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Antifungal Evaluation of Formulated Ointment From *Sphenocentrum jollyanum* Root Extract

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ABSTRACT

In the light of high cost and frequent reoccurrence of current antifungal drugs, there is a need to explore the natural product resources in managing fungal infection, candidiasis, in which *Candida albicans* is the causative agent.
This study is aimed at evaluating the *in vitro* and *in vivo* antifungal activity of *Sphenocentrum jollyanum* in view of the folkloric use in dressing chronic wounds.
The ethylacetate fraction of 70% w/v methanol extract of *S. jollyanum* root (SJRME) was assessed for *in vitro anticandidal* activity using agar dilution method. Five groups of *Candida albicans* infected albino rats were treated with graded concentrations of ethylacetate fraction formulated ointment (50 - 200 mg/mL), with tioconazole cream 1% and normal saline as controls. Skin swabs were taken on days 0, 3, 6, 9 and 12 and placed on tryptone soya broth for three days. Serial dilution of the skin swabs was carried out and fungal loads determined using colony counter. The physical properties of the ointment formulated were evaluated.
The ethylacetate fraction of SJRME was found to be active at tested concentrations against *Candida albicans* with a minimum inhibitory concentration of 12.5 mg/mL. Significant activity was also observed against other species of *Candida* and plant fungi. The formulated ointment had moderate viscosity, smooth texture, bland odour and bright yellow color.
Treatment groups showed a significant reduction in fungal loads of skin swabs and ethylacetate fraction of SJRME possess antifungal activity and may therefore be potent templates in antifungal drug development.

***Keywords*:** *Sphenocentrum jollyanum,* Antifungal properties, *Candida albicans*, Tioconazole.

Introduction

Sphenocentrum jollyanum (Menispermaceae) known locally in Ibibio as Ibong Isong, in Ghana as *Adurokokoo* (Red medicine) or *Okramankote* (dog's penis) and in Yoruba as *Akerejupon*. It is a small erect sparsely branched shrub, growing up to 1.5 m in height with very few branches and glabrous leaves up to 20 cm in length with the breath of 5-12 cm.[1] The brightly yellow roots have sour taste and are therefore useful as chewing sticks, in constipation, as a stomachic, cough syrups, sickle cell crisis, rheumatism and inflammatory conditions.[2-4] The fruits are bright orange colored. The ellipsoid bright yellow or orange fruits are ovoid and occur in clusters which are edible when ripe.[5] Its natural habitat is in the forest zones of southern Nigeria as undergrowth plant. It is a perennial plant that grows naturally along the west coast sub region of Africa with expanse from Cameroon across Nigeria to Sierra Leone. *Sphenocentrum jollyanum* has been reported to possess a wide spectrum of biological and pharmacological properties. Dalziel reported the medicinal importance first where he reported that the leaves decoctions were used as Vermifuge.[6] The plant is also used for the management of jaundice, breast engorgement related to the menstrual cycle, tumors and inflammatory conditions.[3,4,7] The root hairs and other antimalarial plants can be used as recipes for fevers, body pains and rheumatism while the leafy twigs and fruits have been reportedly used for its aphrodisiac activity.[2,3] The antiviral activity of the chloroform and methanol extracts of *S. jollyanum* on cowpea mosaic and polio-viruses has also been reported.[8,9] According to previous studies, the leaves as well as the roots and fruits of the plant possess significant anti-inflammatory, anti-angiogenic and analgesic properties.[3,4,7] They have also been found to be potent against polio type-2 viruses.[4] The plant extract is popular in chronic wound dressing, feverish conditions, cough and as an aphrodisiac.[2,3,6]

The ethylacetate fraction of *Sphenocentrum jollyanum* root has been reported useful ethno-medicinally in the management of several diseases. Hence, this study formulated ointments and evaluated it for antifungal activity. The ethylacetate fraction of the root of *S. jollyanum* was tested against different fungal species. The fungal species understudied were *Candida valida*, *Candida pseudotropicalis*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida albicans*, *Trichophyton rubrum*, *Trichophyton interdigitalis*, *Trichophyton tonsurans*, *Epidermophyton flocossum*, *Aspergilus niger* and *Fusarium spp*.
The rationale for this study is to assess the possibility of identifying and formulating safe, cost effective and potent natural products of plant origin from Nigeria ethno-medical practice that may find use as templates for new antifungal/anticandidal drug discovery.

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Materials and Methods

Plant material

Sphenocentrum jollyanum root was collected from Itak Ikot Akap in Ikono Local Government Area of Akwa Ibom State and authentication of the plant material was by Mr. Adeniji of the Forest Herbarium Ibadan where voucher specimen (FHI 111156) was also deposited.

Preparation of the extract

Eight kilograms (8 kg) of the air dried chopped *Sphenocentrum jollyanum* roots were reduced to smaller particles to obtain a coarse powder which was macerated in 70% w/v methanol for 72 h, decanted, and then macerated for another 48 h, to ensure thorough extraction. The methanol filtrates were combined and concentrated in vacuo using a rotary evaporator with the resulting residue weighed to obtain the yield. Approximately 62.85 g of the residue was later dissolved in 70% w/v methanol and partitioned with equal volume of ethyl acetate. This was repeated three times to allow complete fractionation. The ethylacetate fractions were collected, combined, concentrated *in vacuo* and kept in the refrigerator until ready for use.

Formulation of ointment

The dried ethylacetate fraction was incorporated into Simple Ointment BP to produce formulations yielding 50, 100, and 200 mg/mL *Sphenocentrum jollyanum* ointments by levigation.[16] The preparation was placed in an ointment jar and stored in the refrigerator.

In-vitro antimicrobial assay

The ethylacetate fraction of the *Sphenocentrum jollyanum root* was tested for antimicrobial activity using selected micro-organisms by the standard agar well diffusion bioassay method.[17, 18] Molten sterile nutrient broth (100 mL) was cooled to 50°C and inoculated with 1 mL of overnight culture of test organism, 20 mL quantities of the inoculated medium were each poured into a 9 cm agar plates and allowed to set. Equidistant wells of 6 mm were bored into the agar using a sterile cork borer and the wells were filled with appropriate concentrations of the extracts under test. All extracts were reconstituted in 50% v/v aqueous methanol, which was used as a control while Tioconazole, was used as reference standard. The plates were left at room temperature for 45 min and then incubated at 25°C. After an incubation period of 48 h, the diameters of the zones of inhibition were measured.

Minimum Inhibitory Concentration Determination

The extract (2 g) was weighed and dissolved in 10 mL of methanol to give 200 mg/mL. This was serially diluted to obtain up to 6.25 mg/mL.

The Sabouraud dextrose agar was poured into a plate and allowed to set at 45 – 50°C. A portion (0.2 mL) of the 1/100 mL dilution of the organism was uniformly applied on the surface of the Sabouraud dextrose agar and wells bored. The graded concentrations of the extract were placed into the wells. The positive and negative controls (tioconazole and methanol) were placed into their respective wells. The plates were left to stand for two hours to allow the pre-diffusion process. Incubation was at 26 –28°C for 48 hours. Clear zones were observed and the diameters measured and recorded accordingly. The work was done in triplicate and the mean diameters were determined. The least concentration that showed the clear zones of inhibition was taken as the minimum inhibitory concentration.

Animal studies

Albino rats (180 - 200 g) were purchased from the animal house of the University of Ibadan. In a preliminary pilot study, two albino rats were anaesthetized using thiopental sodium (50 mg/kg). Parts of the skin of the rats were shaved, bruised and exposed. An overnight culture of *Candida albicans* was applied on the exposed bruise of each animal using sterile swab stick.

The rats were dressed using sterile gauze, bandaged and kept in a cage. For the culturing method, a sterile swab was rubbed on the infected skin surface. The swab was streaked on an agar culture medium. The culture was incubated at 37°C for forty-eight hours, to allow development of yeast or bacterial colonies. Twenty-five albino rats (180 – 200 g) were grouped into five groups and fed with standard feeds and water *ad libitum*. Thiopental sodium injection served as an anesthetic agent and was administered at a dose of 50 mg/kg body weight. The hair at the upper back side of the albino rat was carefully removed and the skin part exposed was bruised.

An overnight *Candida albicans* culture prepared was applied on the bruised skin parts of the albino rat and left for the next 2 days. The growth of *Candida albicans* was established on the second day. Each group were treated daily with graded doses of the formulated (50 mg/mL, 100 mg/mL, 2000 mg/mL) formulated ointment. The reference standards were tioconazole cream 1% and normal saline. The skin swabs samples were taken on days 0, 3, 6, 9 and 12 respectively and placed on a tryptone soya broth for 3 days. Appropriate serial dilution was done and the fungal loads were counted using colony counter.

Ethical consideration

The experimental protocol was in conformity with the Ethics Committee Guidelines of the University of Ibadan as well as the US guidelines of internationally accepted principles for laboratory animal use and care. Animals were also treated in agreement with OECD guidelines for testing of chemicals (NIH publication #85-23, 1985).

Statistical Analysis

Data obtained from the study were analysed using Two-way ANOVA (GraphPad Prism® 6 (GraphPad Software Inc., San Diego, USA). *P* values less than 0.05 were considered significant.

Results and Discussion

The minimum inhibitory concentration of the ethyl acetate fraction of the root of *Sphenocentrum jollyanum* shows that it was active at graded concentrations (200 mg/mL - 6.25 mg/mL) on test organisms (Table 1). Sensitivity testing results for tioconazole (reference standard) revealed wider zones of inhibition while methanol was a negative control with no zone of inhibition.

The viscosity of each of the ethyl acetate fraction formulated ointment was also investigated and observed to be moderately viscous (Figure 2). The physical properties of the simple ointment revealed a smooth texture, bright yellow color and bland odor making it cosmetically acceptable.

The fungal load for the formulated ointment at graded concentrations (50 mg/mL, 100 mg/mL and 200 mg/mL) revealed a significant decrease of 18.20 ± 2.06, 71.40 ± 4.14 and 293.20 ± 21.99, respectively of which the lower doses tested were comparable to the reference standard (Tioconazole cream 1% at 17.40 ± 3.57 by day 12). Normal saline (negative control) revealed no significant decrease in fungal load in the *Candida* infected skin of the albino rats (Figure 1). However, the formulated ointment (50 mg/mL) was the most active with a fungal load percentage reduction of 97.55% comparable to 97.65% of the reference standard (Table 2). The observed significant reduction in fungal loads demonstrated by 50 mg/mL formulated ointment could be attributed to saturation whereby the ointment is able to mop-up several *Candida albicans* present in the infected skin of albino rats in that group. Considering enzyme-substrates activities it can be said that the enzymes were able to mop-up all the available substrates when the 50 mg/mL ointment was used while the reverse was the case with formulated ointment at other concentrations tested.

It was observed that the formulated ointment from ethyl acetate fraction of *Sphenocentrum jollyanum* root revealed antifungal activity against various strain of *Candida albicans*, dermatophytes and the plant fungi. This shows that the plant extracts may possess antifungal property which qualifies it as a potential template in antifungal drug discovery. This is supported by reports of other researchers, that medicinal plants or traditional medicine has a critical role in the

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Table 1: Antimicrobial screening of the ethylacetate fraction of the root of *Sphenocentrum jollyanum* using agar well diffusion method

	Conc. (mg/mL)	CV	CP	CT	CG	CK	CA	TR	TI	TT	EF	AN	FS
		Zones of Inhibitions (mm)											
1	200	21	21	23	20	12	20	10	21	19	21	22	21
2	100	19	19	19	18	10	18	-	19	17	19	20	18
3	50	16	17	17	16	-	14	-	17	15	17	18	17
4	25	15	14	15	14	-	12	-	14	13	14	14	14
5	12.5	13	12	12	12	-	10	-	12	12	12	12	12
6	6.25	10	10	10	10	-	-	-	10	10	10	10	10
7	-ve	-	-	-	-	-	-	-	-	-	-	-	-
8	TC	26	28	28	26	28	28	28	26	26	26	28	28

CV - *Candida valida*, CP - *Candida pseudotropicalis*, CT - *Candida tropicalis*, CG - *Candida glabrata*, CK - *Candida krusei*, CA - *Candida albicans, TR-Trichophyton rubrum, TI – Trichophyton interdigitalis, TT - Trichophyton tonsurans, EF - Epidermophyton flocossum, AN –Aspergilus niger*, FS - *Fusarium Spp* from yam, -ve: Negative control (Methanol), TC: 1% Tioconazole cream by Drugfield Pharmaceuticals).

Table 2: The percentage reduction of fungal load after 13 days of the formulated simple ointment, (200 mg/mL, 100 mg/mL, 50 mg/mL), tioconazole cream 0.10 mg/mL and normal saline.

Sample	Concentration mg/mL	% Fungal load reduction after 12 days
Formulated ointments	200	64.95
	100	90.66
	50	97.55
Tioconazole 1%	0.10	97.65
Normal saline	-	-43.13

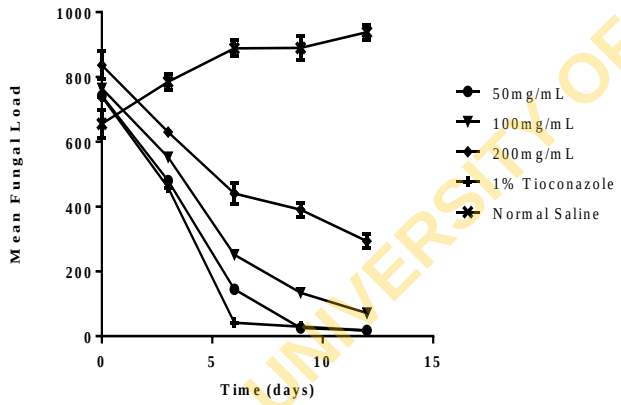

Figure 1: Mean fungal load versus number of days for rats treated with ethylacetate fraction formulated ointments

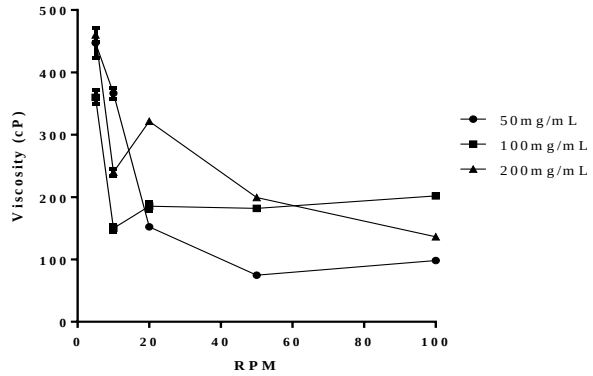

Figure 2: Viscosity of ointment formulations at different shear rates

provision of health care coverage for over 80% of the world population especially in the developing world.[10-12]

In the *in vivo* test of the antifungal activity of the root of *Sphenocentrum jollyanum*, it has been demonstrated that the ethyl acetate fractions of the methanol extract of the root of this plant have antifungal activity against different strains of *Candida*, dermatophytes and other plant fungi.

Of particular interest in this study, is the great significant reduction of the fungal load with the 50 mg/mL ethyl acetate fraction formulated ointment and similar pattern of reduction when compared to the reference standard (1% tioconazole cream) (Figure 1).

The species of *Candida*, dermatophytes, and plant fungi, are observed to be susceptible to formulations from *S. jollyanum* ranging from skin creams, antiseptics/disinfectants, antifungal vaginal creams, vaginal douches and vaginal pessaries. Antifungal infections are emerging health issues particularly in the tropics (*Candida albicans*, the most common species identified (50-60%), *Candida glabrata* (15-20%), *Candida parapsilosis* (10-20%), *Candida tropicalis* (6-12%), *Candida krusei* (1-3%), *Candida lusitaniae* (<5%), *Candida kefyr* (<5%), *Candida guilliermondi* (<5%), *Candida dubliniensis*, primarily recovered from patients infected with HIV.[13]

Further research on other activities of *S jollyanum* extracts and constituents against a wider range of fungi, and bacteria should be encouraged with the aim of drug discovery (systemic or topical) considering the relative safety from the toxicity studies earlier carried out[14] in addition to the relevant bioactive chemical constituents already identified and characterized.[4,15]

Conclusion

This study demonstrated that the formulated ointment from *Sphenocentrum jollyanum* root ethyl acetate fraction may possess anticandidal activities of varying degrees with evidence of significant reduction of *Candida* fungal loads at all doses tested. The formulated ointment may therefore, be effective in inhibiting the growth of

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Candida causative agents of infections. Interestingly, the formulated ointment at the lowest dose of 50 mg/mL revealed highest activity which suggests that the activity could be dose dependent and an indication that ointments formulated from *S. jollyanum*, under good manufacturing practices may be said to be potentially antifungal. *Sphenocentrum jollyanum* may therefore be a promising template in antifungal drug discovery.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Figure 3: Bruised and *Candida* infected skin area of the albino rat before treatment with the ethylacetate formulated simple ointment

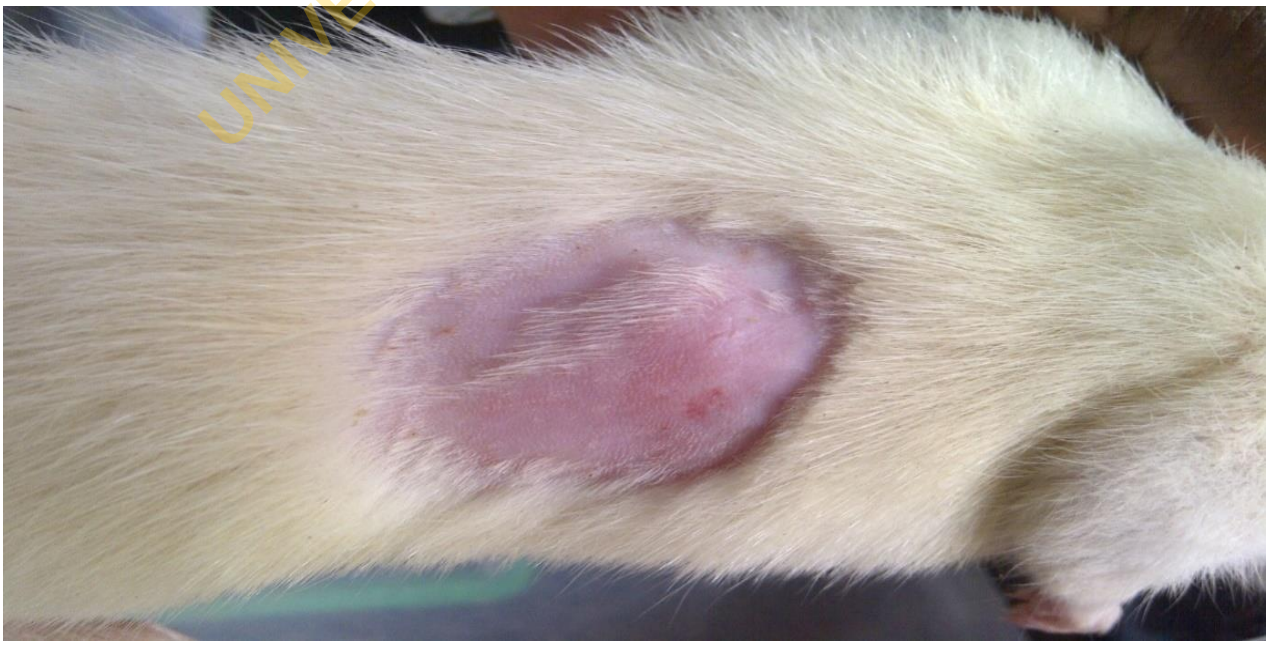

Figure 4: The skin area of the albino rat after treatment with the ethylacetate fraction formulated simple ointment

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References

1. Nia R, Paper DH, Essien EE, Iyadi KC, Bassey AIL, Antai AB, Franz G. Evaluation of the Anti-Angiogenic effects of *Sphenocentrum jollyanum* Pierre. Bioline international Official Site. *http: // www.bioline.org*. 2004.
2. Burkill HM. The Flora of West Tropical Africa. 1985; (4), 2nd Edn., Kew: Royal Botanic Gardens, London.
3. Iwu MM. Handbook of African Medicinal Plants. CRS Press, USA. 1993; 1-13.
4. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpenes constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmcol. 2006; (104):87-91.
5. Neuwinger HD. African Ethno-botany: Poisons and Drugs. Chapman and Hall, London, 1996; 631-632 p.
6. Dalziel JM. The useful plants of west tropical Africa. Crown Agents. London, 1985.
7. Odugbemi T. Medicinal plants by species names: Outlines and pictures of medicinal plants from Nigeria. 2016; Lagos University press.
8. Moody JO, Robert VA, Adeniji JA. Anti-viral effect of selected medicinal plants. I: *Diospyros barteri*, *D. monubutensis* and *Sphenocentrum jollyanum* on polio viruses. Niger J Nat Prod Med. 2002a; 6:4-6.
9. Moody JO, Robert VA, Hughes JA. Anti-viral activities of selected medicinal plants. II: Effect of extracts of *Diospyros monbutensis* and *Sphenocentrum jollyanum* on cowpea mosaic viruses. Pharm Biol 2002b; 40(5):342-345.
10. WHO. Traditional Medicines Growing Needs and Potentials. WHO Policy Perspectives on medicine. World Health Organization, Geneva. 2002; 1-6 p.
11. Scheiman D. The ancient and modern world units to fight HIV/AIDS in Tanga, Tanzania, 2002; Merck: Science in Africa online magazine.
12. Kala CP, Faroquee NA, Dhar U. Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Haranchial, India, J Biodiv Conserv. 2004; (13):453-469.
13. Vazquez JA, Sobel JD. Candidiasis. In Dismukes WE, Pappas PG, Sobel JD, editors. Clinical Mycology. New York: Oxford University Press. 2003. 143-187 p.
14. Mbaka GO, Adeyemi OO, Oremosu AA. Acute and sub-chronic toxicity Studies of the ethanol extract of the leaves of *Sphenocentrum jollyanum* (Menispermaceae). ABJNA ISSN 2151-7517, ISSN Online: 2010; 2151-7525.
15. Ajayi TO, Radhakrishnan S, Nyong EE, Odeniyi MA, Moody JO, Ross SA. Two New Phytoecdysteroids From *Sphenocentrum jollyanum* Pierre Root. Steroids, 2019;150 https://doi.org/10.1016/j.steroids.2019.108456
16. Nyong EE, Odeniyi MA, Moody JO. *In vitro* and *in vivo* Antimicrobial Evaluation of Alkaloidal extracts of *Enantia chlorantha* stem bark and their formulated ointments. Acta Pol. Pharm. ñ Drug Research, 2015; 72(1):147-152.
17. Hugo WB, Russell AD. Pharmaceutical Microbiology, 3rd edn. Blackwell, Oxford 1983. 167 p.
18. Ogundipe OO, Moody JO, Houghton PJ, Odelola HA. Bioactive chemicals constituents from *Alchornea laxiflora* (benth) Pax and Hoffmann J. Ethnopharmacol. 2001; 74:275-280.