

Phytochemical Screening and *In Vitro* Antimicrobial Investigation of the Methanolic Extract of *Croton Zambesicus* Muell ARG. Stem Bark

Reuben, K.D

*Department of Basic Sciences, Adamawa State College of Agriculture
Ganye, Ganye, Nigeria*

F.I. Abdulrahman

Department of Chemistry, University of Maiduguri, Borno State, Nigeria

J.C.Akan

Department of Chemistry, University of Maiduguri, Borno State, Nigeria
E-mail: joechemakan@yahoo.com
Tel: 2348036000506

H. Usman

Department of Chemistry, University of Maiduguri, Borno State, Nigeria

O.A.Sodipo

*Department of Clinical Pharmacology and Therapies, College of Medical Sciences
University of Maiduguri, Maiduguri, Nigeria*

G.O. Egwu

Department of Veterinary Microbiology, University of Maiduguri, Maiduguri, Nigeria

Abstract

The stem bark of *Croton zambesicus* was collected, gabbled, pulverized air dried and subjected to gradient extraction with soxhlet apparatus and the methanol extract (MTE) was screened phytochemically for its chemical components. This revealed the presence of alkaloids, reducing compounds (carbohydrates), cardiac glycosides, flavonoids, saponins, terpenes and steroids in moderate concentration. *In vitro* Antibacterial assay of the extract by the use of agar plate-hole diffusion and nutrient broth dilution techniques revealed the extracts to have broad spectrum activity on gram positive, negative organisms and fungal strain respectively. The highest activity was shown in *S. aureus* and *E. coli* with the same MIC and MBC values of 1.560mg/ml and least activity on *S. pyogens* with the same MIC and MBC values of 50mg/ml showing the extract to be cidal antimicrobial. This study provides some scientific bases for the use of this plant as a remedy for stomach, urinary tract, skin and fungal infections in folkloric medicine whose causative agents are some of the pathogens studies. The activities observed could be attributed to the presence of some of the phytochemicals detected which have been associated with antibacterial activity.

Keywords: *In vitro* Antimicrobial, *Croton zambesicus*, Methanolic Extract, Phytochemical Screening.

Introduction

Croton zambesicus Muell Arg. (*Euphorbiaceae*) (syn. *C. ambilis* Muell Arg.; Syn. *C. gratissimus* Burch) is an ornamental tree grown in villages and towns of Nigeria. It is a Guinea-Congolese species widely spread in tropical Africa. The leaf decoction is used in Benin as antihypertensive and antimicrobial (urinary infections). (Adjanohaun, *et al.*, 1989 and Okokon, *et al.*, 2004, 2005). The Ibibios in Uruan area of Akwa Ibom state of Nigeria use the leaf traditionally as a remedy for malaria. Block *et al.*, (2002) reported that ent-trachyloban-3 β -ol, a trichloban diterpene, isolated from dichloro-methane extract of the leaves has cytotoxic activity on *Hela* cells. The alkaloidal fractions of the leaf have been reported to possess weak activity against *Kelebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus megaterium*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Microsporium* species and *Penicillium* species Abo and Ogunleye, (1999). The alkaloidal fraction of the stem has also been reported to be active against all micro-organisms mentioned above (Abo and Ogunleye, 1999). In spite of the large number of activities studied of *Croton zambesicus*, no detailed report has been advanced of the susceptibility, MIC and MBC. The authors want to give report on the alcoholic fraction got by gradient soxhlet extraction of the stem bark as against alkaloidal fraction reported on, of some of the gram positive and negative microbes and fungal strain.

Materials and Methods

Plant collection and identification

The plant parts of *C. zambesicus* Muel Arg. was collected at long. 13°5'E, Lat 10°15'N Mubi, Adamawa state Nigeria and authenticated by Prof. S. S. Sanusi and voucher specimen with number 190520081/2 deposited at the Biological Sciences Department, University of Maiduguri, Maiduguri, Nigeria.

Preparation and Extraction of Plant material

The stem bark of *C. zambesicus* was gabled for removal of adulterants and pulverized. It was air dried at room temperature and four hundred grams (400g) of the pulverized stem bark was exhaustively defatted with petroleum ether (60-80°) and successively extracted with ethylate then finally methanol using the soxhlet extractor. The extract portion of methanol was concentrated *in vacuo* and a brown mass which weighed 52.023g equivalent to 12.64% (w/w) and coded MTE (methanol extract) was kept aseptically until use.

Phytochemical screening

The methanol extract (MTE) was screened for phytochemical constituents using standard procedures of analysis (Harborn, 1993; Sofowora, 1993 and Trease and Evans, 2002).

Antibacterial activity

The plate-hole diffusion assay as described by (Kudi *et al.*, 1999 and Ogundipe *et al.*, 2000) was used to determine the growth inhibition of bacteria by the plant extract. The following bacteria obtained from Human Clinical cases at the University of Maiduguri Teaching Hospital (UMTH) Maiduguri, Nigeria were used; *S. aureus*, *S. pyogenes*, *S. dysenterea*, *C. albicans*. All bacteria were maintained at

40°C on nutrient agar plates before use. The tests were carried out by using a stock concentration of 500mg/ml prepared by dissolving 1g of the methanol extract (MTE) into 2ml of distilled water. Nutrient agar was prepared and 25ml each was poured into sterile petri dish. This was allowed to solidify and dry. Using a sterile cork-borer of 9mm diameter three equi-distant holes per plate were made in the set agar and were inoculated with 0.5ml over night suspension of the bacteria. There after, the wells (holes) were filled with the extract solution at varying concentrations of 500mg/ml, 400mg/ml and 300mg/ml respectively. This was done in triplicate and the plates were incubated at 37°C for 18hours. The antibacterial activities were observed and measured using a transparent meter rule and recorded if the zone of inhibition was ≥ 10 mm (Vlietink *et al.*, 1995 and Kudi *et al.*, 1999).

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The Vollekova *et al.*, (2001) method modified by Usman *et al.*, (2007) was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and serially diluted (two-fold) to a working concentration ranging from 0.780mg/ml to 200mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37°C, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Minimum Bacterial Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub-culturing to antimicrobial free agar as described by Vollekova *et al.*, (2001) and Usman *et al.*, (2007). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37°C for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

Results and Discussion

The result of the phytochemical screening is presented in Table 1. This reveals moderate concentration of alkaloids, carbohydrate, cardiacglycosides, flavonoids, saponins, terpenes and steroids some of which chemical compounds have been associated to antibacterial activities and thus have curative properties against pathogens (Nweze *et al.*, 2007). The *in vitro* antibacterial activities are shown in Table 2. This showed a wide spectrum activity against most bacteria and fungal strains studied. *Proteus vulgaris* was resistant. The standard antibiotic tetracycline inhibited the growth of the test bacterial and fungal strains under study at the smallest concentration (300mg/ml) except for *P. vulgaris* that was not tested. The inhibition zones produced were significantly higher for the extract as compared to the standard drug used except for *S. pyogenes* for which the zone of inhibition by the extract is also significantly lower than that of the standard drug tetracycline even though the concentrations are not comparable.

Table 1: Phytochemical screening of methanol extract (MTE) of *Croton zambesicus* Muell Arg. stem bark.

S/No.	Constituents	Methanol extract
1	Alkaloids	
	i.Dragendorff's test	++
	ii.Meyer's test	++
2	Carbohydrates	
	i.Mohch's test	+
	ii.Burford's test	-
	iii.Fehling (reducing sugar) test	++
	iv.Fehling (combined reducing sugar) test	++
3	Cardiac glycosides	
	Killer-killanis test	++
4	Flavonoids	
	i.Shinoda's test	-
	ii.FeCl ₃ test	++
	iii.Pew's test	++
5	Saponins	
	Frothing test	++
6	Terpenes and steroids	
	i.Salkowski test	++
	ii.Libarman-Burchard's test	++
7	Tanins	
	i.FeCl ₃ test	-
	ii.Lead acetate test	-

Key:

- = Negative (absent)
- + = Positive (slightly present)
- ++ = Positive (moderately present)

Table 2: *In vitro* Antimicrobial Potential Investigation of the Methanolic Extract of Stem bark of *Croton zambesicus* Muell Arg.

Extract/conc. Mg/ml	Zone of inhibition (mm)							
		S.d.	S.a	S.p	E.c.	P.a	P.v	C.a
Methanol	500	30.0±0.00	30.0±0.00	31.5±0.50	30.0±0.00	31.7±2.89	R	30.0±0.00
Extract	400	27.7±0.58	30.0±0.00	27.3±0.50	28.3±1.53	29.0±1.00	R	29.7±0.00
(MTE)	300	25.0±0.00	28.7±0.58	23.0±0.00	27.0±0.00	26.7±2.89	R	25.3±1.53
TCN	25	10.0±0.00	25.0±0.00	28.0±0.00	12.0±0.00	10.0±0.00	NT	13.0±0.00

Key:

- S.d = *S. dyentirea*; S.a = *S. aureus*; S.p = *S. pyogens*; E. c. = *E. coli*
- P.v = *P. vulgaris*; C.a. = *C. albicans*
- N.T. = Not tested; R = Resistance (-ve); control = TCN (Tetracycline).
- All data except TCN were mean of 3 values (X±SEM)

The minimum inhibitory concentration MIC and minimum bactericidal concentration MBC results are shown in Tables 3 and 4 respectively. These tables reveal that the ranges of activity for both MIC and MBC are 1.560 to 50mg/ml.

Table 3: Minimum Inhibitory Concentration (MIC) values for Bacterial Isolates Against Methanolic Extract (MTE) of Stem bark of *Croton zambesicus* muell Arg.

Bacteria	Extract concentration (mg/ml)								
	0.780	1.560	3.125	6.25	12.5	25	50	100	200
S.d.	-	-	-	-	-	β	+	+	+
S.a	-	B	+	+	+	+	+	+	+
S.p	-	-	-	-	-	-	β	+	+
E.c	-	B	+	+	+	+	+	+	+
P.a	-	-	-	β	+	+	+	+	+
C.a	-	-	-	-	-	β	+	+	+

Key:S.d = *S. dysenteriae*; S.a = *S. aureus*; S.p = *S.pyogens*E.c. = *E. coli*; P.a = *P. aerogenosa*; C.a = *C. albicans*

- = Resistance (growth of bacteria/fungi or turbidity)

+ = Concentrations show no turbidity (inhibition of bacterial growth)

β = least concentration showing no turbidity (MIC)

Table 4: Minimum Bacterial Concentration (MBC) Values for Bacterial Isolates Against Methanolic Extract (MTE) of Stem bark of *Croton zambesicus*

Bacteria	Extract concentration (mg/ml)								
	0.780	1.560	3.125	6.25	12.5	25	50	100	200
S.d.	-	-	-	-	β	+	+	+	+
S.a	-	β	+	+	+	+	+	+	+
S.p	-	-	-	-	-	-	β	+	+
E.c	-	β	+	+	+	+	+	+	+
P.a	-	-	-	β	+	+	+	+	+
C.a	-	-	-	-	-	-	β	+	+

Key:S.d = *S. dysenteriae*; S.a = *S. aureus*; S.p = *S.pyogens*E.c. = *E. coli*; P.a = *P. aerogenosa*; C.a = *C. albicans*

- = Resistance (growth of bacteria/fungi)

+ = Bactericidal/fungicidal concentrations

β = Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

It can also be seen that the MIC and MBC values of the extract on *S. aureus* and *E. coli* is 1.560mg/ml similarly for *S. pyogens*, it is 50mg/ml. Hugo and Rusell, (1998) reported that “with most bactericidal antimicrobials, the MIC and MBC are often near or equal in value” as has typically been observed in this study. It is also observed from these results that the extract (MTE) have wide antibacterial activity against both gram positive, negative and fungal strain under study with highest activity on *S. aureus* and *E. coli* with the same MIC and MBC value of 1.560mg/ml and least MIC and MBC values of 50mg/ml on *S. pyogens*. The results of this investigation are indicative of possible pure active principle of natural origin from the extract with possible high potency which could serve as a lead to the isolation of chemotherapeutic agents. Also, in view of the fact that prevalence of *S. aureus* resistant strains to conventional antibiotics has increased to high levels of some hospitals (Shalit *et al.*, 1989 and Usman *et al.*, 2007) and that *S. aureus* is a pyogenic bacterium known to play significant role in invasive skin diseases including superficial and deep follicular lesion (Srinivan *et al.*, 2007). The extract could serve as a remedy to such resistance folklorically. The extract has also showed the same level of activity against *E. coli* which is the commonest cause of urinary tract infection and accounts for approximately 90% of first urinary tract infection in young women. (Brooks *et al.*, 2002 and Usman *et al.*, 2007). Especially this very low concentration of the extract as MBC against these prominent pathogens, this result therefore gives scientific base and credence for the claims of the therapeutic capabilities and folkloric usage of the various parts of *Croton zambesicus* for the treatment of various ailments. This study also provides some validity for the use of the plant parts in African traditional medicine and as a source of chemotherapeutic agents of grate novelty if studied well to harness the potential in the plant.

Conclusion

This study therefore provided bases to the folkloric use of this plant as a remedy for urinary tract infection, skin disease and other infections caused by the pathogens studied as practiced ethnomedically the world over. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent and we therefore, suggest further, the purification and characterization of the phytochemicals that would be obtained with a view to obtaining useful chemotherapeutic agent.

Acknowledgement

The authors wish to acknowledge and thank Mr. F. Akawu and S. Gamache of the Department of Chemistry, University of Maiduguri and Microbiology, University of Maiduguri Teaching Hospital respectively for their Technical assistance.

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