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Research Article

IN VITRO PROPERTIES OF SOLID LIPID MICROPARTICLES (SLMS) LOADED WITH METHANOLIC EXTRACT OF *GARCINIA KOLA* (HECKEL) SEED

*** SALOME AMARACHI CHIME, IKECHUKWU V. ONYISHI AND GODSWILL C. ONUNKWO**

Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka 410001, Nigeria
Email: emmymarachi@yahoo.com

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ABSTRACT

Objective: The decline in the use of herbal medicine especially in the Western world may be due to lack of readily available market brand formulations and the fact that most herbal remedies are taken as tea, decoctions and infusions. The taste of some of these herbal drugs is not palatable, and some have unpleasant odour and colour hence, the need to formulate these drugs in form of encapsulated dosage forms. The objective of the work was to formulate solid lipid microparticles (SLMs) loaded with the methanolic extract of *Garcinia kola* seed.
Methods: The SLMs containing 1 and 3 % of *Garcinia kola* seed extract were formulated using fat from *Capra hircus* and Phospholipon® 90H (3:1). The particle morphology and size, encapsulation efficiency (EE%), pH, *in vitro* release and the inhibition zone diameter (IZD) of the SLMs were determined.
Results: The results showed that the extract was very bitter while, the encapsulated *G. kola* had slight bitter taste. The pH remained in the acidic region from 1 to 30 days. Particle size of 28.65 ± 1.13 and 29.49 ± 1.24 µm were obtained for SLMs loaded with 1 and 3 % of the extract respectively. SLMs had high EE% of 94 % and also exhibited good release of the extract in simulated intestinal fluid (SIF, pH 7.2). *Garcinia kola*-loaded SLMs had good activity against *Staphylococcus aureus* and no action against *Escherichia coli.*
Conclusion: Therefore, *Garcinia kola* seed extract could be formulated as SLMs in order to mask its bitter taste and improve compliance.

Keywords: Herbal formulation; *Garcinia kola* seed; SLMs; *Capra hircus* lipid

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind [1]. Herbs have been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter and medicine. Many drugs commonly used today are of herbal origin. Some are made from plant extracts; others are synthesized to mimic a natural plant compound [1].

A lot of studies are being carried out on medicinal plant, and the potency of most of these medicinal plants has been established. Therefore, there is need to formulate and standardize these herbal drugs in order to encourage its use in the treatment of various ailments. Formulation, standardization and quality control is needed in order to improve patient acceptability and compliance and to ensure the purity, potency and efficacy of these herbal drugs. Efforts have recently been made in order to formulate herbal drugs into various dosage forms [2,3,4]. The advantages of herbal drugs include low toxicity, easy availability, low cost, highly efficacious, and form the basis for the development of synthetic medicine among others. The decline in the use of herbal medicine especially in the western world may be due to lack of readily available market brand formulations of these products, the fact that most herbal remedies are taken as tea, decoctions and infusions. The taste of some of these herbal drugs is not palatable, and some have unpleasant odour and colour hence, the need to formulate these drugs in form of encapsulated dosage forms.

Solid lipid microparticles (SLMs) attract increasing attention as alternative delivery systems and combine the advantages of different traditional carriers [5]; the solid matrix protects loaded labile substance against degradation and they offer the possibility of controlled drug release and drug targeting [6], and has been investigated as a taste – masking delivery system of some drugs [7].

Lipid based formulations have been shown to enhance the bioavailability of drugs administered orally [8,9,10,11]. The proven safety (biocompatibility) of lipid based carriers makes them attractive candidates for the formulation of pharmaceuticals. Lipid based formulations promotes wetting or solubilization of drugs and enhances permeability or further undergoes intraluminal processing to solubilize the drug [8].

Garcinia kola Heckel (*Guttiferae)* is a tropical plant whose seed is generally known as bitter kola [10]. *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhea [11,12]. The seed is masticatory and also used to prevent and relieve colic, chest colds, cough and can as well be used to treat headache [13, 14]. Iwu [11] reported the use of this plant for the treatment of jaundice, high fever and as purgative. The plant also found usefulness in the treatment of stomach ache and gastritis [13, 15]. The anti-ovulation properties of the seed extract have been reported [16,17]. Phytochemical analysis of extracts from both root, stem and seed of *Garcinia kola* show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1,GB-2), xanthones and benzophenones [18,19,20]. Their antibacterial activities are due to flavonoids especially biflavonoid type GB1 [13], and this has been demonstrated using methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin- resistant *enterococci* (VRE) [21], *Lactobacillus* spp. [22] and *Streptococcus pyogenese* [23].

Garcinia kola seeds possess properties that is of immense ethnomedicinal use to mankind and because of the popular and widespread use of this herbal drug, important technical aspects such as standardization and formulation of a highly efficacious formulation will be of immense benefit in order to enhance their efficacy and improve patient's compliance. Therefore, the aims of the present study were to formulate the methanolic extract of *Garcinia kola* seed into solid lipid microparticle, to study the antimicrobial properties of the formulations and also determine the organoleptic properties of the SLMs.

MATERIALS AND METHODS

Materials

Phospholipon® 90H (Phospholipid GmbH, köln, Germany), Soluplus® (BASF, Germany), activated charcoal (Bio-Lab (UK) limited, London), distilled water (STC UNN, Nigeria), methanol, sorbic acid, sorbitol (Merck, Darmstadt, Germany). *Garcinia kola* seeds were purchased

from a local market in Orba Nsukka, Enugu state, Nigeria in the month of June, 2012 and were authenticated by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethno medicine and Drug Development (InterCEDD) Nsukka and the voucher specimen (no. PC98032) is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka. Goat fat was extracted from *Capra hircus*.

Extraction of goat fat

The goat fat was extracted from *Capra hircus* by grating the adipose tissue prior to boiling with half its weight of water on a water bath for 45 min. The molten fat was separated from the aqueous phase using a muslin cloth. Further purification was carried out by heating a 2% w/w suspension of a 1:9 ratio blend of activated charcoal and bentonite in the lipid at 80 to 90°C for 1 h. Thereafter, the suspension was vacuum-filtered using Buchner funnel [9].

Extraction of *Garcinia kola*

About 5 kg of *Garcinia kola* (Heckel) seed were cut into pieces, sun dried for 3 days and then pulverized using an end runner mill (Pascal Engineering Co Ltd, England). The fine powder (310 g) was extracted with methanol by cold maceration method for 24 hours. The methanol was allowed to evaporate and a yield of 20.3 g (5.63 %) was obtained.

Preparation of lipid matrix

The lipid matrix consisted of Phospholipon® 90H and purified goat fat at a ratio of 1:3. The lipids were weighed and melted together in a crucible using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) at 70 °C and stirred with a glass stirrer until a transparent homogenous white melt was obtained. The lipid matrix was stirred continuously until it solidified at room temperature [5].

Preparation of *Garcinia kola* seed extract-loaded SLMs

The SLMs were formulated by melt homogenization; the details of its composition are shown in Table 1. About 5 g of the lipid matrix was weighed using analytical balance (Adventurer, Ohaus, China), melted in a beaker at a temperature of 70 °C using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) and the appropriate amount of *Garcinia kola* seed extract was dispersed homogenously in the molten lipid. Sorbitol was dissolved in hot distilled water at the same temperature with the lipidic melt together with Soluplus® (surfactant) and sorbic acid (preservative). The hot aqueous phase was transferred into the molten lipid dispersion and immediately subjected to high shear homogenization with Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany) at 5000 rpm for 10 min. SLMs containing extract (bland SLMs) was also formulated [9].

Table 1: Contents of SLMs loaded with the methanolic extract of *Garcinia kola* seed

Batch	*G. kola* seed extract (%)	Lipid matrix (%)	Sorbitol (%)	Soluplus® (%)	Sorbic acid (%)	distilled water q.s (ml)
P1	1	5	4	0.75	0.05	100
P2	3	5	4	0.75	0.05	100
P3	0	5	4	0.75	0.05	100

P1 and P2 contain 1 and 3 % of *Garcinia Kola* extract, batch P3 is the bland SLMs

Determination of organoleptic properties

The odour, taste and colour of the *Garcinia kola* seed extract-loaded SLMs were determined.

Analysis of particle size and morphology

The particle size of the microparticles was determined by computerized image analysis of at least one hundred microparticles. Each of the batches was placed on a microscope slide, covered with a cover slip and imaged under a binocular microscope (Lieca, Germany) attached with a Motic image analyser (Moticam, China), at a magnification of x 100. The particle morphologies were also observed and photomicrographs taken.

The pH studies

The pH of the SLMs was determined in time dependent manner (24 hours, 1week, and 1month) using pH meter (Suntex TS-2, Taiwan).

Encapsulation efficiency and loading capacity

Beer's calibration curve was obtained for *Garcinia kola* extract in simulated intestinal fluid (SIF, pH 7.2) at a concentration range of 0.1 – 1.0 mg/ml at a predetermined wavelength of 285 nm. Approximately 10 ml of the *Garcinia kola* extract-loaded SLMs was added into a centrifuge and separated (Chem. Lab. Instrument, UK) at 1,252 × g for 30 min. About 0.5 g of the sediment was adequately analyzed for drug content using a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA). The drug content was calculated with reference to Beer's calibration curve. The encapsulation efficiency (EE %) of the *Garcinia kola* in the SLMs was calculated from the equation below:

$$\text{Encapsulation Efficiency (EE \%)} = \frac{ADC}{TDC} \text{x} 100 \qquad (1)$$

where ADC is the actual drug content and TDC is the theoretical drug content [24].

The LC was determined using the relationship

$$LC = \frac{Wa - Ws}{Wa - Ws + Wl} \times 100 \qquad (2)$$

Where Wl is the weight of lipid in the formulation, Wa is the weight of *Garcinia kola* extract added to the formulation and Ws is the actual amount of *Garcinia kola* extract encapsulated in the lipospheres [25].

Inhibition zone diameter (IZD) test

The plate agar diffusion method was used for this study conducted 2 weeks after the preparation. This method depends on the diffusion of antibiotics from holes on the surface of the microbial seeded agar. Molten nutrient agar (20 ml) was inoculated with 0.1ml of *Staphylococcus aureus* broth culture. It was mixed thoroughly, poured into sterile Petri dishes and rotated for even distribution of the organism. The agar plates were allowed to set and a sterile cork borer (8 mm diameter) was used to bore three cups in the seeded agar medium. The process was first validated using the crude drug extract in order to confirm the sensitivity of the microbial culture to the inhibitory action of *Garcinia kola* extract then, different concentrations (500, 250 and 125 mg/ml) of the *Garcinia kola* extract, the SLMs and the reference sample (tetracycline), respectively, were prepared. A 0.01-ml volume of each of the samples was added, respectively, into the different cups in each of the plates using Pasteur pipettes. The plates were allowed to stand at room temperature for 15 min to enable the samples to diffuse into the medium before incubating at 37 °C for 24 h. The diameter of each inhibition zone was measured and the average determined [26,27,28]. The procedure above was repeated for *Escherichia coli*.

***In vitro* release of *Garcinia kola* extract from the SLMs**

The USP XXII rotating paddle apparatus (Erweka, Germany) was employed for this release study. The dissolution medium consisted

of 500 ml of freshly prepared simulated intestinal fluid (SIF pH 7.2) maintained at 37 ± 1 °C. The polycarbonate dialysis membrane selected as release barrier was pretreated by soaking in the dissolution medium for 24 h prior to use (MWCO 6000-8000, Spectrum Labs, Brenda, The Netherlands). A 10 ml quantity of the SLMs was placed in the polycarbonate dialysis membrane containing 2 ml of the dissolution medium, securely tied with a thermo–resistant thread and placed in the chamber of the release apparatus. The paddle was rotated at 100 rpm, and at predetermined timed intervals, 5 ml-portion of the dissolution medium was withdrawn, appropriately diluted, and analysed for the content of the extract in a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA) at a predetermined wavelength of 285 nm. Sink condition was maintained by replacing with an equal volume of the withdrawn medium. The amount of extract released at each time interval was determined with reference to Beer's Plot.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 (SPSS Inc. Chicago, IL.USA). Data were analyzed by one-way ANOVA. Differences between means were assessed using student's t-test.

RESULTS AND DISCUSSION

Organoleptic properties

The results of the organoleptic properties of *Garcinia kola* seed extract-loaded SLMs are shown in Table 2 and show that the extract was brown in colour, the bland SLMs had light yellow colour while, the *Garcinia kola*-loaded SLMs were light brown. The extract was very bitter but, the encapsulated *G. kola* had slightly bitter taste. Therefore, the SLMs had taste masking effect and could be a better delivery system for this herbal drug in order to adequately present a suitable dosage form that will be acceptable to patients so as to improve compliance.

Table 2: The organoleptic properties of *Garcinia kola*-loaded SLMs

Batches	Colour	Odour	Taste
P1	Light brown	Sweet smell	Slightly bitter
P2	Light brown	Sweet smell	Slightly bitter
P3	Yellow	Pleasant	Salty
P4 (extract)	Brown	Pleasant	Very bitter

P1, P2 and P3 SLMs contain 1 and 3 % of *Garcinia Kola* extract, batch P3 is the bland SLMs and P4 is the *Garcinia Kola* extract.

The pH of SLMs

The results of the pH of the *G. kola*-loaded and the bland SLMs are shown in Fig. 1 and show that the unloaded SLMs had pH of 6.4, 5.5 and 4.7 at 1, 7 and 30 days respectively. When *Garcinia kola* was incorporated into the SLMs, the pH remained in the acidic region throughout the study period. The results showed that the pH of the formulations were stable. However, the slight pH decline seen in the SLMs may be due to the degradation of the fatty acid components of the individual lipids that made up the lipid matrix, since there was also a decline in the pH of the bland SLMs [25,26].

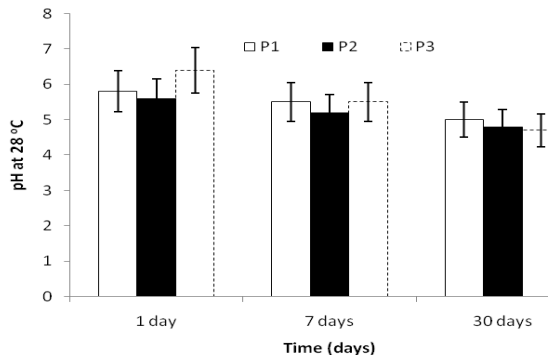


Fig. 1: Time dependent pH stability of *Garcinia kola*-loaded SLMs; P1, P2 and P3 SLMs contain 1 and 3 % of *Garcinia Kola* extract, batch P3 is the bland SLMs and P4 is the *Garcinia Kola* extract.

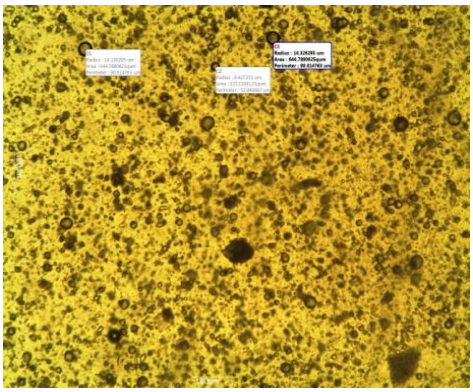
Particle size and morphology

The results of the part size of the SLMs are shown in Table 3, and show that *Garcinia kola*-loaded SLMs had particle size of 28.65 ± 1.13 and 29.49 ± 1.24 µm for P1 and P2 loaded with 1 and 3 % of the extract respectively, while the bland SLMs had particle size of 21.06 ± 1.17 µm. The results therefore, showed that particle size increased with increase in drug loading in agreement with previous works [5,24,29]. However, particle size of SLMs is important because they have direct bearing on the bioavailability of the encapsulated active pharmaceutical ingredient and the site of administration. The results show that the particles were within the acceptable range for microparticles. The results of the particle morphology shown in Fig. 2 showed that the particles were spherical as expected for SLMs.

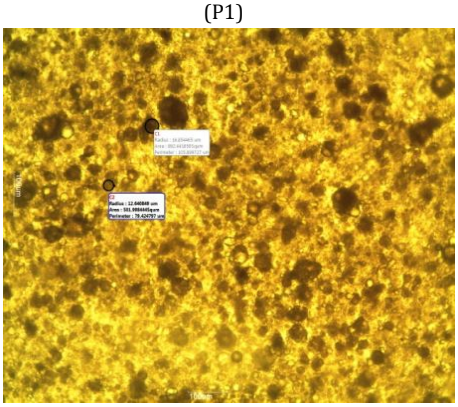
Table 3: Some physicochemical properties of *Garcinia kola*-loaded SLMs

Batches	TDC (%)	ADC (%)*, ††	EE (%)	LC (g PI/100 g)	Particle size (µm*)†
P1	1	0.92 ± 0.32	92.3	1.57	28.65 ± 1.13
P2	3	2.81 ± 0.15	93.5	3.66	29.49 ± 1.24
P3	-	-	-	-	21.06 ± 1.17

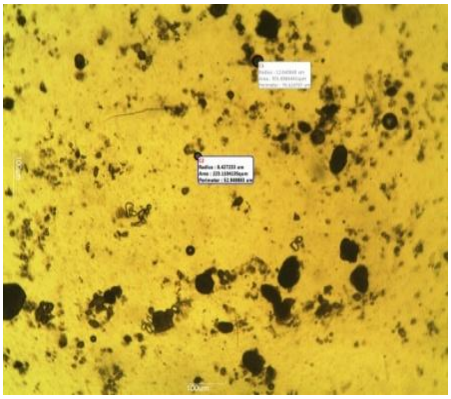
***Mean ± standard deviation, †n = 100; ††n = 3, P1, P2 and P3 SLMs contain 1 and 3 % of *Garcinia Kola* extract, batch P3 is the bland SLMs; TDC: Theoretical drug content, ADC: Actual drug content; EE: Encapsulation efficiency; LC: Loading capacity.**



(P1)



(P2)



(P3)

Fig. 2: Photomicrographs of SLMs, P1, P2 and P3 SLMs contain 1 and 3 % of methanolic extract of *Garcinia Kola* seed, batch P3 is the bland SLMs.

Encapsulation efficiency and loading capacity

The encapsulation efficiency was calculated using the ADC and the TDC with reference to Beer-Lamberts calibration curve obtained for *Garcinia kola* extract in simulated intestinal fluid without enzyme shown in Fig. 3. The results of the encapsulation efficiency shown in Table 3 revealed that the SLMs exhibited high encapsulation of the extract. This formulation technique is good for encapsulating herbal extract in order to mask the taste and odour of these formulations.

The lipid matrix had a loading capacity of up to 3. 7 g API/100 g lipid and increased with increase in the amount of extract incorporated as shown in Table 3.

The ability of the SLM to accommodate active molecules is an important property and this can be expressed by the encapsulation efficiency (EE %) and loading capacity. EE% defines the ratio between the weight of entrapped drug and the total weight of drug added to the dispersion, while LC expresses the ratio between the entrapped drug and the total weight of the lipids [5].

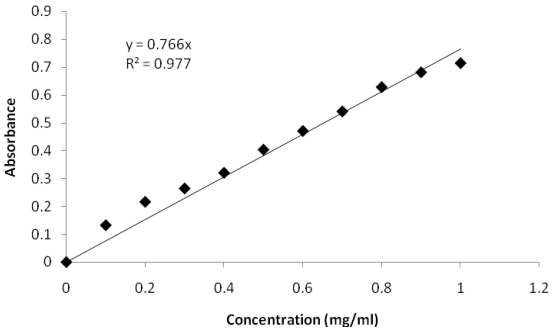


Fig. 3: Standard Beer-Lamberts plot for the extract of *Garcinia kola* seed in SIF, pH 7.2

***In vitro* release**

The results of the *in vitro* release of the extract from the SLMs are shown in Fig. 4 and the results show that batch P1 containing 1 % of *Garcinia kola* extract had 19.5, 42.4 and 59.1 % release while, batch P2 containing 3 % of the extract had 34.4, 37.8, 64.2 % release at 5, 30 and 100 min respectively. The results showed an initial high release at 5 min in both formulations which may be due to the presence of loosely bound extract in the periphery of the SLMs giving rise to an effect like an initial burst. The results showed that the SLMs had good release of the extract in SIF and could be a better delivery system for this herbal extract in order to improve patient acceptability and compliance of this herbal drug.

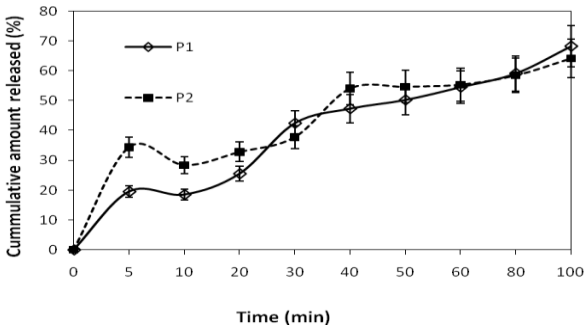


Fig. 4: *In vitro* release of *Garcinia kola* seed extract from SLMs in SIF, pH 7.2

Antimicrobial properties

The results of the inhibition zone diameter of the methanolic seed extract of *Garcinia kola*-loaded SLMs are shown in Table 4 and results show that the SLMs formulations (P1 and P2) had IZDs of about 15 to 15.6 mm. However, the reference sample (tetracycline) had significantly higher IZD than the test SLMs ($p < 0.05$). The results also showed that *Garcinia kola*-loaded SLMs had good activity against *Staphylococcus aureus* and no action against *E. coli.* The results show that the properties of the extract were not destroyed by the production process adopted in the study.

Table 4: Antimicrobial properties of *Garcinia kola* extract-loaded lipospheres

Batches	*Staphylococcus aureus*	*Escherichia coli*
	IZD (mm)*†	
P1	15.0 ± 0.17	-
P2	15.6 ± 0.72**	-
Tetracycline	25.0 ± 0.53	35 ± 0.83
Extract	16.0 ± 0.23	-

Notes: IZD: Inhibition zone diameter; *mean ± SD, †n= 3; ** p < 0.05 was considered to be significantly different from the reference, P1 and P2 SLMs contain 1 and 3 % of *Garcinia Kola* extract.

CONCLUSION

SLMs loaded with methanolic seed extract of *Garcinia kola* had good properties against *S. aureus* and no activity against *E. coli*. The particle size revealed spherical particles within the micrometer range for microparticles. High encapsulation efficiency of up to 94 % was obtained for the extract and had good taste masking properties. This delivery system could be employed in the delivery of herbal drugs in other to enhance oral bioavailability, enhance patient compliance and acceptability of the formulation.

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COMPETING INTERESTS

The authors state no conflicts of interest and have received no funding for the research or in the preparation of this manuscript.

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