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Evaluation of the antibacterial potentials of phytosomal gel of *Cassia alata* leaf extract

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Abstract

Phytosomes are vesicular drug delivery systems which are complexes between herbal material and natural phospholipids proven to be beneficial in providing good absorption and better bioavailability over herbal conventional extracts. This study was carried out to evaluate the antibacterial activity of phytosomal gels formulated with aqueous extract of cassia alata leaf. The crude drug was extracted using cold maceration and concentrated. The crude extract was evaluated for its antibacterial activity using antibacterial sensitivity test against Staphylococcus aureus and Escherichia coli, by measuring the inhibitory zone diameter (IZD). The phytosomal herbal gel was evaluated for viscosity, pH, extrudability and spreadability. The pH values of the formulated gels were in the range of 5.46-7.53, the viscosity ranged between 11,567 to 70,333 mPa s. The 200 mg and 400 mg samples of the crude extract gave IZD values of 10.5 mm and 15 mm for S. aureus and 15mm and 17 mm for E. coli. Corresponding concentrations of phytosomal gels of Cassia alata (PG1-PG3 containing 200mg Cassia alata) and (PG4-PG6 containing 400 mg Cassia alata) gave higher IZD of 21.5-27 mm for S. aureus and 19-24.5 mm for E. coli. Phytosomes of Cassia alata was successfully formulated. The in vitro antibacterial activity of Cassia alata was improved with the incorporation of phytosomes giving better profile than the crude extract and the plain gel against S. aureus and E. coli. This enhanced activity can possibly be harnessed for the treatment of skin condition in which these pathogens are implicated in the aetiology.

Keywords: Antibacterial, *Cassia alata*, Gels, Phytosome, Drug delivery

INTRODUCTION

Phytosomes are vesicular drug delivery system which has proved to be beneficial in providing good absorption and better bioavailability over the herbal conventional extracts (Mubeen and Mannan, 2021; Burjwal *et al*., 2023). They are more advanced herbal products than traditional herbal extracts since each component of the extract is bound to a phospholipid, like phosphatidylcholine, to improve absorption and yield better outcomes (Sharma and Roy, 2010; Manthena and Srinivas, 2010).

Phytosomal drug delivery system can be used to integrate hydrophilic phyto-constituents or standardized plant extracts into lipids to produce lipid-compatible molecular complexes (Fatima, and Shahidulla, 2023). Phytosomes are complexes between a natural product and natural phospholipids. As a result, they show better absorption and improved pharmacological and pharmacokinetic profiles than conventional herbal extract (Pawar and

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Bhangale, 2015; Lu and Qiu, 2019). Whereas the active principle of phytosomes is bound to the polar head of phospholipids and forms a critical component of the membrane, the active principle of liposomes is dissolved in an internal compartment or floats in the layer membrane (Dubey *et al*, 2007; Semalty *et al*, 2007).

Many plant phytoconstituents have trouble passing through cell lipid membranes because of their large molecular size or low solubility in water, which limits their absorption and bioavailability (Sawant and Yadav, 2020). Providing an effective concentration of the active ingredients is necessary for any herbal product to be successful. Another feature of phytosomes is the phospholipids' demonstrated ability to promote health. Many well-known herbal extracts have been formulated as phytosomes, such as ginseng, green tea, milk thistle, grape seed, hawthorn, kushenin, marsupsin, and curcumin.

Phosphatidylcholine can be directly bound by the flavonoid and terpenoid components of these plant preparations via H-bond (Sawant and Yadav, 2020; Pananchery and Gadgoli, 2021). Pharmacokinetic investigations in humans and animals have shown that the phytosomes had a higher bioavailability than the simpler, less complex plant extract. Based on their physiochemical and spectroscopic properties, these chemicals can be regarded as novel entities. To transfer water-soluble ingredients to the skin, phytosomes are currently mostly utilized in cosmetics. This method can also be applied to pharmaceutical formulations meant for oral cavity therapy, where contact durations are quite short. This is because phospholipid increases the product's adherence to surfaces it comes into touch with (Parris and Kathleen, 2005; Gupta *et al*, 2007).

Cassia alata leaf extract have been proven to have antibacterial activity and several researchers have carried out studies on the antimicrobial activity of *Cassia alata* leaf extract either as crude or in topical formulation as well as studies on the quantification and isolation of the active phytoconstituents eliciting its pharmacological activity (Khan *et al*, 2001; Chang *et al*, 2002; Villasenor *et al*, 2002; Makinde *et al*, 2007; Bandyopadhyay *et al*., 2008; Aviello *et al*., 2010; Okwu and Nnamdi, 2010; Aftab *et* al, 2011; Fernand *et al*, 2011; Alalor *et al*., 2012a; Alalor *et al*., 2012b; Tarkang *et al*, 2012; Veeresham, 2012).

Several researchers have carried out studies on the phytosomes loaded with different plant extracts but there is little or no study on the phytosomal formulation of *Cassia alata* extract. Consequently, this study is aimed at exploring the effect of phytosome, a vesicular drug delivery system as a carrier and the possibility of potentiating the antibacterial activity of *Cassia alata* for topical delivery.

MATERIALS AND METHODS

Area of study

The study was carried out in the laboratories of the Faculty of Pharmacy at the Delta State University, Abraka, Nigeria

Collection of samples

The plant material, *Cassia alata* leaves were harvested from the premises of the Federal Government College, Warri, Delta State, Nigeria. The leaves were washed to remove debris and then air dried at room temperature. The dried leaves were coarsely milled and stored in a sealed container.

Preparation of aqueous crude extract of Cassia *alata*

A 400 g quantity of the dried *Cassia alata* leaves was soaked in 2 L of water containing 20 ml of chloroform, it was allowed to stand for 72 hours for proper maceration with intermittent agitation. The plant extract was filtered from the plant material using a muslin cloth and concentrated at 60ºC in a water bath. The crude concentrated extract obtained was put in a container, labelled and stored in a refrigerator at 4ºC. The percentage yield of the crude extract from the dried plant was calculated using the formula below:

$$Percentage\ Yield = \frac{Actual\ Yield}{Weight\ of\ Dried\ Material} * 100 \quad (1)$$

Total ash value determination

A 1g quantity of *Cassia alata* powder was taken into a crucible and it was incinerated at a temperature not exceeding 450ºC until free from carbon. The sample was cooled and weighed. The percentage of ash was calculated with reference to the air dried *Cassia alata* powder.

$$Total\ ash = \frac{\text{Weight of ash}}{\text{Weight of sample}}\ x100 \quad (2)$$

Preparation of phytosomal gel of *Cassia alata* extract

The phytosome loaded with *Cassia alata* extract was first formed using the thin-film method (Fatima, and Shahidulla, 2023), and then incorporated into carbopol gel. The crude extract of *Cassia alata* was weighed in accordance with the quantity specified in Table 1 below and transferred into a 500 mL beaker, lecithin was weighed and transferred into the beaker containing the plant extract, and then chloroform and methanol in a ratio of 1:1 were added. The mixture was heated until a thin film was formed, this was done under a low temperature with the aid of a water bath, 20 mL of phosphate buffer pH 7.4 was transferred into the measuring cylinder containing the thin film in other to hydrate it and it was stirred, distilled water was added in a fine stream using a syringe to make up to 50 mL. The beaker was then transferred unto a magnetic stirrer at a temperature of 25^{0}C and stirred to facilitate the dissolution of lecithin. The phytosome formed was incorporated into carbopol gel base containing methylparaben and neutralized with triethanolamine. This procedure was repeated for the different batches in accordance with the composition in Table 1 below. Batches OG1 and OG2 were conventional gels prepared without the phytosomal suspension but had the crude extract of *Cassia alata* incorporated in them.

Table 1: Composition phytosomal gel of *Cassia alata* extract

Ingredients	PG1	PG2	PG3	PG4	PG5	PG6	OG1	OG2
Cassia alata extract (g)	0.2	0.2	0.2	0.4	0.4	0.4	0.2	0.2
Lecithin (g)	0.1	0.2	0.3	0.2	0.4	0.6	---	---
TCM (10ml):Methanol (10ml) ml)	20	20	20	20	20	20	20	20
Phosphate buffer (ml)	20	20	20	20	20	20	20	20
Carbopol (g)	1	1	1	2	2	2	1	2
Triethanolamine (ml)	2	2	2	4	4	4	2	4
Paraben (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Distilled water (ml) to	100	100	100	100	100	100	100	100

TCM (Chloroform or Trichloromethane)

Phytochemical screening

The preliminary phytochemical screening of the extract of *Cassia alata* was done using standard protocols (Nanna *et al*., 2013; Gul *et al*., 2017; Silva *et al*., 2017; Singh and Kumar, 2017; Uma *et al*., 2017).

Antibacterial screening of *Cassia alata* crude extract

Preparation of overnight broth culture

A 0.65g/ml of freshly prepared nutrient agar was sterilized at 121ºC for 20 minutes at one atmospheric pressure. After sterilization, the nutrient agar was left to cool and transferred into sterile bijou bottles and left to stand in a slanted position to solidify. After solidification, strain of *Staphylococcus aureus* and *Escherichia coli* were inoculated and incubated for 24 hours at 37ºC respectively.

Determination of minimum inhibitory concentration (MIC)

The agar dilution technique was used to determine the minimum inhibitory concentration of *Cassia alata* extract against the test organisms. Ten different concentrations of the extract (0.782, 1.563, 3.125, 6.25, 12.5, 25, 50, 100, 200, and 400 mg mL^{-1}) were used for the determination ranging from 0.782-400 mg mL^{-1}. Sterile petri dishes containing varying volumes of extracts were inoculated with 0.2 mL of the test organisms. A plate without an extract and another without a test organism were used as controls. The plates were incubated at 37°C for 24 h and observed for growth. The plate with the lowest concentration of the extract which showed no growth after incubation was recorded as the MIC.

Antibacterial sensitivity test for crude extract

The cup plate method was utilized to ascertain the antibacterial activity of aqueous extracts of C*assia alata* leaves at concentrations of 12.5, 25, 50, 100, 200, and 400 mg mL^{-1}. The microorganisms were streaked onto the agar in the petri dish using a broth that had been left overnight. Wells of diameter of 6 mm were made by means of a sterile cork borer, which were then filled approximately three-quarters of the way with solutions containing the aqueous extracts of Cassia alata leaves. To facilitate the diffusion of the solutions, the plates were pre-incubated for one hour at room temperature and thereafter incubated for a whole day. We assessed the inhibitory zone diameters (IZD). Propylene glycol and ciprofloxacin were employed as positive and negative controls, respectively.

Antibacterial sensitivity test for herbal phytosomal gels.

The prepared Mueller Hinton agar was aseptically poured into sixteen (16) petri dishes to solidify. Upon solidification the agar were inoculated with the organisms (*S. aureus* into 8 petri dishes and *E. coli* into the remaining 8 petri dishes) with the aid of a sterile swab stick, thereafter two holes were bored in each of the agar plates with a 6 mm cork borer and the plates were labeled respectively. The various batches of the gel (batch 1-8) were aseptically filled into their respective holes with the aid of a Pasteur pipette in accordance with the batches of the gel and each batch was carried out in duplicate in a petri dish and the same process were repeated for the remaining 7 batches. The bacterial inoculated samples were incubated for 24 hours and the inhibition zones diameters (IZD) were measured to determine the activity.

Evaluation of the phytosomal gels

pH measurement

A 1 g sample of phytosomal gel was diluted to 100 ml with de-ionized water in a volumetric flask. The pH of the resulting 1% dispersion was measured using a pH meter. Determinations were carried out in triplicates and the mean result was recorded.

Viscosity measurement

The viscosity of the phytosomal gels were determined using a Brookfield viscometer CAP-2000. Test samples were weighed in a clean and dry 250 ml beaker, and the viscosity of the

test samples was determined using spindle 4 following standard operating procedure at 12 rpm. Samples were measured at 27 ± 1°C (Akanksha *et al.,* 2009)

Spreadability study

The spreadability of the gel was determined by measuring the spreading diameter of 0.2 g of gel between two horizontal plates (20 cm x 20 cm) after one minute. The standard weight applied on the upper plate was 300 g.

Extrudability study

The Extrudability of the gel was determined by measuring the amount of gel extruded out of a collapsible tube on the application of a 500 g load for 1 min. The percentage extrudability was calculated as the ratio of the amount of gel extruded to the amount of gel in the tube (Okafo *et al.,* 2022)

$$\%\ Extrudability = \frac{Weight\ of\ the\ gel\ extruded}{Weight\ of\ gel\ in\ tube}\ x\ 100 \qquad (3)$$

RESULTS AND DISCUSSION

Percentage yield and total ash value

The percentage yield of extract from the dry powder of the leaves of *Cassia alata* was 51%, while the total ash value was 8.5%. The ash values usually represent the inorganic residues such as phosphates, carbonates and silicates present in herbal drugs. These are important indices to illustrate the quality as well as purity of herbal medicine. The percentage yield of 51% is quite high, typical yields from plant source are usually low. The total ash value of 8.5% is reasonably low indicating low contamination.

Phytochemical Screening Test

Phytochemical screening of the aqueous extract of *Cassia alata* was carried out to identify the various secondary metabolites present in the extract. The result as presented in Table 2 below reveal the presence of the under listed phytoconstituents.

Table 2: Phytochemical constituents of extracts of *Cassia alata*

S/N	Phytoconstituents	Observation
1	Saponin	+
2	Tannins	+
3	Flavonoids	+
4	Steroids	+
5	Terpenoids	+
6	Cardiac Glycosides	+
7	Alkaloids	+
8	Reducing Sugar	+

Physicochemical properties of *Cassia alata* herbal Gel

Viscosity of gel plays a vital role in the control of drug permeation through the skin, retention at the site of action as well as making it easily spreadable on the affected area of treatment and extrudable from collapsible tube packaging. The viscosity of the formulations ranged from 11,567 – 70,333 mPa. s as presented in Table 3 below. The plain gels (OG1 and OG2) exhibited higher viscosities of 43,333 and 70,333 mPa. s, than the phytosomal gels PG1-PG6 with viscosities ranging from 11,567-30,833 mPa. s. The addition of the phospholipid (lecithin) in forming the phytosomes probably led to the thinning out of the gels. The phytosomal

formulations also showed better spreadability (3.38-6.23 cm) and extrudability (18.66-26.56%) as against the plain gels with spreadability of 2.85-3.37 cm and extrudability of 8.12-15.96%.

Table 3: Physicochemical properties of *Cassia alata* herbal Gel

Batch	pH	Spreadability (cm)	Viscosity (mPa. s)	Extrudability (%)
PG1	7.42	4.30 ± 0.18	13,200 ± 871.78	26.56
PG2	7.51	6.23 ± 0.39	11,800 ± 458.25	25.70
PG3	7.03	4.85 ± 0.53	11,567 ± 1778.57	23.76
PG4	7.11	3.60 ± 0.66	18,467 ± 611.01	20.20
PG5	6.61	3.38 ± 1.19	30,833 ± 3329.16	19.68
PG6	5.46	3.88 ± 0.33	17,333 ± 577.35	18.66
OG1	7.53	3.37 ± 0.53	43,333 ± 1755.94	15.96
OG2	7.08	2.85 ± 0.31	70,333 ± 763.76	8.12

In vitro antibacterial activity of crude *Cassia alata* extract and *Cassia alata* phytosomal gel

The results of the *in vitro* antibacterial activities of crude extract and phytosomal gel of *Cassia alata* are presented in Tables 4 and 5 below. The MIC of *Cassia alata* extract against the test organisms were found to be 12.5 and 25 mg mL^{-1} for S. *aureus* and *E. coli* respectively as presented in Table 6.

The phytosomal gels of *Cassia alata* gave higher antibacterial activity than the plain gels (without phytosomes) and the crude extract based on the inhibitory zone diameters (IZD) as revealed in the result in Table 5. The enhanced activity may be due to the complex formed by the phytoconstituents and the phospholipid leading to enhanced *in vitro* penetration into the agar in this case. The 200 mg and 400 mg samples of the crude extract gave IZD of 10.5 and 15 mm (*S. aureus*) and 15 and 17 mm (*E. coli*) respectively (Table 4). Corresponding concentrations of phytosomal gels of *Cassia alata* (PG1-PG3 containing 200mg *Cassia alata* and different concentrations of phospholipid) and (PG4-PG6 containing 400 mg *Cassia alata* and different concentrations of phospholipid) gave much higher IZD of 21.5-27 mm for *S. aureus* and 19-24.5 mm for *E. coli* (Table 5).

Table 4: *In vitro* antibacterial activity of *Cassia alata* extract (zone of inhibition in mm)

Bacteria species	50 mg mL^{-1}	100 mg mL^{-1}	200 mg mL^{-1}	400 mg mL^{-1}
S. aureus	3.5	5.5	10.5	15
E. coli	3	4	15	17

Table 5: *In vitro* antibacterial activity of phytosomal gel of *Cassia alata* (zone of inhibition in mm)

Bacteria species	PG1	PG2	PG3	PG4	PG5	PG6	OG1	OG2
S. aureus	21.5	26	26.5	25.5	25	27	12	17
E. coli	19	17	24.5	23	20	18.5	16	18

PG1 (Cassia extract 200 mg/mL + Lecithin 100 mg), PG2 (Cassia extract 200 mg/mL + Lecithin 200 mg), PG3 (Cassia extract 200 mg/mL + Lecithin 300 mg), PG4 (Cassia extract 400 mg/mL + Lecithin 200 mg), PG5 (Cassia extract 400 mg/mL + Lecithin 400 mg), PG6 (Cassia extract 400 mg/mL + Lecithin 600 mg)

Table 6: Minimal inhibitory concentration (MIC) profile of *Cassia alata* extract

Microorganisms	Concentration of extract (mg mL^{-1})									
	0.782	1.563	3.125	6.25	12.5	25	50	100	200	400
S. aureus	+	+	+	+	-	-	-	-	-	-
E. coli	+	+	+	+	+	-	-	-	-	-

DISCUSSION

In this study phytosomes of *Cassia alata* leaf extract were formulated and incorporated into carbopol polymer forming phytosomal gels. The phytosomal gels gave favourable physicochemical properties. The viscosity of the formulations ranged from 11,567 – 70,333 mPa. s with the phytosomal gels showing lesser viscosities than the plain gels due to the presence of phospholipid making the gels more spreadable on the skin and easily extrudable from tubes.

The phytosomal gels of *Cassia alata* gave higher antibacterial activity than the plain gels and the crude extract. The enhanced activity may be due to the complex formed between the phytoconstituents and the phospholipid leading to enhanced *in vitro* penetration. The antibacterial activity of the phytosomal gels increased with increasing concentration of the phospholipid (lecithin) in the complex. The phytosomal gel formulations PG1, PG2 and PG3 containing same concentration of extract (200mg) but increasing phospholipid concentrations of 100 mg, 200 mg and 300 mg respectively showed increasing *in vitro* activity against both *S. aureus* and *E. coli*. This result was corroborated by the findings of some researchers who worked on other plant extracts and concluded that phytosomal formulations of their extracts showed better activities than the crude drugs (Singh *et al*., 2022; Jagtap *et al*., 2023 and Saonere *et al*., 2023)

CONCLUSION

Cassia alata phytosomes were successfully formulated and incorporated into carbopol gel base to form phytosomal gels. The *in vitro* antibacterial activity of *Cassia alata* crude extract was improved with the incorporation of phytosomes giving better profile than the crude extract and the conventional gel against *S. aureus* and *E. coli*. This enhanced activity can be harnessed by formulating phytosomal gels for the topical treatment of skin condition in which these pathogens are implicated.

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