



Phytochemical Composition and Antifungal Activity of the Leaf and Root Extracts of *Costus afer*

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ABSTRACT

This study screened the phytochemical composition and antifungal activity of the leaf and root extracts of *Costus afer* on *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*. The root and leaves of *Costus afer* was collected along the farm in umuofocha Nekede, Owerri. Ten (10g) of the plant powder for leaf and root was extracted with ethanol by soxhlet method. All the extracts were subjected to standard phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites. The antifungal activity test of the plant extracts on *C.albicans*, *A.niger* and *A.flavus* were done using the agar well diffusion method. All the organisms tested were sensitive to both extracts, except *A. flavus* which was 100% resistant to the leaf extract of *Costus afer*. The results of this study showed antifungal potentials of the *Costus afer* leaves and roots and could be used for treatment of diseases associated with tested organisms except *A.flavus* which is resistant to leaf extract of *Costus afer*.

Keywords: Phytochemical; Antifungal; *Costus afer*; Root; Leaf

INTRODUCTION

Some strains of clinically important pathogens have increased in antifungal resistance, thus leading to the emergence of new fungi strains that are multi-resistant. Antifungal resistance has become a global concern. The relationship between human norms and plants is not limited to use of plant for food, shelter, and clothing alone but also include their use for ornamentation and health care. The clinical efficacy of many existing anti fungi's is being threatened by the emergence of multidrug-resistant pathogens.

Researchers are increasingly turning their attention to traditional medicine, looking for new leads to develop better drug against fungal infections. The increasing failure of some of these therapeutic agents has led to screening of these local herbs for potential therapeutic effects. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products either as pure compound or as standardized plant extracts provide opportunities for new drug leads.

Previous report has indicated that *Costus afer* extracts contains some bioactive ingredient that has inhibitory effects on some microorganisms [1]. *Costus afer* is commonly called bush cane. Information on the antimicrobial activities of many wetland plants in the tropics is necessary for utilization of such plants in the manufacture of drugs. This

study was designed to undertake phytochemical screening and in-vitro Antifungal activity of *Costus afer* leaves and root against clinically important pathogens.

MATERIALS AND METHODS

Plant Materials

Fresh leaves and roots of *Costus afer* were collected from the farm along Umuofocha Nekede, Owerri for the study and identified by a Botanist in the Department of Environmental Microbiology, Federal Polytechnic Nekede, Owerri Imo State.

Media Preparation

Sabouraud Dextrose Agar, was used and prepared according to the manufacturer's instructions and was dispensed into 3 sterile petri dishes for culture. The plates were streaked with the selected organisms and then covered to prevent contamination, examined for growth and pure cultures were used for identification. The isolates were observed according to the methods [2].

Source of Test Organisms

Clinical isolates of *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were obtained from Federal medical center Owerri and were confirmed using Gram staining and Biochemical test in the Microbiology Department Federal Polytechnic Nekede. The isolates were maintained on the sabouraud dextrose agar and stored until required.

Extraction of *Costus afer*

The extraction was carried out according to the method described by Harborne [3]. Some of the plant parts collected was air dried and some were sun-dried for 7 days. The plant materials were then ground into powder form using a mechanical grinder.

10g of the ground leaves was weighed out and 10g of the ground root was also weighed out. The extraction was done using the Soxhlet method after which the extracts were stored at 4°C until required. All extracts were filtered through Whatman (No. 1) filter paper and concentrated over a water bath using Soxhlet to recover the solvent.

Phytochemical Screening

The extract was subjected to phytochemical screening using standard procedure as described by Trease et al. [4].

Antifungal Bioassay

The antifungal activity test of the plant extracts was carried out using the agar well diffusion method [5]. Clinical isolates of *A. niger*, *A. Flavus* and *C. albicans* were inoculated separately on the surface of Mueller Hinton agar plates by surface spreading using a sterile cotton swab and each fungus spread over the entire surface of agar plate to obtain a uniform inoculum. Two (2) agar wells of 6mm diameter and 5mm depth were made on the solid agar on each plate using a sterile glass borer and then labeled. Each extract of 0.02 ml were then dispensed into their respective wells on the inoculated plates. After which the set up was allowed to stay for 24 hours. After 24 hours of incubation, the zones of inhibition were measured in millimeters (mm) using a transparent ruler. Oxoid [6] standard susceptibility range was used to classify zones of inhibition as either sensitive (>10 mm) or resistant (≤ 10mm).

RESULT AND DISCUSSION

Phytochemical Constituents of the Extracts

The results of the phytochemical screening of the extract reveal that Anthroquinone, Tannins, Anthranoid and Flavonoid were confirmed present in all the extracts from Ethanol. The test however revealed the absence of Saponin, Phylobatannins, and Alkaloids (Table 1). These constituents were determined by using the methods described by Trease et al [4].

Antifungal Activity of the Ethanolic Extract

The three isolated pathogenic fungi were tested in vitro against *Costus afer* extract on mycelia growth of *A. flavus*, *A. niger* and *C. albicans* (Table 2).

Table 1. Phytochemical constituents of the root and leaf extracts

Constituents	Leaf	Root
Saponins	-	-
Anthroquinone	+	+
Phenol	+	-
Phylobatannins	-	-
Alkaloids	-	-
Tannins	+	+
Cardiac Glycoside	-	+
Anthranoid	+	+
Flavonoid	+	+

Key: +Presence, -Absence

Table 2. Zones of inhibition (mean \pm standard error) of fungi in ethanolic extract. Zones of Inhibition (mm)

Organisms	Leaf	Root
<i>C. albicans</i>	17.00 \pm 0.00	11.00 \pm 0.00
<i>A. niger</i>	18.00 \pm 0.00	15.00 \pm 0.00
<i>A. flavus</i>	0.00 \pm 0.00	10.00 \pm 0.00

Discussion

The phytochemicals detected in the extracts (Anthroquinone, Phenol, Tannins, Cardiac Glycoside, Anthroid and Flavanoid) have been associated with antimicrobial activities [7]. The secondary metabolites could be responsible for antifungal activity of the plant extracts. Flavonoids have been shown to exhibit anti-inflammatory, anti-angonic, anti-allergic effects, and analgesic and antioxidant properties. Tannins have been found to form in irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis [8]. The results for the

antifungal screening have shown that all the extracts have antifungal activity on at least one of the three test organism, *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*. The results of the inhibition of fungi growth have shown that the root and leaf extracts of *Costus afer* were active against fungi. The ethanolic extracts had more inhibitory effect on *Aspergillus niger*, followed by *Candida albicans*, but the leaf extract did not have any activity on *Aspergillus flavus*. This results shows that the organism are sensitive to the extracts while *Aspergillus flavus* is resistant to the leaf extract of *Costus afer*. This result reveals that the extracts had an inhibitory effect on *Aspergillus niger*, and *Candida albicans* which was in line and corroborates an earlier report by Vasantharaj *et al.* [9]. This can be attributed to the fact that the leaf and root of *Costus afer* has antimycotic potentials. The leaf extract of *Costus afer* was resistant on *Aspergillus flavus*. This finding is at disparity with the report by Mary *et al.* [10]. It also reveals that *Aspergillus niger* had the highest inhibitory effect compared to other organisms. This corroborates with the work by Mary *et al.* and Vasantharaj *et al.* However, the leaf and root of *Costus afer* have shown their antimycotic effect as regards to the organisms used in this study.

CONCLUSION

The secondary metabolites detected in the extracts from the plant include Flavonoid, Anthroquinones, Tannins and Anthranoids which are all present in all the extracts. Saponins, Phenol and Phylobatannins were absent in both extracts. *Aspergillus niger* had the highest zone of inhibition in both extracts then followed by *Candida albicans* and finally *Aspergillus flavus* which was resistant to the Leaf extract of *Costus afer*. In other words, the extract exhibited antifungal effect on the organisms. This research may serve as a scientific basis and lend credence that *Costus afer* leaves and Roots have antifungal or antimycotic potentials.

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