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Preliminary Phytochemical Analysis and Antimicrobial Properties of Crude Extract of *Combretodendron macrocarpum* Stem Bark

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ABSTRACT

The antimicrobial properties of the tree bark extract of *Combretodendron macrocarpum* using different solvents were investigated. Extracts of *C. macrocarpum* were prepared using hot water, cold water, diethyl ether and dilute hydrochloric acid (0.02M). The extracts were tested against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* and *Klebsiella pnuemoniae* using well-diffusion method. The plant extracts of dilute HCl and diethyl ether exerted the highest zone of inhibition against all the test microorganisms with *S. aureus* (29.9 mm) and *K. pneumoniae* (27.5 mm). The diethyl ether extract exerted the highest effect against *P. aeruginosa* (21.9 mm) and *K. pneumoniae* (21.8 mm) with no effect on *E. coli*. The cold water extract was not active on any of the bacterial pathogens tested at any of the concentrations of the extract used. The Minimum Inhibitory Concentration (MIC) value of the hot water extract was lowest for *E. coli* (31.25mg/ml) and *K. pneumoniae* (62.5 mg/ml), and the dil. HCl extract also showed a low MIC against *P. aeruginosa* (31.25mg/ml). Preliminary phytochemical screening revealed the presence of alkaloids, saponin, steroids, tannins and terpenoids. These results therefore support and encourage the use of *C. macrocarpum* stem bark for the treatment of conditions that may be of bacterial etiology.

Keywords: *Combretodendron macrocarpum*, phytochemicals, etiology, minimum inhibitory concentration, antimicrobials.

Introduction

The use of plant and plant derived substances to fight against microorganisms is now on the increase, partly because the abuse of traditional antibiotics has led to the development of resistance against chemical antibiotics by microbial strains, occurrence of undesirable side effects from some chemically synthesized drugs, scarcity and high cost of new generation antibiotics (Masibo and He, 2009), biodegradation time of synthetic materials compared to natural or organic substances with faster degradation time (Lewis and Elvin-Lewis, 1995). The effectiveness of most common antibiotics has been limited due to the emergence of multi-drug resistant bacteria strains. *Staphylococcus aureus* is capable of developing resistance to commonly used antibiotics. The Methicillin Resistance Staphylococcus aureus (MRSA) is an emerging bacterial pathogen associated with significant morbidity and mortality (Munyendo *et al*., 2011). Cowan (1999) reported that *Pseudomonas aeruginosa* is also a significant opportunistic human pathogen associated with nosocomial infections. Microorganisms have developed resistance to many antibiotics. This has led to serious clinical problems in the treatment of infectous diseases. The indiscriminate use of commercially available antimicrobial drugs in the treatment of infectious diseases has led to an increase in the resistance of bacteria to antibiotics (Prasad *et al*, 2008; Chanda *et al*, 2011). This development has prompted science to search for new antibiotics to take up the challenge

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of resistance strains of microbial pathogens. The search for antibiotics largely depends on medicinal plants as raw materials (Doughari and Manzara, 2008). Investigations into medicinal plants have revealed the presence of bioactive substances that can be utilized to solve health needs. A variety of plants or materials derived from plants have been used for the prevention and treatment of diseases virtually in all cultures (Adesina *et al*, 2010).

The medicinal potential (antimicrobial, antioxidant, anticancer, antimalaria, immunomodulatory, etc.) attributed to plants has been linked to the presence of bioactive constituents in these plants. These bioactive substances have been found to produce definite physiological action on the human body (Akinmoladun *et al*., 2007). These bioactive substances include alkaloids, essential oils, saponin, tannins, flavonoids, terpenoids and phenolic compounds (Edeoga *et al*., 2005, Mungole *et al*., 2010). *C. macrocarpum* is a plant belonging to the family Lecythidaceae with the common name essia. It is distributed throughout the West African sub-region. It is called *owewe* in Edo state of Nigeria, *abale* in Ivory Coast, *abing* in Cameroon, *abin* in Gabon and *minzu* in Zaire (USDA 2012). There has been reported work on the antimicrobial activity of *C. macrocarpum*, but there are reports on some other uses of the stem bark of this plant, such as its hypotensive property (Ogundaini *et al*., 1983: Preliminary phytochemical studies of *Combretodendron macrocarpum (P. Beauv)* keay with reference to its hypotensive principles, *Journal of Ethnopharmacology* 9 (2 – 3): 337 – 345); as an anti-fertility agent (Benie *et al*., 1990: Combretodendron africanum bark extract as an antifertility agent. I: Estrogenic effects in vivo and LH release by cultured gonadotrope cells) Journal of Ethnopharmacology, 29(1):13 – 23). Therefore, this study is aimed at determining the phytochemical constituents and antimicrobial properties of *C. macrocarpum* using different solvents against some disease-causing organisms.

Materials and Methods

Sample collection

The tree bark of *C. macrocarpum* used for this study was obtained from the bushes of Odighi and Oke in Ovia North East Local Government area of Benin City (6.31760N 5.61450E), Nigeria. This was identified and authenticated botanically at the Department of Botany, University of Benin, Edo Sate, Nigeria.

Test organisms

The bacteria cells used for this study were clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerugenosa* and *Klebsiella pneumoniae*. These isolates were obtained from Irrua Specialist Hospital in Irrua, Edo State.

Sample extraction

Fresh pieces of *C. macrocapum* bark were collected and washed to remove dirt and debris. They were dried in a hot air oven at 35^{o}C for 5 days. The dried tree barks were ground into powder. 10 g of the powdered stem bark was weighed using a weighing balance into a flask. 20 ml of solvent was measured into the flask. This was repeated for other solvent and made to stand for 24 h. The mixture for the hot water extract was heated for 30 min.

Phytochemical screening

Preliminary phytochemical screening involving chemical tests to determine the presence of alkaloids, saponins, tannins, anthroquinones flavonoid and cardiac glycosides were carried out using the methods described by Odebiyi and Sofowora (1999).

Antimicrobial studies

The microorganisms used for this study were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerugenosa* and *Klebsiella pneumoniae*. The agar well diffusion method was used for the antimicrobial activity (Okeke *et al*., 2001). Nutrient agar was used for the study. Gentamicin and Nalixic acid were the commercially available antibiotics used for the study. Using the infusion technique, well holes of 0.9 mm diameter were measured and a two-fold serial dilution of the extracts were prepared by first

reconstituting in 20% dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve a decreasing concentration range of 50 mg ml^{-1} to 8.021 mg ml^{-1}. A 100 µl volume of each dilution was introduced in duplicate wells into nutrient agar (NA) plates already seeded with the standardized inoculum (5×10^5) of the test bacteria cells. The test plates were incubated at 37°C for 24 h. The least concentration of each extract showing a clear zone of inhibition was taken as the MIC.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the various extracts was determined using the method described by Doughari and Manzara (2008) with slight modification. The MIC was determined for each test organisms in triplicates at varying concentrations of 500, 250, 125, 62.5, 31.25, 15.65 mg/ml for each of the test organisms. 1 ml of varying concentrations of the extract were placed in separate test tubes, 2 ml of Nutrient broth and a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard were added. Same procedure was repeated using standard antibiotics. A control experiment was set up using a tube containing Nutrient broth only and seeded with the test organisms. The cultured tubes were then incubated at 37°C for 24 h.

Results and Discussion

The preliminary phytochemical screening of *Combretodendron macrocarpum* revealed the presence of alkaloids, saponins, tannins and terpenoids (Table 1). Alkaloids, as reported by Elekwa *et al.* (2008), have been seen to interfere with cell division which makes them an important plant part to possibly be used as remedy in the treatment of cancer. Noble (1990) corroborated that alkaloids are widely used as cancer chemotherapeutic agent. Cardiac glycosides have been reported to be effective in congestive heart failure (Elekwa *et al.*, 2008). Saponins and glycosides have been reported to have hypertensive and cardiac depressive properties (Trease and Evans, 1985). The antimicrobial studies revealed that the dilute hydrochloric acid extract of *C. macrocarpum* showed inhibitory affects on *E. coli. P. aeruginosa*, *S. aureus* and *K. pneumoniae* with zone of inhibition of 20.8, 25.3, 29.9 and 27.5mm respectively while the cold water extract exhibited no inhibitory effects on these micro-organisms (Table 2). This shows that the extracts of dil. Hydrochloric acid were more effective against the test organisms. It has been documented that the potency of plant

Table 1: Phytochemical profile of plant extracts

	Alkaloids	Flavonoids	Steroids	Tannins	Terpenoids	Cardiac glucoside	Anthraquine
Hot water extract	++	+	+	+	+	-	-
Cold water extract	+	-	-	+	-	-	-
Diethyl ether	+	+	+	+	+	+	-
Dil HCl	++	+	+	+	+	-	-

Table 2: Zone of inhibition of extract with test organisms in mm

	E. coli	*Pseudomonas aeruginosa*	*S. aureus*	*K. pneumoniae*
Hot water extract	22.2	19.8	21.0	21.0
Cold water extract	0.0	0.0	0.0	0.0
Diethyl ether	0.0	21.9	20.6	21.8
Dil. Hydrochloric acid	20.8	25.3	29.9	27.5

extracts depends on the solubility of the bioactive constituents (Nenaah and Ahmed, 2011). The MIC value of *C. macrocarpum* was lowest in the hot water extract (31.25 mg/ml) against *E. coli* and in dil. Hydrochloric acid against *P. aeruginosa* (31.25 mg/ml) (Tables 3 and 4). The result of this study revealed that the hot water extract and dil. HCl extract are more effective against test organisms compared to some standard antibiotic (Gentamycin, Nalixic acid) with a MIC value of 62.5 mg/ml. It could therefore be inferred that the extracts of *C. macrocarpum* are more potent in the fight against bacterial pathogen compared with some standard antibiotics, hence the report of this work support the use of *C. macrocarpum* to treat diseases of bacterial etiology.

Table 3: Antimicrobial activity of *Combretodendron macrocarpum* stem bark

Extraction concentration and diameter zone of inhibition (mm) Bacterial agent																
	62.5				125				250				500			
Test organisms	H.W	C.W	D.E	dil HCl	H.W	C.W	D.E	dil HCl	H.W	C.W	D.E	dil HCl	H.W	C.W	D.E	dil HCl
E. coli	13.2	-	-	15.0	R	-	-	18.0	19.1	-	-	21.2	21.0	-	-	22.2
P. aeruginosa	-	-	-	19.0	17.9	-	11.3	20.0	18.5	-	14.8	27.0	19.7	-	7.6	28.5
S. aureus	11.5	-	-	-	-	-	-	-	-	-	17.6	24.0	19.1	-	19.5	25.0
K. pneumoniae	-	-	11.9	-	16.2	-	20.0	-	-	-	20.5	20.2	19.6	-	20.8	26.9

Among consumers and health professionals, complementary and alternative medicine (CAM) has become sought after and integrated into mainstream provision of medical services. CAM, known as nonallopathic, unconventional, holistic, or natural therapy, encompasses many types of healing practices (Spigelblatt, 1995; Brenuer, *et al.*, 2003). From the Cochrane Collaboration, CAM is a broad domain of healing resources that encompasses all health systems, modalities and practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health systems in a particular society or culture in a given historical period (Zollman, *et al.*, 1999).

Herbal products historically have been the cornerstone of much of the pharmaceutical armamentarium.

Active segments of the plant include leaves, flowers, stems, roots, seeds and berries. The following herbs and other compounds have been evaluated predominantly in the laboratory with mixed results. If available, information on typical doses is cited. Techniques used for extraction of the bioactive ingredients of these herbs are noted to use ethanol or methanol, which may cause some of the anticontraceptive effects. Except for the isolated occasion, many of these herbs have not been studied in people. Multiple studies on the stem bark extracts of *Combretodendron macrocarpum*, *Cola nitida*, *Afrormosia laxiflora*, and *Pterocarpus erinaceus* have shown that they block the estrus cycle of female rats. It is thought that these compounds may bind to steroid receptor sites with a resultant antigonadotropic activity. The most potent competitor for steroid receptors was *C. macrocarpum* extract, followed by *P erinaceus*, *C. nitida* and *A. laxiflora* (Benie, *et al.*, 2003; El Izzi, *et al.*, 1990).

Table 4: The minimum inhibitory concentration mg/ml of *Combretodendron macrocarpum* stem bark extract

Fractions	Organisms	500	250	125	62.5	31.25	15.65	MIC (mg/ml)
H W	*E.col*	-	-	-	-	-	+	31.25
	P. acruginosa	-	-	-	+	+	+	125
	S. aureus	-	-	+	+	+	+	250
	K.pneumoniae	-	-	-	-	+	+	62.5
C W	*E. coli*	+	+	+	+	+	+	ND
	P.aeruginosa	+	+	+	+	+	+	ND
	S.aureus	+	+	+	+	+	+	ND
	K.pneumoniae	+	+	+	+	+	+	ND
D.E								
	E. coli	+	+	+	+	+	+	ND
	P. aeruginosa	-	-	-	+	+	+	125
	S.aureus	-	-	+	+	+	+	250
	K.pneumoniae	-	-	-	-	+	+	62.5
DH	*E. coli*	-	-	-	-	+	+	62.5
	P. aeraginosa	-	-	-	-	-	+	31.25
	S.aureus	-	-	+	+	+	+	250
	K.pneumoniae	-	-	+	+	+	+	250
GEN	*E.coli*	-	-	-	-	+	+	62.5
	P.aeruginosa	-	-	-	-	+	+	62.5
	S. aureus	-	-	-	-	+	+	62.5
	K. pneumonia	-	-	-	+	+	+	125
NAL	*E. coli*	-	-	-	-	+	+	62.5
	P. aeroginosa	-	-	-	+	+	+	125
	S. aureus	-	-	-	+	+	+	125
	K. pneumonia	-	-	-	-	+	+	62.5

HW = hot water, CW = cold water, DE = diethyl ether, DH = dil. HCl, GEN = Gentamycin, NAL= Nalixic acid, ND = not detected

Conclusion

The result of this study showed that the extract of *C. macrocarpum* stem bark exhibited antimicrobial activity. The plant extract contains bioactive principles which are active in the inhibition of some microorganisms. This study, therefore, supports the use of the extract of *C. macrocarpum* as a medicinal plant. However, further investigation needs to be carried out on the antioxidant properties and toxicity of the plant.

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