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POTENTIALITY OF INCORPORATING COCOA LIQUOR IN SKIN CARE COSMETICS

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ABSTRACT

Halal cosmetics, generally derived from plant-based materials, are receiving much interest amongst health-conscious consumers and cosmetic industry. Cocoa liquor, a paste produced from ground cocoa (*Theobrama cacao* L.) beans, is a natural source of antioxidants with potential health benefits. The present study was conducted to determine the prospect of incorporating cocoa liquor in skin care cosmetics complementing its ability in protecting the skin by warding off free radicals from the environment. The amount of total phenolic contents (TPC) in cocoa liquor was determined by Folin-Ciocalteau colorimetric method using gallic acid as a standard, and various concentrations of extract sample were measured at 765 nm. Total flavonoid contents (TFC) were measured by using aluminium chloride colorimetric assay. Rutin was used as a standard and the absorbance was measured at 405 nm. The 1,1-diphenyl-1-picylhydrazyl (DPPH) free radical scavenging activity was estimated and

absorbance was measured at 517 nm. At the highest concentration of 1000 ppm, both TPC and TFC recorded values of 131.97 mg GAE/g and 4.10 mg RE/g dried weight of sample respectively. DPPH free radical scavenging activity was recorded at the highest concentration of 87.99% with EC_{50} value of 30.33 mg/mL. The various concentrations implied different levels of antioxidant properties. The results suggest that cocoa liquor is a potential source of phytochemicals. The study presented scientific validation on phytochemical contents of cocoa liquor showing presence of bioactive compounds with nutritional and therapeutic values which have positive impact on skin health and suggesting its prospective use in value-added products such as skin care cosmetics.

INTRODUCTION

The term "*halal*" is, by and large, associated with food. In recent years, the term has become increasingly affixed to other items such as skin care cosmetics, a preparation used to clean, improve or change skin's appearance. The demand towards *halal* cosmetic products has increased among younger generations of conscious consumers. *"Halal"* certification rests not only on the ingredients that go into the making of the product, but also the packaging, manufacturing, and distribution methods. For a product to be considered *halal*, it must be free of alcohol, not tested on animals, and contains no animal fat or other harmful chemicals [11].

Empirical studies have documented various beneficial effects of cocoa liquor, also called chocolate liquor, unsweetened chocolate, cocoa mass or simply liquor [15]. The thick paste texture of cocoa liquor contains about 55% fats from cocoa butter and cocoa cake [2]. The chemical composition of cocoa liquors depends on cocoa varieties, fermentation and roasting conditions in processing the cocoa beans [14]. Cocoa liquor does not contain any alcohol liquids, *halal* and safe to consume or for use as topical applications for the skin. Its phenolic and flavonoid contents have been documented to be higher than any of other phytochemical-rich foods [20]. The abundance of polyphenols has also been reported by Porter *et al.* (1991) to be specifically in the form of flavonoids.

Previous study by Nichols and Katiyar (2010) reported that topical applications of polyphenols can protect the skin against ultraviolet radiation effectively. Flavanol-rich cocoa has been reported to enhance skin elasticity while improving skin thickness and structure [22]. Long-term effect of ultraviolet radiation causes photo-damage such as wrinkles, dermal connective alteration and accumulation of collagen and can be prevented by similar topical application [10].

Against this background, the present study was conducted to establish the antioxidant contents in cocoa liquor with a view of determining its potentiality in skin care cosmetics or incorporating with other *halal* materials giving the skin an added protection in fighting free radicals from the environment.

MATERIALS AND METHODS

Cocoa Liquor

General

Cocoa liquor was directly purchased from Pusat Inovatif dan Teknologi Koko (Cocoa Innovative and Technology Centre) Nilai, Negeri Sembilan, Malaysia.

Preparation of extracts

Cocoa liquor blocks were melted in an oven at 40℃. Extraction procedures followed the method of Radojčić et al. (2009) with some modifications. Melted cocoa liquor was subsequently defatted with hexane (C_6H_{14}), an organic alkane compound. Cocoa liquor was extracted with 10 mL ethanol in an ultrasonic bath. The supernatant was centrifuged for 10 minutes at 5000 rpm and condensed in a rotary evaporator until dry. The prepared extracts were subjected to total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity determinations of DPPH. All extracts were prepared and analysed in triplicates.

Total phenolic content (TPC) determination

Total phenolic content (TPC) was determined following method described by Kaur and Kapoor (2002) with some modifications using Folin-Ciocalteau (FC) reagent. An extracted sample and a standard were dissolved in ethanol (serial dilution) and 100 µL was pipetted into vials. An amount 500 µL of FC reagent was added to a sample and mixed for about 8 seconds. Sodium carbonate solution (20% w/v) was added at an amount of 1500 µL to the sample mixed and allowed to stand in the dark at 40℃ for an hour. Measurement of phenolic acid content was carried out using an UV-Vis microplate reader at wavelength of 765 nm against gallic acid standard. Results obtained were expressed in mg gallic acid equivalent (GAE)/g sample.

Total flavonoid content (TFC) determination

Total flavonoid content (TFC) was determined following method by *Chang et al*. (2002). An amount 2 g of aluminium chloride was dissolved in 100 mL ethanol resulting in a 2% (w/v) aluminium chloride solution. An extracted sample and standard (serial dilution) of 100 µL each were pipetted into a 96-well microplate and 100 µL aluminium chloride solution was added. The mixtures were incubated for 10 minutes and the absorbance was recorded at wavelength of 405 nm using an UV-Vis microplate reader. Readings of samples were expressed in mg Rutin Equivalent (RE)/g sample.

Determination of DPPH scavenging assay

The DPPH free radical scavenging activity was carried out following method described by Blois (1958) with minor modifications. An extracted sample and a standard were weighed and dissolved in ethanol (serial dilution). Subsequently, 10 mg of 1,1-diphenyl-2-picryhdrazyl (DPPH) was weighed and dissolved in ethanol and the solution was made up to 100 mL in a volumetric flask. An amount 50 µL of the extracted samples and standard were mixed with 150 µL DPPH solution in a 96-well microliter plate and incubated in the dark for about 30 minutes for reaction to occur. Absorbance was measured at wavelength of 517 nm using an UV-Vis microplate reader. A sample control

was prepared by omitting sample extract from DPPH working solutions. All analyses were performed in triplicates. The radical inhibition activity of the extracts was calculated by the reduction of DPPH radical using the following equation (Eq. 1):

$$\text{DPPH Scavenging effect (\%)} = [(A_0 - A_1)/A_0 \times 100] \quad (1)$$

Where,

A_0 = Absorbance of control reaction

A_1 = Absorbance in the presence of sample

The plotted graph of DPPH scavenging effect against concentration of plant extract determined the EC_{50} value, described as the reduction of initial DPPH radical concentration by 50% as affected by the total antioxidant in need.

Statistical analysis

All data were expressed as mean ± standard deviation and independent analyses were performed in triplicates. Data were evaluated using one-way (unstacked) analysis of variance (ANOVA) using Tukey test by Minitab Software version 14.

RESULTS AND DISCUSSION

Total phenolic content

The amount of TPC in extracted cocoa liquor was based on the absorbance of extracted sample and Folin-Ciocalteau reagent mixture at wavelength of 765 nm. The phenolic compounds presented in each of sample was recorded as mg GAE per gram sample. A standard curve was plotted to quantify the phenolic compound in samples as mg GAE per gram samples. The total amount of TPC in sample is presented as mean mg GAE per gram sample (Figure 1).

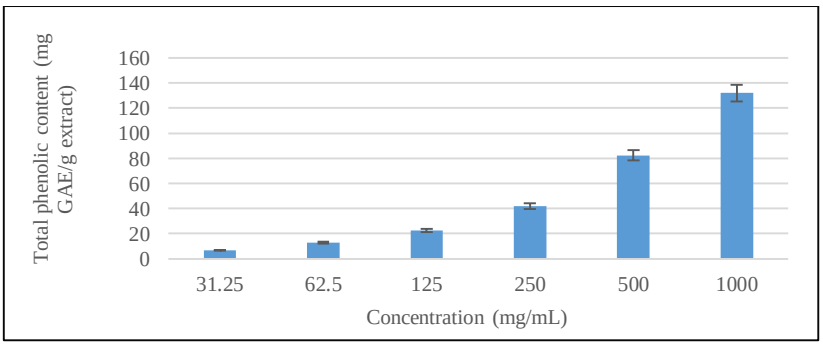

Figure 1. Total phenolic content (TPC) of cocoa liquor extracts. Values are means ± SD (n=3); Means with different letters are significantly different at level of ($p<0.05$) by Turkey test.

Previous studies reported that different extracting solvents influenced antioxidant activity and yield of phenolic content [21]. Among several studies, only one concentration was used to show TPC values. In addition, ethanol and water were safer solvents than methanol and other organic solvents [18]. In the present study, an extraction medium was used for preparing cocoa liquor extracts. Total phenolic contents were recorded in each concentration of extracted cocoa liquor in the order of 1000 > 500 > 250 > 125 > 62.5 > 31.25.

The study recorded significant differences between concentrations ($p<0.005$) using Tukey's test. Higher concentration of cocoa liquor extract showed higher level of phenol in the serial dilution methods which started from highly concentrated to less concentrated suggesting less amount of TPC in the diluted extract compared to concentrated samples. The results of TPC were similar as reported by Brabo de Sousa *et al*. (2018) on fruit of palm species, *Oenocarpus distichus*.

TPC of extracted cocoa liquor recorded in the present study was highest when compared with studies. Radojčić et al. (2009) documented that the order of TPC yield from highest to lowest level were Madagascar > Mexico > Ecuador > Venezuela > Sao Tome > Ghana. A report by Natsume *et al*. (2000) reaffirmed that phenolic content in cocoa liquor varied with countries where the study was conducted.

Total flavonoid content

The amount of TFC in cocoa liquor extract was based on the absorbance of sample using aluminium chloride colorimetric assay at wavelength of 405 nm. Flavonoid compounds present in each concentration of samples was reported as mg RE per gram sample. One standard curve was plotted to quantify the flavonoid compound in the samples as mg Rutin Equivalent per gram sample (mg RE/g).

Compounds in flavanols known as catechin are usually found in cocoa or chocolate [9]. In extracted cocoa liquor, the total flavonoids contents detected in each concentration followed the order of 1000 > 500 > 250 > 125. There were significant differences ($p< 0.05$) between the concentrations using Tukey's test. The highest flavonoid content was in 1000 (mg/L) concentration giving a value of 4.10 mg RE/g sample (Table 1). The results in TFC were in agreement with a study by Zhang et al. (2019) in petioles of sweet potato (*Ipomoea batatas* L.). The amount of flavonoid in the extracted cocoa liquor depicted lower yield due to several factors such as quality of cocoa beans producing the cocoa liquor [3], as well as fermentation process and roasting conditions [20]. These conditions caused phenol levels to be reduced thus affecting flavonoids content.

Table 1: Total flavonoid content from cocoa liquor

Concentration (mg/mL)	**(TFC) (mg RE/g)**
1000.0	4.10 ± 0.000
500.0	-5.65 ± 0.001

250.0	-8.21 ± 0.002
125.0	-12.33 ± 0.001

DPPH scavenging assays

DPPH scavenging assay is the simplest and most widely reported method for screening antioxidant activity in food and many other plant-derived drugs [1]. The change of colour from deep purple fading to light yellow indicated that antioxidant compounds in the extracted cocoa liquor reacted with DPPH radicals by donating hydrogen radicals resulting in bleaching of DPPH solution. Antioxidants assay determines scavenging of stable radical species of DPPH by antioxidants.

In the present study, serial dilution methods were used to determine scavenging activity. At 1000 mg/mL concentration, the highest activity was affected followed by others at lower concentrations. Scavenging activity increased as the concentration of sample extract was increased until a plateau was reached at 500 mg/mL as shown in Figure 2. There were significant differences ($p<0.005$) between the concentrations with the highest recorded at 87.99%. The EC_{50} value was determined from the plotted graph of scavenging activity against the concentration of the extract, which was the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [20]. From the study, the EC_{50} of the sample of extracted cocoa liquor was at 30.33 mg/mL suggesting that reactions occurred to fight free radicals in maintaining skin health.

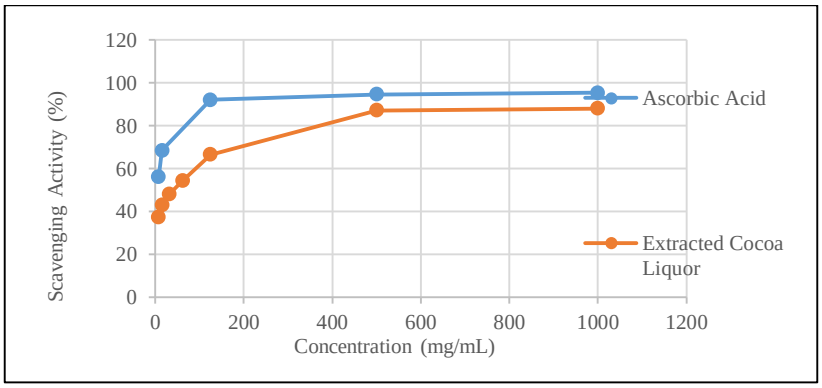

Figure 2. DPPH of cocoa liquor extracts. Values are means SD (n=3); Means with different letters are significantly different at the level of ($p<0.05$) with Turkey test.

CONCLUSION

In the present study, polyphenols level was dependent on fermentation, roasting and quality of cocoa beans resulting in a variation of results. The total phenolic was highest at 1000 mg/mL giving a value of 131.97 mg GAE/g dried weight of sample while the total flavonoid content was 4.10 mg RE/g dried weight of sample. The antioxidant activity's highest percentage was at 1000 mg/mL with 87.99% and EC_{50} of 30.33 mg/mL. The data suggest presence of high antioxidants in cocoa liquor indicating its potentiality in the development of skin care cosmetics.

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