quantity of the flavonoid compounds and its antioxidant activities in coconut husk. Further investigation is also needed to identify and quantify the flavonoid compounds in coconut husk which give the highest antioxidant activities.

5. Acknowledgement

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