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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ESTIMATION OF ANTI OXIDANT POTENTIAL OF PHYTOSOMES FORMULATIONS OF *MORINDA LUCIDA* BENTH

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Abstract

Introduction: *Lucid morinda* (L) A medium-sized evergreen tree of the Morinda genus, the Benth (*M. lucida*, Rubiaceae) is a tropical West African rainforest plant that is also known as the Brimstone tree. In several West African nations, it is utilized in traditional medicine to cure a variety of human ailments. Phytosomes are freshly developed herbal formulations that have superior bioavailability and effects than traditional phyto molecules or botanical extracts because they are more readily absorbed. The goals of the current study were to evaluate ethanolic leaf extract of *M. lucida*'s phytosomes, *in vitro* antioxidant properties, qualitative and quantitative phytochemical analyses, and phytosome formation.
Methods: The ethanolic plant extract and cholesterol-containing phospholipids were used to make the phytosome. The entrapment efficiency, particle size and size distribution, optical microscopic research, stability tests, and *in vitro* dissolution investigations were used to characterize phytosomes. Alkaloids, glycosides, sterols, phenols, terpenoids, tannins, and saponins were found by phytochemical examination.
Results: The ethanolic leaf extract of *M. lucida* contained 0.721 mg of total phenols and 0.464 mg of total alkaloids per 100 mg. Using industry-standard procedures, the ethanolic leaf extract's *in vitro* antioxidant activity was evaluated against the DPPH assay technique. Combining phospholipids and *M. lucida* can have a synergistic impact, which can be measured using the DPPH model for free radical scavenging activity. The optimised batch F10's particle size and entrapment efficiency were found to be 223.30±0.41 nm and 76.46±0.61 nm, respectively.
Conclusion: It can be employed as a targeted medicine delivery system in the future for applications such as liver, brain, heart, etc. protection. The use of novel approaches rather than traditional methods for delivering herbal drugs increases the bioavailability of polar extracts and boosts patient compliance.
Keywords: Characterization, DPPH assay, *Morinda lucida* (L) Benth, phospholipids, phytosome, phytochemical analysis.

INTRODUCTION

All across the world, medicinal plants are used to treat or prevent a variety of ailments. Despite the development of modern medicine, which largely relies on synthetic substances and cutting-edge technology, there has been an alarming rise in the prevalence of various chronic diseases, including cancer, oxidative stress, microbial, parasite, and viral infections[1]. In order to revive regional health customs, it is imperative that adequate recording and assessment programmes be put in place using nanotechnology. Otherwise, this magnificent people's health culture will be lost forever. According to the World Health Organization, the annual demand for medicinal plants is currently projected to be around US$14 billion[2]. According to a WHO estimate, the global market for medical plants is expected to reach $5 trillion in value by 2050. The demand for raw materials derived from medicinal plants is expanding at a pace of 15 to 25% annually[3]. Due to their improved ability to pass lipid-rich biomembranes and eventually reach the blood,

phytosome are more accessible than plain herbal extracts. Phospholipids, especially phosphatidyl-choline, are the lipid-phase components used with phytoconstituents because they are lipid compatible (PC)[4]. All known life forms require phospholipids, which are intricate chemicals, to create their cell membranes. They are the building blocks of the cell membrane, forming the matrix into which a wide range of proteins, including receptors, transport proteins, enzymes, and other biological energy converters, can fit. Phospholipids are used in humans and other higher animals as well. They are well absorbed when taken orally and are miscible in both water and conditions that contain oil or lipids[5]. When compared to triglycerides, which have glycerol bound to three fatty acids, phospholipids only have two fatty acid bonds, with a phosphate group occupying the third location. Numerous studies have consistently demonstrated the presence of numerous biologically active secondary metabolites in medicinal plants that have a variety of pharmacological effects, including analgesic, antitumor, antipyretic, antiplasmodial, antiinflam-matory, antimicrobial, anti-diabetic, antioxidant, and antiviral effects. *Morinda lucida*, sometimes referred to as the Brimstone tree, is a species of medium-sized evergreen tree. Morinda is the Rubiaceae family's most diverse genus. The genus is widely dispersed throughout the tropical and subtropical regions, with more than 131 recognised species of flowering trees, shrubs, and herbaceous plants[6]. It is frequently referred to by the names Sagogo or Bondoukou alongua (Ivory Coast), Ewi or Konkroma (Ghana), Atak ake or Ewe amake (Togo), and Oruwo (Nigeria). Alkaloids, terpenoids, iridoids, fatty acids, anthraquinones, glycosides, essential or volatile oils, tannins, and flavonoids have all been identified as secondary metabolites in *M. lucida*[7]. The secondary metabolites present in crude extracts, fractions, or isolates of Brimstone tree may contribute to the varied antitrypanosomal, anti-inflammatory, antioxidative, antipyretic, anti-cancer, antidiabetic, antibacterial, and anti-plasmodial potencies of various plant sections[8].The current work's goal is to create phytosomes from *M. lucida* leaf extracts in order to create superior antioxidant formulations.

MATERIALS AND METHODS

Vegetal matter

The leaves of *M. lucida* fresh undamaged leaf and fruit samples were harvested from several parts of the innermost canopies of fruiting plants from Emene, Enugu, Enugu State Nigeria in June, 2022. The collected samples were authenticated by Egbuji Jude Victor EJ, a botanist. Only analytical-grade chemicals were utilised in current study.

Plant material fattening

Plant matter from *M. lucida* was crushed up and allowed to air dry at ambient temperature. Soxhlation was used to remove the substance from the shade-dried plants using petroleum ether after it had been coarsely crushed up. The substance was extracted repeatedly until it had been adequately fatted.

Extraction by soxhlation process

Defatted *M. lucida* powder was fully extracted with ethanol using the soxhlation method. The extract evaporated at temperatures higher than their boiling points. The dried crude concentrated extract was weighed in order to calculate the extractive yield. When it was time for analysis, it was then put into glass vials (6 x 2 cm) and kept refrigerated (4°C)[9].

Phytochemical screening

According to the protocols described, phytochemical screening was done to find any bioactive compounds. By visually seeing a colour change or the production of precipitates following the addition of specific reagents to the solution, the tests were recognized[10,11].

Total phenol measurement

The Folin Ciocalteu reagent was employed to calculate the total phenolic substance of the extracts. Gallic acid concentration (20-100 µg/ml) was produced in CH_3OH. 100 µg/ml plant extract concentrations were likewise made in CH_3OH, and 0.5 ml of every sample was added to the test along with 4 ml of 7.5% sodium carbonate and 2 ml of a 10 fold diluted folin Ciocalteu reagent. After para filming the tubes, they were keep warmed at RT for 30 minutes with periodic shaking. The absorbance at 765 nm was calculated against CH_3OH as a vacant. Gallic acid's conventional regression curve was utilized to calculate the content of phenol overall, and the results were given in milligrammes per gramme (mg/gm) of gallic acid[12].

Total alkaloids measurement

Dimethyl sulphoxide (DMSO) was employed to liquefy the plant extract (1 mg), and then 1 ml of 2 N HCl was added and drinkable. This solution was reassigning to a separating funnel, and then 5 ml of phosphate buffer and 5ml of bromocresol green solution were included. The mixture was vigorously agitated with 1, 2, 3, and 4 ml $CHCl_3$ before being collected in a 10ml volumetric flask and $CHCl_3$ was added to dilute to the volume. Atropine reference standard solutions ranging from 50 to 250 µg/ml were created. The absorbance of the test and standard solutions in relation to the reagent blank at 470 nm was measured using a UV/Vis spectrophotometer. Mg of AE/g of extract was used to express the overall alkaloid content[13].

DPPH free radical scavenging assay

The DPPH scavenging activity was tested using a modified methodology[14]. The DPPH scavenging activity was evaluated by the spectrophotometer. The stock solution was prepared to yield an initial absorbance when mixed with 1.5 ml of methanol (6 mg in 100 ml of methanol). When the sample extract was present at different concentrations (10-100µg/ml) for 15 minutes, a decrease in absorbance was observed. The DPPH solution was diluted with methanol to make 3 ml from 1.5 ml, and the absorbance was immediately measured at 517 nm for the control reading. 1.5 ml of DPPH and 1.5 ml of the test sample were added to a series of volumetric flasks at varied concentrations. The final volume was then modified with methanol to 3 ml. We gathered three test samples, and we treated them all the same. Finally, the mean was employed. The absorbance at zero time was used to calculate each

concentration. The DPPH absorbance ultimately reduced with the sample's changing concentration after 15 minutes at 517 nm. The fraction of DPPH radicals that were scavenged was used to determine the 50% inhibitory concentration (IC_{50}), which is how the activity is expressed. With declining IC_{50} values, antioxidant activity levels rise.

Creation of phytosomes formulation

Synthesis of phytosomes

Cholesterol and *M. lucida* were used to make the complex in the following ratios: 0.5:0.3:1, 1:0.6:1, 1.5:0.9:1, and 2:1.2:1. A 100 ml round-bottom flask weighed out with extract, phospholipids, and cholesterol, and 50 ml of methanol was added as the reaction media. The mixture was refluxed for 3 hours while maintaining a temperature of 50°C for the reaction of the complex. After the resulting clear fluid had evaporated, 20 ml of n-hexane was stirred in. To get rid of the traces of solvents, the precipitate was filtered and dried under vacuum. The leftovers were collected, desiccated overnight, and stored in an amber-colored glass bottle at room temperature Table 1.

Table 1: Different formulation of phytosomes.

Batch	Ratio of Phospholipid and Cholesterol	Drug Concentration (%)	Alcohol Concentration (%)
F1	0.5:0.3	1	50
F2	1.0:0.6	1	50
F3	1.5:0.9	1	50
F4	2.0:1.2	1	50
F5	1.5:0.9	0.5	50
F6	1.5:0.9	1.0	50
F7	1.5:0.9	1.5	50
F8	1.5:0.9	2.0	50
F9	1.5:0.9	1.0	25
F10	1.5:0.9	1.0	50
F11	1.5:0.9	1.0	75
F12	1.5:0.9	1.0	100

Characterization

Determining how *M. lucida* interacts with phospholipids

The interaction between *M. lucida* and phospholipids was investigated using a Fourier transform infrared spectrophotometer (FT-IR Spectrometer, Bruker alpha). The KBr technique was used to acquire the IR spectra of the *M. lucida* extract, phospholipids, and their complex and physical combination[15].

Entrapment efficiency

A phytosome preparation was obtained and centrifuged for 30 minutes at 12000 rpm using a cooling centrifuge (Remi). The non-entrapped quercetin was carefully separated from the clear supernatant by syphoning it off, and the absorbance of the supernatant for non-entrapped *M. lucida* was measured at max 230.0 nm using a UV/visible spectrophotometer (Shimadzu 1800). To lyse the vesicles, sediment was treated with 1 ml of 0.1% Triton x 100 before being diluted to 100 ml with phosphate buffer saline (7.2), at which point absorbance measurements were made at 230.0 nm. Gallic acid concentrations in the sediment and supernatant combined to yield a total of *M. lucida* in 1 ml dispersion[16].

Particle size and size distribution

A computerized inspection system was used to measure the particle size, size distribution, and zeta potential of an improved phytosomes formulation using dynamic light scattering (DLS) (Malvern Zetamaster ZEM 5002, Malvern, UK). By infusing the diluted system into a zeta potential measurement device, the electric potential of the phytosomes, including its Stern layer, was ascertained[17].

***In vitro* drug release study**

The sample was tested for *in vitro* drug release using a USP-type II dissolution equipment (Paddle type). The dissolution flask was filled with 900 ml of 0.1N HCl and kept at a constant temperature and rotational speed of 50. Each bowl of the dissolution device was filled with phytosomes equal to 100 mg. Ten hours were given for the device to run. Using a 10 ml pipette, samples weighing 5 ml were taken every hour for up to 10 hours. The same amount of the sample was always added to the brand-new dissolving medium (37^0C). Take 0.5 ml from this, diluted up to 10 ml, and use spectroscopy to measure the absorbance at 230.0 nm[18,19].

Preparations for *M. lucida* phytosomes' ethanolic extract

In this study, we created a combination of *M. lucida* and phospholipids to enhance *M. lucida*'s lipophilic characteristics. We produced the complex using *M. lucida* and phospholipids in various quantities of 0.5, 1, 1.5, and 2. The outcomes demonstrated that the stability of the *M. lucida*-phospholipids complex was worse when the ratio was less than 1. Finally, using a 1:1.5:0.9 ratios of the materials, we built an *M. lucida*-phospholipids complex to produce the best complex while using the least amount of phospholipid possible[20].

RESULTS AND DISCUSSION

After completing each consecutive soxhlation extraction process, the crude extracts were focused on a bath of water by totally evaporating the solvents to achieve the real extraction capitulate. Petroleum ether and ethanol were found to produce extracts from plant

portions called leaves with yields of 1.45 and 9.229%, respectively. Table 2 displays the findings of a qualitative phytochemical examination of *M. lucida* leaves in their natural state. In contrast to petroleum ether extracts, which contained protein and carbs, ethanolic plant extracts contained sterols, phenols, terpenoids, tannins, alkaloids, glycosides, and saponins. Using the equation based on the calibration curve: Y = 0.032 x + 0.004, R^2=0.999, where X is the gallic acid equivalent (GA) and Y is the absorbance, the total phenolic content was estimated as gallic acid equivalent (mg/100 mg).

Table 2: Results of extract phytochemical screening of *M. lucida.*

S. No.	Test	Result	
		Petroleum ether Extract	Methanolic Extract
1	Flavonoids	-ve	-ve
2	Alkaloids	-ve	+ve
3	Phenolic compounds	-ve	+ve
4	Saponins	-ve	+ve
5	Tannins	-ve	+ve
6	Carbohydrates	+ve	-ve
7	Glycosides	-ve	+ve
8	Sterols	-ve	+ve
9	Terpenoids	-ve	+ve
10	Steroidal compounds	-ve	-ve
11	Protein	+ve	-ve

Table 3: Estimation of total phenolic and alkaloid content of *M. lucida.*

Total phenolic content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/100 mg of dried extract)
0.721	0.464

The calculation based on the calibration curve: Y= 0.007 x + 0.007, R2=0.999, where X is the Atropine equivalent (AE) and Y is the absorbance, was used to determine the total alkaloid content as atropine equivalent mg/100 mg. The ethanolic extracts of *M. lucida* leaves yielded a total phenolic estimation with a content value of 0.721. The estimated concentration of total alkaloids in ethanolic extracts of *M. lucida* leaves was 0.464 mg/100 mg (Table 3). There is growing evidence that natural antioxidants found in herbs and medicinal plants may be helpful in mitigating the harmful effects of oxidative stress, and there is a growing interest in the beneficial biochemical actions of natural antioxidants.

Table 4: % Inhibition of ascorbic acid, extract and phytosomes using DPPH method.

S. No.	Concentration	% Inhibition		
		Ascorbic acid	Phytosomes	Ethanolic extract
1	10	36.77419	27.74193	24.51612
2	20	58.06452	36.77419	43.87096
3	40	64.51613	56.12903	46.45161
4	60	76.77419	63.87096	47.09677
5	80	86.45161	83.22580	61.93548
6	100	36.77419	27.74193	24.51612
	IC 50	35.44068	54.89850	73.46154

The DPPH free radical scavenging activity is used to gauge the *M. lucida* ethanolic extract's antioxidant activity. Table 4 lists significant antioxidant activity for the investigated plant extracts. An essential factor in defining phytosomes is entrapment efficiency. The concentration of the lipid, the concentration of the medication, and the concentration of alcohol were all adjusted in order to get the best encapsulation efficiency[21,22]. Table displays the entrapment effectiveness of all the produced formulations. The phytosomes' entrapment effectiveness was observed to range from 50.15±0.42 to 76.46±0.61%. The range of all formulations' particle sizes was 223.30±0.41 to 336.85±0.83 nm. Size of phytosomes is significantly impacted by lipid concentration. The optimum formulation was determined to be F10, which is now being tested for drug release, solubility, and UV spectroscopy. A structural analysis of the best *M. lucida*-phospholipids complex (1.5:0.9) formulation F10 was performed by a drug release research (Table 5). The formulation's optimised zeta potential F10 measured at -34.50 mV[23]. The results of an *in vitro* F10 phytosome dissolving investigation showed that the phytosomes had an extended release dissolution pattern. The phytosomes release after 12 hours at a 98.85% rate. When the regression coefficient values were compared, it was revealed that Korsmeyer Peppas's "r^2" value, which is the highest, was 0.976, suggesting that drug release from formulations was found to follow Korsmeyer Peppas kinetics.

Table 5: Particle size and entrapment efficiency of drug loaded phytosomes.

Batch	Particle size	Entrapment Efficiency
F1	243.52±0.43	50.15±0.42
F2	237.42±0.31	62.13±0.55
F3	278.21±0.40	75.46±0.22
F4	303.43±0.34	64.22±0.35
F5	336.85±0.83	64.55±0.13
F6	283.62±0.64	64.88±0.64
F7	293.42±0.40	54.59±0.46
F8	276.93±0.30	62.02±0.57
F9	268.51±0.21	64.35±0.53
F10	223.30±0.41	76.46±0.61
F11	290.51±0.22	62.22±0.57
F12	289.42±0.11	57.88±0.84

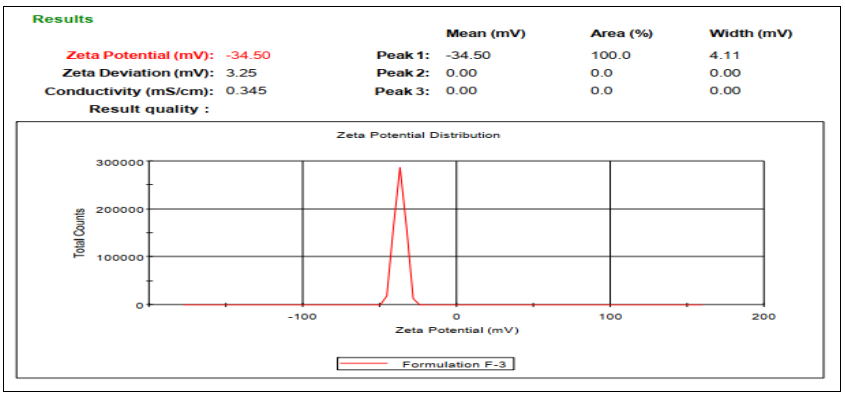

Figure 1: Zeta potential of optimized batch F10.

After that, optical microscopy was used to evaluate the sample. Results of stability testing clearly show that optimised batches of phytosomes were stable for up to 3 months under the selected temperature and humidity settings, as there was no discernible change in their physical characteristics or percentage drug content[24,25].

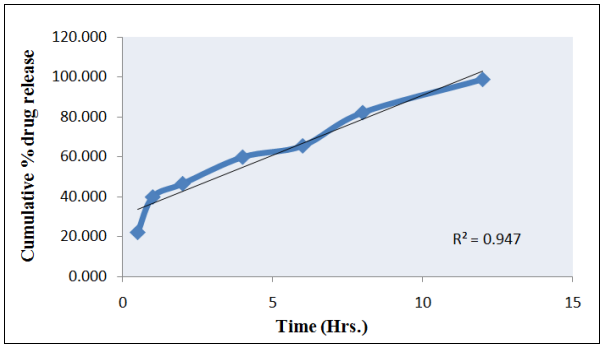

Figure 2: Cumulative % drug released Vs Time of batch F10 formulations.

Table 6: Regression analysis data of optimized formulation F10.

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.947	0.856	0.963	0.976

CONCLUSIONS

The findings mentioned above lead us to the conclusion that phytosomes have superior physical properties to extract. *In vitro* studies indicated that phytosomes had the same antioxidant activity as an ethanolic extract of *M. lucida* leaves. The phytochemical investigation provided important details about the various phytoconstituents found in the plant, which will aid future researchers in choosing a specific extract for additional research isolating the active principle. It also provided insight into the different phytochemicals that have been discovered to have a variety of activities. It is necessary to do additional research to isolate certain chemicals and study there *in vivo* behaviors using various mechanisms.

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AUTHOR'S CONTRIBUTION

Al-Kaf AG: Conceived idea, data collection, data analysis. **Nelson NO:** Design the study, final drafting of manuscript. **Patrick OU:** Literature survey, analysis of data, review. **Peace AN:** Manuscript initial drafting, data interpretations. **Victor EJ:** Literature survey. **Okolie SO:** lab work. **Alexander I:** methodology, investigation. **Dibwan FA:** data analysis, report drafting. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

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