

Antifungal Profiles of Extracts of *Vitellaria paradoxa* (Shea-Butter) Bark

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Abstract

The antifungal profiles of the bark of *Vitellaria paradoxa* were examined against clinical isolates of *Aspergillus niger*, *Aspergillus flavus*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Trichophyton mentagrophytes*. Decoction method was used for the extraction of the active substances from the plant bark with cold and hot water and ethanol as extraction solvents. Agar dilution method was used in the antifungal susceptibility studies while the Minimum Inhibitory Concentration (MIC, mg/ml) and Minimum Fungicidal Concentration (MFC, mg/ml) of the ethanolic extract were determined. Generally, the ethanolic extract was the most effective, followed by the hot aqueous extract. The cold aqueous extract was the least effective against all the test fungi. All the extracts exhibited greater antifungal activity against the dermatophytes than the *Aspergillus* sp. Growth of *T. mentagrophytes* was completely inhibited by both the hot aqueous and ethanolic extracts. The similarity between the low values of the MIC and MFC obtained revealed that the plant bark possesses potent fungicidal components against the test isolates. The rate of kill study showed that with 200mg/ml of the ethanolic extract, 10^6 spores/ml of the *Aspergillus* sp. were reduced by over 50% while spores of *T. . mentagrophytes* were completely killed after 60 minutes contact time. This study therefore suggests that the bark of *V. paradoxa* could contain high concentrations of biocidal substances against the dermatophytes.

Key words: Antifungal profiles, *Vitellaria paradoxa*, Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), dermatophytes.

Introduction

The use of plants for medicinal purposes predates the introduction of antibiotics and other modern drugs (Harkenthal *et al.*, 1999). The potency of herbal remedies soon became an issue of dispute due to lack of qualitative identification of their bioactive components (Sofowora, 1986). The search for more potent chemotherapeutic agents led to the discovery and development of antibiotics (Pelczar *et al.*, 1993). However, as years passed several microorganisms developed resistance to these antibiotics thereby rendering them impotent and otherwise useless (Adeleke, 1979; Montefiore *et al.*, 1983; Olayemi and Oyagade, 1987; Rotimi *et al.*, 1987; Spencer *et al.*, 1986).

Overtime, the economy of producing these antibiotics and subsequent cost of acquiring such orthodox medications was fast getting out of the reach of the common man. Sale of fake and adulterated pharmaceutical drugs, which has been on the increase unfortunately, did not help matters. In recent times, some of these antibiotics have been found to exhibit neurotoxic effects while a few others cause severe liver damage and bone marrow depression (Chong and Pagano, 1997). All these factors led to the re-birth of intensive search for natural products from higher plants, which contain active ingredients of medicinal values.

The plant used in this study is *Vitellaria paradoxa* (synonym: *Butyrospermum parkii*), which belong to the family *Sapotaceae*, and is commonly called Shea butter tree. The plant is a small deciduous tree found commonly growing in the savanna areas of the African continent (Lowe and Soladoye, 1990). The clinical isolates used include *Aspergillus niger*, *A. flavus*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Trichophyton mentagrophytes*. *Aspergillus* species are ubiquitous saprophytes in nature while the pathogenic members of the genus cause a spectrum of diseases called aspergillosis (Austuick, 1974; Thomas, 1993). The genera *Epidermophyton*, *Microsporum* and *Trichophyton* belong to a group of fungi classified as dermatophytes. They infect keratinised surface of the body producing conditions known as tinea or ringworm. *E. floccosum* infects hair shaft follicles and is the commonest cause of ringworm of the groin (Thomas, 1993). *M. audouinii* attacks hair and skin and the cause of epidemic ringworm of the scalp, tinea capitis, in children (Wolfgang and David, 1972). *T. mentagrophytes* is the most common cause of athlete's foot, tinea pedis (Hugo and Ressel, 1998).

The aim of this study is to investigate the antifungal activities of cold and hot aqueous and ethanolic extracts of the plant bark and evaluate the effect of different concentrations of the extracts on the test fungi. The Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentrations (MFC) of the extracts were determined. The rates of kill of the test organisms against the ethanolic extract of the plant bark were also investigated.

Materials and Methods

Plant Collection and Identification

Samples of bark of *Vitellaria paradoxa* were collected from the Permanent Site Campus of the University of Ilorin, Ilorin. The plant samples were identified macroscopically as described by Dalziel (1968) and confirmed at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Voucher specimens were deposited at the Unit.

Preparation of Plant Extracts

The fresh plant materials collected were air-dried for a period of two weeks and they were pre-crushed in a mortar. They were later pulverized into fine powder using electric blender. Extraction was done with cold and hot water, and ethanol. Five grams of the powdered sample were separately suspended each in 25 ml of the extractants. The cold water and ethanol extraction were done on a rotatory shaker at 60 rpm for 24 hours while the hot water extraction was carried out in a water bath at 70 °C for 48 hours. The mixtures were further filtered through sterile 0.45µm millipore filter. The filtrates were evaporated to semi-solid mass and subsequently dried in a beaker on water bath to give a dark brown resinous mass. The dry extracts were later reconstituted with their respective extractants to give a concentration of 200mg/ml for the antimicrobial activity evaluation (Banso and Ayodele, 2001).

Source and Maintenance of Test Microorganisms

The fungi used in this study were *Aspergillus niger*, *Aspergillus flavus*, *Epidermophyton floccosum*, *Microsporum audouinii* and *Trichophyton mentagrophytes*. They were obtained from the stock culture collection of the Department of Microbiology, University of Ilorin, Nigeria. *Aspergillus* species were maintained on Malt Extract Agar while the dermatophytes (*Trichophyton mentagrophytes*, *Microsporum audouinii* and *Epidermophyton floccosum*) were maintained on Sabouraud's Dextrose Agar at 4 °C. Each culture was routinely checked for purity by inoculation on Malt Extract Agar at ambient temperature ($28 \pm 2^{\circ}\text{C}$) for 7 days in the case of *Aspergillus* species and 18 days in the case of dermatophytes.

Antifungal Susceptibility Studies

The agar dilution method was used to assay for the antifungal activity. One milliliter of 200mg/ml concentration of the extracts was separately incorporated into Malt Extract Agar and allowed to gel (Oloke *et al.*, 1988). Mycelial plugs of each test fungus on a 24 hour culture plate were cut with sterile 6.0 mm cork borer from the advancing margin of the fungal colonies. The plug was placed at the center of each agar medium containing the plant extract (Banso, 2005). Agar plates without the plant extracts were inoculated with mycelial plugs as controls. All plates were made in duplicates and incubated at room temperature. The radial growth was measured after seven days for *Aspergillus* species and 18 days in the case of the dermatophytes. The percentage of growth inhibition was calculated using the formula:

$$\text{Percentage of growth inhibition (A\%)} = \frac{W - X}{W} \times 100$$

Where W = Diameter of radial growth on Malt Extract Agar without the bark extracts (control plates) and X = Diameter of radial growth on Malt Extract Agar after exposure to bark extracts.

Minimum Inhibitory Concentration (MIC)

The standard solution (200mg/l) of the extract was diluted to obtain the following concentrations: 200, 150, 100, 85, 80, 75, 70, 65, 60, 55 and 50 mg/ml. One milliliter each of the concentrations of the extracts was mixed with 9ml of the malt extract broth in test tubes. The contents were thoroughly mixed and the tubes were inoculated with 0.1ml of spore suspension (standardized to 10^6 spores/ml) of the test fungi. The tubes were incubated at $28 \pm 2^{\circ}\text{C}$ and examined for growth after 7 days for *Aspergillus* species, and 18 days in the case of the dermatophytes. The least concentration of the bark extracts that did not permit any visible growth of the inoculated test fungus in the broth medium was regarded as the MIC in each case. Test tubes inoculated with the test fungi without the bark extracts served as controls (Black, 1996).

Results and Discussion

Antifungal susceptibility profiles of *V. paradoxa* bark extracts on the test fungi are shown in Figure 1. The extracts exhibited greater inhibitory effects against the dermatophytes than the *Aspergillus* species and the ethanolic extract exerted the greatest inhibitory activity against the test fungi while cold aqueous extract exhibited the least. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the various extracts are presented in Table 1. *T. mentagrophytes* and *E. floccosum* were the most susceptible while the *Aspergillus* sp. showed the highest MIC and MFC values.

The survival profiles of the test fungi against the ethanolic extract are shown in Figure 2. Generally there was a marked reduction in viability of all the test fungi with increase in contact time. There was a 100% loss of viability of *T. mentagrophytes* after an exposure to the extract for 60 minutes while less than 40% loss was recorded for *A. niger* after the same contact time.

Extracts of the bark of *Vitellaria paradoxa* possess antifungal activities against *A. niger*, *A. flavus*, *E. floccosum*, *M. audouinii* and *T. mentagrophytes*. The ethanolic and hot aqueous extracts exhibited higher antifungal activities than the cold aqueous extract. Ethanol is known to dissolve multivariable compounds either polar or non-polar (Cowan, 1999). This may be responsible for its greater antifungal efficacy than water. The extraction of some bioactive chemical constituents that are thermostable might have been enhanced by the hot aqueous extract at 70°C . This suggests the

possibility of an increase in the solubility of active ingredients of the plant material in hot water making more constituents available in the resulting extract. This observation is in agreement with the work of Banso (2005) who reported that there was an enhanced effect of the extracts of *A. ceraceopunctata* when extracted at a temperature as high as 70°C.

Emeruwa (1982) and El-Faraley *et al.* (1983) reported that agents with low antimicrobial activity against an organism would require high concentrations (MIC and MFC) while those with high activity require low concentrations to either inhibit or totally kill such organism. The results in Table 1 indicated that the ethanolic extract was most effective against the dermatophytes. This implies that the bark of *V. paradoxa* would be fungicidal against *Aspergillus* sp. only at higher concentrations than the dermatophytes. However, the corresponding MIC and MFC values, which are generally similar, indicate that the plant bark could possess potent fungicidal components against the test isolates at very low concentrations.

The rate of kill study showed that with 200mg/ml of the ethanolic extract, 10⁶ spores/ml of the *Aspergillus* sp. were reduced by over 50% while spores of *T. mentagrophytes* were completely killed after 60 minutes contact time. The observed trend revealed that exposure time would affect the effectiveness of the bark extract as an antiseptic. These results suggest the possibility that the bark of *V. paradoxa* could contain high amount of biocidal substances at low concentrations especially against clinical dermatophytes.

Though it is common in Nigerian ethno-medicine to use the seed of *V. paradoxa* to treat ringworm infections, this study has proven that the bark of Shea-butter plant is also very effective against the dermatophytes. Most times, the traditional preparation of herbal drugs from medicinal plants often involves cold, hot water or local gin (ogogoro) for the extraction of different organs of the plants such as bark, leaves and roots. However, this work has also shown that ethanol is the extractant of choice because the bioactive substances in the bark of *V. paradoxa* tested are less soluble in cold water than in ethanol. Therefore, using appropriate extractants, the bark of *V. paradoxa* could be purified and manufactured as an antiseptic agent for the treatment of skin infections caused by these groups of fungi.

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Table 1: The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Ethanolic Extract of Bark of *V. paradoxa*.

Test Organisms	MIC (mg/ml)	Control	MFC (mg/ml)	Control
<i>A. niger</i>	80	NI	80	NI
<i>A. flavus</i>	70	NI	75	NI
<i>E. floccosum</i>	65	NI	70	NI
<i>M. audouinii</i>	75	NI	80	NI
<i>T. mentagrophytes</i>	50	NI	55	NI

NI: No Inhibition

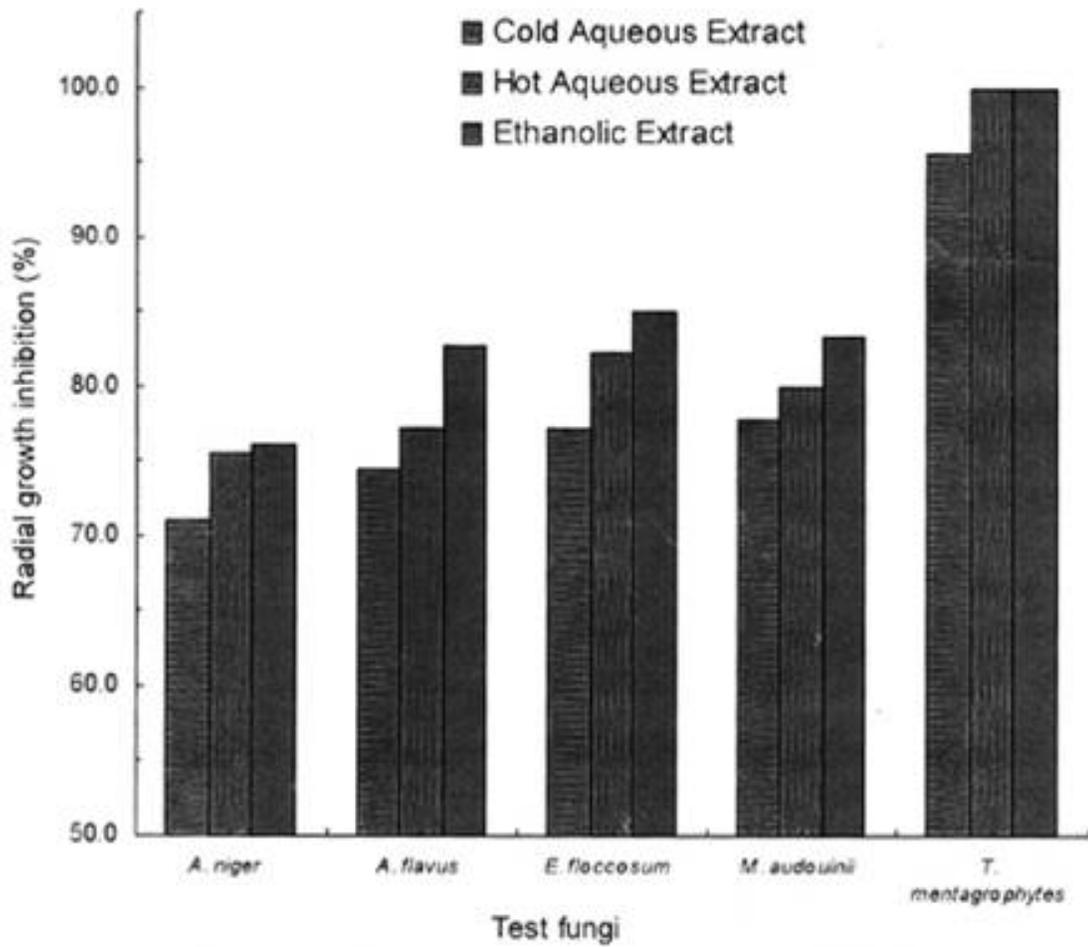


Figure 1: Growth profile of test fungi in 200mg/ml *Vitellaria paradoxa* bark extract

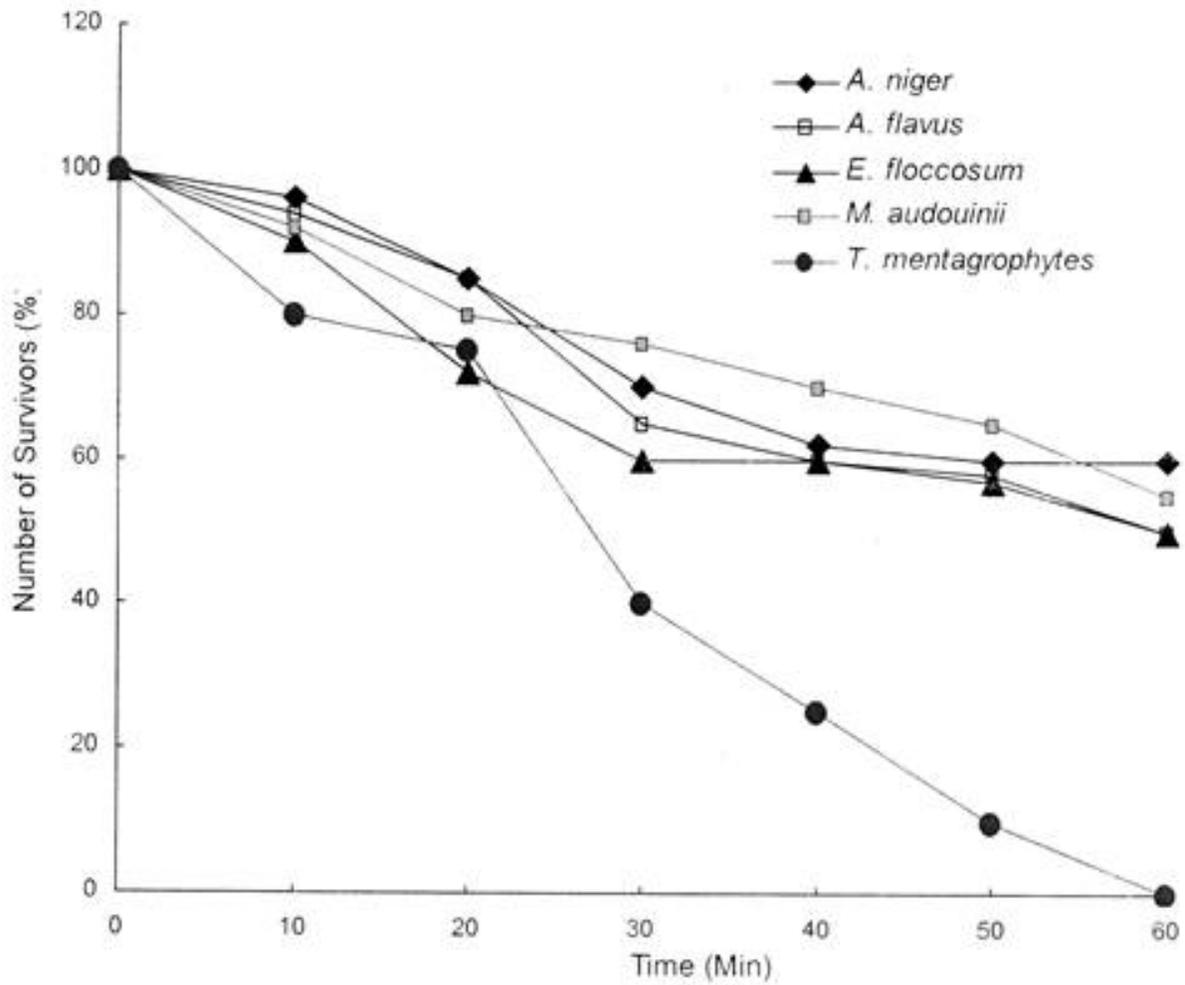


Figure 2: The survival profile of test fungi in 200mg/ml ethanolic extract of *V. paradoxa* bark