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

Stephen Olaribigbe Majekodunmi*, Akanimo Akpan Essien

*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria*

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

**Methods:** Sun-dried 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*, *Exophilia 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
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[3].

The aqueous leaf extract exhibits various pharmacological properties such as anti-bacteria, anti-fungal as well as anti-inflammatory activities[4]. Leaf sap contains a fungicide, chrysophanic acid used to treat several skin ailments[5]. 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[6]. C. alata leaves contain emodin, kaempferol, aloe-emodin, chrysophanol and iso-chrysophanol, rhein, ellagittannin, phenolic acid and cassia xanthone, among other substances. One of the important flavonoids of C. alata leaves is astragalin[7]. Astragalin has lately aroused increased pharmaceutical interest because of its potential as anti-inflammatory agent in addition to having antimicrobial activity[6].

Other scientific names of C. alata are Sena alata, Herpetic alata and Cassia bracteata. In Nigeria, C. alata is a herb commonly used for the treatment of ringworm, eczema, dermatitis, ringworm gonorrhea, 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[8,9].

Many reports and recent studies revealed that C. alata has been proven to be effective against bacteria and fungi species[7,8]. Alalor et al.[2] 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[10,11]. Sule et al.[9] observed that preliminary phytochemical analysis of C. alata showed the presence of phenol, tannins, anthraquinones, saponins and flavonoids. Oladele et al.[12] also corroborated this study and 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 physicin as well as flavonoid and kaempfero[9]. The antiseptic property of C. alata–based herbal soap against common pathogens of the skin has been studied[12]. 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 Coccidiodes immitis (C. immitis), Exophiala dermatis (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 Mueller 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 ≤10 mm, intermediate 11–15 mm and susceptible ≥16 mm[23].

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 1x10^5 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*.

| Test organism | Concentration of extracts (mg/mL) | Nystatin (IU/mL) | Streptomycin (mg/mL) |
|---------------|----------------------------------|-----------------|----------------------|
|               | 25 | 50 | 100 | 200 | 50 000 IU | 0.04 |
| *C. albicans* | – | – | 6.3±0.5 | 6.4±0.5 | 29.0±0.1 | – |
| *C. immitis* | 5.8±0.2 | 6.1±0.1 | 6.2±0.3 | 7.0±0.2 | 34.0±0.1 | – |
| *E. dermatitidis* | 5.5±0.1 | 5.7±0.1 | 5.8±0.1 | 6.1±0.3 | 31.0±0.2 | – |
| *A. fumigatus* | 6.0±0.5 | 6.0±0.1 | 7.2±0.2 | 8.0±0.1 | 36.0±0.1 | – |
| *S. aureus* | 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.

### 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 Alao 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-impetig[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

The authors wish to thank members of technical staff of the Dispensing and Pharmaceutical Microbiology Laboratories, Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Akwa–Ibom State, Nigeria, for their cooperation.

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. The more the concentration of the extract the more the zone of inhibition. Therefore, it could explain the successes claimed folk use of the plant in the treatment of common skin conditions.

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 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 Alalor 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.

References

[1] Kareru PG, Keriko JM, Kenji GM, Thiong’o GT, Gachanja AN, Mukirra HN. Antimicrobial activities of skincare preparations from plant extracts. Afr J Tradit Complement Altern Med 2010; 7(3): 214–218.
[2] Alalor CA, Igwilo CI, Jeroh E. Evaluation of the antibacterial properties of aqueous and methanol extracts of Cassia alata. J Pharm Allied Health Sci 2012; 2(2): 40–46.
[3] Faruq ZU, Rahman UA, Bello M, Obianke M, Atiku FA. Antibacterial activity of the active component of Cassia alata (Linn.) leaves. Niger J Basic Appl Sci 2010; 18(1): 97–100.
[4] Okoro IO, Osagie A, Asibor EO. Antioxidant and antimicrobial activities of polyphenols from ethnomedicinal plants of Nigeria. Afr J Biotechnol 2010; 9(20): 2989–2993.
[5] Lubaina AS, Murugan K. Physiological and biochemical characterization of Senna alata (L.) Roxb. leaf extract—a plant based fungicide against alternaria leaf spot in sesame. World J Pharm Pharm Sci 2013; 2(6): 5790–5801.
[6] Amoa EA, Adeoti TM, Ayandele B, Jimoh AR. Clinical response of broilers on varying levels of aqueous Cassia alata leaf extract. J Med Plant Res 2014; 8(12): 520–522.
[7] Lee HB, Kim EK, Park SJ, Bang SG, Kim TG, Chung DW. Isolation and anti–inflammatory effect of astragalin synthesized by enzymatic hydrolysis of tea seed extract. J Sci Food Agric 2011; 91(13): 2315–2321.
[8] Gaddam SA, Kotakadi VS, Sai Gopal DVR, Rao SY, Reddy AV. Efficient and robust biofabrication of silver nanoparticles by Cassia alata extract and their antimicrobial activity. J Nanostruct Chem 2014; doi: 10.1007/s40097-014-0082-5.
[9] Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, et al. Phytochemical properties and in–vitro antifungal activity of Senna alata Linn. crude stem bark extract. J Med Plants Res 2011; 5(2): 176–183.
[10] Sharma S, Dangi MS, Wadhwa S, Daniel V, Tiwari A. Antibacterial activity of Cassia tora leaves. Int J Pharm Biol Arch 2010; 1(1): 84–86.
[11] Krishnan N, Ramanathan S, Sasidharan S, Munugaiyah V, Mansor SM. Antimicrobial activity evaluation of Cassia spectabilis leaf extracts. Int J Pharmaco 2010; 6: 510–514.
[12] Oladele AT, Dairo BA, Elujola AA, Oyelami AO. Management of superficial fungal infections with Senna alata (“alata”) soap: a preliminary report. Afr J Pharm Pharmacol 2010; 4(3): 98–103.