bioavailability of these formulations over the non-lipid complexed plant extract has been demonstrated by pharmacokinetics and activity studied in animals and humans [25-27].

Lipid-based formulations can be used to influence the absorption of active ingredients through different mechanisms to modify the release of active ingredients thus, improving bioavailability. Lipid formulations, in general, provide increased drug solubilization for water-insoluble drugs. If the drug is dissolved in the lipid matrix of the carrier, the drug absorption is observed to be better. The drug suspended in the lipid matrix has been shown to get absorbed better than the conventional solid dosage forms [28-30]. This could be due to the ease of wetting of the hydrophobic drug particles in the presence of lipid matrix [30].

Herbospheres are majorly solid lipid microparticles loaded with extracts of natural plants and have been investigated as a delivery system of *G. kola and G. latifolium*; results showed the taste masking potentials and also enhanced the oral bioavailability of the extracts [22]. The aim of the study is to encapsulate *V. amygdalina* extract in herbospheres using lipid matrix based on Phospholipon 90H and goat fat and to evaluate the antimicrobial properties of the formulations, in addition to taste masking and other *in vitro* properties.

METHODS

The following materials were procured from their local suppliers and used without modifications: Solutol® HS 15 (macrogol 15 hydroxystearate - polyoxyl-15-hydroxystearate), Soluplus® (polyethylene glycol-polyvinyl caprolactam-polyvinylacetate copolymer) (BASF, Germany), Phospholipon® 90H (Phospholipid GmbH, köln, Germany), activated charcoal (Bio-Lab (UK) Limited, London), distilled water (STC UNN, Nigeria), methanol, sorbic acid, and sorbitol (Merck, Darmstadt, Germany).

Collection and identification of plant material

The fresh leaves of *V. amygdalina* (bitter leaf) were collected from Franco garden, University of Nigeria, Nsukka. The leaf was authenticated by Mr. A.O Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka and the voucher specimen is deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka.

Extraction of *V. amygdalina*

V. amygdalina leaves were washed with water, dried under shade for 7 days and subsequently milled using a blender (500# grinder/Fuyu Metal, Linyi Fuyu Metal Products Co., Ltd., China). The powdered leaf (290 g) was extracted by cold maceration using methanol as extracting solvent. The leaves were soaked in 1 L of analytical methanol for 48 h with constant shaking in the first 6 h. The cold macerate was filtered using a muslin cloth embedded with cotton wool and attached to a filter funnel. The residue was discarded, and the filtrate was transferred into a stainless steel tray to evaporate the methanol and was allowed to dry. The percentage yield of the extract was found to be 11.0 6% [5].

EXTRACTION AND PURIFICATION OF GOAT FAT

The adipose tissues of *Capra hircus* were obtained from an abattoir in Nsukka market, Enugu, Nigeria. The adipose tissue was grated and subjected to moist heat by boiling with about half its weight of water in 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 blend of activated charcoal and bentonite (1:19) in the lipid at 80°C for 1 h. Thereafter, the suspension was vacuum filtered using a Buchner funnel [31].

Phytochemical analysis

Phytochemical studies were carried out on the aqueous extract of bitter leaf for the presence of alkaloids, carbohydrates, saponins, reducing sugars, steroids, proteins, tannins, glycosides, terpenoids, flavonoids, resins, oils, and acid compounds using the standard procedures of analysis [32-34].

Preparation of lipid matrix carrier

The lipid matrix was prepared using the goat fat (70%) and Phospholipon 90H, purified and completely hydrogenated soy phosphatidylcholine (30%) by fusion method. The lipids were melted together in a beaker using a magnetic stirrer hot plate (SR1 UM 52188, Remi Equip., India). It was stirred until they melted completely and was allowed to solidify.

Preparation of herbospheres

The herbospheres were prepared by melt homogenization using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany). Soluplus® and Solutol® HS 15 were used, respectively, as the surfactants, and the effect of the individual surfactants was studied. Details of the composition of the formulations are shown in Table 1. Sorbitol was used as a stable cryoprotectant, and sorbic acid was used as a preservative. The lipid matrix was melted using the magnetic stirrer hot plate, and the extract was dispersed in the molten lipid. A solution of sorbitol, sorbic acid, and the surfactant at the same temperature with the lipid was transferred into the dispersion of the extract and homogenized using an Ultra-Turrax homogenizer (T25 Basic, Digital, Ika Staufen, Germany) at 5000 rpm for 5 min. The herbospheres (o/w) were formed by phase inversion and were stored in an airtight bottle for further studies.

Characterization of Herbospheres

Analysis of taste of herbospheres

A panel consisting of 10 normal human volunteers with age range of 22–48 years was used for taste evaluation of the herbospheres. Each volunteer was given 100 mL of the formulation and was retained in the oral cavity for 2 min. After each determination, the oral cavity was rinsed with water and a washout period of 1 h was allowed between determinations. Response of each volunteer was recorded and scored as follows:
+++ Strongly bitter
++ Bitter
+ Slightly bitter
0 Palatable
– Tasteless.

Particle size and morphology studies

Table 1: Composition of the herbospheres

Batch	Extract (%)	Lipid matrix (%)	Sorbitol(%)	Solutol® HS 15 (%)	Soluplus® (%)	Sorbic acid (%)	Distilled water q. s (%)
A	1	5	4	0.75	-	0.05	100
B	3	5	4	0.75	-	0.05	100
C	5	5	4	0.75	-	0.05	100
D	1	5	4	-	0.75	0.05	100
E	3	5	4	-	0.75	0.05	100
F	-	5	4	0.75	-	0.05	100
G	-	5	4	-	0.75	0.05	100

Batches A, B, and C contain 1, 3, and 5% w/w of *V. amygdalina* extract, respectively, and Solutol, while batches D and E contain 1 and 3% w/w of crude extract, respectively, and Soluplus®. Batches F and G are bland herbospheres formulated with Solutol® and Soluplus®, respectively

The particle size of the herbospheres was determined by computerized image analysis using a microscope (Wetzlar, Germany) attached with a digital image analyzer (Moticam, China). The particle diameter and the morphology were determined [31]. The mean particle size for each batch was calculated.

Analysis of encapsulation efficiency (EE) and loading capacity

Quantitative determination of the amount of extract encapsulated in each formulation was determined using ultraviolet (UV)-spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA) at a predetermined wavelength of 283 nm. A 10 mL quantity of the herbospheres was centrifuged at 1,252×g for 30 min (Chem. Lab. Instrument, UK). The supernatant was diluted and analyzed in a spectrophotometer (UNICO 2102 PC UV/Vis Spectrophotometer, USA). The actual amount of extract encapsulated was determined by subtracting the actual mass extract in the supernatant (Wf) from the total amount of extract incorporated into the formulation (Wi).

EE % was calculated from the equation:×

$$EE\ (\%) = \frac{Wa\text{-}Ws}{Wa\text{-}Ws\text{+}W1} \times 100 \qquad (1)$$

LC was determined using the formula:

$$LC = \frac{Wa\text{-}Ws}{Wa\text{-}Ws\text{+}W1} \times 100 \qquad (2)$$

Where Wl is the weight of lipid in the formulation, Wa is the weight of bitter leaf added to the formulation, and Ws is the actual amount of extract encapsulated (Ws = Wi - Wf) in the herbospheres.

Analysis of pH of herbospheres over time

The pH was determined using a pH meter (Suntex TS-2, Taiwan) at 1, 7, and 30 days.

Inhibition zone diameter (IZD) test

The plate agar diffusion method was used for this study. Molten nutrient agar (20 mL) was inoculated with 0.1 mL of *Staphylococcus aureus* broth culture or *Escherichia coli* as the case may be. 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 herbospheres, bitter leaf extract, and tetracycline, respectively, were prepared. About 0.01 mL of each of the samples was added, 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 and lather incubated at 37°C for 24 h. The IZD was measured [30].

Statistical analysis

Data were analyzed by one-way ANOVA. Differences between means were assessed using Student's t-test, p<0.05 was considered significant.

RESULTS

Phytochemical constituents

The results of the phytochemical constituents of *V. amygdalina* leaf extract are shown in Table 2 and show that it contains alkaloids, carbohydrates, saponins, reducing sugars and steroids in very high concentrations, proteins, tannins, glycosides, terpenoids, flavonoids in moderate concentrations, and resins in low concentrations, while oils and acid compounds were absent.

Taste masking properties

The results of the taste masking potentials of herbospheres are shown in Table 3 and show that the extract was strongly bitter, while the bland formulations (F and G) were palatable. However, the herbospheres containing 1 and 3% of extracts (A, B, D, and E) were slightly bitter, while Batch C containing 3% of the extract was bitter. Therefore, herbospheres masked the terribly bitter taste of bitter leaf as shown in Table 3.

Particle size and morphology

The results of the particle morphology of herbospheres containing bitter leaf extract are shown in Fig. 1 and show that the particles were spherical in shape as expected for herbospheres. The results of particle size of herbospheres are shown in Fig. 2 and show that particle size increased with increase in amount of extract loaded in the herbospheres and ranged from 12.13±0.10 to 28.50±0.71 μm for batches A and C formulated with Solutol® as their surfactant and 6.90±0.2 to 11.70±0.4 μm for batches E and D, respectively, containing 3 and 1% of extract and Soluplus® as the surfactant.

The pH of the herbospheres

The results of the pH stability of the bitter leaf extract-loaded and unloaded herbospheres are shown in Fig. 3 and show that the pH of the bland (unloaded) formulations ranged from 6.3 to 4.4 at 1 and 30 days for batch F, formulated with Solutol, while batch G containing Soluplus had pH of 6.4 and 4.7 at 1 and 30 days. The pH of the bitter leaf

Table 2: Phytochemical constituents of *V. amygdalina* leaf extract

Constituents analyzed	Remarks
Alkaloids	+++
Tannins	++
Carbohydrates	+++
Resins	+
Saponins	+++
Proteins	++
Reducing sugars	+++
Steroids	+++
Glycosides	++
Oils	-
Terpenoids	++
Flavonoids	++
Acid compounds	-

+++: Highly concentration, ++: Moderate concentration, +: Low concentration, - Absent. *V. amygdalina: Vernonia amygdalina*

Table 3: Taste masking properties of *V. amygdalina* herbospheres

Batches	Score	Taste
A	+	Slightly bitter
B	+	Slightly bitter
C	++	Bitter
D	+	Slightly bitter
E	+	Slightly bitter
F	0	Palatable
G	0	Palatable
Extract (Et)	+++	Strongly bitter

Batches A, B, and C contain 1, 3, and 5% w/w of crude extract of *V. amygdalina* respectively and Solutol®, while batches D and E contain 1 and 3% w/w of the crude extract, respectively, and Soluplus®. Batches F and G are bland formulations containing Solutol® and Soluplus®, respectively. *V. amygdalina: Vernonia amygdalina*

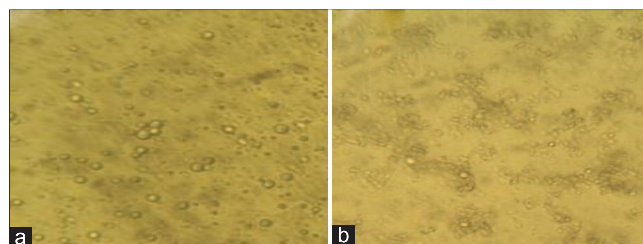

Fig. 1: (a and b) Photomicrographs of *Vernonia amygdalina* herbospheres containing 1% of extract; batches a and b were formulated with Solutol® and Soluplus®, respectively

herbospheres had pH of 5.6–5.9 at day 1 and 4.1–4.4 at 30 days. The results of bitter leaf herbospheres showed a significant decrease over time and remained in the acidic region from preparation to 30 days ($p<0.05$).

EE and loading capacity

The results of the EE are shown in Fig. 4 and show that highest EE of 90–92% was obtained for herbospheres loaded with 3 and 1% of bitter leaf extract and formulated with Soluplus as a surfactant. However, herbospheres containing Solutol had EE% range of 44 to 87% for batches C and B containing 5 and 3% of extract, respectively.

The results of the loading capacity of the lipid matrices are also shown in Fig. 4 and show that loading capacity increased with increase in the concentration of the extract loaded, with maximum LC of 64 mg extract/100 mg lipid matrix for batch C containing 5% of the extract.

Antimicrobial properties of bitter leaf herbospheres

The results of the IZD of the bitter leaf herbospheres are shown in Fig. 5 and show that the herbospheres showed good antibacterial properties against *Staph. aureus and E. coli.* The results were comparable to that of the reference drug-tetracycline used for the studies. Bitter leaf herbospheres showed higher inhibition of *Staph. aureus* than *E. coli* ($p < 0.05$). Batches B and E showed significantly higher inhibition of *Staph. aureus* than tetracycline ($p < 0.05$).

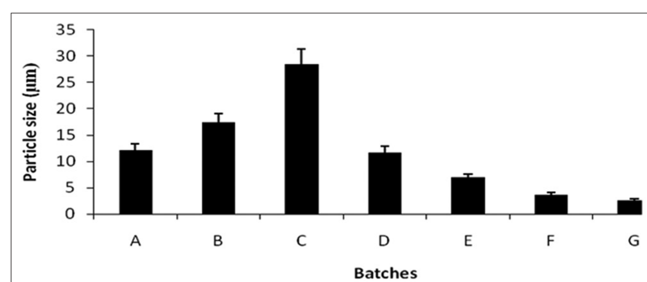

Fig. 2: Particle size of bitter leaf extract-loaded herbospheres; batches A, B, and C contain 1, 3, and 5 % w/w of *V. amygdalina* extract, respectively, and Solutol®, while batches D and E contain 1 and 3% w/w of extract and Soluplus®. Batches F and G are bland formulations with Solutol® and Soluplus®, respectively

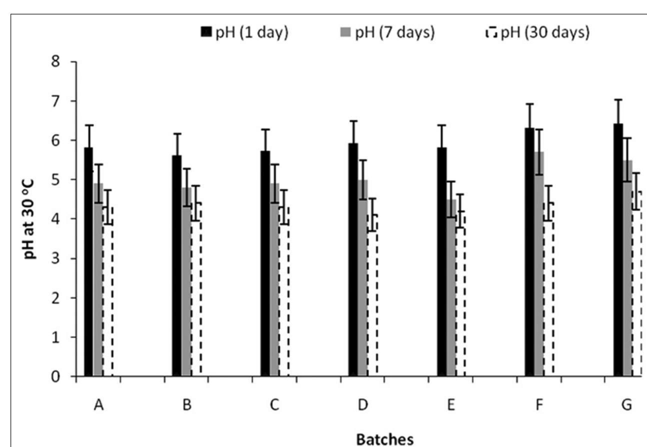

Fig. 3: pH stability of *Vernonia amygdalina* leaf-extract herbospheres; batches A, B, and C contain 1, 3, and 5% w/w of *V. amygdalina* extract, respectively, and Solutol® while batches D and E contain 1 and 3% w/w of crude extract, respectively, and Soluplus® as a surfactant. Batches F and G are bland formulations with Solutol® and Soluplus®, respectively

DISCUSSION

The results of the phytochemical screening of bitter leaf indicated the presence of alkaloids, saponins, tannins, saponins, carbohydrates, reducing sugars, protein, steroids, flavonoids, and cardiac glycosides in substantial quantities. Acid compounds and oils were, however, absent. The presence of alkaloids in high concentration explains the traditional use of the plant for the treatment of malaria. The medicinal plants that are moderately rich in alkaloids and tannins have potential health-promoting effects [35]. Similarly, saponins have anti-carcinogenic properties and other health benefits [22]. Alshawsh *et al.* [36] reported that tannins may have antiplasmodial activity. The stem bark of the plant also contains glycosides. Cardiac glycosides are used to treat heart problems that may result from a severe malaria attack. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals substances to protect themselves, and they are also believed to protect humans against certain diseases [22].

The results of the taste masking properties of the herbospheres show that encapsulating bitter leaf extract into herbospheres significantly reduced the bitterness of this all-important herbal drug. This is particularly important because bitter leaf macerate, tonic or tea would be very

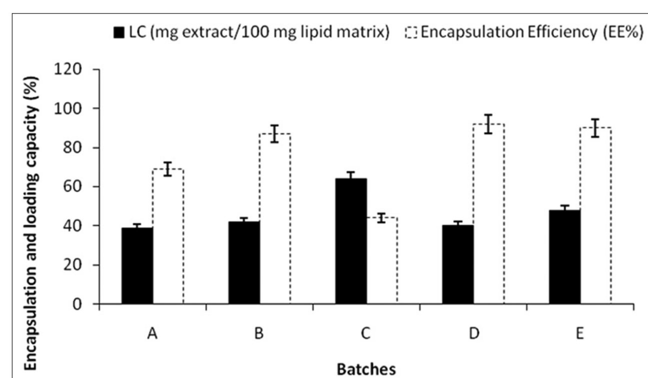

Fig. 4: Encapsulation efficiency and loading capacity of *Vernonia amygdalina* leaf-extract herbospheres; batches A, B, and C contain 1, 3, and 5% w/w of *V. amygdalina* extract, respectively, and Solutol® while, batches D and E contain 1 and 3% w/w of extract, respectively, and Soluplus® as a surfactant.

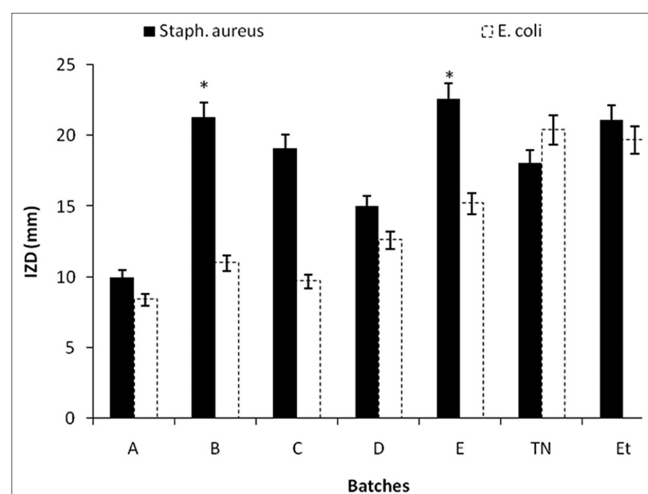

Fig. 5: Antimicrobial properties of *Vernonia amygdalina* leaf-extract herbospheres; batches A, B, and C contain 1, 3, and 5% w/w of *V. amygdalina* extract, respectively, and Solutol® while, batches D and E contain 1 and 3% w/w of extract, respectively, and Soluplus®; TN: Tetracycline, Et: Extract

difficult for any patient to swallow; therefore, this herbal remedy is best administered as an encapsulated dosage form. Encapsulating of herbal formulation into solid lipid microparticles has been shown to mask the bitter properties of the extract and also enhance oral bioavailability [30]. Therefore, this novel herbospheres formulation strategy would enhance patient compliance in addition to enhanced bioavailability.

The particle size of the herbospheres was found to lie within micrometer range and agrees with the results of previous works [22]. Particle size significantly increased with the incorporation of the drug compared to the bland formulations ($p<0.05$). However, the increase was not proportional to the quantity of extract loaded as shown by batch E containing 3% of extract which showed smaller particle size than batch D containing 1% of the extract. The results also varied significantly within the sub-batches with batch C showing the highest mean particle size of 28.5 µm ($p<0.05$). The photomicrographs of the solid lipid microspheres revealed that they are crystalline in nature, spherical and have uniform smooth surface. Particle size was significantly affected by the type of surfactant used ($p<0.05$). Batches A, B, and C formulated with Solutol® had higher particle sizes than batches C and D formulated with Soluplus®.

The results of the pH of the herbospheres showed a significant decline in storage ($p<0.05$). However, the bland formulations also showed a significant decline in pH suggesting that pH decrease may be due to release of free fatty acids from the individual lipids. Therefore, pH decline was not due to degradation of encapsulated extract since there was also a corresponding decrease in the pH of the unloaded formulations.

The results of EE% of the herbospheres show that the formulations generally exhibited high EE. However, the EE of the herbospheres was affected by the type of surfactant used. Herbospheres formulated with Soluplus® generally exhibited higher EE% than those formulated with Solutol®. The Soluplus® being a copolymer of polyethylene glycol-polyvinyl caprolactam-polyvinyl acetate had higher surface tension reduction effect than Solutol® which is macrogol 15 hydroxystearate - polyoxyl-15-hydroxystearate, hence enhanced encapsulation of the extract.

The results of the loading capacity of the lipid matrix showed that LC increased with increase in the loading of the extract in agreement with previous researches [30].

The *in vitro* antimicrobial properties of the herbospheres show that bitter leaf extract formulations exhibited significantly higher inhibition of *S. aureus* than tetracycline pure sample used as the reference drug. The activities were, however, comparable to that of the extract against *S. aureus* and *E. coli*. The results show that the formulations had good antibacterial properties in addition to taste masking potentials. These results also revealed that the active constituents in the extract were not denatured by the processes of formulations and/or the excipient used in the formulation.

CONCLUSION

Herbospheres is a novel and efficient drug delivery system for herbal drugs. The advantages of presenting herbal drugs inform of herbospheres include enhanced palatability, improve patient acceptability of herbal formulations due to enhanced esthetic appeal and taste masking, better disease management due to improvement in patient compliance and improve bioavailability due to enhanced absorption caused by the presence of surfactants in the formulations and presence of lipids. This formulation approach should be scaled up to further encourage the use of the natural drug as a curative in the treatment of diseases.

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COMPETING INTERESTS

The authors state no conflicts of interest.

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