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Evaluation of the Antifungal Properties of *Cassia alata* Based Herbal Ointments Formulated in Different Ointment Bases

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Original Research Article

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Abstract: Superficial fungal infections are generally more prevalent in tropical environments because of the enhancement of their growth by the high temperatures and high humidity conditions prevalent in these regions. The aim of this study is to investigate the antifungal potency of herbal ointments formulated with aqueous extract of *Cassia alata*. The *in vitro* antifungal activity of crude extract of leaves of *Cassia alata* as well as herbal ointments were determined against *Candida albicans, Trichophyton mentagrophyte, Aspergillus niger* and *Penicillium* using the Agar cup plate method. Herbal ointments were prepared by incorporating the crude extract of *Cassia alata* (10 % w/w) into ointment bases and evaluated for their *in vitro* antifungal efficacy. The crude extract showed moderate antifungal activity against *Candida albicans, Trichophyton mentagrophyte and Aspergillus niger* with zones of inhibition of 23 mm, 21 mm and 19 mm respectively at 200 mg/ml but no activity against *Penicillium*. The antifungal activity was retained in the herbal ointments with higher zones of inhibition for corresponding concentrations. The formulation containing *Cassia alata* extract in aqueous cream showed comparatively better antifungal activity. The herbal ointment also compared favourably with a commercial brand of Miconazole cream used as standard. This study shows that *Cassia alata* possesses antifungal activity and also has high potential as antifungal 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, hence the need to have herbal topical dosage forms.

Keywords: *Cassia alata,* antifungal properties, *Candida albicans, Trichophyton mentagrophyte, Aspergillus niger* and *Penicillium,* herbal ointments.

INTRODUCTION

Fungal infections can be classified into superficial mycoses located on the skin, hair, nails and mucosal membranes, deep or subcutaneous mycoses affecting the subcutaneous tissues and systemic mycoses primarily affecting internal organs. The fungi implicated in superficial mycoses are yeasts, dermatophytes and moulds [1]. Superficial fungal infections are a common diagnosis in everyday dermatological practice and their prevalence are generally more in developing than in developed countries [2].

Fungi are of special significance in tropical environments because their growth is encouraged by high temperatures and high humidity conditions prevalent in these regions [3]. Dermatomycoses such as dermatopyhte infections, Tinea and scabies are among the common human infections in the developing world [4, 5].

For most patients, topical treatment with antifungal ointment or cream may suffice when applied twice daily for 6 to 8 weeks. For onychomycosis, tinea capitis, and extensive dermatophyte disease, systemic treatment is often necessary [6].

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 [7].

An ointment is a semisolid preparation applied topically on the skin and the mucus membranes of the eye, vagina, anus, and nose. An ointment may or may not be medicated. 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 [8].

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 bracteate* [9]. *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 [10].

Traditionally, the leaves are pounded and rubbed on the skin to cure skin infections [11, 12]. Reports have shown that some *Cassia* species contain antimicrobial substances, particularly *Cassia alata* [13, 14]. Recent studies revealed that *Cassia alata* has been proven to be effective against bacteria and fungi species [15]. In another study it was observed that preliminary phytochemical analysis of *Cassia alata* showed the presence of phenol, tannins, anthraquinones, saponins, flavonoids, alkaloids and cardenolides [16, 17]. 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 [18, 19]. This present study was carried out to evaluate the antifungal properties of the aqueous extract of *Cassia alata* in different ointment bases.

MATERIALS AND METHODS

The leaves of *Cassia alata* (*Fabaceae*) were collected from the premises of Federal Government College, Warri, Delta State, Nigeria. The plant sample was identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, Delta state University, Abraka, Delta State, Nigeria. The collected leaves were cleaned of unwanted foreign materials, sun-dried for a week. The microorganisms used for the study were *Candida albicans, Trichophyton mentagrophyte, Aspergillus niger and Penicillium*.

Preparation of aqueous 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 distilled water 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.

Evaluation of antifungal activity of Extract

The antifungal activity of the extract was determined at concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml. A molten Sabouraud Dextrose agar stabilized at 45°C was seeded with 0.1ml of test organism (*Candida albican, Trichophyton mentagrophytes, Aspergillus niger and Penicillium)* containing approximately 10^8 sfu /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 aqueous extract of the leaves of *Cassia alata*. Clotrimazole was used as control. The zones of inhibition were also measured. This method depends on the diffusion of the various extracts from a cavity through the solidified agar layer of Petri dish. Strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant stains have smaller zones of inhibition or grow up to the edge of the disc. The experiments were run in duplicate and the zones of inhibition were determined and recorded (mean± SD, $n = 2$). The *in vitro* fungal 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 [20].

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 aqueous 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 [21]. The prepared herbal ointments were put in ointment jars, labelled and were stored at room temperature pending the evaluation.

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

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

Formulations	Ingredients	Concentration (%w/w)
F1	Extract	10
	Wool fat	5
	Cetostearyl alcohol	5
	Hard paraffin	5
	White soft paraffin	85
F2	Extract	10
	Liquid paraffin	20
	Emulsifying wax	30
	White soft paraffin	50
F3	Extract	10
	Emulsifying ointment	30
	Chlorocresol	0.1
	Purified water	69.9

F1 (*Cassia alata* 10%w/w in simple ointment B.P), F2 (*Cassia alata* in emulsifying ointment B.P) and F3 (*Cassia alata* 10%w/w in aqueous cream B.P)

In vitro antifungal efficacy of formulated ointments

The cup-plate method was also used to assess the relative antifungal efficacy of the formulated herbal ointments prepared with the extract of *Cassia alata*. A molten Sabouraud Dextrose agar stabilized at 45°C seeded with 0.1ml of test organism (*Candida albican, Trichophyton mentagrophytes, Aspergillus niger and Penicillium)* containing approximately 10^8 sfu /ml was used. A commercial brand of Miconazole cream (Daktarin^R, Janssen Pharmaceuticals Ltd) 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*).

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.

RESULTS

Table-2: Preliminary *in vitro* antifungal activity of crude extract of *Cassia alata* (Zone of inhibition in mm)

	Concentration of Extract			
Test Organism	50mg/ml	100mg/ml	150mg/ml	200mg/ml
C albicans	14.00 ±0.00	16.50±1.15	17.00±0.0	22.00 ±0.5
T. ment.	12.50 ±0.82	14.50±0.96	17.50±1.89	23.00±0.96
A. niger	12.00 ±0.00	14.50±1.89	16.00±1.41	19.00±0.82
Penicillium	0.00	0.00	0.00	0.00

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

	F1		F2		F3	
Test Organism	**100mg/ml**	**150mg/ml**	**100mg/ml**	**150mg/ml**	**100mg/ml**	**150mg/ml**
C albicans	17.00±0.45	19.00±0.5	17.00±0.82	19.00±0.00	21.00±0.67	23.00±0.58
T. ment	15.00±0.33	17.50±1.29	15.00±0.00	17.00±0.00	20.00 ±0.82	21.00±0.00
A. niger	12.00±0.67	14.00±0.82	13.00±1.83	14.00±0.96	18.00±0.50	18.50±0.50

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

	Zone Of Inhibition (mm)	
Test Organism	*Cassia alata* (10% w/w) in aqueous cream	Miconazole cream
C albicans	21.00 ± 0.58	30.00 ± 0.00
T. mentagrophyte	20.00 ± 0.50	27.00 ± 0.00
A. niger	18.00 ± 0.58	24.00 ± 0.50

Table-5: pH evaluation of different formulation of ointments

Time period (days)	Ointment formulation	pH
0	F1	6.1±0.74
	F2	6.3±0.21
	F3	7.0±0.53
15th	F1	6.5±0.28
	F2	6.3±0.09
	F3	6.8±0.64
30th	F1	6.2±0.69
	F2	6.4±0.38
	F3	7.0±0.25

DISCUSSION

The preliminary *in vitro* antimicrobial activity of the aqueous extract of *Cassia alata* presented in Table-2 showed activity against *Candida albicans, T. mentagrophyte and A. Niger* ($p < 0.05$) but there was no activity against *Penicillium.* The *in vitro* activity was also found to be concentration dependent as revealed by the zone of inhibition (Table-2).

The *Cassia alata*-based herbal ointments demonstrated antifungal activity (Table-3). The order of antifungal activity of *Cassia alata* in the topical bases was as follows: F3 > F2 > F1. 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.

F3 compared favourably with Miconazole cream for its antifungal activity against *Candida albicans, T. mentagrophyte and A. Niger*. But the standard drug, Miconazole showed better antifungal activity than the herbal ointments (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).

CONCLUSION

This study shows that *Cassia alata* has antifungal activity and has high potential as antifungal agent when formulated as ointment or cream 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 the test organism could be harnessed in the treatment of Ringworm and other fungal infections.

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