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Novel Anti-Ulcer Phytosomal Formulation of Ethanol Extract of *Pentaclethra macrophylla* Stem-Bark

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ABSTRACT

Pentaclethra macrophylla, a medicinal plant widely used for ulcer treatment in Nigeria by herbal practitioners, is limited by poor lipid solubility, resulting in poor absorption and bioavailability. Phytosomes, a novel dosage form that are better absorbed and produce better results than conventional herbal extracts, could be employed to enhance its antiulcer activity. The objective of this study was to formulate *Pentaclethra macrophylla* stem-bark extract as phytosomes by forming complexes with phospholipid and compare the antiulcer activity with omeprazole, a standard anti-ulcer drug. Phytosomal formulations of ethanol extract of *Pentaclethra macrophylla* stem-bark and Phospholipon® 90G (P90G) (extract:P90G ratios of 1:1, 1:3, 1:5) were prepared following established method. Their physicochemical properties, *in vitro* drug release in simulated intestinal fluid (SIF, pH=7.4) and simulated gastric fluid (SGF, pH=1.2) and anti-ulcer properties on aspirin-induced ulcer using Wistar rats were determined and compared with omeprazole. Phytosomes with spherical smooth particles with size range 0.106-0.217 µm and good encapsulation efficiencies (range = 67.61-72.8%) were obtained. Drug release increased with time irrespective of phospholipid concentration or dissolution medium. The extract possessed antiulcer activity (23.33%) which was increased to 33.33, 43.33 and 56.67% by formulating it as phytosomal formulations containing extract:P90G ratios of 1:1, 1:3, 1:5, respectively. However, omeprazole and its formulations gave significantly ($p<0.05$) greater antiulcer activity when compared with both the ethanol extract and phytosomes. *Pentaclethra macrophylla* stem-bark possessed antiulcer activity, which was improved via phytosomal formulation. This would serve as potential safer and cheaper alternative therapeutics for ulcer given the side-effects associated with omeprazole.

Keywords: *Pentaclethra macrophylla*, Phytosome, Aspirin-induced ulcer, Omeprazole, Ulcer inhibition, Phospholipon® 90G.

Introduction

Drug bioavailability is a well-known challenge in drug and bioactive delivery in the pharmaceutical sector. For good bioavailability, natural products must have a good balance between hydrophilicity (for dissolving into body fluids) and lipophilicity (to cross lipid bio-membranes).[1,2] Many approaches have been developed to improve the oral bioavailability of herbal/plant-based products such as inclusion of solubility and bioavailability enhancers, structure modifications, and entrapment with lipophilic carriers.[3,4]
A recent strategy based on phytosome technology (a patented dosage form developed by Indena) is a breakthrough model for marked enhancement of bioavailability, significantly greater clinical benefits and assured delivery to tissues without compromising nutrient safety.[5-8] Phytosomes are prepared by reacting natural or synthetic phospholipids with active components like bioflavonoid, flavolignan and polyphenolic constituents.[6] Solvent evaporation method is the most common technique used for preparation of phytosomes (e.g. ginsenoside, puerarin and kushenin), though mechanical dispersion method can also be used (e.g. marsupsin-phospholipid complexes) in which case, phospholipids is dissolved in a suitable solvent and active ingredient is added drop by drop while sonicating the solution. Phospholipid complex is sometimes prepared under reflux and stirring conditions to effect complete interaction. Curcumin phospholipids complexes are prepared by adding phospholipids to ethanol solution of the hydroalcoholic extract of turmeric rhizomes, under reflux and with stirring. Prepared complex called phytosome can be isolated by precipitation with nonsolvent, lyophilization, spray drying or vacuum drying.[9] Behavior of phytosomes in both physical and biological system is governed by factors such as physical size, membrane permeability, percent entrapped solutes, chemical composition as well as quantity and purity of the starting materials. Therefore, phytosomes are characterized for physical attributes as well as percentage drug capture, percentage drug released and chemical composition. Novel drug delivery systems have also employed this technology of inclusion of phospholipids to generate micro/nanoparticles based on polymers or

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lipids,[10-12] which have produced good products in the market for cure of difficult-to-treat disease conditions.

Pentaclethra macrophylla (Mimosaceae) is used in African traditional herbal practice for the treatment of disorders of both veterinary and human diseases.[13] It is commonly known as the African oil bean tree;[14] also locally called "apara apagha" (Yoruba), "okpaghan" (Urhobo),"okpagha" (Bini), "ugba" (Ibo).[15] The plant is mostly found in the forests of Eastern, Western and Central Africa.[16] The bark is grayish to dark reddish brown, thin and patchy with irregular pieces flaking off. All parts of the plant are used for various human ailments. The bark, fruits, seeds and leaves are used as anthelmintic, for gonorrhea, convulsion and as analgesic,[17,18] whereby whole leaves are given to domestic or wild animals and ruminants while the aqueous extract of the leaves is administered to man orally. Antimicrobial property and the fixed oil extracted from the seeds are used in the preparation of formulation against pruritus, worms and dysentery.[19-21] Extracts from *P. macrophylla* plant have the abilities to maintain the integrity (or prevent the lysing) of the cell membranes skeletal protein. This could therefore support the anti-inflammatory (anti-allergy/anti-itching) property of this plant.[13,20,22] The ripe fruits are applied externally to heal wounds.[16] Although phytosomes have been employed to enhance the bioavailability of several bioactives,[23,24] there is paucity of information in the literature on phytosomes of *P. macrophylla*.

Consequently, this study was carried out to validate the folkloric use of *Pentaclethra macrophylla* stem bark in ulcer treatment among Nigerian herbalists and improve its activity via phytosomal formulation. Phytosomes were formed from complexes of ethanol extract of the stem bark with phospholipids and were investigated as anti-ulcer agents alongside standard anti-ulcer drug, omeprazole.

Materials and Methods

Chemicals and reagents

The materials used include absolute ethanol (%) CAS No. (Sigma-Aldrich Germany.), Phospholipon® 90G (P90G) (Phospholipids GmbH, Germany), distilled water (Lion, Nigeria), sodium chloride, potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide and hydrochloric acid (BDH, England), omeprazole pure sample 7.5% w/w and commercial omeprazole (AC Pharmaceutical PLC Enugu, Nigeria), These materials were used as procured from the manufacturers without further purification. All other reagents were of analytical grade and used as such.

Animals

The animal experiments complied with the regulations of the Committee on Ethics on the Use of Laboratory Animals of the University of Nigeria (approval no. FPSRE/UNN/17/00022) in accordance with the Federation of European Laboratory Animal Science Association and the European Community Council Directive of November 24, 1986 (86/609/EEC). Male Wistar rats (*Rattus norvagicus*) (100 - 150 g) were kept in the experimental animal house of University of Nigeria, Nsukka, with free access to food and water. The animals were allowed to acclimatize for 14 days prior to their use for the experiment.

Collection and preparation of plant materials

The stem bark of *Pentaclethra macrophylla* was collected in February, 2013 from Ovoko in Igbo-Eze South Local Government Area of Enugu State, Nigeria by a herbal practitioner. Botanical identification and authentication were done by Mr. Ozioko, an analyst in Botany Department, University of Nigeria Nsukka and the voucher specimen (voucher number PHE-08-2009) was deposited in the Department of Pharmacognosy, University of Nigeria, Nsukka. The stem barks were then cut into small pieces, sun-dried, pulverized and properly stored until used.

Extraction of plant materials

Powdered stem bark of *Pentaclethra macrophylla* (1 kg) was macerated in 4 L of ethanol for 72 h and afterwards, filtered using a filter paper. The ethanol extract was evaporated using rotary evaporator to get dry concentrate. The dry extract was weighed and percentage yield calculated.

Phytochemical analysis

Qualitative assay for the presence of secondary plant metabolites such as alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the ethanol extract of the stem-bark following standard procedures.[25]

Preparation of phytosomes

Established technique was employed with modifications for the preparation of the phytosomes.[23,24] Dry ethanol extract of the stem-bark of *Pentaclethra macrophylla* and graded concentrations of Phospholipon® 90G (P90G) complexes were formulated in the ratios of 1:1, 1:3, and 1:5. In the formulation, appropriate quantities of extract were weighed and dissolved in ethanol. The ethanol solutions of the extract were then mixed with corresponding ratios of P90G to obtain extract-phospholipids complexes. The complexes formed were isolated by vaporizing ethanol at 40 ± 2°C in a humidity chamber having relative humidity of 75 ± 5% to obtain thin film of the extract-phospholipids complexes (phytosomes). The phytosomes formed were further dried in desiccators and then crushed in mortars to obtain fine powders. Omeprazole-loaded P90G (1:1) was also formulated following the procedure described above.

Morphology and particle size analysis

Aliquot samples of the various phytosomes were prepared in ethanol from where a drop was placed on a microscopic slide which 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 ×400. The average particle sizes of the phytosomes were determined and the particle morphologies also observed. This procedure was repeated for ethanol stem-bark extract.

Thermal analysis

Thermal analysis of ethanol extract of *Pentaclethra macrophylla*, P90G, phytosomes (1:1, 1:3, 1:5), pure omeprazole and omeprazole-loaded P90G were studied using differential scanning calorimeter (NETZCH DSC 204 F1, Germany) at a heating rate of 10°C/min over a temperature range of 30-360°C under an inert nitrogen atmosphere with flow rate of 20 mL/min.

Encapsulation efficiency

The encapsulation efficiency (EE%) of each of the formulation was determined. An aliquot (1 mg/mL) of each phytosome formulation was prepared using alcoholic buffer (pH 1.5) as the solvent as well as 1 mg/mL of omeprazole-loaded P90G. The different solutions were filtered using a filter paper. The absorbance of phytosomal filtrate was taken at a wavelength of 320 nm spectrophotometrically (Jenway 6305, Germany) while that of omeprazole-loaded P90G filtrate was taken at a wavelength of 310 nm spectrophotometrically (Jenway 6305, Germany). The actual drug content of each formulation was computed, and encapsulation efficiency was calculated using the equation below.[10,12]

$$\% \text{ EE} = \frac{Actual\ Drug\ content}{Theoritical\ Drug\ content} \times 100 \quad \ldots\ldots\ldots\ldots \quad \text{Equation 1}$$

Preparation of biorelevant media for in vitro release studies

Simulated gastric fluid (SGF, pH 1.2) without pepsin, simulated intestinal fluid (SIF, pH 6.8) without pancreatin and alcoholic buffer (pH 1.5) were prepared following standard procedures.[26,27]

In vitro release studies

The magnetic stirrer method was adopted in this study. The dissolution medium consisted of 200 mL of freshly prepared SGF (pH 1.2) maintained at 37°C as acceptor phase. The dialysis membrane was pre-treated by soaking in the dissolution medium 24 h *ab initio*. Three hundred milligram of each phytosome formulation (1:1; 1:3 and 1:5),

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300 mg of omeprazole-loaded P90G, 150 mg of ethanol extract and 150 mg of pure omeprazole were analyzed separately by placing each of them in a dialysis membrane and dissolution achieved with 5 mL of SGF (donor compartment). The dialysis membrane was securely tied with a thermo-resistant thread attached to a clamp and suspended into the recipient compartment. The stirrer was set at 50 rpm and a 5 mL aliquot was removed for analysis and replaced with an equal volume, 5 mL of fresh dissolution medium at different sampling intervals of 0.5, 1, 2, 4, 6, 8, 10, 12 h. The absorbances of the samples were taken at a wavelength of 315 nm spectrophotometrically. The procedure above was repeated using SIF (pH 7.4) and wavelength of 320 nm except for the pure omeprazole and omeprazole-loaded P90G in which absorbances were taken at wavelength of 310 nm.

Pharmacodynamic studies

In vivo investigation of ulcer

Clinically healthy male Wistar rats weighing 200 ± 10 g were used for the experiment. Eight groups of rats (n = 5) were used to study the antiulcer activity of all formulations. The rats were fasted for 16 h, but allowed free access to water before commencement of the experiments. Group 1 received normal saline 10 mL/kg, group 2 was given ethanol extract 300 mg/kg while groups 3-5 received 300 mg/kg each of 1:1, 1:3 and 1:5 phytosomal formulations. Group 6 received 10 mg/kg of pure omeprazole sample, group 7 received 10 mg/kg omeprazole-loaded P90G and group 8 received 10 mg/kg of commercial sample of omeprazole. One-hour post administration, all rats were given 200 mg/kg of aspirin p.o and 2 h later, they were all sacrificed using ether as the anaesthetic. Their stomachs were isolated, cut along the greater curvature and washed with normal saline; and each stomach was then viewed with ×10 magnifying lens and the ulcer scores calculated as thus: ≤1mm = 1, >1mm but ≤ 2mm = 2, >2mm = 3. The scores were summed, divided by ×10 magnification and averaged by the number of animals to get the mean ulcer indices from where the percentage ulcer inhibition was calculated using the equation shown below.

$$\% \text{ UI} = \frac{Ulcer\ index\ (control) - Ulcer\ index(test)}{Ulcer\ index(control)} \text{ x } 100 \quad \ldots \quad \text{Equation 2}$$

Statistical analysis

All experiments were performed in replicates (at least n = 3) for validity of statistical analysis. Results were expressed as mean ± SD. ANOVA and Student t-tests were performed on the data sets generated using SPSS. Differences were considered significant for P values < 0.05.

Results and Discussion

Percentage yield and phytochemical analysis of the extract

The percentage yield of the extract was 8.84% w/w. The poor yield (less than 10% w/w) may be as a result of the nature of solvent used. A previous study reported that methanol or acetone gave better yield when used in extraction.[28] The phytochemical analysis of the stem-bark of *Pentaclethra macrophylla* revealed significant abundance of tannins, glycosides, cyanogenic glycosides and phenols. Alkaloids and saponins were found in small amount while flavonoids, sterols and triterpenes were absent.

Morphology

The phytosomes were smooth and spherical while the ethanol extract appeared as uniformly distributed discrete particles (Figure 1). The 1:1 phytosomal particles appeared singly while 1:3 and 1:5 phytosomal particles were in clusters, though more pronounced in the 1:5 phytosomes.

Particle sizes

The mean particle size of the ethanol extract was less than that of 1:1 phytosome but greater than those of 1:3 and 1:5 phytosomes (Table 1). The mean particle sizes can be represented in decreasing order as follows: 1:1 phytosome > ethanol extract > 1:5 phytosome > 1:3 phytosome. The decreasing order of the particle sizes may suggest a decrease in crystalline habit of the particles and also a decrease in particle size growth since it was independent of phospholipid concentration, which is in agreement with previous reports.[23,24]

Table 1: Physicochemical properties of the phytosomal formulations

Formulations	Parameters	
	Particle size (μm ± SD)^n	**Encapsulation efficiency (%)^n**
Ethanolic extract	0.175 ± 0.094	-
1:1 Phytosomes	0.217 ± 0.036	67.6 ± 0.3
1:3 Phytosomes	0.106 ± 0.014	77.7 ± 0.5
1:5 Phytosomes	0.118 ± 0.006	85.8 ± 0.2
Omeprazole-loaded P90G	-	72.8 ± 0.9

Key: n = 3, SD is standard error of mean.

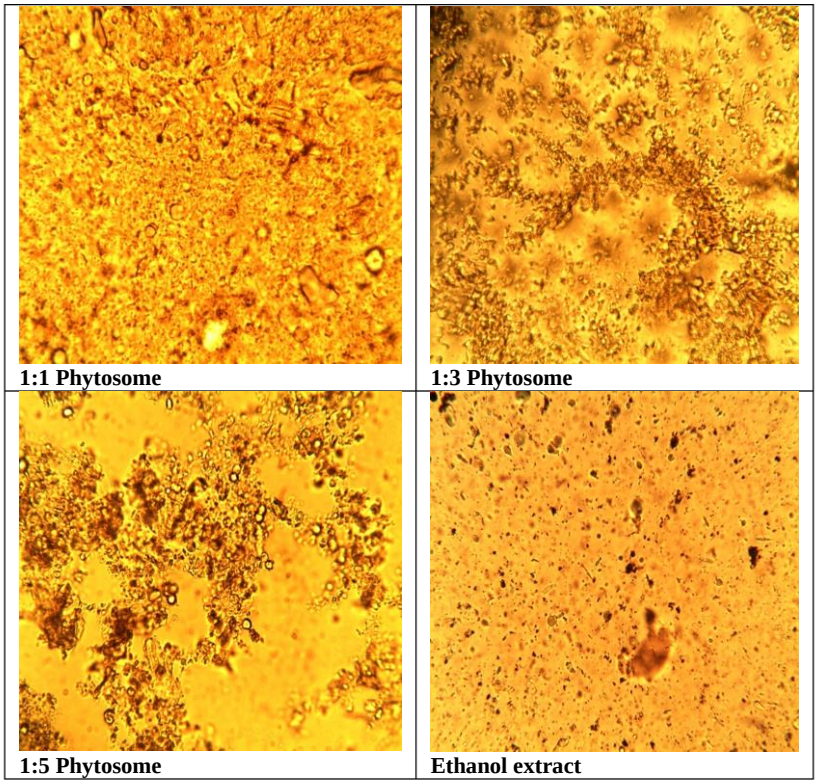

Figure 1: Photomicrograph of formulations (magnification ×400).

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Encapsulation efficiency

Encapsulation efficiency (EE%) of the phytosomes increased as the proportion of phospholipid increased in the formulations; hence the 1:5 phytosome had the highest EE% followed by 1:3 phytosome while 1:1 phytosome had the least EE%. The 1:1 omeprazole loaded phospholipids had a higher encapsulation efficiency compared to 1:1 phytosome (Table 1). Generally, encapsulation efficiency is a function of the amount of drug or substances that was entrapped or encapsulated in the lipid base compared to the total weight of the drug.[10-12]

Thermal analysis

Differential scanning calorimetry (DSC) result of ethanol extract of the stem-bark of *Pentaclethra macrophylla* showed two melting peaks at 67.7 and 301.7^{o}C with corresponding enthalpies of -5.099 and -0.174 mW/mg (Figure 2A). However, the more stable modification which melted lastly had the less enthalpy (-0.174 mW/mg), even though it had higher melting temperature (301.7^{o}C). The phospholipid (P90G) gave three melting peaks (112.3, 140.3, and 260.9^{o}C) with corresponding enthalpies of 11.51, -10.59 and -8.94 mW/mg (Figure 2B). The presence of multiple melting temperatures could be due to presence of impurities or unstable entities.[10]

When P90G was employed to formulate the phytosomes, the DSC trace of the formulations showed 1:1 phytosome as having two melting peaks (75.8 and 261.5^{o}C) with corresponding enthalpies of -2.59 and -1.567 mW/mg (Figure 2C). The 1:3 phytosome had a melting peak of 61.3^{o}C, and an enthalpy of -0.8204 mW/mg (Figure 2D) while the 1:5 phytosome gave a DSC trace of 60.5^{o}C, and enthalpy of -2.471 (Figure 2E). Generally, enthalpy values are low, which shows that samples were somewhat not crystalline. However, the 1:5 phytosomes had the highest value for enthalpy (-2.471 mW/mg) which may suggest a more crystalline arrangement in the sample when compared to 1:1 and 1:3 phtytosomes of lower enthalpies (-1.567 and -0.8204 mW/mg), respectively. This perhaps may be suggestive of instability as a result of having more clusters or particle growth which may lead to drug expulsion upon long storage. However, the 1:3 phytosome with the least enthalpy (-0.8204 mW/mg) also possesses the least particle size (0.106 ± 0.014 µm) suggesting less crystallinity and possibility of retention of entrapped drug. This is because lower enthalpy suggests less crystallinity and the possibility for retention of entrapped drug over time.[10]

Omeprazole thermogram showed three melting peaks (144.2, 192.3 and 223.0^{o}C) with enthalpies of -9.241, -17.09 and -10.8 mW/mg, respectively (Figure 2F). This melting point value deviated from the literature value (156^{o}C) probably due to impurities. When P90G was loaded with omeprazole (Figure 2G), the thermal history traced three melting peaks of 122.6, 185.3, and 573.3^{o}C. It could be seen that the presence of omeprazole in P90G lowered the melting temperature from 223.0 to 209.1^{o}C and also decreased enthalpy from -10.08 to -5.733 mW/mg indicating that the omeprazole-loaded P90G had a less crystalline arrangement.

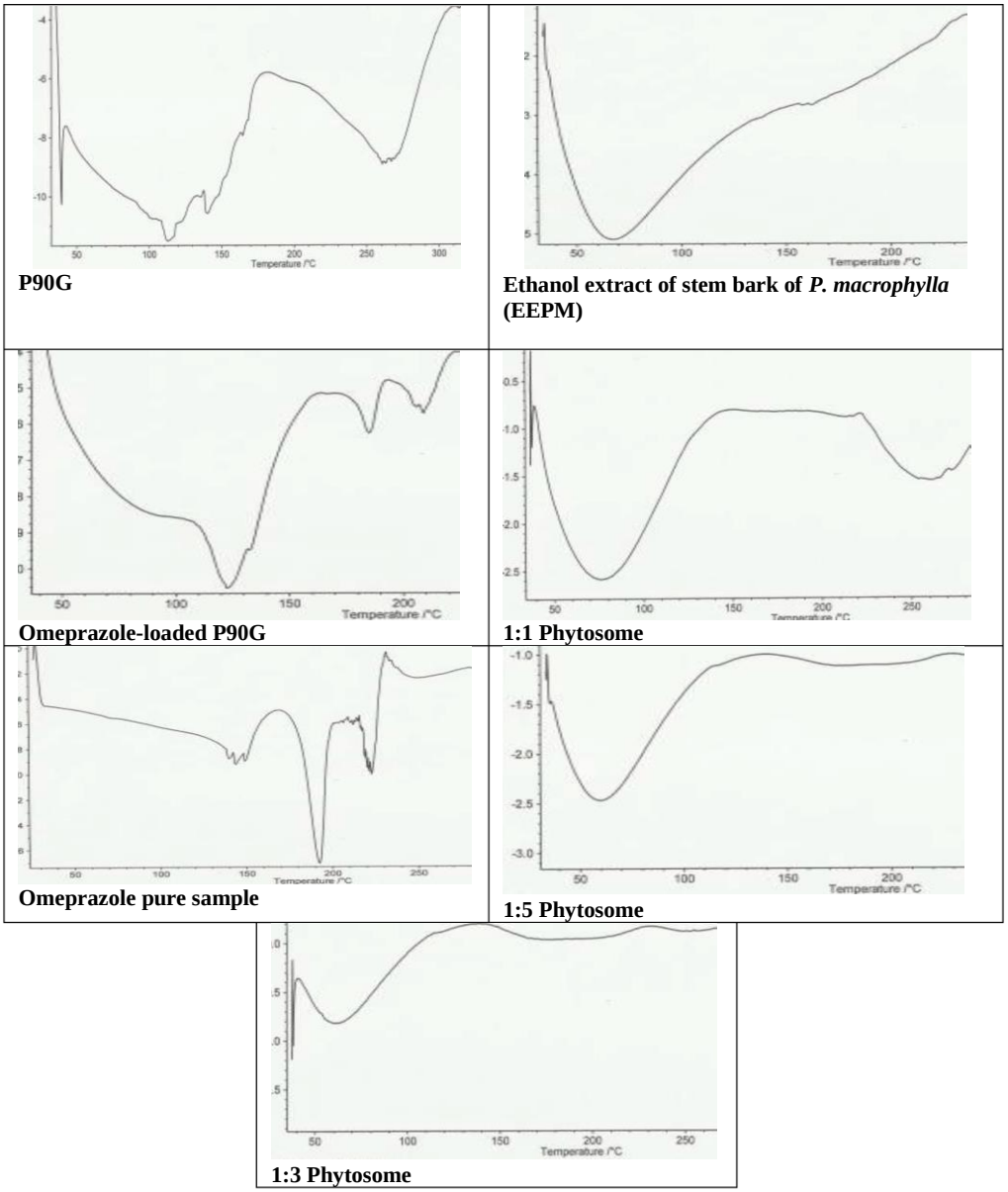

Figure 2: Differential scanning calorimetry (DSC) thermograms of the formulations

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In vitro drug release

Figures 4 and 5 illustrate the *in vitro* release profile of the various formulations in SGF and SIF, respectively. It was observed that the amount of drug released in both SGF and SIF increased as the proportion of phospholipid increased in all the formulations. Overall, the 1:5 phytosome gave the highest release, followed by 1:3 phytosome and 1:1 phytosome while the ethanol extract had the least release. The effect of phospholipids on drug release could also be observed in the release profile of omeprazole-loaded P90G which also had higher release compared to omeprazole pure sample. Comparing the release profile in SGF and SIF, all formulations (ethanol extract, 1:1, 1:3, 1:5 phytosomes, omeprazole pure sample, and omeprazole-loaded P90G) had higher release in SGF than in SIF which may suggest higher protection against gastric ulcer than intestinal ulcer. Generally, drug release was affected by the nature or design of the delivery system and the medium used in the release study. Hydrogen ion concentration, pH, was the most important factor that affected drug release followed by agitation, viscosity, temperature of the medium as well as stirring speed of the apparatus used in the release study. This is in agreement with the study of Attama *et al*.[29]

In vivo investigation of ulcer

Figure 6 represents the effect of the phytosomal formulation on ulcer index while Figure 7 shows the percentage ulcer inhibition by the various formulations on aspirin-induced gastric ulceration. It was observed that pretreating the rats with ethanol extract of the stem bark of *Pentaclethra macrophylla* (300 mg/kg), phytosomes (300 mg/kg each of 1:1 and 1:3), and pure omeprazole (10 mg/kg) failed to produce significant statistical reduction of ulcer index ($p > 0.05$) in comparison to control. However, the 1:5 phytosome (300 mg/kg), omeprazole-loaded P90G (10 mg/kg) and commercial omeprazole sample (10 mg/kg) exhibited significant statistical reduction of ulcer index compared to the control group ($p < 0.05$). This means that percentage ulcer protection by phytosomes increased as phospholipid content increases and was significant at 1:5 phytosome ($p < 0.05$). This agrees with an earlier observation, that production of phytosomes by binding individual component of herbal extract to phosphatidylcholine results in a product that is better absorbed and produces improved therapeutic efficacy and bioavailability than the conventional herbal extracts.[3,24] Phospholipids therefore, vastly improve absorption and utilization of standardized herbal extracts due to inherent surfactant action which aids higher adhesion of the product itself to molecules with cell structure. Phytosomes therefore have added dimension of proven health-giving activity of the phospholipids themselves.[24] The antiulcer activity of the ethanol extract of *Pentaclethra macrophylla* could probably be due to the presence of tannin which is believed to have antiulcer activity.[30]

Omeprazole pure sample (10 mg/kg) which was the reference drug provided the animal with ulcer inhibition of 43.33% while omeprazole-loaded P90G provided the animals with ulcer inhibition of 66.67%. Increase in the ulcer protection by omeprazole from 43.33 to 66.67% indicated enhanced effect of phospholipids in drug delivery in terms of increased drug solubility and absorption at body epithelial surfaces. Commercial omeprazole sample provided higher ulcer inhibition effect (76.67%) perhaps due to the fact that it is formulated as enteric-coated product and thus protected from degradation at the low pH of the gastric environment (pH 1.2) which was the pH of the release medium used in the study. However, given the unwanted side effects of conventional anti-ulcer drugs such as omeprazole, the enhanced anti-ulcer activity obtained from herb-phospholipid complex may imply that co-administration of this herb with oil that is rich in phospholipid is encouraged among the populace as this would help in amelioration of ulcer cases especially among the underprivileged in the society.

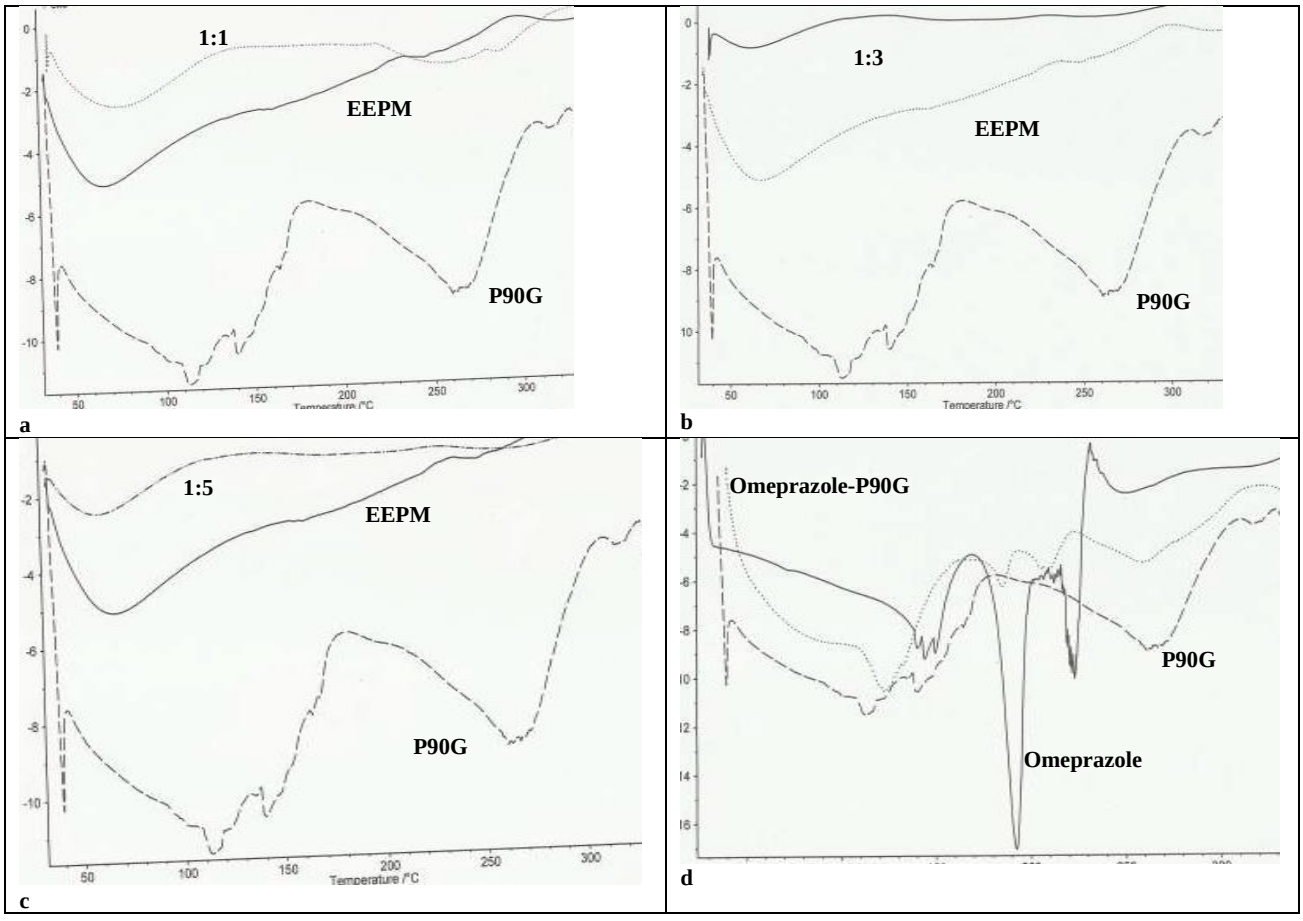

Figure 3: Comparative DSC thermograms of (a) P90G/EEPM/1:1 (b) P90G/EEPM/1:3 (c) P90G/EEPM/1:5 and (d) Omeprazole/P90G (Omeprazole-loaded P90G) formulations in superposition.
Key: EEPM is Ethanol extract of stem bark of *P. macrophylla*, P90G is Phospholipon® 90G, 1:1, 1:3 and 1:5 phytosomes containing 1:1, 1:3 and 1:5 herb:phospholipid complex, respectively, while Omeprazole-P90G is omeprazole-phospholipid complex.

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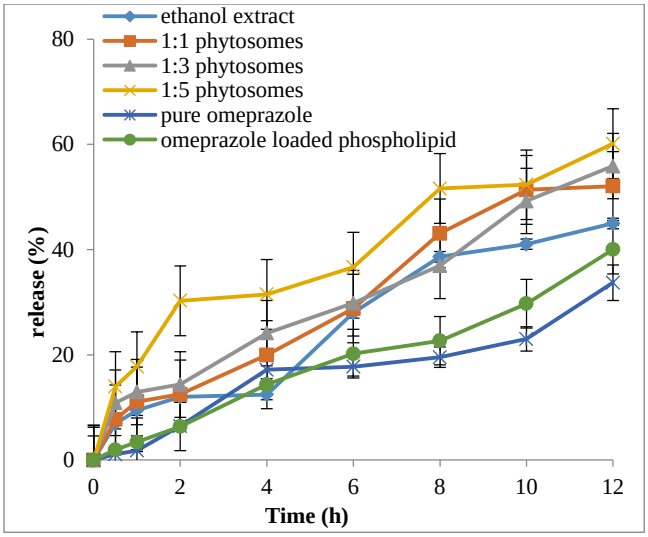

Figure 4: Percentage drug release from the formulations in simulated gastric fluid (SGF, pH 1.2).

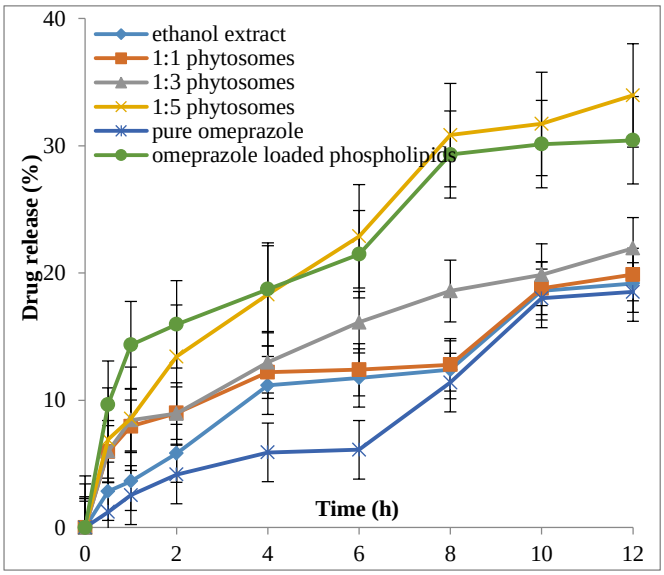

Figure 5: Percentage drug release from the formulations in simulated intestinal fluid (SIF, pH 7.4).

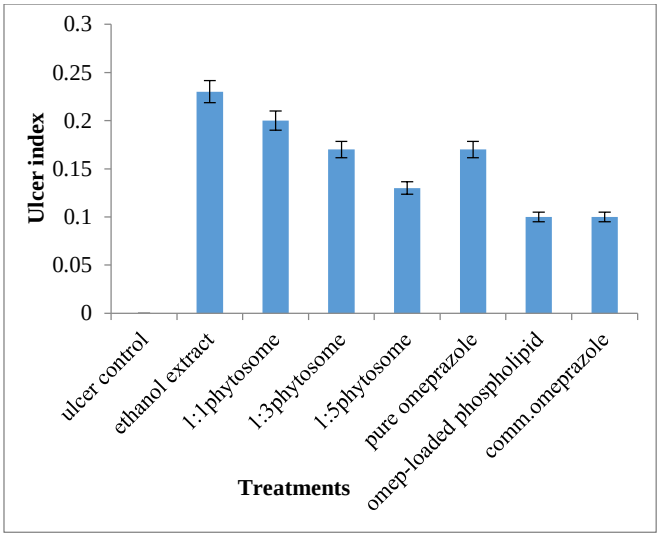

Figure 6: Effect of formulations on ulcer index in rats.

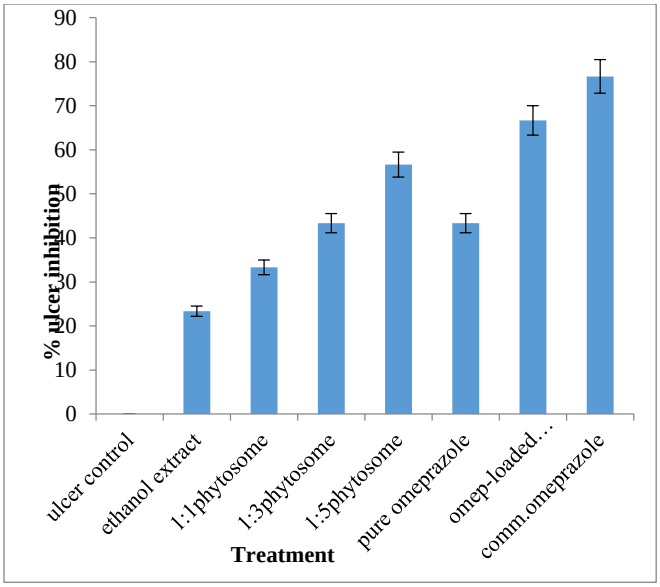

Figure 7: Percentage ulcer inhibition caused by the formulations in rats.

Conclusion

The result of the present study suggests that ethanol extract of *Pentaclethra macrophylla* stem-bark had antiulcer activity which was significantly ($p < 0.05$) improved when combined with Phospholipon® 90G in the form of phytosomes. Overall, percentage ulcer inhibition provided by these phytosomal formulations increased with increase in phospholipid concentration because phospholipids have the ability to enhance absorption and bioavailability of water-soluble phytoconstituents. Further work is recommended to isolate and characterize the secondary metabolite responsible for the antiulcer activity, verify therapeutic merits and reveal the exact mechanism of action even as the administration of this herbal extract with oil rich in phospholipid should be encouraged.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: Food sources and bioavailability. Am J Clin Nutr. 2004; 79:727–747.

2. Scalbert A and Williamson G. Dietary intake and bioavailability of Polyphenols. J Nutr. 2000; 130:2073S-2085S.
3. Fasinu P, Pillay V, Ndesendo VMK, Choonara E. Diverse approaches for the enhancement of oral drug bioavailability. Biopharm. Drug Dispos 2011; 32(4):185-209.
4. Venkatesan N, Babu BS, Vyas SP. Protected drug carriers for prolonged systemic circulation-a review. Indiana J Pharm Sci. 2000; 62:327-333.
5. Rossi R, Basilico F, Rossoni G, Riva A, Morazzoni P, Mauri PL. Liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry of bilobalide in plasma and brain of rats after oral administration of its phospholipidic complex. J Pharm Biomed Anal. 2009; 50:224–227.
6. Parris K and Kathleen H. A review of the bioavailability and clinical efficacy of milk thistle phytosome: a silybin-phosphatidylcholine complex. Altern Med N Rev. 2005; 10(3):193-203.
7. Gupta A, Ashawat MS, Saraf S. Phytosome: A novel approach towards functional cosmetics. J Plant Sci. 2007; 2(6):644-649.
8. Ravarotto L. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B_1 in broiler chicks. Poult Sci. 2004; 83:1839-1843.
9. Gabetta G, Bombardelli E, Pifferi G. Complexes of flavolignans with phospholipid, preparation thereof and associated pharmaceutical composition. Inverni Della Beffa S.P.A., Milan, Italy. US Patent 4764508, 1998.
10. Nnamani PO, Kenechukwu FC, Dibua EU, Ogbonna CC, Monemeh UL, Attama AA. Transdermal microgels of gentamicin. Eur J Pharm Biopharm. 2013; 84:345-354.
11. Attama AA, Reichl S, Müller-Goymann CC. Sustained release and permeation of timolol from surface modified solid lipid nanoparticles through bio-engineered human cornea. Curr Eye Res. 2009; 34(8):698-705.
12. Attama AA, Reichl S, Müller-Goymann CC. Diclofenac sodium delivery to the eye: *in vitro* evaluation of novel solid lipid nanoparticle formulation using human cornea construct. Int J Pharm. 2008; 355:307-313.
13. Imafidon EK and Okunrobo LO. The effects of aqueous extracts of the leaves of *Hibiscus rosasinensis* Linn on renal function in hypertensive rats. Afr J Biochem Res. 2010; 4(2):043-046.
14. Akah PA, Aguwa CN, Agu RU. Studies on the antidiarrhoeal properties of *Pentaclethra macrophylla*. Phytother Res.1999; 13:292-295.
15. Gill LS. Ethnomedicinal uses of plants in Nigeria. University of Benin press, Nigeria. 1992; 184-185 p.
16. Keay RWJ, Onochie CEA, Stanfield DP. Nigerian Trees. Department of Forest Research, Ibadan. 2000: 119-120 p.
17. Okorie CC, Oparaocha ENT, Adewunmi CO, Iwalewa EO, Omodara SK. Antinociceptive, anti-inflammatory and cytotoxic activities of *Pentaclethra macrophylla* aqueous extracts in mice. Afr J Trad Compl Altern Med. 2006; 3(1):44-53.
18. Gugnani HC and Ezenwanze EC. Antibacterial activity of extracts of ginger (*Zingiber officinale*) and African oil bean seed (*Pentaclethra macrophylla*). J Commun Dis. 2003; 17:233-236.
19. Ugbogu OC and Akukwe AR. The antimicrobial effect of oils from *Pentaclethra macrophylla* Bent, *Chrysophyllum albidum* G. Don and *Persea gratissima Gaerth* F on some local clinical bacteria isolates. Afr J Biotech. 2009; 8(2):285-287.
20. Folefoc GN, Bisseck JP, Fomum ZT, Bodo B. Constituents from the roots of *Pentaclethra macrophylla*. Biochem Syst Ecol. 2005; 33:1280-1282.
21. Atindehou K, Koné C, Terreaux D, Traore K, Dosso M. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. Phytother Res. 2002; 16:497-502.
22. Milner JA. New insights into mechanism of action of antioxidants. New Engl J Med. 2000; 234:196-202.
23. Rathee S and Kamboj A. Optimization and development of antidiabetic phytosomes by the Bpx-Behnken design. J Liposome Res. 2018; 28(2):161-172.
24. Bombardelli E, Curri SB, Della LR. Complexes between phospholipids and vegetable derivatives of biological interest. Fitoter, 1999; 90(1):1-9.
25. Trease GE and Evans WC. Pharmacognosy. Tindall Press: London, 2000. 307-702 p.
26. European Pharmacopoeia. 7th edition. Strasbourg: Council of Europe, 2007.
27. Unites States Pharmacopoeia. Rockville: United States Pharmacopoeia Conventions, 2007.
28. Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants? J Ethnopharmacol. 1998; 60(1):1-8.
29. Attama AA, Onugu CO, Nnamani PO, Adikwu MU, Nzekwe IT. The use of self-emulsifying systems in the delivery of diclofenac. Int J Pharm. 2003; 262:23-28.
30. Gadekar R, Singour PK, Chaurasiya PK, Pawer RS, Patil UK. A Potential of some medicinal plants as an antiulcer agent. Pharmacogn Rev. 2010; 4(8):136-146.