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Research Article

# PHYTOSOMES ENHANCED THE ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF *LANTANA CAMARA*

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**ABSTRACT**

**Aim:** The aim of the work was to formulate *Lantana camara* phytosomes to improve the antimicrobial properties.

**Methods:** *L. camara* phytosomes were prepared by solvent evaporation using soy lecithin (Phospholipon® 90H). The effect of surfactant Poloxamer 188 was carried out. The qualitative and quantitative analysis of the phytoconstituents was analyzed. The encapsulation efficiency and loading capacity were studied. Furthermore, the *in vitro* release profile was studied in ethanolic buffer. The inhibition zone diameter (IZD) was evaluated against three bacterial and two fungi.

**Results:** The results showed that saponins were the most dominant phytochemical with about 7% on the plant leaves. The highest EE of 82.80% was obtained. *In vitro* release showed about 23% drug release at 60 min. The IZD results showed that *L. camara* had significantly higher activity against *Escherichia coli* and *Listeria ivanovii* than *Staphylococcus aureus* ($p<0.05$). The results also showed that for *Candida albicans*, *L. camara* phytosomes had significantly higher IZD than the extract ($p<0.05$). However, the *L. camara* extract and the formulations showed no activity against the *Aspergillus flavus*.

**Conclusions:** Phytosomes enhanced the antimicrobial properties of *L. camara* and could serve as a good delivery system for this herbal drug.

**Keywords:** Herbal formulation, Drug delivery, *Lantana camara*, Inhibition zone diameter, Lipids, Phytosomes.

## INTRODUCTION

The use of herbal remedies for the treatment of bacterial and fungal infections continues to increase in developing countries. About 80% of African populations use some form of traditional herbal medicine, and the worldwide annual market for herbal products was up to US$ 60 billion [1]. Herbal medicines have been found to be very useful in the treatment of some drug-resistant microorganisms causing various illnesses [2]. Some microbes develop resistance to some synthetic drugs using different mechanisms. Some patients abuse the use of synthetic antibiotics by not taking their drugs according to the dosage regimen or underdosing, leading to microbial resistance to those synthetic drugs, thereby making them less effective in the treatment of various infections. In most countries with rich biodiversity of plants, most patients are more confident in using plant products to treat infections. Antimicrobial resistance has triggered research in developing of herbal dosage forms [3-5], thereby standardizing plant extracts and constituents for the benefit of preventing and treatment of various infections and diseases of human [1].

*Lantana camara* Linn. (Verbenaceae) is a flowering plant native to Central and Northern South America and the Caribbean and has spread to other countries including Mexico, Brazil, the United States of America, Nigeria, and other African countries [6]. This plant has been found in more than 60 countries of the world where it is utilized by herbal medicine practitioners in treating various ailments. The leaves of *L. camara* have vast pharmacological activities, namely, antibacterial, antimotility, wound healing, antifungal, hepatoprotective, antidiabetic, larvicidal, nematicidal, antimalarial, antihemorrhoidal, antifilarial, insecticidal, antitumor, anti-inflammatory, antiulcer, anti-asthmatic, and antioxidant activities [6-21].

Phytosomes (herbosomes) are structures resulting from the stoichiometric reaction of the phospholipids with the standardized extract or polyphenolic constituents in a non-polar solvent [22]. They have better oral bioavailability than herbal extracts [22]. Phytosomes have several advantages, namely, the active components in the crude extracts are shielded away from being destroyed by the gut bacteria and gastrointestinal secretions, it is very simple to prepare, requiring less equipment, space, and relatively cheap materials. These attributes make scale-up feasible. They are made with biodegradable lipids with generally regarded as safe status [23]. Phytosomes enhance the bioavailability of active phytochemical constituents as they are readily permeable and cross the lipid-rich biomembranes with ease [24,25]. The aim of this work was to formulate the phytosomes of the methanolic leaves extract of *L. camara* and to study the antimicrobial properties of the formulations.

## METHODS

Poloxamer® 188 was a gift from BASF (Ludwigshafen, Germany); Phospholipon® 90H was a kind gift from Phospholipid GmbH (Köln, Germany); and methanol, sodium hydroxide, and potassium dihydrogen phosphate were from Merck KGaA (Darmstadt, Germany). Nutrient agar, nutrient broth (Lab M), Sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) were purchased from Merck KGaA (Darmstadt, Germany). All other reagents were of analytical grade.

### Extraction of the phytoconstituents of *L. camara*

*Collection and authentication of plant*

The fresh leaves of *L. camara* were collected from Amokwe village, Enugu, Nigeria, in the month of July 2018 and authenticated by Mr. A.O. Ozioko, a taxonomist at the International Center for Ethnomedicine and Drug Development (InterCEDD). The leaves were washed with clean water to remove sand materials and dirt. They were dried under the shade for several days and then milled into powder. A 400 g quantity of the powdered leaves was extracted with methanol in a Soxhlet extractor after which the extract was exposed and the solvent allowed to evaporate.

**Phytochemical analysis and quantification**
Qualitative and quantitative phytochemical analysis was evaluated using standard procedures earlier reported. The presence and the quantity of the following phytochemicals were analyzed, namely, alkaloids, flavonoid, phenol, saponin, tannin, and glycoside using standard methods [26].

**Preparation of phytosome complexes**
A digital magnetic stirrer hot plate 3810001 (IKA® RCT Basic, Germany) maintained at 32 ± 1°C was employed. The appropriate quantities of the materials were weighed; details are shown in Table 1. The extract and Phospholipon® 90H were separately dissolved in 10 ml of methanol using a beaker placed on the magnetic stirrer. The lipid phase and the extract were then mixed with agitation from the stirrer until homogeneous. Batch E was prepared with a surfactant Poloxamer® 188. The solvent was allowed to evaporate completely after which the phytosomes were stored appropriately.

**Encapsulation efficiency and loading capacity**
A 2 ml quantity of the phytosomes suspension was centrifuged at 10,000 rpm for 90 min at room temperature using a Vivaspin microcentrator (Vivascience, Hanover, Germany). The supernatant was diluted with methanol, filtered with a filter paper (Whatman no. 1) and their absorbance readings were recorded at a predetermined wavelength of 246 nm using spectrophotometer 2102 PC UV/Vis (UNICO, USA). The actual content of the extract was calculated with reference to Beer–Lambert's law and the EE was determined using Equation 1:

$$EE\% = \frac{\text{Total extract concentration} - \text{actual extract in supernatant}}{\text{Total extract co}} \times 100 \quad (1)$$

The LC was calculated as follows:

$$LC = \frac{\text{Amount of extract encapsulated}}{\text{Weight of lipid}} \times 100 \quad (2)$$

***In vitro* release**
The release was carried out in 500 ml of ethanolic buffer. A 100 mg quantity of the complexes was weighed and placed in a dialysis membrane MWCO 5000–8000 (Spectrum Labs, Brenda, The Netherlands) soaked for 24 h in the ethanolic buffer before use. The paddle was rotated at 150 rpm and at intervals, 5 ml was withdrawn from the medium followed by its replacement with another 5 ml to maintain sink condition; this was done for 1 h and repeated. The absorbance of the withdrawn sample was determined at a wavelength of 246 nm. The amount of extract released and percentage released was calculated using the equation adequately.

**Fourier transform infrared analysis**
Compatibility studies were analyzed using Fourier transform infrared (FTIR) spectrum (Netzch, Germany) and were recorded for Batches A and E using Fourier transform infrared spectrophotometer.

**Antimicrobial evaluation**
Exactly 100 mg of each sample was dissolved in 5 ml of sterile distilled water containing 10% dimethyl sulfoxide. This resulted in the stock concentration of 20 mg/ml. From the stock, further two-fold dilutions were made to 20, 10, 5, and 2.5 (mg/ml) of the extracts. Five test isolates were used to assay the antimicrobial activity of the extracts. The isolates included bacteria (2 g positive: *S. aureus, Listeria ivanovii* and 1 g negative: *Escherichia coli*) and two fungi (*Candida albicans* [yeast] and *Aspergillus flavus* [mold]). Two-week-old bacterial isolates were inoculated in nutrient broth tubes and incubated overnight. The resulting cell turbidity was adjusted to 0.5 McFarland standard and (equivalent to $0.5^{-1} \times 10^{8}$ cfu/ml) using normal saline. Freshly prepared *C. albicans* and *A. flavus* on SDA and PDA, respectively, were inoculated into test tubes containing nutrient broth and incubated for 48–72 h. The cell suspensions were adjusted to 0.5 McFarland's standard (equivalent to $0.5–10^{6}$ cfu/ml).

The standardized organisms were inoculated using sterile swab sticks and spread on the entire surface of already prepared Mueller-Hinton agar (38 g/l) on culture plates. Wells of 6 mm in diameter were bored into the agar plates using a sterile cork borer. Each concentration

**Table 1: Constituent of the phytosomes**

| Batch code | Phospholipid:extract:Poloxamer |
|---|---|
| A | 1:1:0 |
| B | 1:2:0 |
| C | 2:1:0 |
| D | 3:1:0 |
| E | 1:1:1 |

**Table 2: Results of phytochemical screening of a methanol extract of *L. camara* leaves**

| Parameters | Methanol extract of *L. camara* |
|---|---|
| Protein | ++ |
| Carbohydrate | ++ |
| Fixed oil | + |
| Tannin | + |
| Glycoside | ++ |
| Alkaloid | ++ |

+: Little presence, ++: Moderately present, +++: Abundantly present.
*L. camara*: *Lantana camara*

**Table 3: Results of phytochemical quantification of *L. camara* leaves**

| S. No. | Parameters | Methanol extract of *L. camara* (%) |
|---|---|---|
| 1. | Saponin | 7.00 |
| 2. | Tannin | 0.072 |
| 3. | Flavonoids | 3.00 |
| 4. | Glycoside | 0.68 |
| 5. | Alkaloids | 1.5 |
| 6. | Terpenes | 0.5 |

*L. camara*: *Lantana camara*

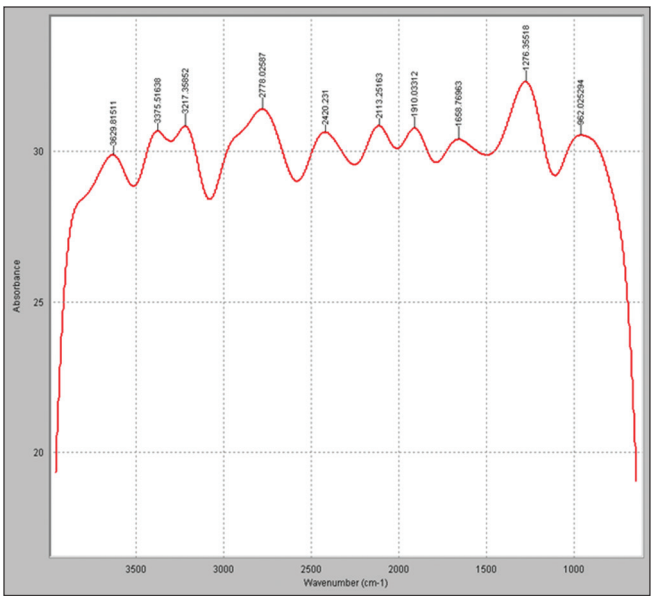


**Fig. 1: Fourier transform infrared of *Lantana camara* phytosomes (Batch A)**

of the already diluted samples was transferred into the wells in the volumes of 20 µL, while gentamicin 40 mg/L served as positive control for antibacterial. The plates were then incubated at 37°C for 16–18 h (bacteria) and 48–72 h (for fungi). The results were read by measuring the inhibition zone diameter (IZD in mm) across the wells.

**Table 4: Encapsulation efficiency (EE%) and loading capacity**

| Batch | TEC (mg) | AEC (mg) | EE (%) | Loading capacity (%) |
|---|---|---|---|---|
| A | 40.00 | 33.12 | 82.80 | 41.40 |
| B | 40.00 | 28.06 | 70.15 | 46.77 |
| C | 40.00 | 23.74 | 59.35 | 19.78 |
| D | 40.00 | 24.93 | 62.33 | 15.58 |
| E | 40.00 | 26.80 | 67.00 | 22.33 |

A-B: *L. camara* phytosomes; TEC: Total extract loaded, AEC: Actual extract entrapped, EE: Encapsulation efficiency

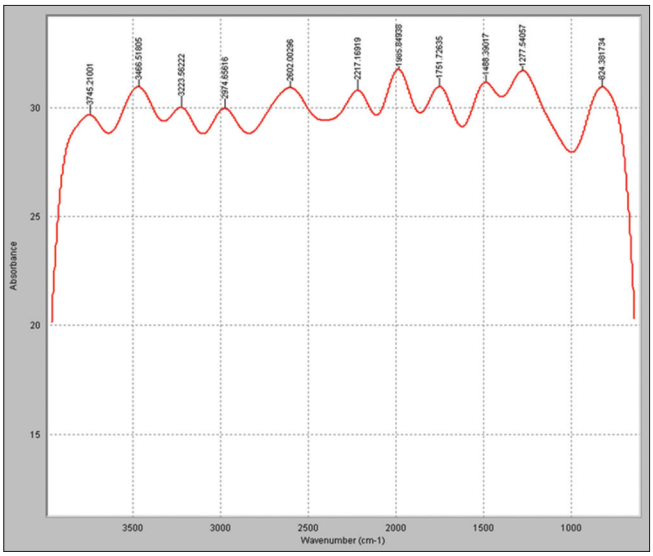


**Fig. 2: Fourier transform infrared of *Lantana camara* phytosomes (Batch E)**

## RESULTS

### Phytochemical constituent

The results of the phytochemical constituents of *L. camara* leaves extract are shown in Table 2 and showed that alkaloids, proteins, carbohydrates, and glycosides were moderately present, while the tannins and fixed oils were present in little amounts.

The antifungal activity of extracts could be explained by the presence of saponins, tannins, glycosides, and alkaloids. The mechanism of the action of alkaloids is based on their ability to bind proteins, thereby inhibiting cell protein synthesis.

### Phytochemical quantification

The results of the phytochemical quantification of flavonoids, tannins, saponins, glycosides, terpenes, and alkaloids are represented in Table 3. Saponin exhibited the highest concentration of 7% followed by flavonoids (3%) and then alkaloid (1.5%). Tannins and terpenes were present in small quantity, as shown in Table 3.

Saponin is a major component of plant that acts as antibacterial secondary metabolite. The mode of action of its antibacterial effects may involve membranolytic effect and not just by altering the surface tension of the extracellular medium. Tannins have the ability to inactivate enzymes, prevent microbial adhesions and cell envelope transport proteins [27,28].

### Encapsulation efficiency and loading capacity

The results of the EE of *L. camara*-loaded phytosomes are shown in Table 4 and showed that Batch A having 1:1 ratio of the extract and Phospholipon had the highest EE of 83%. Increasing the extract concentration beyond this level significantly reduced the EE, as shown in Table 4 ($p<0.05$). Phytosomes containing surfactant (Batch E) also had low encapsulation efficiency of 67%. Hence, surfactant also significantly reduced the EE% of *L. camara.*

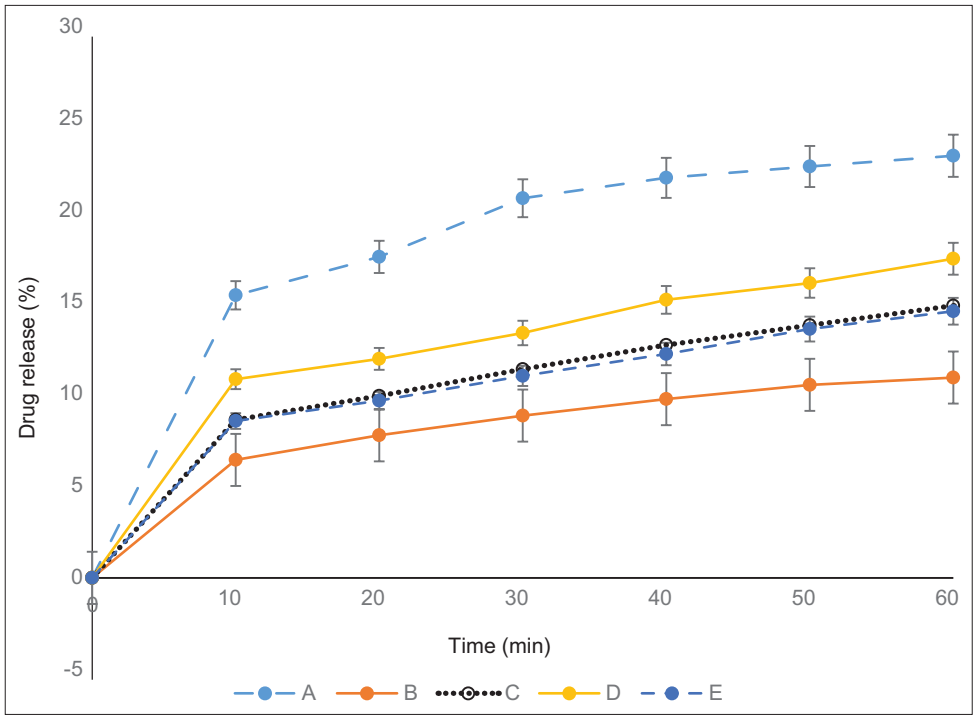


**Fig. 3: *In vitro* release profiles of *Lantana camara* phytosomes in ethanolic buffer. Batch A contained 1:1, B contains 1:2, C contained 2:1, D contained 3:1 of Phospholipon 90H:extract; Batch E was a replica of Batch A with added surfactant**

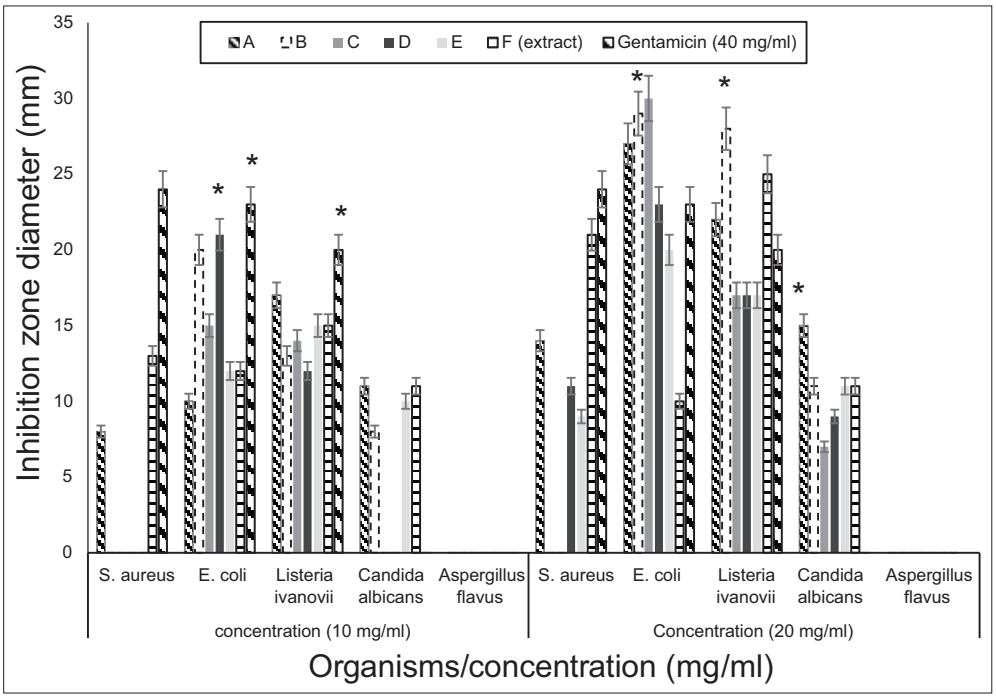


**Fig. 4: Antimicrobial properties of *Lantana camara* phytosomes**

The results of the LC are also shown in Table 4 and showed that LC increased with increase in extract concentration. Batch B having higher extract concentration exhibited the highest LC of 46%. The results are in agreement with the previous works [3,29].

**FTIR**

The result of the FTIR of the phytosomes of *L. camara* is shown in Figs. 1 and 2, respectively. The results revealed that all the materials used in formulating the phytosomes were compatible with the extracts. At ranges 3500–4000, the broadening of the characteristic alcoholic (–OH) band for A signifies the presence of H-bonding. The broad nature is not in E due to the presence of Poloxamer which has a high hydrophilic/hydrophobic molar ratio. The Poloxamer breaks the hydrogen bond making the extract/phospholipid water soluble and increasing their miscibility. This compatibility would enhance the shelf life of the product and ensure the efficacy of the formulation.

***In vitro* release**

The results of the release profiles of *L. camara* phytosomes are shown in Fig. 3 and showed that Batch A had significantly higher release ($p<0.05$) at time interval than other batches. Hence, addition of Poloxamer 188 did not enhance drug release here. At 60 min, batch formulated with phospholipid:extract 1:1 had 22.7% release of the extract, while Batch E containing phospholipid: extract:Poloxamer 188 (1:1:1) 14.5% release. The results showed that the formulations had good release profiles and also showed that the best combination for the preparation would be phospholipid: extract 1:1. No burst release was seen; hence, phytosomes had good stability and could protect loaded extracts as revealed by their release profiles.

**Antimicrobial properties**

The results of the antimicrobial properties of *L. camara* phytosomes are shown in Fig. 4. The results showed that the *L. camara* phytosomes at 20 mg/ml concentration exhibited IZD of 30 mm for *E. coli*, the extract had IZD of 10 mm while gentamycin (40 mg/ml) had IZD of 23 mm. Hence, for *E. coli*, the *L. camara* phytosomes had significantly higher antibacterial properties than the extract and gentamicin at concentrations used ($p<0.05$). For *L. ivanovii*, *L. camara* phytosomes at 20 mg/ml showed IZD of 28 mm, the extract (Batch F) had 23 mm, while gentamicin had IZD of 20 mm. The results showed that *L. camara* had higher activity against *E. coli* and *L. ivanovii*, than *S. aureus*. The results showed that for *C. albicans*, *L. camara* phytosomes had significantly higher IZD than the extract ($p<0.05$). However, the *L. camara* extract and the formulations showed no activity against the *A. flavus.*

## CONCLUSIONS

Phytosomes enhanced the antimicrobial activities of *L. camara* in adding to enhanced stability provided by shielding the extract from various degradation agents. The medicinal properties of this plant would be enhanced due to the standardization of the herbal drug. There would be enhanced compliance and increased patients' acceptability.

## ACKNOWLEDGMENT

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## CONFLICTS OF INTEREST

We state no conflicts of interest.

## REFERENCES

1. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: An ethical analysis. Bull World Health Organ 2008;86:594-9.
2. Ionescu MI. Are Herbal Products an Alternative to Antibiotics? Intechopen; 2017. Available from: https://www.intechopen.com/books/bacterial-pathogenesis-and-antibacterial-control/are-herbal-products-an-alternative-to-antibiotics. [Last Accessed on 2019 Jul 03].
3. Chime SA, Ugwu CE, Ugwuoke CE, Akuwara BU, Onunkwo GC. Formulation and evaluation of novel herbospheres delivery system of leaf extract of *Vernonia amygdalina* del (*Asteraceae*). Innov J Ayurvedic Sci 2018;6:10-5.
4. Chime SA, Onyishi VI, Ugwoke PU, Attama AA. Evaluation of the properties of *Gongronema latifolium* in phospholipon 90H-based solid lipid microparticles (SLMs): An antidiabetic study. J Diet Supplo 2014;11:7-18.
5. Chime SA, Brown SA, Ugwu CE, Agubata CO, Obidike TC, Onunkwo GC. Effect of binder type and concentration on the *in vitro* properties of *Alstonia boonei* tablets. Int J Pharm Sci Rev Res 2012;16:5-9.

6. Kalita S, Kumar G, Karthik L, Venkata K, Rao B. A review on medicinal properties of *Lantana camara* Linn. Res J Pharm Technol 2012;5:711-5.
7. Ganjewala D, Sam S, Khan KH. Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. Eur Asian J Biosci 2009;3:69-77.
8. Kalita S, Kumar G, Loganathan K, Rao B. Phytochemical composition and *in vitro* haemolytic activity of *Lantana camara* L. (*Verbenaceae*) leaves. Pharmacologyonline 2011;1:59-67.
9. Bhakta D, Ganjewala D. Effect of leaf positions on total phenolics, flavonoids and proantho-cyanidins content and antioxidant activities in *Lantana camara* (L). J Sci Res 2009;1:363-9.
10. Tripathi S, Rawat K, Dhyani S, Pant H. Potential of *Lantana camara* Linn. Weed against wood destroying fungi. Indian Forest 2009;135:403-11.
11. Ganesh T, Sen S, Thilagam E, Thamotharan G, Loganathan T, Chakraborty R, *et al*. Pharmacognostic and anti-hyperglycemic evaluation of *Lantana camara* (L.) var. Aculeate leaves in alloxan-induced hyperglycemic rats. Int J Res Pharm Sci 2010;1:247-52.
12. Nayak BS, Raju SS, Eversley M, Ramsubhag A. Evaluation of wound healing activity of *Lantana camara* L.-a preclinical study. Phytother Res 2009;23:241-5.
13. Abdulla MA, Hassandarvish P, Ali HM, Noor S, Mahmoud FH Bashah NS, *et al*. Acceleration of wound healing potential by *Lantana camara* Leaf extract in experimental rats. Res J Med Sci 2009;3:75-9.
14. Sagar L, Sehgal R, Ojha S. Evaluation of antimotility effect of *Lantana camara* L. var. Acuelata constituents on neostigmine induced gastrointestinal transit in mice. BMC Complement Altern Med 2005;5:18.
15. Dua VK, Pandey AC, Dash AP. Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. Indian J Med Res 2010;131:434-9.
16. Venkatachalam T, Kumar VK, Selvi PK, Maske AO, Anbarasan V, Kumar PS, *et al*. Antidiabetic activity of *Lantana camara* Linn fruits in normal and streptozotocin-induced diabetic rats. J Pharm Res 2011;4:1550-2.
17. Misra N, Sharma M, Raj K, Dangi A, Srivastava S, Misra-Bhattacharya S. Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. Parasitol Res 2007;100:439-48.
18. Thamotharan G, Sekar G, Thangavel G, Sen S, Chakraborty R, Kumar NS, *et al*. Antiulcerogenic effects of *Lantana camara* Linn. Leaves on *in vivo* test models in rats. Asian J Pharm Clin Res 2010;3:57-60.
19. Gidwani BK, Bhargava S, Rao SP, Majoomdar A, Pawar DP, Alaspure RN. Analgesic, anti-inflammatory and antihemorrhoidal activity of aqueous extract of *Lantana Camara* Linn. Res J Pharm Technol 2009;2:378-81.
20. Kumar MS, Maneemegalai S. Evaluation of larvicidal effect of *Lantana Camara* Linn. Against mosquito species *Aedes aegypti* and *Culex quinquefasciatus*. Adv Biol Res 2008;2:39-43.
21. Badakhshan MP, Sasidharan S, Rameshwar NJ, Ramanathan S. A comparative study: Antimicrobial activity of methanol extracts of *Lantana camara* various parts. Pharmacognosy Res 2009;1:348-51.
22. More MS, Shende MA, Kolhe DB, Jaiswal NM. Herbosomes: Herbo phospholipid complex an approach for absorption enhancement. Int J Biol Pharm Res 2012;3:946-55.
23. Chime SA, Onyishi VI. Lipid-based drug delivery systems (LDDS): Recent advances and applications of lipids in drug delivery. Afr J Pharm Pharmacol 2013;7:3034-59.
24. Singh A, Saharan V, Singh M, Bhandari A. Phytosome: Drug delivery system for polyphenolic phytoconstituents. Iran J Pharm Sci Autumn 2011;7:209-19.
25. Shalini S, Ram KR. Phytosomes: An emerging technology. Int J Pharm Res Dev 2011;2:1-7.
26. Ajuru MG, Williams LF, Ajuru G. Qualitative and quantitative phytochemical screening of some plants used in ethnomedicine in the Niger delta region of Nigeria. J Food Nutr Sci 2017;5:198-205.
27. Wadhai VS. Antibacterial activity of and comparative phytochemical analysis of flower and leaves of *Lantana camara.* Bionano Front 2012;5:143-5.
28. Aliyu AB, Musa AM, Abdullahi MS, Ibrahim MA, Tijjani MB, Aliyu MS, *et al*. Activity of saponin fraction from *Anisopus manni* against some pathogenic microorganisms. J Med Plant Res 2012;5:6709-13.
29. Chime SA, Onyishi IV, Eze IO. Application of molecularly structured Ben oil in gentamicin entrapped lipospheres. J Pharm Sci Ther 2019;5:262-70.