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Phytochemical Composition and Antimicrobial Activity of Aqueous and Ethanol Leaf Extracts of *Eremomastax speciosa* Against Clinical Pathogens

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ABSTRACT

Background: The continuous spread of drug resistance underscores the urgent need for new antimicrobial agents. Given the serious burden of bacterial and fungal infections, this study investigated the phytochemical constituents and antimicrobial activities of Eremomastax speciosa (Acanthaceae), a Nigerian medicinal plant widely used ethnomedically for treating microbial infections.

Methods: Air-dried leaves were pulverized and macerated in distilled water and 70% ethanol to obtain aqueous and ethanol extracts. Antimicrobial activity against Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, and Candida albicans was evaluated using the agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) were determined, while phytochemical screening of both extracts was carried out using standard qualitative procedures.

Results: The extracts produced measurable inhibition zones and demonstrated concentration-dependent antimicrobial activity against all test organisms. At the highest concentration of 1600 mg/ml, the ethanol extract showed greater inhibitory effects than the aqueous extract. Staphylococcus aureus was the most susceptible organism, whereas Candida albicans was the least susceptible. Standard antimicrobial agents were included as controls, with levofloxacin exhibiting the highest activity against the bacterial isolates. MIC values ranged from 200 to 6.25 mg/ml, with Candida albicans showing inhibition at the lowest concentrations. The MBC/MFC values fell within the corresponding MIC ranges, indicating time-dependent bactericidal and fungicidal effects. There was significant ($P<0.05$) difference in the inhibition zone diameters across all the microorganisms, with Staphylococcus aureus being most susceptible with the highest IZD (29.6mm), while candida albicans was the least susceptible with IZD of 19.3mm. Both extracts showed similar microorganisms susceptible profile

Conclusion: The findings suggest that aqueous and ethanol leaf extracts of E. speciosa contain bioactive compounds with significant antibacterial and antifungal properties and may serve as potential sources of antimicrobial agents. This supports its continued ethnomedicinal application in Nigeria.

Keywords: Antimicrobial, clinical pathogens, Eremomastax speciosa, extracts, phytochemical

1. INTRODUCTION

Over the years, various classes of antibiotics ranging from beta-lactams, sulfonamides, aminoglycosides, chloramphenicol, macrolides, oxazolidinones, ansamycins, glycopeptides to quinolones, tetracyclines, nitroimidazoles, etc has been discovered and synthesize [1]. The use of herbal medicinal products and supplements has however increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare [2]. Traditional medicine is the sum of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health and the prevention, diagnosis, improvement or treatment of physical and mental illness [3]. The term "Medicinal plant" refers to a variety of plants that have medicinal properties. These plants are used as a medical resource in almost all cultures and are a rich source of compounds that can be used to develop drug synthesis [4]. Subsequently, countless active compounds have been

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separated from natural products. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations [3]. Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established, many of them remain untested and their use is either poorly monitored or not even monitored at all [2]. The consequence of this is an inadequate knowledge of their mode of action, potential adverse reactions, contraindications and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents [2]. *Eremomastax speciosa* (Hochst.) Cufod. Of the Family Acanthaceae is widely distributed in the tropics of Africa. It is a robust, polymorphous shrub that grows up to 2m long and has a characteristic quadrangular stem and violets on the underside of leaves. It is known commonly in southern Nigeria (Ibibio) as Edem ididuot ("golden seal" or "African blood tonic"). In addition to its application in treating female infertility and menstrual cramps, the plant has also been used in treating anaemia, dysentery, urinary tract infection, haemorrhoids and gastric ulcers. This study aimed to evaluate the phytochemical constituents and antimicrobial activities of aqueous and ethanol leaf extracts of *Eremomastax speciosa* against selected clinical bacterial and fungal pathogens.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials

Fresh leaves of Eremomastax speciosa,

2.1.2 Chemicals and reagents

70% ethanol, distilled water, mannitol salt Agar, salmonella-shigella Agar, Sabouraud Dextrose broth, 0.5 MC Farland Standard, Mueller Hinton Agar, Antibiotic discs, Fluconazole

2.1.3 Equipment and other materials

Laboratory bench, weighing balance, 2000 ml conical flask, sterile muslin cloth, filter paper, beakers, aluminium foil.

2.2 Methods

2.2.1 Plant collection and identification

Fresh leaves of *Eremomastax speciosa* were collected in the month of September, 2023. The plants were identified and authenticated by Mrs. E. G. Udoma, a Taxonomist at the Faculty of Pharmacy Herbarium, University of Uyo. *Eremomastax speciosa* was identified and authenticated with Voucher Number UUPH 1(b).

2.2.2 Plant extraction and collection

2.2.2.1 Extraction

2.2.2.1.1 Aqueous Extraction

One kilogramme (1 kg) of fresh leaves Eremomastax speciosa was pulverized into smaller sizes and dried on a laboratory bench at room temperature. Five hundred grams (500 g) of dried Eremomastax speciosa leaves was weighed and separately macerated in 1000ml of distilled water contained in a 2000ml conical flask with intermittent stirring for 24 hours. The samples were thereafter filtered separately using sterile muslin cloth and filter paper to obtain the filtrate/marc. The marc was concentrated by evaporating to dryness using a water bath at40 °C for about 5 days. The aqueouscrude extract obtained was 28.87 g (5.77% yield), The concentrated extracts were transferred to beakers, sealed with aluminium foil, labelled appropriately and stored in the refrigerator pending presentation for analysis.

2.2.2.1.2 Ethanol Extraction

About 500g each of dried Eremomastax speciosa leaves was weighed and separately macerated in 1000ml of 70% ethanol contained in a 2000ml conical flask with intermittent stirring for 72 hours. The samples were thereafter filtered separately using sterile muslin cloth and filter paper to obtain the filtrate/marc. The marc was concentrated by evaporating to dryness using a water bath at 40 °C for about 3 days. The ethanol crude extract obtained was 16.68 g (3.34 % yields). The concentrated extracts were transferred to beakers, sealed with aluminum foil, labeled appropriately and stored and preserved in the refrigerator.

2.2.3 Phytochemical Screening

The qualitative tests were carried out in the Department of Pharmacognosy and Natural medicine, Faculty of Pharmacy, University of Uyo. The aqueous and ethanol extracts were analyzed for the presence or absence of the following secondary metabolites, using modified methods of [5], [6]and [7]. Saponins, tannins, terpenoids, Cardiac Glycosides, Flavonoids, Alkaloids, Steroids, Phlabotannins, Phenols

2.2.4 Collection, characterization and maintenance of test organisms

Four clinical pathogenic microbes were obtained from the laboratory unit of the University of Uyo, Health centre and the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Uyo. The microbial isolates were Gram-positive bacteria, Staphylococcus aureus, Gram-negative bacteria, Salmonella typhi and Shigella dysenteriae and one fungal isolate, Candida albicans. The test organisms were isolated and purified by subculturing to obtain the pure cultures. The Gram-positive bacteria isolate was cultured on Mannitol salt agar while the Gram-negative bacteria were sub cultured on Salmonella-Shigella agar which served as both differential and selective media for the bacteria isolates. All bacteria cultures were incubated at 37°C for 24 hours. The fungal isolate was cultured on Sabouraud Dextrose Agar and incubated at 28°C for 72 hours. Standardization of Test organism before Inoculation. Standard microbial suspensions were obtained by preparing an overnight broth of the bacterial and fungal isolates. The overnight broth was prepared by subculturing a loopful of each bacterial culture in 5ml of nutrient broth and incubating at 37 °C for 24 hours while the fungal culture was incubated in 5ml of Sabouraud dextrose broth at 28 °C for 72 hours. Before use, 0.1 ml of each overnight broth was diluted in 9.9 ml of distilled water to obtain an isolate suspension equivalent to 0.5 McFarland standard [8].

2.2.5 Antimicrobial Assay

2.2.5.1. Antimicrobial assay of the Aqueous and Ethanol leaf extracts of Eremomastax speciosa

The aqueous and ethanol leaf extracts of the aforementioned plants were analyzed against the standardized test organisms using agar well diffusion technique [9]. Two-fold dilution technique, 1600 mg/ml, 800 mg/ml, 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml of each plant's aqueous and ethanol extract were prepared aseptically using distilled water. Thereafter, sufficient amount of Mueller Hinton Agar and Sabouraud Dextrose Agar (SDA) were prepared using the manufacturer's instructions and autoclaved at 121°C for 15 minutes. 2 drops of diluted and standardized bacterial and fungal cultures were put in sterile labeled agar plates before 20 ml of warm (about 27 °C) Mueller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) respectively was measured into each plate and swirled to disperse the inoculums. When the seeded plates solidified, a sterile cork borer of 5mm was used to bore holes in the agar plates and about 0.1 ml of each extract concentration was used to fill the appropriate hole. The plates were allowed to stand for one hour to allow the extracts diffuse into the medium before being incubated at 37°C for 24 hours and 28°C for 72 hours for bacteria and fungi respectively. After incubation, the antimicrobial activity of each extract was determined by measuring the inhibition zone diameters (IZD) in millimeters. The analysis was carried out in triplicates.

2.2.5.2. Antimicrobial Assay of Standard Drugs

Antibiotic discs impregnated with twelve (12) standard drugs of known concentration were also evaluated in comparison with the antimicrobial activities of the extracts. The Antifungal Drug Fluconazole at a concentration of 15mg/ml was also used as the standard drug in comparison with the antifungal activity of the extracts.

2.2.6 MIC and MBC/MFC determination

2.2.6.1 Determination of Minimum Inhibitory Concentration (MIC)

The MICs were determined using Broth dilution method with the extract concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were aseptically added to 5 ml of nutrient broth and Sabouraud Dextrose broth by two-fold dilution technique. Thereafter, two loopfuls of the specific diluted microbial suspensions was added to each broth and mixed. The test tubes were incubated at 37°C for 24 hours and 28°C for 72 hours for bacteria and fungi respectively. The least concentration that did not permit the growth of inoculated organisms was recorded as the minimum inhibitory concentrations for the extract on the specified test organisms.

2.2.6.2. Determination of Minimum Bacteriocidal Concentration (MBC) by Plate Method

After 24 hours, nutrient agar plates were seeded with a loopful of inoculum from all bacterial test tubes that showed no growth and incubated at 37°Cfor24hours.The concentration that showed no growth was taken as the minimum bacteriocidal concentration for the extract against the specified test organisms.

2.2.6.3. Determination of Minimum Fungicidal Concentration (MFC) by Plate Method

After 72 hours, appropriately labelled Sabouraud dextrose agar plates were seeded with a loopful of inoculum from all fungal test tubes that showed no growth and incubated at 28°C for 72 hours. The concentration that showed no growth was taken as the minimum fungicidal concentration for the extract against C. albicans.

2.3 Statistical analysis

Statistical analysis were expressed as mean ± SD and differences between sets obtained were determined using ONEWAY ANOVA followed by Duncan post Hoc Test with the use of SPSS v 20 software. Differences were considered significant at $p < 0.05$

3. RESULTS

Table 1: Qualitative Phytochemical Screening of the Aqueous and Ethanol Leaf extracts of Eremomastax speciosa

S/N	Phytochemical composition		Eremomastax speciosa Leaf extract	
			Aqueous	Ethanol
1.	Saponins		+	+
2.	Tannins		+	+
3.	Terpenoids		+	+
4.	Alkaloids		-	-
5.	Cardiac glycosides		+	+
6.	Phenols		-	-
7.	Phlobatannins		-	-
8.	Steroids		-	-
9.	Flavonoids		+	+
10	Anthraquinones	Free	-	-
		Combined	-	-
11	Glycosides		-	-

KEY: - (phytochemical absent) + (phytochemical present)

Table 2: Antimicrobial Activity of *Eremomastax speciosa* Leaf extracts

S/N		*Eremomastax speciosa* Aqueous Leaf Extract							*Eremomastax speciosa* Ethanol Leaf Extract						
		Mean Inhibition Zone Diameter (IZD) in mm							Mean Inhibition Zone Diameter (IZD) in mm						
	Concecnration mg/mL	1600	800	400	200	100	50	25	1600	800	400	200	100	50	25
	Test Organisms														
1	*Staphylococcus aureus*	29.6	19.3	14.3	11.3	9.67	-	-	31.6	21.3	14.3	11.6	9.6	-	-
2	*Salmonella typhi*	21.3	16.6	11.3	6.6	5	-	-	26.0	19.3	16.6	14.3	11.3	6.5	-
3	*Shigella dysenteriae*	26	18.3	13.6	11	7	-	-	31.0	22.6	17.3	14.6	9.6	-	-
4	*Candida albicans*	19.3	13.6	10.3	8.0	-	-	-	16.3	12.0	10.3	8.0	-	-	-

KEY: – (no inhibition); IZD - Inhibition Zone Diameter

Table 3: Antimicrobial Activity of Standard Drugs against Clinical Pathogens

S/N	Test Organisms	Standard Antibiotics Mean Inhibition Zone Diameter (in mm)														
		AUG 30ug CTX 25ug	CTX 25ug	CRO 45ug	IMP 10ug	CXM 30ug	OFX 5ug	ERY 15ug	GN 10ug	AZN 15ug	ZEM 5ug	LBC 5ug	CIP 10ug	NF 300ug	NA 30ug	ACX 10ug	FLU 15 µg
1	*Staphylococcus aureus*	7.33	NT	16.67	29.33	-	-	43.33	13.33	15.33	18	18	37.33	31.33	-	-	-
2	*Salmonella typhi*	-	19.33	35.67	-	-	31.67	NT	20.67	-	14.33	36.33	-	8.67	-	-	-
3	*Shigella dysenteriae*	-	19.33	35.67	21.33	-	25.67	NT	11.67	-	21	27	-	-	-	-	-

KEY: - No inhibition, AUG - Amoxicillin Clavulanate, CTX- Cefotaxime, CRO- Ceftriaxone-Sulbactam-Disodium, IMP - Imipenem/Cilastatin, CXM- Cefuroxime, OFX- Ofloxacin, ERY - Erythromycin, GN- Gentamicin, AZN- Azithromycin ZEM - Cefixime, LBC- Levofloxacin, CIP- Ciprofloxacin, NF - Nitrofurantoin, NA-Nalidixic Acid, ACX- Ampiclox, FLU- Fluconazole

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Table 4: Minimum Inhibitory Concentration (MIC) of Eremomastax speciosa Leaf extracts against the Test organisms

S/N		*Eremomastax speciosa* Aqueous Leaf Extract						*Eremomastax speciosa* Ethanol Leaf Extract					
		Minimum Inhibitory Concentration						Minimum Inhibitory Concentration					
	Concentration (mg/mL)	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25
	Test Organisms												
1	*Staphylococcus aureus*	–	+	+	+	+	+	-	-	-	+	+	+
2	*Salmonella typhi*	–	–	+	+	+	+	-	-	+	+	+	+
3	*Shigella dysenteriae*	–	+	+	+	+	+	-	+	+	+	+	+
4	*Candida albicans*	–	–	–	–	+	+	-	-	-	-	-	-

KEY: - (growth absent) + (growth present)

Table 5: Minimum Bactericidal/ Fungicidal Concentration (MBC) of Eremomastax speciosa Leaf extracts against the Test organisms

S/N	Test Organisms	*Eremomastax speciosa* Aqueous Leaf Extract	*Eremomastax speciosa* Ethanol Leaf Extract
		Minimum Bactericidal/Fungicidal Concentration (mg/ml)	Minimum Bactericidal Concentration (mg/ml)
1	*Staphylococcus aureus*	200	200
2	*Salmonella typhi*	200	100
3	*Shigella dysenteriae*	200	50
4	*Candida albicans*	25	25

4. DISCUSSION

The results from the phytochemical screening of the aqueous and ethanol leaf extracts of E. speciosaare presented in Table 1. Following the standard chemical procedures, saponins, tannins, terpenoids, flavonoids and cardiac glycosides were present in both plant extracts. However, other secondary metabolites like alkaloids, phenols, phlabotannins, steroids, free and combined anthraquinones as well as glycosides were absent in both. These results are in accordance with the findings obtained by other researchers such as [10], [11] who reported the presence of saponins and tannins in the aqueous and ethanol extracts of E. speciosa; the presence of flavonoids and absence of glycosides reported by Oben et al., 2006 in E. speciosa aqueous leaf extract as well as the presence of flavonoids and absence of free anthraquinones and phlabotannins in E. speciosa ethanol leaf extract mentioned by [11]. Furthermore, the results contradict some findings by these researchers who reported the presence of alkaloids and absence of cardiac glycosides in E. speciosa aqueous leaf extract [10] as well as the absence of cardiac glycosides and presence of alkaloids and combined anthraquinones in E. speciosa ethanol leaf extract highlighted by [11].The variation in observation could be due to differences in geographical location as well as the processing and extraction methods employed. The antimicrobial activities of E. speciosa aqueous and ethanol leaf extracts against the test isolates are presented on Tables 2. The antimicrobial activity of E. speciosa aqueous leaf extract as reported indicates that S. aureus recorded the highest inhibition zone diameter of 29mm, followed by S. dysenteriae with 26 mm, S. typhi with 21mmand C. albicans with 19mm. Also, all four isolates were sensitive against E. speciosa ethanol leaf extract with S. aureus recording the highest inhibition zone diameter of 32mm, followed by S. dysenteriae with 31mm, S. typhi with 26mmand C. albicans with 16mm. When the extract was deployed in a range of concentrations from 25 mg/ml to 1600 mg/ml, it was discovered that 1600 mg/ml, 800 mg/ml, 400 mg/ml and 200 mg/ml demonstrated highest inhibition radii in the increasing order of concentration against the test organisms, thus indicating dose dependent effect and antimicrobial potency. The standard antibiotic discs employed against the bacterial isolates contained twelve (12) different antibiotics of varying concentrations while the antifungal drug (Fluconazole) was employed at a dose of 15mg/ml. Each antibiotic inhibited the bacterial growth by different degrees as indicated by the mean inhibition diameter recorded in Tables 3. Ofloxacin, Ceftriaxone Sulbactam and levofloxacin were active and sensitive against all the bacterial isolates. This implies that they are very effective in the treatment of diseases caused by these bacterial isolates. Furthermore, the standard antifungal drug, Fluconazole was active against C. albicans at the 15 mg/ml concentration used. According to the results of the minimum inhibition concentration (MIC)

tests shown in Table 4,E. speciosa aqueous extract had the inhibitory activity against the test organisms (Staphylococcus aureus, Salmonella typhi, Shigella dysenteriaeand Candida albicans) at the concentrations of 200 mg/ml, 100 mg/ml, 200mg/ml and 25 mg/ml respectively, while the MIC of E. speciosa ethanol extract was recorded against the organisms at 50mg/ml,100mg/ml,200mg/ml and 6.25mg/ml respectively. The minimum bacteriocidal (MBC) and minimum fungicidal concentration (MFC) of the extracts are recorded on Table5 with the aqueous extract having an MBC of 200 mg/ml against the bacterial isolates. The MFC of both extracts was 25mg/ml. The results recorded indicate that S. typhi was most resistant to nine (9) of the standard antibiotics, S. dysenteriae was resistant to seven (7) of the antibiotics while S. aureus was resistant to only five (5) antibiotics. C. albicans was sensitive to the standard drugs, fluconazole at the concentration used. Also, Shigella dysenteriae recorded the highest mean inhibition zone diameter across both plant extracts used while C. albicans had the least. This implies that although there is a level of activity, C. albicans has higher resistance to the plant extracts than other test isolates. Furthermore, compared to the aqueous extract, E. speciosa ethanol extract had the highest inhibition diameters across all test. Also, the ethanol extract inhibited microbial growth at much lower concentrations than the aqueous extract. Conversely, C. albicans which was highly resistant with less inhibition zone diameters to the plant extracts exhibited the least MIC and MFC among the four microbial isolates.

5. CONCLUSION

The antimicrobial activity of the aqueous and ethanolic extracts of E. speciosa was evaluated and compared against one Gram-positive bacteria, two Gram-negative bacteria and one fungal isolate. This study showed that both aqueous and ethanolic extracts of E. speciosa have antibacterial activity against all the test organisms with S. typhi as the most susceptible Gram-negative bacteria. Also, E. speciosa ethanol leaf extracts had relatively higher antimicrobial activity than E. speciosa aqueous leaf extracts. This study shows that E. speciosa leaves can be exploited for the development of new potent antibiotics which will help combat the problem of antibiotic-resistance. When properly formulated, the plant can be used for the treatment of bacterial infections caused by the test organisms such as, urinary tract infection, neonatal meningitis, respiratory tract infection, enteric (typhoid) fever, oral or vaginal candidiasis, etc. This further potentiates the use of plants as a base for the development of a medicine (a natural blueprint for new drug development) and also as a phytomedicine used for the treatment of diseases.

DECLARATIONS

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Contribution of Authors

EmemAbasi Jackson; investigation, project administration. Motunrayo P. Agboke andAkeem A. Agboke; Conceptualization, supervision. Sifon G. Umoh and Egenu S. Chikezie; writing- original draft preparation, Formal analysis, manuscript writing - Review and Editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, publication of this article.

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