Extraction and Formulation of Oil in Water (O/W) Emulsion with Coco plum (*Chrysobalanus icaco*) Seed Oil: Physicochemical and Microbiological Evaluations

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***Abstract*—** The emulsion instability and macroscopic phase separation of oil in water (O/W) emulsion have become a significant concern globally as it poses lots of threat to human skin. Several mechanisms involved in emulsion instability include the rupture of oil globules or coalescence of the internal water droplets. The present study aimed to formulate oil in water (O/W) emulsion by entrapping 5% of cocoplum oil extract in increasing order (0,10,15 and 20) into the oil phase of the emulsion. The base form contains the paraffin oil (20,10,15 and 0) in decreasing order. Rosemary extract was incorporated as a fragrance to the emulsion. Both the base (B) and formulations (FA, FB, and FC) were stored at 8°C (in a refrigerator) and at 30^{o}C (room temperature) for 35 days to investigate their stability, color, smell, phase separation without agitation, phase separation under centrifugation, liquefaction, pH. The microbiological evaluations (Aerobic plate count) were investigated with an acceptable standard. The findings indicate that the formulation with only cocoplum oil extract (FC) shows good resistance to stability and macroscopic phase separation. The microbiological evaluation indicates no significant growth of microorganisms at dilution 10^{-4} and 10^{-6} after 48hrs of the incubation period. Further sub-cultured within 35 days produces 0.2log10CFU/g, which does not exceed 6.9log10CFU/g recommended by ISO NF-21149, 2009 for cosmetics analysis. The newly formulated oil in water (O/W) emulsion has a promising property that might eradicate skin diseases. Also, it may open new opportunities for the formulation of more active, safe, and cost-effective cosmetics and pharmaceutical formulations.

***Keywords*—** cocoplum oil, stability, organoleptic, (O/W) emulsion, rosemary extract.

I. Introduction

Recently, skin infections originated from adulterated skincare products have led scientist into searching for therapeutic compounds with antioxidant properties for the formulation of cosmetics emulsion. Efforts have included the formulation of cosmetics emulsion with oil extract from the seed plant. Such as oil extract from almond, walnut, castor olive, olive oil, and African nutmeg [1]–[3]. Most of these skincare products are either oil in water (O/W) or water in oil (W/O) emulsions. Oil in water emulsion (O/W) is used for cosmetics formulations and drug bases, whereas water in oil emulsion (W/O) is for emollient creams formulation [3]. The oil commonly used for the preparation of emulsions are the paraffin oil, extracted from the fractional distillation of petroleum. The paraffin oil only moisturizes but adds no nutrient to human skin and could be contaminated during processing. The oil can block skin pores, thereby causing lots of skin disorders. Therefore, the formulation of cosmetic emulsion or skincare products with paraffin oil poses many threats to human skin. Itching, rashes, eczema, acne, irritation, and other skin inflammation are skin disorders associated with paraffin oil-based emulsion. The skin care products such as sunscreens creams, emollient creams, night creams, face oil, tinted moisturizers, skin lightening creams, anti-aging creams, eye creams, face moisturizers, and cleansing oil contain emulsion formulated with paraffin oil. These skincare products are marketed globally to satisfy social benefits such as providing cleansing and protective barriers against damaging sunlight from the environment. Also, it is expected that the formulation should give a pleasant odor, make the skin feel good on application but instead, most formulations cause infections on human skin. And this is as a result of adulterated and unpleasant oil used during emulsion formulations.

An emulsion can be defined as a complex mixture of two immiscible phases (internal and external) where droplets of the internal phase are entrapped or dispersed within the sheets of the external phase forming a miscible mixture. In the formulation of cosmetics emulsions, amphiphilic emulsifiers are needed to reduce the interfacial tension and formation of barriers between the two phases [3], [4]. The emulsifiers are surfactants that prevent microscopic separation during emulsion formulation. A suitable emulsifier possesses two moieties (hydrophobic and hydrophilic) that aids adsorption unto water-oil phases. The hydrophobic character is adsorbed by the oil phase to form O/W emulsion, while the aqueous phase adsorbs the hydrophilic character and forms the W/O emulsion [5], [6]. In selecting emulsifiers for cosmetic emulsion, the hydrophilic and lipophilic balance (HLB) is an essential parameter detecting the type of emulsion involved. The HLB relates to solubility, size, and strength of moieties of surfactant molecules. The HLB scale ranges from 0-20. For example, the suitable HLB for O/W emulsion ranges from 8.0 to

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18.0 (preferentially obtained with hydrophilic molecules), whereas W/O emulsion ranges from 3.5 to 6.0 (obtained with hydrophobic molecules). Thus, high HLB value becomes soluble in water, whereas low HLB values are oil-soluble [7]. An emulsion is thermodynamically unstable but can kinetically be stabilized with emulsifiers [1].

Emulsifiers must maintain the life-time stability of the type of emulsion involved. Thus, the formulation of emulsion with the oil that contains antioxidant properties enhances the shelf life of the products. The emulsion instability is determined by the composition and emulsifiers used during formulation and is the primary cause of skin disorder in humans [8], [9]. The emulsion instability causes emulsion defects, including sedimentation, flocculation, coalescence, and Ostwald ripening [10], [11]. Therefore, the formulation of emulsion with antioxidant properties would assist in eradicating skin defects or disorders. Cosmetic industries have increased their interest in the oil with antioxidant and therapeutic properties for emulsion formulations. These oils will improve the appearance of skin such as skin texture, radiance, and reduce wrinkles as a result of old age. Incorporation of antioxidant oil, in particular, coco plum seed oil, would avoid oxidation, which causes emulsion instability and as well as adding nutrients to human skin.

Cocoplum (*Chrysobalanus icaco L.)* (CP) is an anthocyanin and polyphenol-rich seed found in tropical areas around the globe, such as the Bahamas and the Caribbean, including the Northern region of the Brazilian Amazon forest [12], [13]. CP has therapeutic properties that are beneficial to health, including reducing inflammation and skin disorder caused by oxidative stress and fake skincare products. The cocoplum leaves have antifungal, analgesic, and hypoglycemic properties [14], which can also be incorporated in the pharmaceutical ointment for fungi infection. The evergreen tree in the family *Chrysobalanus* that has sweet-smelling flavors and the fruit is edible. The tree grows up to 9 m (30 feet) tall, and it has roundish shiny green leaves and clusters of white flowers. Reports have shown that cocoplum has many health benefits, such as improving the functionality of the human eye, cancer treatment, reduces the risk of stroke and heart attack [14], [15]. Cocoplum seed is useful for pregnant women, and consumption after childbirth purifies the blood. It contains high amount of vitamin C and antioxidants, which may be beneficial to human skin, such as combating dark spots, curing acne, and make the skin look smooth and radiant. Due to its antioxidant properties, the oil extract may preserve the shelf life of emulsion and cream bases. The significant problems of emulsion are the growth of microorganism or pathogens that causes emulsion instability and could be invasive when used on human skin.

This study investigates the effects of emulsion stability, shelf life, and microbial activities using cocoplum oil extract in emulsion formulation. We hypothesize that the incorporation of oil extract in the emulsion will eradicate the growth of pathogens, enhance the longevity of emulsion, and prevent emulsion instability. The use of cocoplum oil extract would have provided more efficient, safe, and cost-effective skin oil to formulate either oil in water (O/W) or water in oil (W/O) emulsion. And to create awareness on the health benefits of the cocoplum seed oil to human skin.

II. Materials and Methods

A. Herbarium Certification

Identification of seed plant and certification were taken into consideration during the study. Cocoplum seed (*Chrysobalanus icaco*) was purchased at the Agbara market in Ogun State and certified at Herbarium at the University of Lagos, Akoka, Nigeria.

B. Chemicals

Paraffin oil, Tween 20, Tween 80, Carbopol 940, Triethylamine (TEA), and Rosemary extract were purchased from Merck chemicals (Hayward, CA, USA) of Esota store Oshodi, Lagos State, Nigeria. All the chemicals were of analytical grades and had the highest possible purity. The distilled water was obtained from the Chemistry department laboratory, Covenant University Ota, Ogun State, Nigeria.

C. Apparatus and laboratory glassware

Soxhlet extractor (60 ml capacity), Rotary evaporator (Chemglass CG-1334-X60), heating mantle and stirrer, thermometer, orbital shaker (MXBAOHENG GS-30), magnetic bar, refrigerator, Inco shake incubator (LABOTEC), benchtop pH meters (S213), Centrifuge (Beckman model, TJ- 6), Digital weighing scale (EMB 1200-1 Kern) and autoclave sterilizers (3D model), conical flasks, beakers, round bottom flask, test tubes, Petri dish and laboratory mortar and pestle.

D. Extraction of cocoplum oil

10kg of cocoplum seed sample (*Chrysobalanus icaco*) was air-dried at ambient temperature for five weeks. The seed was grounded into powdered form. The oil was extracted with normal hexane as a solvent within 48 hours using soxhlet extraction equipment and concentrated with a rotary evaporator at 40-50^{o}C for few minutes. The oil was kept in an amber bottle to avoid further oxidation. The oil sample obtained was subjected to an experimental procedure.

E. Formulation of emulsions

Oil in water (O/W) emulsions were prepared using a modified method of Henrietta, 1995. The formulation was prepared at a different concentration by adding an aqueous phase to the oil phase through continuous agitation. Four samples of oil in water emulsions (B, FA, FB, and FC) were prepared using tween 20, tween 80, Carbopol 940, triethylamine paraffin, and cocoplum oil at different concentration. The formulations were prepared in three steps. Firstly, formulation with paraffin oil (B), secondly, the formulation of different concentration varying the paraffin oil and cocoplum oil (FA and FB), and the third step was the formulation of emulsion with only cocoplum oil (FC) with the addition of emulsifiers and other chemicals as shown in table 1. The oil phase consists of paraffin oil, Tween 20 and 80, Carbopol 940, and triethylamine was heated up to 75^{o}C. At the same time, an aqueous phase was also heated at the same temperature. After heating, the aqueous phase was added to the oil phase drop by drop using a magnetic stirrer with constant stirring at 100rpm for 5 hrs. Two to three drops of rosemary oil were added during stirring at a temperature of 55^{o}C to give fragrance to the emulsion. After stirring for 5 hours, the speed of the stirrer was reduced to 50 rpm for 2 hrs in other to achieve complete homogenization. Agitation was

maintained until the emulsion was cooled at room temperature.

TABLE I: EMULSION FORMULATIONS AT DIFFERENT CONCENTRATIONS (%W/W) VARYING PARAFFIN OIL AND COCOPLUM OIL.

Materials	Base	Formulation A (FA)	Formulation B (FB)	Formulation C (FC)
Cocoplum oil	0	10	15	20
Paraffin oil	20	10	5	0
Tween 20	5	5	5	5
Tween 80	5	5	5	5
Carbopol 940	4	4	4	4
Rosemary extract	1	1	1	1
Triethylamine (TEA)	5	5	5	5
Distilled water	40	40	40	40

III. Experimental analysis

A. Physical analysis

The emulsions were subjected to a set of organoleptic (feel, thickness, color, look), creaming, and phase separation throughout the study period.

B. *Emulsion stability*

The stability of emulsion was determined by centrifuging the formulated emulsions (B, FA, FB, and FC) ranging from Fresh, 24 h, 7, 14, 21, 28, and 35 days of preparation. The samples were poured into a beaker and homogenized for 20 minutes at 50rpm. Each formulation was then transferred into 100ml of measuring cylinder and kept at room temperature of the emulsions obtained was determined at the most stable emulsion and the base (B) and the formulation (FC). The samples were poured into the beaker and homogenized for 10 minutes at 25rpm. The emulsions were then transferred into a 100ml of measuring cylinder and kept at room temperature for 2 hrs. The readings were taken as follows:

$$\text{Emulsion stability} = \frac{VA-VB}{VB} \times 100\%$$

Where: VA = the volume of the aqueous phase after emulsification.

VB = the volume of the aqueous phase before emulsification.

C. pH determination of emulsion

Determination of pH of emulsions was conducted using digital pH- meter. It was measured by dipping the probe of the equipment into the beaker filled with the sample. The test was repeated after 7, 14, 21, and 28 and 35 days of preparation.

D. *Microbiological study*

Microorganisms contamination was accessed by dispersing 5ml sterile solution containing 0.15% tween 80. Appropriate dilution was made on the emulsions, and 0.1 ml was plated on a tangible medium using the surface viable method. Emergent colonies were counted after 48 hrs incubation period at 37^{o}C. All operations were carried out in duplicates (ISO NF- 21148, 2005).

a. Aerobic plate count

Aerobic plate count (APC) were investigated by inoculating 0.1 ml of the homogenate sample onto triplicate sterile plates of pre-poured and dried standard agar method using surface spread technique. The plate was incubated for 48 hrs for 37^{o}C (ISO NF-21149, 2006). Duplicates of each dilution (1 ml) of neutralized and non-neutralized samples were pour-plated using standard methods agar and incubated at 37 ± 1°C for 48 ± 4 hrs. The method used was done on serial dilution 10^{-2}, 10^{-3}, 10^{-4}, 10^{-6} on the four samples prepared. Plates containing 10 to 200 colonies were selected and counted, and the average number of CFU/ml was calculated.

IV. Result and discussion

A. Stability of the emulsions

Cosmetics creams and pharmaceutical formulations represent oil in water (O/W) or water in oil (W/O) that evolve with time. These formulations must penetrate deep into the skin to prevent or cure skin diseases. They are thermodynamically unstable and can separate into two distinct layers visible to the human eye. Emulsion instability could be physicochemical destabilization, such as sedimentation, flocculation, coalescence, and phase inversion. Lack of appropriate composition of emulsifiers contributes to emulsion instability [4], [16]. In this study, formulations were placed in two storage conditions; at 8°C in a refrigerator and 30^{o}C at room temperature for 35 days. The samples were observed for liquefaction, change in color, and phase separation, as represented in table 2. The findings revealed that freshly prepared emulsion of FA, FB, and B were white while FC was yellow, which may be due to the color of oil extract. The color change of FA, FB, and B were observed between 12th to 14th days and persisted throughout the study period, but FC remains yellow throughout the study period. The change in color was due to oil phase separation attributed to high temperature. Phase separation or breaking is attributed to time and temperature process after emulsion formulations, leading to a change in viscosity [17]. It increased liquefaction, which is equally a sign of instability. The phase separation was observed on B and FA within the seven days (7 days) of preparation on the two storage conditions. On the 14th day, the physical separation was seen on FB at the storage temperatures and persisted throughout the study period. The phase separation on centrifugation indicates that sample B, FA and FB are not suitable for cosmetic formulations that will last more than 21 days.

TABLE II: PHYSICAL CHARACTERISTICS OF BASE AND FORMULATIONS (FA, FB, FC) AT DIFFERENT STORAGE TIME

Parameter	Temp. (°C)	Fresh	24 hrs	7 days	14 days	21 days	28 days	35 days
		B FA FB FC	B FA FB FC	B FA FB FC	B FA FB FC	B FA FB FC	B FA FB FC	B FA FB FC
Liquefaction	8	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
	30	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
Colour	8	w w w y	w w w y	w w w yw	w yw yw yw	w yw yw yw	yw yw yw yw	yw yw yw yw
	30	w w w y	w w w y	w w w y	y y yw yw	y y yw yw	y y y yw	y y y yw
Phase separation	8	- - - -	- - - -	+ + - -	+ + - -	+ + + -	+ + + -	+ + + -
	30	- - - -	- - - -	+ + - -	+ + - -	+ + + -	+ + + -	+ + + -
PS on centrifugation	8	- - - -	- - - -	- - - -	+ + - -	+ + + -	+ + + -	+ + + -
	30	- - - -	- - - -	- - - -	+ + - -	+ + + -	+ + + -	+ + + -

(- = No changes; + = Slight changes; W = White; YW = Yellowish white; Y = Yellow).

The centrifugation test is a separation technique used to the dispersed emulsion and other formulations in the cosmetics industry. It is used to accelerate stability analysis by involving gravitational stress on the samples [18]. It also predicts the shelf life of emulsion. In this study, phase separation on centrifugation was recorded in the samples (B, FA, and FB) kept at different storage temperatures between the 14th to the 21st days of preparation. Sample FC kept at both storage temperature experiences, no phase separation on centrifugation throughout the study period. This indicated that samples FC was stable due to the oil, suitable emulsifiers, and proper homogenization during the formulation.

B. *Determination of pH*:

pH is a useful parameter for the determination of emulsion stability [19]. The suitable pH of human skin ranges from 4 to 6.5, and the average pH value of dry skin is 5.5. Adopting skincare products or pharmaceutical formulations depends on the pH value, which is between 4.0 - 6.5. The pH of emulsions (B, FA, FB, and FC) within 48 hrs was 7.30, 6.40, 5.91, and 4.28 at 8°C and 7.30, 7.45, 6.80, and 5.20 at 30°C respectively. Thus, the pH of the emulsions kept at 8°C and 30°C can decrease with time. The decrease in pH resulted from the diffusion of water from dispersed to the continuous phase and the fatty acid and other ingredients present in the oil extract [20].

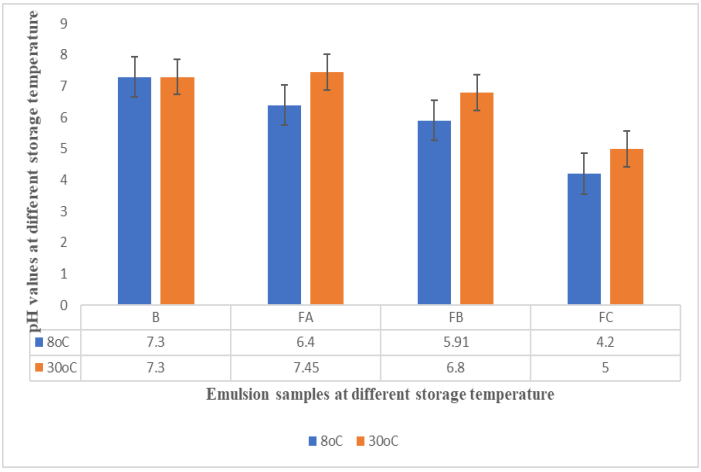


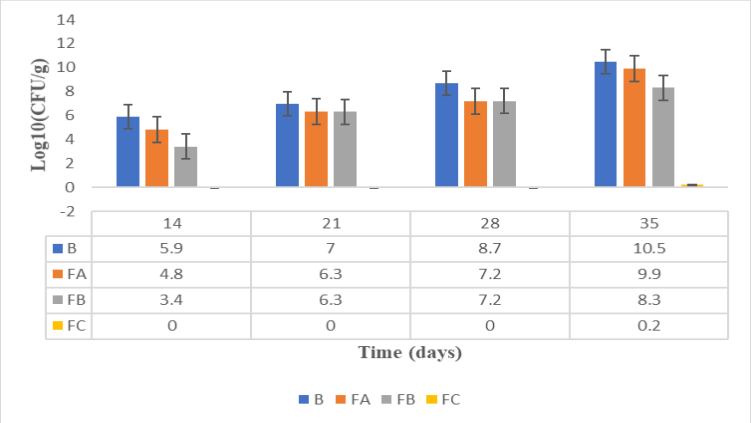
Fig 1: The pH value of freshly prepared emulsions (B, FA, FB, and FC) at different storage temperatures.

C. *Microbiological evaluation*

a. Aerobic plate count

Bacterial contamination on emulsion causes lots of skin diseases such as itching, tetchiness. This contamination is due to the increase in storage time, promoting the growth and proliferation of microorganisms on the emulsion. In this study, microbial evaluation on the emulsion samples was carried out between 14th to 35 days of the formulation. It proves that B, FA, and FB produced positive *cocci* and *staphylococcus spp* on grams stain. The average log mean count or aerobic plate count of the sample B, FA, FB, FC

kept at two storage conditions within 14 days were 5.9log10CFU/g, 4.8log10CFU/g, 3.4log10CFU/g, 0log10CFU; 7.0log10CFU/g, 6.9log10CFU/g, 6.3log10CFU/g, 0log10CFU for 21 days; 8.5log10CFU/g, 7.9log10CFU/g, 7.2log10CFU/g, 0log10CFU for 28 days and 10.5log10CFU/g, 9.9log10CFU/g, 8.3log10CFU/g and 0.2log10CFU/g for 35 days as shown in figure 2. The dilutions 10^{-6} and 10^{-8} of the FC sample were further sub-cultured in a sterile plate for 24 hrs at 37°C. The aerobic plate count of sample FC maintained 0.2log10CFU/g. It did not exceed the limit of 6.9log10CFU/g recommended by ISO NF-21149 (2009) for cosmetics analysis. It indicates that sample FC is safe for human skin. The absence of microbes on sample FC may be due to the antimicrobial properties of the cocoplum seed oil. This calls for more study on the effect of antimicrobial to microorganism's test.



	14	21	28	35
B	5.9	7	8.7	10.5
FA	4.8	6.3	7.2	9.9
FB	3.4	6.3	7.2	8.3
FC	0	0	0	0.2

Fig 2: Average Aerobic plate count (APC) of B, FA, FB, and FC at 37°C

V. Conclusion

The findings indicated that the formulation of oil in water (O/W) emulsion with cocoplum oil would be a promising oil for cosmetics and pharmaceutical formulations. Incorporation of cocoplum seed oil would prevent emulsion instability, increase the shelf, and eradicate the growth of microorganisms irrespective of preparation time. The pH value obtained on the two storage conditions proves that it is suitable for human skin and can decrease with time. The resistance of sample FC to phase separation, even on centrifugation and absence of liquefaction, makes it safe for human delivery. In this study, the prolonged time of emulsion stability may be achieved with suitable and more quality emulsifiers. The aerobic plate count of FC (0.2log10CFU/g) during microbiological evaluation indicated that the formulation of emulsion with cocoplum oil is safe for human skin.

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