Bioscience Research Communications
Vol. 15, No. 3, June 30, 2003
Printed in Nigeria

0795-8072/2003 $12.00 + 0.00

http://www.klobexpublishers.com

BRC 2002076/15309

Antimicrobial activity of some local herbs on common skin pathogens

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(Received November 1, 2002)

ABSTRACT: Extracts from *Anogeissus leoicarpus, Daniellia diveri* and *Xylopia aethiopica* were screened for antimicrobial activity against *Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Trichophyton mentagrophytes* and *Aspergillus fumigatus* using disc and well diffusion methods. Both aqueous and ethanolic axtracts of the three plants showed antimicrobial activities against at least two microorganisms. None of the extracts showed activity against *Candida albicans*. The three medicinal plants were found to be *Bacteriostatic, bacteriocidal, fungistatic* and *fungicidal*. Preliminary phytochemical screening indicated presence of tannins, saponnins, phenols and anthraquinones.

Key words: Antimicrobial agents; Medicinal herbs; Skin pathogens.

Introduction

The skin is a haven for many microbes. The predominant 'resident' microorganisms of the skin are aerobic and anaerobic diptheroid bacilli (e.g. *Corynebacterium*); non-haemolytic aerobic staphylococci (*S. epidermidis, Peptococcus*), gram positive aerobic spore forming bacilli which are ubiquitous in the air, water and soil namely:α-hemolytic Streptococcus (*S. viridans*) and Enterococci (*S. faecallis*), and gram negative coliform bacilli and *Acinetobacter* (Jawets et al., 1978). Fungi and yeasts are often present in skin folds, while acid fast, non-pathogenic mycobacteria occur in areas rich in sebaceous secretions (genitalia, external ear) as normal skin flora. These organisms do no harm to the skin where they reside, not until integrity of the skin is breached by trauma (accidental or surgical), burns, foreign body or primary skin diseases (Badame, 1988).

Hyman mycotic infection have been grouped into superficial, subcutaneous and deep (systemic) mycoses. Superficial fungal infections of skin, hair and nails may be chronic and resistant to treatment but rarely found to be pathogenic to man causing skin infections. *Microsporium audounini, M. canis, M. fulum, Trychophyton tonsurans, T. scuneuleini, T. mentagrophytes, T. rubrum, T. violaceum, Candida albicans, Epidermophyton flocossum, Coccidiodes immitrs, Cryptococcus neoformans, Histoplasma capsulatums*. Others are *Aspergillus spp*. and *Fusarium spp.* (Rona, 1986). In skin and soft tissue infections, the commonest bacterial agents are *Staphylococcus aureus, Streptococcus pyogenes* (Group A

haemolytic*Streptococcus*), *Clostridium perfringes* and the bacteriodes group. Others are *Mycobacterium tuberculosis, M. leprae, Neisseria gonorrhea, Pasturella tulurensis, Bacillus antracis, Pseudomonas aeruginosa* and many more factors other than trauma and primary skin disease have been identified as contributory to skin infections. These include immune deficiency disease, diabetes mellitus and systemic or topical use of steroids (Jawetz et al., 1978).

Medicinal plants have been used in traditional treatment of skin diseases worldwide. *Acalypha wilkesiana* is a common ornamental plant in southern Nigeria with a wide range use as a herbal remedy for the treatment of undefined skin infections in children (Alade and Irobi, 1993). Other herbs known to be used for treatment of skin infections include *Quisqualis indica, Cormelina benghalensis, Amaranthus spinosus, Ramunculus scleratus* Cassia alata (Sofowora, 1986, Damodaran and venkataraman, 1994). The barks of *Anogeissus leiocarpus* Guil and Peri (Combretaceae) and *Daniella diveri* Hutch (*Caesalpinaceae*) as well as fruit extracts of *Xylopia aethiopica* Rich-Holl (Anonaceae) have also been used as remedy to skin infections (Asuquo, 1976, Malcom and Sofowora, 1969). Thomas (1965) and Irvine (1961) reported that the leaves, roots and bark of *Xylopia aethiopica* contain 17% tannin and infusion made from them is traditionally applied to wounds and skin diseases in addition to their uses for treatment of fever and tapeworm as well as in curing stomach ache, dysentery and stomach ulcer (Keay, 1989).

Herbalists in Nigeria treat wounds and skin infections with concoctions whose recipee includes infusion of *Anogeissus leiocarpus, Daniella diveri* and fruit extracts of *Xylopia aethiopica.* This present study was undertaken to investigate the antimicrobial activities of extracts of these plants on ring worm causing *Trychophyton mentageophytes, Candida albicans, Aspergillus fumigatus, Pseudomonas aeruginosa* and Pathogenic *Staphylococcus aureus* with a view to confirming their use as a remedy for skin disease.

Materials and Methods

Collection of Plant Samples

The dried plant materials *Anogeissus leoicarpus* (bark), *Daniella diveri* (bark) and *Xylopia aethiopica* (fruits) were purchased from markets in Lagos, Nigeria. They were identified by Prof. D. Olowokudejo of Botany Department of the University of Lagos. Voucher samples were also deposited at the same Department.

Extract Preparation

A 20g quantity of the marcerated bark of *Anogeisus leiocarpus, Daniella diveri* and dried fruits of *Xylopia aethopica* were soaked separately in 150ml distilled water and ethanol respectively for seven days. Each mixture was stirred at regular intervals. The extracts were later filtered through Whatman Filter paper No. 1 (Whatman Limited, England). The filterate obtained were concentrated in vacuorotatory evaporator (RE 100). The residue was dried in the oven at 60°C to give a final coloured crystalline compound.

Test Organisms

The test organisms used for screening the antimicrobial activity of the extracts were fungal isolates identified as *Trychophyton mentagrophytes, Candida albicans, Aspergillus fumigatus.* They were obtained from Central Medical Laboratory Services, Yaba, Lagos and were cultured on Sabouraud medium maintained at 25°C for 72h. Other test organisms employed were clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital, Idi-Araba, Lagos. They were cultured overnight at 37°C on Mueller Hinton Agar.

Screening for Antimicrobial Activity

Each plant extract was reconstituted in sterile distilled water and diluted to a concentration of 100mg/,l. Diluted broth cultures (containing 10^6/ml bacterial cells or 10^3 fungal spores/ml) were asceptically poured and spread on sets of Mueller Hinton agar plates (for bacteria) and Sabouraud's dextrose agar plates (for

fungi). Surplus suspension was decanted from the surface of the agar plates which were allowed to dry at room temperature for 3 to 5 minutes. Later 5mm diameter wells were dug on the surface of the agar with the aid of sterile cork borer. Into each of the well was delivered 100µl distilled water in place of plant extract. The bacterial plates were incubated overnight at 37°C while the fungal plates were incubated at 25°C for 72h.

The disc diffusion method was also employed. Agar plates were flooded with test organisms and were allowed to dry. dried filter paper discs impregnated with plant extracts were placed firmly on the agar plates seeded with the test organisms. For a series of test *E. coli* NCTC 10418 and *S. aureus* ATCC 29213 were used as sensitive control strains. All plates were observed for zones of inhibition of microbial and fungal growth and the diameter of the zones were measured. Different combinations of the tree extracts were also tested in order to establish synergism in antimicrobial activity.

Minimum Inhibitory Concentrations (MIC)

The MIC of the extracts was determined by incorporating various amounts (3.12-50 mg/ml) of the reconstituted extracts solutions into sets of test tubes containing culture media. Using a micropipette, 0.01 ml of the standard test bacterial and fungal suspension were added to each of the test tube. The tubes were then incubated at 37°C for 24h (bacterial isolates) and 25°C for 72h (fungal isolates) respectively. A positive control containing only the growth medium and each of the organism was also set up. The MIC was determined as the lowest concentration of the extract which inhibited the organism.

Minimum Bactericidal and Minimum Fungicidal Concentrations (MBC and MFC)

Samples from the test tubes in the MIC determination above which did not show any visible growth after the period of incubation was streaked unto freshly prepared Mueller Hinton agar and Sabouraud agar respectively to determine their MBC and MFC.

Phytochemistry

The powdered bark of *Anogeissus leiocarpous*, *Daniella diveri* and powdered fruits of *Xylopia aethiopica* were screened for biologically active secondary metabolites. These include alkaloids, tannins, saponins and quinines. The modified method of Odebiyi and Sofowora (1978) and Sofowora (1986) were employed.

Results

The three plant materials produced coloured extracts. *D. diveri ox-blood* crystals, *A. leiocarpus* brown crystals and *X. aethiopica* light brown crystals. Table 1 shows the antimicrobial activity of the three plant extracts determined by well and disc diffusion methods. Both aqueous and ethanolic extracts of the plant exhibited antimicrobial activities on almost all the tested organisms except *Candida albicans* while *Trichophyton mentagrophytes* was resistant to both extracts of *D. diveri* and *X. aethiopica*. generally, the aqueous extract showed greater antimicrobial activity than the corresponding organic extracts against the tested organisms. Results obtained also showed that the well diffusion method was more effective than the disc diffusion method with zones of inhibition ranging from 8mm to 29mm as against 4mm to 13mm observed in the disc diffusion method.

Table 2 shows the results of the different combinations of the extracts on test organisms. Comparatively, larger inhibition zones were observed in the combined extracts, indicating greater anti-microbial activities of the extracts in the combined state. Again, *C. albicans* was resistant to the various extract combinations. The result of the minimum inhibitory concentration of the three plant extracts against bacteria and fungi is shown in Table 3. The range of MIC for bacteria was 12.5 – 25.0mg/ml while that of fungi was 6.25 – 25.00mg/ml. None of the extracts was however able to inhibit *C. albicans* even at high concentrations used. Similar result was obtained for the minimum bactericidal and fungicidal determination. The range of MBC for bacteria was 25 – 50mg/ml while 12.5 – 50.0mg/ml was recorded as minimum fungicidal concentration (Table 4). Phytochemical analysis showed the presence of Tannins and

Anthraquinones in *D. diveri* while *A. leiocarpus* contained Saponins in addition. However, *X. aethiopica* contained alkaloids and phenols in addition to Tannins and Anthraquinones (Table 5).

Table 1: Antimicrobial activity of aqueous and ethanolic extracts of three African plants.

Plant	Zone diameter of inhibition (mm)																			
	A				B				C				D				E			
	aq.		eth.		aq.		eth.		aq.		eth.		aq.		eth.		aq.		eth.	
	d	w	d	w	D	w	d	w	d	w	d	w	d	w	d	w	d	W	d	w
Daniellia diveri	4	10	<19	29	10	12	11	12	0	0	0	0	0	0	0	0	12	15	10	<10
Anogeissu leoicarpus	10	12	8	11	11	17	11	19	0	0	0	0	13	22	11	16	13	15	14	15
Xylopia aethiopica	<10	8	9	10	7	<10	<10	10	0	0	0	0	0	0	0	0	14	22	10	14
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

A = *Staphylococcus aureus*
B = *Pseudomonas* sp.
C = *Candida albicans*
D = *Trychophyton mentagrophytes*
E = *Aspergillus mentagrophytes*
aq = aqueous
eth = ethanol
d = disk diffusion zone diameter
w = well diffusion zone diameter

Table 2: Antimicrobial activities of equimixture of extracts of three African plants.

Equi-mixture Extracts	Zone diameter of inhibition (mm)									
	A		B		C		D		E	
	aq.	eth.	aq.	eth.	aq.	eth.	aq.	eth.	aq.	eth.
1 + 2	13.0	13.0	19.0	15.0	0.0	0.0	0.0	0.0	18.0	13.0
1 + 3	10.0	12.0	11.0	16.0	0.0	0.0	19.0	16.0	22.0	23.0
2 + 3	11.0	< 10.0	17.0	18.0	0.0	0.0	18.0	18.0	14.0	23.0
1+2 +3	17.5	10.0	21.0	19.0	0.0	0.0	14.0	14.0	27.0	17.5

1 = *Daniella diveri*
2 = *Anogeissus leoicarpus*
3 = *Xylopia acthiopica*
A = *Staphylococcus aureus*
B = *Pseudomonas* sp.
C = *Candida albicans*
D = *Trychophyton mentagrophytes*
E = *Aspergillus mentagrophytes*

Table 5: Phytochemical composition of the extracts.

Plant	Tannins	Saponins	Anthra-quinones	Flavonoids	Alkaloids	Phylo-batanni	Phenol
Daniella diveri	+	–	+	–	–	–	–
Anogeissus leoicarpus	+	+	+	–	–	–	–
Xylopia aethipica	+	–	+	–	+	–	+

– = not detected; + = detected

Discussion

A fairly good yield of the extract was obtained from the barks and fruits of the plants resulting in the formation of crystalline coloured compounds which were readily soluble in water and ethanol. Both aqueous and organic solution of extracts produced inhibitory activity against Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa* with water extract producing larger zones of inhibition. This is an indication that water was a better solvent in extracting the anti-microbial agent. The extracts were also effective against *Aspergillus fumigatus* and *Trychophyton mentagrohytes*. Both fungi have been implicated in cases of dermatomycosis. These findings indicated the presence of active anti-microbial agents in these plants thereby justifying their use for treatment of microbial infections. Indeed, Oliver (1959) and Irvine (1961) had described *A. leicorpus* as remedy for sorefeet, tooth ache, cought and diarrhoea, while *X. aethiopica* was said to contain Xylopia acid and diterpene (Boakye-Yiadem et al., 1977) which are antiseptic. Although not much has been reported previously about the anti-microbial properties of *D. diveri* this present study confirms its anti-microbial properties. Also the synergistic activity of the extracts was confirmed when equimixtures of two or more extracts produced larger inhibition zones in all the susceptible organism. This explains why the herbalist usually combine the extracts in the preparation of skin remedies. Indeed, other extraneous matters including gun powder, sulphur, camphor etc. are known to be added to enhance the curative value. The physiological explanation for synergistic activity may be that the combine extracts have sequential effects in inhibiting a particular metabolic pathway of their target organisms. However, none of the extracts or their combination was able to inhibit *Candida albicans* the principal causative agent of candidiasis. This may probably be due to the fact that the extracts were in sub-optimal doses to effect the inhibition of the organism. Moreover the test organisms used being a clinical isolate, may have been a highly resistant strain.

The phytochemistry of the plant extracts revealed the presence of Tannins, Saponins, Anthraquinones, Alkaloids and phenols. Previous report by Okogun (1986) implicated these and other chemicals in the curative values of many Nigerian medicinal plants in use. Thus, from the overall results obtained, it is evident that the three plants screened possess anti-microbial agents active against some pathogenic organisms associated with skin infections. They therefore justify their popular use by local herbalist in the treatment of skin diseases.

ACKNOWLEDGEMENT: We wish to express our appreciation and thanks to Dr. D.K. Olukoya, Dr. S.I. Smith, Mr. K.A. Akinsehinde, Mr. M. Bamidele, Mr. J. Iyanda, Mrs. F.O. Baiyewunmi and Mr. M.B. Kadiri-Alaga all of Nigerian Institute of Medical Research, Yaba, for their collaboration in this work.

References

Alade, P.J. and Irobi, O.N. (1992). Anti-microbial activities of crude leaf extracts of *Acalypha wilkesiana*. Journal of Ethnopharmacology 39, 171 – 174.

Asuquo, B.I. (1996). Identification and Characteristics of Local Chewing Stick. B.Sc. Dissertation, submitted to the University of Ibadan, p. 34 – 37.

Boakye, Yaidem, K.; Fiagbe, N.Y. and Aderemi, S.K. (1977). Anti-microbial properties of some West Africa Medicinal Plants. IV. Anti-microbial action of *Xylopia* and other constituents of the fruit of *X. aethiopica*. Lloydia, 40, 543.

Badame, A.I. (1980). Incidence of skin diseases in rural Jamaica. International Journal of Dermatology, 27, 109 – 111.

Damodaram, S. and Venkaraman, S. (1994). A study on the therapeutic efficacy of Aski alaba Linn. Leaf extract against Pityriasis versicolor. Journal of Ethno-pharmacology. 42, 19 – 23.

Irvine, F.R. (1961). Woody plants of Ghana with special references to their uses. Oxford University Press, p. 69 – 75.

Jawetz, E.; Janet, S.; Nicholas, L. and Edwards, E. (1978). Skin micro organism. In: Medical Microbiology. Lange Internationa, N.Y., p. 25 – 27.

Malcolm, S.A. and Sofowora, E.A. (1969). Anti-microbial activity of selected Nigerian folk remedies and their constituent parts. Journal of National product. 32, 512 – 526.

Odebiyi, O.O. and Sofowora, E.A. (1978). Phytochemical screening of Nigerian Medical Plants. Llyodia, 41, 234 – 236.

Okogun, A. (1986). The Chemistry of Nigeria Medicinal Plants. In: The state of medicinal plants in Nigeria (Ed.) Abayomi Sofowora, Ibadan University press, Nigeria, p. 31 – 52.

Rona, M.M. (1986). Clinical Dermatology. 2nd ed. Oxford University Press, London, p. 24 – 34.

Sofowora, A. (1986). The state of medicinal plants research in Nigeria. Ibadan University Press, Nigeria, p. 100 – 120.

Thomas, S.G. (1965). Chemical basis of drug action. In: Drug Plants of Africa. Publications of University of Pennsylavanus, p. 120 – 133.