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Full Length Research Paper

Antifungal activity of aqueous and ethanolic extracts of *Picralima nitida* seeds on *Aspergillus flavus, Candida albicans* and *Microsporum canis*

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Aqueous and ethanolic extracts of *Picralima nitida seeds* were tested for their antifungal activities using *Aspergillus flavus, Candida albicans* and *Microsporum canis* as test organisms. Phytochemical analysis revealed the presence of some plant metabolites which have been reported to have antimicrobial effects. Assays were performed using extract concentrations of 25, 50, 100 and 200 mg/ml and the agar well diffusion technique was employed. Results obtained, revealed a significant difference (P<0.05) in inhibition zone diameter between *A. flavus,* and *C. albicans* and between *C. albicans* and *M. canis*. However, there was no significant difference (P>0.05) in inhibition zone diameter between *A. flavus* and *M. canis.* With the aqueous seed extract, a significant difference (P<0.05) in inhibition zone diameter was observed between *A. flavus* and *C. albicans* and between *A. flavus* and *M. canis* but there was no significant difference (P>0.05) in inhibition zone diameter between *C. albicans* and *M. canis.* The inhibitory activity of the ethanolic extract on each test organism was compared with that of the aqueous extract and in all cases the observed difference was significant (P<0.05). These findings indicate the potentials of the seeds of *P. nitida* as panacea for some fungal infections.

Key words: Antifungal activity, aqueous extract, ethanolic extract, *Picralima nitida* seeds.

INTRODUCTION

Humans everywhere in the world are still plagued by a myriad of ailments and infections with microorganisms (including fungi) causing a good number of them. Inspite of the availability of drugs for treatment, diseases of microbial origin remain a scourge. The use of herbal medicine in the treatment of infection with microorganisms predates the introduction of antibiotics (Owoyale et al., 2005). Herbalists have claimed that certain ailments and infections which have defied western medicine can be cured with local herbs. As such they have used different plant parts in the treatment of various infections. Discovery of active principles in plants have given credence to the idea that integration of traditional medicine into the health care delivery would be very promising and should therefore be encouraged. Several medicinal plants have been screened for their activity on different species of microorganisms. Ibrahim and Osman (1995) screened *Cassia alata* from Malaysia for antimicrobial activity. Their report indicated that the plant demonstrated high activity against dermatophytic fungi, including *Microsporum canis* (MIC: 25 mg/ml). Owoyale et al. (2005) evaluated the antifungal and antibacterial activities of alcoholic extracts of *Senna alata* leaves. Wokoma et al. (2007) reported on the *in vitro* antifungal activity of *Allium sativum* and *Allium cepa* extracts against dermatophytes and yeast. The antimicrobial activity of ethanolic and aqueous extracts of *Sida acuta* on microorganisms from skin infections has been documented by Ekpo and Etim (2009).

Picralima comprises a single specie-*Picralima nitida and* belongs to the family Apocynaceae. It is a tree that grows up to 35 m tall with white latex in all parts. The leaves are opposite, simple and entire. It is restricted to Africa and throughout its distribution area, the seeds, stem bark and roots have a reputation as a febrifuge and remedy for malaria. They are also extensively used for

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pain relief and to treat chest and stomach problems, pneumonia and intestinal worms. The biological activities of the plant, *P. nitida* have been reported by researchers such as Nkere and Iroegbu (2005) and Okokon et al. (2007). Although as aforementioned, so much has been documented on the biological activities of the different parts of *P. nitida*, but there is a paucity of information on its activity against *Aspergillus flavus* (one of the causative agents of aspergillosis), *Candida albicans* (the most common cause of candidiasis) and *Microsporum canis*, a tinea causal organism.

This study was therefore designed to evaluate the inhibitory effect of crude ethanolic and aqueous extracts of the seeds of *P. nitida* on *A. flavus, C. albicans* and *M. canis*.

EXPERIMENTAL

Collection of plant materials

The seeds of *P. nitida* used in this study were obtained from Anua Obio in Uyo Local Government Area of Akwa Ibom State. The plant was authenticated at the Department of Botany and Ecological Studies, University of Uyo and a voucher specimen with herbarium number Ubulom UUH 875 (Uyo) was deposited in the herbarium of the same department for further referencing.

Preparation of extracts

The plant parts were first of all dried on laboratory tables at room temperature (27 ± 2°C). They were later pulverized using the crusher machine in the pilot plant unit of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. 100 g each of the pulverized seeds were macerated separately in distilled water and 50% ethanol, for 72 h, with periodic stirring. Each extract was filtered repeatedly using a sterile muslin cloth, cotton wool and filter paper. This was to get rid of the marc. All aqueous filtrates were concentrated using a lyophilizer at the freeze drying unit of NIPRD, Abuja. Ethanolic filtrates were concentrated in vacuo at 40°C, using a rotary evaporator. All extracts were stored in a refrigerator at 4°C until used for the experiments reported in this study. The percentage yield of each extract was also determined.

Phytochemical screening

The extracts of *P. nitida* seeds were screened for their phytochemical components, using the methods described by Harborne (1984), Evans (2002) and Sofowora (2006). The plant metabolites that were tested for were alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, phlobatannins, tannins and terpenes.

Test microorganisms

Test organisms *A. flavus, C. albicans* and *M. canis* used in this study were laboratory isolates obtained from the Department of Microbiology, University of Uyo. These fungal specimens were separately plated out on sterilized Sabouraud Dextrose Agar (Biomark). They were purified after isolation through repeated subculturing and characterized using the methods of Collins and Lyne (1970) and Cruickshank et al. (1975). They were subsequently stored in agar slants in the refrigerator at 4°C, until used for the experiments reported in this study.

Antifungal assay

The extracts were screened for antifungal property using the agar well diffusion technique. Standardized inoculum (1×10^6 cfu/ml) of each test fungus was spread on to sterile Sabouraud dextrose agar plate so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 6 mm was used to bore wells in the agar plates. From a stock solution, different concentrations of the extracts were prepared (200, 100, 50 and 2 5 mg/ml) and were separately introduced into the wells. Each extract concentration was replicated thrice. In all cases of preparation of extract solution, appropriate volumes of the solvent dimethyl sulphoxide (DMSO) were used to achieve solubilisation and required concentrations of the extracts. The plates were allowed to stand for 1 h for diffusion to take place and then were incubated at room temperature (27± 2°C), for 48 h. The external diameters of visible zones of growth inhibition were measured after incubation. A positive control was set up using separate plate. The assay in this case consisted of each test organism and the drug, ketoconazole at a concentration of 30 mg/ml. Negative control consisted of test organism each in separate plate and distilled water. These plates were also incubated at room temperature (27± 2°C) for 48 h. These controls were set up in triplicate too. The mean inhibition zone diameter was determined in each case.

Statistical analysis

Data obtained from this study were statistically analyzed using one way analysis of variance (ANOVA) and t-test.

RESULTS

The yield of the ethanolic and aqueous extracts of *P. nitida* seeds were 8.76 and 11.08% w/w respectively.

Phytochemical analysis of the extract of *P. nitida* seeds revealed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenes (Table 1).

The inhibitory activities of extracts of P. nitida on the fungal species tested are shown in Figure 1. Generally, the zone of inhibition increased with increase in concentration of extract. The aqueous extract of *P. nitida* seeds inhibited *A. flavus* with inhibition zone diameter ranging from 8 to 15 mm. For *C. albicans* it was 8 to 13 mm, but the least extract concentration (25 mg/ml) did not show any inhibition. For *M. canis,* the zone of inhibition ranged from 6 to 18 mm. The least extract concentration (25 mg/ml) also did not inhibit the growth of *M. canis.* However, significant inhibition was observed in all cases with ethanolic extract of *P.nitida* seeds. The zone of inhibition ranged from 11 to 20, 5 to 15 and 13 to 25 mm for *A. flavus, C. albicans* and *M. canis* respectively, but the least extract concentration (25 mg/ml) did not inhibit the growth of *M. canis*. Inhibition of test fungal species was observed in all cases with the use of the standard drug (ketoconazole) as positive control (Figure 1). No inhibition was observed in the negative control. In conclusion, the fungal species used in this study showed

Table 1. Phytochemical constituents of the extract of *P.nitida* seeds.

Phytochemical constituent	Test	Seed extract
Alkaloids	Dragendorff's	+
Anthraquinones		
free Anthraquinones	Borntrager's	-
combined anthraquinones	Borntrager's	-
Cardiac glycosides	Salkowski's	+
Flavonoids	Shinoda's reduction test	+
Saponins	Froth	+
Tannins	Ferric chloride	+
Phlobatannins	Hydrochlonc acid	-
Terpenes	Liebermann – Burchard	+

Legend: + = Detected, - = Not detected.

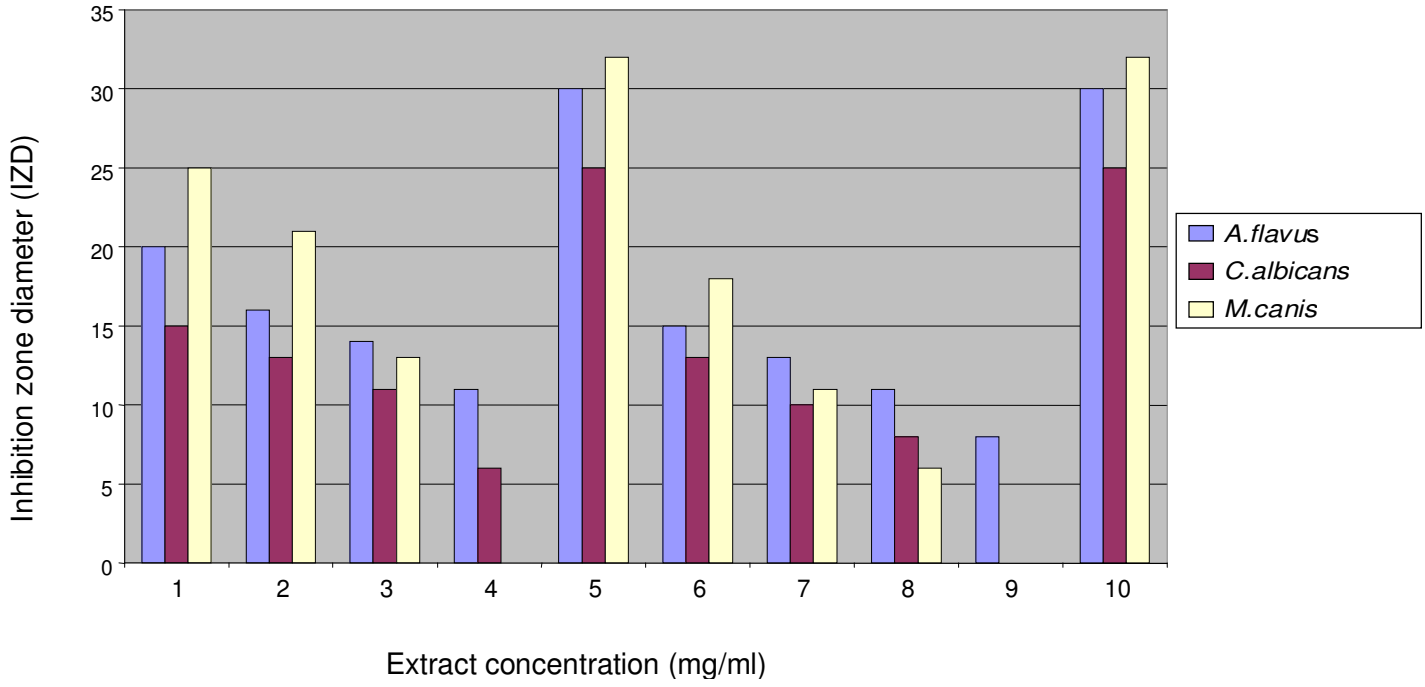

Figure 1. Effect of different concentrations of aqueous and ethanolic extracts of *P. nitida* seeds on test organisms. Key: 1 – 5 show results obtained due to the activity of ethanolic seed extracts. 6 – 10 show results obtained due to the activity of aqueous seed extracts. 1 = 200 mg/ml, 2 = 100 mg/ml, 3 = 50 mg/ml, 4 = 25 mg/ml, 5 = ketoconazole (30 mg/ml), 6 =200 mg/ml, 7 =100 mg/ml, 8 = 50 mg/ml, 9 =25 mg/ml, 10 = Ketoconazole (30 mg/ml).

variable sensitivity to extracts of *P. nitida*.

Results obtained from this study were statistically analyzed using one way ANOVA and t-test. ANOVA was used to determine the significance of difference in inhibitory activity of the extracts on the test organisms. T-test was used to compare the effect of the extracts on each test organism. Analysis of results of antifungal assays involving the ethanolic extract revealed that there was significant difference ($P<0.05$) in inhibition zone diameter between *A. flavus* and *C. albicans* and between *C. albicans and M. canis*. However, there was no significant difference ($P>0.05$) in inhibition zone diameter between *A. flavus* and *M. canis*. With the aqueous extract significant difference ($P<0.05$) in inhibition zone diameter IZD was observed between *A. flavus* and *C. albicans* and between *A. flavus* and *M. canis*. However, there was no significant difference ($P>0.05$) in inhibition zone diameter IZD between *C. albicans* and *M. canis.* Also the inhibitory activity of the ethanolic extract was compared with that of the aqueous extract, using t-test. For *A.*

flavus, the difference in activity between the ethanolic and aqueous extract was very significant ($P<0.05$). The difference between the inhibitory activity of the ethanolic and aqueous extracts was also significant ($P<0.05$), for *C. albicans* and *M. canis.*

DISCUSSION

The use of water and alcohol for extraction in this research is in consonance with folkloric practice. The percentage yield of extracts used in this study varied with the solvent used for extraction. However, the yield was low when compared with the amount of pulverized plant part used for extraction. This is attributable to the method of extraction employed (maceration). Maceration has been reported to result in low extract yield compared to soxhlet and other methods of extraction (Ibrahim et al., 1997), but it was preferred in this research because it does not require heating, thus preserving thermolabile components. The aqueous extract had higher yield than the ethanolic extract, suggesting a higher proportion of water-soluble components in the seeds of *P. nitida*.

Phytochemical screening revealed the presence of certain plant metabolites (Table 1), which have been reported in other studies to elicit inhibitory effect on microorganisms (Leven et al., 1979). These phytochemicals may have caused the observed inhibitory effect either singly or in synergy with each other. This requires further investigation. Also, Reyes–Chilpa et al. (2009), reported that flavonoids possess antifungal pro-perty. Earlier, Baba-Moussa et al. (1999) reported that tannins present in some plant species possess antifungal property. It is hoped that the elucidation of the structure of the active principle(s) and its/their subsequent use in antifungal investigations would give better results.

In this study, the ethanolic extract demonstrated higher activity on the test organisms than the aqueous extract. This suggests that the active principle(s) were more soluble in ethanol than in the aqueous medium.

It was observed that the fungal isolates used in this research exhibited varying degrees of susceptibility to the extracts. Thus, the values obtained for the zones of inhibition differed, for each test organism (Figure 1). These results corroborate the findings of Anani et al. (2000) and Rajakaruna et al. (2002). Results of this study also agree with the report of Karou et al. (2007). The difference in susceptibility observed in this study could be attributed to the inherent resistance factor of the test organisms among other factors (Ekpo and Etim, 2009). The significant difference ($P<0.05$) observed bet-ween the inhibitory activity of the ethanolic and aqueous extracts agrees with the research findings of Nkere and Iroegbu (2005) and Ekpo and Etim (2009).

This study has revealed that the extracts of *P. nitida* seeds hold antifungal potential, which can be further explored in the treatment and control of some fungal infections.

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