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SENSITIVITY PATTERN OF CLINICAL ISOLATES OF *CANDIDA ALBICANS* FROM HIV/AIDS PATIENTS TO COMBINED *PHYSCIA GRISEA* EXTRACT AND TIOCONAZOLE

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ABSTRACT

This study was carried out to investigate the sensitivity pattern of clinical isolates of *C. albicans* from HIV/AIDS patients to combined *P. grisea* extract and tioconazole. Twenty isolates of *C. albican*s were obtained from high vaginal swab (HVS) from HIV/AIDS patients in Bishop Shanahan Hospital, Nsukka after their confirmatory test. The sensitivity pattern of these isolates to *P. grisea*, tioconazole, and *P. grisea* combined with tioconazole was carried out using agar diffusion technique. The result of the *in vitro* study showed that *P. grisea* extract decreases the MIC of tioconazole thereby increasing the efficacy of the agent. The test microorganism, *C. albicans* was highly sensitive to *P. grisea* combined with tioconazole but less sensitive to the *P. grisea* extract alone. Overall, the antimicrobial activity of both the single and combined agents was found to be concentration dependent. This shows that in the treatment of candidiasis in HIV/AIDS patients, *P. grisea* extract combined with tioconazole may be used.

KEY WORDS: Sensitivity, Clinical isolates, *Candida albicans*, *Physcia grisea* and Tioconazole

INTRODUCTION

Over the centuries, plants have served man as sources of drugs for the treatment of microbial infections. At present, medicinal plants are increasingly being projected as suitable alternative source of antimicrobial agents (Esimone *et al,* 2005). Thus, medicinal plants have provided opportunities for new drugs because of their matched less availabilities of chemical diversity (Abad *et al* 2007). Virtually, all phyla from thallophytes to the higher phyla have come under investigations (Chah *et al*, 2005). Lichens are thallophytes with abundant antimicrobial substances and is thus worthy of further studies hence this work.

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. The colour develops a greenish tinge when the plant is wetted (Nicholson, 1966). Since treatment of candidiasis with fungicidal compounds such as nystatin and amphotericin B has toxicity on humans and other shortcomings, plant-derived chemotherapeutic agents could give better treatment options in managing fungal infections at large and candidiasis in particular (Nweze *et al,* 2005).This study looked at the antifungal activities of the lichen, *P. grisea* in both single and in combined cases.

MATERIALS AND METHODS

Test organisms: the test microorganisms used for this experiment were clinical isolates of *C. albicans* from twenty HIV/AIDS patients.

Reagents: The following reagents were used: tioconazole (Drugfield Pharmaceuticals, Nigeria), *P. grisea* extract. The culture media were Sabouraud agar, Sabouraud broth, nutrient agar and nutrient broth (Oxoid).

Sources of Samples

The lichen, *P. grisea* used for this work was obtained from the back of *Dialum guinense* tree in Ezimo, Udenu Local Government Area of Enugu State. The *P. grisea* was identified in the Botany Department, University of Nigeria, Nsukka.

Preliminary Sensitivity Test

The preliminary sensitivity tests of the *P. grisea* extract both in a single and in a combined dose, were evaluated by the bore plate and agar diffusion method as described by Agboke *et al* (2005) to determine the sensitivity pattern of the isolates of *C. albicans*.

Determination of the IZD of the Extracts on *C. albicans*

The procedure used for the determination of MIC of the *P. grisea* extract is as follows:

Sterile Petri dishes were aseptically seeded with 0.1 ml of freshly prepared suspension of *C. albicans* using a sterile pipette. A 20 ml aliquot of a sterile molten Sabouraud agar at 45 ^{0}C in McCartney bottle was poured into each plate and swirled clockwise and anti-clockwise for even distribution of the organism. After solidifying, the agar plates were marked into four sections representing the four two-fold dilution of the extract and labelled 1-4 with an indelible marker. Using a sterile 6 mm cork-borer, cups were made in each of the four divisions. The two fold dilutions of the *P. grisea*

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extracts were aseptically added in the cups using standard sterile dropper starting with the highest concentration of the *P. grisea* extract to that with lowest concentration of the extract.

The plates were incubated at 37 ^{0}C for 24 hours and then, the zones of inhibition were measured. This was repeated and the average values of the zones of inhibition were determined.

The graph of the inhibition zone diameter square against the logarithm of the concentrations of the dilutions used was plotted. The MIC of the extract was then determined from the graph.

Determination of IZD of Tioconazole

A 250 mg of tioconazole was dissolved in 10 ml of sterile distilled water to make 25 mg/ml solution of the drug solution. Then, three two-fold serial dilutions were made from the 1 in 10 dilution and their concentrations noted.

A 0.1 ml of culture suspension of *C. albicans* was aseptically seeded in the sterile Petri dish using a sterile pipette. A 20 ml quantity of sterile molten Sabouraud agar in a McCartney bottle was poured into the seeded plate. This was swirled both clockwise and anticlockwise to obtain an even distribution of the organism in the agar. After solidifying, the agar plates were marked into four sections and numbered 1-4. Using a sterile 6 mm cork-borer, a cup was made in each of the four sections in the agar plate.

The dilutions of the tioconazole were aseptically added into the cups using a sterile dropper starting with the highest concentration of the tioconazole to the lowest concentration.

The plates were incubated at 37 ^{0}C for 24 hours and the zones of inhibitions determined. The graph of the inhibition diameter against the logarithm of the concentrations of the dilutions used was plotted and the MIC of the tioconazole calculated.

Determination of IZD of Combined *P. grisea* extract and tioconazole

The seeding, preparation of agar and formation of cups were done as described above. Then four two fold dilutions of tioconazole and *P. grisea* extract were also done and labelled 1-4 as described above. The four two fold dilutions of the extract were first dropped in the holes using sterile pipette according to the labelled numbers. Then, the same dilutions of tioconazole according to the labelling were also dropped on already dropped extract. The plates were then incubated at 37 ^{0}C for 24 hours and the zones of inhibitions were determined.

The graph of the inhibition zone diameter square against the logarithm of the concentrations of the dilutions used was plotted. The MIC of the combined agents was calculated from the graph.

Statistical Analysis

Data were analyzed by analysis of variance to determine whether differences in the mean diameters of the zones of inhibitions were statistically significant (Genstat, 2003).

RESULTS

The results of the culture and sensitivity tests of *P. grisea*, and tioconazole both in single and in combined form are presented in table 1.

The preliminary sensitivity test revealed that *C. albicans* was highly sensitive to combined *P. grisea* extract and ticonozole. These observations are consistent with the findings of other workers. Esimone *et al*, (2005) have extensively investigated on medicinal plants. Their findings revealed that medicinal plants can be projected as suitable alternative sources of antimicrobial agents.

Table 1: Table of culture and sensitivity tests.

a)	Antimicrobial Agents	*C. albicans*
1	*P. grisea* extract	**+**
2	Commercial tioconazole	**++**
3	Combined *P. grisea*	
4	Extract and tioconazole	**+++**

+ *C. albicans* was moderately sensitive to the *P. grisea* extract used

+ + *C. albicans* were intermediately sensitive to tioconazole.

+ + + *C. albicans* was highly sensitive to the combined *P. grisea* extract and tioconazole

The minimum inhibitory concentrations (MICs) were calculated from the graph of inhibition zone diameter square against the log concentrations of the agents used. The minimum inhibitory concentration (MIC) of the antimicrobial agents used is shown in Table 2. The results showed that the MIC of the *P. grisea* extract on *C. albicans* is 3.13 (mg/ml), the MIC of tioconazole on *C. albicans* is 0.020 (mg/ml) and the MIC of the combined *P. grisea* extract and tioconazole on *C. albicans* is 0.0063 (mg/ml).

Table 2: Table of MIC'S of *P. grisea on C. albican,* tioconazole on *C. albican and* combined *P. grisea* and tioconazole on *C. albican.*

S/N	Agents	Organism	MIC (Mg/ml)
1	*P. grisea*	*C. albicans*	3.130
2	Tioconazole	*C. albicans*	0.020
3	Combined *P. grisea* and tioconazole	*C. albicans*	0.0063

The reductions in MICs of the combined *P. grisea* and standard agents were in line with Ofokansi and Esimone (2005). Their findings revealed that the application of lichen extract (*Ramalina farinacea*) was generally better, in terms of rapidity of action when combined with standard drugs.

Table 3: Table of means values of concentration, zones of inhibition and log concentration of *P. grisea* extract (P) on *C. albicans*

s/n	Conc mg/ml	Zones of inhibition (cm)	Zones of inhibition (mm)	Log of conc (mg/ml)	$1ZD^2$
1	25.00	2.40	24.00	1.398	576
2	12.50	2.20	22.00	1.097	484
3	6.25	1.80	18.00	0.796	324
4	3.125	0.00	0.00	0.495	0.0

Table 4: Table of means values of concentration, zones of inhibition and log concentration of tioconazole (T) on *C. albicans*

S/n	Conc. (mg/ml)	Zones of inhibition (cm)	Zones of inhibition (mm)	Log of Conc (mg/ml)	$1ZD^2$
1	25.00	3.60	36.00	1.398	1296
2	12.50	3.40	34.00	1.097	1156
3	6.25	3.20	32.00	0.796	1024
4	3.125	2.80	28.00	0.495	784

Table 5: Table of means values of concentration, zones of inhibition and log concentration of the combined *P. grisea* extract and tioconazole (PT) on *C. albicans*

S/n	Conc. (mg/ml)	Zones of inhibition (cm)	Zones of inhibition (mg/ml)	Log of conc (mg/ml)	$1ZD^2$
1	50.00	3.8	38.00	1.699	1444
2	25.00	3.6	36.00	1.398	1296
3	12.50	3.4	34.00	1.097	1156
4	6.25	3.0	30.00	0.796	900

The result of the analysis of variance of concentration (conc) and the inhibition zone diameter (IZD) showed that the IZDs were statistically significant as shown in Table 6

Table 6: Table of analysis of variance of inhibition zone diameter of *P. grisea,* ticonazole and *P. grisea* combined with tioconazole

Variate: IZD_

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
AGENT	2	1696.3333	848.1667	5356.84	<.001
CONC	2	647.3333	215.7778	1362.81	<.001
Agent CONC	6	227.6667	37.9444	239.65	<.001
Residual	12	1.9000	0.1583		
Total	23	890.839583			

Variate: IZD_

Grand mean 27.83

AGENT	P.	PT.	T.
	16.00	34.75	32.75

CONC	A	B	C	D
	32.67	31.33	28.00	19.33

Agent CONC	A	B	C	D
P	24.00	22.00	18.00	0.00
PT	38.00	37.00	34.00	30.00
T	36.00	35.00	32.00	28.00

*** Standard errors of differences of means ***

Table	AGENT	CONC	AGENT CONC
rep.	8	6	2
d.f.	12	12	12
s.e.d.	0.199	0.199	0.398

*** Least significant differences of means (5% level) ***

Table	AGENT	CONC_	AGENT CONC_
rep.	8	6	2
d.f.	12	12	12
l.s.d.	0.433	0.501	0.867

DISCUSSIONS AND CONCLUSION

Extensive use of antimicrobial drugs, has favoured the emergence of resistant strains. Thus, overuse and misuse of antimicrobials have lead to the death of sensitive strains leaving resistant strains to survive, multiply, and infect new hosts (Cheesbrough, 1985).

At present, a worldwide increase in the incidence of candidiasis has been observed as well as a rise in the resistance of some species of *Candida* to different antifungal agents used in medical practice. This is why our society has witnessed a rise in the incidence of life threatening *Candida* infections. The challenge has been to develop effective drugs for the treatment of candidiasis; considering the increase in opportunistic fungal infections in HIV/AIDS patients and in the others who are immuno compromised.

In rational drug therapy, the concurrent administration of two or more antimicrobial agents is often essential and sometimes mandatory in order to achieve the desired therapeutic (Attama et. al, 2005 and Aguwa, 1996). This is why the *P. grisea* extract was combined with other antimicrobial agent like tioconazole in this work.

The combinations of these agents could lead to extension of the anti-microbial (antibacterial and antifungal) spectrum because it is possible that two or more infectious microorganisms with different sensitivity patterns have to be dealt with as in HIV cases (Agboke *et al,* 2005). This can be seen in combined activities carried out in this work. The treatment of infections with two antimicrobial agents enhances the biostatic or biocidal effect of the antimicrobial agents on the organisms (Hugo and Russel, 1993). Besides, treatment with some antimicrobial agents like *P. grisea* can help to maintain or restore balance of the normal flora since it has both antifungal and antibacterial properties (Eze, 2007).

In this study, the *P. grisea* extract was found to have antifungal property but not as that of the standard agent. The differences between the zones of inhibition of the extract and that of standard drug (tioconazole) were not much. What made the standard drug more effective may be other agents added during their formulations while the low zone of inhibition of the extract may be because of the solvent used both in single dose and in a combined dose. From the results obtained, there was a decrease in the MIC of the combined case. Thus, since the decrease in the MIC increases the efficacy of the antimicrobial agents, it means that the combination of the extract with tioconazole will give more reliable therapeutic effect in the treatment of candidiasis caused by C*. albicans*.

It is recommended that more studies should be done on *P. grisea* to evaluate its toxicity on human cells and tissues for suitable human application in clinical settings.

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