International Journal of Chemistry and Pharmaceutical Sciences

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R E S E A E C H A R T I C L E

Cosmetics Emulsion from African Nutmeg Oil (Monodora Myristica): Formulation, Chemical Evaluation and Microbiological Analysis

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A B S T R A C T

Globally, adulterated skin care products have been on distribution in the cosmetic market which causes alot of skin infections which has become a major concern in the global world. The addition of African nutmeg oil extract as a suitable method of eradicating the threat of skin diseases was employed in this work. The formulation containing 5% of African nutmeg oil was developed by entrapping it in oily phase of oil in water (o/w) emulsion. The African nutmeg oil was added in increasing order of 0, 10, 20 and 30 w/w % and formulation with paraffin oil was investigated. Rosemary oil was incorporated to improve the fragrance. Both base and formulations were stored at room temperature for 28 days for investigation. The more effective formulation were evaluated for physical analysis, specific gravity, Electrical conductivity, Emulsion stability test, centrifugation test, pH determination, microbial analyses were evaluated using acceptable standard methods. The optimized formulation of emulsion with African nutmeg oil showed good resistance to phase separation on centrifugation under storage condition. The finding indicates that there was no significant growth of harmful microorganism at dilution 10^{-4} and 10^{-6} using pour plate method after 48hrs of incubation at 37^0C. Culture yielded no fungal growth, pseudomonas aeruginosa. The species of staphylococcus present were found to be normal flora of skin. The finding indicates that the African nutmeg oil of oil in water (o/w) emulsion is stable and proved to be suitable and exhibit the attribute that might open a new opportunity for the preparation of more efficient skincare, pharmaceutical and cosmetics products.

Keyword: African nutmeg oil, paraffin oil, tween 20 and 80, stability, pH determination, organoleptic test.

A R T I C L E I N F O

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PAPER-QRCODE

A R T I C L E H I S T O R Y: Received 15 March 2018, **Accepted** 28 April 2018, **Available Online** 27 May 2018

Citation: Ezeuko Adaora Stella, *et al. Cosmetics Emulsion from African Nutmeg Oil (Monodora Myristica): Formulation, Chemical Evaluation and Microbiological Analysis*. *Int. J. Chem, Pharm, Sci.*., 2018, 6(5): 151-156.

C O N T E N T S

1. Introduction

Cosmetics and pharmaceutical preparations for the treatment of skin infection and diseases are usually supplied in form of emulsion. Creams, lotions, massage oil and compresses are all external preparation used in treatment of skin problems such as bruises, rashes, acne and pains, all these products are oil and water preparations.

An emulsion can be defined as an immiscible liquid where droplets of one phase (disperse or internal phase) are entrapped within sheets of another (continuous or external phase). An emulsion needs Emulsifiers to stabilize the system[1]. The main actions of emulsifiers are to reduce the interfacial tension between the phases and forming a barrier between the phases. Emulsifiers also stabilize the system by forming a thin film around the globules throughout the second phase (continuous phase). The hydrophilic-lipophilic balance (HLB) of an emulsifier should be considered before preparation of emulsion. This hydrophilic –lipophilic balance (HLB) of emulsifiers relates to the solubility of emulsion. The emulsifier having low HLB tends to be oil soluble whereas the emulsifiers with high HLB are water soluble. Blends of emulsifiers can be used be used and may have the same HLB number yet exhibit different characteristic.

"Oil in water" (o\w) or "water in oil" (w/o) emulsion represents the majority of pharmaceutical and cosmetics creams evolve with time. A system which consist of oil droplets dispersed in an aqueous phase is oil in water emulsion while a system that consist of water droplets dispersed in oil phase is called water in oil emulsion[2]. Oil in water emulsion is used as general cosmetics product and drug bases while water in oil emulsion are used as emollient for treatment of dry skin. Also it can be used for cleansing of oil soluble dirt from skin. They are thermodynamically unstable, splitting into two phases[3]. This instability of emulsion depends on the composition and concentration of the emulsifiers used, some of these emulsion defects includes, sedimentation, flocculation, coalescence, and Ostwald ripening.

Cosmetics products such as pharmaceutical ointments contain emulsion which must penetrate deep into the skin to reduce skin defects. These creams should have storage stability at ambient temperature and should be marketed as quickly as possible. The shelf life assessment of oil in water (O/W) or water in oil (W/O) emulsion has been time consuming and required a great physical or mental effort of the industrial scientist. Their final objective is to save time by predicting weather the emulsion is unstable before breakage and separating into two layers which is visible by naked eyes. The numerous applications of emulsion include cosmetics, foods, medicine, paints, hydraulic fluids, asphalt, polymerization, fibre production, printing, metal and wood processing.

Supplementary values can be conferred to the formulations by adding active ingredients such as antioxidant from plant extract or natural source to combat the effect of oxidative stress and skin diseases. The use of plant extract in cosmetics and pharmaceutical creams to cure tropical skin disease is as a result of beneficial and therapeutic properties of the plant. Their derived products have been incorporated in form of emulsion in modern day's cosmetics, pharmaceutical formulations and preparations[4]. Cosmetics and pharmaceutical industries have increased their interest in replacing synthetic raw materials with plant extract which improves the appearance by providing nutrients necessary for healthy skin[5]. They are able to improve skin texture, radiance, tone and reduce wrinkle.

The number of ingredients which is derived from plant origin such as olive oil, castor oil, coconut oil, almond and olive oil have been used greatly in the preparation of cosmetics and pharmaceutical emulsion. Due to therapeutic and healing properties of African nutmeg (Monodora myristica) which are well known since ancient time, it was employed in this study to create the awareness of its efficacy in oil in water emulsion. In addition to its application as food spices or additive and active pharmaceutical ingredients, it also has great potential cosmetics ingredients. It is locally called ehuru ofia in Igbo, ariwo in Yoruba.

It is a perennial plant that belongs to the family of Annonaceae. It is cultivated in India, Sri lanka and Southern part of Nigeria. It is found in deciduous forest of tropical African country. The seeds are embedded in a white sweet smelling pulp of a fruit that can be 20cm long by 15cm in diameter[6]. In the olden days the juice pressed out of the fresh bark of the seed is used in treatment of itching and various eye diseases. Report has shown that the oil contains some important compounds which can be used in treatment of headache, migraine and cold. The oil can be applied externally as oily pomade to a sore especially those caused by guinea worm.

The African nutmeg oil extract is not only beneficial to human skin but also capable of preserving the shelf life of oils within emulsions and cream bases. Cosmetics can be contaminated by microorganisms and pathogens which may spoil the emulsion and causes serious skin disease to the users. It can be invasive when applied to a blemish or break in the skin[7]. This study was undertaken to investigate the potential gain effects of using African nutmeg oil in place of paraffin oil in preparation of cosmetics emulsion for construction of more efficient, safe and cost effective skin care products.

2. Materials and Methods

Materials

5kg of African nutmeg seed (Monodora myristica) was purchased at Agbara market in Ogun State and certified at Herbarium in University of Lagos, Akoka, Nigeria. Paraffin oil, Tween 20, Tween 80, Carbopol 940, Triethylamine (TEA), Deionise distilled water was obtained from Esota Chemical Store Oshodi, Lagos State.

Methods

Preparation of the African nutmeg oil (Extraction):

5kg of African nutmeg seed sample (Monodora myristica) were air dried at ambient temperature for five weeks. The seed were grounded using moulter and pestle. After grinding, it was transported to the laboratory where the oil was extracted with normal hexane as a solvent within 48 hours using soxhlet extraction unit. The oil mixed with the solvent was poured into a schlink flask, set up in a Rotary extractor at 40- 50^{o}C until the volume was reduced. The oil concentrated was store in amber bottles to avoid oxidation. The oil sample obtained was subjected to experimental procedure.

Preparation of Emulsion:

Oil in water (O/W) emulsions were prepared using a modified method of Henrietta, 1995, the formulation were prepared at different concentration by addition of an aqueous phase to the oil phase through continuous agitation.

Preparation of Base:

The oil phase consists of paraffin oil, Tween 20 and 80, carbopol 90, triethylamine were heated up to 75^{o}C. At same time, an aqueous phase which is the deionised distilled water was also heated to 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 10rpm for 2 hours, Two to three drops of rosemary oil were added during stirring period when the temperature reached 55^{o}C to give fragrance to emulsion. After stirring for 2 hours, the speed of the stirrer was reduced to 5rpm for 30 minutes in other to achieve complete homogenization. Agitation was maintained until the emulsion was cooled at room temperature.

Preparation of Emulsions:

Three oil in water emulsions (FA, FB and FC) were prepared using tween 20, tween 80, carbopol 940, triethylamine paraffin and African nutmeg oil at different concentration. The formulations were prepared in two steps. Firstly, formulation with paraffin oil and African nutmeg oil at different concentration with the emulsifiers and deionised distilled water.

Secondly the formulation of African nutmeg oil with the emulsifiers without the paraffin oil as shown in table 1. The deionised water heated at the same temperature with the oil phase, added drop by drop to the mixture of African nutmeg oil, tween20 and 80, carbopol 940 and hours. Two to three drops of Rosemary oil was added to give fragrance to the emulsion when the temperature reached 55^{o}C. After the addition of aqueous phase, the speed of the stirrer was reduced to 5rpm for 30 minutes to achieve homogenization. Agitation was also maintained until the emulsions were cooled at room temperature.

Experimental

Physical analysis:

The emulsion prepared were subjected to a set of organoleptic (feel, thickness, colour, look, creaming) and phase separation.

Emulsion stability

Stability 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 4 rpm. The emulsions were then transferred into a 100ml of measuring cylinder and kept at room temperature for 2 hours. The readings were taken as follows:

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

Where:

VB is the volume of aqueous phase before emulsification.

VA is the volume of aqueous phase after emulsification.

Centrifugation test:

The centrifugal tests were carried out on freshly prepared emulsions. The test was repeated on the emulsions after 24hours 7, 14, 21 and 28 days of preparation. They were performed at 2000 rpm at 27^{o} C for 15 minutes by placing 10g of sample in a centrifuge tube.

pH determination:

The pH of freshly prepared emulsions was determined using digitalpH- 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 days of preparation.

Electrical conductivity:

Electrical conductivity of each emulsions were measured using conductivity meter by dipping the probe of the equipment into the samples kept at 27^{o} C for 28 days[8]. It is carried out to determine the nature of the emulsions and to control the stability with time [9].

Specific gravity:

The specific gravity of the emulsions was measured using 20ml specific gravity bottle at room temperature. The empty bottle was weighed and the value was recorded. 20ml of the sample were filled into the bottle and weighed. The different between the values were divided by the weight of 20ml of water measured in the same bottle. The values were recorded.

Microbiological analysis:

In order to access the degree of contaminations, nutrient agar and plate count agar method were employed in this work. Both media were prepared according to manufacturer's specification. Serial dilution was carried out on sample of base (B), FA, FB, FC up to the sixth dilution (10-6) using pour plate method after 48 hours at 37^{o}C. The dilution 10^{-2} and 10^{-3} were sub cultured in a sterile on the sample kept in an incubator for 48 hours at 37^{o}C. Grain stain was done in the microorganisms that appeared on the plate after 24 hours of incubator at 37^{o}C for identification and characterization.

Aerobic plate count:

Aerobic plate count (APC) was determined using a pour plate method. The samples were inoculated on the sterile plate and dried standard methods using surface spread

techniques. The plates were placed in an incubator for 48 hours at 37^{o}C[10].The method used was done on serial dilution 10^{-2}, 10^{-3}, 10^{-4}, 10^{-6} on the four samples prepared. Plate counting colonies observed were between 2 to 128 and the average number of CFU/ml was calculated.

3. Results and Discussions

Stability of formulated emulsions :

"Oil in water" (O/W) or "water in oil" emulsions is a major constituent of most cosmetics and pharmaceutical creams which evolve with time. These types of emulsions are thermodynamically unstable, splitting into two immiscible phases manifested at different time rates caused by physicochemical destabilize processes such as sedimentation or creaming, flocculation, coalescence. In this study, formulations were placed at 30^{o}C for 28 days. The samples were investigated for change in colour, liquefaction, and phase separation as represented in Table 2. It was observed that emulsions FC were stable throughout the 28 days of storage at 30^{o}C. While emulsion FA, FB and B separated into two distinct layers visible by naked eyes. Several mechanisms may be the cause of this instability including the rupture of the oil layers and also the storage temperature.

The study revealed that the freshly prepared emulsion FA, FB were white in colour but later changes to yellowish white before the 28 days of preparation while freshly emulsion FC and B maintained yellowish white and white up to 28 days of observation. The changes in colour of FA and FB appeared on the 14th day and persisted up to 28 days of observation and analysis. This change in colour of these emulsions was due to oxidation or exposure of the oil globules.

Temperature and time process affects the separation of emulsion leading to decrease in viscosity which increases the liquefaction. One of the causes of instability is liquefaction which is due to passage of water from the internal phase to the external phase. The present study reveals no liquefaction on the emulsions prepared and stored at room temperature throughout the 28 days of observation. The absence of liquefaction shows that the emulsions are stable under investigation. Several techniques were employed in the study for further investigation of stability of the emulsions which also includes phase separation and centrifugation.

Phase separation:

Creaming and sedimentation give rise to phase separation. Phase separation depends on the density difference and the viscosity of emulsion prepared. In other to reduce phase separation on emulsion, a gelling agent is added to increase the viscosity of the continuous phase. In this context, carbopol 940 were added to reduce the phase separation on the four set of emulsion prepared at different concentration. No phase emulsion was observed throughout the study period of 28 days.

Centrifugation:

Centrifugation test is the most important and widely applied in determining emulsion stability. It involves using a centrifugal force to separate two or more liquid into distinct layers which will be visible by naked eye. It is used to access the shelf life of emulsion[4]. Phase separation occurred on centrifugation on the emulsion B, FA, FB on the 21st day up till the 28th day of experimental period of the samples kept at room temperature. Sample FC kept at room temperature recorded no phase separation on centrifugation. This indicated that samples FC are stable at the storage temperature. This is due to the type of the oil used with surfactant which produces small droplet during homogenization.

pH determination: An important parameter used to determine the effectiveness of emulsion stability[12]. The suitable pH of human skin ranges from 4 to 6.5 and the average pH value of dry skin with high alkaline is 5.5. The pH of freshly prepared B, FA, FB and FC of set of emulsions was found to be 7.51, 7.30, 6.91 and 5.98 respectively. Thus the pH of the emulsions kept at different storage days at room temperature decreases with time (Table 2) and it continued till the 28th days of investigation. This decrease in pH was as a result of diffusion of water from dispersed to continuous phase and the fatty acid and other ingredients present in the oil extract[13].

Electrical conductivity:

Electrical conductivity value of formulations and base of fresh emulsion kept at 30^{o}C was determined. Electrical conductivity was found in B, FA and FB. No electrical conductivity was found in FC throughout the experimental period. This indicates the absence of instability in FC throughout the experimental period of 28 days at 30^{o}C.

Specific gravity:

Specific gravity is a number assigned to represent how dense or mass per unit volume of a substance in relation to water. The specific gravity of FC is 1141 g/cm^{3}

Microbiological analysis :

Microbial contamination on emulsion constitutes a threat to human skin when applied. It causes itching, tetchiness and a lots of skin diseases. In this study, microbial analysis of the emulsions prepared was investigated. Growth was not observed after 24 hours on the plate with dilution 10^{-2} and 10^{-3}. The dilutions 10^{-4} and 10^{-6} of FC sub cultured in a sterile plate for 48hrs at 37^{o}C produced no significant growth on gram stain. But emulsion B, FA and FB produced positive cocci and staphylococcus spp on grams stain. Also the culture yielded no fungal growth on FC after 7 days of incubation at 25^{o}C. The absence of microbes on FC may be due to the antimicrobial properties found in the oil of African nutmeg seed.

Aerobic plate count:

The further sub culture of the sterile plates for 48 hrs which was done by inoculating 0.1ml of homogenates samples produces significant growth of species of staphylococcus or colonies which resulted in aerobic plate count. The log mean count recorded on the B, FA, FB and FC samples on the 1st day are 4.5log 10CFU/g, 3.8log10CFU/g, 4.2log10CFU/g and 0log10CFU. On the 28 day of the study period, the long mean count reached 7.5log10CFU/g for the base (B), 6.9log10CFU/g for FA, 7.1log10CFU/g for FB and 0.2log10CFU/g for FC. The aerobic count on FC on the 28 days did not exceed the limit of 6.9log10CFU/g recommended by ISO NF – 21149, 2009 for cosmetics analysis. This indicates that FC is safe for human skin.

Table 1: Formulation of Emulsions at different concentrations varying paraffin oil and African nutmeg oil

Amount of materials (% w/w)				
Materials	**Base (B)**	**Formulation A (FA)**	**Formulation B (FB)**	**Formulation C (FC)**
African nutmeg oil	0	10	20	30
Paraffin oil	30	20	10	0
Tween 20	5	5	5	5
Tween 80	5	5	5	5
Carbopol 940	5	5	5	5
Triethylamine(TEA)	5	5	5	5
Deionised distilled water	50	50	50	50

Table 2: Physical characteristics of Base (B) and Formulations (FA, FB, and FC) at different concentration kept at ambient temperature (30^oC)

Parameter	**Fresh**				**24 hrs**				**7 days**				**14 days**				**21 days**				**28 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
Liouification	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-
Colour	w	w	w	yw	w	w	w	yw	w	w	W	yw	w	yw	yw	yw	w	yw	yw	yw	w	yw	yw	Yw
Phase separation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Centrifugation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-

(-) No changes; (+) Slight changes; (W) White; (YW) Yellowish white

Table 3: pH values of formulations at 30^oC

Formulations	**Fresh**	**7 days**	**14 days**	**21 days**	**28 days**
B	7.51	7.20	7.15	7.05	6.93
FA	7.30	6.93	6.43	6.30	6.25
FB	6.91	6.61	6.11	6.05	6.00
FC	5.98	5.68	5.18	5.10	4.90

4. Conclusion

The O/W emulsion containing African nutmeg oil was evaluated for physical stability. The result of this study indicates that sample FC with 5% African nutmeg oil possesses good stability, adequate resistance to phase separation and microbiological stability which is safe for human delivery. No liquefaction was observed during the 28 days of experimental period at 30^oC. The pH value of emulsion (FC) investigated different days at 30^oC decrease with increase in time. Microbiological assay reveals that sample FC was stable during storage conditions at 25^oC and is free from any contaminations.

5. Acknowledgements

The authors are greatful to the entire staff and management of Chemistry department of Covenant University, Ota, Ogun state. And also to their respective families who contributed immersely for the completion of this research. God bless you all.

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