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PHYTOCHEMISTRY AND ANTIMYCOTIC ASSAY OF *Senna alata* and *Borreria verticilliata* LEAVES EXTRACTS on DERMATOPHYTES

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ABSTRACT : Phytochemical screening and biological evaluation of the efficacy of the ethanol extract of the leaves of *Senna alata* and *Borreria venticiliata,* locally used for the treatment of dermatophytosis in Akwa Ibom State, Nigeria was carried out. The phytochemical screening confirms the presence of bioactive secondary metabolites: saponins, tannins, anthraquinones, flavonoids, phlobatannins, terpenes and cardiac glycosides while alkaloids were absent. *In vitro* antimycotic assay of the plant extract revealed that *Senna alata* exhibited a higher potency against the dermatophytes tested. The presence of pharmacologically active antimycotic agents in the plants extracts justifies the use of these plants by traditional medicine practitioners in the treatment of dermatophytoses.

INTRODUCTION

The demand for medicinal plants has increased globally due to the resurgence of interest in and acceptance of herbal medicine. Most of the demand are being met through collection of large quantities of medicinal plants and plant parts from wild populations. Each medicinal plant species has it own nutrient composition besides having pharmacologically important and phytochemicals.

Plants have in their arsenal amazing array of thousands of chemicals noxious or toxic to bacteria, fungi, insects, herbivores, and even humans.Fortunately, this chemical diversity also includes many compounds that are beneficial to human. Vitamins, nutrients, antioxidants, anti carcinogens and many other compounds with medicinal value (Noval and Haslberger, 2000).

Senna alata and *Borreria verticilliata* are known for their importance as source of drugs in medicine and pharmacology.Medicinal plants play a significant role in providing primary health care services to rural people and are used by about 80% of the marginal communities around the world (Prajapati and Prajapati, 2002; Latif *et al.*, 2003; Shinwari *et al.*, 2006). The plants are commonly used as cure for dermatophytosis, (Shinwari *et al.*, 2006).

Dermatophytosis is a group of superficial fungal infections affecting the keratinized layer of the skin and its appendages the hairs and nails are the commonest sites of infection (Rippon, 1974). These mycotic infections are caused by a group of morphological related fungi collectively known as dermatophytes. It was thought worthwhile investigating the *in-vitro* sensitivity of representative isolates to *Senna alata* and *Borreria verticilliata* plants extract commonly used for the treatment of dermatophytes in Akwa Ibom State.

Antimycotic have been marketed in Nigeria in the forms of cream and ointments for several years, and have been successfully tried for the therapy of dermaphytosis.

Itah (1996) tested and confirmed the efficacy of aqueous and ethanol extract of local plants namely: *Hibiscus Sarrathensis, Dracaena manii, Erythrinia senequalensis, Laccoidiscus Pseudostipularis and Sterculia tragacantha* were tested against known pathogenic micro-

organisms like *E. coli, Staph aureus, Candida albicans, Klebsiella pneunmoniae,Proteus mirabilis* and *Nesseria gonorrhoae.* The extracts from *H. sarrathensis* strongly inhibited growth of all the tested organisms followed by *Erythrinia senequalensis.*

The present study was designed to determine the phytochemistry of the plants used for the treatment of dermatophytosis in Akwa Ibom State and to study the susceptibility pattern of the isolates to the plants extract.

MATERIALS AND METHOD

Isolation of Test Organisms

All those with dermatophytic lesions and other superficial mycotic features were identified for sampling. Scrapings of mycological examination were collected from the lower layers of the skin to minimize contamination. Pre-sterilized forceps and blunt scrapping blade were used. The specimens were collected into sterile envelops and put in a cellophane bags labeled and transported to the laboratory for examination and isolation using standard mycological techniques (Umana *et al.* 2012). Pure cultures of the isolates were identified based on the cultural and biochemical characteristics as described by de-Hoog *et al.* (2000).

Extraction of plant materials.

Cold extraction technique described by Sofowora (1982) was adopted. During the process the leaves of *Senna alata* (Adaya Okon) and *Borreria verticilliata* (Abia Ikana) were collected and dried under the sun. The leaves were milled and marcerated with ethanol in a cornical flask and allow to stand for 72 hours (3 days). The liquid ethanol extract was evapourated to dryness in vacuo at 40^{o}C. The concentrated extracts were covered with aluminium foil and stored in the refrigerator until when needed.

Antifungal Assay of Plant Extracts

The disc diffusion method (Nester *et al.*, 1995) was employed. Lawns of the test isolates were separately prepared by spreading 24 hr –old broth culture of the isolates on freshly prepared Sabouraud Dextrose Agar (SDA) plates. Punched circular discs from Whatman No. 1 filter paper were sterilized in a hot air oven for 1 hour. The paper discs were impregnated (soaked) with the plant extracts, air dried for few minutes and transferred aseptically on to the surface of the inoculated plates. The plates were incubated at room temperature for 5 days and zones of inhibition that appeared on the plates were measured.

RESULTS AND DISCUSSION

The phytochemical screening of *Senna alata* and *B. verticilliata* (Table 1) show the presence of saponins, tannins and flavonoides in both plants while anthraqinones were present in *S alata* but absent in *B. verticilliata* and phlobatannins were absents in *S. alata* but present in *B. verticilliata.* The findings of the phytochemical analysis indicated the presence of some active drug compounds which have medicinal potency as evidenced in the various uses of *S. alata* and *B. verticilliata* in treating dermatophytes by traditional medicine practitioners in Akwa Ibom State.

Table 1: Phytochemical properties of the plant extracts used in the antimycotic assay

Properties	*Senna alata*	*Borreria verticilliata*
Alkaloids	-	-
Saponins	+	+++
Tannins	+	++
Antraquinones	+++	-
Flavonoids	+	+++
Phlobatannins	-	+
Terpenes	-	++
Liber man's		-
Keller Kiliani		+

Key: + = Trace ++ = Moderate
+++ = Strongly positive- = Negative

Saponins are glycosides components often reffered to as "natural detergent" because of their foamy nature (Seigler, 1998). Saponins have been known to posses both beneficial and deleterious properties depending on its concentration in the sample (Seigler, 1998; Oakenful and Sidhu, 1989). Seigler (1998) reported that saponins have anticarcinogenic properties, immune modulation activities and regulation of cell proliferations as well as health benefits such as inhibition of the growth of common cells and cholesterol lowering activity.

Flavonoids have also been reported to excrete multiple biological effects including antibacterial, antiviral, antitoxic and anti inflammatory activities (Cook and Samman, 1996). Many of these alleged effect of flavoniods have been linked to their known functions as strong antioxidants, free radicals scavenger and metal chelators(Nakayama *et al.* 1993).

Based on a standard diameter of inhibition of 1.0cm the most effective extract was obtain from *Senna alata* with a inhibition zone of 4cm against *Microsporum audounii*. Some of the isolates exhibited high resistance to some of the test extracts. For instance *Epidermophyton floccosum* was resistant to the two plant extract while *Trichophyton rubrum* was sensitive to *Senna alata* with zone of inhibition of 1.6cm. Typical inhibitory activities of the effective and non effective extracts are presented in Plates 1 and 2 while the antibiogram of the isolates is presented in Table 2.

Table 2: Antibiogram of the ringworm pathogens using extracts from selected medicinal plant parts

Isolates Code	Species of Dermatophytes	*Senna alata* (cm)	*Borreria verticilliata* (cm)
RWP 1	*Microsporum canis*	S	S
RWP 2	*Microsporum audouinii*	S	S
RWP 3	*Trichophyton tonsurans*	S	S
RWP 4	*Trichophyton rubrum*	S	R
RWP 5	*Epidermophyton floccosum*	R	R
RWP 6	*Trichophyton schoenleinii*	S	S
RWP 7	*Trichophyton soudanense*	S	S
RWP 8	*Candida albicans*	S	R
RWP 9	*M. nanum*	S	S
RWP 10	*Candia tropicalis*	R	S

Key: R - Resistance
S - Sensitive

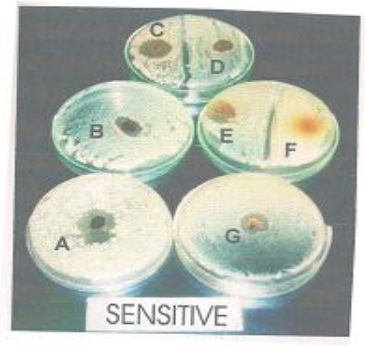

Plate 1: A typical inhibitory activities of the ethanolic extracts of the test plants against the ringworm pathogens

Plate 2: Resistance of *Epidermophyton* species against the plant extract. Notice profuse growth on isolate over the filter paper containing plant extract.

Key: (a) *Microsporum audouinii* on *Senna alata*
(b) Candida tropicalis on *Borreria verticilliata*
(c)M-nanum on *Borreria verticilliata*
(d) Trichophyton tonsurans on *Borreria verticilliata*
(e) Microsporum audouinii on *Borreria verticilliata*
(f) Epidermophyton floccosum on *Senna alata*
(g) Trichophyton schoenleinii on *Senna alata*

The antimycotic assay revealed that *Senna alata* exhibited the most active anti microbial activity against the fungal isolates. Its effect against the different aetiological agents was wide and very remarkable. It inhibited the growth of nearly all the species of dermatophytes tested.

The highest mean diameter of inhibition (4cm) was recorded against *Microsporum audounii*, *Borreria verticilliata* also exhibited a high antimicotic activity with a 3.8 inhibitory zone against *M. nanum*.

Table 3:Antimycotic potential of the medicinal plant parts against test isolates.

Isolates Code	Species of Dermatophytes	*Senna alata* (cm)	*Borreria verticilliata* (cm)
RWP 1	*Microsporum canis*	2	1.6
RWP 2	*Microsporum audouinii*	4	2.6
RWP 3	*Trichophyton tonsurans*	2.6	2.4
RWP 4	*Trichophyton rubrum*	1.6	0
RWP 5	*Epidermophyton floccosum*	0	0
RWP 6	*Trichophyton schoenleinii*	3.2	2.1
RWP 7	*Trichophyton soudanense*	2.6	1.6
RWP 8	*Candida albicans*	2.1	1.2
RWP 9	*M. nanum*	3.1	3.8
RWP 10	*Candida tropicalis*	1.4	2.0

NB: point of sensitivity was considered from 1.5cm in diametre

CONCLUSION

The phytochemical screening of *S. alata* and *B. verticilliata* revealed the presence of important pharmacological bioactive substance as well as the medicinal potential in the leaves. The plants contain antimycotic bioactive agents which were able to inhibit almost all the dermatophytic isolates. It is thus suggested that more studies on concentrations of active ingredient and toxicity level be carried out.

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