ANTIMICROBIAL ACTIVITY OF STEM BARK EXTRACTS OF *CEIBA PENTANDRA*

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Summary

Aqueous and ethanol stem bark extracts of *Ceiba pentandra* were screened for the presence of bioactive components and tested for antimicrobial activity using the filter paper disc diffusion method. Results revealed the presence of anthraquinones, alkaloids, saponins, tannins, glycosides and phenolics. The active components exerted inhibitory effects on *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Shigella dysentriae, Candida albicans iand* Aspergillus flavus with the ethanol extracts demonstrating higher activity than the aqueous extracts. The minimum inhibitory and minimum bactericidal concentrations of the extracts against the test bacteria ranged between 6.25-50 mg/ml, while the minimum fungicidal concentration of the plant extracts against the test fungi ranged between 50-100 mg/ml. The plant can be used in treating urinary and gastrointestinal tract, wound and mycotic infections associated with the test organisms.

Key words: Antimicrobial activity, *Ceiba pentandra,* minimum inhibitory concentration, minimum bactericidal concentration.

Introduction

Antimicrobial agents inhibit the growth and multiplication of microorganisms (pathogenic or non-pathogenic) and are therefore used in control of infectious agents. Quite a number of antimicrobial substances exist and they are gotten from diverse sources including plants, microorganisms, animals and chemicals (1) The use of medicinal plant is accepted as the most common form of traditional medicine in the world. Among the entire flora, it is estimated that 35,000 to 70,000 species have been used for medicine purposes and some 5,000 of these have been studied in biomedical research (2). One of the earliest records of the use of herbal medicine is that of Caulmoogra oil from the species of *Hydracapus guertr*, which was known to be effective in the treatment of leprosy, such use was recorded in the pharmacopoeia of emperor Shen Nung of China between 2730 and 3000BC (3) Similarly, *Ricirus cosmunis* L. seeds were excavated from some ancient Egyptian tombs, which indicated their use in that part of Africa as far back as 1500BC. The plant extracts are usually used in their crude forms in that they are not chemically purified. However, some therapeutic agents have been identified in medical plants and these includes alkaloids, tannins, cardiac glycosides, saponins, phlobatamins, mucilages and resin (3,4).

Many plants have been screened for their antimicrobial activities to provide scientific basis for their use in the treatment of many infectious diseases of microbial origin and have been found promising. Antibacterial and antifungal activities of alkaloids isolated from *Erytolapsis sanguno* in Kinshasa, Zaire, has been reported. Similarly antimicrobial activities of *Psiduim guajava* and *Citrus aurantifolia* have been reported (5-8). These plants may be an insignificant number compared to many other plants that have been used as sources of antimicrobial agents in different parts of the world especially in the tropics. The potential use of many other plants therefore has remained largely undiscovered (9).

Ceiba pentandra (Bombacaleae) commonly called Kapok tree or silk cotton (English), Rimi (Hausa, Nigeria) Vamber (Tiv, Nigeria), Kĭng (Yandang, Nigeria) is grown chiefly in Asia and Indonesia and is an important product of Java. The plant has a spreading crown, huge trunk and large palm shaped leaves. The fibre sometimes referred to as silk cotton is lustrous, yellowish brown in colour, and light in weight. The mature tree bears hundreds of pods, up to 15 cm (6 inches) long filled with fibrous seeds. The pods are either cut down or gathered when they fall are broken open with mallets. Individual fibres are 0.8-3.2 cm (0.3 to 1.25 inches) long, averaging (0.7 inches) with diameters of 30 to 36 μm and are moisture-resistant, quick drying-resistant and buoyant. The plant is known to contain isoflavones and glycosides, lignin cellulose, and carbohydrate. The seeds may be processed to obtain oil for soap manufacture, and the residue used as fertilizer and cattle feed. The stem bark of *Ceiba pentandra*, is used locally as myriad of effects on medical conditions such as treatment of wounds, cough, high blood pressures and tumours. It is therefore interesting to investigate the antimicrobial activity of the stem bark extracts of the plant against some pathogenic bacteria.

Collection and preparation of plant samples

The stem bark of *Ceiba pentandra* was obtained from Golf training field along Federal University of Technology-Mubi road Adamawa State. The plant was subsequently identified by Mr. Bashir Pola of the Botany programme, Biological Sciences Department, Federal University of Technology, Yola.

The plant bark was shade-dried to constant weight for 5-7 days. The dried samples were grounded and sieved in order to obtain the powdered form. This was done to enhance the penetration of the extracting solvents, thus facilitating the release of active constituents. The powdered sample was kept in closed bottles under dried condition for further use (10).

Extraction of phytoconstituents and preliminary screening of plant extracts for phytochemicals

Ten grammes of grounded dried samples of the bark of the plant were soaked separately in 250 ml of distilled water and ethanol contained in a 500 ml capacity conical flask. The flask was plugged with cotton wool wrapped in aluminum foil, shaken vigorously and allowed to stand in the refrigerator for 24 h. The extracts were evaporated to dryness (semi-solid) and stored in the refrigerator in reagent bottles for further use.

Presence of phytoconstituents was screened using the methods described elsewhere (11). The plant extracts were screended for tannins, saponnins, phenolics, alkaloids, glycosides, anthraquinones, flavonoids and steroids.

Test for saponins
Two grammes (2 g) of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtered sample was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously to observe for the formation of emulsion.

Test for Tannins and Phenolics
Zero point five grammes (0.5 g) of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. 1 ml of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for alkaloids
To test for alkaloids, 1 ml of the aqueous plant extracts was treated with 2 ml of picric acid solution in a test tube. The formation of orange coloration indicated the presence of alkaloids in the extract.

Test for glycosides
Into two different beakers was added 1 g each of coarsely powdered plant material. To one of the beakers was added Sulphuric acid (5 ml) and to the other equal volume of water was added. The two beakers were heated for 3 min and the contents were filtered into labeled test tubes, the filtrate was made alkaline with 1 ml of Sodium Hydroxide and then heated with 5 ml of Fehling's solution for 3 min. The presence of reddish-brown precipitate was an indication of the presenceof glycosides in the extract.

Test for anthraquinones
For anthraquinones, 3 g of the powdered sample was soaked into 10 ml of benzene in a conical flask. The mixture was allowed to stand for 10 min then filtered. 5 ml of 10% ammonia solution was added to the filtrate and shaken for 30 sec. Pink, red or violet colour in the ammonia phase indicated the presence of anthraquinones in the extracts.

Test for flavonoids
In this method, 5 ml dilute ammonia solution was added to 5 ml of the aqueous filtrate followed by addition of 2-3 drops of conc. H_2So_4. The formation of a yellow coloration in the extract was an indication of the presence of flavonoids.

Test for steroids
To test for steroids, 2 ml of acetic anhydride was added to 0.5 g of ethanol extracts of the sample with 2 ml H_2So_4. Colour change from violet to blue or green indicated the presence of steroids.

Test organisms

Four bacterial isolates namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeroginosa* and *Shigella dysentriae* and two fungal isolates namely *Aspergillus flavus* and *Candida albicans*, were obtained from the Microbiology Laboratory of the Federal Medical Centre, Yola and taken to the Microbiology Laboratory of the Department of Microbiology, Federal University of Technology, Yola. The bacteria and *Candida albicans* were maintained on Nutrient Agar (NA, Sigma UK) slants, while *Aspergillus flavus* was maintained on Sabouraud Dextrose Agar (SDA, Sigma UK)) slants. For this work all the isolates were purified by subculturing into NA (for bacteria) and SDA (for fungi) 2-3 times (12).

Antimicrobial susceptibility testing

The cup plate agar diffusion method as earlier described (13), was used for this purpose. Three holes were bored on the surface of prepared Mueller Hinton agar (MHA, Oxoid, New York) plates (for *Candida albicans* and bacteria) and SDA, (Sigma UK) (for fungi) and seeded with the test organisms (0.5 McFarland turbidity standard for bacteria and *Candida albicans* and 10^5 spores/ml for fungi) using 6.0 mm cork borer at the intervals of 4.0 cm. 0.1 mm of 200 mg/ml of the extracts was placed into each of the holes with the exception of one hole in which glycerol was used instead of the extract to serve as negative control and two other holes with 25 µg/ml of cotrimoxazole and 50 µg/ml griseofulvin to serve as positive controls for bacteria and fungi respectively. The plates were then incubated at 37^{o}C for 24 h (*Candida albicans* and bacteria) and at room temperature for 2-3 days (fungi). After incubation antimicrobial activity was determined by measurement of zone diameter of inhibition (mm) against the test organisms.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extracts

The MIC of the extracts against the test organisms was determined using the broth dilution method using nutrient broth (NB) for bacteria and Sabroud Dextrose Broth (SDB) for fungi (14). Briefly, 1.0 ml of the extract solution at concentrations of 200 mg/ml was added to 1 ml of NB or SDB to obtain extract concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml in different test tubes. 1 ml of an 18 h culture adjusted to 0.5 McFarland turbidity standard (1.0 x 10^8 cfu/ml) was inoculated in each test tube. It was mixed thoroughly on a vortex mixer. The tubes were then incubated at 37^{o}C for 24 h (*Candida albicans* and bacteria) and at room temperature for 2-3 days (fungi). After incubation, the tube with lowest dilution with no detectable growth was considered the MIC.

To obtain the MBC or MFC, two loopfuls of broth culture was taken from the tubes that showed no growth in the test tubes used for MIC determination and inoculated on to agar plates (NA for bacteria and *Candida albicans*, SDA for fungi). The plates were incubated at 37^{o}C for 24 h (for *Candida albicans* and bacteria) and at room temperature for 2-3 days (for fungi) and then observed for possible growth. Concentrations that did not show any visible growth on the agar plates after incubation were regarded as MBC or MFC as the case may be (15).

Effect of pH and temperature on antimicrobial activity of plant extract

This was determined as earlier described (15) but with slight modifications. 2.0 g each of powdered plant extracts was dissolved in 6 ml of distilled water and filtered. 2 ml of the suspension was pipetted into four different test tubes and treated at 4°C in the refrigerator and 30 (ambient), 60 and 100°C in a water bath respectively for 1 h and tested for antimicrobial activity. To determine the effect of pH, the extract was treated at pH ranges of 3.0 and 10 using 1N HCl and 1N NaOH solutions respectively in a series of test tubes for 1 h. After 1 h of treatment, each of the extracts was neutralized (pH 7) once again using 1 N HCl and 1 N NaOH as the case may be and then tested for antimicrobial activity. Control tubes containing untreated extracts in each case were also incubated for 1 h and then tested for antimicrobial activity.

Results

Phytochemical screening of crude extracts of stem bark of *ceiba pentendra*

The result of phytochemical screening of the stem bark of *Ceiba pentandra* are shown in Table 1. The stem bark contained Anthraquinones, saponins, glycosides, tannins, phenolics, alkaloids and flavonoids.

Table 1. Phytochemical constituents of ethanol stem bark extracts of *Ceiba pentandra*

Chemical constituents	Aqueous extracts	Ethanol extracts
Anthraquinones	-	+
Saponins	-	+
Glycosides	-	+
Tannins	+	+
Phenolics	+	+
Alkaloids	+	+
Flavonoids	-	+
Steroids	-	-

Key : + = present; - = absent

Antimicrobial activity of plant extracts on test bacteria

Table 2 shows the results of the antimicrobial susceptibility testing of the extracts against the test organisms. Results revealed that water and the ethanol extracts showed higher activities than the aqueous extracts. The activity of the ethanol stem bark extracts against the test organisms were 18 mm (zone diameter of inhibition) against *Shigella dysenteriae,* 17 mm against *Escherichia coli*, 16 mm against *Pseudomonas aeroginosa* and 14 mm against *Staphylococcus aureus* followed by that of aqueous extracts 12 mm (zone diameter of inhibition) against *Pseudomonas aeroginosa,* 11 mm against *Shigella dysentriae* and 10 mm in each case against *Escherichia coli* and *Staphylococcus aureus* respectively.

For antifungal activity, only the ethanol extracts demonstrated activity against *Candida albicans* (8 mm zone diameter of inhibition) and *Aspergillus flavus* (5 mm zone diameter of inhibition). The aqueous extracts did not show any activity against both the test fungi (Table 2).

Effects of pH and temperature on the antimicrobial potentials of stem bark extracts of *Ceiba pentandra*

Results of the effects of pH and temperature on the antimicrobial potentials of stem bark extracts of *Ceiba pentandra* is shown in Table 2. Results revealed that the activity of the extracts increased as the pH treatment was adjusted towards acidity and the temperature increased. At a pH of 4.2 (untreated) the activity of the extracts against *Escherichia coli*, and *Pseudomonas aeroginosa* were 17 and 16 mm respectively, but this increased to 33 and 30 mm respectively as the pH was adjusted to 2 and at room temperature (untreated) the initial activity of the extracts (17 and 16 mm respectively) increased to 20 and 18 mm respectively with increase in temperature to 60ºC, and further to 24 and 22 mm respectively as the temperature was once again increased to 100ºC. A similar response was recorded with the fungal isolates.

Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extracts

Results of MIC, MBC and MFC are shown in Table 3. Results showed that the MIC and MBC values for the test bacteria ranged between 6.25-50 mg/ml while the MIC and MFC values for the test fungi ranged between 50-100 mg/ml.

Table 2. Antimicrobial activity of stem bark extracts of *Ceiba pentandra* (200 mg/ml) against the test organisms.

Test Organisms	Zone diameter of inhibition (mm)		Effect of pH and temperature on antimicrobial activity of extracts						
			pH			Temperature (ºC)			
	AE	EE	*4.2	2.0	10.0	*30	4	60	100
Escherichia coli	10	17	17	33	25	17	17	20	24
Pseudomonas aeroginosa	12	16	16	30	25	16	16	18	22
Staphylococcus aureus	10	15	15	20	16	15	15	15	17
Shigella dysentriae	11	18	18	27	24	18	18	18	20
Aspergillus flavus	-	5	5	23	16	5	5	5	7
Candida albicans	-	6	6	26	19	6	6	6	10

Key: - = no measurable zone inhibition; AE = acetone extracts; EE = ethanol extracts

Table 3. Minimum inhibitory concentration (MIC mg/ml), minimum bactericidal concentration (MBC mg/ml) and minmum fungicidal concentration (MFC mg/ml) of ethanol stem bark extracts of *Ceiba pentandra* against the test organisms

Organisms	MIC (mg/ml)					MBC (mg/ml)					MFC (mg/ml)					*Cot	**Grv	Gly
	6.25	12.5	25.5	50	100	6.25	12.5	25.5	50	100	6.25	12.5	25.5	50	100			
Escherichia coli	+	+	-	-	-	+	+	+	-	-	x	x	x	x	x	-	-	+
Pseudomonas aeroginosa	-	-	-	+	+	+	+	+	-	-	x	x	x	x	x	-	-	+
Staphylococcus aureus	-	-	-	-	+	+	+	+	+	-	x	x	x	x	x	-	-	+
Shigella dysentriae	-	-	-	-	-	-	-	-	-	-	x	x	x	x	x	-	-	+
Aspergillus flavus	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-	+
Candida albicans	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-	+

Key: - = no visible growth, + = visible growth; x = not applicable; *Cot = cotrimoxazole (both MIC & MBC); Grv = griseofulvin (both MIC/MFC)

Discussion

Results of this study revealed the presence of the phytoconstituents, anthraguinoues, saponins, glycosides, tannins phenolics, alkaloids and flavonoids in the ethanol extracts and tannins, alkaloids and phenolics only in the aqueous extracts of *Ceiba pentandra*. Several plants which are rich in alkaloids, tannins and glycosides have been shown to possess antimicrobial activity against a number of microorganisms (16-18). Ethanol extracts contained a higher number of phytoconstiutuents than the aqueous extracts. Different phytoconstituents in different solvents used for extraction of phytoconstituents of leaves of *Euzenia uniflora* was also reported earlier (16). The stem bark extracts demonstrated antimicrobial activity against both Gram-negative bacteria and fungi used in this study with the ethanol extracts demonstrating a higher activity. In this work, ethanol extracted more of the phytoconstituents than water, thus demonstrating higher activity. The differences in concentration of phytoconstituents in the different solvents is responsible for the differences in antimicrobial potency between the two solvents (19). Its been reported that different solvents have different ability of extracting phytoconstituents depending on their polarity (12). All the test bacteria and fungi were susceptible to the extracts. This is also

an indication that the plant contains antibiotic substances that have broad spectrum of activity including antifungal activity. This is actually a very significant discovery giving hope for the possible development of a novel antibiotic from this plant that can be effective in controlling multidrug resuistant bacteria and a variety of other microbial disease agents.
The test bacteria used for this study are associated with a variety of human and animal infections; *Esherichia coli* (urinary tract, dysentery and gastroenteritis) *Pseudomonas aeroginosa* (wound infections), *Staphylococcus aureus* (urinary tract infections) and *Shigella dysentriae* (shigellosis, gastroenteritis), *Candida albicans* (Candidiasis) and *Aspergillus flavus* (systemic mycosis and spergiloma) (20, 21). The activity of the extracts increased with increased acidity. This is also an indication that oral administration of any antibiotic developed from the plant may withstand inactivation by the acidic fluids (Hydrochloric acid and pancreatic juice) of the stomach. Results of MIC and MBC ranged between 6.25-50 mg/ml and that MIC and MFC ranged between 50-100 mg/ml. The low values recorded is an indication that when the active substances are further purified, can be very potent as antibiotics. The result also revealed that the plant possesses both bactericidal and bacteristatic activity. MIC, MBC/MFC values are measures of efficacy of any antimicrobial substance (22,23).

Conclusion

This study has shown *Ceiba pentandra* to posses antimicrobial property against *Staphylococcus aureus*, *Escherichi coli, Shigella dysentriae*, *Pseudomonas aeroginosa, Candida albicans* and *Aspergillus flavus*. It therefore suggests that constituents of the plant could serve as a source of individual drugs useful in the chemotherapy of microbial infections including urinary tract infections, gastroenteritis, dysentery, wound infections and some mycotic infections. It also justifies the traditional usage of this plant as health remedy for various medical ailments. The future prospect for effective utilization of this plant for the development of novel antibiotics will however depend on identification of the various chemical components of the phytoconstituents, purification of the components and the determination of their toxicity level with a view to establishing the biosafety of the plant as source of drug for human consumption.

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