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Original Research Article

Evaluation of Properties of *Garcinia kola* (Heckel) Seed Extract in Lipospheres Based on Fat from *Capra hircus*: An Antimicrobial Study.

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Abstract

The aims of the study were to evaluate the antibacterial properties of *Garcinia kola* seed extract-loaded lipospheres and to mask its bitter taste in order to improve palatability and enhance patients' acceptability of this herbal drug. The methanolic extract of *Garcinia kola* seed-loaded lipospheres were prepared using lipid matrix consisting of mixture of goat fat and Phospholipon® 90H (3:1). The lipospheres were characterized by determining the organoleptic properties, particle size and morphology, pH and encapsulation efficiency (EE%). The antibacterial properties were also determined. The results showed that the lipospheres had a taste masking effect on the extract. The particles sizes of the lipospheres were within the acceptable range for lipospheres. Maximum EE% of 92.2, 93.6 and 95.7 % were obtained for the lipospheres loaded with 5, 1 and 3 % of extract respectively. The pH remained in the neutral range at day one, but reduced to acidic pH at 7 and 30 days. The lipospheres had Inhibition zone diameter (IZD) of about 20 ± 0.91 mm against *Staphylococcus aureus* but, had no action against *Escherichia coli.* The reference drug (tetracycline) however, had significantly higher IZD of both organisms than the *Garcinia kola* seed extract-loaded lipospheres ($p < 0.05$). *Garcinia kola* seed-loaded lipospheres had good antimicrobial properties, improved palatability and could be used to enhance patient's compliance to this herbal drug.

Keywords: *Garcinia kola seed extract, lipospheres, lipids, antimicrobial properties.*

1. Introduction

Garcinia kola Heckel (*Guttiferae)* is a tropical plant whose seed is generally known as Bitter kola [1], and in Nigeria it is commonly called "*Namiji goro*" in *Hausa,* "*Aguinu*" in Igbo [1, 2] and *"orogbo"* in Yoruba [3].The plant has been referred to as a "wonder plant" because every part of it has been found to be of medicinal importance [4, 5]. *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhea [6, 7]. The seed is masticatory and also used to prevent and relieve colic, chest colds, cough and can as well be used to treat headache [4, 8].

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Iwu [6] reported the use of this plant for the treatment of jaundice, high fever and purgative. The plant also found usefulness in the treatment of stomach ache and gastritis [4, 9]. Phytochemical analysis of extracts from both root, stem and seed of *Garcinia kola* and other members of the genus show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1,GB-2), xanthones and benzophenones [10,11,12]. 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) [14], *Lactobacillus* spp. [15] and *Streptococcus pyogenese* [16]. Similar study by Afolabi et al, [17] showed its antibacterial effects on *Streptococcus mutans* another

important organism involved in plaque formation. The anti-ovulation properties of the seed extract has been reported [18, 19]. Because of the medicinal importance of this herbal drug a suitable drug delivery system is necessary in order to improve its efficacy and oral bioavailability. Also due to the bitter taste of *Garcinia kola* seed a suitable delivery system that could mask its taste is needed in order to improve the palatability and compliance to this herbal drug. Lipid-based formulations are typically reputed to improve the solubility and bioavailability of orally administered drugs [20, 21, 22, 23]. Fundamentally these formulation techniques promote wetting or solubilization of drug and enhance permeability or further undergo intraluminal processing to solubilize the drug [24]. Intraluminal processing is facilitated by bile secretion which creates a pool of cholesterol/phospholipid/bile salt complex with surfactant property which facilitates dissolution of poorly soluble drugs and lipophilic drug formulations [21].

Lipospheres were first reported as a particulate dispersion of solid spherical particles between 0.2-100 µm in diameter consisting of solid hydrophobic fat core such as triglycerides or fatty acids derivatives, stabilized by monolayer of phospholipids [25]. The internal core contains the drug dissolved or dispersed in solid fat matrix. Liposphere drug delivery system is an emerging carrier for both hydrophilic and hydrophobic drugs and has several advantages over other delivery systems in terms of physical stability, low cost of ingredients, ease of preparation, and scale-up, high dispersibility in aqueous medium, high entrapment efficiency, and extended release of entrapped drug [26, 27]. Most biotech drugs and plant-derived bioactive compounds can be formulated as lipospheres [28, 29]. Liposphere formulation could be a better way to formulate plant extracts for increased bioavailability [29]. The aim of the work was to formulate *Garcinia kola* seed extract–loaded lipospheres in order to mask the bitter taste of this herbal drug, improve its palatability thereby, enhancing patients compliance and acceptability, and also to improve its oral bioavailability.

Experimental

Materials

Solutol® (BASF, Germany), Phospholipon® 90H (Phospholipid GmbH, köln, Germany), activated charcoal (Bio-Lab (UK) limited, London), distilled water (STC UNN, Nigeria), methanol, sorbic acid, sorbitol (Merck, Darmstadt, Germany). Goat fat was extracted from *Capra hircus* and *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 Ethnomedicine and Drug Development (InterCEDD) Nsukka and the voucher specimen (no. PC98032) is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka.

Extraction of goat fat from *Capra hircus*

The fat was extracted 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 [21].

Extraction of *Garcinia kola*

About 5 kg of *Garcinia kola* (Heckel) seeds were cut into pieces, sun dried for 3 days and then pulverized using an end runner mill. The fine powder (310 g) was extracted with methanol by cold maceration method for 24 hours. The extract was further filtered and allowed to evaporate to a semi-solid residue (20.3 g) which afforded a percentage of 5.63 %.

Preparation of lipid matrix

The lipid matrix was prepared by fusion using Phospholipon® 90H and purified goat fat at a ratio of 1:3. The lipids were weighed and melted together in a beaker placed on a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) at 70 ^{o}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 [23].

Preparation of *Garcinia kola* extract-loaded lipospheres

The lipospheres were prepared by melt homogenization using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany). Details of the composition of the lipospheres are shown in Table 1. In each case 5 g of the lipid matrix was weighed using analytical balance (Adventurer, Ohaus, China), melted in a beaker at a temperature of 70 ^{o}C using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India) and the appropriate amount of *Garcinia kola* seed extract was incorporated into the lipidic melt. Sorbitol was dissolved in hot distilled water at the same temperature with the lipidic melt together with Solutol® and sorbic acid. The hot aqueous phase was poured into the lipidic melt and immediately subjected to high shear homogenization with Ultra-Turrax at 5000 rpm for 10 min. Lipospheres containing no drug (unloaded lipospheres), which served as the control was also formulated [23].

Determination of organoleptic properties

The organoleptic properties of all the batches of the lipospheres were examined by checking the odour, observing the colour, and tasting to determine the taste.

Determination of particle size and morphology

Small amount of lipospheres were placed on a microscope slide, the slide was covered with a cover slip and imaged under a Hund® binocular microscope (Weltzlar, Germany), attached with a motic image analyzer (Moticam, China) at a magnification of x100.

The pH studies

The pH of the lipospheres 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 lipospheres was added into a centrifuge and separated using a centrifuge (Chem. Lab. Instrument, UK) at 1,252 × g for 30 min. About 0.5 g of the sediment was adequately analyzed for drug content in 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 lipospheres was calculated from the equation below:

$$\mathbf{EE}\ (\%) = \frac{\mathbf{Wi-Wf}}{\mathbf{Wi}}\mathbf{x\ 100} \qquad \textbf{(1)}$$

Where Wi is the mass of *Garcinia kola* extract added to the formulation, while Wf is the actual mass of *G. latifolium* encapsulated in the lipospheres.

The LC was determined using the relationship

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

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 [30].

Inhibition zone diameter (IZD) test

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.* 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. Different concentrations (500, 250 and 125 mg/ml) of the *Garcinia kola* extract, the lipospheres and the reference sample, 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 ^{o}C for 24 h. The diameter of each inhibition zone was measured and the average determined [31, 32]. The procedure above was repeated for *Escherichia coli*.

Statistical analysis

Data were analyzed 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 lipospheres loaded with the extract of *Garcinia Kola* are shown in Table 2. The results show that the colour of the extract was brownish, while the colour of the bland lipospheres was yellowish. However, when the *Garcinia Kola* extract was incorporated into the lipospheres, the colour was brownish and the intensity varied directly with the concentration of the extract loaded. Ordinarily *Garcinia Kola* extract is very bitter and most patients find it difficult to take this extracts either as infusion or macerates and this affects patient compliance leading to patient's not following therapy and as result poor treatment outcome. However from the results, the lipospheres had a taste masking effect on the extract thus, this will enhance compliance to *G. kola* formulation and invariably lead to good therapeutic outcomes.

Particle morphology and size

The results of particle morphology are shown in Fig. 1, and the results show that the lipospheres were spherical in shape. The results of the particle size are shown in Table 3 and the results show that the particles size of the lipospheres were within the acceptable range for lipospheres and increased with increase in drug content. The bland lipospheres had the smallest particle size, while the batch G3 containing the 5 % of *G. kola* extract had highest particle size. Particle size of lipospheres is important because they determine the route of drug administration and also affects the bioavailability of the drug. Small particle size of liposphere (< 20 µm) is hypothesized to be well tolerated by a single cell contact, where as large particle size (> 50 µm) are much more reactive due to attractive forces (e.g. Van der waals) [22, 32].

Encapsulation efficiency and loading capacity

The results of EE% and LC of *Garcinia kola* extract-loaded lipospheres are shown in Table 3 and show that high EE of 92.2, 93.6 and 95.7 % were obtained for G3, G1 and G2 loaded with 5, 1 and 3 % of extract respectively. EE% was generally high as shown in Table 3. LC increased with increase in the amount of *Garcinia kola*-loaded lipospheres.

The pH of *Garcinia kola* extract-loaded lipospheres

The results of the pH of *Garcinia kola*-loaded and the bland lipospheres are shown in Fig. 2 and show that the bland lipospheres (G4) had a neutral pH of 6.3 and reduced to acidic pH on the 7^{th} and 30^{th} day. However this pH reduction was significant at day 30 compared to the pH at day 1 ($p < 0.05$). Also when *Garcinia kola* was loaded into the lipospheres, the pH remained in the neutral range at day one, but also reduced to acidic pH at 7 and 30 days. Batches G1 and G2 loaded with 1 and 3 % of the extracts exhibited significant pH decrease at day 30 ($p < 0.05$), however, batches G3 loaded with 5 % of the extract had a stable pH over time. This may be due to saturation of the lipid matrix with higher drug loading leading to lower presence of unloaded lipids and free fatty acids. The pH decrease over time was not attributed to degradation of *Garcinia kola* extract in the lipospheres since there was also a slight decline in pH in the unloaded lipospheres. The decline in pH may be due to the release of free fatty acids from the lipid matrix [21-23,30].

Antimicrobial properties of *Garcinia kola* seed extract-loaded lipospheres

The results of the inhibition zone diameter are shown in Table 4 and the results show that the *Garcinia kola*-loaded lipospheres had good antibacterial properties against *S. aureus* with

about 20 ± 0.91 mm inhibition for batch G3 containing 5 % of *Garcinia kola* extract. However *Garcinia kola*-loaded lipospheres had no action against E. coli as shown in Table 4. The reference drug however, had higher inhibition zone diameter significantly higher than the *Garcinia kola*-loaded lipospheres ($p < 0.05$). The results show that batch G3 containing 5 % of the extract showed higher IZD than the other formulations, this may be due to the presence of loaded extract in the periphery of the lipospheres, leading to faster release of the extract.

Conclusion

The results of the study revealed that lipospheres could be a better delivery system for herbal extract such as bitter kola seed extract in order to enhance its oral bioavailability and at the same time mask the bitter and or unpleasant taste of *Garcinia kola* seed extract. The *in vitro* studies showed a high encapsulation efficiency of the herbal extract. The results also showed that the encapsulated *Garcinia kola* seed extract had good antibacterial properties against *S. aureus*, therefore, the preparation technique adopted did not destroy the properties of the extract. However, further research into this area is highly encouraged in order to scale up this formulation and make the product available in the market.

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Authors' Contributions

Authors AAA, SAC and GCO designed the study, wrote the protocol, and supervised the entire work. Authors SAC, IVO and ACA managed the analyses of the study and carried out statistical analysis of the study. Authors SAC and IVO managed the literature searches. All authors read and approved the final manuscript.

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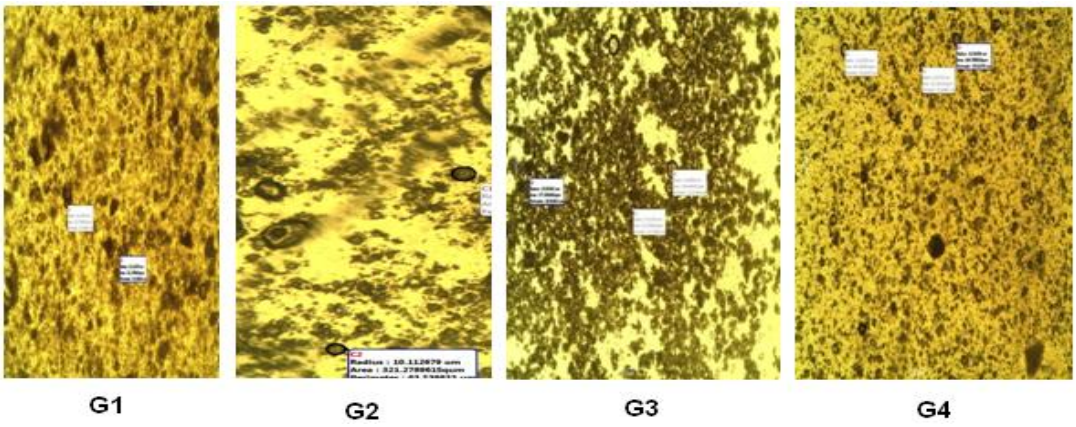

Fig. 1: Photomicrographs of lipospheres loaded with *Garcinia Kola* extract. G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres.

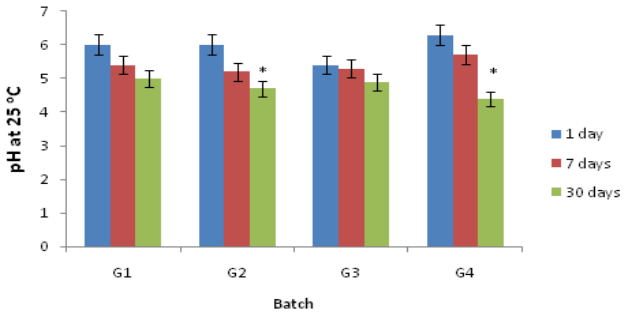

Fig. 2: The pH of *Garcinia kola* extract-loaded lipospheres over time. G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres; *significant reduction compared to day 1 at $p < 0.05$.

Table 1. Composition of *Garcinia kola* seed extract-loaded lipospheres.

Batch	*G. kola* seeds extract (%)	Lipid matrix (%)	Sorbitol (%)	Solutol® (%)	Sorbic acid (%)	distilled water q.s (ml)
G1	1	5	4	0.75	0.05	**100**
G2	3	5	4	0.75	0.05	**100**
G3	5	5	4	0.75	0.05	**100**
G4	**0**	**5**	**4**	**0.75**	**0.05**	**100**

G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres and G5 is the *Garcinia Kola* extract.

Table 2. The organoleptic properties of the lipospheres.

Batches	Colour	Odour	Taste
G1	Light brown	Sweet smell	Slightly bitter
G2	Light brown	Sweet smell	Slightly bitter
G3	Brown	Pleasant	Slightly bitter
G4	Yellow	Pleasant	Salty
G5 (extract)	Brown	Pleasant	Very bitter

G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres and G5 is the *Garcinia Kola* extract.

Table 3. Physicochemical properties of *Garcinia kola*-loaded lipospheres.

Batches	TDC (%)	ADC (%)*	EE (%)*	LC (mg API/100 mg)	Particle size (µm*)†
G1	1	0.93 ± 0.32	93.6	1.96	20.23 ± 0.11
G2	3	2.87 ± 1.80	95.7	2.53	22.75 ± 0.17
G3	5	4.61 ± 0.41	92.2	7.24	28.09 ± 0.24
G4	-	-	-		16.86 ± 0.19

ADC: Actual drug content, TDC: Theoretical drug content, EE: Encapsulation efficiency, LC: Loading capacity; batches G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres; *mean ± SD; †n = 100.

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

Batches	***Staphylococcus aureus***	***Escherichia coli***
	IZD (mm)*†	
G1	18 ± 0.72**	-
G2	16 ± 0.43**	-
G3	20 ± 0.91	-
Tetracycline (reference)	25 ± 0.53	35 ± 0.83

Notes: IZD: Inhibition zone diameter; *mean ± SD, †n= 3; G1, G2 and G3 contain 1, 3 and 5 % of *Garcinia Kola* extract, batch G4 is the bland lipospheres; ** $p < 0.05$ was considered to be significantly different from the reference.