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## Development and evaluation of antimicrobial herbal formulations containing the methanolic extract of *Cassia alata* for skin diseases

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### PEER REVIEW

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#### Comments

The authors evaluated the development of antimicrobial herbal formulation using plants available locally like *C. alata* for skin diseases. The research showed that skin infections caused by *S. aureus* can be treated by the ointment formulated with active ingredient in *C. alata*. It is of interest that ointment can be formulated from locally readily available natural resources, giving easy accessibility to the treatment.

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### ABSTRACT

**Objective:** To explore the antifungal and antibacterial activity of methanolic extract of *Cassia alata* (*C. alata*) and its formulations for skin diseases.

**Methods:** Sundried leaves of *C. alata* Linn. were extracted using different solvents as follows: water, methanol, ethanol, *n*-hexane and lastly with acetone. The crude extract was investigated for antifungal and antibacterial activities using disc diffusion method against *Coccidioides immitis*, *Exophiala dermatitidis*, *Aspergillus fumigatus* and human pathogenic fungi *Candida albicans* and a group of bacteria, *Staphylococcus aureus*. The minimum inhibitory concentrations of the methanolic extract were determined using the agar dilution method. Herbal ointments were prepared by incorporating the methanol extract of *C. alata* into emulsifying ointment to obtain different concentrations of 25, 50, 100, and 200 mg/mL.

**Results:** The methanol extraction gave the maximum extraction. The formulated *C. alata* ointment when compared with standard drugs nystatin and streptomycin *in vitro* was more effective against the microorganisms.

**Conclusions:** This study showed that *C. alata* had antifungal and antibacterial activities when formulated as ointment for topical use and could, therefore, explained its folkloric use for the treatment of dermatitis.

### KEYWORDS

*Cassia alata*, Antifungal, Antibacterial properties, Ointment

## 1. Introduction

Herbal remedies for skin care with antibacterial and antifungal activities are prepared from a variety of plant parts such as leaves, stem, root, bark and fruits. These medicines are administered topically and may be applied in the form of cream, lotion, soap, sap, solvent extract and ointment, and have been established to possess antimicrobial properties[1].

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. Along with other dosage forms, herbal drugs are also formulated in 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.*, protectants, antiseptics, emollients, antipruritic keratolytics and astringents. Ointment bases are mainly anhydrous and generally contain one or more medicaments in suspension, solution or dispersion. Ointment bases may be hydrocarbon (oleaginous), absorption bases, water removable and water soluble type[2].

*Cassia alata* family Fabaceae (*C. alata*), is a pan tropical

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ornamental shrub, 2–3 m high, widely distributed in tropical countries, stretching from Tropical America to India, Fiji, Indonesia, Malaysia and Africa. It is common in villages, wastelands and clearings and chiefly in the forest regions of Nigeria<sup>[3]</sup>.

The aqueous leaf extract exhibits various pharmacological properties such as anti-bacteria, anti-fungal as well as anti-inflammatory activities<sup>[4]</sup>. Leaf sap contains a fungicide, chrysophanic acid used to treat several skin ailments<sup>[5]</sup>. The leaves of *C. alata* L. are used as an effective treatment against ringworm and also against other skin diseases such as eczema and chronic skin impurities<sup>[6]</sup>. *C. alata* leaves contain emodin, kaempferol, aloe-emodin, chrysophanol and iso-chrysophanol, rhein, ellagitannin, phenolic acid and cassia xanthone, among other substances. One of the important flavonoids of *C. alata* leaves is astragalins<sup>[7]</sup>. Astragalins has lately aroused increased pharmaceutical interest because of its potential as anti-inflammatory agent in addition to having antimicrobial activity<sup>[6]</sup>.

Other scientific names of *C. alata* are *Senna alata*, *Herpetica alata* and *Cassia bracteata*. In Nigeria, *C. alata* is a herb commonly used for the treatment of ringworm, eczema, dermatitis, ringworm gonorrhoea, scabies, itchiness, and internally as expectorant for bronchitis, for alleviation of asthma symptoms, as a laxative to expel intestinal parasites, for stomach problems and weight loss<sup>[8,9]</sup>.

Many reports and recent studies revealed that *C. alata* has been proven to be effective against bacteria and fungi species<sup>[2,6]</sup>. Alalor *et al.*<sup>[2]</sup> observed that the minimal inhibitory concentration values of methanolic extracts of the leaves of *C. alata* against *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* were 10.0 mg/mL and 2.5 mg/mL, respectively<sup>[10,11]</sup>. Sule *et al.*<sup>[9]</sup> observed that preliminary phytochemical analysis of *C. alata* showed the presence of phenol, tannins, anthraquinones, saponins and flavonoids. Oladele *et al.*<sup>[12]</sup> also corroborated this study and they further stated that the plant also had alkaloids and cardenolides. The leaves of *C. alata* have been qualitatively analyzed for the presence of anthraquinones: rhein, aloe-emodin, chrysophanol, emodin, and physcion as well as flavonoid and kaempferol<sup>[9]</sup>. The antiseptic property of *C. alata*-based herbal soap against common pathogens of the skin has been studied<sup>[12]</sup>. However, to the best of our knowledge, there were scanty reports on the activity of *C. alata* in the form of herbal ointment preparation. Thus, this present study was carried out to evaluate the antibacterial properties of the methanolic extract of *C. alata* in formulated ointments.

## 2. Materials and methods

### 2.1. Collection/Identification of the plant (*C. alata*) sample

Fresh *C. alata* leaves were collected at Ikot Udo Village, Nkwot 1, ward 6 in Ikono L.G.A., Akwa Ibom State. Identification was carried out by Mr. Etefia, a herbalist/laboratory foreman in the Department of Pharmacognosy

and Natural Medicine, University of Uyo, Uyo, Nigeria. The collected leaves were cleaned of unwanted foreign materials and sun-dried for a week. Dried leaves were then pulverized into fine particles with the use of manual grinder.

### 2.2. Test microorganisms

Four broth cultures of mould isolates of *Coccidioides immitis* (*C. immitis*), *Exophiala dermatitidis* (*E. dermatitidis*), *Aspergillus fumigatus* (*A. fumigatus*), a yeast *Candida albicans* (*C. albicans*) and the only bacteria, *S. aureus* used in this study were obtained from Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, University of Uyo.

### 2.3. Microbiological media, chemicals and standard drug

Mueller Hinton agar gel, the media for the fungi and bacteria, was obtained from the chemical store of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. Streptomycin was given as a gift by Drugfield Ltd., Lagos. All other chemicals were of analytical grade and used without further purification.

### 2.4. Preparation of solutions of the extract with varying concentrations

Each extract was weighed (2 g) and stored into a sterilized bottle and 10 mL of sterile water was added to obtain a stock solution of 200 mg/mL. A portion of the 200 mg/mL was diluted with an equal volume of sterile water to obtain a 100 mg/mL. The double dilution procedure was continued to obtain lower concentrations of the extract.

### 2.5. Evaluation of antifungal and antibacterial activity of extracts

The media used for this work was a molten Meller Hinton agar at 45% inoculated with 0.1 mL of a broth culture of standard drugs nystatin for fungi (*C. albicans*, *C. immitis*, *E. dermatitidis*, *A. fumigatus*) and streptomycin for bacteria *S. aureus*. *C. alata* at three concentrations of 25, 50 and 100 mg/mL were added into the broth culture. The plates were incubated at room temperature for 1 h initially to allow for the diffusion of the mixture and then incubated for 24 h in a sterile Petri dish and allowed to set. Wells of 6 mm diameter were created with a sterile cork borer and filled to about three-quarter full with different concentrations of solutions of the extracts of *C. alata*. The plates were pre-incubated for 1 h at room temperature to allow for the diffusion of the solution and then incubated for 24 h. The *in vitro* bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows: resistant  $\leq 10$  mm, intermediate 11–15 mm and susceptible  $\geq 16$  mm<sup>[2]</sup>.

### 2.6. Preparation of ointment

Constituents of emulsifying wax BP, namely, soft paraffin,

emulsifying wax and liquid paraffin 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 at 70 °C for about 5 min and then cooled with continuous stirring. Formulation of ointments was done by incorporating 10 g of the various semisolid methanolic extracts of *C. alata* into the ointment base by triturating in a ceramic mortar with a pestle to obtain 10 g of herbal ointment. The obtained ointment was collected in jars, labelled and stored at room temperature pending the evaluation.

### 2.7. *In vitro* antifungal and antibacterial efficacy of formulated ointments

The cup–plate method was used to assess the relative antibacterial efficacy of the formulated herbal ointments prepared with the extract of *C. alata*. A molten Mueller Hinton agar with 0.1 mL 24 h broth culture containing approximately  $1 \times 10^8$  CFU/mL was used to test the organisms (*C. albicans*, *C. immitis*, *E. dermatitidis*, *A. fumigatus* and *S. aureus*) at 45 °C. Wells of 6 mm diameter were created and filled to three-quarters full with the topical products of *C. alata* extract. The plates were pre-incubated for 1 h at room temperature to ensure adequate diffusion and finally incubated at 37 °C for 24 h. Commercial brands of nystatin and streptomycin, and blank ointment were used as standard and control, respectively. The clear zones of inhibition for the extract were measured in millimeters using a meter rule.

### 2.8. Statistical analysis

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

## 3. Results

The results of the antifungal and antibacterial assays of the methanol leaf extract are shown in Table 1, which showed excellent growth inhibition against *C. albicans*, *C. immitis*, *E. dermatitidis*, *A. fumigatus* and *S. aureus*.

**Table 1**

Antifungal and antibacterial assay of methanolic leaf extract of *C. alata* showing zones of inhibition in mm (*n*=5).

Test organism	Concentration of extracts (mg/mL)				Nystatin 50000 IU	Streptomycin (mg/mL) 0.04
	25	50	100	200		
<i>C. albicans</i>	–	–	6.3±0.5	6.4±0.5	29.0±0.1	–
<i>C. immitis</i>	5.8±0.2	6.1±0.1	6.2±0.3	7.0±0.2	34.0±0.1	–
<i>E. dermatitidis</i>	5.5±0.1	5.7±0.1	5.8±0.1	6.1±0.3	31.0±0.2	–
<i>A. fumigatus</i>	6.0±0.5	6.0±0.1	7.2±0.2	8.0±0.1	36.0±0.1	–
<i>S. aureus</i>	12.0±0.2	15.0±0.1	16.0±0.1	19.0±0.4	–	34.0±0.1

The result of the *in vitro* antimicrobial activity of the methanolic extract of *C. alata*-based herbal ointments are presented in Table 2. They demonstrated excellent

antibacterial activity.

**Table 2**

*In vitro* antifungal efficacy of the methanolic extract of *C. alata* incorporated in emulsifying ointment B.P against standard antifungal agent (zone of exhibition in mm).

Test organism	Ointment % (w/w)					Standard nystatin 50000 IU
	10	15	20	25	30	
<i>C. albicans</i>	0.00	5.60±0.20	9.70±0.12	13.60±0.30	14.50±0.30	37.00±0.10
<i>C. immitis</i>	0.00	5.60±0.30	10.00±0.10	13.90±0.11	14.80±0.12	37.00±0.20
<i>E. dermatitidis</i>	0.00	5.70±0.10	9.80±0.14	15.00±0.15	15.00±0.10	38.00±0.11
<i>A. fumigatus</i>	0.00	6.00±0.13	10.00±0.10	16.00±0.10	16.00±0.50	39.00±0.30

## 4. Discussion

In the preliminary *in vitro* antimicrobial sensitivity screening, the methanolic extract of *C. alata* showed excellent activity against *C. immitis*, *E. dermatitidis*, *A. fumigatus*, *C. albicans* and *S. aureus*. This finding was in line with the works of Kareru *et al.*[1] and Alaor *et al.*[2]. Most of these organisms were natural flora of the skin and the genitals, and also known as etiologic agents of several skin and mucous membrane infections of man. The extract did not show any activity against *C. albicans* at 25 and 50 mg/mL. The activity was concentration dependent as revealed by the zone of inhibition. The more the concentration of the extract the more the zone of inhibition.

The *C. alata*-based herbal ointment demonstrated an excellent antifungal activity. The order of antibacterial of *C. alata* in the emulsifying ointment base at 30% (w/w) is *A. fumigatus*>*E. dermatitidis*>*C. immitis*>*C. albicans*. The results also revealed that the extracts incorporated into the ointment bases showed better activity than that of the crude extract of *C. alata*. This implied that there might have been better diffusion of drug for the herbal ointments than for the crude extract. The activity against *S. aureus* is of significant interest because it was commonly found on the hands, face and on deep layers of the skin and was perhaps the most widely encountered and very undesirable. *S. aureus* is not easily eliminated especially in the deeper skin layers, sweat gland, sebaceous gland, and hair follicles by routine washing and scrubbing even with some antiseptic soap[12]. It is implicated as the commonest etiologic agent of boils, carbuncles, breast abscess and infantile-impetigo[12]. All the herbal ointments did not show any activity at the lowest concentration of 10% (w/w).

This study showed that *C. alata* had antifungal and antibacterial activity and possessed high potential as antifungal and 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.

### Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgements

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## Comments

### Background

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. Along with other dosage forms, herbal drugs are also formulated in form of ointment. Topical application of medication allow the medication to concentrate at the targeted area for therapeutic effectiveness.

### Research frontiers

This research work has shown the effectiveness of topical use of *C. alata* as ointment in fungal and bacteria skin infections, an microbiological exploration of the antifungal and antibacterial activity of methanolic extract of *C. alata* and its formulations for skin disease. The leaf extracts of *C. alata* L. were confirmed useful in the treatment of fungal and some bacterial skin infections such as eczema and chronic skin infections caused by *S. aureus* as shown in the minimal inhibitory concentrations of the extracts and the formulated ointments against used microorganisms.

### Related reports

This finding was in line with the works of Kareru *et al.* and Alaor *et al.* Most of these organisms were natural flora of the skin and the genitals, and also known as etiologic agents of several skin and mucous membrane infections of man. The extract did not show any activity against *C. albicans* at 25 and 50 mg/mL. The activity was concentration dependent as revealed by the zone of inhibition. The more the concentration of the extract the more the zone of inhibition.

### Innovations and breakthroughs

This study has shown that plants like *C. alata* and other natural herbal formulations can be used in the treatment of skin infections most especially bacterial skin infections as ointments. Most of the recent research works tend to explore the use of natural products as antimicrobial agents and this should be encouraged.

### Applications

*C. alata* has antifungal and antibacterial activity. And it also possesses high potential as antifungal and antibacterial agent when formulated as ointment for topical use. Therefore, it could explain the successes claimed folk use of

the plant in the treatment of common skin conditions.

### Peer review

The authors evaluated the development of antimicrobial herbal formulation using plants available locally like *C. alata* for skin diseases. The research work showed that skin infections caused by *S. aureus* can be treated by the ointment formulated with active ingredient as *C. alata*. It is of interest that ointment can be formulated from locally readily available natural resources, giving easy accessibility to the treatment.

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