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Preliminary Phytochemical Screening and *In vitro* Antimicrobial Investigation of the Stem Bark Petroleum Ether Extract of *Croton zambesicus* Muell Arg.

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ABSTRACT

The petroleum ether extract of *Croton zambesicus* Muell Arg. was subjected to preliminary *Phytochemical* screening and *in vitro* antimicrobial tests. The *Phytochemical* tests were conducted using standard methods of analysis and the extract revealed the presence of cardiac glycosides and steroids. Antimicrobial effects of the plant extract were assayed using the plate-hole agar disc diffusion and nutrient dilution techniques. Test micro-organisms were *Shigella dysenteriae, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Candida albicans*, were all clinical isolates. The extract inhibited all the tested organisms at various concentrations except *Proteus vulgaris* and *C. albicans*. It showed a minimum inhibitory concentration (MIC) of 25 mg/ml against *S. dysenteriae, S. aureus* and *E. coli* while MIC against *S. pyogenes* and *p.aeruginosa* were 100 and 50 mg/ml respectively. The minimum bacterial concentration (MBC) was 50 mg/ml against *S. dysenteriae, S. aureus* and *E. coli* while MBC against *S. pyogenes* and *P. aeruginosa* was 100 mg/ml. Therefore it was concluded that this study laid credibility for the use of the plant against stomach and urinary tract infections whose causative agents are most of the organisms used in this study.

Key words: Antimicrobial, phytochemical, *Croton zambesicus*, extract. Euphorbiaceae

INTRODUCTION

Natural substances of plant origin have been used and are being used through out the world for human and animal health care (Saxena, 2003). The development of resistance to synthetic antimicrobials by micro-organisms necessitated the search for natural products as therapeutic alternatives (Olutimayin *et al.*, 2001). Most herbal medicines are found to be safe and affordable by the commom man (Balentine et al., 1999) thus the high demand for herbal medicines, which has increased the growing interest in medicinal plant research.

Croton zambesicus Muell Arg. (Keay, 1989; Arbonnier, 2004) commonly known as *Koriba* or *Icen maser* in Hausa, *Ajekokofole* in Yoruba, *Mfam* in Ekoi languages (Agishi and Shehu, 2004) and *"Moramora"* in kilba language belongs to *Euphorbiaceae* family. It is a shrub of 10 - 16 m high, often branching low down with a spreading crown and characteristic hanging leaves, silvery beneath. The bark is whitish to pale gray, slash thin and yellowish with a strong pharmaceutical smell. Flowering usually at the beginning of the dry seasoning, it is found in Sudan and Guinea Savannah zones and distributed from Cameroon to tropical Africa (Arbonnier, 2004).

The leaf decoction of *Croton zambesicus* is used in the treatment of urinary tract infection, as antihypertensive and to treat fever associated with malaria. The leaf alkaloidal fraction has also been reported to have broad spectrum antibacterial property (Arbonnier, 2004 and Okokon *et al.*, 2005). This study therefore, was aimed at investigating the preliminary phytochemical constituent and antimicrobial properties of petroleum ether extract of the stem bark of this plant.

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MATERIALS AND METHODS

Plant material

The stem bark of *Croton zambesicus* Muell Arg. was collected in March at latitude 10º15'N, longtitude13º15'E, Mubi, Adamawa State and was authenticated by a botanist, Prof. S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Nigeria.

Extraction and preparation of plant material

The stem bark of *C. zambesicus* (5 kg) was gabled for removal of adulterants and then air dried at room temperature (37ºC) for ten days and later pulverized with wooden pestle and mortar. Four hundred grams (400 g) of the powdered material was exhaustively and successively extracted with petroleum ether (60 - 80^{0}C), using the Soxhlet extractor. The extract was concentrated *in vacuo* and a brownish-yellow oily paste which weighed 17.197 g (4.30% w/w) and coded PEE was kept aseptically in a desiccator until use.

Phytochemical screening

The petroleum ether extract (PEE) was screened for the presence of chemical constituents using standard procedures of analysis (Harbone, 1983; Sofowora, 1993; Trease and Evans, 2002).

Test organisms

The bacterial and fungal organisms used include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Candida albicans*. All species were clinical isolates obtained from the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria.

Susceptibility tests

The plate-hole assay of susceptibility testing described by Onoruvwe and Olorunfomi (1998), Kudi *et al.* (1999) and Ogundipe *et al.* (2000) was used to determine the growth inhibition of bacteria by the plant extract (PEE). The tests were carried out by using a stock concentration of 500 mg/ml prepared by dissolving 1 g of the extract into 2 ml of distilled water. The dilution ratio for grams positive and negative bacteria were 1:1000 and 1:5000 respectively using peptone water (Usman *et al.*, 2005, 2007) while for *C. albicans*, Sabourand dextrose broth was used and incubated for 48 h. Nutrient agar was prepared and 25 ml each was poured into sterile Petri-dish. This was allowed to solidify and dry. Using a sterile cock-borer of 9 mm diameter, three holes per plate were made in the set agar and were inoculated with 0.5 ml suspension of the bacteria. There after, the wells were filled with the extract at varying concentrations of 500 mg/ml, 400 mg/ml and 300 mg/ml respectively. This was done in triplicate and the plates were incubated at 37^{0}C for 24 h. The diameter of zones of inhibition were measured and recorded; if the zone was greater or equals to 10 mm, it was considered as having antibacterial activity (Vlietink *et al.*, 1995; 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. The method of Vollekova *et al.* (2001) with little modification by Usman *et al.* (2007) was employed in this assay. In this method, the tube dilution technique was utilized where the plant extract was prepared to the highest concentration of 500 mg/ml in sterile distilled water and serially diluted (two-fold) to a working concentration ranging from 0.780 mg/ml to 200 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 24 h of incubation at 37^{0}C, the tubes were then observed for the presence of turbidity. The lowest concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration.

Minimum bacterial concentration (MBC)

The MBC defined as the lowest concentration where no bacterial growth is observed and 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), Usman *et al.* (2005, 2007).

The contents of the test tubes from MIC was streaked using a sterile wire loop on antimicrobial free agar plate and incubated at 37^{0}C for 24 h to observe bacterial growth. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC value.

Statistical analysis

The data was expressed in mean and standard deviation of the mean using statistical analysis software (SAS) system.

RESULT AND DISCUSSION

The result of preliminary phytochemical screening of petroleum ether extract (PEE) is shown in Table 1. This revealed the presence of cardiac glycosides and steroids. These compounds are associated with antibacterial activity and thus have curative properties against pathogens (Nweze *et al.*, 2004; Usman *et al.*, 2007; Nwaogu *et al.*, 2007). The *in vitro* antimicrobial activities of PEE are shown in Table 2. It shows that the extract has activity against *Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus, Shigella dysenteriae* and *Streptococcus pyogenes* and none against *Proteus vulgaris* and *Candida albicans* at the tested concentrations. The extract at the lowest concentration showed greater zones of inhibition on three of the microbes; *S. dysenteriae, E. coli* and *P. aeruginosa* than shown by the standard drug tetracycline.

Table 1. Phytochemical analysis of petroleum ether extract (PEE) of the stembark of *C. zambesicus*

S/No.	Constituent and test	Petroleumether extract
1.	***Alkaloids***	
	Dragendorff's test	–
	Meyer's test	–
2.	***Carbohydrates***	
	Molisch's test	–
	Barfoed's test	–
	Fehling's (combined and reducing sugar) test	–
	Fehling's (combined reducing sugar) test	–
3.	***Cardiac glycosides***	
	Keller-Killani's test	++
4.	***Flavonoids***	
	Shinoda's test	–
	$FeCl_3$ test	–
	Pew's test	–
5.	***Saponins***	
	Frothing test	–
6.	***Steroidal nucleus***	
	Salkowski test	++
	Libarman-Burchard's test	++
7.	***Tannins***	
	$FeCl_3$ test	–
	Lead acetate test	–

– = absent; + = present

The minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) results are shown on Tables 3 and 4, respectively. The extract exhibited broad activity against grams positive and negative organism with highest activity shown on *S. dysenteriae*, *S. aureus* and *E. coli* with MIC value of 25 mg/ml and MBC of 50 mg/ml. Its lowest activity is on *S. pyogenes* with MIC of 50 mg/ml and MBC of 100 mg/ml. This activity was indicative of the possible means of finding pure active principles from natural source with possible high potency that could serve as a lead to the pharmaceuticals, in view of the fact that prevalence of *S. aureus* resistant strains to conventional antibiotics has increased to high levels in some hospitals (Shalit *et al.*, 1989 and Usman *et al.*, 2007) and that *S. aureus* is a pathogenic bacterium known to play significant role in invasive skin diseases including superficial and deep follicular lesion (Srinivasan *et al.*, 2001, Usman *et al.*, 2005, 2007). The extract also have shown the same activity against *E. coli* and *S. dysenteriae* which are the common cause of urinary tract infection and diarrhea and accounts for approximately 90% of first urinary tract infection in young women (Brooks *et al.*, 2002; Usman *et al.*, 2007). This plant extract if studied and harnessed well, could be an answer to the peoples yearning for a better therapeutic agent from natural sources, which can remedy cases of urinary tract and skin infection

Table 2. *In vitro* antimicrobial activity of petroleum ether extract (PEE) of *C. zambesicus* stembark

Extract/ drug	Organisms/zones of inhibition (mm) (mean ± SD*)						
	Conc. (mg/ml)	*E. coli*	*P. aeruginosa*	*P. vulgaria*	*Staph aureus*	*Sh. dysenterae*	*Strep. pyrogenes*	*C. albicans*
PEE	500	24.30 ± 1.15	21.30 ± 2.08	–	20.00 ± 1.00	29.30 ± 1.00	33.30 ± 0.58	–
	400	23.00 ± 2.16	19.00 ± 1.73	–	17.00 ± 0.05	25.30 ± 0.58	30.00 ± 0.00	–
	300	19.70 ± 0.58	14.70 ± 0.58	–	15.20 ± 0.29	22.60 ± 0.58	27.00 ± 0.00	–
Tetracycline	25	12.00 ± 0.00	10.00 ± 0.00	–	25.00 ± 0.00	10.00 ± 0.00	28.00 ± 0.00	13.00 ± 0.00

* All data were average of 3 values (× ± SEM)

Table 3. Minimum inhibitory concentration (MIC) of *C. zambesicus* stembark petroleum ether extract (PEE) on some bacterial organisms

Bacteria	Concentration (mg/ml)					
	6.25	12.50	25.00	50.00	100.00	200.00
Escherichia coli	+	+	β	–	–	–
Pseudomonas aeruginosa	+	+	+	β	–	–
Staphylococcus aureus	+	+	β	–	–	–
Shigella dysenterae	+	+	β	–	–	–
Streptococcus pyrogenes	+	+	+	+	β	–

+ = growth; – = no growth; β = MIC

Table 4. Minimum bacterial concentration (MBC) of *C. zambesicus* stembark petroleum ether extract (PEE) on some bacterial organisms

Bacteria	Concentration (mg/ml)					
	0.25	12.50	25.00	50.00	100.00	200.00
Escherichia coli	+	+	+	B	–	–
Pseudomonas aeruginosa	+	+	+	+	B	–
Staphylococcus aureus	+	+	+	B	–	–
Shigella dysenterae	+	+	+	B	–	–
Streptococcus pyrogenes	+	+	+	+	B	–

+ = growth; – = no growth; B = MBC

CONCLUSION

This study therefore, laid credence for the folkloric use of this plant as a remedy to urinary tract infections, skin diseases, stomachache and diarrhea as practiced ethno-medically the world over.

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