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MICROBIAL SENSITIVITY TO EXTRACTS OF *Mitracarpus scaber*

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ABSTRACT

Leave extract of *Mitracarpus scaber* were investigated for phytochemistry and in vitro antimicrobial activities by agar – diffusion and broth diffusion techniques. Phytochemisry revealed that water extract had alkaloids, glycosides, saponins, flavonoids, reducing sugars, polyphenols, phlobatanins, anthraquinones and hydroxymethylanthraquinones with the exception of tannins Ethanol extract had seven of the ten phytochemical components screened for with the exception of glycosides, tannins, and flavonoids. Both extracts of *Mitracarpus scaber* produced definite antimicrobial activities against tested isolates with the exception of *Aspergillus fumigatus* and *Mucor* species. The zones of inhibition produced by fungal isolates against both water and ethanol extracts ranged from 7.5 to 17.5mm and compared favourably with that of the control antifungal agent, ketoconazole The zones of inhibition produced bacterial isolates against both leave extracts ranged from 7 5 to 20mm Studies revealed that the water extract was more potent than the ethanol extract. The minimum inhibitory concentration (MIC) for fungal isolates ranged from 0 03 to 4.0mg/ml and from 0.03 to 12 0mg/mlfor bacterial isolates Minimum fungicidal concentrations (MFC) ranged from 5 0 to 25 0mg/ml while minimum bacterial concentration (MBC) ranged from 10 0 to 45mg/ml These results indicate the fungistatic nature of the extract a probable alternative source of medication for the treatment of superficial infections caused by these agents

KEYWORDS. Antimicrobial sensitivity; *Mitracarpus scaber. Rubiaceae*

INTRODUCTION

The use of medicinal herbs in the treatment of infections is perhaps as old as recorded human history (Irobi and Daramola 1993). *Mitracarpus scaber Zucc* (Rubiaceae) is a herb that is indigenous to many African countries including Nigeria, Senegal, Ghana and Gambia. The plant is an annual woody weed. *Mitracarpus scaber (M scaber)* is used extensively in traditional medicinal practice in Africa for the treatment of ringworm, eczema and craw-craw (Dalziel, 1936) The plant is also used for the treatment of sore throat and leprosy in Senegal (Ekpendu *et al.*, 1993) In Nigeria, the plant is used by major tribes and in northern Cross River state for various purposes ranging from treatment of parasitic infection, as antidote to arrow poison and anti-inflammatory agent to treatment of skin infections such as eczema and ringworm (Dalziel, 1936). Application of the herb could be topical or ingested.

The composition of the volatile parts of *M scaber* investigated showed that the plant contains eleven free fatty acids as the major components. Hexadecanoic acid was pentadecanoic, (Z) – Octadeca-9-noic, octadecanoic and dodecanoic acids. Minor constituents included six fatty acid esters, two aldehydes and six oxygenated monoter-penoids (Ekpendu *et al.*, 1993). The anti-inflammatory and antibacterial activities of *M. scaber* extracts has been studied by Akah *et al.* (1994) who discovered that the extracts progressively reduced oedema in rat hind paws

This work investigates the phytochemical properties of *M. scaber*, which is non-existent, and the general antimicrobial activities of the water and ethanolic extracts of its leaf.

MATERIALS AND METHODS

Microbial Isolates:

The fungal isolates used for this study included yeast *Candida parapsilosis, C tropicalis, C glabrata* and *Trichosporon inkin* and moulds *Trichophyton rubrum Fusarium solani, Aspergillus fumigatus* and *Mucor* species Bacterial isolates were *Pseudomonas aeruginosa, Escherichia coli, Streptococcus faecalis Staphylococcus aureus, Proteus* species, and *Shigella flexner* All these isolates were obtained from the Department of Medical Microbiology and Parasitology, University of Calabar Teaching Hospital Fungal isolates were stored on sabouraud dextrose agar slant and bacterial isolates in nutrient agar and kept at 4°C prior to use

Collection of Plant and Extraction:

Mitracarpus scaber plant was collected from a farmland in Ikom in Northern Cross River State and authenticated in the Department of Botany, University of Calabar The leaves were shade dried at ambient temperature (25°C), and then crushed with the aid of a mortar and pestle into fine powder Extraction of the powdered leaves was carried out using water and ethanol as extracting solvent

Water Extraction:

The leaf powder (50 gm) was put into a soxhlet extractor The soxhlet extractor was fitted onto a previously weighed round bottom flask Into the round bottom flask 500ml of water was introduced and the apparatus was set on a heating mantle As the content of the flask was heated to boiling the water evaporated it condensed and dropped into the soxhlet where the water soluble fraction was extracted into the round bottom flask leaving behind the water insoluble fraction The extraction was removed and passed through a

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Whatman filter No. 1 (Whatman, U K) and concentrated by drying in an oven at 80°C.

Ethanol Extraction:

The water insoluble fraction obtained after water extraction was dried and 50gm was put into the soxhlet extractor fitted on a round bottom flask into which 500ml of ethanol has been added and the apparatus was set on a heating mantle at 78°C. As the content of the flask was heated, ethanol soluble fraction was extracted The extract was removed and passed through a filter paper and concentrated by drying in an oven at 80°C

Phytochemical Studies

Phytochemical studies of the plant was undertaken to know the basic chemical components of the plant and active ingredients present. The extracts were screened for the presence of flavonoids, polyphenols, hydroxymethyl anthraquinones according to methods by Guilei (1982). Phytochemistry of tannins, saponins alkaloids, glycosides, phlobatanins were screened according to the method of Sofowora, (1984) while anthraquinones was screened according to method of Trease and Evans, (1984).

Preparation of Test Inocula

Sets of 24hr culture of the isolates were used to prepare the inocula The organisms were suspended in %ml of sterile normal saline and turbidity adjusted to 0.5 McFarland's standard, which is an equivalent of 1×10^6 to 5×10^6 CFU/ml. This was further diluted 1 2000 to provide a final test inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml (Espinel-Ingroff *et al*, 1999)

Preliminary Screening for Antimicrobial Acitivity

Preliminary screening test of the leaf extracts were carried out by a modified agar diffusion method described by Irobi and Daramola (1993). Three different concentrations of leaf extracts viz 100,200, and 300mg/ml was prepared by adding 10ml of distilled water to 1g, 2g and 3g respectively to assess antibacterial activities. Same dilutions were constituted with the ethanol extract using glycerol as diluent. To assess anti-fungal activity, a 100mg/ml concentration was prepared as above and futher diluted by two fold dilution to give 50 and 25mg/ml of leaf extract A drop (0.05ml) of each of the diluted extract concentrations prepared was introduced into wells (5mm) bored with a sterile cork borer on the surface of potato dextrose agar (PDA) and nutrient agar (NA) seeded with prepared fungal or bacterial suspension respectively. The plates were incubated for 2-4 days at 25°C for fungal isolates and 24 hours at 35°C for bacterial isolates. Time of incubation was controlled by appearance of growth around extract free control well Seeded plates with no extract were set up as controls. The presence of zones of inhibition around the wells was interpreted as indication of microbial inhibition by the extracts

Antimicrobial Susceptibility Testing

A modified agar diffusion method as above was employed to determine the susceptibility of microbial isolates to extracts of *M. scaber* leaves Susceptibility studies were carried out using extract concentrations of 25, 50, 100mg/ml for fungal isolates, and 100, 200, 300 mg/ml for bacterial isolates One drop (0 05ml) of each extract concentration was introduced into wells of pore size 2mm on the surface of agar plates The antifungal drug, ketoconazole (2mg/ml) and antibacterial drug, amplicillin (2 5mg/ml) were used as drug control while agar plates without drugs/extract were used as growth control After incubation at 25°C for 3-4 days for antifungal activity and 24 hours at 35°C for antibacterial acitivity, zones of inhibition were measured.

Minimum Inhibitory Concentration (MIC) Testing

The MIC of the extracts was determined by adding 0 5ml of extract solution into tubes containing 2ml of sabouraud broth (for fungal isolates) and nutrient broth (for bacterial isolates) This was diluted serially from 20 to 0.0013mg/ml and from 60 to 0 004mg/ml for fungal and bacterial isolates respectively Two drops of each microbial suspension was separately introduced into each set of test tubes Test tubes of the control drugs were similarly treated All the test tubes were incubated for 2-4 days at 25° for fungi and 24hrs at 35°C for bacteria The MIC was regarded as the lowest concentration of extract that did not permit any visible growth when compared with the drug free broths containing either fungal or bacterial suspension

Minimum Fungicidal Concentration (MFC) and Minimum Bactericidal Concentration (MBC)

The tubes with mixture of the organism and extract in MIC studies which showed no visible growth after 2-4 days or 24hrs incubation, respectively were subcultured onto a PDA and NA and incubated for 2-4 days at 25°C for fungal, or 24hrs at 35°C for antibacterial activity. The MBC was regarded as the lowest concentration of the extract that did not yield a single bacterial colony during subculture on NA plate after 24hrs incubation period, while the MFC was taken as the lowest concentration of the extract that did not show any fungal colony growth after 2-4 days incubation with PDA.

Table 1: Phytochemical Composition of *M. scaber* Extract

Phytochemical Components	Water	Ethanol
Alkaloids	+	+
Glycosides	+	-
Saponins	++	+
Tannins	-	-
Flavonoids	+	-
Reducing sugars	++	+
Polyphenols	+++	+
Phlobatanins	++	+
Anthraquinones	+	+
Hydroxymethyl anthraquinones	+	+

KEY:

+	=	Present
++	=	Present in excess
+++	=	Very much present in excess
-	=	Absent

RESULTS

Results in Table 1 show that water extract of *M scaber* contains alkaloids, glycosides, saponins, flavonoids, reducing sugars, polyphenols, anthraquinones and hydroxymethyl anthraquinones with the absence of tannins Ethanol extract however, contains seven out of the ten phytochemical components screened for with the absence of glycosides, tannins and flavonoids The result also shows that water extract of *M scaber* contains more saponins, reducing sugars, polyphenols and phlobatanins than the ethanol extract of the plant The signs (+) show that 65% of the leaf extracts contain alkaloids, (++) 75% saponins, (+++) 90% polyphenols and (-) 0% of tannins.

Results presented in Tables II a and b show that water and ethanol extracts of *M scaber* possess antimicrobial activities against the organisms tested with exception of *Aspergillus fumigatus* and *Mucor* species *Candida tropicalis* and *Candida glabrata* were the most sensitive fungi to extracts of *M. scaber*, while *Pseudomonas aeruginosa* appeared to be the most sensitive bacteria to extracts of *M scaber*

Table IIa: Preliminary Antibacterial Activity of *M. scaber* Extracts

	ZONES OF INHIBITION(MM)					
Organisms	**Water Extract (mg/ml)**			**Ethanol Extract (mg/ml)**		
	100mg	200mg	300mg	100mg	200mg	300mg
Pseudomonas aeruginosa	15.0	17 5	20.0	10 0	12 5	15 0
Escherichia coli	5.0	10 0	15.5	5 0	7 5	10 0
Staphylococcus aureus	7.5	10.0	15.0	7.5	10.0	12 5
Proteus Species	5.0	7.5	10.0	2.5	5.0	7 5
Shigella flexneri	5.0	7.5	10.0	5.0	7 5	10 0
Streptococcus faecalis	10.0	15.0	17.5	7 5	10 0	15.0

Table IIb: Preliminary Antifungal Activity of *M. scaber* Extracts

	ZONE OF INHIBITION(MM)					
	Water Extract (mg/ml)			**Ethanol Extract (mg/ml)**		
Organisms	100mg	200mg	300mg	100mg	200mg	300mg
Candida parapsilosis	5.0	10.0	15.0	2.5	7.5	10 0
C. tropicalis	5.0	10.0	16.0	5.0	7.5	10.0
C. glabrata	2.5	5.0	10 0	2 5	5 0	7 5
Trichosporon inkin	-	-	10 0	-	-	7 5
Trichophyton rubrum	10 0	7 5	10 0	10 0	12 5	15 0
Fusarium solani	5 0	7.5	10 0	5 0	7 5	10 0
Aspergillus fumigatus	-	-	-	-	-	-
Mucor species	-	-	-	-	-	-

Susceptibility studies (Tables III a and b) show that the mean zone of inhibition obtained for ethanol extract ranged between 7.5 ± 0.03 and 12.5 ± 0.07mm while the mean zone of inhibition for water extract was between 10.0 ± 0 03 and 17.5 ± 0 07mm The mean zone of inhibition produced by ketoconazole (2mg/ml) was between 7.5 ± 0 03 and 15 0 ± 0 03mm for fungal isolates For bacterial isolates, the mean zone of inhibition obtained for the extract was between 7 5 ± 0.03 and 20.0 ± 0.03mm while that produced by ampicillin (2.5mg/ml) ranged between 7 5 ± 0 03 and 15 0 ± 0 05mm

The MIC values as shown in Table IIIa indicate that *E coli* and *Staphylococcus aureus* had MIC values of 0 10mg/ml for water extract; *P aeruginosa* had MIC value of 0.04mg/ml, *Streptococcus faecalis* 0 02mg/ml while *Proteus* species and *Shigella flexneri* which were less sensitive had MIC value of 2.4mg/ml Ampicillin (the antibacterial control drug) had MIC values that ranged between 0.02 and 0 04mg/ml. MIC values of 0.03mg/ml were obtained for *Candida parapsilosis*, *C glabrata* and *Trichophyton rubrum* while 0.8mg/ml was obtained for *C tropicalis* and 0 2mg/ml for *Fusarium solani* *Trichosporon inkin* was the least sensitive with MIC value of 4mg/ml water extract (Table IIIb) Ketonconazole (then antifungal control drug) had MIC values of 0 02mg/ml for *C parasilosis*, *Trichosporon inkin* and *Trichophyton rubrum*, 0 004mg/ml for *C glabrata* and 0 08mg/ml for *Fusarium solani*

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) as shown in Table IIIa and IIIb respectively indicates higher values than MIC values

DISCUSSION

This study shows that ethanol and water extracts of *M. scaber* possess antimicrobial activity against microorganisms tested *M scaber* possess some antifungal activities against *Candida parasilosis*, *C tropicalis*, *C glabrata* *Trichosporon inkin*, *Trichophyton rubrum*, *Fusanum solani* This observation is partly in agreement with a previous report by Akah *et al.*, 1994 that alcohol extract of *M scaber* produced antifungal activity against *C albicans*. It is further shown here that water extract of *M. scaber* was more potent than ethanol extract with mean diameter of inhibition of 17 5 ± 0.07mm while mean diameter of inhibition for ethanol extract was 12 5 ± 0.03mm for fungal isolates For bacterial isolates, the mean diameter of inhibition by water extract was 20 0 ± 0 03mm while the mean diameter of inhibition by ethanolic extract was 15 0 ± 0 03mm. Also phytochemical analysis of a closely related species *M. villosus* shows that it contains phenolics and sequinterpenes (Irobi and Daramola, 1993) Ethanol may modify the phenolic content of the extract thereby affecting its

Table IIIa: Susceptibility Studies of M. scaber extract on bacterial isolates

Bacterial isolates	Water Extract 300g/ml	Ethanolic Extract 300g/ml	Ampicillin 2.5mg/ml	MIC Water	MIC Ethanol	MIC Ampicillin	MBC Water	MBC Ethanol	MBC Ampicillin
Pseudomonas aeruginosa	20.0 ± 0.03	15.0 ± 0.03	NT	0.04	0.10	NT	5.9	10.0	NT
Escherichia coil	15.0 ± 0.03	10.0 ± 0.07	15.0 ± 0.05	0.10	2.40	0.04	10.0	20.0	5.0
Staphylcoccus aurues	15.0 ± 0.03	12.5 ± 0.05	NT	0.10	0.60	NT	10.0	15.0	NT
Streptococcus faecalis	17.5 ± 0.05	15.0 ± 0.05	NT	0.02	0.10	NT	10.0	10.0	NT
Proteus species	10.0 ± 0.07	7.5 ± 0.03	7.5 ± 0.03	2.4	12.0	0.02	25.0	45.0	15.0
Shigella flexneri	10.0 ± 0.07	10.0 ± 0.07	10.0 ± 0.05	2.4	2.4	0.02	15.0	20.0	10.0

Mean ± SEM zone of inhibition (N= 4 Assays)
NT = Not tested
Ampicillin 2.5mg/ml stock solution was serially diluted from 0.5mg/ml to 0.0008mg/ml

Table IIIb: Susceptibility Studies of M. scaber extract on fungi isolates

Bacterial isolates	Water Extract 300g/ml	Ethanolic Extract 300g/ml	Ampicillin 2.5mg/ml	MIC Water	MIC Ethanol	MIC Ampicillin	MFC Water	MFC Ethanol	MFC Keto
Candida parapsilosis	20.0 ± 0.03	15.0 ± 0.03	NT	0.03	0.20	0.02	5.0	10.0	10.0
Candida tropicalis	15.0 ± 0.03	10.0 ± 0.07	15.0 ± 0.05	0.8	0.8	0.04	15.0	15.0	5.0
Candida glabrata	15.0 ± 0.03	12.5 ± 0.05	NT	0.03	0.8	0.04	5.0	15.0	5.0
Trichosporon inkin	17.5 ± 0.05	15.0 ± 0.05	NT	4.00	4.0	0.02	20.0	25.0	10.0
Trichosporon rubrum	15.0 ± 0.05	12.5 ± 0.07	10.0 ± 0.03	0.03	0.2	0.02	10.0	15.0	10.0
Fusarium solani	15.0 ± 0.05	12.5 ± 0.05	7.5 ± 0.03	2.4	2.4	0.02	15.0	20.0	10.0

KEY
Keto=Ketonoconazole
Mean ± SEM zone of inhibition (N= 4 Assays)
NT = Not tested
Ampicillin 2.5mg/ml stock solution was serially diluted from 0.5mg/ml to 0.0008mg/ml

potency. This observation may explain the efficacy of the local application of squeezed exudates of fresh leaves of this plant in water rather than in ethanol. The exudates of fresh leaves of the plant may also be applied directly without water to the affected part of the body for the same therapeutic efficacy.

Pseudomonas aeruginosa was the most sensitive bacterium to both water and ethanol extracts of *M. scaber*. Only a few known antimicrobials can inhibit *P. aeruginosa*. The reason for this appears to be the recalcitrant nature of the Pseudomonads that easily degrade and destroy drugs (Sadoff and Sanford, 1983). It is suggested here that *M. scaber* in crude extract form may offer some hope to the inhibition problem possed by *P. aeruginosa* as the crude active ingredient may be shielded transiently thus allowing it time to act before destruction by the organism. Furthermore, this work validates previous report on highest antibacterial activity of *M scaber* on *P. aeruginosa* by Akah *et al*., (1994) who also reported antibacterial activity of *M. scaber* extracts against *Staphylococcus aureus* and *Escherichia coli*.

The sensitivity of the dermatophyte fungi, *Trichophyton rubrum* to the extracts noteworthy as the local populace use the plant leaves for fungal skin infections. The extract also inhibited the growth of *Fusarium solani* which has been implicated in fungal infections such as keratitis and mycetoma (Midgley *et al*., 1978), suggesting that *M. scaber* extracts may be effective in the treatment of not only superficial infection but also subcutaneous infections.

The values obtained in MBC and MFC studies were greater than those of MIC studies. In addition, some colonies of test microorganisms were recovered after sub-culturing the content of the tubes containing 25mg/ml of water extract in MFC assays and 100mg/ml in MBC assays These observations indicate both fungistatic and bacteriostatic nature of the extracts

The fact that the extracts of *M. scaber* inhibited commonly encountered microorganisms explains the popularity of the plant among the local folks in the treatment of some infections *Candida* species are ubiquitous opportunistic fungi associated with the pathogenesis of skin diseases, urinary tract infections, endocarditis, vulvovaginitis and oral thrush (in children and elderly patients), hence the therapeutic rationale in the treatment of oral thrush by rural Nigerian herbalist Phytochemical screening of the plant showed a wide range of organic compounds, which have been reported to possess some degree of antimicrobial action Many disinfectants used in hospitals such as lysol, cresol and dettol contain phenols as their active ingredient The active ingredient in the extract may be acting by the mechanism of these organic phenol derivative as previously suggested by Hagerman and Butler (1981)

It is a common clinical experience that many microorganisms are acquiring resistance to the routine antimicrobial agents with broader therapeutic spectrum

potency, and efficacy. Plants continue to provide rich soumces of therapeutic agents and the results of this study has added yet new potential antimicrobial agents.

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