

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *CEIBA PENTANRA* AGAINST SOME CLINICAL PATHOGENIC BACTERIA

Banso A. and Ajayi M.A.

Department of Biological Sciences, Federal Polytechnic Bida, Niger State, Nigeria

*Corresponding Author Email Address: drbanso@yahoo.com

ABSTRACT

Many of the plant materials used in traditional medicine are readily available in rural areas and thus have made traditional medicine relatively cheaper than modern medicine. Plants generally produce many secondary metabolites and these constitute an important source of microbiocides, pesticides and many pharmaceutical drugs. *Ceiba pentandra* is naturally present in equatorial Africa and naturalized in all the humid tropics including Nigeria. Ethyl acetate, ethanol and aqueous leaf extract of *Ceiba pentandra* were assayed against clinical isolates of *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumoniae* by agar diffusion method. All the extracts showed antimicrobial activity against all the test organisms. *K. pneumoniae* was the most susceptible to the plant extracts while *E. coli* was the most resistant. The minimum inhibitory concentration of the plant extracts against the test organisms ranged between 15µg/mL to 30µg/mL. The minimum bactericidal concentration of the extracts was within the ranges of 15µg/mL and 35µg/mL. Ethyl acetate extract of leaf of *Ceiba pentandra* appeared to be more effective than aqueous or ethanol extract as antimicrobial agent against all the test bacteria. The results obtained may suggest that the plant extracts possess useful antimicrobial properties.

Keywords: Pharmaceutical, bactericidal, susceptible, drugs, *Ceiba pentandra*.

INTRODUCTION

Ceiba pentandra known as "araba" among the Yoruba of Western Nigeria belong to the family Malvaceae. The plant is commonly called riimiiin "Hausa" and "Okpu" in igbo. The plant is naturally present in equatorial Africa and naturalized in all the humid tropics including Nigeria after human introduction (Pezzini *et al.*, 2001). It has a long history for herbal medicine all parts of the plant are used traditionally as remedy for various ailments. The leaves are used for the relieve of fever and headache (Friday *et al.*, 2021). The fruit is a capsule containing seeds surrounded by long wooly. Non-wettable hairs called kapok (Dick *et al.*, 2007); Moris *et al* 2002). The seed contain about 24 to 28% fat depending on the extraction method (Answer *et al.*, 2014; Saliman and Kadir, 2005). Kapo seed fiber is covered by a hydrophobic waxy layer (Peng *et al.*, 2021). Many of the plant materials used in traditional medicine are readily available in rural areas and thus have made traditional medicine relatively cheaper than modern medicine. Over sixty percent of Nigeria rural population depends on traditional medicine for their healthcare need (Apalu *et al.*, 1994). Mpiana *et al.* (2007) reported the anti-bacterial activity of aqueous and ethanol extract of *Ceiba pentandra*. The stem bark extract reduced the parasitaemia in mice experimentally infected with *Trypanosoma brucei* when the mice was treated with aqueous extract *Ceiba pentandra* at the dose of 100mg/kg body weight intraperitoneally two times daily for three

days. Anti-inflammatory activity of activity of the bark, xylem of stem and root of *Ceiba pentandra* in carrageenan- induced paw oedema in rats has been reported (Lin *et al.*, 1992). Aqueous extract of *Ceiba pentandra* exhibited hypoglycaemic properties in streptozotocin-induced diabetic rat (Bizimana *et al.*, 2006). Methanol stem extract of *Ceiba pentandra* showed inhibitory activity in the tube like formation induced by human umbilical venous endothelial cells in the in vitro angiogenesis assay (Nam *et al.*, 2003).

Plant generally produce many secondary metabolites and these constitute an important source of microbiocides, pesticides and many pharmaceutical drugs (Nam *et al.*, 2003). Noreen *et al.* (1998) isolated new isoflavone glucoside vavain 3-o-beta-glucoside and its aglycon vavain from the bark of *Ceiba pentandra*. Rao *et al.* (1993) also isolated two new sesquiterpenes lactones showing moderate antimicrobial activity from the root bark of *Ceiba pentandra*. A new naphthaquinone 2,7-dihydroxy-8-formyl-7-hydroxy-5-quinone has been isolated from the heartwood of *Ceiba pentandra* (Rao *et al.*, 1993) Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Hence the aim of this study is to investigate the efficacy of leaf extract of *Ceiba pentandra* against some pathogenic bacteria to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

The leaves of *Ceiba pentandra* used in this study were obtained from Bida, Niger State Nigeria. The plant was authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan; in accordance with the criteria stipulated by International Committee for Botanical Nomenclature (ICBN). Voucher specimens of *Ceiba pentandra* was deposited in the herbarium at the Department of Plant Biology, University of Ilorin, Nigeria with the herbarium number UNILORIN135.

Test microorganism

Clinical isolates of *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia* used in this study were obtained from Federal Medical Centre, Bida Niger State, Nigeria. Biochemical and Gram staining tests were used to confirm the identity of the organisms. The biochemical characterization of the organisms was done by indole test, methyl red test, Voges-Proskauer test, citrate utilization test, catalase test and protease test. The cultures were subcultured and maintained on nutrient agar slants. They were stored in the refrigerator at 4°C until required.

Extraction of plant leaves

Aqueous extraction

Ten grammes of powdered air dried leaf sample of *Ceiba pentandra* was weighed into 100mL of distilled water and boiled for 6h. At interval of 2h it was filtered through a membrane filter with pore size

0.45µm into different conical flasks and centrifuged at 500 x g for 15 mins. The supernatant was collected and concentrated using a rotary evaporator and stored in refrigerator in reagent bottles. The extract was tested for contamination by plating them on nutrient agar at 37°C for 24h. When no growth was observed visually in the extract, it was then assayed against the test organisms for antimicrobial activity.

Solvent extraction

Ten grammes of powdered air dried leaf sample of *Ceiba pentandra* was weighed separately into 100mL of organic solvent (petroleum ether or 80% ethanol) contained in 500mL capacity flasks and kept in a rotary shaker (190-220 rpm) for 30 mins. The extracts were filtered through a membrane filter with pore size 0.4µm into different conical flasks and centrifuged at 5,000 x g for 15 mins. The supernatant was collected and evaporated into a dry mass using a rotary evaporator and stored in refrigerator in reagent bottles. Each extract was tested for growth/ contamination by plating them on nutrient agar at 37°C for 24h. When no growth was observed visually in the extract, it was then assayed against the test organisms for antimicrobial activity (Olorundare *et al.*, 1992).

Phytochemical analysis

The extract were subjected to phytochemical analysis as adopted by Banso *et al.* (2020).

Antimicrobial assay

Agar diffusion assay of Nair and Chanta (2005) was adopted to test for the effect of the leaf extracts against the test organisms. Clinical isolates of *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia* cells were inoculated on different nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5mm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of forceps sterilized by flaming. To each well were introduced graded concentrations (5µ/mL, 10µ/mL, 15µ/mL, 20 µ/mL, 25 µ/mL, 30 µ/mL, and 35 µ/mL) of the extract. The graded concentrations were obtained by first reconstituting the extracts in 20% dimethyl sulphoxide (DMSO). They were then diluted in sterile distilled water to achieve graded concentrations of 5, 10, 15, 20, 25, 30 and 35µ/mL. Control experiments comprising inoculums without the plant extracts were set up. Tetracycline (25µ/ml) was used as a positive control while 20% dimethyl sulphoxide (DMSO) served as a negative control. The plates were allowed to stand for 1h at room temperature (28±2°C) for diffusion of the extracts to proceed before the growth of organisms commenced. The plates were incubated for 24h. The zones of inhibition were then measured and recorded.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of the extracts was determined by broth dilution method. Various concentrations of the plant extracts were introduced into different test tubes; each tube was inoculated with an overnight culture of *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia* diluted to give a final concentration of 10⁶cells/ml. The tubes were incubated at 37°C for 24h. The least concentration of the plant extracts that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration in each case (Banso and Olutimayin, 2001).

Determination of minimum bactericidal concentration

After culturing the test organisms separately in nutrient broth containing graded concentrations of the extracts, the broth was inoculated onto freshly prepared nutrient agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24h. The lowest concentration of extract that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC (Alade and Irobi, 1993).

RESULTS

Crude leaf extract of *Ceiba pentandra* contain alkaloid, glycoside, tannin, saponin and flavonoid (Table 1). The extract exhibited antimicrobial activity against *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia* (Table 2). Antimicrobial activity of the plant extracts increase with increase in the concentration of the extracts. The largest diameter of inhibition ranging from 7.5 ±0.01mm to 8.6±0.1mm were obtained from assay containing ethyl acetate extract (35µg/mL), while the lowest ranges of mean diameter of zones inhibition (3.0±0.1mm to 3.6±0.2mm) were obtained with aqueous extract (10µg/ml). *Escherichia coli* appears to be the most resistant to the effect of the leaf extracts of *Ceiba pentandra* while *Klebsiella pneumonia* which has higher ranges of diameter of zones of inhibition (3.6±0.2 to 8.6±0.1) appeared to be the most susceptible (Table 1). The MIC of *Ceiba pentandra* leaf extract against the test bacteria ranged between 15µg/mL and 30µml). Ethyl acetate extract has the lowest MIC when assayed against the test bacteria while aqueous extract has the highest MIC (Table 2). The MIC of aqueous, ethyl acetate and ethanol extract of the leaves of *Ceiba pentandra* against *Klebsiella pneumoniae* was 30µ/ml, 23µ/ml and 30µ/ml respectively (Table 2). The minimum bactericidal concentration of the leaf extract of *Ceiba pentandra* proved to possess more bactericidal action against the bacterium (Table 3). This was indicated by the low value obtained in assay with ethyl acetate and ethanol leaf extracts of *Ceiba pentandra* against *Staphylococcus aureus* (Table 3).

DISCUSSION

Antibacterial assay of aqueous, ethanol and ethyl acetate extracts of crude leaf extract of *Ceiba pentandra* showed that the leaf extract exhibits antibacterial activity against *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia*. It was indicated in this study, that increase in antibacterial activity of the extract was enhanced by increase in the concentration of the extract. This finding agrees with the report of Banso and Olutimayin (2000) that higher concentration of antimicrobial substances show appreciation in growth inhibition. The results of this study showed that leaf extracts of *Ceiba pentandra* exhibit antibacterial properties. This finding justifies its traditional use as a medicinal plant. This may be due to the presence of secondary metabolites in the plant leaf. Plants generally produce many secondary plant metabolites which constitute an important source of microbiocides and many pharmaceutical drugs (Ogundipe *et al.*, 1998). Minimum inhibitory concentration values of the plant extracts against the test organisms showed that bacteria vary widely in the degree of their susceptibility to antimicrobial agents. This agrees with the report that antimicrobial agents with low activity against an organism have a high minimum inhibitory concentration while a highly antimicrobial agent has a low minimum inhibitory concentration (Prescott, 2000). The antibacterial substances contained in the crude leaf extract of *Ceiba pentandra* were bacteriostatic at lower concentrations while becoming bactericidal at higher concentrations of the extracts.

Similar observation has been reported by Bansa and Mann (2006). This present study showed that the leaf extracts of *Ceiba pentandra* could be useful as a source of antimicrobial agent.

Table 1: Phytochemical constituents of *Ceiba pentandra*

Chemical constituent	<i>C. pentandra</i>
Alkaloid	+
Glycoside	+
Steroid	-
Phenol	-
Tannin	+
Saponin	+
Flavonoid	+
Anthroquinone	-

Table 2: Antimicrobial spectrum of leaf extracts of *Ceiba pentandra*

Concentration(mg/ml)	zone of inhibition (mm)±SD															
	Aq				EtOH				EtOAc				Tet			
	Sa	Pv	Ec	Kp	Sa	Pv	Ec	Kp	Sa	Pv	Ec	Kp	Sa	Pv	Ec	Kp
5	3.0±0.1	3.4±0.2	2.5±0.1	3.6±0.2	3.5±0.2	5.0±0.3	3.4±0.1	4.7±0.1	4.8±0.2	5.5±0.1	4.5±0.1	5.4±0.1	10.5±0.2	13.0±0.1	12.5±0.2	15.5±0.1
15	3.4±0.2	3.9±0.2	2.9±0.1	3.9±0.3	3.6±0.2	5.0±0.1	3.7±0.1	5.1±0.1	5.0±0.1	6.0±0.2	6.1±0.2	5.8±0.2	NT	NT	NT	NT
20	3.6±0.2	4.4±0.1	3.6±0.2	4.4±0.1	3.9±0.2	5.9±0.2	4.7±0.1	5.5±0.1	5.7±0.2	6.5±0.2	5.8±0.1	6.4±0.1	NT	NT	NT	NT
25	3.8±0.1	4.7±0.2	3.9±0.1	4.8±0.1	4.7±0.2	5.9±0.2	4.3±0.1	6.5±0.1	6.5±0.2	7.3±0.2	6.4±0.3	7.0±0.2	NT	NT	NT	NT
30	4.6±0.1	5.2±0.2	4.8±0.3	5.2±0.1	5.0±0.1	6.5±0.2	5.9±0.2	6.9±0.1	6.8±0.2	7.9±0.1	7.0±0.1	8.2±0.1	NT	NT	NT	NT
35	5.0±0.1	5.8±0.3	5.2±0.1	5.9±0.2	5.7±0.1	6.8±0.1	6.4±0.2	7.9±0.1	7.5±0.2	8.5±0.3	7.9±0.1	8.6±0.1	NT	NT	NT	NT

Sa = *S. aureus*, Pv = *P. vulgaris*, Ec = *E. coli*, Kp = *K. pneumonia*

Aq = Aqueous extract, EtOH = Ethanol extract, EtOAc = Ethyl acetate extract

Negative control (20% dimethyl sulphoxide) showed no zone of inhibition

Tet = Tetracycline (Positive control)

NT = Not tested

Table 3: Minimum inhibitory concentration of leaf extracts of *Ceiba pentandra* µg/ml

Extract	Organism			
	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>K. pneumonia</i>
Aq	25	30	25	30
EtOAc	15	20	20	25
EtOH	20	25	20	30

Aq = Aqueous extract

EtOAc = Ethyl acetate extract

EtOH = Ethanol extract

Table 4: Minimum bactericidal concentration of leaf extract of *Ceiba pentandra* µg/ml

Extract	Organism			
	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>K. pneumonia</i>
Aq	30	35	30	30
EtOAc	15	20	25	30
EtOH	25	30	25	35

Aq = Aqueous extract

EtOAc = Ethyl acetate extract

EtOH = Ethanol extract

Conclusion

Ceiba pentandra is naturally present in equatorial Africa and naturalized in all the humid tropics including Nigeria. The leaf of the plant is used traditionally as remedy for various ailments. The

leaves are used to treat fever and headache. Extracts of the plant showed exhibited antimicrobial activity against *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia*. An increase in the concentration of the extracts enhanced the antibacterial activity. The antibacterial substances contained in the extracts were bacteristatic at lower concentrations while becoming bactericidal at higher concentrations of the extracts. Leaf extract of *Ceiba pentandra* could be useful as a source of chemotherapeutic agent.

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