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Evaluation of antibacterial activity of leaf and stem extracts of *Combretum calobotrys*

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ABSTRACT

Antibacterial activity of the extracts and fractions of leaf and stem of Combretum calobotrys Engl. & Diels (Combretaceae) against clinical isolates of Bacillus subtilis, Pseudomonas aeruginosa, Salmonella paratyphi, Eschericia coli, Staphylococcus aureus and Klebsiella pneumonia were evaluated using the agar diffusion method. Results showed that with the exception of the stem methanol fraction, all the extracts and fractions, elicited antibacterial activity. The leaf methanol fraction had minimum inhibitory concentration (MIC) of 0.32 mg/ml against K. pneumoniae while the stem ethylacetate fraction had MIC of 0.46 and 0.78 mg/ml against P. aeruginosa and S. aureus respectively. The results justify the ethnomedicinal use of C. calobotrys in Southeastern Nigerian to manage bacterial infections.

Keywords: *Combretum calobotrys*, antibacterial activity, minimum inhibitory concentration.

INTRODUCTION

Combretum calobotrys (Combretaceae), a shrub widely distributed in the tropics, thrives in the savannah areas and secondary forest and is popularly used in Nigeria as an antibacterial agent. The morphology of the plant has been described [1]. Combretum species are used in the traditional medicine of many regions of the world to treat variety of diseases including infections. Though a lot of research have been carried out in other members of the species, there is no report of pharmacological study on *C. calobotrys.*

This study investigates the antibacterial properties of the leaf and stem extracts of *C. calobotrys* using *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Eschericia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*.

EXPERIMENTAL SECTION

Preparation of extract

Fresh stems and leaves of *C. calobotrys* were collected in March from Orba, Enugu State, Nigeria. The plant material was identified and authenticated by Mr. A. Ozioko of International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State. The plant parts were separately cut into pieces, dried and reduced to coarse powder using an electric blender. Each of the leaf powder (256.2 g) and stem powder (218.18 g) was subjected to successive extraction in a soxhlet at 50 ^{0}C using hexane, ethylacetate and methanol. The different extracts were concentrated in a rotary evaporator at 40 - 50^{o}C under reduced pressure to obtain 6.74 g of the leaf n- Hexane fraction (LHF; 2.63%), 0.50 g of the leaf ethylacetate fraction (LEF; 0.20%), 16.58 g of the leaf methanol fraction (LMF; 6.47% w/w), 2.00 g of stem n-hexane fraction (SHF; 0.92%), 4.50 g of stem ethylacetate fraction (SEF; 2.06%) and 0.55g of stem methanol fraction (SMF; 0.25%) respectively.

Also, a fresh batch of leaf powder (85.43 g) and stem powder (72.73 g) were each subjected to continuous methanol (100%) extraction in a soxhlet at 50^{0}C to yield 6.90 g of the leaf methanol extract (LME; 8.08%) and 0.90 g of the stem methanol extract (SME; 1.24%) respectively.

Bacterial culture

Clinical isolates of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Eschericia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* obtained from Bishop Shanahan Hospital, Nsukka were used for the tests.

Bacterial Sensitivity test

This was done using the agar diffusion technique as reported by Lovian [2]. Briefly, sterile Muller Hinton agar plates were flooded with 1 × 10^6 cfu/ml suspension of the test microorganisms. Each extract (0.03 ml of 100 mg/ml) was placed in the wells made on the seeded agar plates and left to diffuse. Control tests were carried out with dimethylsulfoxide (DMSO) and gentamicin (1.4 mg/ml). The plates were allowed 30 min for diffusion and incubated in an inverted position at 37ºC for 24 h; bioactivity was determined by measuring the inhibition zone diameter (IZD). All tests were done in triplicate. With the exception of SMF, all extracts and fractions showed antibacterial activity; hence they were subjected to MIC test.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of the extracts with IZD > 5mm were determined using the agar well diffusion technique [2]. Agar plates were seeded with the test organisms. Each extract (100 mg/ml) was subjected to series of two-fold serial dilutions to obtain a final concentration of 6.25 mg/ml. Each concentration (2 drops) was transferred to a corresponding well. About 30 min was allowed for diffusion, followed by incubation at 37ºC for 24 h. The value of the MIC was extrapolated from a plot of the corrected IZD (square of the inhibition zone radius) vs log concentration.

RESULTS AND DISCUSSION

All the extracts and fractions, with the exception of SMF, exhibited concentrated-dependent antibacterial activity against the test gram +ve and gram –ve bacteria as shown by the zones of inhibition and MIC values (Tables 1 and 2). The LEF, LMF and SEF exhibited broad spectrum of activity as shown by their antibacterial activity against all test organisms. The SEF showed the most potent antibacterial activity with greatest sensitivity against *P. aeruginosa*, *B.subtilis* and *S.*

aureus, and second most potent against *E. coli*, *S. paratyphi* and *K. pneumoniae*. The SEF was most potent against *S. aureus* (gram +ve cocci), *B. subtilis* (gram +ve rod) and *P. aeruginosa* (gram –ve rod) with MIC of 0.78, 1.94 and 0.47 mg/ml respectively, while LMF was the most potent against *E. coli* (gram –ve) and *K. pneumoniae* (gram –ve rod) with MIC of 2.05 and 0.32 mg/ml respectively. The MIC of SEF against *P. aeruginosa* was comparable to that of the standard antibiotic, gentamicin.

Antibacterial activity of *C. calobotrys* was evaluated *in vitro* against gram –ve and gram +ve bacteria known to be pathogenic to man and the results lend credence to the ethnomedicinal use of the plant as antibacterial agent. Furthermore, it also highlights the potential benefit of the plant in the management of wounds, since *E. coli*, *K. pneumonia*, *P. aeruginosa* and *S. aureus* are among the microbes involved in the progression of wounds and sores of diverse etiology [3,4].
Medicinal plants constitute an important source of lead compounds for the development of new and improved antibacterial drugs for the control of pathogenic organisms. The increase of antibiotic resistance by pathogenic microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective drugs for the control infectious diseases; *E. coli* and *P. aeruginosa* are known to be multi-resistant to drugs, hence *C.calobotrys* extracts may be beneficial in such resistant cases.

Table 1: Bacterial sensitivity of *C. calobotrys* extracts

Extract	Inhibition Zone Diameter (IZD) (mm)					
	B.subtilis	*P.aeruginosa*	*S.paratyphi*	*E.coli*	*S. aureus*	*K. pneumonia*
LME	7±0.01	12±0.27	4±0.02	13±0.13	0	0
LHF	11±0.04	9±0.01	16±0.09	6±0.01	0	0
LEF	10±0.01	13±0.16	9±0.01	13±0.16	8±0.12	7±0.03
LMF	10±0.06	13±0.12	9±0.16	13±0.01	26±0.11	11±0.09
SME	2±0.01	13±0.12	11±0.01	16±0.11	0	0
SHF	5±0.03	9±0.06	9±0.11	8±0.11	16±0.06	19±0.11
SEF	14±0.15	14±0.08	11±0.12	13±0.15	31±0.12	20±0.16
SMF	0	0	0	0	0	0
Gentamicin (1.4 mg/ml)	32±0.06	13±0.16	14±0.03	22±0.03	30±0.01	20±0.01

n=3; 0 = no inhibition; IZD = observed IZD – diameter of cork borer (7mm); extracts with IZD ≤ 5.0mm were not subjected to MIC determination; LME = leaf methanol extract; LHF = leaf hexane fraction; LEF = leaf ethylacetate fraction; LMF = leaf methanol fraction; SME = stem methanol extract; SHF = stem hexane fraction; SEF = stem ethylacetate fraction; SMF = stem methanol fraction

Table 2: Minimum Inhibitory Concentration (MIC) of extracts against sensitive bacteria

Extract	Minimum Inhibitory Concentration (MIC) (mg/ml)					
	B. subtilis	*P. aeruginosa*	*S.paratyphi*	*E. coli*	*S. aureus*	*K. pneumonia*
LME	7.94	11.60	NS	4.30	NS	NS
LHF	9.73	5.27	2.27	26.41	NS	NS
LEF	4.78	10	15.84	7.94	11.08	5.25
LMF	2.05	1.23	3.98	2.05	2.05	0.32
SME	NS	5.15	2.15	3.59	NS	NS
SHF	NS	5.99	2.85	8.80	12.24	11.64
SEF	1.94	0.46	2.39	2.51	0.78	1.07
SMF	NS	NS	NS	NS	NS	NS
Gentamicin	0.02	0.40	0.36	0.05	0.04	0.06

n=3; NS = not sensitive; LME = leaf methanol extract; LHF = leaf hexane fraction; LEF = leaf ethylacetate fraction; LMF = leaf methanol fraction; SME = stem methanol extract; SHF = stem hexane fraction; SEF = stem ethylacetate fraction; SMF = stem methanol fraction

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