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Characterization of microemulsions formulated from naturally and chemically extracted oils from *Cocos nucifera Linne*

O. A. ADETUNJI*[1A-F], B. ADESANWO[1BCF], O. A. ODENIYI[2A-C,EF]

[1]Department of Pharmaceutics & Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria
[2]Department of Microbiology, University of Ibadan, Ibadan, Nigeria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Oil derived from *Cocos nucifera Linne* (CNL) has been used in formulating creams; however, its use in formulation of microemulsion is not well documented in literature.

Objective: Oils obtained from CNL were characterized, used in the formulation of microemulsions and were evaluated for their antimicrobial properties in comparison with Castor oil BP (CO) and commercially available coconut oil (CACO).

Methodology: Oil from CNL was extracted by maceration in hot – water and petroleum ether to yield naturally extracted oil (NEO) and chemically extracted oil (CEO) respectively. The NEO and CEO were used in formulating microemulsions containing pre-determined ratios (3:7, 1:1, 9:1) of oil to surfactant mix (Smix) [containing polyethylene glycol: Tween 80® at ratio 2:1] Characterization of NEO, CEO and microemulsions were carried out using elemental constituents, rheology, physico-and phyto-chemical analysis, thermal stability and antimicrobial profiles as parameters. Statistical analysis was done using ANOVA at $p<0.05$

Results: The NEO and CEO contained antraquinones and terpenoids, but were devoid of lead, copper, zinc, alkaloids, saponins, cardiac glycosides and flavonoids. Viscosity profiles were in the order CO>CEO>CACO>NEO. The microemulsions were in the size range 54.24±0.26-89.08±0.07 µm. Microemulsions of oil:Smix (3:7) were the most stable. Inhibition ranking was *Candida* sp.D25 (CEO:Smix>CACO: Smix>NEO: Smix>NEO>CO>CEO), *Candida* sp.D33 (CEO = CO>CACO: Smix=NEO>CEO:Smix=NEO:Smix), *Staphylococcus* sp.DS2 (NEO>CO>CACO:Smix>NEO: Smix>CEO: Smix=CEO) and *Pseudomonas* sp.DP8 (NEO:Smix>CO>CACO:Smix>CEO:Smix=NEO > CEO).

Conclusion: Oil derived from CNL has good potentials as an excipient in the formulation of microemulsions and the method of oil extraction had a significant effect on the antimicrobial activities and on the microemulsions formulated using the oils.

Keywords: Coconut oil, extraction method, castor oil, microemulsions, antimicrobial properties

INTRODUCTION

Microemulsions can be defined as clear, thermodynamically stable, isotropic liquid mixtures containing oil, water and surfactant, frequently in combination with a cosurfactant (Fouad, *et al*.,2013). The aqueous phase may contain salts or other ingredients and the oil phase may be complex mixtures of different hydrocarbons and olefins (Vinod *et al*, 2011). In contrast to ordinary emulsions, microemulsions form after simple mixing of the components and do not require the high shear conditions generally used in the formation of ordinary emulsions. Three basic types of microemulsions: Direct (oil dispersed in water), Reversed (water dispersed in oil) and Bi-continuous (interspersed lamellar formation of micro domains of oil and water) have been identified (Boonme, 2017). It has been estimated that approximately half of approved drugs are lipophilic and have poor absorption characteristic and bioavailability when administered from oral route (Shalviri*et al*, 2011). To meet with such challenge, drug delivery programs proffered better routes for drug administration with new drug delivery platforms (Hashem *et al*, 2011), including the use of microemulsions as effective vehicles for drug delivery across the skin (Vikramjeet *et al*, 2012), with very promising results as solubility enhancers, modified drug permeability in topical administration and quick penetration of drug in skin (Hashem *et al*, 2011).
Topically administered microemulsion systems have been shown to provide improved drug stabilization and improved dermal absorption due to their sizes when compared with conventional topical emulsions (Birus *et al*, 2007). The three main components of microemulsions include the oil phase, surfactant and the aqueous phase. If a co-surfactant is used, it may sometimes be presented at a fixed ratio to the surfactant and treated as a single "pseudo component". The relative amounts of these three components can be represented in a ternary phase diagram. Gibbs phase diagram (Fig.1) can be used to show the influence of changes in the volume fractions of the phases within the microemulsion system. Coconut oil is obtained from *Cocos nucifera Linne* (Family: *Arecaceace*) and due to its high content in saturated fatty acids (exemplified by lauric acid and myristic acid), it is used in the synthesis of most cosmetic detergents., and has been applied topically to promote wound healing (Nevin and Rajamohan, 2010). Some of the most promising research has been in the area of using lauric acid or monolaurin, both derived from coconut oil, in treatment of antibiotic resistance (Abbas *et al*, 2017). Research shows that coconut oil is a good topical agent for fighting germs (Marina *et al*, 2009). Lans (2007) reported that *Cocos nucifera* was also used in ethno-medicine to treat minor cuts, injuries and swellings. However, the use of coconut oil in the formulation of microemulsions has not been well documented in literature. Thus, in this present work, the oil obtained from *Cocos nucifera* (extracted using two different methods) were characterized, assessed for their pharmaceutical properties and used in the formulation of microemulsions whose properties were compared with Castor oil BP and commercially available coconut oil. The microemulsions were also evaluated for their antimicrobial properties.

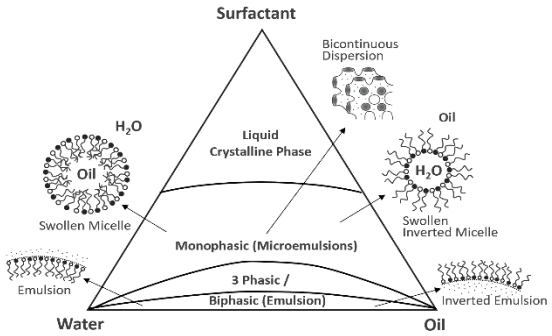


Figure 1: Phase diagram of microemulsion formation (https://en.wikipedia.org/wiki/Microemulsion)

METHODOLOGY

Materials

Pharmaceutical grade petroleum ether, polysorbates 80 (Tween 80® and polyethylene glycol (PEG) were obtained from Analar Laboratory reagents, Sussex UK, Castor oil B.P (CO), (*Ricinum communis*, Family: *Euphorbiaceae*) was obtained as a gift from Drugfield Pharmaceuticals Ltd., Nigeria, while a registered brand of commercially available coconut oil (CACO) was procured from a registered pharmacy in Ibadan, Nigeria. Ultra-pure water (UPW) from Milli-Q system (Millipore, USA) was used. Coconut fruits (*Cocos nucifera;* Family: *Arecaceace)*, were procured from Association of Crop Farmers in Oyo State, Nigeria and authenticated at the department of pharmacognosy herbarium, University of Ibadan, Nigeria. The test organisms (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans)* were obtained from the pharmaceutical microbiology laboratory stock of the University of Ibadan, Ibadan, Nigeria. All other reagents used were of analytical grade.

Methods

Water extraction of coconut oil

The method of Oseni *et al* (2015) was adopted for this extraction method. The fresh endosperms of coconut were manually separated from the shells and then washed to remove dirt. The washed endosperms were cut into small pieces, weighed and milled using a Kenwood laboratory blender (BL 440A, Woking, UK). The resulting mass was mixed with UPW (70 ^{0}C) and filtered using muslin cloth. The process was repeated severally to ensure thorough extraction of the milky liquid oil which was heated (100 ^{0}C) for a period of 15 min. The cooled liquid oil was then decanted and finally re-filtered using muslin cloth to obtain the naturally extracted oil (NEO), which was stored in an air-tight container.

Chemical extraction of coconut oil

The fresh endosperm of coconut was separated manually from the shells and then washed to remove dirt. The washed endosperms were cut into small pieces and dried in the hot air oven at 50 ^{0}C for 48 h. The dried endosperms were weighed, milled using a Kenwood laboratory blender (BL 440A, Woking, UK) and soaked in petroleum-ether for 48 h. It was then filtered using muslin cloth to extract the oil. The filtrate (oil) was left to settle for 30 min before it was decanted to collect the chemically extracted oil (CEO) (Oseni *et al*, 2015)

Digestion of extracted oil

Exactly 2 g of each extracted oil sample (NEO and CEO) was placed in separate polytetrafluoroethylene (PTFE) flask, each containing nitric acid (65 %) and hydrogen peroxide (30 %) in a ratio of 2:1. up to the 20 mL mark, and kept at 27 ± 2 ^{0}C for 1 h. The PTFE flask was then placed in a microwave oven for 5 min to remove the excess acid before diluting the content with 0.2M nitric acid up to the 50 mL mark. The resulting mixture was filtered using muslin cloth and stored in air-tight containers (Jin *et al*, 2015)

Determination of elemental constituents of oils

Exactly 20 g of each digested oil sample (NEO or CEO) was weighed into a 350 mL Vycor dish and charred on a full-heat controlled hot plate. Carbonization of the digested oil was conducted at a temperature of 300 ^{0}C for 24 h. After charring, the dish was ignited in a lined muffle furnace at 500 ^{0}C for 16 h and the resulting ash was then dissolved in 10 mL of 5 % sulphuric acid. Acid digestion of the ash was carried out on a steam plate for 30 min and the sample was carefully washed with UPW (Jin *et al*, 2015). The procedure was repeated for CACO and CO.

Free fatty acid determination

Exactly 20 g of each digested oil sample (NEO or CEO) was weighed into a 250 mL conical flask and 50 mL of 95% alcohol (neutralized with 0.1N sodium hydroxide) was added. The content was heated to boiling and shaken thoroughly to dissolve the free acid completely. The solution was then cooled and titrated with 0.1N alcoholic caustic potassium hydroxide solution with constant shaking using phenolphthalein as indicator until a pink colour persists after vigorous shaking. The solution was kept hot to prevent precipitation of the oil (Jin *et al*, 2015). The procedure was repeated for CACO and CO.

Peroxide value determination

Exactly 1g of potassium iodide powder was added to 20 g of each digested oil sample (NEO or CEO) followed by the addition of 20 mL of the solvent mixture acetic acid and chloroform (1:1). The resulting liquid was then boiled and transferred quickly into a flask containing 5% potassium iodide solution. The mixture was then titrated with standard 0.002M sodium thiosulphate solution, using 1 % corn starch solution as an indicator until a colourless solution was obtained indicating the end point (Selin, 2019). The procedure was repeated for CACO and CO.

Determination of electrical conductivity

Exactly 2g of each digested oil sample (NEO or CEO) was weighed and diluted 10 folds using UPW. The sample was then analyzed with a multi-parameter machine H19828 (Instruments Inc. Woonsocket USA). The procedure was repeated for CACO and CO (Selin, 2019).

Phytochemical screening of extracted oil

The phytochemical chemical tests that were conducted on the extracted oils (NEO and CEO) were to determine the presence of carbohydrates (Molisch's test), reducing sugars (Fehling's test), ketones (Selivanoff's test), alkaloids (Wagner's and Dragendorff's tests), saponins, anthraquinones, tannins, glycosides, mucilages and flavonoids (AOCS, 2009)

Rheological properties of NEO and CEO

Viscosity of the digested oil sample (NEO or CEO) was carried out at 50 rpm and 100 rpm using spindle size 5 of a Brookfield Viscometer DV-II+ Pro, Brookfield Engineering Laboratories USA. The readings were done in triplicates and the procedure was repeated for CACO and CO (AOCS, 2009)

Formulation of microemulsions

The formulation design for the microemulsions prepared is presented in Table 1. A total of 250 mL of each microemulsion batch was prepared according to the ratios indicated in the formula. The ratios were determined based on preformulation studies. The mixture, which was melted in a crucible placed on a water bath, was transferred into a plastic beaker, and a homogenizer (Heavy duty laboratory mixer/emulsifier; Silver Machine Ltd, England) was used in formulating the microemulsions, which were later kept in air tight containers.

Table 1: **Formulation design of microemulsions on a ratio basis**

Composition of microemulsions				
NEO	**CEO**	**CACO**	**CO**	**Surfactant mix Polyethylene glycol: Tween 80 (2:1)**
1	1	1	1	1
3	3	3	3	7
9	9	9	9	1

Characterization of microemulsions

Determination of hydrogen potency

The hydrogen potency (pH) of each microemulsion formulation was analyzed using a digital pH meter (Delta 340, Germany). The readings were taken in triplicates (AOCS, 2009)

Rheological properties

Viscosities of the microemulsions (20 mL) were determined (in triplicates) at 50 rpm and 100 rpm (at a temperature of 27 ± 2 ºC) using spindle size 5 of a Brookfield Viscometer DV-II+ Pro, Brookfield Engineering Laboratories USA.

Globule size determination

The globule sizes of the formulated microemulsions were determined using a Malvern Zetasizer Nano-ZS90 microscope (Malvern Instruments, UK), that makes use of dynamic light scattering technique.

Determination of conductivity of microemulsions

Exactly 10 mL of each microemulsion formulation was measured and diluted with UPW to obtain a 1:10 dilution factor (Kogan, 2007). The sample was then analyzed for electrical conduction using a multi-parameter machine H19828 (HANNA) Instruments Inc. Wisconsin, USA.

Physical stability studies

Exactly 50 mL of each microemulsion formulation was allowed to stand at 27 ± 2^0C in an air-tight transparent container for forty two (42) hours and physically examined at specific intervals for phase separation, colour changes and changes in odour.

Microbial analysis of oil and microemulsions

Preparation of microbial culture and culture media

An overnight culture of each bacterial organism (*Staphylococcus aureus and Pseudomonas aeruginosa)* was prepared. Each organism in the stock was aseptically sub-cultured into freshly prepared nutrient broth using a flamed wire loop and then incubated at 37± 0.2 ^{0}C for 24 h. For the fungal test organism (*Candida albicans),* sterile wire loop was used to transfer a loopful of fungi spore to the surface of sabouraud dextrose agar (SDA) and streaked. They were then incubated at 28 ± 2 ºC until they grew and produced spores (5-7 days). The spores were harvested and used for sensitivity test.

Preparation of culture media

Nutrient broth was prepared. Volumes (5 mL) were distributed into test tubes bottles and sterilized by autoclaving at 121 ºC for 15 min. The nutrient agar was similarly prepared following the manufacturer's instructions. It was also distributed into 20 mL aliquots into universal bottles and sterilized in an autoclave at 121ºC for 15min (Ogbolu *et al*, 2007)

Evaluation of antimicrobial activity

The antibacterial activities of the oil (NEO, CEO, CACO or CO) and microemulsions were carried out by screening them against the test microorganisms using the agar well diffusion method. Freshly sterilised agar culture media (15-20 mL) were poured into 9 mm disposable petri dishes and allowed to set by solidifying. The agar surfaces in the plates were swabbed (using sterile swab sticks) with overnight broth cultures of the test organisms containing 0.5 McFarland standard, equivalent to 10^6 colony forming units per millilitre (cfu/mL) of cell broth. Wells, (8mm diameter), were bored into the agar mat and 100 µL of the oil and microemulsion samples were introduced into each well using sterile micropipette tips. After this, the bacterial plates and fungi plates were incubated at 37 ± 0.2 ºC and 25 ± 0.2 ºC respectively or 24 h and then observed for zones of clearance around wells. The diameter of the zones of microbial inhibition around each well (taken as measure of antimicrobial activity) was measured in mm and recorded. Readings were considered positive if greater than 1mm (Kazemipoor *et al*, 2012). The experiment was performed in triplicates and the mean values were determined.

RESULTS AND DISCUSSION

Percentage yield and elemental constituents

Several factors such as age of coconut endosperm and drying process have been documented to affect the yield of oil from *Cocos nucifera* (Hamied *et al*, 2011). The percentage yield of the oil shows that the method of extraction is significant. The naturally extracted oil using UPW (NEO) had a higher yield (63.1 %w/w) than the chemically extracted oil (CEO) with a yield of 52.4 %w/w. Coconut is an edible fruit and the elemental analysis of NEO and CEO shows the absence of lead, copper and zinc which is comparable with the results obtained for castor oil BP and the commercially available coconut oil (CACO), thus further ascertaining the non-toxicity of the oils and applicability for topical formulations.

Peroxide index, fatty acid and electrical conductivity

The peroxide index is used to characterize oils and fats in order to determine the tendency of the oil to undergo oxidation (Bernadi *et al*, 2011). The AOCS (2009) gives a peroxide value limit of 15meq/kg for virgin oils in general, and classified products with peroxide values between 1 and 5 meq/kg as low oxidation rate oil, between 5.1 and 10 meq/kg as moderate oxidation rate oil and above 10 meq/kg as high oxidation rate oil. The peroxide values of 1.78, 2.14, 1.88 and 0.68 meq/kg obtained for NEO, CEO, CACO and CO respectively is thus, an indication of the low tendency for the oils under investigation to go rancid (Kitagawa *et al*, 2011). The use of chemicals in the extraction process of CEO probably led to a lower value of free fatty acid when compared with the NEO. Electrical conductivity values have a direct relationship with the water content of a substance (Hathout and Nasr, 2013) as observed in the electrical conductivity result which was ranked CO (40.0) > NEO (34.0) > CACO (29.1) > CEO (15.0).

Phytochemical analysis, rheological properties and pH of oils

Active principles such as alkaloids and glycosides are of utmost significance when dealing with natural products as their presence can exert some pharmacological activities and could also be used in identifying adulteration of coconut oil (Manikantan *et al*, 2016). The phytochemical analysis of the oil showed the absence of alkaloids, flavonoids, glycosides, saponins and tanins, while the presence of antraquinones and terpenoids were confirmed. Rheology is the science of deformation and the study of the manner in which materials respond to applied stress or strain (Koop *et al*, 2012). The viscosity of a solution can give primary information on the internal consistency of such a system. For topical applications of microemulsions, low fluidity at a high shear rate is the goal (Shalviri *et al*, 2011). The rheological properties of the oil samples showed viscosity ranked as CO > CEO > CACO > NEO at both 50 and 100 rpm (Table 2). However, values obtained at 100 rpm were greater, thus showing a higher viscosity as the shear rate increased, which will favor topical application (Koop *et al*, 2012). The presence of ricinoleic acid, which consists of mono unsaturated 18- carbon fatty acid with hydroxyl group, could be responsible for the high value obtained for the CO (Geller and Goodrum, 2010)

The pH values obtained from aqueous titration of the oils were found to be 4.4 ±0.03, 4.1 ±0.08, 4.9 ±0.13 and 4.3 ±0.01 for CO, CACO, NEO and CEO respectively, indicating that NEO had the highest pH. Lambers *et al* (2006) reported that the resident flora of the skin will be kept intact if the pH of the skin is kept acidic. Thus, it is expected that the pH of NEO and CEO will support flora retention.

Table 2: Viscosity of oil samples at 50 rpm and 100 rpm

Samples	Viscosity/cps at 50rpm	Viscosity/cps at 100rpm
NEO	52.0 ±0.18	81.0 ±0.16
CEO	68.0 ±0.02	95.0 ±0.23
CACO	79.0 ±0.02	108.0 ±0.07
CO	684.0 ±0.06	687.0 ±1.17

(n=3 ±SD)

Rheological properties and pH of microemulsions

The rheological properties of the microemulsions at a temperature of 25 ± 2ºC are shown in Table 2, while the pH values obtained for the microemulsions are shown in Fig. 2 (a and b). The rheological properties of the microemulsions showed that there was an increase in the viscosity of the microemulsions as the shear rate increased (Table 3). The ranking of the viscosities was CO: Smix< NEO: Smix< CACO: Smix< CEO: Smix Generally, the microemulsions containing higher proportions of the oil extracted by water (NEO) had lower viscosities than microemulsions containing CACO and CEO. This observation is similar to the result obtained by Basheer *et al* (2013) where the authors reported the influence of the presence of water in lowering the viscosity of microemulsions prepared using isopropyl palmitate.

All the microemulsions formulated were acidic in nature (Fig. 2) and were ranked CACO: Smix> CO: Smix> NEO: Smix> CEO: Smix for ratios 3:7 and 1:1 and CO: Smix> CACO: Smix> CEO: Smix> NEO: Smix for ratio 9:1, thus showing that the ratio of the oil to surfactant mix has a significant effect on the pH of the microemulsions. The stability of

microemulsions could be dependent on the occurrence of chemical reactions, which could be monitored by the use of pH profiles (Chen *et al*, 2007). A significant change in pH could lead to alteration of the therapeutic efficacy of the final product. Geller and Goodrum (2010) documented that vegetable oils are prone to experience a reduction in pH value due to degradation products containing fatty acid esters as a result of hydrolysis. However, retention of dermal flora will be favoured by an acidic pH (Lambers *et al*, 2006)

Table 3: Viscosity values of microemulsions (n = 3±SD)

Samples	Viscosity/cps at 50rpm	Viscosity/cps at 100rpm
NEO: Smix (3:7)	86.0±0.01	114.0±0.07
CEO: Smix (3:7)	109.0±0.05	120.0±0.07
CO: Smix (3:7)	65.0±0.01	86.0±0.12
CACO: Smix (3:7)	97.0±0.01	113.0±0.02
NEO: Smix (1:1)	16.0±0.01	42.0±0.07
CEO: Smix (1:1)	39.0±0.12	81.0±0.06
CO: Smix (1:1)	15.0±0.05	33.0±0.09
CACO: Smix (1:1)	23.0±0.11	47.0±0.17
NEO: Smix (9:1)	307.0±0.07	340.0±0.12
CEO: Smix (9:1)	364.0±0.02	391.0±0.03
CO: Smix (9:1)	148.0±0.11	201.0±0.05
CACO: Smix (9:1)	312.0±0.18	336.0±0.07

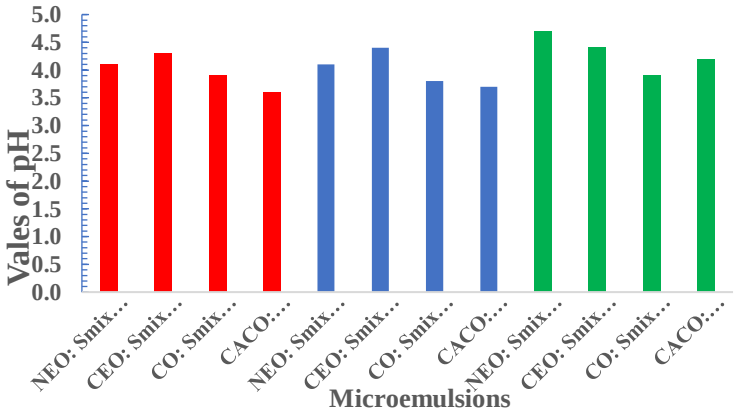


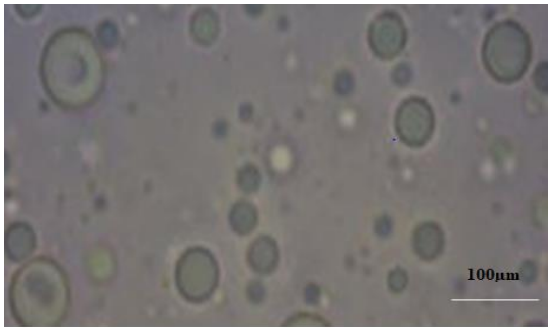
Figure 2. Plot showing the pH values of different microemulsions

Globule Size and conductivity of microemulsions

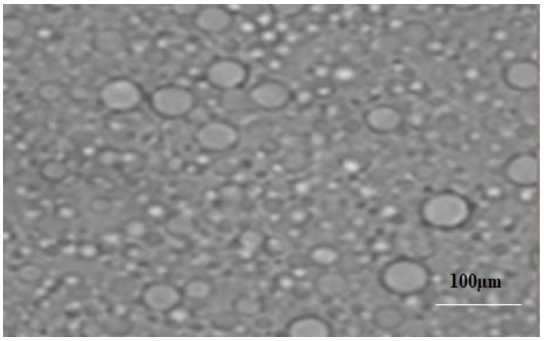
The globule sizes and conductivity of the microemulsions are shown in Table 4. The mean droplet diameter of microemulsions is an important parameter that affects physical stability (Bernadi*et al*, 2011). Generally, the globule size was ranked in the order CO: Smix > CACO:Smix > NEO: Smix > CEO: Smix. The presence of the surfactant mix had a significant effect on the globule size of the emulsions; as the ratio of the surfactant mix decreased, there was an increase in the globule size of the microemulsions. It was observed from the representative photomicrographs (Fig. 3) that the globules of the microemulsions containing CEO: Smix (3:7) are more tightly packed together, while those of NEO: Smix (3:7) are well spaced. Shalviri *et al* (2011) reported that while the reduction in globule size does not necessarily lead to increased stability, uniform sized globules are expected to have a better dispersion. The conductivity was ranked NEO: Smix > CEO: Smix > CO: Smix > CACO:Smix, thus showing that the presence of water has a direct relationship with conductivity values. Seo-Jin*et al* (2015) also confirmed that the conductivity of microemulsions containing fenofibrate was enhanced by dilution with water.

Table 4: Globule Size and Conductivity

Samples	Globule Size (µm) (Mean ± SD)
NEO: Smix (3:7)	61.73±1.04
CEO: Smix (3:7)	54.24±0.26
CO: Smix (3:7)	85.19±1.11
CACO: Smix (3:7)	75.27±1.08
NEO: Smix (1:1)	65.13±0.05
CEO: Smix (1:1)	65.08±1.04
CO: Smix (1:1)	75.07±0.01
CACO: Smix (1:1)	71.23±0.11
NEO: Smix (9:1)	87.08±1.04
CEO: Smix (9:1)	86.83±0.18
CO: Smix (9:1)	89.08±0.07
CACO: Smix (9:1)	84.27±1.06



(a) NEO: Smix (3:7)



(b) CEO: Smix (3:7)

Figure 3: Representative plots of photomicrographs

Physical stability of microemulsions

The results obtained from the physical examination of the different ratios of the formulated microemulsions are shown in Table 5. Generally, the microemulsions containing oil:Smix (3:7) were observed to be the most stable after forty two days as there was no significant change in the consistency of the microemulsions. The implication of this is that the presence of the Smix (Polyethylene glycol: Tween 80®) had a positive effect on the stability of the emulsions. Marina *et al* (2009) observed a direct relationship between the viscosities of microemulsions containing Tween 20 as surfactants with an increased stability of the microemulsions when the surfactant was mixed with polyphenol.

Table 5: Stability of microemulsions

Samples	Summary of Appearance			
	Day 0	**Day 14**	**Day 28**	**Day 42**
NEO: Smix (3:7)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Transparent emulsion with single layer	Transparent emulsion with single layer
CEO: Smix (3:7)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Transparent emulsion with single layer
CO: Smix (3:7)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer
CACO: Smix (3:7)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer
NEO: Smix(1:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Dark yellow-coloured emulsion with reversible creaming.	Dark yellow-coloured emulsion with irreversible creaming
CEO: Smix (1:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Dark yellow-coloured emulsion that has creamed	Dark yellow-coloured emulsion with irreversible creaming
CO: Smix (1:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with reversible creaming	Yellow-coloured emulsion with reversible creaming
CACO: Smix (1:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with reversible creaming	Yellow-coloured emulsion with reversible creaming
NEO: Smix (9:1)	Transparent emulsion with single layer	Transparent emulsion with single layer	Transparent emulsion with single layer	Dark yellow-coloured emulsion that has creamed
CEO: Smix (9:1)	Transparent emulsion with single layer	Transparent emulsion with single layer	Yellow-coloured emulsion with two layers indicating onset of creaming	Dark yellow-coloured emulsion with reversible creaming
CO: Smix (9:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with two layers indicating onset of creaming	Yellow-coloured emulsion with two layers indicating onset of creaming
CACO: Smix (9:1)	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with single layer	Yellow-coloured emulsion with two layers indicating onset of creaming

Microbial analysis of microemulsions

Microemulsions containing oil: Smix (9:1) showed inhibitory activities against the test microorganisms except for *Candida* sp. D33 which was resistant (Fig. 4). The coconut oil samples (CEO and NEO) had inhibitory activities against all the test organisms. In general, the inhibition was ranked in the following order: *Candida* sp. D25 (CEO: Smix> CACO: Smix> NEO: Smix > NEO > CO > CEO), *Candida* sp. D33 (CEO = CO> CACO: Smix = NEO > CEO: Smix = NEO: Smix), *Staphylococcus* sp. DS2 (NEO >CO> CACO: Smix>NEO: Smix> CEO: Smix = CEO) and *Pseudomonas* sp. DP8 (NEO: Smix>CO> CACO: Smix> CEO: Smix = NEO > CEO).

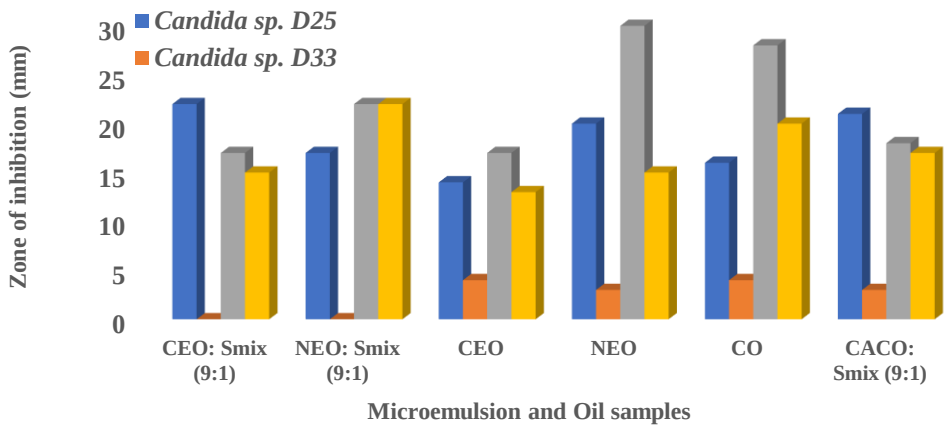


Figure 4: Representative plot showing the zones of inhibition

CONCLUSION

The two oil extraction methods (involving the use of ultra-pure water and the use of petroleum ether) from the endosperms of coconut were economically feasible, and yielded oils that were safe for use as vehicles in topical delivery without altering the natural flora of the skin. The `microemulsions formulated using the extracted oils also showed promising antimicrobial activities. Further research into alteration of the surfactant mix is necessary to conclude on the significance of the ratio of the mix that will give the optimum value as adjuvants in topical microemulsions containing coconut oil.

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*Address for correspondence: Dr. O. A. Adetunji
Department of Pharmaceutics & Ind. Pharmacy,
University of Ibadan, Ibadan
Telephone: +234-8055412280
E-mails: adetunjioladapo@gmail.com

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