International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Utilization and Anti Bacterial Assessment of *Chrysophyllumalbidum* Seed Oil on Skin Cream Formulation

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Abstract: ***Seed crops are vital sources of oils and the plant-derived oils from underutilized seed crops such as Chrysophyllumalbidum could be useful in cosmetic and pharmaceutical applications especially for the formulation of creams, soaps, emulsions, ointments and for wound disinfection and management. Three batches of creams were, formulated following the melt emulsification method of oil and aqueous phase. Batch A consist of (extracted C. albidum seed oil alone), batch B (olive oil used alone) and batch C (extracted oil and olive oil combined in a 1:1 ratio), then the physiochemical and anti-bacterial properties determined and compared with gentamicin a reference standard. Dilution tests of the formulated creams reveals an oil in water type of emulsion with pH values: batch A (6.85±0.08), batch B (7.13±0.04) and batch C (8.34±0.11). The formulated creams were smooth and non-greasy in appearance with viscosity (Cp) at 28.7^{O}C of batch A (1.13±0.02) batch B (3.04±0.04) and batch C (4.02±0.03) There was no phase separation upon centrifugation at 3000rpm but on thermal analysis at 60^{O}C, creams of batch A liquefied within 35 minutes , batch B- 79 minutes and batch C- 28 minutes. Anti-microbial activities reveals high susceptibility of test organisms (Staph aureus, P. aerugnosa, E. coli, P. vulgaris, K. pneumonia and B. cereus) to formulated creams with that of batch C being comparable to gentamicin followed by batch A then batch B.***

Keywords: *Chrysophyllumalbidum*, oil, Cream, utilization, anti- bacteria

1. Introduction

Oils are substances or materials, which occurs mainly, as liquid or semi- solid and could be of plant, animal or mineral origin.
Natural oils especially those sourced from plants could be either volatile or fixed and the volatile ones have been reported to have immunological and physiological effects [1].

Plant oils have been applied in cosmetics and pharmaceuticals as some of the plant derived oils could be useful in making soaps, hair and body creams, emulsions, laxatives, suppositories and even for industrial and technological applications as coating, polymers, lubricants, leather processing, pesticides/herbiciides and biofuel [2].

Seed crops are vital sources of oils, which could of nutritional pharmaceutical and industrial importance. The characteristics of the oils from the diverse sources, is dependent mainly on their compositions, where no oil from a single source can be satisfactorily useful for all purposes [3].

Reference to this diverse usefulness therefore, is the need for further investigation of plant oils of underutilized seeds in order to employ them medicinally, pharmaceutically and industrially for ointment and cream production and for wound disinfection and management [4].

Presently, the quest for natural vegetable oils has increased immensely because of the ever-growing world population and their use for industrial purposes. One of the possible alternative crops is *Chrysophyllumalbidum* also known as African star apple. Its seeds are a source of oil, which is used for diverse purposes [5].

The species *Chrysophyllumalbidum* G.Don, belongs to the kingdom Plantae and family Sapotaceae. It is a small to medium-buttressed tree up to 25 – 36m in height. The plant is commonly found in the rainforest region of West Africa and in Nigeria it is commonly called agbalumo, udala and agwaliba by the Yoruba, Ibo and Hausa tribes respectively [6].

The plant produces seeds which are about 1.0 – 1.5 x 2cm, bean like, shiny when ripe, compressed with one sharp edge and a star – shaped arrangement in the fruit. The seed coats are hard, bony, shiny and dark brown which when broken reveals a white colored cotyledons.

The *Chrysophyllumalbidum* seed oil is characterized by presence of tri-glycerol, glycol-lipids, and phospholipids and is also rich in both linoleic acid and oleic acid.

The oil could be extracted from the seed using various processes including, pressurized solvent extraction, mechanical pressing, sox let extraction, ultra- sonic extraction and aqueous enzymatic oil extraction [7].

The medicinal properties of the plant received a great interest due to its low toxicity, pharmaceutical activities and economic viability [8].

In a screening test under taken by some researchers, ethanolic extracts from the seed of *Chrysophyllumalbidum* were found to be effective against some microorganisms including *Staphylococcus aureus*, *Escherichia Coli*, *Pseudomonas aerugenosa*, *Proteus vulgaris* and

Micrococcus varians. The result suggest that the crude extracts obtained showed strong activity against most of the tested bacterial strains when compared to Gentamicin standard and hence can be applied in the pharmaceutical industries as an anti-microbial agent [9].

Creams

These are viscous semi– solids, which are either oil- in – water emulsions (aqueous creams) or water- in- oil emulsions (oily creams). Certain water- miscible bases that have a complex matrix- like physical structure are referred to as creams [10]. The ideal properties of creams includes: high affinity, rapid onset of action, bio compatibility, free from grittiness, smooth to touch, readily washable, non-irritant, non- allergic, non- toxic, physically and chemically stable.

Cream bases

Aqueous creams are usually oil- in- water emulsions. By the selection of anionic, cationic or non- ionic emulsifying agents, it is possible to formulate aqueous creams, which are compatible with most active pharmaceutical substances. The water – in – oil emulsions, form oily creams and are frequently used for their emollient and occlusive properties [11].

Rheology and stability of Creams

Emulsified creams are usually non- Newtonian systems, their rheological properties vary with the shear forces applied, and hence viscosity and flow characteristics may change in accordance with the degree to which the system are homogenized or with the amount of shear applied during processing either in mechanical devices or by a slight agitation [11].

Cream instability

Instability of creams occurs when dispersed phase droplets of the emulsion collide and coalesce, producing corresponding larger emulsions droplets, the presence of which destabilizes the system. The large droplets may further collide, coalesce, and compete for interaction space, which might eventually lead to cream destabilization. The instability of creams may thus be classified as sedimentation, creaming, coalescence, breaking, miscellaneous physical and chemical changes and phase inversion [12].

Classification of creams

Pharmaceutical or skin creams are classified based on contents and functions as: cleansing and cold creams, foundation and vanishing creams, night and massage creams, hand and body creams then all purpose and general creams. Creams therefore, are used, for their cleansing, vanishing, provision of barrier to skin perspiration, retention of moisture, drug administration, prevention of skin burn and emollient effect [13].

The objective of the study is to formulate pharmaceutical/ cosmetic creams using the extracted *Chrysophyllumalbidum* seed oil and assess the stability and anti-microbial activity relative to olive oil and Gentamicin as standards.

Materials Used

Extracted *Chrysophyllumalbidum* seed oil (Pharmaceutical Technology laboratory, University of Port Harcourt, Nigeria), olive oil, staeric acid, Tween 80, Peptone water nutrient agar and Muller Hunton agar (Titian Biotech, India), photo colorimeter, Table centrifuge (PEC Medicals, USA), pH meter (Helmreason PHS – 25, England).

Method

Formulation of Cream

Emulsifying Ointment was prepared using emulsifying wax, white soft paraffin and liquid paraffin [14].

Three batches of an oil in water (o/w) cream of 30g weight each of batch 1 consisting of extracted *Chrysophyllumalbidum* oil 21%, batch 2 - olive oil 21% and batch 3 a 1:1 ratio of extracted oil and olive oil was formulated with the given formula.

Table 1: Formula for cream formulation

Oil Phase	
Glycerine	5.6%
Stearic Acid	8.5%
Emulsifying Ointment	2.5%
C. *albidum*Oil / Olive Oil	21.0%
Aqueous Phase	
Water	53.5%
Methyl Paraben	0.5%
Sodium Lauryl Sulphate	2.0%
Tween 80	3.5%
Sodium Carboxy Methyl Cellulose	2.4%
Sodium Meta bisulphite	1.0%

The oil phase was prepared by mixing the ingredients: Glycerin (5.6%), stearic acid (8.5%), emulsifying ointment (2.5%), and the oil (s) (21.0%) in a beaker. This mixture was melted to a temperature of 70-75^{o}C.

The aqueous phase was prepared by incorporating the ingredients in a beaker with water. These included water (53.59%), methyl paraben (0.5%), sodium lauryl sulphate (2.0%), tween 80 (3.5%), sodium carboxymethyl cellulose (2.4%) and sodium meta bisulphite (1.0%). They were introduced into a hot water bath and stirred continuously until they attained a temperature of 70-75^{O}C as the oil phase.

While stirring, the oil phase was gradually added to the aqueous phase, still in the water bath at the same temperature. The mixture was then homogenized and passed through a stream of cold water to cool and solidify.

Cream Evaluation

Anti-bacterial Property of the cream

Susceptibility testing: Anti-microbial activity of the oils was carried out using the Mueller Hinton agar. Agar plates were inoculated with 0.1 ml broth culture of test organisms (E.coli, P.aerugenosa, P. vulgaris, K. pneumonia and B. cereus), and spread with an L-shaped glass rod. Sterile cork borer was used to make agar wells on the media and 2 drops of the creams were introduced into the wells. The plates were allowed to stand for 1h for pre diffusion of the creams to occur then incubated, at 37^{O}C for 24 h. The inhibition

zone diameters were measured in millimeters (mm) [15]. This test was conducted for all the batches of creams formulated and that of gentamicin used as a reference standard.

Organoleptic
The appearance of the cream was judged by its color using a lovibond colorimeter, the pearlscence and roughness were also graded accordingly [16].

pH Evaluation of the Cream
A10ml aqueous solution using 1.0g of the sample from each batchwas made in a beaker and the electrode inserted into it and the reading recorded when stable and in triplicates then the mean taken.

Determination of the cream viscosity
The sample (cream) to be evaluated was introduced into a clean 600 ml beaker. A temperature probe was attached to spindle guard leg and the viscometer lowered into the beaker until the spindle becomes fully immersed in the sample.

The procedure was repeated at different temperatures ($25^{O}C$, $40^{O}C$ and $50^{O}C$) and the displayed viscosity was recorded in Centipoise (Cp), rpm and %Torque on the viscosity data sheet

Physical observation of the Cream
A 14 man volunteers consisting of 8females and 6 male within the environment of study were contracted to evaluate the feel, homogeneity, wetness, emolliency and smear of the formulated creams.

The formulations were tested for the homogeneity by visual appearance and by touch to determine the extent of smoothness or grittiness of the cream.

The wetness was carried out to know whether it could impact any moisturizing effect, the type of smear was evaluated by noting the ease at which the creams can be washed off after application on the skin. The emolliency, slipperiness and amount of residue of the cream was determined on 12 of the volunteers suspected to have dry skin and the extent to which the cream lubricated or softened the skin was noted [17].

Determination of Emulsion type

Dilution test: The emulsion (cream) is diluted either with water and oil using two separate beaker. The emulsion type was determined by observing for stability or breaking of the cream globules in the different medium.

Dye test: This was also carried out to determine the extent of spread of the dye (methyl blue) on the creams.

Conductivity Test of the Cream
This was done using the Probe –Conductometer and this involves the insertion of the electrode into a beaker containing the aqueous sample and connected to a power source. The reading when the sensitive spindle swing became stable was taken and in triplicates while the mean of the readings was calculated and recorded

Centrifugation Test of the Cream
Centrifugation test was carried out for the three batches of creams after preparation. The tests were performed using the table centrifuge (PEC medicals USA) at 3000rpm for 10 minutes and at room temperature after infusing 5.0g of the samples in the centrifuge tubes.

Refractive Index of the Cream
This was done using the refractometer consisting of an incident and refractive prism [18]. A 1%w/v aqueous solution of formulated cream (sample) was made and few drop of the sample to be tested was placed on the polished surface of the lower Refracting Prism.

The hinged upper Incident Prism was closed and locked into place with the knob, so that the liquid becomes evenly distributed on the face of the Refracting Prism.

The lower adjustment knob was scanned until a light and dark divided image was seen.

The dispersion was adjusted, using the upper smaller dispersion correction knob, until a sharp light/dark boundary was seen.

The boundary in the crosshairs of the telescope was centered using the lower large adjustment knob and the refractive index noted from the green scale below the boundary.

Thermal / Stability test of the Cream
The cream samples were subjected to various temperatures ($27^{o}C$ and $60^{o}C$)so as to determine the appropriate temperature for storage and stability assuming the formed cream is subjected to varied environmental conditions.

Spreadability of the cream
Spreadability of the cream formulated was determined by measuring the spreading diameter of 1.0g of the sample between two horizontal glass plates (10cm x 20cm). The standard weight applied to the upper plate was 25g. The time in which the upper glass slide moves over the plate to cover a distance of 10cm is noted. Each formulation sample was tested in triplicates

The spreadability (S) was determined using the relation
S = M x L/T where S = spreadability, L= length moved on the lower glass slide
T = time taken (minutes), M = weight applied to upper plate

Free alkali test
A 5.0g each of the cream samples was placed in a 250ml flask and dissolved in 100ml distilled water after gentle heat application. The resultant solution was cooled to room temperature and 3 drops of 0.1% methyl orange indicator was added and titrated with 0.05M H_2SO_4 [20].

$$\text{Free alkali (expressed as } Na_2O) = \frac{V \times 100 \times 0.0031\%}{W}$$

Where V = volume (ml) of 0.05M H_2SO_4 solution, W = weight (g) of the sample

2. Results

Figure 1: Cream formulated with Olive Oil

Figure 3: Cream made using equal ratio (1:1) of extracted *C. albidum* seed
Oil and Olive Oil

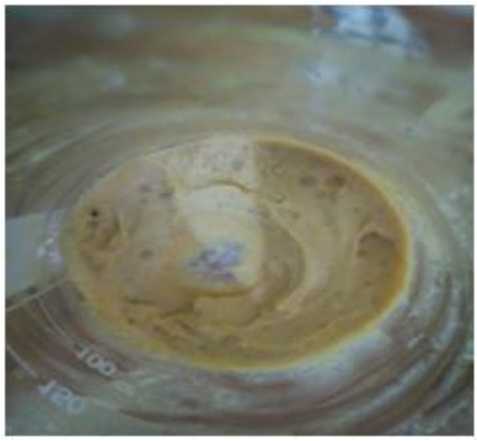

Figure 2: Cream of extracted *C.albidum*seed Oil

Evaluation of the Formulated Creams

Table 2: Physical Characterization of the Formulated Creams

Physical parameter	Observation		
	Cream A	Cream B	Cream C
Appearance	Cream colour	White colour	Light Brown colour
pH	6.86±0.08	7.13±0.04	8.34±0.11
Homogeneity (By Touch and Visual)	Homogeneous, Smooth and consistent	Homogeneous Smooth and consistent	Homogeneous Smooth and consistent
Viscosity (Cp)	1.13±0.02 at 28.7^{o}C	3.04±0.04 at 27.6^{o}C	4.02±0.03 at 28.3^{o}C
Dilution Test	O/W type of emulsion	O/W type of emulsion	O/W type of emulsion
Robustness (Spreadability and Wetness)	Easily Spreadable and Moisturises Skin Surface	Easily Spreadable and Moisturises Skin Surface	Easily Spreadable and Moisturises Skin Surface
Type of smear	Non-greasy	Non-greasy	Non-greasy
Emolliency	No residue left	No residue left	No residue left
Refractive Index	1.33±0.00	1.33±0.00	1.34±0.01
Conductivity mhos/cm	650.67±4.04 at 29.2^{o}C	920.67±60.48 at 28.5^{o}C	1266±4.00 at 29.9^{o}C

Cream A = Formulated Cream of *C.albidum* seed oil, Cream B = Formulated Cream of Olive Oil and Cream C = Formulated Cream of *C.albidum* and Olive Oil in a 1:1 ratio

Table 3: Centrifugation Test for Formulated Creams

Sample	Observation
Cream A	No Phase Separation Occurred
Cream B	Phase Separation Occurred
Cream C	No Phase Separation Occurred

Table 4: Thermal Test for Creams

Temperature	Cream A	Cream B	Cream C
Room Temp (29^{O}C)	Stable	Stable	Stable
60^{o}C	Liquefied within 35 mins	Partially Liquefied within 79 mins	Liquefied within 28 mins

Result of Microbial Analysis

Table 5: Anti- microbial effects of oils on some bacterial isolates

	Agents	*E coli*	*S. aureus*	*P. aerugnosa*	*P. vulgaris*	*B. cereus*	*K. pneumonea*
Zone of inhibition (cm)	Gentamicin	30	25	19	40	35	20
	Olive oil	16	17	23	20	20	18
	Extracted *C. albidum* oil	18	30	30	30	49	25
	Combined Olive oil and extracted *C. albidum* oil (1:1) ratio	15	25	20	25	30	22

Table 6: Free Alkali Test

Products			
	Cream A	Cream B	Cream C
Mean titre volume (ml)	1.2	0.1	0.2

3. Discussion

Three batches of creams were formulated with difference in the actual content of the oil phase, where batch A and B were formulated with the extracted *C.albidum* seed oil and olive oil, respectively, whereas batch C contain equal proportion of the extracted *C. albidum* seed oil and olive oil in a 1:1 ratio.

Some similarities and differences occurred amongst the three batches as shown in Table 2. Such similarities included the homogeneity, emulsion type as detected after adopting the dilution test method, robustness, type of smear and emolliency as also shown in Table 2.

Based on physical observations, all the three batches of the cream formulation have cosmetically appealing appearance. They had a pH that was close to neutrality hence the product outcome could be safe upon application on the skin without any possible association of corrosive or deleterious effect. The third batch had the greatest consistency and that reflected on the outcome of refractive index and electrical conductivity result as obtained from the test conducted on the cream [19].

Reference to the antimicrobial screening result of the extracted oil, the formulated creams were also tested against both Gram negative and Gram positive bacteria which included *Escherichia coli, Staphylococcus aureus, Pseudomonas aueruginosa, Proteus vulgaris, Bacillus cereus* and *Klebsellapneumoniae* as in Table 5. Activities of the three batches of creams formulated from the oils (extracted *C.albidum* seed oil, olive oil and combined 1:1 ratio of extracted oil and olive oil), against the chosen bacterial isolates was compared to that of gentamicin. Greater anti-microbial activity than gentamicin was observed for the various cream formulations on most of the isolates except for*E. coli* and *P.vulgaris.* Olive oil cream inhibited the growth of the bacterial isolates but its activity was lower than the cream formulated using *C. albidum* seed oil. The zones of inhibition produced by cream formulated using the combination of the oils was lower than that made using only the extracted *C.albidum* seed oil. Thus there was no synergistic effect rather the presence of the olive oil could have influenced the reduced anti microbial activity of the resultant cream from the batch. The observed antibacterial activity of the *C. albidum* seed oil, as demonstrated by the study can be a frontier for its use in the formulation of pharmaceutical creams useful for wound disinfection and management [4].

In the stability assessment under varied temperatures as in Table 4, the three batches were stable at room temperature but batch C was the least stable especially as the temperature rises to about 60^{o}C. This implies that the creams should be stored at below or room temperature (29.38^{o}C) as their consistency is gradually affected by temperature and environmental changes especially for that consisting of olive oil and extracted seed oil. Another stability test carried out on the cream was centrifugation. The batch formulated with only olive oil separated into two different phases (Table 3). This instability occurred as a result of the inappropriate pressure/condition under which the cream formulated with olive oil was subjected but such was not observed in the other batches of creams (batch A and B) consisting of *C. albidum* oil. This suggests therefore that the cream formulated with olive oil content alone is liable to ease of deterioration with pressure during movement or transportation hence should be handled with care upon storage. The outcome of this study therefore suggests an appreciable - disinfectant, durability and stability profile of *C. albidum* seed oil, as it was able to impact anti- microbial effect on some microbial isolates, withstand reasonable increase in temperature and maintain stability over increased pressure application hence recommended to be incorporated as an ingredient in pharmaceutical cream formulation.

4. Conclusion

An oil in water cream was, successfully formulated using the extracted oil from *C.albidum* seed. The seed oil exerted synergestic activity with olive oil when used in a 1:1 ratio forming a smooth, non-greasy cream with anti-microbial effect comparable to gentamicin, although the effect of the extracted oil was higher than that of olive oil when used alone in the cream formulation.

The formulated creams using *C.albidum* seed oil was of neutral pH comparable to olive oil but of low conductivity hence could be compatible with most ingredients for use in cream formulation and to the human skin and the cream formulated with the seed oil also maintained stability and durability over appreciable temperature and pressure changes. Interest should therefore, be geared towards the advancement and incorporation of oil from natural waste material such as *C.albidum* seed in pharmaceutical cream formulation to discourage the neglect of natural waste materials but rather enhance the advancement and economic growth of the developing countries.

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