Research Article

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Phytochemical Properties and Antimicrobial Activity of Natural Colorant Extracted from Mesocarp and Exocarp of *Cocos nucifera*

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Abstract

Phytochemical analysis was carried out on the natural colorant extracted using the microwave-assisted extraction which performed at a microwave power of 300 watts for 2 minutes. The pigment compound was extracted from mesocarp and mesocarp of *Cocos nucifera* by using 0.1 M NaOH as extracting agent. The qualitative analysis that was carried out for these natural colorants confirmed that flavonoids, terpenoids, cardiac glycosides and phenol/tannins were present in both plant parts (mesocarp and exocarp) tested whereas alkaloids, saponins, steroids and anthraquinone were not detected. Quantitative analysis showed that tannin content was significantly ($p < 0.05$) higher (134.82 mg TAE/g) in the mesocarp extract compared to the exocarp extract (33.90 mg TAE/g). The amount of flavonoids was low in both extracts (mesocarp: 15.48 mg QE/g and exocarp: 28.45 mg QE/g, respectively). The antimicrobial study discovered that the mesocarp and the exocarp extract were not effective as antibacterial agents.

Keywords: Coconut palm, Natural dye, Microwave-assisted extraction (MAE)

Introduction

The world demand for natural dyes is estimated to be 10,000 tonnes, which is equivalent to 1% of the world synthetic dyes consumption. This is expected to grow tremendously in the near future [1]. Rising demand for eco-friendly and non-toxic colorants particularly for food coloration and children toys and textile could be seen following a growing concern about the safety of some commonly use synthetic dyes [2][3]. The safety of goods containing synthetic dyes has been a point of debate for decades owing to the claims of their toxicity and carcinogenic effects [4]. Additionally, the applications of synthetic dyes have been associated with detrimental effects on the environment and health [5] whereas non-toxic of natural dyes have recently re-emerged as a potential viable 'Green chemistry' option as an alternative

to synthetic dyes [6][7].

Since time immemorial, products which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine, thus indicating that every part of a plant may contain important active compounds [8]. Phytochemicals are natural bioactive compounds which are present in plants. These natural compounds work with nutrients and dietary fibers to protect animals and man against diseases. Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [9][10].

Cocos nucifera (coconut) is a member of the family

Arecaceae (palm family), an ornamental tree grew in the rural and urban area in Malaysia [11]. In Malaysia, coconut is the fourth most important plantation [12]. The fruit is a three-sided drupe. Its wall, or pericarp, consists of three distinct layers: a smooth thin rind or exocarp, which is green to begin with, but then changes to brown or reddish-brown on maturity; a large brown fibrous middle region (or the mesocarp) forming the husk; and, a hard stony endocarp which forms the coconut shell, enclosing the seed inside [13]. Coconut husk corresponds to around 85% of the fruit weight and disposal is also problematic [14]. Conversion of these residues to natural dye can provide an alternative strategy for managing agronomic waste disposal. In Malaysia, there is an abundance of coconut wastes which should be recycled rather than discarded and this issue has long been overlooked. Keeping this in view, the present research was performed to identify the phytochemical constituents in natural colorant extracted from mesocarp and exocarp of *Cocos nucifera* and to explore the antimicrobial properties of these extracts against selective gram positive and negative bacteria.

Materials & Methods

Sample preparation

The exocarp (outer layer) and mesocarp (fibrous husk) of *Cocos nucifera* (coconut) were obtained from Tanjung Karang, Selangor (Malaysia). The exocarp was separated from the mesocarp, cut into a small fragment and dried in an oven at 60°C for 24 hours. The samples were then ground using a grinder, sieved to pass through a 0.5 mm sieve and kept in a clean airtight plastic container.

Methods of extraction

Microwave-assisted extraction: Mesocarp and exocarp alkaline extract were recovered using a microwave assisted extraction technique. About 10 g of each mesocarp and exocarp samples were transferred into a conical flask containing 200 ml of 0.1 M sodium hydroxide (ratio of 1:20). The mixtures were then heated in a microwave (Samsung, Korea) at 300W for 2 minutes [15]. After microwave heating, the mixtures were allowed to be cooled to room temperature before filtered using filter paper (150 mm) (CHM, Germany). All the filtrates were kept at 4°C in the dark prior to analysis. Experiments were performed in triplicates.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of alkaline extracts of mesocarp and exocarp was conducted according to the standard of procedures [16][17]. Tests were conducted to detect the presence of secondary metabolites, such

as alkaloids, saponins, flavonoids, steroid, cardiac glycosides, tannins, terpenoids and anthraquinone.

Quantitative phytochemical analysis

The phytochemicals which were present in the alkaline extracts of mesocarp and exocarp were determined and quantified by standard procedures as follow.

Determination of Total Phenolic Content: Approximately, 200 µL sample was added to 1.5 mL of diluted Folin-Ciocalteu reagent (1:10, v/v) and was incubated for 5 minutes at room temperature [18]. The mixture was then added with 1.5 mL of 0.566 M Na₂CO₃. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Genesys 20, United State) after 90 minutes of incubation. The standard gallic acid range 0-125 mg/ml was constructed and the same analysis procedure as samples was conducted. The result was expressed as mg of GAE per amount of sample in g.

Determination of Tannins: The percentage composition of tannin in the extract was determined using Swain's method [19] with some modifications. One ml of sample extract was pipetted into a 50 ml volumetric flask consist of 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na₂CO₃. The mixture was made up to mark to 50 ml with distilled water, mixed well and allowed to stand for 20 minutes until bluish-green coloration developed. Standard tannic acid solutions of range 0-500 ppm were treated similarly as the sample above. The absorbance of the tannic acid standard solutions as well as the samples was read using a spectrophotometer (Genesys 20, United State) at a wavelength of 760 nm after the bluish-green color was fully developed. Tannin content was determined by a tannic acid standard curve and expressed as mg of tannic acid equivalence (TAE) per 100 g of dried sample.

Determination of Flavonoid Content: Aluminium chloride colorimetric method [19] was used with some modifications to determine flavonoid content. One milliliter of the extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 minutes. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. The absorbance was measured at 420 nm. Quercetin was used as standard (1 mg/ml). Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of the extracted compound).

Determination of Cardiac Glycosides: Cardiac glycosides were determined according to Ibraheem and Maimako [20]. Relatively, 2 g of ground plant parts were weighed and mixed with 20 ml of 0.1 M NaOH. The mixtures were incubated in an orbital shaker incubator (Stuart, United Kingdom) with an agitation speed of 300 rpm for 6 hours at room temperature. The mixtures were filtered using Whatman No. 1 filter papers before transferred into 1 L volumetric flasks. Each flask was added with 500 ml of distilled water and then followed by 100 ml of 12.5% lead acetate. Volumes were made up to 800 ml with distilled water and vigorously shaken (300 rpm for 10 minutes) before 200 ml of 4.77% disodium hydrogen phosphate (Na_2HPO_4) solution was added. Resultant solutions were filtered separately through Whatman No. 1 filter papers to give a clear filtrate. The filtrates were then evaporated to dryness. The percentage of cardiac glycosides content was calculated as follows:

$$\% \text{ Cardiac glycosides} = \frac{\text{Weight of dried extract, g} \times 100}{\text{Weight of dried ground plant sample, g}}$$

Determination of Terpenoid: For this purpose, a method described by Indumathi et al. [21] was adopted. For each 200 μL of extract, 1.5 mL of chloroform and 100 μL sulphuric acids was added before the mixture was vortexed thoroughly. The mixture was then incubated at room temperature for 2 hours in the dark. At the end of incubation time, a reddish brown precipitation was formed and the solution mixture was gently and carefully decanted without disturbing the precipitation. Approximately, 1.5 mL of 95% methanol was added and vortexed thoroughly until all the precipitation was completely dissolved. Subsequently, absorbance was read at wavelength 538 nm. Linalool standard was also prepared (100 mg/200 μL to 1 mg/200 μL) and treated as the sample but incubation was performed not more than 5 minutes according to standard procedure. In serial dilution of standard, a total volume of 200 μL was made up by addition of 95% (v/v) of methanol. In this analysis, methanol was used as a blank.

Antimicrobial study

Bacterial Strains and Culture Conditions: Antibacterial activity of *Cocos nucifera* extracts was tested against three Gram-positive – *Bacillus subtilis*, *Bacillus cereus*, Methicillin-resistant *Staphylococcus aureus*; and three Gram negative-*Salmonella thypi*, *Salmonella enteritidis*, *Escherichia coli*. The bacterial strains were obtained from Forest Research Institute Malaysia (FRIM)

and Institut Penyelidikan Produk Halal (IPPH) at Universiti Putra Malaysia. Broth containing the bacteria strains were separately streaked onto selective agar and incubated for 24 hrs at 30°C before further cultured in Tryptic soy broth in order to get uniform cultures.

Preparations of Test Disks: Extracts of mesocarp and exocarp were dissolved into several concentration (250 mg/mL, 500 mg/mL & 1000 mg/mL) using dimethylsulfoxide (DMSO). Then sterile discs (6 mm in diameter) were impregnated with 30 μL of each extract. Discs impregnated with DMSO were prepared as negative controls and chloramphenicol (30 μg) was used as the positive control.

Antibacterial Susceptibility Test: The concentration of broth containing bacteria strains which was earlier cultured in Tryptic soy broth was adjusted to 10^8 cfu/mL with a sterilized saline solution in order to match 1.0 McFarland standards [22]. Antimicrobial activity of the mesocarp and exocarp extracts was evaluated by using disc-diffusion method [23]. Approximately 20 mL of sterilized Mueller Hinton agar was poured into a sterile petri dish. After solidification, 100 μL of microbial inoculum were swabbed on the respective plates. By using a sterile forcep, the impregnated discs were placed on cultured plates. The plates were incubated at 37°C for 24 hrs. The inhibition zone of each microorganism by each plant extract was then measured.

Statistical analysis

Experiments were carried out in triplicates, and the results were expressed as means $\pm$ SD (standard deviation). One way ANOVA was used to analyze the experimental data and a value of $p < 0.05$ was considered statistically significant.

Results and Discussions

Qualitative phytochemical analysis

The phytochemical characteristics of natural colorant extracted from mesocarp and exocarp of *Cocos nucifera* were summarized in table 1. The results revealed the presence of bioactive compounds in the extracts studied. From the result, it could be seen that flavonoids, cardiac glycosides, terpenoids, phenols and tannins were present in all the extracts. Phenolics, tannins, flavonoids, terpenoid and cardiac glycosides which were present in both extracts are important secondary metabolites. Whereas, alkaloids, saponins, steroids and anthraquinone were absent in all samples.

A study has reported that ethanolic extracts of coconut fiber (mesocarp) revealed the presence of phenols, tannins, flavonoids, triterpenes, steroids and alkaloids while butanol extracts recovered triterpenes, saponins and tannins [13]. Quantity and quality of phytochemicals vary from one plant to another although they vary from the same species. This may be associated with lots of variations such as the geographical location of the plant, season, soil topography and nutrients content, plant age; as plants do possess different levels of secondary metabolites subject to their metabolic state that is well connected to their cellular, developmental and environmental statuses [24]. Besides, different extracting solvent used for the extraction might be slightly affected the result obtained.

Table 1: Phytochemical analysis of natural colourant extracted from mesocarp and exocarp of *Cocos nucifera*.

Compounds	Mesocarp	Exocarp
Alkaloids	-	-
Saponins	-	-
Flavonoids	+	+
Steroid	-	-
Cardiac glycosides	+	+
Phenol	+	+
Tannins	+	+
Terpenoids	+	+
Anthraquinone	-	-

Note: + = Present; - = Absent

Quantitative phytochemical analysis

The amounts of phytochemicals available in the natural colorant of mesocarp and exocarp of *Cocos nucifera* were quantitatively determined by standard procedures. The quantitative phytochemical estimation present in natural colorant showed that the mesocarps are very rich in total phenolic content, tannin and terpenoid (Table 2). While colorant extract from exocarps was slightly higher in cardiac glycosides and flavonoids.

Tannin Content: Tannins have been reported to be the most important ingredients in natural dyes, especially to brown shades [25]. As displayed in table 2, the total tannin was significantly higher in mesocarp (134.82 mg/g) compared to exocarp (33.90 mg/g). According to the result, total tannin content of mesocarp and exocarp is comparable with a very well known natural standard, green tea, which reported to be (42.1 mg TAE/g) [26] and methanolic extract of oil palm leaves that exhibited

165.00 mg TAE/g [27]. However, tannins are dietary anti-nutrients that are responsible for the astringent taste of foods and drinks [28]. Tannins also bind to both proteins and carbohydrates which later affect the human absorption of both nutrients. Their presence can cause browning or other pigmentation problems in both fresh foods and processed products. Based on these finding, natural colorant (mainly mesocarp) might not suitable to be applied as natural food colorant unless the addition of tannin to the product is generally for preservation purposes. Furthermore, other studies have been reported that tannin possesses antibacterial and antiviral effect [29][30], stimulate the wound healing and burns [31] and effectively protecting kidneys [32].

Total Phenolic Content: Phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Analysis of the mesocarp and exocarp extracts depicted the presence of constituents which are known to exhibit medicinal as well as antioxidant properties such as phenolic compounds (Table 2). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [33][34]. The total phenolic content of the natural colorant from mesocarp was significantly different from exocarp which was 33.23 mg GAE/g and 8.63 mg GAE/g dry weight of plant material, respectively. Chalinee et al. [35] reported the total phenolic content of *Cocos nucifera* was 2.21 mg GAE/g in ethanol extract and 4.36 mg GAE/g in aqueous extract. However, other studies 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 [36][37].

Flavonoid Content: As depicted in table 2, flavonoid content was significantly higher in exocarp (28.45 mg QE/g) compared to mesocarp (15.48 mg QE/g). Flavonoids are secondary metabolites of low molecular weights that are produced in the cytosols and vacuoles of many plant cells [38]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms in vitro. In fact, they are also an effective antioxidant and showed strong anticancer activities [32][39][40].

Cardiac Glycoside: Based on the quantitative study (Table 2), this colorant has a moderate quantity of cardiac glycosides in both parts of the plant (mesocarp: 43.7%

and exocarp: 44.4%). A similar outcome was reported by Odenigbo and Otisi [41] who claimed that cardiac glycoside was in a moderate amount in aqueous and n-hexane extracts of Nigerian coconuts, but the values were not reported. Glycosides are known to lower the blood pressure [42]. Recent studies have reported that cardiac glycosides are used in the treatment of cardiac congestion and a few types of cardiac arrhythmias. In fact, preclinical research has shown that cardiac glycosides can inhibit both cancer cell proliferation at very low concentrations and induce potent anticancer effects in mice [43].

Terpenoid Content: The terpenoids are the primary constituents of most essential oils. They are widely used in traditional medicines for aromatherapy [44]. Table 2 shows the terpenoid content of mesocarp and exocarp which are relatively high (1535.61 mg/g and 1057.20 mg/g, respectively). Terpenoids are reported to have anti-inflammatory, anti-bacterial, anti-viral, and anti-malarial activity and ability to inhibit cholesterol synthesis [45][46]. This present result has an agreement with the finding

from Parker et al. [47] who studied the endosperm of *Cocos nucifera*.

Antimicrobial activity

Antimicrobial activity of dye extracts was examined based on the diameters of clear inhibition zones surrounding the paper disks. If there is no inhibition zone, it implicit that the dye extract did not possess antimicrobial activity towards the studied bacterial strains. From the results obtained, dye extract from mesocarp and exocarp did not show any antimicrobial activity against the six bacteria tested (Table 3). The current study did not show any inhibition zone may be due to the low concentration of the bioactive compound in the crude extract of the mesocarp and the exocarp. Plant extracts which did not show any antibacterial activities did not indicate that the plant and their bioactive constituents are absent. Active constituents could be present in small amount in the crude extracts and caused negative activity due to the low dose concentration [48]. In addition, the extracts may be active against other bacterial species which were not tested in this study [49]. Besides, bacteria shows unpredictable

Table 2: Quantitative analysis of phytochemicals in natural colourant extracted from mesocarp and exocarp of *Cocus nucifera*.

Sample	Phytochemical constituents				
	Total Phenolic Content (GAE/g)	Cardiac glycoside (%)	Flavonoid (QE/g)	Tannin (TE/g)	Terpenoid (LE/g)
Mesocarp	33.23a±0.247	43.73a±1.007	15.48b±1.876	134.82a± 2.16	1535.61a± 2.16
Exocarp	8.63b ±0.164	44.43a±3.21	28.45a±0.79	33.90b ± 0.19	1057.20b± 4.75

Values are represented as means ± standard deviation for each phytochemical constituents, means that do not share the same letter in the same column were significantly different at $p < 0.05$. GAE: gallic acid equivalent; QE: quercetin equivalent; TE: tannic acid equivalent; LE: linalool equivalent

Table 3: Antimicrobial activity of dye extracts from mesocarp and exocarp of *Cocus nucifera* against the bacterial strains tested based on disc-diffusion method.

Microorganism	Antimicrobial activity by agar diffusion							
	Mesocarp extract (µL/mL)			Exocarp extract (µL/mL)			Control	
	250	500	1000	250	500	1000	CH	DMSO
Bacillus cereus	–	–	–	–	–	–	++	–
Methicillin-resistant Staphylococcus aureus	–	–	–	–	–	–	++	–
Salmonella thypi	–	–	–	–	–	–	+	–
Salmonella enteritidis	–	–	–	–	–	–	++	–
Escherichia coli	–	–	–	–	–	–	++	–

Note: (–): no inhibition; (+): diameter of inhibition zone > 10 mm; (++): diameter of inhibition zone > 10 mm; CH: Chloroamphenicol (30 ug/disk) was used as the positive reference standard; DMSO: Dimethylsulfoxide was used as negative reference standard.

sensitivity to chemical substances related to different resistance level between strains. Furthermore, there are also differences in the antimicrobial effects of plant groups, mainly due to phytochemical properties and differences among species [50].

Conclusion

The results obtained exhibited that the colorant extract (mesocarp and exocarp) have constituents of flavonoids, cardiac glycosides, terpenoids, phenols and tannins. From this research, the presence of phenolic compounds such as terpenoids and flavonoids may contribute to the antioxidant activities of this colorant. However, both extracts did not demonstrate antimicrobial activity due to no inhibition zones surrounding both extracts against all tested bacteria. It is suggested that further work should be done to establish the cytotoxicological activities of these phytochemicals in order to ascertain its biosafety for human use.

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