Antifungal Properties of the Crude Extracts of *Bauhinia thonningii* Schum. (Caesalpiniaceae) and *Sarcocephalus esculentus* Afzel. (Rubiaceae) from a Tropical Forest in Nigeria

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Ethanol and water crude extracts of *Bauhinia thonningii* and *Sarcocephalus esculentus* barks were investigated individually for *in vitro* antifungal activity by disc diffusion agar technique. The phytochemical properties of the extracts were also assayed. The plant crude extracts had significant antifungal activity on *Aspergillus niger, Candida albicans, Microsporum audouinii* and *Trichophyton rubrum* which were previously isolated from patients with skin diseases. The water extract of the plants showed more antifungal activity than the corresponding ethanol extract. The water extract of *B. thonningii* had the highest zone of inhibition (16.70±0.19 mm) on *Trichophyton rubrum* culture plate. The water extract of *B. thonningii* had a higher zone of inhibition on the cultures of *Candida albicans, Microsporum audouinii* and *Trichophyton rubrum* than the antibiotics Nystatin and Fulcin at 10µg/ml. The crude extracts of *B. thonningii* and *Sarcocephalus esculentus* contained the alkaloid, anthocyanin, anthraquinone, betacyanin, flavonoid, saponin and steroid.

Keywords: Antifungal activity, Crude extracts, Skin diseases, Phytochemical properties, Tropical rain forest, Medicinal plants.

Introduction

The tropical rain forest is known for its abundant natural resources. This include raw materials for clothing, furniture, fuel, source of food, industrial enzymes, organic acids, paints and medicine (Sofowora, 1982). In many developing countries some 80% of available medicines are obtained from medicinal plants, while in the western developed countries the plants mainly constitute raw materials for industrial processing or preparation of the pure chemical derivatives (Owonubi, 1998). Due to ever increasing prices of drugs, especially in developing countries a need to search for economical drugs from natural sources has become imperative.

Several studies on antifungal activity of medicinal plants have been conducted by a number of investigators such as Irobi and Daramola (1993) on *Mitracarpus villosus;* Alade and Irobi (1993) on *Acalypha wilkesiana;* Swets and Zeitlinger (1995) on *Cassia alata;* Saxena and Mathela (1996) on *Nepeta leucophylla;* Rana *et al.,* (1997) on *Aegle marmelos;* Adekunle (2000) on the crude extracts of *Brachystegia eurycoma* and *Richardia brasililience* etc.

Some tropical rain forest trees are used as medicinal plants to cure fungal skin diseases, by the natives in the southwestern Nigeria. The plants include the barks of *Bauhinia thonningii* and *Sarcocephalus esculentus*. *B. thonningii* in the family Caesalpiniaceae is called *Abafe* in Yoruba. It is a shrub or small tree of upto 7.5 m in height. The bark has been shown to contain tannin with traces of alkaloid (Burkill, 1997). The

powdered bark or the fresh inner bark is used as a dressing for wounds and ulcers. A warm infusion of the bark of the leaves relieves toothache. The young leaves and bark are believed to have expectorant activity and are used as infusion or merely chewed for chest complaints. The cold infusion of the bark is an astringent for diarrhoea and dysentery (Sofowora, 1982). The bark, root or leaf of *B. thonningii* is used for occasional application in the native treatment of leprosy and smallpox (Burkill, 1997).

Sarcocephalus esculentus in the family Rubiaceae is called *Egbesi* in Yoruba. It is a tropical tree, perennial, about 40 m high and 4.5 m grith. The young leaves contain traces of toxic alkaloid. The bark's cold infusion is taken as a relief for indigestion and vomiting. The bark and root together are pulverized and applied to wounds (Dalziel, 1937).

The antifungal activity and phytochemical data of *B. thonningii* and *S. esculentus* has not been reported in literature. As a continuation of studies in this laboratory on antifungal components and phytochemical properties of Nigerian medicinal plants (Adekunle, 2001), antifungal and phytochemical analysis of the crude extracts of the barks of *Bauhinia thonningii* and *Sarcocephalus esculentus* are presented here.

Materials and Methods

Source of Plant Materials

The plant materials, the barks of *Bauhinia thonningii* and *Sarcocephalus esculentus* were collected from Olokemeji forests, a tropical rain forest in the South-western Nigeria (Lat. 3°N Long, 13.5°E). The plant parts were shade dried at room temperature (28-30°C) for 14 days. Samples of the plants were authenticated by Dr. O.T. Ogundipe of the Department of Botany and Microbiology, University of Lagos, Nigeria, as well as using the text on vernacular names of Nigerian medicinal plants by Gbile (1984). Voucher samples (LUH 200063 and LUH 200064) have been deposited at the University of Lagos, Nigeria.

Source of Micro-organisms

Aspergillus niger, Candida albicans, Microsporum audouinii and *Trichophyton rubrum* were obtained from the culture collection unit of the Department of Biotechnology, Federal Institute of Industrial Research (FIIRO), Oshodi, Nigeria, previously collected from patients with skin diseases at the Federal health laboratory, Yaba, Lagos, Nigeria. All the cultures were maintained on Sabouraud's dextrose agar (SDA) slants in the refrigerator at 4°C prior to use.

Extraction

The dried barks of *B. thonningii* and *S. esculentus* were ground to a 60-mesh diameter powder using an electric blender. Six hundred grams (600 g) of each plant part was soaked in 1.2 litres of 70% aqueous ethanol for 24 hours. Another 100 g of each plant part was soaked in 1.2 litres of distilled water for 24 hours. There after each plant extract was passed through Whatman filter paper No. 1823, and concentrated by evaporating in a rotatory evaporator at 40°C, procuring the ethanol and aqueous extracts of each plant. The two concentrates or extracts of each plant were stored in the refrigerator at 4°C prior to use.

Antifungal Testing

The antifungal activity testing was carried out using the disc agar diffusion method of Irobi and Daramola (1993). Spore or conidia suspension of 10^5-10^7 cells of the 4 fungal species, counted with haemocytometre were made. About 10 ml of previously prepared Sabouraud's dextrose agar (Oxoid) were poured into Petri dishes and allowed to solidify. A micropipette was used to introduce 0.1 ml of the spore or conidia suspensions onto the agar plate, and spread with glass spreading rod under sterile conditions. Sterilised discs (6 mm Whatman No. AA2017006) were soaked in each of the extracts (100 µg/ml extracts were diluted with 30% aqueous methanol, being assayed for 6 hours). Four of these soaked discs were placed on a fungal spore or conidia seeded plates with the help of sterile forceps, three plates were prepared for each fungus used per plant extract. There were two types of controls: one contained the fungal inoculum but with discs that were soaked in sterile distilled water. The second type of control had the discs soaked in orthodox antibiotics. There were two antibiotics used, Fulcin and Nystatin (10 µg/ml) separately. All the plates containing the discs were then incubated at 28°C-31°C. Zone of inhibition was measured after 72 hours of incubation.

A concentration gradient or minimum inhibition concentration (MIC) of the antifungal extracts was determined by varying the concentration of reconstituted extract solution (0.01-1000 µg/ml). The antifungal activity testing results were statistically analysed as described by Parker (1979).

Preliminary Phytochemical Studies

Preliminary phytochemical studies were carried out using the methods described by Fadeyi *et al.*, (1987) and Harborne (1998). The bark extracts of *B. thonningii* and *S. esculentus* were screened for the presence of alkaloid, anthocyanin, anthraquinone, butacyanin, flavonoid, phlobatannins, saponin, steroid and tannin.

Results

The powdered bark of *Bauhinia thonningii* rendered 48 g of ethanol extract and 126 g aqueous extract while the bark of *Sarcocephalus esculentus* rendered 43 g of ethanol extract and 83 g of aqueous extract. From Tables 1 and 2 it was observed that the crude extracts had significant antifungal activity on the four fungi tested. The zone of inhibition of the plant extracts were above 10 mm diameter except the ethanol extract of *Bauhinia thonningii* which had a zone of inhibition 8.30±0.21mm on *Aspergillus niger* (Table 2). The water extract of the plants showed more antifungal activity than the corresponding ethanol extract. The water extract of *B. thonningii* had the highest zone of inhibition (16.70±0.19mm) against *Trichophyton rubrum* while the ethanol extract of *B. thonningii* had the least inhibition against *Aspergillus niger,* among the extracts of the 2 plants. The antibiotics Fulcin and Nystatin, had significant zone of inhibition compared to the control (distilled water), above 10 mm, on all the fungi except Fulcin that did not inhibit the growth of *Microsporum audouinii.* The water extract of *B. thonningii* had a greater zone of inhibition than the antibiotics, i.e. the zone of inhibition against the fungi increased as the concentration of the antifungal active plant extracts or antibiotic increased.

The crude extracts (ethanol and water) of the 2 plants contained alkaloid, anthocyanin, anthraquinone, butacyanin, flavonoid, saponin and steroid. Tannin and phlobatannins were absent in the extracts of *Sarcocephalus esculentus* but present in the extracts of *Bauhinia thonningii* (Table 3).

Discussion

The ethanol and water extracts of *Bauhinia thonningii* and *Sarcocephalus esculentus* possess antifungal activity against *Aspergillus niger, Candida albicans, Microsporum audouinii* and *Trichophyton rubrum.* This confirms previous reports by Alade and Irobi (1993) that water and ethanol extracts of some medicinal plants possess antifungal properties.

It is of interest to note that the water extract of *B. thonningii* showed more antifungal activity than the check antibiotics, Nystatin and Fulcin, against *Candida*

TABLE 1
Antifungal activity of ethanol extracts of the bark of two tropical forest plants

Extracts or solution	Zone of inhibition (mean±S.E.M) produced by extracts Fungi			
	Aspergillus niger	*Candida albicans*	*Trichophyton rubrum*	*Microsporum audouinii*
Control (distilled water)	0.00±0.00a*	0.00±0.00	0.00±0.00	0.00±0.00
Fulcin	16.30±0.33c*	12.80±0.38d	14.00±0.18d	0.00±0.00a
Nystatin	15.65±0.74c	20.25±0.12b	15.30±0.11c	28.30±0.31b
Bauhinia thonningii	8.30±0.21f	12.50±0.13e	10.50±0.13e	11.80±0.11e
Sarcocephalus esculentus	12.00±0.00e	13.87±0.24d	13.65±0.47	12.00±0.15e

* Samples with similar alphabet show no significance at p = 0.01.
Samples with different alphabets show significant difference at p = 0.01.