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Evaluation of the Emulsifying Property of Solid Phase Purified Garcinia kola Seed Oil

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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: Crude seed oils consist essentially of naturally occurring mixtures of triacylglycerols (>95%). They are purified to improve their overall quality. Oil purification facilitates elimination of impurities and toxicants.
Objective: The objective of the study is to purify *Garcinia kola* seeds crude oil and to evaluate the purified oil for its emulsifying character.
Materials and Methods: Crude seed oil of *G. kola*, obtained from the dried, powdered seed, was purified by passing the crude oil in *n*-hexane through silica gel and charcoal arranged in tandem. The crude and refined oils were evaluated by thin layer chromatography and infra-red spectroscopy. Emulsions of the refined oil at 30% and 20% concentrations were prepared and evaluated.
Results: With the purification process, there was removal of dissolved polar solutes and pigments in the crude oil relative to the purified oil as revealed by the thin layer chromatographic profile and infra-red spectra. At 20% concentration of the refined oil, a lotion with viscosity of 25.67 ± 1.20 cP was formed while a creamy emulsion was produced with the refined oil at 30% concentration with viscosity of 46.00 ± 1.16 cP. This suggests the suitability of the purified oil as a base in the formulation of lotions and creams.
Conclusion: The purified oil of *G. kola* possesses emulsifying property and could find applications in cosmetic and pharmaceutical industries.
Keywords: *Garcinia cola*, seed oil, solid phase purification, emulsifying property

INTRODUCTION

Seed oils consist essentially of naturally occurring mixtures of triacylglycerols (>95%). They have a wide variety of fatty acids, the composition of which is characteristic of the family to which the plant source belongs. The minor, non-triacylglycerol components include phosphatides, glycolipids, free fatty acids and dissolved organic compounds (oxidation products: peroxides, aldehydes, ketones; unsaponifiable matters: tocopherols, sterols, hydrocarbons, pigments) (Gunstone, 2004). The occurrence and quantity of these non-triacylglycerol components vary relative to the oil source, seasons of collection from the source, adopted extraction and purification processes. Extracted seed oils are subjected to purification to remove unwanted substances and impurities with minimal damage to the triacylglycerols and other desirable constituents. Purification improves the overall quality of crude extracted oils (Boskou, 2006; Medina-Juarez *et al*., 2011).

Garcinia kola Heckel (Guttiferae) is a medium sized tree that is widely distributed throughout West and Central Africa. The seeds, otherwise called bitter kola, are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders, and bronchitis

(Hutchinson, 1956; Iwu, 1982). The seeds contain unsaturated fatty acids - oleic and linoleic acids- in abundance (71.29 % of total fatty acids) with lesser amounts of linolenic acid, myristic acid, palmitic acid and stearic acid. In addition, the seeds consist of 29.2 g/kg dry weight (DW) of neutral lipids, 10.0 g/kg DW glycolipids and 5.9 g/kg DW phospholipids relative to the total lipids (45.3 g/Kg DW) content (Adeyeye, 1991; Essien *et al*., 1995; Eleyinmi *et al*., 2006).

While numerous studies have been carried out on *G. kola* seeds, there is no documented information about the emulsifying property of the seed oil. This study therefore, sets out to purify *G. kola* seeds oil by solid phase purification protocol, evaluate the physicochemical and molecular changes that took place because of the purification, and then assess its emulsifying character.

Material and Methods

Materials

Seeds of *G. kola* (bought at Oja-Oba market, Ibadan, Oyo State and authenticated at the Botany Department, University of Ibadan), silica gel 60 G (for thin-layer chromatography, Merck, Germany), pre-coated thin-layer chromatography (TLC) aluminium sheets (0.2 mm, silica gel, F_{254}, Merck, Germany), methanol, *n*-hexane, acetone, ethyl acetate, dichloromethane (Merck, Germany), iodine crystals, activated charcoal (BDH Chemicals, England).

Crude Oil Extraction

G. kola seeds were first dried for two days at room temperature after which the thin outer coats were peeled off. The seeds were chopped into smaller pieces; air-dried for a week and subsequently milled into powder. The powdered seed material (1 kg) was then packed into a cotton cloth bag and the oil component extracted by cold maceration using *n*-hexane (2L, for 24h). Next, the *n*-hexane was removed and the dried marc re-extracted with fresh *n*-hexane (2L, for 24h). The extracts were pooled, filtered and concentrated at 40°C. The recovered oil in a clean glass container was dried *in vacuo* at 40°C for 48h and then stored in a refrigerator for subsequent use. The oil was tagged '*G. kola* crude oil' (GKCO).

Solid Phase Purification

The solid phase purification of GKCO was conducted by using the method of Idowu *et al.,* (2009). Briefly, silica gel solid phase extraction (SPE) cartridges and activated charcoal SPE cartridges were prepared in the laboratory by packing silica gel 60 G (3 g) and activated charcoal (2 g) respectively with the aid of vacuum line, into 10 mL plastic injection syringe barrel, with cotton wool plug. The GKCO was diluted with *n*-hexane to make a stock solution of 10% (v/v). 25 mL of this was filtered, in turn, through one silica gel and one activated charcoal cartridges arranged in tandem. The *n*-hexane in the filtrate was removed by rotary evaporation at 40°C. The recovered oil, tagged '*G. kola* purified oil' (GKPO), was dried and stored as was done with GKCO.

Thin Layer Chromatographic Profiles of GKCO and GKPO

About 0.1 mL of the GKCO and GKPO samples were each diluted to 1 mL with ethyl acetate. Aliquots of these were spotted on TLC plates which were later developed using dichloromethane/acetone (8:2) as the mobile phase. After developments, the plates were examined under UV light (254 nm and 365 nm) and by exposure to iodine vapour in an iodine saturated tank.

Infrared Spectroscopic Analysis of GKCO and GKPO

Fourier transform infrared (FT-IR) spectroscopic absorption spectra of the oils were recorded on a Perkin Elmer Spectrum BX spectrophotometer in the range 4000-350 cm^{-1}. A drop of the oil samples was spread on potassium bromide (KBr) discs separately and placed in the sample compartment of the IR spectrophotometer to enable the radiation from the light source to pass through it.

Preliminary Study of Emulsifying Properties - Formulation I

To gain insight into the emulsifying behaviour of GKCO and GKPO, oil-in-water emulsions were prepared as shown in Table 1 using formulation I (Aderibigbe *et al*., 2011). Part A was prepared by melting the emulsifying wax and adding the oil after lowering the temperature to 60°C. Oleic acid was then added. Part B ingredients were mixed in water and heated to 60°C. Part A was then added to part B at the same temperature while stirring with a glass rod until the emulsion was formed. The prepared emulsions of GKCO and GKPO were then macroscopically evaluated. Here, about 10 mL of each of the emulsions were poured into separate 10 mL measuring cylinders, covered with aluminium foil to prevent evaporation, and allowed to stand at room temperature for 5 days. In both cases, a daily visual observation was done to note any change in

general appearance, colour, odour and emulsion consistency.

Purified Oil Emulsions - Formulations II and III
Based on the outcome of the preliminary study, new emulsions of GKPO were prepared in accordance with the above method with slight modification using formulations II and III (Table 1). This was followed by photo-micrographic evaluations of the prepared emulsions. Their viscosity was determined using Brookfield viscometer (Model – DV– 11 + pro, Brookfield Engineering laboratory Midddlebro, MA, USA) at a speed of 50 rpm with spindle no 2.

Table 1 Formula for the preparation of *G. kola* oil emulsions

	FORMULATIONS		
	I	**II**	**III**
Part A			
INGREDIENTS	**QUANTITY(%v/v)**		
• *G. kola* oil	32.0	30.0	20.0
• Oleic acid	2.0	2.0	2.0
• Emulsifying Wax	8.0	8.0	8.0
Part B			
INGREDIENTS	**QUANTITY (%v/v)**		
• Triethanolamine	1.0	1.0	1.0
• Glycerol	4.0	4.0	4.0
• Distilled Water	53.0	55.0	65.0

Results and Discussion

Crude Oil Extraction

The extraction of powdered *G. kola* seeds using *n*-hexane yielded 24 g/kg dry weight of *G. kola* crude oil (GKCO). This yield is moderate although it is lower than that reported by Adeyeye (1991) and Essien *et al*., (1995). The variance in yield might be due to different in the extraction procedures adopted in each study; Adeyeye (1991) employed soxhlet extraction, while cold maceration was employed in this study.

Solid Phase Purification
The yield arising from the solid phase purification is 20.5 g/kg dry weight. Relative to the GKCO which was dark-brownish in colour, the solid phase refinement of GKCO yielded clear, pale yellow purified oil (GKPO). It was observed that the *n*-hexane stock solution of GKCO was cloudy, an indication that the oil contains dissolved components that were incompletely soluble in the non-polar *n*-hexane. This was confirmed by allowing a sample of the stock solution to settle down, after which a small brownish residue was observed at the bottom of the volumetric flask. The residue dissolved completely in 5ml methanol (a polar solvent) but relatively insoluble in same volume of ethyl acetate (a less polar solvent). The silica gel and charcoal used in the purification process were polar sorbents; they adsorbed dissolved polar molecules and pigments in the GKCO respectively. It is suggestive that their removal enhanced the emulsifying behaviour of the GKPO.

Spectroscopic and Chromatographic analysis of GKCO and GKPO
The thin layer chromatographic profile of the oils is shown in Fig. 1a. The profile showed many spots, indicating the various dissolved compounds in the oils. Eight spots were seen with GKCO while two

spots were seen towards the solvent front for the GKPO. From the profile, it was observed that the more polar constituents of the GKCO had been removed by the purification leaving only the less polar ones which are basically lipids. The iodine vapour employed for detection purpose identified the presence of unsaturated compounds including unsaturated lipids in the oils.

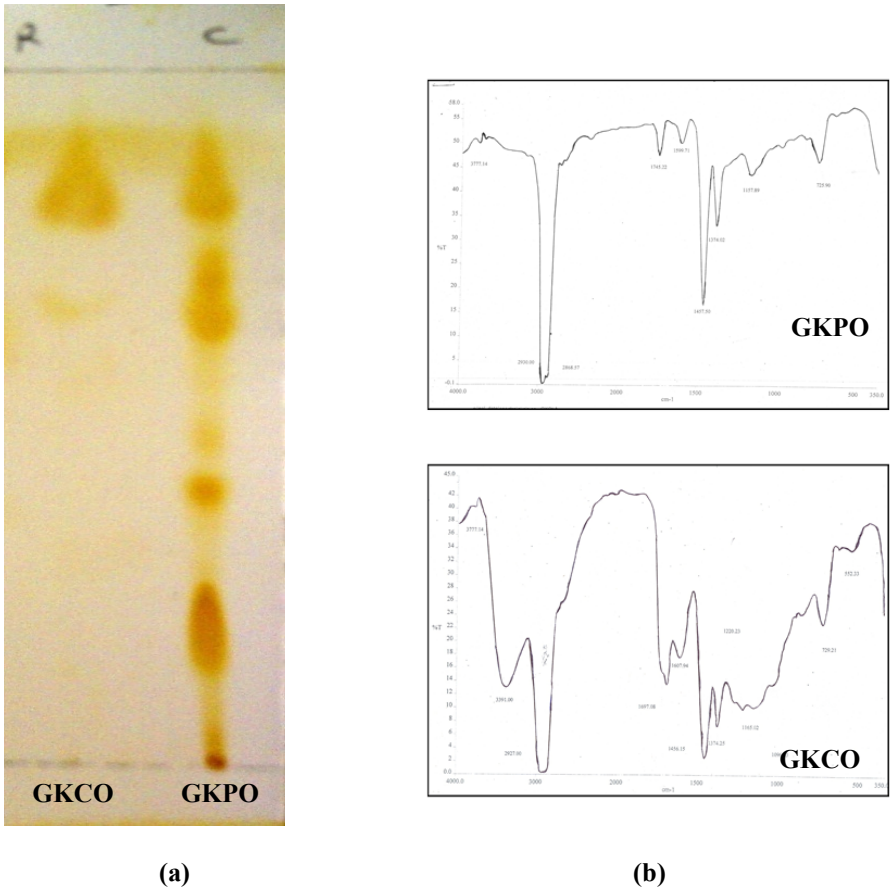

Figure 1. Digital images of TLC profile (a) and IR spectra (b) of GKCO and GKPO

The FT-IR spectra of the GKCO and GKPO are shown in Fig. 1b. The different spectra of the oils gave further indication of removal of some dissolved compounds because of the purification process. Specifically, the overall profiles of the two spectra were quite different. The GKCO shows strong carbonyl stretching (C = O, 1697.08 cm^{-1}) and N-H stretching (3391.08 cm^{-1}) possibly coming from alkaloids; while these were conspicuously absent from GKPO. In both spectra, C-H stretching (2930 cm^{-1}, 2927 cm^{-1}) and C-H bending (1456.15 cm^{-1}, 1457.50 cm^{-1}) were retained. The weak carbonyl peak of 1745.22 cm^{-1} observed in the GKPO spectrum could be coming from a reduced presence of such compounds in the purified oil (Ogundaini, 2000).

Preliminary Study of Emulsifying Properties - Formulation I

GKCO emulsion was observed to be brownish in colour, less viscous, less creamy and could be poured easily while that of GKPO was light yellow in colour, viscous with a creamy consistency. There was no visible change in colour, odour or texture of the two emulsions over the period of five days. There was also no observation of physical instability such as breaking or coagulation in both formulations. The relative difference in the number of dissolved solutes in the oils may account for the dissimilarity in the emulsifying property of the oils. The enhanced emulsifying property as observed with the GKPO suggests that the purification process enriched the oil with emulsifying agents through the removal of interfering dissolved organic solutes in the GKCO. Previous studies by Essien *et al*. (1995) and Eleyinmi *et al*. (2006) found that the seeds of *G. kola* contain

phospholipids which are known emulsifiers used in the food and pharmaceutical industries .

Purified Oil Emulsions - Formulations II and III

For the GKPO emulsions prepared using formulation II and III, at the concentrations of the oil used, products with lubricating and emollient properties were formed. However, the viscosities of the preparations differ. At 20% v/v, a lotion with viscosity of 25.67 ± 1.20 cP was formed. A creamy emulsion was produced with the oil at 30% v/v with viscosity of 46.00 ± 1.16 cP. This suggests the suitability of GKPO as a base in the formulation of lotions and creams. Suitability of materials as bases in liquid formulation is determined by stability of the therapeutic agent and capacity for water (Jones, 2008). The capacity for water was found to increase as the concentration of the refined oil increased in the formulation. The results of the photomicrographic analysis of the emulsion are shown in Table 2, Figures 2 and 3. The particle size and distribution analysis of GKPO emulsions showed the 20 % emulsion to have closer inter-particulate interaction, smaller size and of less cluster morphology than 30 % emulsion. This is probably responsible for the creamy consistency obtained with the 30% emulsion preparation.

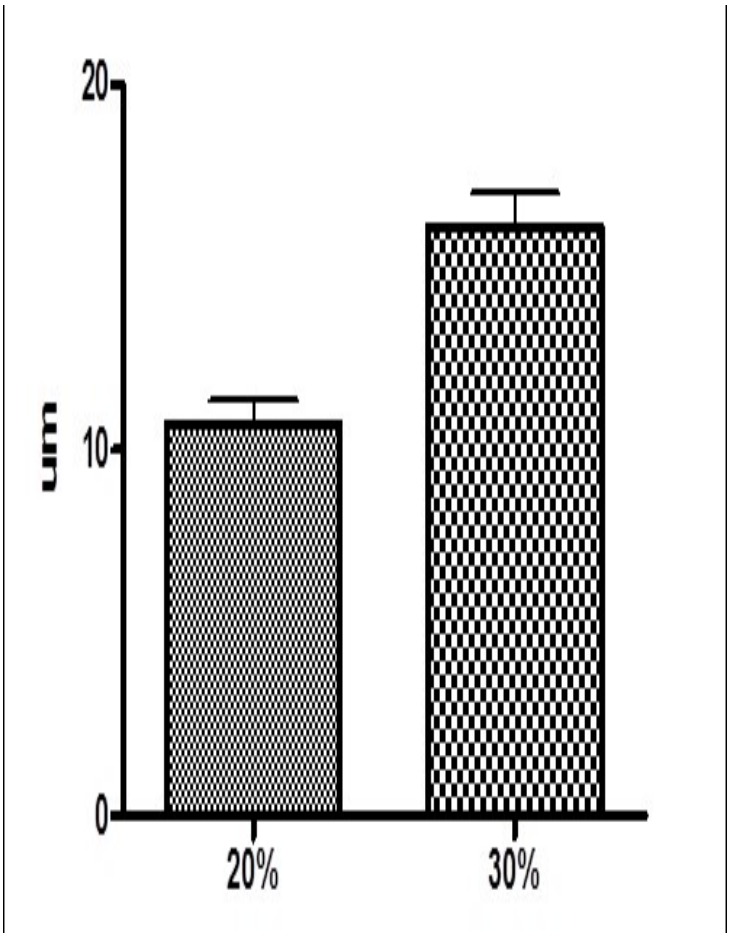

Figure 2. Bar chart of the particle size analysis of GKPO emulsions (20 % and 30 %)

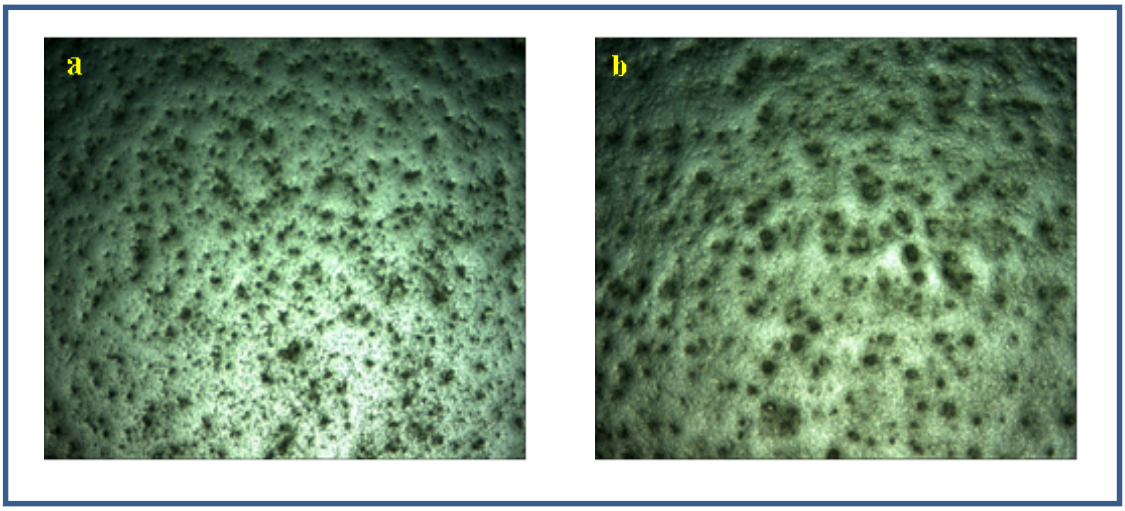

Figure 3. Photomicrographic images of GKPO emulsions: (a) 20% and (b) 30% at 40X magnification

Table 2 Statistics of the photomicrographs of the GKPO prepared emulsions

STATISTICS		20% GKPO Emulsion	30% GKPO Emulsion
No.	**Valid**	100	100
	Missing	0	0
Mean		10.70	16.09
Std. Error of Mean		0.65	0.95
Median		9.62	14.82
Mode		6.22	3.16[a]
Std. Deviation		6.53	9.54
Variance		42.65	90.95
Minimum		2.58	2.67
Maximum		35.15	54.96

a. Multiple modes exist. The smallest value is shown

Conclusion

Purification of *G. kola* seed oil yields yellow oil devoid of most of the polar constituents and pigments. The purified oil is suitable for formulation of lotion and cream at 20% and 30% concentrations respectively. It could also find applications in cosmetic and pharmaceutical industries .

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