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PROSPECTIVES OF COCOA LIQUOR FACIAL MASK AS A SKIN CARE AND COSMETIC PRODUCT

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Abstract

Cocoa liquor is a natural source of antioxidants with potential health benefits. The present study was conducted to determine the stability, level of toxicity and efficacy of cocoa liquor facial mask (CLFM) in protecting the skin by warding off free radicals. A centrifugal study on CLFM was conducted with minimal separation compared with pure cocoa liquor. Microbiological tests on colony-forming units showed the formation of yeast and molds were <1 and <10 cfu/g., respectively. Heavy metals levels in CLFM including arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) were recorded at <0.05, 6.0, 0.2, and <0.01 mg/kg., respectively, below established permissible levels. Efficacy tests on CLFM on the skin significantly decreased skin roughness (SEr), skin scaliness (SEsc) and skin wrinkles (SEw) and increased skin smoothness (SEsm). Skin thickness was observed to increase following the slowing down of degradation of collagen and elastin in the skin. The study provides scientific validation on the phytochemical contents of cocoa liquor, demonstrating the presence of bioactive compounds with nutritional and therapeutic values that may have a positive impact on skin health and recommending its potential use in value-added products such as skin care and cosmetics. The present study confirms not only that CLFM does what it is intended to do but, above all, that it is safe to use for consumers.

INTRODUCTION

Cosmetics and skincare products are widely consumed worldwide. Their consumption has been on the increase every year due to their frequent use, thus consciously and unconsciously increasing the exposure of consumers to various benefits as well as detrimental chemical compounds [1]. Cosmetic products refer to any substance or preparation intended to be applied, by topical administration, directly to various external parts of the human body to treat various ailments of the skin [2,3]. Unfortunately, exploitation of

cosmetic ingredients by adding harmful chemicals such as mercury, parabens, arsenic, tretinoin and lead has caused affected skin to become more sensitive to elements such as ultraviolet radiation [4]. Natural facial masks give specific treatments to the skin such as soothing, hydrating, repairing, pore cleansing and others. The different functionality of each facial mask on the skin derives from using different active ingredients usually from different phytochemical sources. For instance, fruits, vegetables, green tea and sugar cane. There are several types of facial masks on the market such as cream masks, clay masks, sheet masks and gel masks and

it takes about 20 minutes to deliver the bioactive compounds to the skin [5]. In previous studies by Norliza et al., (2014) and Azila et al., (2014) they developed cosmetic formulations using cocoa bean clones as well as cocoa pod extracts as the main active ingredients for cosmetic applications. In the present study, cocoa liquor, a paste produced from ground highly nutritious cocoa nibs made from cocoa beans, was selected due to its various beneficial effects as reported in various studies. Literature has it that the bioactive compounds found in cocoa liquor such as catechin, epicatechin, tannin, theobromine and caffeine [8,9] aid in increasing skin hydration, elasticity, wrinkle reduction and improving skin thickness [2]. Cocoa liquor does not contain alcohol liquids and is halal and safe for consumers to use as topical applications on the skin. Against this background, the study was conducted to establish the product's stability, toxic metal content and efficacy as a facial mask with the view of determining its potential in skin care cosmetics giving the product an added ability to protect the skin in fighting free radicals from the environment.

MATERIALS AND METHODS

Materials

Cocoa liquor was sourced from Malaysian Cocoa Boards's (MCB) Cocoa Innovative and Technology Centre (CITC) Nilai, Negeri Sembilan, Malaysia. MCB CITC undertakes various research and development in cocoa downstream activities involving personal care products, cosmetics, pharmaceuticals, as well as research on biotechnology for the commodity. All chemicals used in the present study were of analytical, cosmetics and food grades. Ingredients that made up Cocoa Liquor Facial Mask (CLFM) emulsifier, emollient, wetting agent, white clay powder and preservatives were purchased from Gattefossé, USA.

Formulation of Cocoa Liquor Facial Mask (CLFM)

Ingredients in each phase of the study were weighed following the formulation presented in Table 1. The ingredients were mixed in a beaker using a homogenizer

(Heindolph, Germany) at 1000 rpm for about 40.0 ± 5.0 minutes. Fresh mixed ingredients were left to rest for 24 hours before further processing.

Centrifugal Procedures

Centrifugation is a technique where separation of particles from a solution occurs according to their density, viscosity and size of the medium and rotor speed used. A centrifugal force is generated on the particles in the suspension due to rotational effects. Principally, lighter density in the solution separates slower than heavier density [10]. Subsequent to the procedure described in the formulation of cocoa liquor facial mask (CLFM), the mixed ingredients were centrifuged following the methods of MakingCosmetics. The CLFM ingredients were heated to 50 °C and an amount of 15 g was weighed and poured into the centrifuge tube. The ingredients were centrifuged for 30 minutes at 3000 rpm and the resultant product was inspected for signs of creaming. The CLFM ingredients were prepared in triplicates and the data was expressed as a mean \pm standard deviation.

Microbiological Determinations

Verification of microbial contamination of the processed product was done by determining the presence of microorganisms at 30°C and yeast and molds at 25°C. CLFM was prepared in diluted peptone saline. An aliquot (1mL) of the initial suspension or dilution of suspension was transferred to sterile petri dishes and each dilution was duplicated. Each dilution (1 mL) was pipetted and aseptically spread on agar plates for microorganisms and Chlortetracycline agar for yeast and molds and allowed the mixture to solidify with the plate standing on a cool horizontal surface. The agar plates were incubated for 72 hours (microorganisms) and 4 days (yeast and molds). Colonies were counted for each dot once. A marker can be used to point to each counted colony on the back of the petri dishes, following which colony-forming units from each sample were recorded [11].

Table 1. Formulation List of CLFM

Ingredients		Amount (%)
Oil Phase	Cocoa liquor	55
	Emulsifier	5
	Emollient	<i>q.s</i>
	Wetting agent	<i>q.s</i>
Dry Phase	White clay powder	20
Preservatives		<i>q.s</i>

CLFM: Cocoa liquor facial mask

q.s: *quantum satis* (Latin term, meaning the amount which is enough)

Heavy Metals Determinations

Determinations on the levels of heavy metals including arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) were carried out. Elements such as lead, arsenic and cadmium were screened for detection using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), following procedures by Puziah et al., (2009). About 5 - 10 g of CLFM extracts were weighed and dried in a sand bath on slow-heat. The ash of the extracts was dried at 450 °C for about 4 hours. About 10 ml of 10% nitric acid was added and filtered. The digested CLFM samples were used to detect the elements of interest by using Perkin-Elmer PE Analyst 300 optima's inductive couple plasma-optical emission spectrometer (ICP-OES).

The determination of mercury in CLFM was accomplished by using a Mercury Analyzer (Milestone, U.S.A). Hydrochloric acid solution at 0.1M was prepared to obtain a standard calibration curve. Measurement was carried out at 253.66 nm wavelength for empty metal sample container. Subsequently, 5 mg of CLFM was placed in the sample container for measurement.

Efficacy of CLFM

For studies on the efficacy of CLFM, a total of 10 subjects, all women between the ages of 25 and 46 years were selected from MCB CITC as volunteers for the study. The selected subjects were verified to be in good health conditions and free from skin infections. Consent forms were duly signed by all volunteers. Prior to the efficacy tests, a qualified dermatologist from the health services examined the subjects for any serious skin diseases or related health issues especially on the cheeks. The subjects were required to personally and topically administer the CLFM on the right as well as the left cheek at a dose of 600 mg for three times per week in the comfort of their homes. The subjects performed the procedure at night on their cheeks for a period of 2 months and reported for facial assessments on Day 0, 1 month and 2 months thereafter at 10 a.m. at the MCB facilities. They were allowed to wash their faces 30 minutes prior to taking measurements and to briefly take a short rest for body stabilization. Measurements were taken in a controlled room set at 25 ± 1 °C temperature and 45 ± 2 % relative humidity.

Surface Evaluation of Living Skin (SELS)

Subjects' skins were evaluated using Visio Scan® VC98 (Courage and Khazaka, Germany). The high technology scan with a UV-A light video camera and high image resolution directly captured the subjects' skin surfaces. Graphic images of the skin displayed four clinical parameters: skin roughness (SER); skin smoothness (SEsm); skin scaliness (SEsc); and skin wrinkles (SEw). These

parameters corresponded to the conditions of the subjects' skin surfaces. Measurements were taken three times ($n=3$) per subject and data were expressed in means \pm standard deviations.

High Resolution Ultrasound Skin-Imaging

Subjects' skin thickness was measured using the DermaLab® Series SkinLab Combo. The ultrasound scan probed the skin with gel applied to the film. The probe was placed on the skin area and massaged with the gel. As the "start" button clicked, ultrasound images emerged giving measurements of skin thickness, intensities, age bands and distances. These parameters corresponded to the conditions of the skin surface of interest. Measurements were taken three times ($n=3$) and data were expressed in means \pm standard deviations.

Statistical Analysis

All data was expressed as means \pm standard deviations. 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

Formulation of CLFM

The formulation of CLFM was developed in the form of a moisturizing cream clay mask. After homogenizing with high shear and pressure, the resultant product was a cocoa cream clay mask with therapeutic values.

Centrifugal Study

The results of the present study were visually evaluated. Cocoa liquor, as an indicator and at room temperature, was in solid form. To formulate CLFM, it requires cocoa liquor to be in soft mud form. As such, some selected ingredients were added to maintain the softness of CLFM. When cocoa liquor consists of cocoa butter and cocoa mass, when melted, separation between oil and residue of cocoa liquor occurs. Figure 1 shows the results from a 30 minute centrifugation of CLFM. Figure 1a shows cocoa liquor while Figure 1b shows formulated CLM. No separation in cocoa liquor was observed. However, in the formulated CLFM, there was minimal separation due to ingredients used in softening the cocoa liquor's texture. There was no excessive use of added ingredients as suggested. Stable facial mask formulation allows minimal separation to occur beside pH, viscosity and spread ability values close to each replication, suggesting that the formulation of CLFM in the present study was stable with minimal separations.

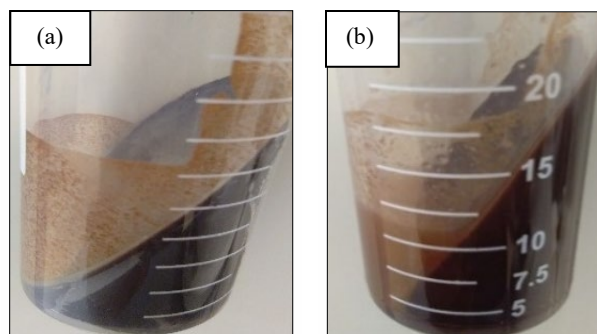


Figure 1. (a) Cocoa liquor and (b) Cocoa liquor face mask (CLFM) with minimum separation

Microbiological Determination

Microbial contamination of cosmetics products generally occurs during the handling and preparation of raw materials and ingredients. Spoilage could affect the organoleptic properties of the formulations in terms of color, pH and odor as well as degrading bioactive compounds in the products. Thus, microbial contaminations contribute risks of infections to consumers including dermatological problems such as skin allergy [12,13]. Prior to release of the products to the public, manufacturers are generally required to conduct clinical tests in terms of microbiological stability to avoid unwanted incidences. In the present study, the purpose of microbiological analyses was to ensure the formulated CLFM was free from pathogenic microorganisms. Chemical changes and spoilage in CLFM could occur with the existence of microorganisms. Preservatives were used to avoid growth of these unwanted microorganisms that could compromise bioactive compounds in CLFM. Hence, CLFM was analyzed in 3 different temperature conditions to detect yeast, mold and microorganisms from Day 0 - Day 28. Identifications of yeast, mold and microorganisms in the present study were based on colony formation on the agar medium. Results were comparable with ASEAN limits of contaminants for cosmetics which was less than 1000 cfu/g sample as shown in Table 2, verifying that CLFM was safe to use as topical applications.

Heavy Metals Determination

Toxic metals such as mercury, lead, arsenic, cadmium, nickel, chromium and cobalt are usually found in many cosmetic products [14,15,1]. Topical application delivers these toxic metals in the products to be absorbed into the skin and reach the bloodstream thus exerting toxic effects [16]. Beyond the maximum limits suggested legally by the government, these metals have adverse effects on human health such as skin problems, kidney dysfunction, lung disease, cancer, issues with the reproductive systems and many more [17,18]. Therefore, in order to produce harmless cosmetic products, toxic metals analyses must be conducted to ensure toxic metals are below the maximum limits. In

Malaysia, heavy metal limits in such products are referred to the Malaysian Cosmetic Guidelines as well as the ASEAN Guidelines on Limits of Contaminants for Cosmetics. The maximum limits for heavy metals have been set as follows: mercury (Hg) <1 mg/kg; lead (Pb) <20 mg/kg; arsenic (As) <5 mg/kg and cadmium (Cd) <5 mg/kg. Table 3 presents levels of heavy metal in CLFM, determined by ASEAN specifications. The detection levels for arsenic, lead, cadmium and mercury levels in the present study were less than 0.05 mg/kg, 6.0 mg/kg, 0.2 mg/kg and less than 0.01 mg/kg, respectively, lower than the maximum level allowed.

Surface Evaluation of Living Skin (SELS)

Skin surfaces of subjects were measured by direct non-invasive method using Visioscan® VC98 and software SELS 2000. The SELS is based on image depicted from living skin under special illumination, and image assessment by electronic processing, referring to four sets of parameters [10]. The four parameters of skin surface included skin roughness (SEr) which calculated the grey levels above the threshold in comparison with full image; skin smoothness (SEsm) calculated wrinkles depth and width; skin wrinkle (SEw) which was calculated from number and average width of horizontal and vertical wrinkles, and skin scaliness (SEsc) that identified the dryness of keratin and defined as the ratio of all pixels greater than the threshold color value and total pixels [19, 20]. Average results of the four parameters (SELS of SEr, SEsc, SEsm and SEw) from ten subjects are presented in Table 4. Skin roughness values decreased from 0 hour, followed by 1 month and 2 month applications for both right and left cheeks. The decreasing skin roughness indicates that the skin had become smoother. The level of dryness decreased in the right cheek while the left slightly increased after 2 months of application. The scaliness of the skin was recorded to decrease and hydration increased in line with other findings [21]. The smoothness of the skin was noted to increase for both cheeks. The values for skin smoothness increased suggesting that CLFM had moisturizing and anti-aging effects (Visioscan® VC 98). The CLFM consisting of cocoa butter and cocoa powder that contained powerful antioxidant compounds was able to reduce skin wrinkles

Table 2. Microbiological Analysis of Cocoa Liquor Facial Mask (CLFM)

Temperature Storage	Colony Forming Units yeast and mold/ g sample	Colony Forming Units microorganisms/ g sample
Oven	$< 10 \pm 0.02$	$< 1 \pm 0.01$
Room Temperature	$< 10 \pm 0.01$	$< 1 \pm 0.02$
Chiller	$< 10 \pm 0.01$	$< 1 \pm 0.03$

Data are expressed as means \pm standard deviations, triplicates of three independent samples.

Table 3. Heavy Metals in Cocoa Liquor Face Mask (CLFM)

Heavy Metal	Results (mg/kg)
Arsenic (As)	$0.05 \pm 0.02^{(1)}$
Lead (Pb)	6.0 ± 0.06
Cadmium (Cd)	0.2 ± 0.03
Mercury (Hg)	$0.01 \pm 0.01^{(1)}$

⁽¹⁾ Limit of quantitation (LOQ)

Data are expressed as means \pm standard deviations, triplicates of three independent samples.

Table 4. Values of SE_R, SE_{Sc}, SE_{Sm}, SE_W before and after application of Placebo (P) and Cocoa Liquor Facial Mask (CLFM)

	Parameter		0 Hour	1 Month	2 Months
SE _R	P	Left cheek	0.37 ± 0.06	0.03 ± 0.01	0.03 ± 0.08
	CLFM	Right cheek	0.04 ± 0.00	0.01 ± 0.01	0.01 ± 0.00
SE _{Sc}	P	Left cheek	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
	CLFM	Right cheek	0.06 ± 0.00	0.02 ± 0.01	0.03 ± 0.02
SE _{Sm}	P	Left cheek	16.77 ± 0.56	16.82 ± 0.53	16.81 ± 0.65
	CLFM	Right cheek	17.54 ± 0.09	17.44 ± 0.11	18.76 ± 0.15
SE _W	P	Left cheek	42.07 ± 0.82	42.15 ± 0.04	42.11 ± 0.10
	CLFM	Right cheek	45.68 ± 1.97	43.49 ± 2.59	40.27 ± 0.33

- SE_R: Skin roughness; SE_{Sc}: Skin scaliness; SE_{Sm}: Skin smoothness; and SE_W: Skin wrinkles.
- Data expressed as mean \pm standard deviation, triplicates of three independent samples.
- Significant differences between concentrations ($p < 0.005$) using Tukey's test.

with applications within 2 months, resulting in skin slowly becoming firmer due to fibroblast in the dermis which is responsible for stimulation of elastin and collagen synthesis aided to reduce wrinkles [22].

High Resolution Ultrasound Skin-Imaging

Thickness of dermis of volunteers were examined according to procedures by DermaLab® Series SkinLab Combo, specifically on high resolution ultrasound skin-imaging. The principle of ultrasound skin imaging is based on measuring the acoustic response from the skin. When the acoustic pulse in forms of very low energy hits under the skin structures, the pulse is reflected and captured by ultrasound transducer. The cross-sectional images shown on the screen are the results of reflected signals. The penetration of ultrasound

into the skin could be reached until 3.4 mm whilst image resolution displayed recorded at 60 x 200 micrometer (DermaLab® Series SkinLab Combo). Data on skin thickness of average results from ten volunteers are shown in Table 5, measured after 0 hour, 1 month and 2 months of applications of CLFM on both right and left cheeks. Significant ($p < 0.005$) improvement in skin thickness was observed at 1 month and 2 months for ten subjects. The average results from ten subjects showed their collagen density in skin slowly increasing. Unhealthy environment such as UV radiation and age were factors in degradation of collagen and causing elastin to be less springy [23]. Skin thickness indicates collagen density of the skin [24], suggesting that CLFM aided in collagen boosting by slowing down the degradation processes of collagen and elastin.

Table 5. Skin Thickness Before and After Application of Placebo (P) and Cocoa Liquor Facial Mask (CLFM)

Volunteer			0 Hour	1 Month	2 Months
1 - 10	P	Left cheek	21.65 ± 0.05	21.15 ± 0.12	22.3 ± 0.13
	CLFM	Right cheek	21.00 ± 0.14	25.00 ± 0	27.10 ± 0.01

- Data expressed as mean ± standard deviation, triplicates of three independent samples.
- Significant differences between concentrations ($p < 0.005$) using Tukey's test

CONCLUSION

In the present study, cocoa liquor facial mask (CLFM) was stabilized with minimum separation. The levels of toxic metals including arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) in CLFM were below the ASEAN limits of contamination in cosmetics. In terms of efficacy, CLFM had high potential in decreasing skin roughness (SEr), skin scaliness (SEsc) and skin wrinkles (SEw) while increasing skin smoothness (SEsm) and skin thickness, suggesting that CLFM had high potential in the development of skin care cosmetics.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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