

An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides*

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Abstract: Chloroform, ethanolic, methanolic, ethyl acetate and aqueous root extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* were investigated *in vitro* for antifungal activities against *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* species, *Microsporum audouinii* and *Trichophyton rubrum* using radial growth technique. The plant extracts inhibited the growth of all the test organisms. The minimum inhibitory concentration (MIC) of the extracts ranged between 0.03µg/ml and 0.07µg/ml while the minimum fungicidal concentration ranged between 0.04µg/ml and 0.08µg/ml. *Anogeissus leiocarpus* appears to be more effective as an antifungal agent than *Terminalia avicennioides*. Ethanolic extracts of the two plant roots were more effective than the methanolic, chloroform, or aqueous extracts against all the test fungi.

Key words: *Anogeissus leiocarpus*, *Terminalia avicennioides*, extracts, inhibitory, fungicidal, Nigeria

Introduction

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine (Apulu *et al.*, 1994). Over Sixty percent of Nigeria rural population depends on traditional medicine for their healthcare need (Apulu *et al.*, 1994). Medicinal properties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols which are the bioactive bases responsible for the antimicrobial property (Ebana *et al.*, 1993).

Medicinal plants contain pharmacologically active principles which over the years have been exploited in traditional medical practice for the treatment of various ailments (Adebanjo *et al.*, 1983). Villegas *et al.* (1988) studied the constituents of African medicinal plants and found that a petroleum ether extract of *Heteromopha trifoliata* leaves had antifungal properties against *Cladosporium cucumerinum*. A number of *Combretaceae* species tested for antifungal activity showed promising results. Among the seven species tested, *Combretum nigrum* was strongly active on dermatophytes (Baba-Moussa, 1998). Sodipo *et al.* (1991) carried out susceptibility test using *Garcinia kola* and found the seed to have fungistatic action on *Aspergillus niger*. Amer *et al.* (1991) conducted test on the effect of aqueous garlic extract on the growth of dermatophytes and the extract was found to have inhibitory effect on the growth of *Microsporum* species, *Trichophyton verrucosum* and *Trichophyton rubrum*. The ethanolic extract of *Tagetes lucida* showed antifungal activity against *Candida albicans* (Caceres *et al.*, 1998). Sodipo *et al.* (1991) reported that the es-

sential oil of *Aframomum melagolatus* fruit inhibited the mycelia growth of *Trichophyton mentagrophytes* at a pronounced rate but the inhibitory concentration obtained against *Aspergillus niger* was lower.

Since medicinal plants play a paramount role in the management of various ailments in rural communities, there is therefore, a need for scientific verification of their activities against fungi. Currently, there is little evidence on the antimicrobial properties of these plants under investigation against majority of fungi. The objective of this study was to assay the root extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* for their antifungal activities.

Materials and Methods

Plant material

The roots of *Anogeissus leiocarpus* and *Terminalia avicennioides* used in this study were collected in Bida, Niger State, Nigeria. The plants were identified by Muhammad Musa of the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria and Ibrahim Muazzami of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria; in accordance with the criteria stipulated by international committee for botanical nomenclature. Voucher specimens of *Anogeissus leiocarpus* and *Terminalia avicennioides* were deposited in the Herbarium at the Department of Biological Sciences, ABU, Zaria, Nigeria and NIPRD, Idu Abuja, Nigeria with the Herbarium numbers ABUH167 and NIPRDH5735, respectively.

Test microorganisms

The microorganisms used in this study were *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium* species

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and were isolated from patients at Federal Medical Centre, Bida, Niger State, Nigeria. *Microsporium audouinii* (PTCC 5060) and *Trichophyton rubrum* (PTCC 5069) were obtained from Microbiology Laboratory, Federal Polytechnic, Bida.

Drying of plant parts and preparation of the plant extracts

The plant parts were air dried at room temperature for two weeks. The dried plant parts were ground into powder form, sieved and packaged into polyethylene bags until when needed. 50g sample of the powdered dried roots of *Anogeissus leiocarpus* and *Terminalia avicennioides* were weighed separately into 200ml ethanol, methanol, chloroform, distilled water and ethyl acetate and then percolated for 24hrs. The extracts were then filtered through a millipore filter into different conical flasks. The extracts obtained were evaporated to dryness using a rotary evaporator. The extracts were assayed against the test organisms to determine the antifungal properties.

Determination of antifungal properties of extracts

The antifungal properties of the extracts were determined using the radial growth method (Banso *et al.*, 1999). 0.02mg/ml (w/v) of the extracts were introduced into McCartney bottles containing 18ml of sterile potato dextrose agar. The mixture was poured into different petri dishes and allowed to solidify. The plates were inoculated with 5mm diameter of the fungal culture. Control experiment was performed without the extracts. Plates were incubated at 25°C for 72 hours. Antifungal activities were expressed in terms of diameter of growth.

Determination of Minimum Inhibitory Concentration (MIC)

Various concentrations (0.01µg/ml, 0.02µg/ml, 0.03µg/ml, 0.06µg/ml and 0.08µg/ml) of the extracts were prepared. Each of these was added to 18ml of malt extract in test tubes. Each tube was then inoculated with 0.1ml of the spore suspension of *A. niger*, *A. fumigatus*, *Penicillin* species, *M. audouinii* and *T.*

rubrum diluted to give a final spore suspension of 10^6 spores per ml. The tubes were incubated at $28^\circ\text{C} \pm 2^\circ\text{C}$ and examined for growth after 7 days. The least concentration of the plant extracts that does permit any visible growth of the inoculated test organism in the broth medium was regarded as the MIC in each case. Control experiments were performed without the plant extracts.

Determination of Minimum Fungicidal Concentration (MFC) of the extracts

The contents of the tubes that showed no visible fungal growth or turbidity in the minimum inhibitory concentration experiment were cultured into fleshy prepared potato dextrose agar plate to assay for the fungicidal effect of the extracts. The plates containing the test organisms were incubated at 25°C for 15days. The minimum fungicidal concentration was regarded as the lowest concentration that did not yield any fungal growth on the solid medium used.

Results

Aqueous, ethanolic, methanolic, chloroform and ethyl acetate extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* exhibited antifungal activity against *A. niger*, *A. fumigatus*, *Penicillium* species, *M. audouinii* and *T. rubrum* (Tables 1 and 2). The largest growth diameters ranging from $10.5 \pm 0.2\text{mm}$ to $19.2 \pm 0.1\text{mm}$ were obtained from the assay containing aqueous extracts (Table 1), while the lowest ranges of mean diameters of growth ($5.2 \pm 0.2\text{mm}$ to $13.3 \pm 0.1\text{mm}$) were obtained with ethanolic extracts. *T. rubrum* appeared to be the most resistant to the effect of the root extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* while *Penicillium* species which had lower ranges of mean diameter of growth ($5.2 \pm 0.2\text{mm}$ to $14.0 \pm 0.3\text{mm}$) appeared to be the most susceptible (Table 1). Lower ranges of mean growth diameters ($5.2 \pm 0.2\text{mm}$ to $18.1 \pm 0.5\text{mm}$) were recorded against *Anogeissus leiocarpus* while higher ranges of mean growth diameters ($7.01 \pm 0.1\text{mm}$ to $19.2 \pm 0.1\text{mm}$) were obtained against *Terminalia avicennioides* and *Anogeissus leiocarpus*.

Table 1: Antifungal activities of root extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides*

Test organism	Mean diameter of growth (mm) ±SD										
	<i>Anogeissus leiocarpus</i>					<i>Terminalia avicennioides</i>					
	Co	EtOH	MeOH	CHCl ₃	Aq	EtOAc	EtOH	MeOH	CHCl ₃	Aq	EtOAc
<i>A. niger</i>	18.0±0.01	9.5±0.1	14.2±0.5	13.30	15.4±0.5	17.2±0.1	10.5±0.1	15.0±0.2	15.5	18.2±0.1	16.2±0.2
<i>A. fumigatus</i>	16.5±0.10	7.2±0.1	10.4±0.5	10.40	12.3±0.1	14.5±0.5	8.6±0.2	11.5±0.2	12.2	16.5±0.1	14.4±0.1
<i>Penicillium spp.</i>	17.0±0.02	5.2±0.2	10.2±0.6	9.40	10.5±0.2	13.0±0.5	7.4±0.1	10.5±0.1	10.0	14.0±0.3	12.5±0.1
<i>M. audouinii</i>	19.5±0.02	8.3±0.2	13.3±0.5	12.00	16.3±0.6	17.5±0.6	10.2±0.2	14.6±0.1	13.5	18.4±0.2	17.2±0.1
<i>T. rubrum</i>	20.0±0.10	12.4±0.2	15.2±0.7	14.30	14.5±0.4	18.1±0.5	13.3±0.1	16.2±0.1	16.0	19.2±0.1	16.8±0.1

Ethanol extract = EtOH; Methanolic extract = MeOH; Chloroform extract = CCl₃; Aqueous extract = Aq; Ethyl acetate extract = EtOAc; Control = Co; Griseofulvine antibiotic was assayed against all the test fungi as a positive control

The MIC of *Terminalia avicennioides* root extract against the test fungi ranged between 0.03µg/ml and 0.07µg/ml. Ethanol extract has the lowest MIC when tested against the test fungi while aqueous extract has the highest MIC (Table 2). The MIC of methanolic, chloroform and ethyl acetate extracts of the root of *Terminalia avicennioides* against *A. fumigatus* was 0.03µg/ml, 0.04µg/ml and 0.04µg/ml respectively (Table 2).

The minimum fungicidal concentration of the extracts of *T. avicennioides* proved to possess more fungicidal action against *Penicillium* species when the extract was assayed (Table 3). This was indicated by the low values obtained in assays with methanolic, chloroform, aqueous and ethyl acetate root extracts of *T. avicennioides* against *Penicillium* species (Table 3). Aqueous root extract of *T. avicennioides* possess the lowest fungicidal action against *A. niger* among the

Table 2: Minimum inhibitory concentration (MIC) of root extracts of *T. avicennioides* and *Anogeissus leiocarpus* against some fungi

Test organism	Minimum inhibitory concentration [µg/ml]									
	<i>Terminalia avicennioides</i>					<i>Anogeissus leiocarpus</i>				
	EtOH	MeOH	CHCl ₃	Aq	EtOAc	EtOH	MeOH	CHCl ₃	Aq	EtOAc
<i>A. niger</i>	0.05	0.04	0.05	0.06	0.05	0.04	0.03	0.04	0.05	0.04
<i>A. fumigatus</i>	0.03	0.03	0.04	0.05	0.04	0.03	0.03	0.04	0.04	0.03
<i>Penicillium spp.</i>	0.03	0.04	0.04	0.06	0.05	0.03	0.04	0.03	0.05	0.04
<i>M. audouinii</i>	0.05	0.04	0.05	0.07	0.06	0.03	0.04	0.04	0.06	0.05
<i>T. rubrum</i>	0.05	0.03	0.05	0.06	0.05	0.04	0.03	0.03	0.05	0.04

Ethanol extract = EtOH; Methanolic extract = MeOH; Chloroform extract = CCl₃; Aqueous extract = Aq; Ethyl acetate extract = EtOAc

The MIC of *Anogeissus leiocarpus* root extracts against the test fungi ranged between 0.3µg/ml and 0.06µg/ml. The MIC of ethanol, methanolic, chloroform, aqueous and ethyl acetate extracts of *Anogeissus leiocarpus* against *M. audouinii* were 0.03µg/ml, 0.06µg/ml and 0.05µg/ml respectively (Table 2).

extracts assayed against the organism with minimum fungicidal concentration of 0.08µg/ml (Table 3).

Methanolic extract of *A. leiocarpus* was the most active fungicidal against *A. niger* with minimum fungicidal concentration of 0.04µg/ml. Relatively higher value of minimum fungicidal concentration

were obtained in assays with ethanolic, chloroform, aqueous and ethylacetate root extracts of *A. leiocarpus* (Table 4)

When the broth culture of the extract and the test organisms used in the minimum inhibitory concentration tests were subcultured on a solid medium for the assessment of the minimum fungicidal concentration

Table 4: Minimum fungicidal concentration (MFC) of root extracts of *A. leiocarpus*

Test organism	Minimum fungicidal Concentration [$\mu\text{g/ml}$]				
	EtOH	MeOH	CCl ₃	Aq	EtOAc
<i>A. niger</i>	0.05	0.04	0.05	0.06	0.05
<i>A. fumigatus</i>	0.04	0.04	0.05	0.05	0.04
<i>Penicillium species</i>	0.04	0.05	0.04	0.06	0.05
<i>M. audouinii</i>	0.04	0.05	0.05	0.07	0.06
<i>T. rubrum</i>	0.05	0.04	0.04	0.06	0.05

Ethanolic extract = EtOH; Methanolic extract = MeOH; Chloroform extract = CCl₃; Aqueous extract = Aq; Ethyl acetate extract = EtOAc

Discussion

Antifungal test of aqueous, ethanolic, methanolic, chloroform and ethyl acetate extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* showed that the plants exhibit antifungal activity against *A. niger*, *A. fumigatus*, *Penicillium species*, *M. audouinii* and *T. rubrum*. It was revealed in this study, that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. This finding agrees with the report of Bansa *et al.* (1999) that higher concentration of antimicrobial substance showed appreciation in growth inhibition.

The fact that the results of this study showed that root extracts of *Terminalia avicennioides* and *Anogeissus leiocarpus* exhibit antifungal properties justifies their traditional use as medicinal plants. This may be due to the presence of active principles in the plant materials. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Oguntipe *et al.*, 1998, Ibrahim *et al.*, 1997). Plant products still remain the principal source of pharmaceutical agents used in orthodox medicine (Ibrahim *et al.*, 1997; Oguntipe *et al.*, 1998).

The minimum inhibitory concentration values of the plant extracts against the test organisms showed that fungi vary widely in the degree of their susceptibility to antifungal agents. This agrees with the report that antimicrobial agents with low activity against an organism have high minimum inhibitory concentration while a highly antimicrobial agent has a low minimum inhibitory concentration (Bansa *et al.*, 1999; Prescott *et al.*, 2002).

of the extracts, the result indicated that the minimum fungicidal concentration of the extracts were obtained at higher concentrations than in the minimum inhibitory concentration studies. This observation therefore suggests that the antifungal substances contained in the extracts were fungistatic at lower concentrations while becoming fungicidal at higher concentrations of the extracts. Similar observations have been reported by Bansa & Adeyemo (2000).

In conclusion, the results obtained from this study shows that the ethanolic, methanolic, ethylacetate, chloroform and aqueous root extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* used in this study exhibit antifungal effect on the test organisms. Extracts of the plant used in this study could be useful in the treatment of fungal infections.

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