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EVALUATION OF ANTIMICROBIAL ACTIVITIES OF *COMBRETUM MICRANTHUM* L.

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ABSTRACT
The antibacterial and antifungal activities of extracts of Combretum micranthum L. (root) were tested against Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Salmonella typhi, Staphylococcus aureus, Escherichia coli and Candida albicans respectively. The plant (root) extracts were obtained using ethanol, chloroform, ethyl acetate and distilled water solvents. All the test organisms were studied using the Agar Diffusion Method (ADM) and were susceptible to crude and ethyl acetate extracts but Escherichia coli and Candida albicans were resistant. The extracts of Combretum micranthum L. had broader spectrum of activity than the commercial antibiotics tested against isolates. Results of the study confirmed the chemotherapeutic values in ethnomedicine of extracts of this plant in the treatment of various ailments.

Key words: Antimicrobial, Extracts, Combretum micranthum

INTRODUCTION

Over many centuries, man uses herbs for the treatment of various diseases all over the world (Geelhoed *et al*, 1994). According to Sofowora (1982), there are about 149 plants that are commonly used in traditional medicine in Africa. In Nigeria, a lot of the rural populace still relies much on herbs for their health care needs. *Citrus aurantifolia* has been used in various Nigerian homes because of its antimicrobial activities (Oboh,*et al*, 1995). Reports in the literature (Watt, 1962; Gelfand, et *al*,1985)indicate that traditional healers throughout Africa have confined themselves almost exclusively to the use of species from the genus *Combretum* and to a lesser extend, the *Terminalia* in the treatment of a wide range of melodies. Although the use of the leaves and barks from *Combretum* is widespread, the winged fruits, which are produced in greater abundance, are never used in medicine (nor are they eaten by wild animals because of their reported toxicity to humans). The medicinal value of plant is due to the presence of certain substances such as amino acids, phenanthrenes and dihydrophenanthrenes, gum and glycosides (Panzini *et al*., 1993; Melan *et al*., 1993; Anderson 1991and Jossang *et al*.,1998). Therefore, a great number of antimicrobial agents exist for various purposes; there is need for the search of new ones, which should be a continuous exercise because microorganisms often evolve new genetic forms, which subsequently become resistant to existing agents. The study is aimed at investigating the antimicrobial effects of *Combretum micranthum L.* on some selected microbial isolates.

MATERIALS AND METHODS

Plant Materials

The plant samples (root of *Combretum micranthum)* were collected from Yako in Kiru Local Government Area of Kano State, Nigeria and were identified colloquially by Baba Ali of the Department of Biological Sciences and further confirmed by comparison with herbarium voucher specimen.

Test Microorganisms

The seven microbial species: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Candida albicans* were obtained from the Microbiology Laboratory of Murtala Mohammed Specialist Hospital, Kano, Nigeria. Stock cultures were maintained on nutrient agar (oxoid) slants at 4^0C.

Extraction procedure

The dried roots of *C. micranthum* were macerated with pestle in a clean mortar and treated with 95% ethanol, chloroform, ethyl acetate and distilled water in a ratio of 1:2 (w/v). The mixtures were allowed to stand with occasional stirring at room temperature (28 ± 2^0C) for 24 hours, after which they were filtered. The initial concentration of crude extracts was determined by the methods of Morris *et al* (1996) and Fatope *et al* (1993). Each of the liquid extracts was separately evaporated to dryness in a weighed crucible. The weight of the crude and dry weight of dry extract to sterile broth gave the concentration in μ g/ml.

Susceptibility Testing

The Disk Diffusion (DD) method was used to test organisms for susceptibility to the plant extracts. The test organisms were separately inoculated into nutrient broth (oxoid), which was incubated at 37^0C for 18 – 20 hours. The broth culture was then diluted with peptone water (oxoid) to achieve a final inoculum of 10^5 colony forming unit per milliliter (CFU/ml). The surface of Mueller – Hilton sensitivity test agar (oxoid) plates were then flooded with the inoculum and drained. 100 sterile disks from Whatman N0. 1 filter paper, 6.25mm were soaked in 1.0ml solution of the plant extracts in screw-capped bottles for 30 minutes. The impregnated disks were then placed on the surface of inoculated agar plates with sterile forceps. Sterile disks were soaked in sterile distilled water and used as control. The plates were incubated aerobically at 37^0C for 24 hours after which zones of inhibition were observed and measured.

RESULTS

The plant extracts showed antimicrobial activity against all the seven microbial isolates viz: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Salmonella typhi, Staphylococcus aureus, Escherichia coli* and *Candida albicans* (Table 1). The susceptibility of the test organisms to the plant extracts varied, the zones of inhibition ranging from 7.0mm to 17.00mm (Table 1). The extracts from the plant root of *C. micranthum* exhibited either bactericidal or bacteriostatic effects on susceptible organisms (Table 1). The crude fraction exhibited an appreciable antimicrobial activity on all the test organisms except *E. coli* and *C. albicans* (Table 1). Table 2 shows the sensitivity of the test organisms to the various concentrations of the inter-phase fraction. This fraction was only active against *Klebsiella pneumoniae* and *S. aureus.* At the concentration of 1000 μ g/ml, the chloroform fraction showed antimicrobial activity against *K. pneumoniae* and *P. aeruginosa* at all concentrations tested (Table 3). Table 4 shows antimicrobial activity of water fraction. This fraction had the highest activity against *S. aureus, P. aeruginosa* and *S. pneumoniae* while *E. coli* and *C. albicans* showed a complete resistance.

Table 1: Antimicrobial Activity of Crude Fraction of the Root of *C. micranthum*

	Zones of Inhibition (millimeters)			
Concentration of Fraction (μ g/ml)	**1000**	**100**	**10**	**Control**
Test organisms				
Pseumonas aeruginosa	11.50	10.00	09.50	07.00
Klebsiella pneumoniae	13.50	10.00	09.00	07.00
Streptococcus pneumoniae	09.50	08.50	07.00	00.00
Salmonella typhi	11.50	09.50	08.50	07.00
Staphylococcus aureus	13.00	11.00	10.00	00.00
Escherichia coli	00.00	00.00	00.00	00.00
Candida albicans	00.00	00.00	00.00	00.00

Table 2: Antimicrobial Activity of Inter-phase Fraction of the Root of *C. micranthum*

	Zones of Inhibition (millimeters)			
Concentration of Fraction (μ g/ml)	**1000**	**100**	**10**	**Control**
Test organisms				
Pseumonas aeruginosa	00.00	00.00	00.00	00.00
Klebsiella pneumoniae	10.00	07.00	06.00	00.00
Streptococcus pneumoniae	00.00	00.00	00.00	00.00
Salmonella typhi	00.00	00.00	00.00	00.00
Staphylococcus aureus	09.00	00.00	00.00	00.00
Escherichia coli	00.00	00.00	00.00	00.00
Candida albicans	00.00	00.00	00.00	00.00

Table 3: Antimicrobial Activity of Chloroform Fraction of the Root of *C. micranthum*

	Zones of Inhibition (millimeters)			
Concentration of Fraction (μ g/ml)	**1000**	**100**	**10**	**Control**
Test organisms				
Pseumonas aeruginosa	01.00	08.00	07.00	00.00
Klebsiella pneumoniae	11.00	00.00	00.00	00.00
Streptococcus pneumoniae	00.00	00.00	00.00	00.00
Salmonella typhi	00.00	00.00	00.00	00.00
Staphylococcus aureus	09.00	00.00	00.00	00.00
Escherichia coli	00.00	00.00	00.00	00.00
Candida albicans	00.00	00.00	00.00	00.00

Table 4: Antimicrobial Activity of Water Fraction of the Root of *C. micranthum*

Concentration of Fraction (μ g/ml)	Zones of Inhibition (millimeters) 1000	100	10	Control
Test organisms				
Pseumonas aeruginosa	14.00	10.00	08.00	07.00
Klebsiella pneumoniae	10.50	10.00	08.00	07.00
Streptococcus pneumoniae	10.50	10.00	09.00	00.00
Salmonella typhi	12.50	11.50	11.00	07.00
Staphylococcus aureus	17.00	14.00	12.50	07.00
Escherichia coli	00.00	00.00	00.00	00.00
Candida albicans	00.00	00.00	00.00	00.00

DISCUSSION

This study has shown that extracts of *C. micranthum* possessed antimicrobial properties. *Escherichia coli* and *C. albicans,* which are increasingly becoming resistant to most extracts. The crude extracts were more active against test organisms then followed by water extracts. This is probably due to the active ingredients of the medicinal plants. The water extracts of the root of *C. micranthum* (a transport organ) had stronger and a wider spectrum of activity than the extracts of chloroform. The research may thus be a sort of enthomedicinal breakthrough in reducing the incidence of enterobacterial and urinary tract infections. It was however confirmed that the fractions used for this investigation did not demonstrate any lethal or inhibitory activity on both the *E. coli* and *C. albicans.* It is worthy to note that the four extracts of the root of *C. micranthum* tested against *E. coli* and *C. albicans* did not show antimicrobial activity. Further detailed study is being undertaken on these medicinal plants.

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