IN-VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF OINTMENT CONTAINING *Physcia grisea* EXTRACT ON *Candida albicans*

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ABSTRACT

The in-vitro evaluation of antimicrobial activity of ointment containing Physcia grisea extract on clinical isolates of Candida albicans was carried out using Agar Cup Diffusion Technique. The result of the in vitro evaluation showed that P. grisea ointment has antifungal activity on C. albicans. The efficacy of the P. grisea ointment was also compared with tioconazole ointment which is a synthetic antifungal cream. The results of the comparative test showed that P. grisea ointment has a moderate activity on the C. albicans. This means that in the treatment of candidiasis, ointment containing P. grisea may be used, if properly utilized.

Keywords: Antimicrobial activity, *Physcia grisea* ointment, *Candida albicans*

INTRODUCTION

In many parts of the world, the use of plant products in treating various infections and disorders have been will documented (Ivoke, 2005). Quinine and penicillin drugs are good examples of the medicinal products from plants that have been used successfully in the treatment of human infections. Thus, there is a need to investigate plants with medicinal properties. Virtually, all phyla from thallophytes to the higher phyla have come under investigation (Chah *et al*., 2005). Lichens are thallophytes with abundant antimicrobial substances. *P. grisea* is lichen that its diversity in medicinal uses has received early attention in studies carried out on HIV/AIDS patients (Eze *et al.,* 2009). Available information on *P. grisea* showed that it has good antifungal and antibacterial property (Eze, 2007).

P. grisea is lichen found on walls, rocks and trees, attached by short threads which grow from the underside and are white with black tips. The plant is light grey or slightly brownish grey, and is almost always covered, at least near the tips of the lobes with a very fine white powder (Nicholson, 1996). *P. grisea* possesses a wide broad spectrum of antimicrobial actions and could represent a novel source of antifungal drugs belonging to a wide range of structural classes that can be used in the treatment of candidiasis.

Candidiasis is an opportunistic fungal infection in persons with an underling pathological process or deficiency state (Sobel, 1986). It is caused by oval budding yeast that produces a pseudomycelium in culture, tissues and exudates known as *Candida albicans.* Most superficial *C. albicans* infections are treated with antifungal ointments despite their potential toxicity on humans and other shortcomings because of the antifungal compounds used in their productions. This study is aimed at developing a better ointment containing *P. grisea* extract that has little or no toxicity on humans which will be used in the treatment of candidiasis.

MATERIALS AND METHODS

The test microorganism used for this experiment was clinical isolate of *C. albicans* obtained from the Department of Pharmaceutical Microbiology, University of

Nigeria, Nsukka. The standard drug for treatment of *C. albicans* was tioconazole ointment (1%) from Drugfield Pharmaceuticals, Nigeria, and the culture media were Sabouraud Dextrose agar and Sabouraud Dextrose broth.

Sources of Samples: The lichen, *P. grisea* used for this work was obtained from the back of *Dialum guinense* tree in Ezimo-Uno, Udenu LGA, Enugu State. The *P. grisea* was identified (Nicholson, 1996) in the Botany Department, University of Nigeria Nsukka.

Preparation of Antimicrobial Ointment using *P. grisea* Extract: This was prepared using soft white paraffin as the ointment base, *P. grisea* extract as the active ingredient and dimethyl sulphoxide (DMSO) as the solvent. One gram of *P. grisea* extract was dissolved in 10 ml of DMSO to get a concentration of 100 mg/ml. The solution was gradually added with continuous stirring into 15 g of soft paraffin on a clean tile, until they were properly mixed together. The prepared *P. grisea* ointment was packaged into a collapsible tube, sealed and labelled as *P. grisea* ointment.

Susceptibility of *C. albicans*: The method used in evaluating the susceptibility of *C. albicans* to the *P. grisea* ointment was agar cup diffusion technique (Agboke *et al.,* 2005).

Determination of Inhibition Zone Diameter (IZD) of *P. grisea* Ointment on *C. albicans:* Sabourand dextrose agar (SDA) was prepared, sterilized and allowed to cool to 45 ^{0}C. About 0.5 ml of the suspension of *C. albicans* was pipetted into a sterile Petri dish. Twenty millilitre of the prepared SDA was poured into the plate and swirled three times in clockwise and in anticlockwise directions to ensure an even distribution of the test organism. It was then allowed to set or gel. Four millilitre of the ointment was dissolved in 2.5 ml of DMSO and 2 fold serial dilution of the dissolved ointment was done. Then, 4 ml of the *P. grisea* ointment solution was introduced into a sterile test tube and labelled one. Two millilitre of DMSO each was measured into three other sterile test tubes, labelled 2, 3, 4 respectively. Then, 2 ml from the test tube labelled one was taken aseptically and introduced into the test tube labelled 2 and mixed thoroughly; two millilitre from the solution labelled 2 was taken and introduced aseptically into the test tube labelled 3 and mixed thoroughly. Finally, 2 ml of solution 3 was collected aseptically and introduced into the test tube labelled 4 and thoroughly mixed. These different concentrations were prepared for the sensitivity testing.

The agar plate was divided into four sections using a marker and labelled 1, 2, 3, and 4 representing the different concentrations (40 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml) got from the serial dilution above. Using a cork borer of diameter 8 mm, cups were made at the centre of each of the four sections. Then, 0.05 ml each of the dilution of ointment was aseptically introduced into the cups staring from the lowest concentration to the highest. The plate was labelled and incubated at 35 ^{0}C for 24 hours and the zones of inhibition (IZD) were measured using a metre rule. The result was tabulated and a graph of IZD square against the logarithm of concentration was plotted. The MIC was determined from the graph.

Determination of IZD of Tioconazole Ointment on *C. albicans:* The preparation of agar, seeding of plate and making of agar cups were done using the same method as described in *P. grisea* ointment above. Ten gram of 100 mg/ml of tioconazole ointment was dissolved in 10 ml of DMSO to get the initial concentration and was labelled. Four millilitre of the dissolved tioconazole was introduced aseptically into a sterile test tube labelled 1 and thereafter, three-two fold serial dilution were carried out using three other test tubes labelled 2, 3 and 4. The inhibition zone diameter (IZD) was then determined and the MIC calculated.

Statistical Analysis: Data were analyzed using analysis of variance to determine if the differences between the IZD of the crude (*P. grisea* ointment) and the standard (tioconazole ointment) were significant (Genstat, 2003).

RESULTS AND DISCUSSION

The sensitivity of *C. albicans* to *P. grisea* ointment indicated that *C. albicans* was moderately sensitive to *P. grisea* ointment when compared with tioconazole ointment (Table 1).

Table 1: Sensitivity of *C. albicans* to antimicrobial agents

Antimicrobial Agent	*C. albicans*
***P. grisea* ointment**	++
Tioconazole ointment	+++

++ = C. albicans was moderately sensitive to P. grisea ointment; +++ = C. albicans was highly sensitive to tioconazole ointment.

The MIC of *P. grisea* ointment was 0.0447 mg/ml while that of the tioconazole ointment was 0.0063 mg/ml (Tables 2 and 3). The results of the analysis of variance showed that the differences in the IZDs were statistically significant ($P < 0.05$).

Worldwide increase in the incidence of candidiasis has been reported as well as increase in the resistance of some species of candida to different antifungal agents used in medical practice (Eze *et al.,* 2009). This may have resulted from prolonged use and adaptability of the candida species to antimicrobials or antifungal agents used in the treatment of *Candida* infections. The challenge has been to develop a low cost antifungal drug that will be effective and with stable antifungal activities that will be used for the treatment of candidiasis. One approach may be the *in-vitro* evaluation of medicinal plant derived ointments. The use of such crude drug therapy may broaden the antifungal spectrum, attain fungicidal activity and lower the risk of resistance (Polak, 1990). Besides, medicinal plants have provided opportunities for new drugs because of their matched less availabilities of chemical diversity (Abad *et al*., 2007). Quinine, penicillin, cephalosporin and several others are good examples of plant-derived drugs that have been used effectively in the treatment of microbial infections of man and animals. Thus, herbal medicinal products are becoming increasingly popular (Brevoort, 1998; Eisneber *et al.,* 1998). In this case, *P. grisea* could represent a led source of antimicrobial drugs. However, only little work has been done on *P. grisea*.

In this study, the *in vitro* evaluation of ointment containing *P. grisea* ointment showed a reasonable antimicrobial activity on *C. albicans*, though comparatively lesser in action than the standard antimicrobial ointment (ticoconazole ointment) used ($P < 0.05$). The differences may have resulted from the crude nature of *P. grisea* ointment. This can also be seen in the MIC of *P. grisea* ointment which was higher than that of tioconazole with higher concentration of active ingredient (Tables 2 and 3). However, the IZDs of the *P. grisea* ointment were statistically significant ($P < 0.05$). Thus, plant derived chemotherapentic agent like *P. grisea* ointment if properly harnessed may give alternative treatment option in managing candidiasis and other superficial fungal infections. Besides, treatment with *P. grisea* can help to maintain or restore balance of the normal flora since it has both antifungal and antibacterial properties (Eze, 2007). Therefore, *P. grisea* ointment may give reliable therapeutic effect in the treatment of candidiasis caused by *C. albicans* if its toxicity on cells and tissues is suitable for human application in clinical settings.

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Table 2: Effects of different concentrations of *P. grisea* ointment on inhibition zone diameter of *C. albicans*

Concentration (mg/ml)	IZD (mm)	Logarithm of Concentration (mg/ml)	IZD^2 (mm^2)	MIC (mg/ml)
40.00	10.00	1.602	100.000	0.0447
20.00	9.00	1.301	81.000	
10.00	8.00	1.00	64.000	
5.00	7.00	0.699	49.000	

Values are means of three replicates from three trails after 24 hours of incubation

Table 3: Effects of different concentrations of tioconazole ointment on inhibition zone diameter of *C. albicans*

Concentration (mg/ml)	IZD (mm)	Logarithm of Concentration (mg/ml)	IZD^2 (mm^2)	MIC (mg/ml)
10.00	21.00	1.000	441.00	0.0063
5.00	19.00	0.699	361.00	
2.50	17.00	0.398	289.00	
1.25	16.00	0.097	256.00	

Values are means of three replicates from three trails after 24 hours of incubation.

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