REPORT

ANTIMICROBIAL ACTIVITY OF ROOT EXTRACT AND CRUDE FRACTIONS OF *CROTON ZAMBESICUS*

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ABSTRACT
The root extract and fractions of *Croton zambesicus* were screened for antimicrobial activity against some typed and pure cultures of bacterial and fungal species. These were carried out by the Plate -hole diffusion method on Mueller – Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungi. The Minimum Inhibitory Concentrations (MICs) of test samples found to be active by the diffusion test were determined based on the macrodilution method. The crude extract as well as chloroform and n-hexane fractions had activity against *B. subtilis* only. While ethyl acetate fraction had a wide spectrum of activity against all the bacteria organisms tested with a promising minimum inhibitory concentrations. However, the crude extract and the fractions were inactive against all the fungal species tested. This result confirms its ethnomedicinal use in the treatment of microbial infections.

Keywords: Antimicrobial, antibacterial, *Croton zambesicus*.

INTRODUCTION

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo – Congolese species widely spread in tropical Africa. Ethnobotanically,the leaf decoction is used in Benin as anti hypertensive and anti- microbial (urinary infections) (Adjanohoun *et al.*, 1989) and in parts of Nigeria as antidiabetic and malarial remedy (Okokon *et al.,* 2005a, 2006). The roots are used as antimalarial and antidiabetic by the Ibibios of Niger Delta region of Nigeria. The root is also used in Sudan for menstrual pain (El-Hamidi, 1970) and as aperients (Ngadjui *et al.,* 1999). Boyom *et al.,* (2002) studied the composition of essential oils from the leaves, stem and roots of *Croton zambesicus* and found the three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes, while that of the root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen-containing compounds, with spathulenol and linalool as major components. Block *et al.,(*2002) isolated entrachyloban - 3β-ol, an ent-trachylobane diterpene from dichloromethane extract of the leaves and reported that the diterpene has a cytotoxic activity on HeLa cells. Also two new trachylobane – and one isopimarane type diterpenoids; ent-18-hydroxy-trachy-loban-3-one, ent-trachyloban-3-one, isopimara-7, 15-dien-3β-ol, together with transphytol, β-sitosterol, α-amyrin and stigmaterol have been isolated from the leaves (Block *et al.,* 2004). Crotonadiol, a labdane diterpenoid, clerodane, crotocorylifuran and two trachylobanes; 7β-acetoxytrachyloban – 18 – oic acid, trachyloban - 7β, 18 – diol, lupeol, β - sitosterol and its 3-β-glucopyranosyl derivative were isolated from the stem bark (Ngadjui *et al,* 1999). Ngadjui *et al.,* (2002) further isolated three clerodane diterpenoids, crotozambefurans A, B and C from the stem bark. Studies have reported on the antimicrobial properties of the leaf and stem (Abo *et al.,* 1999, Reuben *et al*., 2008). The ethanolic leaf and root extracts have been reported to possess antiplasmodial (Okokon *et al.*, 2005a; Okokon and Nwafor, 2009a), antidiabetic (Okokon *et al.,* 2006), anti-inflammatory, analgesic and antipyretic activities (Okokon *et al.,* 2005b) as well as antiulcer activities (Okokon and Nwafor, 2009b). Information on biological activity of the root is scarce, we therefore investigated the antimicrobial activity of the root extracts of the plant to ascertain the folkloric claim of its use in the treatment of antimicrobial infections.

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MATERIALS AND METHODS

Plant materials

The plant part (roots) was identified by a taxonomist in the Department of Botany, University of Uyo, Uyo. The roots were collected from compounds in Uyo metropolis and were authenticated. A voucher specimen (DPNM.31c) of the plant was deposited at hebarium of Department of Pharmacognosy and Taditional Medicine, University of Uyo, Uyo.

Extraction

The roots were washed and shade dried for 2 weeks. The dried roots were further chopped into small pieces and reduced to powder. The powdered root (2kg) was divided into two parts, one part (1kg) was macerated in 97% ethanol (3L) for 72 hours to give the crude ethanolic

extract while the other part (1kg) was successively and gradiently macerated for 72 hours in 3L of each of these solvents; n-hexane, chloroform, ethyl acetate and methanol to give the corresponding gradient fractions of these solvents (Okokon and Nwafor, 2009a). The liquid filtrates were concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The yield of each extract was calculated. The dry extracts were stored in a refrigerator at -4°C until use for the proposal experiment.

Phytochemical ccreening

Phytochemical screening of the crude root extract was carried out employing standard procedures and tests (Trease and Evans, 1989, Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

Microorganisms

Typed and pure cultures of some bacterial and fungal species were obtained from Pharmaceutical microbiology unit of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo and maintained at 40°C on nutrient agar plates before use.

Evaluation of antibacterial and antifungal activities of the extract

Plate-hole diffusion test

The evaluation of antimicrobial activity of the extract/fractions were carried out by the Plate -hole diffusion method (Berghe and Vlietinck, 1991) on Mueller – Hinton agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for the fungi. Solutions of the extract and fractions were prepared in 10% Tween 80 to concentrations of 100, 50, 25 and 12.5mg/ml.

The innocula of the microorganisms were prepared separately from 12h broth cultures (Mueller-Hinton broth for bacteria and the Sabouraud dextrose broth for the fungi) and incubated at 37°C. All culture media and distilled water were sterilized at 121°C for 15min in an autoclave. These innocula were diluted with sterilized distilled water to obtain a density corresponding approximately to 0.5 of Mc Farland standard turbidity scale (10^8 colony forming unit "CFU" per ml for the bacteria and 10^3 spores per ml for fungi) (Berghe and Vlietinck, 1991). 0.5ml of each innoculum was introduced into the corresponding fluid agar medium homogenized and 25 ml of it poured into sterile plastic petri dishes. The petri dishes were allowed on the flat slab top for the medium to solidify within 30min. A standard cork borer of 5mm in diameter was used to cut four equidistant uniform wells per plate on the surface of different plates into which was added 50µl solution of each extract/fraction at varying concentration 12.5, 25, 50 and 100mg/ml. The reference drugs were Gentamicin, batch 20070402 (0.4mg/ml) and Nystatin batch 04D05 (500µg/ml). The plates were incubated at 37°C for 24 and 48h for the bacteria and fungi respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition around the hole. Each test concentration had three replications. The results were recorded as the mean diameter of the zones of growth inhibition surrounding the discs (Lyudmila *et al.,* 2003).

Determination of minimum inhibitory concentrations (MIC) using macrodilution method

The Minimum Inhibitory Concentrations (MICs) of test samples found to be active by the diffusion test were determined based on the macrodilution method (Berghe and Vlietinck, 1991) with some modifications as follows. The test extract/fractions were dissolved in 10% Tween 80 to give a stock concentration of 100mg/ml and serially diluted (two-fold) in a seres of test tubes to a working concentration ranging from 1.560 to 100mg/ml using nutrient broth supplemented with 10% glucose and 0.05% phenol red (colour indicator). These were later inoculated with 0.2ml suspension of the test organisms. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

RESULTS

Phytochemical screening

Phytochemical screening of the ethanolic root extract of *Croton zambesicus* revealed the presence of compounds like saponins, alkaloids, terpenes, cardiac glycosides, and anthraquinones. Flavonoids were found to be absent.

Antimicrobial activity of the crude extract and fractions

Table 1 shows the diameters of the zones of inhibition exhibited by the crude extracts and fractions at various concentrations employed. The ethyl acetate fraction showed a broad spectrum activity against all gram positive and gram negative bacteria tested with highest activity against *E. coli* (ATCC10418), *E. coli* (ATCC 25923), *B. subtilis* (NCTC 8853), *Staph. aureus* (ATCC 25923) and *Kliebsiella pneumoniae.* The activity of the ethyl acetate fraction was comparable to that of the standard, streptomycin. The crude ethanolic extract, chloroform and n-hexane fractions were only active against *B. subtilis* (NCTC 8853).The crude extract and all the fractions were inactive against all fungal species tested.

The results of minimum inhibitory concentrations (MIC) of the crude extract/fractions are shown in table 2. The lowest MICs of ethyl acetate fraction (3.125mg/ml) were recorded against *B. subtilis* (NCTC 8853) and *Staph.*

Table 1: Antimicrobial activity of *Croton zambesicus* root extract and fractions

Fraction/Drug Microorganism	Zone of inhibition in mm							
	Conc/ ml Mg/ml	Ethanolic crude	Ethyl acetate	Chloroform	N-hexane	Methanol	Streptomycin 0.4 mg/ml	Nystatin 500µg/ml
E. coli ATCC 10418	100	-	25.0	-	-	-	15.0	-
	50	-	22.0	-	-	-		
	25	-	18.0	-	-	-		
	12.5	-	15.0	-	-	-		
Staph .aureus NCTC 6571	100	-	16.50	-	-	-	20.0	-
	50	-	14.0	-	-	-		
	25	-	10.0	-	-	-		
	12.5	-	-	-	-	-		
B. subtilis NCTC 8853	100	20.0	25.0	25.0	20.0	-	25.0	-
	50	16.50	20.0	20.0	18.0	-		
	25	14.0	18.0	18.0	-	-		
	12.5	10.0	16.0	16.0	-	-		
Pseudomonas aeruginosa ATCC 27853	100	-	20.0	-	-	-	27.0	-
	50	-	18.0	-	-	-		
	25	-	-	-	-	-	-	
	12.5	-	-	-	-	-	-	
Klebsiella. Pneumonia	100	-	26.0	-	-	-	22.0	
	50	-	24.0	-	-	-		
	25	-	18.0	-	-	-		
	12.5	-	14.0	-	-	-		
Salmonella typhi	100	-	20.0	-	-	-	21.0	
	50	-	18.0	-	-	-		
	25	-	15.0	-	-	-		
	12.5	-	12.0	-	-	-		
E. coli ATCC 25923	100	-	26.0	-	-	-	16.0	
	50	-	24.0	-	-	-		
	25	-	20.0	-	-	-		
	12.5	-	15.0	-	-	-		
Staph. aureus ATCC 25923	100	-	28.0	-	-	-	16.0	
	50	-	25.0	-	-	-		
	25	-	23.0	-	-	-		
	12.5	-	21.0	-	-	-		
Aspergillus niger	100	-	-	-	-	-		30.0
	50	-	-	-	-	-		
Candida albicans	25	-	-	-	-	-	-	30.0
	12.5	-	-	-	-	-		
A. fumigatus	100	-	-	-	-	-	-	30.0
	50	-	-	-	-	-		
	25	-	-	-	-	-		
	12.5	-	-	-	-	-		
Tinea capitus	100	-	-	-	-	-	-	30.0
	50	-	-	-	-	-		
	25	-	-	-	-	-		
	12.5	-	-	-	-	-		

aureus (ATCC 25923). Chloroform fraction also inhibited *B. Subtilis* at the same concentration (3.125mg/ml).

DISCUSSION

The phytochemical screening of the root extract revealed the presence of alkaloids, saponin, tannins, anthraquinones and cardiac glycosides and their differential distribution in the fractions. Alkaloids were found in all the fractions and crude extract. Ethyl acetate fraction was predominantly composed of alkaloids; this was confirmed during the confirmatory test for alakaloids. All the bacterial strains tested (Gram-positive and Gram-negative) were sensitive to the ethyl acetate fraction. In

contrast to the weak antibacterial activity reported by Abo *et al*. (1999) on the leaf extract against some cultures like *Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Proteus mirabilis, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus* , the root exerted a significant activity against these organisms. However, the antibacterial activity of the root and that reported of the stembark extract (Abo *et al*., 1999; Reuben *et al*., 2008) were comparable. Besides, the leaf and stembark extracts had been reported to possess weak and active antifungal activities(Abo *et al*., 1999) respectively, the root extract in this study did not exert any antifungal activity. In this study, the alkaloidal rich ethyl acetate fraction had the highest activity against the bacterial species tested. This result is consistent with that of Abo *et al*. (1999), who reported of broad spectrum activity of the leaf and stembark extract of *C. zambesicus* especially in alkaloidal rich fraction of the extracts. The weak activities of the ethanolic crude extract, chloroformic and n-hexane fractions are understood and probably could have resulted from antagonistic interactions of compounds from different groups in them. The MIC obtained varied from 3.125 to 65 mg/ml for ethyl acetate fraction, while MIC values of 3.125, 12.5 and 65 mg/ml were obtained for chloroform fraction, ethanolic crude extract and n-hexane fraction respectively for *B. subtilis*. The differential MIC of the chloroform and n-hexane crude fractions against *B. subtilis* could have resulted greater concentration of the active ingredient in chloroform than n-hexane fraction despite their similar composition. The MIC values and the antimicrobial spectrum of ethyl acetate indicate the remarkable antimicrobial potency of this fraction which can be considered as promising antimicrobial agents.

Table 2: Minimum inhibitory concentration of crude extract and fractions of *C. zambesicus* (mg/ml)

Organism	Gradient crude extract/fractions			
	Ethanolic	Ethyl Acetate	Chloroform	N-Hexane
E. coli ATCC10418	-	6.25	-	-
Staph. aureus NCTC 6571	-	50	-	-
Bacillus subtilis NCTC 8853	12.5	3.125	3.125	65
Pseudomonas aureginosa ATCC 27853	-	70	-	-
E. coli ATCC 25922	-	6.25	-	-
Staph. aureus ATCC 25923	-	3.125	-	-
Kliebsiella pneumoniae	-	6.25	-	-
Salmonella typhi	-	6.25	-	-

In conclusion, the results of this study show that the root extract of *Croton zambesicus* possess significant antimicrobial activity which justifies its use in traditional medicine in the treatment of microbial infection. The alkaloids present in these fractions and extract may in part be responsible for the antimicrobial activity observed in this study. Therefore, it will be interesting if the alkaloids are isolated and characterised for future beneficial use.

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