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Full Length Research Paper

The efficacy of seven ethnobotanicals in the treatment of skin infections in Ibadan, Nigeria

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In southwestern Nigeria, ethnobotanical investigation confirmed that *Afzelia africana, Alstonia boonei, Azadirachta indica*, *Ficus exasperata*, *Senna alata*, *Tetrapleura tetraptera* and *Xylopia aethiopica* are commonly used in the treatment of skin infections. Therefore, this study examined the *in vitro* antimicrobial activity of crude methanol extracts of the ethnobotanicals against clinical isolates of *Candida albicans, Staphylococcus aureus* and *Streptococcus pyogenes* associated with skin infections. The isolates (1×10^{-1} – 1×10^{-6} cfu/ml) were tested against plant extracts (500 mg/ml) using agar-well diffusion method. The Minimum Inhibitory Concentration (MIC) was determined using broth dilution method. At 10^{-4} cfu/ml inoculum concentration, *C. albicans* was significantly susceptible ($P \leq 0.05$) to methanol extracts of *S. alata* and *X. aethiopica* with 25.0 mm zone of inhibition, while *T. tetraptera* was the most active on *S. aureus* with 21.5 mm and *S. pyogenes* was significantly susceptible to *A. africana*, *A. boonei*, *S. alata* and *T. tetraptera* with 25.0 mm zone of inhibition. The significant antimicrobial activities exhibited by the methanol extracts of the ethnobotanicals confirmed their therapeutic potentials in the treatment of skin infections. Also soap, cream and ointment could be prepared from these ethnobotanicals for topical application in the treatment of skin infections. However, their toxicity tests will ascertain safety in administration.

Key words: Skin pathogens, antimicrobial screening, methanol extract, indigenous recipes Ethnobotanicals, Nigeria.

INTRODUCTION

Skin infection refers to disorders of the superficial layers of the skin (WHO, 2005). They are inflammations of the skin that are caused by disease causing organisms and allergic reaction to something that irritates the skin that may itch or seep. The World Health Organization's 2001 report (Mathers, 2006) on global burden of disease indicated that skin infections were associated with mortality rates of 20,000 in sub-Saharan Africa in 2001. This burden was comparable to mortality rate attributed to meningitis, hepatitis B, obstructed labour and rheumatic heart disease in the same region. The main skin conditions at community level include: scabies, superficial mycoses, pyoderma, pediculosis, eczema or dermatitis, HIV- related skin disease, pigmentary anomalies and acne (Hay et al., 2007). There have also been several reports on the frequency of skin infections (mainly pyoderma and scabies) in specific population groups. Street-children in Kenya (prevalence of skin infections is 50.9%), child workers in Nigeria (skin infections is 12%) and refugee camp in Sierra Leone where scabies occurred in 77 - 86% of children (Ayaya and Esamia, 2001; Omokhodiou and Omokhodiou, 2001; Terry et al., 2001). Some skin infections with their causative organisms include: pyodermis (*Staphylococcus aureus*), folliculitis (*S. aureus*), furuncles/carbuncles (*S. aureus*), absecesses/phlemons (*S. aureus*/*Streptococci* spp.), dermatitis (due mainly to *Streptococci* spp.) and candidiasis (*Candida albicans*) (Neugebauer, 1983). *Afzelia africana, Alstonia boonei, Azadirachta indica*, *Ficus exasperata*, *Senna alata*, *Tetrapleura tetraptera* and *Xylopia aethiopica* have been found to be effective in the treatment of various skin infections by the indigenous people of Ibadan, southwestern Nigeria (Asuzu and Anaga, 1991; Crockett et al., 1992; Akah et al., 2007; Mshana et al., 2008). This study investigated the indige-

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nous treatment of skin infections and examined the *in vitro* antimicrobial activities of methanol crude extracts of seven ethnobotanicals against clinical isolates of skin pathogens (*C. albicans*, *S. aureus* and *Streptococcus pyogenes*) to confirm the efficacy of their ethnotherapeutic use in the treatment of skin infections.

MATERIALS AND METHODS

Ethnobotanical investigation

50 respondents comprising herb-sellers and herbalists were informally interviewed in three local markets in Ibadan, Oyo State, Nigeria. The markets visited Bode, Oje and Oja-oba. The population of respondents comprised of 20% male and 80% female, the interview was conducted in Yoruba language. They were questioned on their knowledge of management and treatment of skin infections. Recipes were documented. The local name, parts of plant used, method of preparation and mode of administration were also recorded (Sofowora, 1982).

Medicinal plant materials

Fresh parts of the test plants were collected from the botanical garden and nursery of the Department of Botany, University of Ibadan in July, 2010. All plant samples were identified in the herbarium of Forest Research Institute of Nigeria (FRIN), Ibadan, where the plant materials were deposited and voucher numbers were issued for the samples. The plant samples were air-dried for two weeks, powdered and stored in air-tight glass containers for further use.

Preparation of extracts

200 g each of the powdered plant samples was separately extracted in 1000ml of 90% methanol at room temperature for 14days. The mixture was then filtered and the filtrate was dried in vacuum using a rotary evaporator at 40°C. The extract was refrigerated at 4°C prior to use.

Microorganisms and culture media

The microorganisms were clinical isolates of skin pathogens (*C. albicans, S. aureus* and *S. pyogenes*) obtained from the medical microbiology laboratory, University College Hospital, Ibadan. The organisms were maintained on nutrient agar and malt extract agar (FLUKA, USA) at 4°C. The organisms were sub-cultured in nutrient broth and malt extract broth, while Mueller Hilton Agar (MHA) was used for the sensitivity test.

Sensitivity test

The antimicrobial activity of the extracts was determined using agar-well diffusion method (Jennie et al., 2003). The Minimum Inhibitory Concentration (MIC) of the extracts was also determined using broth dilution method. The isolates were grown in sterile broth (18-36h) before use. For the initial antimicrobial screening, the inoculum suspensions were standardized to give a range of concentrations ($1 \times 10^{-1} - 1 \times 10^{-6}$ cfu/ml) and the methanol extract concentration was varied between 250 and 1000 mg/ml. The least concentration of extract active on the test organisms (500 mg/ml) was used for the screening in each MHA medium. The plates were incubated at 35 ± 2°C for 18-36 h after which zones of inhibition were measured. A control plate containing the test organism without any plant extract was also incubated. Standard drugs (1 - 5 mg/ml) were also used as the control experiment for the determination of MIC. All experiments were carried out under sterile condition and each experiment replicated three times for all isolates.

Statistical analysis

Analysis of variance and comparison of means were carried out on all data using Statistical Analysis System (SAS). Difference between means was assessed for significance at $P \leq 0.05$ by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The indigenous recipes are presented in Table 1. The test plants were used singly or combined in crude drug formulation. *X. aethiopica* was the most frequent in the recipes. The profile and voucher numbers of the test plants are shown in Table 2. The plants are from different families; their habit is tree except *S. alata* (shrub). The methanol extracts of plant samples gave various percentage yields. The highest yield (36.8%) was recorded for *X. aethiopica*, followed by *A. boonei* (20.0%) and the least (9.3%) was recorded for *T. tetraptera* (Table 3). Table 4 shows the inhibitory activity of methanol extracts of various plant samples against test organisms. The methanol extracts of *S. alata* and X. *aethiopica* was significantly active against *C. albicans* with 25 mm zone of inhibition and least activity (10.8 mm) was recorded for *T. tetraptera* at 10^{-4} cfu/ml inoculum concentration. The highest (21.5 mm) inhibitory activity against *S. aureus* was recorded for *T. tetraptera* and the least (15.5 mm) was recorded for *A. indica* at 10^{-4} cfu/ml inoculum concentration. The extracts of *A. africana*, *A. boonei*, *S. alata* and *T. tetraptera* were significantly active against *S. pyogenes* at 10^{-4}cfu/ml with 25.0 mm zones of inhibition. Table 5 shows the MIC of the various extracts (1 – 5 mg/ml) and standard drug (1 – 5 mg/ml), the extracts of *A. indica, F. exasperata* and *X. aethiopica* gave a MIC value of 1 mg/ml against *C. albicans*, a value lower than the MIC of metronidazole (5 mg/ml). The least MIC value (1 mg/ml) against *S. aureus* was recorded for *X. aethiopica*.

The results of the present study are in line with the findings of other authors except for variations obtained in the plant parts and extraction solvent used for experiments: Akinpelu et al. (2008) reported that the crude 70% methanol extract of stem bark of *A. africana* exhibited antimicrobial activities at a concentration of 25 mg/ml against twenty-one of the bacterial isolates comprising both Gram positive and Gram negative strains. The volatile oil of *A. boonei* leaves showed antibacterial activity against *Escherichia coli, S. pneumonia, S. aureus* and *Proteus mirabilis* (Okwu and Ighodaro,

Table 1. Herbal recipes for the treatment of skin infections in Ibadan, Nigeria.

S/N	Recipes and dosage	Method of preparation
1	*Xylopia aethiopica* fruit is ground into coarse powder and soaked in warm palm oil. The oil is applied to the affected part of the skin.	Oil
2	*Alstonia boonei* leaves or bark is boiled in water with small quantity of *Xylopia aethiopica* fruit. 200ml of the herbal preparation is taken after food twice daily.	Decoction
3	*Senna alata* leaves, *Tetrapleura tetraptera* fruits and *Xylopia aethiopica* fruits are powdered. The herbal powder is mixed with native soap and used for bathing twice daily.	Soap
4	Leaves of *Ficus exasperata* and *Azadirachta indica* are soaked in coconut oil for 2 weeks and then allowed to boil for 15 min. The cooled oil is added to shea-butter. The herbal preparation is applied to the affected part of the skin twice daily.	Ointment
5	*Azadirachta indica* leaves and *Tetrapleura tetraptera* fruits are boiled in water for 15 minutes. 200ml of the herbal preparation is taken after food twice daily.	Decoction
6	*Xylopia aethiopica* fruit and bark of *Khaya ivorensis* are boiled in water for 15 min. 200ml of the herbal preparation is taken after food twice daily.	Decoction
7	*Afzelia africana* bark is ground into fine powder. Half teaspoonful of the powder is taken in pap once daily after food.	Powder

Table 2. Profile of ethnobotanicals used in the management and treatment of skin infections in Ibadan, Nigeria.

Botanical name	Family	Common name	Habit	Part used	Voucher number
Afzelia africana	Fabaceae	African mahogany	Tree	Leaf	FHI108884
Alstonia boonei	Apocynaceae	Stool wood	Tree	Leaf	FHI108885
Azadirachta indica	Meliaceae	Neem	Tree	Leaf	FHI108886
Ficus exasperata	Moraceae	Sand paper tree	Tree	Leaf	FHI108887
Senna alata	Caesalpinaceae	Ringworm shrub	Shrub	Leaf	FHI108888
Tetrapleura tetraptera	Leguminosae	-	Tree	Fruit	FHI108889
Xylopia aethiopica	Annonaceae	Ethiopian pepper	Tree	Fruit	FHI108890

2010). Crude water extracts of *A. indica* (leaf) showed antibacterial activity against *Escherichia coli, Enterococcus faecalis, Proteus mirabilis, Bacillus cereus* and *S. aureus*, its activity was weak against *Klebsiella pneumonia*, while *Neisseria gonohorroea* was found to be relatively resistant to all forms of neem extract (Pritima and Pandian, 2008). Natarajan et al. (2003) reported that the changes in growth curve of the treated dermatophytes with extracts (ethanol, ethyl acetate and hexane) of the leaves and seeds of *A. indica* were found to be statistically significant with reference to the untreated fungi. Igoli et al. (2005) and Gbadamosi (2008) reported the antibacterial and anticandidal activities of ethanol leaves extracts of *S. alata*, respectively. Nwaiwu and Imo (1999) reported the fungi-toxic activity of fruits of *X. aethiopica* essential oil against mycelial growth of three food-borne fungi (*Aspergillus fumigatus, A. nidulans* and *Mucor hiemalis*). The essential oil of *X. aethiopica* fruits from Nigeria showed significant antifungal activity against *Stellocapella madis, C. albicans, Aspergillus flavus, A. ocheraccus* and *Fusarium oxysporum* (Asekun and Adeniyi, 2004).

The fresh and dried fruits, leaf stem bark and root bark of *X. aethiopica* essential oil showed various degrees of activity against *C. albicans* and Gram positive/negative bacteria (Fleischer et al., 2008).

The significant antimicrobial activities of *A. africana, A. boonei, A. indica, F. exasperata, S. alata, T. tetraptera* and *X. aethiopica* against skin pathogens have justified their ethnotherapeutic use in the treatment of skin infections, contributed to the importance of traditional knowledge of management and treatment of skin infections. Although, the plants may be good alternatives to expensive orthodox medicines, the toxicity tests of their active constituents would ascertain their safety in administration.

Table 3. Percentage yield of methanol extracts of plant samples used in this study.

Botanical name	Percentage yield
Afzelia africana	16.0[b]
Alstonia boonei	20.0[b]
Azadirachta indica	17.5[b]
Ficus exasperata	14.7[b]
Senna alata	20.0[b]
Tetrapleura tetraptera	9.3[c]
Xylopia aethiopica	36.8[a]

Values followed by the same letter are not significantly different ($P>0.05$) from each other. They differ significantly ($p\leq0.05$) with values that do not share a similar letter.

Table 4. *In-vitro* Inhibitory activity of methanol extracts of plant samples on test organisms.

Plant extracts (500 mg/ml)	Zones of inhibition (mm)		
	C. albicans **(10^{-4} cfu/ml)**	***S. aureus*** **(10^{-4} cfu/ml)**	***S. pyogenes*** **(10^{-4} cfu/ml)**
A. africana	20.5[a] ± 10.0	20.5[a] ± 10.0	25.0[a] ± 10.0
A. boonei	20.5[a] ± 10.0	20.5[a] ± 10.0	25.0[a] ± 10.0
A. indica	15.5[a] ± 10.0	15.5[a] ± 10.0	18.0[a] ± 10.0
F. exasperata	10.8[a] ± 9.5	18.5[a] ± 10.0	19.5[a] ± 10.0
S. alata	25.0[a] ± 10.0	20.0[a] ± 10.0	25.0[a] ± 10.0
T. tetraptera	15.5[a] ± 10.0	21.5[a] ± 10.0	25.0[a] ± 10.0
X. aethiopica	25.0[a] ± 10.0	20.5[a] ± 10.0	20.5[a] ± 10.0

Values are mean of three readings ± standard deviation. Values in the same column followed by the same letter are not significantly different ($p>0.05$) from each other. They differ significantly ($p\leq0.05$) with values that do not share a similar letter.

Table 5. Minimum Inhibitory Concentration (mg/ml) of test plants and standard antibiotics against test organisms.

Plant extracts/ antibiotics	Zones of inhibition (mm)		
	C. albicans **(10^{-6} cfu/ml)**	***S. aureus*** **(10^{-6} cfu/ml)**	***S. pyogenes*** **(10^{-6} cfu/ml)**
A. africana	5.0	2.0	2.0
A. boonei	ND	2.0	ND
A. indica	1.0	5.0	5.0
F. exasperata	1.0	2.0	2.0
S. alata	5.0	2.0	5.0
T. tetraptera	5.0	5.0	5.0
X. aethiopica	1.0	1.0	5.0
Metronidazole	5.0	ND	ND
Tetracycline	ND	1.0	ND
Ampicillin	ND	ND	1.0

ND – Not determined.

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