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RESEARCH ARTICLE

Evaluation of the Antibacterial activity of Herbal ointments formulated with Methanolic extract of *Cassia alata*

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ABSTRACT

In this study, the antibacterial potency of herbal ointments formulated with methanolic extract of *Cassia alata* was evaluated. The preliminary *in vitro* antibacterial activity of the methanolic extracts of sun-dried leaves of *Cassia alata* was determined against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* using the Agar cup plate method. The antibacterial activity of the extract was predominantly against Gram-positive organisms. Herbal ointments were prepared by incorporating the methanolic extract of *Cassia alata* (10 % w/w) into aqueous cream, emulsifying ointment and simple ointment bases and evaluated for their *in vitro* antibacterial efficacy. The formulation containing *Cassia alata* extract in aqueous cream showed comparatively better antibacterial activity than the other formulations in the following order: aqueous cream > emulsifying ointment > simple ointment > crude extract. The herbal ointment also compared favourably with a commercial brand of Gentamicin ointment. This study shows that *Cassia alata* has antibacterial activity and also has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions.

KEYWORDS: *Cassia alata,* antibacterial properties, *Bacillus subtilis*, *Staphylococcus aureus*; *Escherichia coli*, *Pseudomonas aeruginosa,* herbal ointments.

1. INTRODUCTION

The delivery of drugs through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and non- invasive nature of the treatment [1].

Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucus membranes of the eye, vagina, anus, and nose. An ointment may or may not be medicated. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Ointments are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. Ointment bases are mainly anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oligeanous), absorption bases, water removable and water soluble type [2].

Cassia alata is categorized under the family *Fabaceae,* a pan tropical ornamental shrub, 2-3m high, widely distributed in tropical countries, stretching from Tropical America to India, Fiji, Indonesia, Malaysia and Africa. Other scientific names of *Cassia alata* are *Senna alata, Herpetic alata* and *Cassia bracteata* [3]. *Cassia alata* is a herb commonly used in Nigeria for the treatment of ringworm, eczema etc. Some of the local Nigerian names are Ilesko and Rinji in Yoruba and Hausa respectively [4].

Traditionally, the leaves are pounded and rubbed on the skin to cure skin diseases [5, 6]. Many reports have shown that some *Cassia* species contain antimicrobial substances, particularly *Cassia alata* [7, 8, 9, 10, 11]. Recent studies revealed that *Cassia alata* has been proven to be effective against bacteria and fungi species [3, 12, 13]. Alalor *et al*. [14] observed that the minimal inhibitory concentration (MIC) values of methanolic extracts of the

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leaves of *Cassia alata* against *Staphylococcus aureus* and *Bacillus subtilis* were 10mg/ml and 2.5 mg/ml respectively. Idu *et al*. [15] observed that preliminary phytochemical analysis of *Cassia alata* showed the presence of phenol, tannins, anthraquinones, saponins and flavonoids. Odunbaku and Lusanya [16] also corroborated this study; they further stated that, the plant also had alkaloids and cardenolides. The leaves of *Cassia alata* have been qualitatively analyzed for the presence of anthraquinones: rhein, aloe-emodin, chrysophanol, emodin, and physcion as well as the flavonoid, kaempferol [17, 18].

There are scanty reports on the activity of *Cassia alata* in the form of herbal ointment preparation to the best of the authors' knowledge. The antiseptic property of *Cassia alata*-based herbal soap against common pathogens of the skin has been studied [13].

This present study was carried out to evaluate the antibacterial properties of the methanolic extract of *Cassia alata* in formulated ointments.

2. MATERIALS AND METHODS

Plant material

The leaves of *Cassia alata* (*Fabaceae*) were collected from the premises of Federal Government College in Warri, Delta state, Nigeria in March, 2010. The plant sample was identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria. The collected leaves were cleaned of unwanted foreign materials, sun-dried for a week.

2.1. Test microorganisms

The microorganisms used for the study were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.. In this study, multi – drug resistant wound isolates bacteria from hospital patients at the Lagos University Teaching Hospital Lagos, (LUTH) Nigeria were used. The bacterial strains were grown and maintained on Mueller Hinton agar at 37 °C.

2.2. Microbiological media, chemicals and standard drugs

Mueller Hinton Agar (MHA) and Nutrient broth was obtained from the Chemical store of the Department of Pharmaceutics, Faculty of Pharmacy, University of Lagos, Nigeria Gentamicin ointment (1mg of Gentamicin in the form of Gentamicin Sulphate), product of Drugfield Ltd, Lagos, Nigeria was kindly supplied by the company and all other chemicals were of analytical grade and used without further purification..

2.3. Preparation of methanolic extract of *Cassia alata* leaves

The sun-dried leaves of *Cassia alata* was powdered using a laboratory mill (Kenwood Ltd, Hertfordshire, UK). 140g of milled leaves of *Cassia alata* was extracted with methanol by maceration for 48 hr. The extract was filtered and concentrated using a Buchi V-801 rotary evaporator at 35°C to obtain semisolid extract. The extract was stored in a refrigerator. A stock concentration of 400mg/ml was prepared from which working concentrations of 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml were prepared.

2.4. Evaluation of antibacterial activity of Extract

The antibacterial activity of the methanolic extract of the leaves of *Cassia alata* at concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*B. subtilis, E. coli, P. aeruginosa and S. aureus*) containing approximately 10^8 cfu / ml in a sterile petri dish and allowed to set. Wells of 6mm diameter were created with a sterile cork borer and filled to about three-quarters full with solutions of the methanolic extract of the leaves of *Cassia alata*. The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, *n=2*). Streptomycin and propylene glycol were used as positive and negative controls respectively.

The *in vitro* bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows; resistant: 10mm and below, intermediate: 11-15mm and susceptible: 16mm and above [19].

2.5. Preparation of Ointments

Three topical ointment bases of varying degrees of aqueous/anhydrous character (Table 1), namely: simple ointment BP, emulsifying ointment BP and aqueous cream BP were prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70°C for about 5 minutes and then cooled with continuous stirring. Formulation of ointment was done by incorporating 10 g of the semisolid methanolic extract of *Cassia alata* into the various bases by triturating in a ceramic mortar with a pestle to obtain 100 g of herbal ointments containing 10 % w/w of *Cassia alata* extract [20]. The prepared herbal ointments were put in ointment jars, labelled and were stored at room temperature pending the evaluation.

2.6. Physical evaluation of formulated Ointments

Physical assessments were carried out on the ointments and cream over a period of 30 days using the following parameters: Appearance, Odour, Texture and Colour.

The pH of various formulations was determined by using Digital pH meter. 0.5g of the weighed formulation was dispersed in 50 ml of distilled water and the pH was measured [21].

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Homogeneity: All the developed ointments were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps [21].

Formulations	Ingredients	Concentration (%w/w)
Formulation 1	Extract	10
	Wool fat	5
	Cetostearyl alcohol	5
	Hard paraffin	5
	White soft paraffin	85
Formulation 2	Extract	10
	Liquid paraffin	20
	Emulsifying wax	30
	White soft paraffin	50
Formulation 3	Extract	10
	Emulsifying ointment	30
	Chlorocresol	0.1
	Purified water	69.9

Table 1: Preparation of medicated formulations with methanolic extract of *Cassia alata*

In vitro antibacterial efficacy of formulated ointments

Formulation 1 (*Cassia alata* 10%w/w in simple ointment B.P), Formulation 2 (*Cassia alata* in emulsifying ointment B.P) and Formulation 3 (*Cassia alata* 10%w/w in aqueous cream B.P)

The cup-plate method was used to assess the relative antibacterial efficacy of the formulated herbal ointments prepared with the extract of *Cassia alata*. A molten Mueller Hinton agar stabilized at 45 °C, seeded with 0.1 ml of a 24 h broth culture of the test organism (*Bacillus subtilis* and *Staphylococcus aureus*) and containing approximately 10^8 cfu / ml was used. Wells of 6mm diameter were created and filled to three-quarters full with the topical products of *Cassia alata* extract. The plates were pre-incubated for 1 hr at room temperature to ensure adequate diffusion and finally incubated at 37 °C for 24 h. A commercial brand of Gentamicin ointment (Drugfield Pharmaceutical Ltd, Lagos) was used as standard while blank ointment base was used as control. The experiments were run in duplicate and the zones of inhibition were determined and recorded (mean± SD, *n = 2*).

2.7. Statistical Analysis

Data obtained was expressed as mean ± SD (standard deviation). The ANOVA test was used to assess if there were any difference in the data obtained. P-values less than 0.05 were considered statistically significant.

3. RESULTS

The preliminary *in vitro* antimicrobial activity of the methanolic extract of *Cassia alata* presented in Table 2 showed excellent activity against *Staphylococcus aureus and Bacillus subtilis.* The *in vitro* antimicrobial activity of the methanolic extract of *Cassia alata*-based herbal ointments presented in Table 3. They demonstrated excellent antibacterial activity. The *in vitro* antibacterial efficacy of the methanolic extract of *Cassia alata* incorporated in aqueous cream base (10 % w/w) against standard antibacterial agent is shown in Table 4 while the pH evaluation of different formulation of ointments is presented in Table 5.

Test organism	Concentration of Extract			
	50mg/ml	100mg/ml	150mg/ml	200mg/ml
S. aureus	16.50±0.58	18.00±0.00	20.00±1.41	22.00 ± .00
B. subtilis	12.50±0.50	13.00±1.29	14.00±0.50	17.00±0.50
E. coli	0.00	8.00±1.20	8.20±0.5	0.00
P. aeruginosa	0.00	0.00	0.00	0.00

Table 2: Preliminary *in vitro* antibacterial activity of methanolic extract of *Cassia alata* (Zone of inhibition in mm)

Test organism	Zone of Inhibition (mm)	
	Formulation 3	Gentamicin ointment (1%w/v)
S. aureus	24.00 ± 0.82	21.00 ± 0.00
B. subtilis	19.50 ± 0.96	20.00 ± 0.50
E. coli	9.00±1.50	27.00± 0.00
P. aeruginosa	0.00	30.00± 1.00

Table 4: *In vitro* antibacterial efficacy of the methanolic extract of *Cassia alata* incorporated in aqueous cream base (10 % w/w) against standard antibacterial agent (Zone of Inhibition in mm)

Time period (days)	Ointment formulation	pH
0	Formulation 1	6.2±0.74
	Formulation 2	6.4±0.21
	Formulation 3	7.0±0.53
15^{th}	Formulation 1	6.4±0.28
	Formulation 2	6.3±0.09
	Formulation 3	7.3±0.64
30^{th}	Formulation 1	6.2±0.69
	Formulation 2	6.1±0.38
	Formulation 3	7.0±0.25

Table 5: pH evaluation of different formulation of ointments

4. DISCUSSION

In the preliminary antimicrobial sensitivity screening, the methanolic extract of *Cassia alata* showed excellent activity against *Staphylococcus aureus and Bacillus subtilis* ($p < 0.05$) (Table 2). Most of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man. The extract did not show any activity against *Pseudomonas* contrary to observations of El mahmood

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and Doughari [17]. The activity of the extract was concentration-dependent as revealed by the zone of inhibition.

	Formulation 1		Formulation 2		Formulation 3	
Test Organism	100mg/ml	150mg/ml	100mg/ml	150mg/ml	100mg/ml	150mg/ml
S. aureus	17.00±0.5	18.00±0.00	18.00±0.00	20.00±0.00	23.00±0.00	24.50 ±1.91
B. subtilis	15.00±1.00	15.50±0.58	16.50 ±1.41	17.00 ±0.50	19.00 ± 0.55	21.00±0.33
E. coli	0.00	4.00±1.41	0.00	6.50±0.00	6.50 ± 0.00	8.50±0.50
P. aeruginosa	0.00	0.00	0.00	0.00	0.00	0.00

Table 3: In vitro Antibacterial activity of the methanolic leaf extract of *Cassia alata* (10 % w/w) incorporated in different topical bases (Zone of inhibition in mm)

The *Cassia alata*-based herbal ointments demonstrated excellent antibacterial activity (Table 3). The order of antibacterial activity of *Cassia alata* in the topical bases was as follows: Formulation 3 > Formulation 2 > Formulation 1. The results also revealed that the extracts incorporated into the ointment bases showed better activity than that of the crude extract of *Cassia alata*. This implied that there might have been better diffusion of drug for the herbal ointments than for the crude extract. The activity against *Staphylococcus aureus* is of significant interest because it is commonly found on the hands, face and in deep layers of the skin and is perhaps the most widely encountered and very undesirable. *Staphylococcus aureus* is not easily eliminated especially in the deeper skin layers, sweat gland, sebaceous gland, and the hair-follicles by routine washing and scrubbing even with some antiseptic soap [22]. It is implicated as the commonest etiologic agent of boils, carbuncles, breast abscess and infantile-impetigo [23].

Formulation 3 compared favourably with Gentamicin ointment for its antibacterial activity against *S. aureus* and *B. subtilis.* Formulation 3 showed a slightly higher activity than Gentamicin ointment against *S. aureus*, and lower activity against *B. subtilis. Cassia alata* herbal ointment did not show any activity against *Pseudomonas* while the standard drug Gentamicin ointment showed relatively high activity against *E. coli* and *P. aeruginosa* (Table 4).

The prepared formulations show a smooth and homogeneous appearance. The pH values of all the prepared formulations ranged from 6.1 to 7.3, which are considered acceptable to avoid the risk of irritation upon application to the skin. The pH values of the formulations are within the normal pH range of the human skin (6.8 ± 1). From the study, the ointments showed no changes in pH, consistency and phase separation after keeping for 30 days (Table 5).

5. CONCLUSION

This study shows that *Cassia alata* has antibacterial activity and has high potential as antibacterial agent when formulated as ointment for topical use and could therefore explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. The potency of the *Cassia alata* herbal ointment against *Staphylococcus aureus* could be harnessed in the containment of the organism implicated as the commonest etiologic agent of boils, carbuncles, infantile-impetigo and wounds.

This investigation suggests the use of both hydrophilic and hydrophobic ointments containing *Cassia alata* as antibacterial ointment preparation. Aqueous cream, a water-miscible topical base, was possibly a better vehicle for the release of the antibacterial compounds present in *Cassia alata* extract.

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Conflict of Interest: None Declared

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