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COMPARATIVE ANTIMICROBIAL ACTIVITIES OF THE LEAVES OF COMBRETUM MICRANTHUM AND C. RACEMOSUM

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ABSTRACT

The leaves of *Combretum micranthum* and *C. racemosum* are used as herbal remedy to treat diarrhoea and various skin diseases. The ethanol extract, n-hexane, chloroform, ethylacetate, n-butanol and aqueous fractions of *C. micranthum* and *C racemosum* leaves were evaluated for *in vitro* antimicrobial activities by agar-diffusion and tube-dilution methods to validate the ethnobotanical uses of the two species and to compare activities. The ethanol extract and ethylacetate fraction of *Combretum micranthum* inhibited the growth of all the organisms tested, with the ethylacetate fraction showing milder activity. The chloroform, n-butanol and aqueous fractions also showed significant activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans* and *Trichophyton rubrum.* They showed more potent activity against *S. aureus and P. aeruginosa* than did the standard drug. N-hexane fraction yielded the least activity. Conversely, the ethanol extract of *Combretum racemosum* showed no activity against any of the test organisms. However, all the fractions exhibited some fairly reasonable activity, with n-hexane fractions yielding the broadest spectrum of activity and ethylacetate fraction the least. The minimum inhibitory concentration of the ethanol extract and the fractions of *Combretum micranthum* ranged from 0.62-15mg/ml, while the fractions of *C. racemosum* yielded values from 2.5-10mg/ml. *Combretum micranthum* afforded far greater antimicrobial activity than *C. racemosum.*

KEYWORDS: *Combretum micranthum, C. racemosum, antimicrobial activity.*

INTRODUCTION

Combretum is an herbaceous plant with short, erect stem, arising from a woody rootstock. *Combretum micranthum* G. Don (Combretaceae) is a spreading shrub with dark reddish-brown leaves and fruits, and white petals; while *Combretum racemosum* P. Beaux (Combretaceae) is a scandent shrub or forest liane with bracteate leaves more commonly white than pink, and petals and stamens obviously dark red (Hutchinson and Dalziel, 1958)

Plant: *Combretum micranthum* G. Don and *Combretum racemosum* P. Beaux (Combretaceae) fresh leaves were collected in August, 1999, at Uyo and Ikono areas of Akwa Ibom State, Nigeria, respectively, and authenticated by a Taxonomist in the department of Botany, University of Uyo, Uyo, Nigeria.

Uses in traditional medicine: *Combretum micranthum and C. racemosum* leaves are used in the treatment of diarrhoea, severe skin wounds and other various skin diseases. [2,10] The antibacterial activity of *C. micranthum* has been reported. [11] The stem of C. micranthum has also been demonstrated to posses antimicrobial activity.[9] However, no antifungal activity has been reported for *C. racemosum* as well as for the leaves of *C. micranthum.*

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Previously isolated classes of constituents: Tannins and catechin – a pseudotanin – have been detected in the leaves of *Combretum micranthum* [11], while a trace of alkaloid has been found in the roots. [1] The leaves and branches of *C. racemosum* have been reported to contain dulcite and tannins. Also, the leaves contain sterol, flavonoid and flavonic glycoside, holoside. [8,10] Flavonoid and tannins are found in the bark too. [5]

Tested material: The fresh leaves (1kg) of each species were air-dried and reduced to powder. The powder was macerated in ethanol for 72h. The liquid extract obtained was concentrated *in vacuo* at 40°C to yield dry ethanol extract (50g). The day ethanol extract of each species was subjected to phytochemical screening[12, 13] to reveal the presence of tannins, flavonoids and terpenes for *C. micranthum,* and the presence of tannins, saponins, and cardiac glycosides for *C. racemosum.* The dry ethanol extract of each species was also dissolved in distilled water and partitioned successively with n-hexane, chloroform, ethylacetate and n-butanol. All fractions were concentrated to dryness yielding 5g, 6g, 6.5g, 8g respectively, and 9g for aqueous residue.

Studied activity: The extract and the fractions were separately redissolved with sterile distilled water to obtain concentrations of 5mg/ml, 10mg/ml and 15mg/ml. The various concentrations were introduced into each of the three wells (11mm) borne on the surface of an agar plate, which has been inoculated separately with one of the test organisms. A control antibiotic disc containing Ampiclox was placed in each of the plates seeded with bacteria, while the plate seeded with fungi bore hole containing Nystatin as a control. The bacteria were incubated at 37°C for 24h while fungi were incubated at 25°C for 7 days. The antimicrobial activity was determined by measuring the diameter of the zone of inhibition in millimetre. [4]

The minimum inhibitory concentration (MIC) of the extract and fractions was determined for susceptible micro-organisms by incorporating various concentrations (0.31-20mg/ml) of the redissolved extract and fractions into sets of test tubes containing culture media. 50ml of the standard test bacterial and fungal broth cultures were added into each of the test tubes. The sets of test tubes with their contents were incubated at 37°C for 24h and 25°C for 7 days respectively for bacteria and fungi. A set of positive control test tubes containing only the growth medium and each of the organisms were also set up. The MIC was regarded as the lowest concentration of the extract and fractions that did not permit any visible growth when compared with that of the control tubes.

Used micro-organism: The fungal species used – *Candida albicans and Trichophyton rubrum* – were obtained from St. Luke's Hospital, Anua, Uyo, stock culture units. The isolates were stored on a Sabouraud's dextrose agar (Oxoid) slants at 4°C before use. The bacterial species used were clinical isolates obtained from the same source, viz: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa.* They were maintained on blood agar slants at 4°C before use.

RESULTS

Variations occurred in the distribution of classes of chemical constituents in the two species of Combretum. Though the two species showed the presence of tannins in much quantity, C.*micranthum* indicated the presence of saponins, flavonoids and terpenes in trace amounts, in variance with the presence of cardiac glycoside shown by C. *racemosum.* The ethanol extract of C. *micranthum* exhibited antimicrobial activity against all the organisms tested, but the fractions of the ethanol extract of the same species showed no activity against at least two organisms. However, n-butanol fraction showed very significant activity against the susceptible organisms. This activity

is comparable to the standard drugs. Conversely, ethanol extract of C. *racemosum* showed no activity at all, while the n-butanol fraction possessed mild activity. Only the aqueous and n-hexane fractions showed reasonable activity against different organisms. Generally, activity appears to be purification and dose – dependent (Table 1).

TABLE I: ANTIMICROBIAL ACTIVITY OF THE EXTRACT AND FRACTIONS OF COMBRETUM MICRANTHUM AND COMBRETUM RACEMOSUM

	Micro Organisms	zone of Inhibition in MM*						
Extract & Fractions	Concentration (mg/ml)	*B. subtilis*	*S. aureus*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*C. albicans*	*T.rubrum*
C.micranthum.								
ETHANOL	5	2	5	9	7	8	9	6
	10	3	8	12	7	9	14	8
	15	4	10	12	8	9	15	11
N-HEXANE	5	-	14	5	-	-	9	9
	10	-	17	7	-	-	10	12
	15	-	19	10	-	-	10	14
CHLOROFORM	5							
	10	-	14	8	-	-	4	2
	15	-	17	12	-	7	8	4
	5	-	19	16	-	9	10	7
ETHYLACETATE			8	13		5	3	4
	10	-	19	14	-	8	5	7
	15	3	20	15	8	9	10	9
N-BUTANOL	5	-	16	12	-	-	7	8
	10	-	20	17	-	4	12	12
	15	-	22	21	-	6	14	16
AQUEOUS	5	-	13	8	-	5	7	3
	10	-	19	10	-	7	9	4
	15	-	20	12	-	9	11	8
C.racemosum					-			
ETHANOL	5	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-
N-HEXANE	5	-	5	-	-	5	5	2
	10	-	9	-	-	6	6	4
	15	-	11	-	-	7	8	4
CHLOROFORM	5	-	-	-	-	-	-	1
	10	-	-	2	-	-	5	6
	15	-	-	5	-	-	8	7
ETHYLACETATE	5	-	-	7	-	-	-	-
	10	-	-	17	-	-	-	-
	15	-	-	19	-	-	-	-
N-BUTANOL	5	-	-	3	-	-	-	2
	10	-	-	4	-	-	-	2
	15	-	-	11	-	-	-	4
AQUEOUS	5	-	-	8	-	-	-	-
	10	4	4	13	-	-	-	-
	15	6	6	15	-	-	-	-
AMPLICOX	5	3	3	4	7	14	NT	NT
	10	5	5	9	12	16		
	15	6	6	12	14	18		
NYSTATIN	5	NT	NT	NT	NT	NT	12	14
	10						14	17
	15						19	22

KEY: *Mean of four assays; NT-Not tested

TABLE 2: MINIMUM INHIBITORY CONCENTRATION VALUES OF THE EXTRACT AND FRACTIONS OF *C. MICRANTHUM* AND *C. RACEMOSUM*

EXTRACT & FRACTIONS	MICRO ORGANISMS CONCENTRATION (mg/ml)						
	B. subtilis	*S.aureus*	*P. aeruginosa*	*E. coli*	*K.pneumoniae*	*C. albicans*	*T. rubrum*
C. micranthum ETHANOL	5	1.25	5	0.62	0.62	0.62	1.25
N-HEXANE		5	5			1.25	10
CHLOROFORM			5	5	10	10	5
ETHYL ACETATE	15	0.62	5	15	5	5	5
N-BUTANOL		5	5		10	5	2.5
AQUEOUS		0.62	5		5	1.25	5
C. racemosum ETHANOL							
N-HEXANE		2.5				5	5
CHLOROFORM			10			10	5
ETHLY ACETATE			5				
N-BUTANOL			5				5
AQUEOUS	10	10	5				

MIC value of 0.62.15.0mg/ml were obtained for the ethanol extract and fractions of C. *micranthum* for bacterial assay, while a range of 0.62-10mg/ml was obtained for the fungal assay. The fractions of the ethanol extract of C. *racemosum* showed MIC values ranging from 2.5 – 10mg/ml and 5.0 – 10mg/ml for bacterial and fungal agents respectively (Table 2).

Conclusion

The leaf of C. *micranthum* exhibited a lot more significant antimicrobial activity against some of the micro organisms implicated in the pathogenesis of human infections (*S. aureus, T.rubrum, C. albicans*) than did that of the C. *racemosum.* The phytochemical screening revealed the presence of polar bioactive compounds – phenolics, saponins – to which may have been imputed the antimicrobial activity observed in this study.

This study has validated the ethnobotanical use of the plants as panacea for microbial infections. However, given our results, *Combrethum micranthum* is recommended for use in preference to *C. racemosum.*

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