



## RESEARCH ARTICLE

### Phytochemical and antimicrobial activity of *Ceiba pentandra* against some pathogenic bacteria

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#### ABSTRACT

Many plant ingredients used in traditional medicine are widely available in rural regions, making it cheaper than modern medication. Several secondary metabolites from plants are used to make microbiocides, insecticides, and pharmaceuticals. *Ceiba pentandra* grows natively in equatorial Africa and other humid tropics, including Nigeria. The agar diffusion method was used to test ethyl acetate, ethanol, and *C. pentandra* leaf extract against clinical isolates of *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumoniae*. All extracts were antibacterial against test organisms. *K. pneumoniae* was most vulnerable to plant extracts, and *E. coli* was most resistant. The plant extracts had a minimum inhibitory concentration of 15 µg/ml to 30 µg/ml against the test organisms. The minimum bactericidal concentrations of extracts ranged from 15 to 35 µg/ml. The ethyl acetate leaf extract of *C. pentandra* was more efficient than the aqueous or ethanol extract against all test microorganisms. The data may indicate that plant extracts are antibacterial.

**Keywords:** antimicrobial activity, *Ceiba pentandra*, phytochemicals, pathogenic bacteria.

#### INTRODUCTION

*Ceiba pentandra*, called “araba” by Western Nigerian Yoruba, is a Malvaceae plant. In Igbo, the plant is called riimiin “Hausa” and “Okpu”. The plant is native to equatorial Africa and naturalized in the humid tropics, including Nigeria, after human introduction (Pezzini et al., 2001). Traditional herbal medicine uses all parts of the plant to treat various ailments. Fever and headache are relieved by the leaves (Friday et al., 2021). Its capsule contains seeds wrapped in long wool. Kapok hairs are non-wettable

(Dick et al., 2007). The seed has 24–28% fat, depending on the extraction method (Answer et al., 2014; Saliman and Kadir, 2005). Kapo seed fibre has a hydrophobic waxy coating (Peng et al., 2021).

Several plant extracts used in traditional medicine are widely available in rural regions, making them cheaper than modern medication (Pandian et al., 2020; Ambiga et al., 2021; Ashokkumar et al., 2021a & b; Ashokkumar et al.,

2023). Traditional medicine serves around 60% of rural Nigerians (Apalu et al., 1994). Mpiana et al. (2007) found antibacterial activity in *C. petandra* aqueous and ethanol extracts. The stem bark extract reduced parasitaemia in Trypanosoma brucei-infected mice treated with aqueous *C. petandra* extract at 100mg/kg body weight intraperitoneally twice daily for three days. *C. petandra* bark, stem xylem, and root have anti-inflammatory properties in carrageenan-induced paw oedema in rats (Lin et al., 1992). Streptozotocin-induced diabetic rats showed hypoglycaemia using *C. petandra* aqueous extract (Bizimana et al., 2006). In the in vitro angiogenesis assay, *C. petandra* methanol stem extract inhibited tube-like development by human umbilical venous endothelial cells (Nam et al., 2003).

Secondary metabolites from plants provide microbiocides, insecticides, and numerous medicinal medications (Nam et al., 2003). From *C. petandra* bark, Noreen et al. (1998) extracted novel isoflavone glucoside vavain 3-o-beta-glucoside and its aglycon vavain. Rao et al. (1993) identified two novel sesquiterpene lactones with mild antibacterial activity from *C. petandra* root bark. A novel naphthaquinone 2,7-dihydroxy-8-formyl-7-hydroxy-5-quinone was obtained from *C. petandra* heartwood (Rao et al., 1993). Plant chemicals may be safer or more effective than manufactured antimicrobials. To determine why *C. petandra* leaf extract is used in traditional medicine, this study will test its efficiency against pathogenic bacteria.

## MATERIALS AND METHODS

This study used *C. petandra* leaves from Bida, Niger State, Nigeria. The Forestry Research Institute of Nigeria (FRIN) Ibadan verified the plant according to ICBN standards. The University of Ilorin Department of Plant Biology herbarium holds voucher specimens of *C. petandra* under the herbarium number UNILORIN135. Clinical isolates of *S. aureus*, *P. vulgaris*, *E. coli* and *K. pneumonia* used in this study were obtained from the federal medical Centre, Bida Niger State, Nigeria.

### Extraction of plant leaves

#### Aqueous extraction

Ten grams of air-dried *C. petandra* leaf powder was cooked for six hours in 100ml of distilled water. It was filtered through a membrane filter into conical flasks every 2 hours and centrifuged at 500 x g for 15 minutes. The supernatant was concentrated in a rotary evaporator and refrigerated in reagent bottles. The extract was plated on nutrient agar at 37°C for 24h to check for contamination. The extract

was tested for antibacterial efficacy against test organisms after no visible growth.

#### Solvent extraction

Ten grams of powdered air-dried *C. petandra* leaf sample was weighed separately into 100ml of organic solvent (petroleum ether or 80% ethanol) in 500ml flasks and shaken at 190-220 rpm for 30 minutes. The extracts were membrane-filtered into conical flasks and centrifuged at 5,000 x g for 15 min. Using a rotary evaporator, the supernatant was dried and kept in reagent bottles in the fridge. Each extract was plated on nutrient agar at 37°C for 24h to test for growth/contamination. When no growth was visible in the extract, it was tested for antibacterial activity against test organisms (Olorundare et al., 1992). The extract was phytochemically analysed (Banso et al., 2020).

#### Antimicrobial assay

The leaf extracts' effect on test organisms was tested using Nair and Chanta (2005) agar diffusion experiment. *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia* clinical isolates were inoculated on nutrient agar plates and dispersed evenly with a sterile glass spreader. With a sterile cork borer, 5mm wells were created in nutritional agar. Flame-sterilized forceps delicately removed the chopped agar discs. Graded concentrations of extract (5µ/ml, 10µ/ml, 15µ/ml, 20µ/ml, 25µ/ml, 30µ/ml, and 35µ/ml) were added to each well. Fires reconstituted the extracts in 20% dimethyl sulphoxide to reach graded quantities. The samples were diluted in sterile distilled water to reach concentrations of 5, 10, 15, 20, 25, 30, and 35 µ/ml. Control trials used inoculums without plant extracts. Tetracycline (25µ/ml) was employed as a positive control and 20% DMSO as a negative control. Plates were left at room temperature (28±2°C) for 1 hour to complete extract diffusion before organism development began. For 24 hours, plates were incubated. The inhibitory zones were measured and recorded.

#### Determination of minimum inhibitory concentration

The extracts' MIC was measured by broth dilution. An overnight culture of *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia* diluted to 10<sup>6</sup> cells/ml was injected into test tubes with varied plant extract concentrations. Tubes were incubated at 37°C for 24h. The minimal inhibitory dose of plant extracts that prevented observable growth of the infected test organism in broth culture was determined in each case (Banso and Olutimayin, 2001).

### Determination of minimum bactericidal concentration

To assess the bactericidal action, the test organisms were cultured separately in nutrient broth with graded extract concentrations and inoculated onto freshly prepared nutrient agar plates. Culture was incubated at 37°C for 24h. The lowest concentration of extract that did not develop colonies on a solid medium after incubation was MBC (Alade and Irobi, 1993).

### RESULTS

*C. pentandra* leaf extract contains alkaloids, glycosides, tannins, saponins, and flavonoids (Table 1). The extract inhibited *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia* (Table 2). The antimicrobial activity of plant extracts increases with concentration. Ethyl acetate extract (35µg/ml) produced the highest inhibition diameter (7.5±0.01mm to 8.6±0.1mm), while aqueous extract (10µg/ml) produced the lowest inhibition diameter (3.0±0.1mm to 3.6±0.2mm). According to Table 1,

*E. coli* is the most resistant to *C. pentandra* leaf extracts, while *K. pneumonia* is the most sensitive due to its wider inhibitory zones (3.6±0.2 - 8.6±0.1). *C. pentandra* leaf extract had a MIC of 15-30µg/ml against the tested microorganisms. Ethyl acetate extract has the lowest MIC against test bacteria, while aqueous extract has the highest (Table 2). Aqueous, ethyl acetate, and ethanol extract leaves had MICs of 30µ/ml, 23µ/ml, and 30µ/ml against *K.*

*pneumoniae*. *C. pentandra* leaf extract had higher bactericidal activity against the bacteria at the lowest concentration. Ethyl acetate and ethanol leaf extracts of *C. pentandra* had reduced *Staphylococcus aureus* test values (Table 3).

### DISCUSSION

The crude *C. pentandra* leaf extract inhibited *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia* in aqueous, ethanol, and ethyl acetate extracts. This study found that extract concentration increased antibacterial activity. This supports Banso and Olutimayin (2000)'s discovery that increased antibacterial concentrations hinder growth. This study found antibacterial activity in *C. pentandra* leaf extracts. This supports its traditional therapeutic usage. This may be attributed to plant leaf secondary metabolites. Secondary plant metabolites are key sources of microbiocides and medicinal medicines (Ogundipe et al., 1998).

Minimum inhibitory concentration values of plant extracts against test organisms demonstrated that bacteria's antimicrobial susceptibility varies greatly. This supports the finding that low-activity antimicrobials have a high minimum inhibitory concentration, while highly active ones have a low one (Prescott. 2000). Antibacterial compounds in *C. pentandra* crude leaf extract were bacteriostatic at low concentrations and bacteriocidal at high quantities. This was also noted by Banso and Mann (2006). This study found that *C. pentandra* leaf extracts may be antibacterial.

**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)	Mean diameter of 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* grows in equatorial Africa and the humid tropics, including Nigeria. The plant leaf is traditionally used to treat many diseases. The leaves relieve fever and headache. The plant extracts inhibited *S. aureus*, *P. vulgaris*, *E. coli*, and *K. pneumonia*. Antibacterial activity increased with extract concentration. The extracts' antibacterial components were bacteriostatic at low concentrations and bactericidal at high ones. *C. pentandra* leaf extract may be used as a chemotherapeutic agent.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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