

Original Research Article

Phytolipid delivery of *Pentaclethra macrophylla* (Fabaceae) extract: *In vitro* and *in vivo* activities

Calister E Ugwu^{1*}, Christopher E Ugwoke², Iheanacho O Enyum¹

¹Department of Pharmaceutical Technology and Industrial Pharmacy, ²Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (UNN), 410001, Nigeria

*For correspondence: **Email:** calister.ugwu@unn.edu.ng

Sent for review: 4 March 2025

Revised accepted: 21 July 2025

Abstract

Purpose: To investigate the physicochemical properties and *in vivo* anti-inflammatory activity of phytosome complexes prepared from *Pentaclethra macrophylla* seeds.

Method: *Pentaclethra macrophylla* seeds were extracted using Petroleum ether. Wavelength of maximum absorption (λ) of bioactive compounds (BCs) was determined. Phytochemical constituents were quantified and formulated as phytosome complexes in different ratios of lipid-based phospholipon complexes (LPCs; 1:1, 1:2, and 2:1 as LPC1, LPC2, and LPC3, respectively). Formulated phytosome complexes were evaluated for percentage yield, encapsulation efficiency (EE), and Fourier transform infrared spectroscopy (FTIR). *In vitro* drug release was done using 10 mg LPCs in simulated intestinal fluid (SIF, pH 6.8). A total of 40 female Wistar rats, randomized into 5 groups ($n = 8$ per group), were used. Group 1 (received 100 mg/kg of pure lipid bioactive compounds), group 2 (received 100 mg/kg LPC formulation (LPC3 optimized complex, (selected because it has the highest EE), group 3 (received 10 mg/kg of Indomethacin (positive standard), group 4 (received distilled water equivalent to 70 mg/kg (0.64 mL), and group 5 (received no treatment (negative control). Anti-inflammatory effect was evaluated using rat paw oedema model.

Results: Yield was 33.43 %, alkaloid was high (2.80 %), λ was 234 nm, EE in LP3 was 93 ± 0.09 %. *In vitro* drug release of all LPCs demonstrated a gradual and retarded drug release within 22 – 38 % after 1 h. Also, LPC3 has significantly higher anti-inflammatory activity (98 %) compared to controls ($p < 0.05$).

Conclusion: Formulating *Pentaclethra macrophylla* as a lipid-based phospholipid complex improves entrapment efficiency, retards drug release, and significantly increases anti-inflammatory activity.

Keywords: *Pentaclethra macrophylla*, Phospholipon 90 H, Phytosomes, Anti-inflammation, Phytolipid

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Globally, there is a vast knowledge of traditional herbal medicine. This has not been translated into tangible products due to numerous problems, which include variable bioavailability, erratic absorption, and reduced drug efficacy when orally administered. Therapeutic efficacy of

any drug is also subject to the efficiency of the delivery system [1]. A novel drug delivery system delivers an appropriate amount of the active entity into target sites. Numerous novel drug delivery systems have been developed to improve these challenges. This approach has achieved tremendous success in making phytoconstituents more bioavailable with high

stability. Such approaches include varieties of vesicular drug delivery systems like liposomes, noisomes, transferosomes, phytosomes, among others [2].

Phytosome was selected because it is a novel drug delivery system for hydrophilic and hydrophobic bioactive compounds, having advanced herbal complex of active drugs entrapped in phospholipid with better absorption properties compared to conventional herbal extracts [1,2]. Phytosomes are mixtures of appropriate quantity of phospholipid (phosphatidylcholine) interacting with an extract in a nonpolar medium [3]. Phytosomes are also known as herbosomes [4]. This new technology protects the active compound from degradation by stomach acidic secretions and intestinal bacteria due to the gastro-protective quality of phosphatidylcholine (an emulsifying compound) which enhances bioavailability of herbal extracts [3,5].

Phosphatidylcholine is a component of phospholipid that incorporates choline as the hydrophilic part and phosphatidyl moiety as the lipophilic bio-membrane with enhanced bioavailability [1-2, 5-6]. Phytosomes are applicable in cosmetic science and pharmaceutical drug delivery. However, phosphatidylcholine is a bioactive agent with clinical efficacy in liver disease [3,7]. Phytosomes present good pharmacokinetic and pharmacodynamic profile compared to conventional herbal medicine [8]. Most biologically active components of plants are hydrophilic molecules. At the same time, hydrophilic soluble phytocomponents, including flavonoids, tannins, glycosidic aglycones, and so on, are poorly absorbed. This is due to large molecular size which is not absorbed through passive diffusion and lack of lipophilic solubility that seriously limits their capacity to cross the oil-rich bio-membranes, leading to poor bioavailability [9]. Phytosomes convert water-soluble phytocomponents to a lipophilic-compatible molecular complex. This is because, in oral administration, the phosphatidylcholine component of phospholipids, a biological component of lecithin and the principal molecular building block of cell membranes, is miscible in the hydrophilic and hydrophobic phases with good absorption.

Pentaclethra macrophylla Benth (George Benth) is a medicinal and nutritional plant from *Fabaceae* family. It is commonly called African oil bean and locally known as 'Ukpaka' in Igbo and 'Apara' in Yoruba. The tree is commonly found in the Tropics with characteristic

low branches, open crown with compound leaves, usually about 20-45 cm long, and covered with rusty hairs. Its fruits are large woody pods which split open explosively as edible seeds that produce the oil [10]. The seed has been investigated for a lot of pharmacological activities, including antimicrobial activity on a wide range of microorganisms, antinociceptive, anti-inflammatory, and cytotoxic effects [11]. There is paucity of information on the use of phytosomes for improving bioavailability and erratic absorption. Hence, this study investigated the physicochemical properties and *in vivo* anti-inflammatory activity of phytosome complexes prepared from *Pentaclethra macrophylla* seeds.

EXPERIMENTAL

Materials

Phospholipon 90H (American Lecithin Company ALC), Kolliphor® HS 15 (BASF), and *Pentaclethra macrophylla* seed were purchased from Ogige market, Nsukka, Nigeria. All reagents used were of analytical grade and were obtained from reliable suppliers. They were used without further purification.

Collection of plants

Plant seeds were authenticated and assigned herbarium no. PM2345 by a taxonomist at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Enugu State. Seeds of *Pentaclethra macrophylla* were spread to dry under shade for two weeks.

Lipid extraction

The milled seed (400 g) was extracted with 750 mL of Petroleum ether using a Soxhlet extractor. Thereafter, the extract was exposed to enable the solvent to evaporate. Yield (Y) of the lipid bioactive compound of *Pentaclethra macrophylla* was calculated using Eq 1.

$$Y = (W_e/W_s) \times 100 \dots\dots\dots (1)$$

Where W_e is the weight of extract obtained and W_s is the weight of powdered seed

Phytochemical screening

Quantitative and qualitative phytochemical evaluations

Phytochemical screening of alkaloids (Mayer's test), anthraquinone (Bontrager's test), flavonoid (Shindo's test), phenol, saponin (Frothing test),

tannin (Wohler's test), and glycoside in lipid BCs of *Pentaclethra macrophylla* was done using standard methods [12].

Alkaloid determination

The extract (10 mL) was transferred into a beaker (250 mL capacity). Acetic acid (20 %; 200 mL) in ethanol was incorporated and left for 4 h. The sample was filtered and concentrated using a thermoregulatory water bath. Concentrated ammonium hydroxide was added drop-wise into the concentrated extract until the reaction was completed. This dispersion was left undisturbed, decanted and precipitate filtered and weighed.

Tannin determination

Lipid BCs (10 mL) were transferred into a calibrated rubber bottle, diluted with distilled water (50 mL) and shaken for about 60 min using a mechanical shaker and filtered. Then, 5 mL filtrate was collected, mixed with 3 mL 0.1 M FeCl_3 in 0.1 N HCl and 0.008 M Potassium Ferrocyanide and assayed within 10 min using a UV spectrophotometer at wavelength of 120 nm. A pure sample was prepared with tannic acid to obtain 100 ppm and used as the blank.

Flavonoid determination

At 25 °C, 100 mL of methanol (80 %) was used as solvent to dissolve 10 mL of BCs. It was thereafter filtered with Whatman filter paper (125 mm). The filtrate was evaporated using a thermoregulatory water bath and then weighed.

Saponin determination

Ethanol (200 mL of 20 %) was used in dispersing 10 mL of BCs and heated at 55 °C with continuous stirring over a hot water bath for 4 h. Another extraction process was done on the residue after filtration with the same volume of 20 % ethanol. The filtrate was concentrated at 90 °C in a water bath and separated using a 250 mL separating funnel. Diethyl ether (20 mL) was incorporated and shaken to separate the water and ether layers. The aqueous layer underwent further purification repeatedly with 60 mL n-butanol, washed twice with approximately 10 mL aqueous NaCl (5 %). This was evaporated and dried by heating over a water bath to a fixed weight, and the percentage saponin content was determined.

Phenol evaluation

The petroleum ether (50 mL) was added to 10 mL of BCs and boiled for 15 min. An equivalent

of 5 mL extract, distilled water (10 mL), and ammonium hydroxide solution (2 mL) were poured into a 100 mL flask, respectively, before incorporation of 5 mL concentrated amyl alcohol and then allowed to react for 30 min for colour change. Absorbance was read at 234 nm with a Spectrophotometer (Spectrum lab, 752s, Netherlands).

Preparation of phytosome complexes

The lipid phospholipid complexes were prepared with appropriate quantities of the lipid extract: Phospholipon 90H of different combined ratios 1:1, 1:2, and 2:1. The complexes were prepared with slight modification of a previous method [2]. The complexes were formulated by weighing an equivalent quantity of the extract (based on the ratio) into a 50 mL beaker. The sample was dissolved with 9.5 mL of Petroleum ether and 0.5 mL Kolliphor® HS 15 (surfactant) incorporated and then stirred with a magnetic stirrer to facilitate dissolution and dispersion. In another beaker, the required amount of Phospholipon 90 H (based on the ratio) was dissolved with 10 mL of ethanol at 32 °C in a magnetic stirrer, incorporated into the extract mix and stirred for 120 s. The complexes were refluxed for 10 min, evaporated under vacuum, and denoted as lipid-based phospholipon complexes, LPCs (LPC1, LPC2, and LPC3 as lipid bioactive compounds with Phospholipon 90H at different ratios of 1:1, 1:2, and 2:1, respectively).

Wavelength of maximum absorption

The BCs (0.86 mol/dL) were prepared by collecting 1 mL of extract and diluting it with an appropriate quantity of Petroleum ether (100 mL). The wavelength of maximum absorption was obtained via a UV-visible spectrophotometer (Spectrum Lab, 752s, Netherlands).

Determination of the morphology

A smear of each formulated complex was prepared on a slide and the morphology was captured using an optical microscope (Hund, Wetzler, Germany) and a digital camera.

Encapsulation efficiency (EE)

The LPCs (10 mg; LPC1, LPC2, LPC3) were weighed in triplets, dissolved uniformly in 10 mL simulated intestinal fluid (SIF, pH 6.8) using a magnetic stirrer and filtered. Thereafter, a 100-fold dilution was made for each solution. The absorbance was measured using a UV-visible spectrophotometer (Spectrumlab 752, Netherlands) at 234 nm (predetermined). The

drug content (DC) and loading capacity (LC) were determined using Eqs 2 and 3, respectively.

$$DC = (Ad/Td)100 \dots\dots\dots (2)$$

$$LC = (Ad/Ac)100 \dots\dots\dots (3)$$

Where Ad is the amount of drug obtained, Td is the total amount of drug loaded and Ac is the amount of complex

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the lipid extract of *Pentaclethra macrophylla* and phospholipid-based complexes using formulation LPC3 (selected based on the highest % EE) were obtained to determine the structure and chemical stability with an FTIR spectrophotometer (IRAffinity-1, Shimadzu FTIR-8400, Germany). The Potassium bromide pellet method was adopted and the background spectrum was obtained under similar situations. Spectra were determined from single mean scans obtained in the region within 400 - 4000 cm^{-1} with a spectral resolution of 2 cm^{-2} and ratio against background interferogram [13].

In vitro drug release study

The phospholipid-based complexes (10 mg each of LPC1, LPC2 and LPC3) were weighed and encapsulated each in a gelatin capsule and then dropped into a beaker containing 250 mL simulated intestinal fluid (SIF, pH 6.8) with the help of a centralizing bag assembled on a magnetic stirrer set at 50 rpm and 37 ± 0.5 °C. After every 10 min, 5 mL of the medium was collected and substituted with the same medium and volume at the same temperature to maintain sink conditions. The procedure was repeated thrice for 60 sec. Withdrawn samples were assayed with a spectrophotometer (Spectrumlab 752, Netherlands) at 234 nm. Average drug release was obtained and % drug released was calculated and plotted against time.

Animals

Rats used in this study were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. A total of 40 experimental animals weighing between 150 - 180 g were kept in standard animal cages under standard conditions (12 h light / dark cycle, room temperature) to acclimatize for one week. They had unrestricted access to food and water *ad libitum* prior to commencement of the experiment. The study was approved by the

Animal Ethics Committee, University of Nigeria (FPSRE/UNN/18/00020) and conducted in accordance with the EU Directive 2010/63/EU for animal experiments [14].

In vivo anti-inflammatory effect

The animals were randomly assigned to 5 groups ($n = 8$) in each group. Group 1 received 100 mg/kg of pure lipid BCs, group 2 received 100 mg/kg LPC formulation (LPC3 optimized complex, (selected because it has the highest % EE), group 3 received 10 mg/kg of indomethacin (positive standard) group 4 received distilled water equivalent to 70 mg/kg (0.64 mL), and group 5 received no treatment (negative control). All treatments started same time in different groups, oral administration once daily after checking the baseline paw sizes. Baseline paw sizes were measured using a digital vernier caliper before egg albumin administration and treatment. Thereafter, 0.2 mL of egg albumin was injected into the sub-plantar right paw of each rat to induce inflammation after 30 min of treatment. After 1, 2, 4, and 6 h, the rats' paws perimeters were measured using a digital Vernier caliper to determine oedema inhibition *vis-à-vis* inflammation inhibition and then % oedema inhibition (OI) was calculated using Eq 4 [15].

$$OI (\%) = \{(m-n)/n\}100 \dots\dots\dots (1)$$

Where m is the mean thickness of the paw size control, and n is the mean thickness of the paw size of tested rats.

Statistical analysis

Data were analyzed using Statistical Packages for Social Sciences version 16.0 (SPSS Inc., Chicago, IL, US) and one-way analysis of variance (ANOVA). Results were expressed as mean $\pm$ standard deviation (SD), and compared using a two-tailed Student T-test. $P < 0.05$ was considered statistically significant.

RESULTS

Yield and wavelength of maximum absorption of BCs of *Pentaclethra macrophylla*

Percentage extract yield was 133.7 g (33.43 %) for lipid BCs. Wavelength of maximum absorption was 234 nm (Figure 1) which was used in the spectrophotometric analysis.

Phytochemical constituents

Qualitative and quantitative analysis of phytochemical constituents of lipid bioactive

compounds (BCs) revealed high amounts of alkaloids (2.8 ± 0.10 mg) and glycosides (0.4 ± 0.20 mg; Table 1).

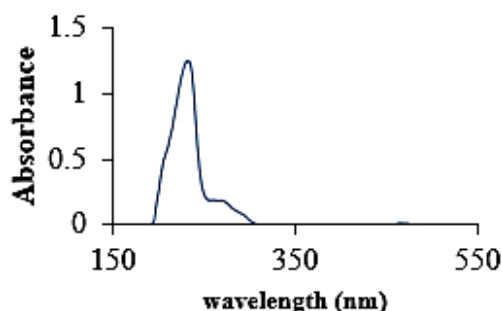


Figure 1: Wavelength of maximum absorption of lipid bioactive compound of *Pentaclethra macrophylla*

Table 1: Phytochemical constituents of lipid BC of *Pentaclethra macrophylla*

Phytoconstituent	Quantity (mg/g)
Alkaloids	2.80
Flavonoids	0.10
Phenols	0.20
Glycosides	0.40

Characterization of the complex

Morphology of the complexes

Morphology of the complexes indicated that phospholipon entrapped the drug particles to form spherical vesicles.

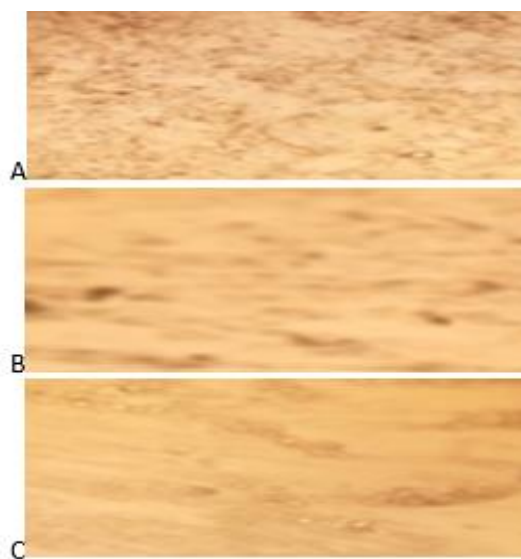


Figure 2: Morphology of the lipid phospholipid-based complexes. A, B, and C represent complexes containing lipid bioactive compounds: Phospholipon 90H 1:1, 1:2, and 2:1 for LPC1, LPC2, and LPC3, respectively

Encapsulation efficiency (EE) and loading capacity (LC)

Percentage entrapment efficiency (% EE) of the complexes was 76.79 ± 0.10 , 90.19 ± 0.15 , and 92.99 ± 0.09 % for LPC1, LPC2, and LPC3, respectively.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of the LPC (LPC3) exhibited major peak ranges of 1165.04 - 1157.33, 1458.23 - 1450.52, 1743.71 - 1651.12, 2924.18 - 2854.74, and 3410.26 - 3379.40 cm^{-1} corresponding to alkyl substitute (CH-), aromatic ring stretch (C=C), carboxyl (C=O), alkane (CH₂), and (alcohol, OH), functional groups respectively.

In vitro drug release

The *in vitro* release from the 3 LPCs was between 22– 38 %. The formulations showed retarded and controlled drug release pattern since high drug content (87.79 - 93.00 %) was yet to be released within the experimental period.

In vivo anti-inflammatory effect

The lipid phospholipon complex (LPC3) showed significant efficacy compared to indomethacin and control group ($p < 0.05$). The LPC complex (LPC3) showed significantly higher anti-inflammatory activity (98 %) compared to the standard (95 %) and extract (79 %).

DISCUSSION

Yield of the lipid bioactive compounds (BCs) of *Pentaclethra macrophylla* was fairly high. This signifies that the adopted Soxhlet extraction method was good and reliable. Phytochemicals are secondary metabolites of plants that are not used by the plant but are synthesized by plants and have physiological effects on humans and animals. These phytoconstituents account for different pharmacological activities of *Pentaclethra macrophylla* seed extracts which include antimicrobial, antifungal, anti-inflammatory, antihypertensive, antinociceptive, and anticytotoxic. Lipid-based phospholipon complexes (LPCs) were observed to have well-formed vesicles with the BC entrapped in these vesicles. This may be as a result of better complexation with the non-polar part of Phospholipon 90H. This is in tandem with previous report that phytosomal complexes enhanced extract entrapment in an organized structure of phospholipon forming vesicles [16].

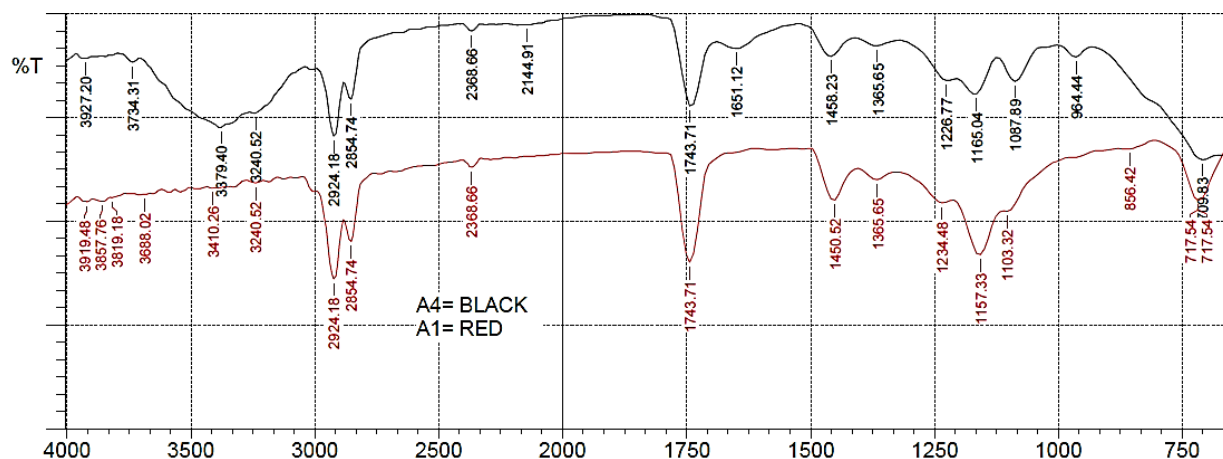


Figure 3: FTIR of lipid bioactive compound and LPC complex. A2 (red, pure lipid bioactive compound of *Pentaclethra macrophylla*), A3 (black, LPC2: (containing complex of lipid bioactive compound: Phospholipon 90H 1:2)

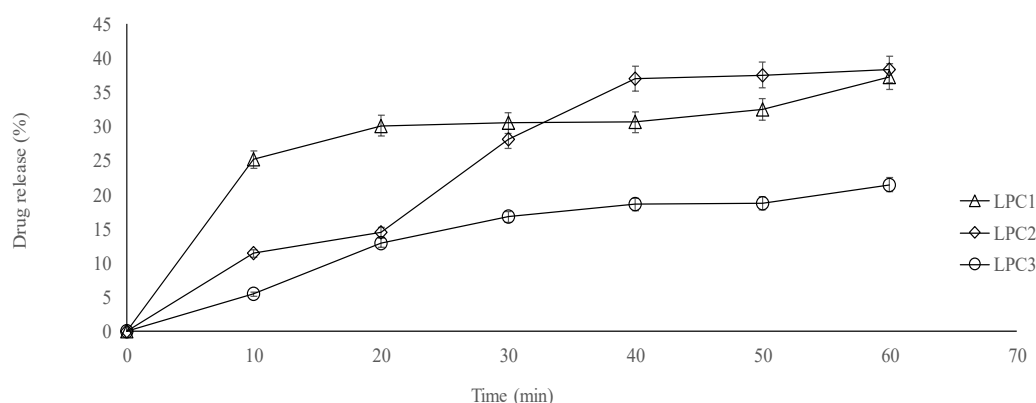


Figure 4: An *in vitro* drug release of lipid-based phospholipid complexes (LPCs). LPC1, LPC2, and LPC3 (containing complex of lipid bioactive compound: Phospholipon 90H 1:1, 1:2, and 2:1, respectively)

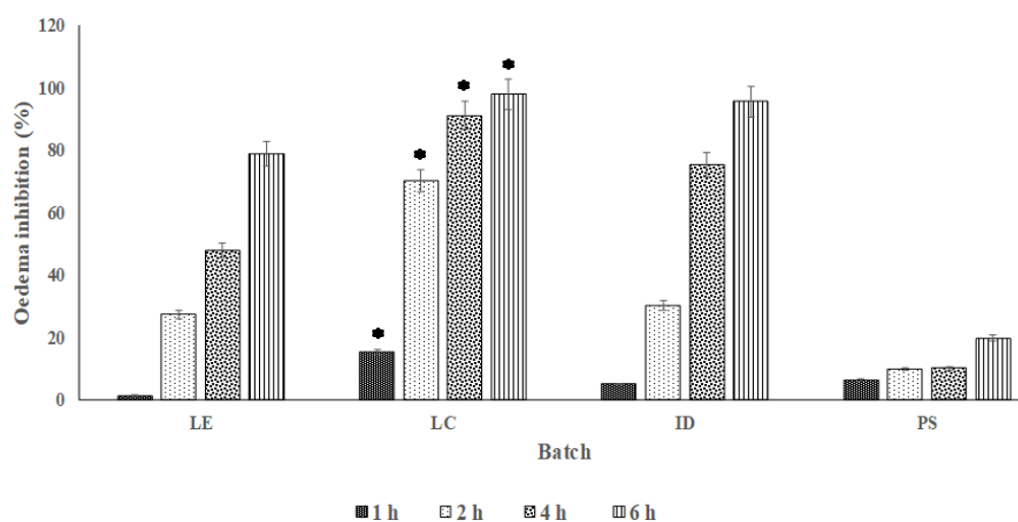


Figure 5: Percentage of oedema inhibition of LPCs. LE (Group treated with 100 mg/kg of pure lipid bioactive compound); *LC ($p < 0.05$; Group treated with 100 mg/kg LPC complex 2:1); ID (Group treated with 100 mg/kg Indomethacin); PS (Group treated with pure solvent)

High drug encapsulation efficiency (% EE) for all LPCs may be due to the presence of oil-based BCs that are readily solubilized with Phospholipon 90 H. There was no shift or disappearance of bands and functional groups detected in the pure BCs. Thus, no molecular interaction between the extracted BCs and constituents leading to good complex formation and hence, no loss of activity. The LPC formulations may have formed a good and stronger complex with Phospholipon 90H which led to retarded drug release. This is supported by the morphological results showing well-entrapped drug vesicles. This retarded and slow drug release is in agreement with previous work [2]. A similar result has been reported that Phospholipid retards drug release from oral dosage form [17].

Previous reports have found that the availability of surfactant and combination ratios of the BCs: Phospholipon are crucial factors in determining good drug release in phytosomes [2,5]. The high *in vivo* anti-inflammatory activity provided by the LPCs is a result of better or more effective novel drug delivery offered by phytolipids (e.g., Phospholipid). This good anti-inflammatory property of *Pentaclethra macrophylla* formulated as LPCs may have been reinforced by the presence of alkaloids, flavonoids, Phenols, and glycosides. Earlier reports showed improved drug absorption of phytosomes to pure extract [5,18]. Other reports have shown that phytosomes produce microspherical vesicles that protect active pharmaceutical ingredients from degradation by digestive secretions and gut bacteria, unlike normal herbal extract [19]. Similarly, it has been indicated that Phospholipid complexes get absorbed through lymphatic transport, thereby bypassing intestinal degradation and hence employed in drug targeting [5,20].

CONCLUSION

Lipid phospholipon complex of *Pentaclethra macrophylla* demonstrates high encapsulation efficiency, retards drug release, and improves *in vivo* anti-inflammatory activity. *Pentaclethra macrophylla* phytosomes are a promising formulation for anti-inflammatory activity, and further large-scale studies are necessary to validate these findings.

DECLARATIONS

Acknowledgement

The authors acknowledged the technical assistance received from Ahmadu Bello

University (ABU), Zaria, for FTIR analysis and all the technical staff of Pharmaceutical Technology and Industrial Pharmacy for their assistance.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Calister E. Ugwu and Enyum Iheanacho conceived and designed the experiments, analyzed, interpreted the data, and wrote the paper. Calister E Ugwu and Christopher E. Ugwoke contributed reagents, materials, and analytical tools. Enyum Iheanacho performed the experiments. All authors read and approved the final manuscript

REFERENCES

1. Bombardelli E. Phytosome: New cosmetic delivery system. *Boll Chim Farm* 1991; 130: 431-388.
2. Ugwu CE, Momoh MA, Ezeugwu RS. *In vivo* antiulcer activity of phospholipid-based complexes of *Musa paradisiaca* (Musaceae) peel extract for improved oral drug delivery. *IJNDD* 2019; 11 (1): 20-29.
3. Patel J, Patel R, Khambholja K, Patel N. An overview of phytosomes as an advanced herbal drug delivery system: An overview of phytosomes. *Asian J Pharm Sci* 2009; 4(6): 363-367.
4. Pandey PS, Patel K. Phytosomes: technical revolution in phytomedicine. *Int J Pharm Tech Res* 2010; 1: 627-631.
5. Ugwu C, Ugorji L, Diovu E, Nwajie C, Amadi B, Ezeigbe A, Ugwu N. Formulation and evaluation of *Morinda lucida*-based phytosome complexes for malaria treatment. *Pharmacol Online* 2020; 3: 345-357.

6. Gandhi A, Dutta A, Pal A, Bakshi P. Recent trends of phytosomes for delivering herbal extract with improved bioavailability. *J Pharmacog Phytochem* 2012; 1(4): 6-14.
7. Mascarella S, Giusti A, Marra F. Therapeutic and anti-lipoperoxidant effects of silybin-phosphatidylcholine complex in chronic liver disease: preliminary results. *Curr Ther Res* 1993; 53: 98-102.
8. Goyal A, Kumar S, Nagpal M, Singh I, Arora S. Potential of novel drug delivery systems for herbal drugs. *Ind J Pharm Edu Res* 2011; 45(3): 225-235.
9. Kumar VS, Asha K. Herbosomes. A novel carrier for herbal delivery. *Int J Cur Pharm Res* 2011; 3: 37-41.
10. Akah PA, Aguwa CN, Agu RU. Studies on the anti-diarrhoeal properties of *Pentaclethra macrophylla* leaf extracts. *Phytother Res J* 1999; 13: 292-295
11. Alinnor IJ, Ozes R. Chemical evaluation of the nutritive value of *Pentaclethra macrophylla* Benth. (African Oil Bean) seeds. *Pak J Nutr* 2011; 10(4): 355-359
12. Trease GE, Evans WC. *Trease and Evan's Textbook of Pharmacognosy*, 13th Edition, London: Cambridge University Press, 1989: 546.
13. Patel A, Tanwar Y, Rakesh S, Patel P. Phytosome: phytolipid drug delivery system for improving bioavailability of herbal drug. *J Pharm Sci Bio Scientif Res* 2013; 3: 51-57.
14. European Community Council Directive on the ethics of experiments involving laboratory animals (86/609/EEC) 1986.
15. Sumana S. Evaluation of the anti-inflammatory activities of *Quillaja saponaria* Mol. Saponin extract in mice. *Tox Rep* 2016; 3: 1-3.
16. Vandijk C, Driessen A, Recourt K. The uncoupling efficiency and affinity of flavonoids for vesicles. *Biochem Pharmacol* 2000; 60: 1593-1600.
17. Raffin RP, Colombo P, Sonvico F. Soft agglomerates of pantoprazole gastro-resistant microparticles for oral administration and intestinal release. *J Drug Del Sci Tech* 2007; 17: 407-413.
18. Saha S, Sharma A, Saikia P, Chakrabarty T. Phytosome: A brief overview. *Sch Acad J Pharm* 2013; 2(1): 12-20.
19. Murray M. Phytosomes increase the absorption of herbal extract. Available at: www.doctormurray.com/articles/silybin.htm Accessed Sept 28, 2008.
20. Kalepu S, Manthina M, Padavala V. Oral lipid-based drug delivery systems – an overview. *Acta Pharm Sin B* 2013; 3(6): 361-372.