

Antioxidant Activity of Natural Pigment from Husk of Coconut

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ABSTRACT

Coconuts grow abundantly in the coastal areas of tropical countries. About 33-35% of the coconut is made of husk which includes mesocarp and exocarp. In Malaysia, the coconut husk is available in large quantities as the residue from coconut production. In previous works, natural pigments from the exocarp and mesocarp were extracted using microwave-assisted extraction. The current study was aimed at investigating the antioxidant activities of these pigments extracts. Quantitative determination of total phenolics and antioxidant capacities of these extracts were assayed for their ability to scavenge DPPH radicals and chelate ferrous ion. The total phenolic content, expressed as mg of gallic acid equivalent (GAE) per gram of extract, was found to be 32.24 mg GAE/g and 8.63 mg GAE/g in the mesocarp and exocarp respectively. The radical scavenging activity measurement, expressed in terms of mmol Trolox equivalent (TE) per gram of extract, was significantly ($p < 0.05$) higher in the mesocarp (119.96 mM TE/g) compared with the exocarp (55.27 mM TE/g). Meanwhile, the reducing ability showed significantly ($p < 0.05$) higher value in the mesocarp extract (751.89 mM Fe^{2+} /g) compared with the exocarp extract (264.36 mM Fe^{2+} /g). Thus, this study indicated the possible use of pigment extract as a source of natural antioxidant, which has great potential in the food industry and medicinal applications.

Keywords: Antioxidant, exocarp, mesocarp, microwave-assisted extraction and natural pigment

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INTRODUCTION

Cocos nucifera (coconut palm) is a member of the Arecaceae family and is cultivated mainly in the tropical areas which have high humidity, sandy soil, and regular rainfall. Countries such as India, Sri Lanka, Indonesia, and the Philippines are major

producers of coconut (Probir et al., 2013). In Malaysia, the coconut palm is known as *kelapa*, and is the fourth important industrial crop after oil palm, rubber and paddy in terms of total planted area and is one of the oldest agro-based industries (Saif et al., 2015). Coconut fruit has three layers: mesocarp, exocarp, and endocarp. The exocarp (outer layer) and mesocarp (fibrous husk) make up the husk of coconut (Victor, 2013). Coconut is made up of 33-35% of husk, and in Malaysia, it was estimated that 5280 kg of dry husks become available per hectare per year (Tan et al., 2007). Hence, it is possible to make better use of this abundant and cheap agricultural waste to be converted into natural pigment.

Recently, with public awareness and focus on health as well concerns over eco-safety, environmental friendly and nontoxic bio resource products are regaining popularity in different aspects of our lives. This offers a good chance for the reintroduction of natural dyes that could be considered as an alternative to synthetic dyes, which have been known to cause health problems due to their carcinogenic effects (Prusty et al., 2010). Natural pigments are complex organic molecules that give variety of colours to plants and foods. In addition, dyes are also part of the ingredients in cosmetic, pharmaceutical, paper, textile and leather industries (Shahid et al., 2009). the plant dyes are also responsible for significant plant functions. Colour is an essential factor for the choice of the final product among consumers, especially for the pigments used in food industry (Boo et al.,

2012). Among all natural dyes, plant-based pigments provide a huge range of medicinal values (Monika et al., 2013). Plants consist of various antioxidants including tannins, flavonoids, and lignin precursors, which act as radical oxidative stress-scavenging compounds (Paramita & Camelia, 2016). For this reason, in vitro antioxidant activities of the mesocarp and exocarp pigment extracts were determined in order to examine any antioxidant component available in the pigment extracts that might have potential to become a natural colorant in food or non-food system. The aim of the current study is to compare the polyphenolic contents and antioxidant activities of sample extracts with standard commercial antioxidants.

MATERIALS & METHODS

Preparation of Samples

C. nucifera was collected from Tanjung Karang, Selangor. Mesocarps and exocarps of the brown-coloured coconuts were utilised in this study. The exocarp was separated from the mesocarp prior to cutting it into smaller pieces and oven dried at a temperature of 60°C for 24 hours. The samples were ground, sieved using a 0.5 mm sieve and kept in a clean plastic container, away from heat and moisture prior to conducting the experiment.

Methods of Extraction

Microwave-assisted Extraction. Extraction of the samples followed Asma Fhadhila et al. (2016). Microwave assisted extraction was performed in an experimental microwave

oven (Samsung, Korea). About 2 g of mesocarp and exocarp samples from the same batch were transferred into a conical flask containing 40 mL of 0.1 M NaOH (ratio of 1:20), each, and heated at 300 W for 2 minutes. The samples were prepared in triplicates. After heating the mixtures in the microwave, they were placed in conical flasks and were allowed to cool down at room temperature and filtered using a filter paper (150 mm [CHM, Germany]). All the filtrates from both treatments were kept at 4°C in the dark prior to analysis.

Determination of Antioxidant Capacity

Total Phenolic Content (TPC). Total phenolic content was determined according to the method described by Santas et al. (2008). A volume of 200 µL extract was mixed with 1.5 mL of Folin-Ciocalteu reagent (1:10 v/v with distilled water) prior to incubation at room temperature for 5 minutes. Later, 1.5 mL of sodium carbonate (Na₂CO₃, 0.566 M) was added to the sample and mixed thoroughly. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Genesys 20, USA) after 90 minutes of incubation in the dark. Standard gallic acid within the range of 0.000-0.125 mg/mL was treated similarly as the 200 µL of sample extract. The results were expressed as mg of gallic acid equivalents per gram sample.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay. The DPPH free radical scavenging activity was determined according to the method proposed by Brand-William et

al. (1995). A volume of 60 µM of DPPH solution in methanol was prepared prior to mixing 3.9 mL of the solution with 0.1 mL extracts. The samples were kept in the dark for 30 minutes at room temperature (27°C), and the absorbance was measured using a spectrophotometer (Genesys 20, US) at 517 nm with methanol as the blank. Standard Trolox with the range of 100–500 µM/mL was treated similarly as 0.1 mL of the sample extract. The results were expressed as mg of Trolox equivalents per gram sample. The DPPH radical scavenging activity was also expressed as the inhibition percentage (IP) of free radical by the sample and was calculated based on the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_1} \times 100$$

A₀ refers to the absorbance of the control reaction containing all reagents except the tested compound, and A₁ refers to the absorbance of the test compound.

Ferric Ion Reducing Antioxidant Power (FRAP). The FRAP assay was performed according to the method described by Benzie and Strain (1999) with slight modifications. Reagents included 300 mM acetate buffer with pH 3.6, 40 mM hydrochloric acid, 10 mM TPTZ solution (dissolved in 40 mM HCL) and 20 mM ferric chloride solution. The working FRAP reagent was freshly prepared on the day of analysis by mixing acetate buffer (100 mL), TPTZ solution (10 mL) and ferric chloride solutions (10 mL) in the ratio of 10:1:1 before incubation at 37°C. As for the blank, 3.0 mL of working

FRAP reagent was mixed with 100 μ L of 0.1 M NaOH (solvent used to extract the sample). An amount of 100 μ L sample was mixed with 3.0 mL of working FRAP reagent, and the absorbance at time zero (A_0) and after 4 min (A_4) was recorded using spectrophotometer (Genesys 20, USA) at 593 nm. The calculated differences in the absorbance are proportional to the ferric-reducing properties of the antioxidants present in the extracts. For quantification, a calibration curve of ferrous sulphate was prepared with dilutions from 0.1–1 mM. The final results were expressed as mM Fe^{2+} equivalents per gram sample.

Statistical Analysis

All results in this study were expressed as mean \pm standard deviation of three replicates. The statistical significance was evaluated using Student’s t-test and set at <0.05 . Statistical analysis was conducted using SPSS 12 software package for Windows (SPSS Inc. USA.)

RESULTS & DISCUSSIONS

Total Phenolic Content (TPC)

The results of total phenolic content in the mesocarp and exocarp extracts are presented in Figure 1. The content of phenolic compound was significantly ($p<0.05$) greater in the mesocarp (33.24 mg GAE/g) compared with the exocarp (8.63 mg GAE/g). However, studies by Amin and Chew (2006) and Yapo et al. (2013), showed that the cocoa shells and cocoa pod husk were found to contain extremely higher total phenolic content of 112.9 mg GAE/g and 69.0 mg GAE/g respectively compared with mesocarp and exocarp. In addition, walnut husk extracts contained 32–74 mg GAE/g of total phenolic content (Oliveira et al., 2008). However, the phenolic content of corn husk (Dong et al., 2014) and Thai rice husk (Butsat, and Siriamornpun, 2010) was moderately lower than mesocarp and exocarp with a value of 2.98 mg GAE/g and 2.2 mg GAE/g respectively. Total phenolic content of green tea was in a range of 16.02 to 233.68 mg GAE/g (Shrififar et al., 2003).

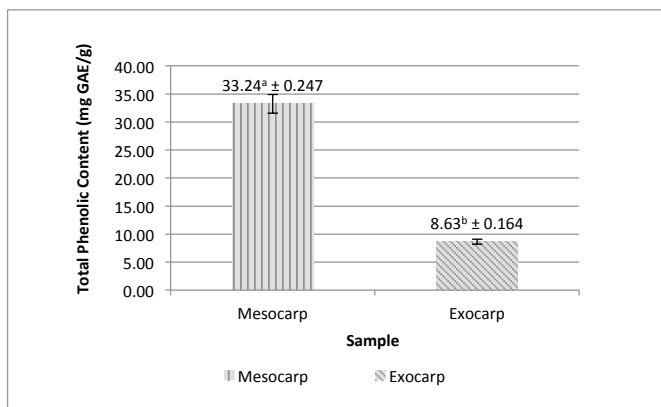


Figure 1. Total phenolic content of mesocarp and exocarp pigment extracts
 Note: Values marked with different superscript letters indicate significant differences between mesocarp and exocarp (independent t-test, $p<0.05$)

Chalinee et al. (2016) reported that ethanol and aqueous extracts of coconut (dried fruit) had total phenolic content of 2.21 mg GAE/g and 4.36 mg GAE/g respectively. Pigment extracts of the mesocarp and exocarp have relatively higher values compared with dried fruit coconut. Although the different extraction medium used may contribute to discrepancies between the present result and previous research, the higher total phenolic content of mesocarp and exocarp can be attributed to the extraction method used in this study, which employed microwave-assisted extraction.

Theoretically, microwave radiation loosens the cell wall matrix, causing severed parenchymal cells (Kratchanova et al., 2004). This in turn initiates rapid and extensive opening of the skin tissues, thus leading to improved interaction between the extracting agent and bioactive compound in the extraction procedure. As a result, permeation of the extracting medium solution will be enhanced, leading to effective increase in the yield of bioactive compound being extracted. Improved extraction yields due to microwave heating have also proven for the extraction of flavonoids (Zhang et al., 2013), anthocyanins (Liazid et al., 2011) and phenolic compounds (ballard et al., 2010).

In fact, sodium hydroxide as an extracting medium in this present study may influence the extraction process. As pointed out by Saxena and Raja (2014), many dyes have low water solubility allowing only water-soluble dye components to be extracted, causing low yields of dyes using

aqueous extraction. On the other hand, as dyes usually occur in the form of glycosides extractable under an alkaline condition, the alkaline extraction is said to be suitable for dyes having phenolic groups since they are soluble in alkali, thus improving the dye yield. The results are similar to those of Naczki and Shahidi (2006) who reported that the recovery of polyphenols from plant materials is affected by solubility of its phenolic compounds in the solvent used for the extraction procedure. Moreover, solvent polarity contributes to the degree of phenolic solubility.

Phenolic compounds are recognised as antioxidant and scavenging agents against free radicals related to oxidative damage (Ferguson et al., 2006). The oxidation process is one of the most essential routes for producing free radicals in drugs, food and even living systems (Pourmorad et al., 2006). Free radicals cause many human diseases including atherosclerosis, arthritis, Alzheimer's, cardiac reperfusion abnormalities, cancers, neurodegenerative disorders and aging (Sarma et al., 2010). Notably, Yu et al. (2003) pointed out that phenolics have been found to strong antioxidants to hinder the influence of free radicals and reactive oxygen species (ROS), which is the basis of several chronic human infections. Furthermore, there is significant focus in the consumption of certain foods to prevent illness. Thus, diets rich in phenolic compounds can be recommended to improve human health due the effects of phenolic antioxidants (Naczki & Shahidi, 2004).

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The antioxidant ability and radical scavenging properties of plants are related to their medicinal properties. In this study, the antioxidant activities of the mesocarp and exocarp pigment extract were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The DPPH radical is a

stable organic nitrogen radical, and the test is quickly and simple which may explain its common use in antioxidant screening (Madhujith & Shahidi, 2006). The mesocarp extract showed significantly ($p < 0.05$) higher antioxidant activity (119.96 mM TE/g) compared with the exocarp, which showed 55.27 mM TE/g (Figure 2).

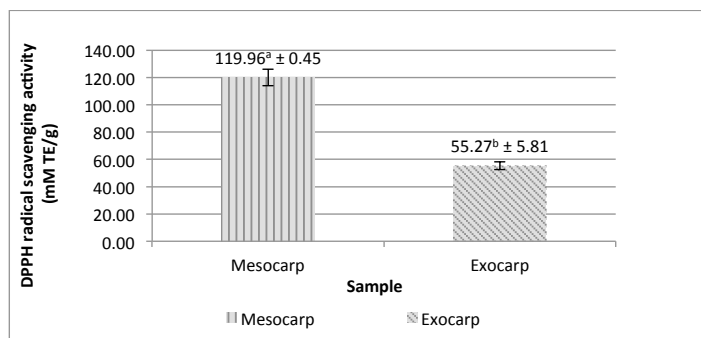


Figure 2. DPPH radical scavenging activity of mesocarp and exocarp pigment extracts

Note: Values marked with different superscript letters indicate significant differences between mesocarp and exocarp (independent t-test, $p < 0.05$)

However, the findings from a previous study by Martinez et al. (2012) contradicted with this study. In the earlier study, the DPPH levels of cocoa pod husks and cocoa bean shells were considerably lower than the mesocarp and exocarp with 0.033 mM TE/g for the cocoa pod husk and 0.004 mM TE/g for the cocoa bean shells. High antioxidant activity exhibited in the mesocarp and exocarp may have been due to the existence of colouring pigment in the extract. The presence of colouring pigment in the sample could be due the bioactive compounds such

as chlorophyll, carotenoids, and phenolics (Lancaster, 1997).

The antioxidant activities of the mesocarp and exocarp pigment extracts are also expressed and quantified in terms of inhibition percentage (IP). In the present study, the mesocarp extract proved to be a few times less powerful to scavenge DPPH radicals compared with the antioxidant activity of pure standard, Trolox. However, due to the low value of inhibition percentage, results revealed that the exocarp extract was not considered as an effective DPPH radical

scavenger either when compared with Trolox (Figure 3). The higher the amount of antioxidants in the extract, the more the DPPH reduction. High drop of DPPH is

connected to the high scavenging activity presented by a particular sample. At a higher concentration, these extracts may display significant free scavenging activities.

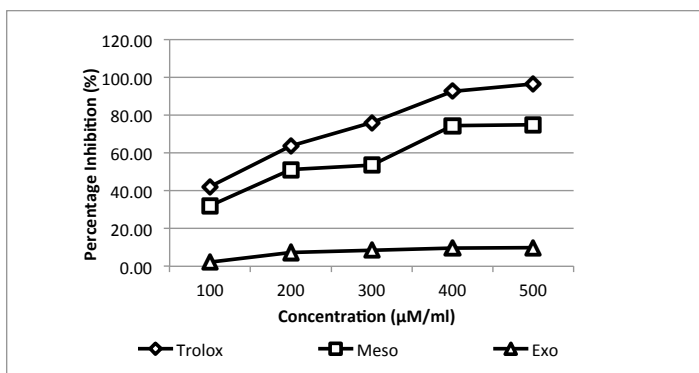


Figure 3. DPPH inhibition of pigment extracts mesocarp and exocarp compared with Trolox

Ferric ion Reducing Antioxidant Power (FRAP)

As shown in Figure 4, the mesocarp pigment extract has significantly ($p < 0.05$) higher antioxidant capacity using the FRAP method that exhibited 751.89 mM Fe²⁺/g compared with the exocarp extract, which showed 264.36 mM Fe²⁺/g. Dong et al. (2014) reported the FRAP value of cornhusk extracted by 80% ethanol was only 0.002 mM Fe²⁺/g, whereas the FRAP value of Thai rice husk was in the range of 0.012 to 0.028 mM Fe²⁺/g (Butsat & Siriamornpun, 2010). On the other hand, Xiang et al. (2016) had isolated compound from Chinese

hickory husks and the findings pointed to antioxidant activity in the FRAP assay with moderate values of 10.34–10.91 mM FeSO⁴/g. Results of the current study indicated that the values of the mesocarp and exocarp pigment extracts were higher than FRAP values of the other types of husk. In addition, these also show that the husk from coconut provides significant antioxidant activity. This means that the husk can no longer be regarded as a worthless part of the coconut. The high antioxidant activities of the mesocarp and exocarp pigment extracts suggest their use in folk medicine.

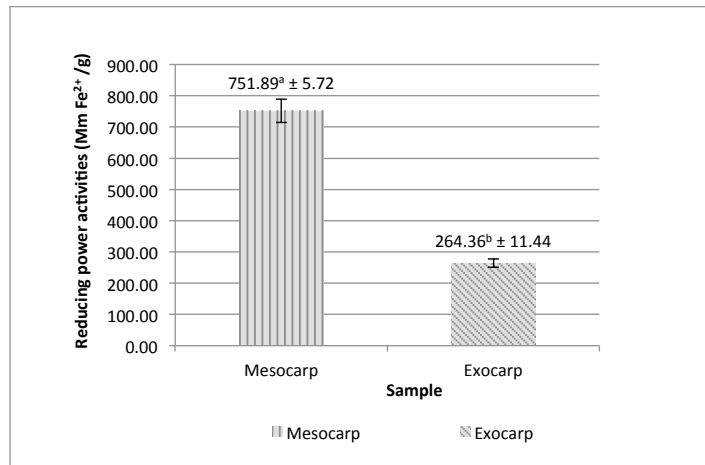


Figure 4. Reducing power activity of mesocarp and exocarp pigment extracts

Note: Values marked with different superscript letters indicate significant differences between mesocarp and exocarp (independent t-test, $p < 0.05$)

Generally, the reducing properties are related to the existence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom. The FRAP assay determines the change in absorbance at 593 nm due to the formation of a blue-coloured complex of ferrous ion (Fe^{2+}) and 2,4,6-tripyridyl-s-triazine, (TPTZ). In addition, a colourless ferric ion (Fe^{3+}) gets oxidised to ferrous ion (Fe^{2+}) by the action of electron-donating antioxidant (Sumitra et al., 2013). The formation of blue colour evaluated spectrophotometrically at 593 nm is taken as linearly connected to the total reducing capacity of electron-donating antioxidants (Mohd et al., 2013).

The different values among antioxidant assays were attributed to the different chemistry principle as the basis of each method. The DPPH and FRAP methods are based on a single electron transfer (SET) reaction in which antioxidants are oxidised by oxidants, namely metal (Fe III) or a

radical (DPPH). As a result, a single electron is transferred from the antioxidant molecule to the oxidant (Mohd et al., 2013). This study pointed to the ability of the mesocarp and exocarp pigment extracts either to quench or to reduce the radicals generated in the assays.

CONCLUSION

This study examined the antioxidant utility of coconut husk, which might be useful in establishing its therapeutic values. The mesocarp and exocarp extract from the husk of coconut showed antioxidant efficacy in all three analyses, namely TPC, DPPH, and FRAP. Thus, coconut husk can be an effective antioxidant despite it being commonly viewed as waste. The study suggests that this extract is a possible source of natural antioxidant that could be of great importance to counter age-associated illness and free radical-related disease.

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