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Assessment of antimicrobial possibilities of stem bark concentrates of *Cochlospermum planchoni*

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Abstract

Antimicrobial activity of root extracts of *Cochlospermum planchoni* against some pathogenic bacteria and fungi were investigated using the filter paper disc diffusion method. Phytochemical studies revealed the presence saponins, tannins, glycosides and aikaloid as phytochemicals. Methanol extracts (40 mg/ml) exhibited the highest activity (16 - 30 mm zone diameter of inhibition, MIC and MBC values 2.5 - 22.5 mg/ml) against the test organisms. Chloroform extracts demonstrated the least activity. The activity of the extracts increased with increase in temperature (4 - 100ºC) and increasing acidity (pH 2.5 - 6), but alkaline pH (pH 10) neither enhanced nor depreciated the activity of the extracts. The plant can be used to source newer antibiotic substances and can be used for the treatment of typhoid fever, dysentery, urinary tract and wound infections and mycotic infections.

Keywords: Antimicrobial activity, disc diffusion method, extracts, infections, pathogenic, phytochemicals.

INTRODUCTION

The investigation of traditionally used plants as a guide to biologically active extracts has been well documented (Cox, 1990). Complications of the use of antibiotics in the treatment of infections encouraged the search for effective medicinal plants as alternatives. Many of these plants have been used to cure many infectious diseases in many countries. They are available locally, inexpensive, and very popular (Odebiyi and Sofowora, 1987; Voravuthikunchai et al., 2008; Mann et al., 2008). In developing countries where antibiotics can be used on an unrestricted basis, the emergence of antibiotic resistant pathogenic bacteria has caused considerable public health concern. More recently resistance to new antimicrobial agents such as linezolid, quinupristin, and daffopristin has already occurred (Leclercq, 2002). The appearance of multi-drug resistant pathogens has threatened antimicrobial chemotherapy. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996; Scazzocchio et al., 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El- Seedi et al., 2002; Rojas et al., 2003; Duraipandiyan et al., 2006). The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. Besides, the more current and most effective antibiotics are very expensive and out of reach of many Africans, majority of whom reside in the rural areas. These antibiotics are also associated with some serious side effects (Adesokan et al., 2007).The investigation of certain indigenous plants may yield useful results. A large number of plants indeed were used to combat different diseases and known to posses antimicrobial activity (Arora and Kaur, 1999) . For example the crude methanol extracts of neem plant have been shown to have strong antibacterial activity (Okemo et al., 2001). Water extract of garlic and clove possesses antimicrobial activity. Some bacteria showing resistance to certain antibiotics were sensitive to extracts of both garlic and clove (Arora and Kaur, 1999). *Cochlospermum planchoni* Hook f.ex. (Cochlospermaceae) commonly called Yasubiyar, Bálágándéé (Hausa, Nigeria) and Avongongon (Jukun, Nigeria) is a shrub growing to about 2 - 4 m tall, leaves scabrid and tri-lobed with small stem, brownish stem bark and reddish-yellow leaves (Blench, 2007). The flowers have 5 yellow petals which produced globose

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fruits. The plant is found in waterlogged areas and is readily available in summer but hard to find during winter. *C*. *planchoni* has various medicinal applications in different parts of Africa. In Senegal, the plant is used in the treatment of jaundice, intestinal worms, bilharzias and hepatitis (Blench, 2007). In Ivory Coast the root is used to treat Schistosomiasis, jaundice, fever and back pains, while in Mali, powder or decoction of the macerated leaves and concoction of the roots and that of *Entada Africana* and *Erythrina senegalensis* are used for the treatment of malaria, neuralgic malaria, fever and jaundice. The fresh root of the plant is used as a concoction with fresh stem bark of *E. senegalensis* for the treatment of stomach disorder, typhoid fever and urinary tract infections (Togola et al., 2008). Although this plant has found wider applications traditionally, there is no documented information on its antimicrobial effects. The purpose of this work was to investigate the antimicrobial potentials of this plant species against some pathogenic microorganisms and to screen for the presence of phytochemicals.

MATERIALS AND METHOD

Test organisms

Clinical isolates of *Candida albicans*, *Staphylococcus aureus*, *Salmonella typhi*, and *Streptococcus pyogenes* were obtained from the Microbiology Laboratory of the Specialist Hospital Yola, Adamawa State, while laboratory isolates of *Aspergillus fumigatus*, *Aspergillus flavus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Microbiology Laboratory of the Department of Microbiology, Federal University of Technology Yola, Nigeria.

Collection of plant samples

Fresh roots of *C. planchoni* were collected from Avri ward in Wukari town, Wukari Local Government Area of Taraba State and were identified and authenticated by Mr. D. A. Jauro of the Forestry Department, Federal University of Technology Yola, Nigeria. The plant parts were chopped into pieces and shade dried at ambient condition to constant weight for five (5) days. The dried plant parts were coarsely pounded using pestle and mortar and then grinded into fine powder using an electric blender (El Astal et al., 2005).

Preparation of extracts

This was carried out as described by El Astal et al. (2005) with slight modification. 20 g of each powder were soaked separately in 100 ml of distilled water, chloroform, and methanol in different sets of 250 ml sterile conical flasks at ambient condition for 24 h. The mixtures were then filtered using a clean white cloth and then Whatman No 1 filter paper. The filtrates were then concentrated under vacuum at 40ºC.

Preliminary phytochemical analysis

Presence of phytochemical components in the extracts was determined as described by Sofowora (1993).

Assay of antimicrobial activity

This was carried out using the filter paper disc diffusion method as described by Voravuthikunchai et al. (2008). Sterile filter paper discs (1.5 mm in diameter) were sterilized by autoclaving and then soaked in 1 ml of different concentrations of extracts (so that each disc was impregnated with extract concentrations ranging from 2.5 - 50 mg/disc) and then allowed to dry. Filter paper discs soaked in sterile distilled water and allowed to dry were used as negative control, while paper discs soaked in ciprofloxacin (2.5 mg/disc) and griseofulvin (40 mg/disc) were used as positive controls. To test for susceptibility, 0.5 ml McFarland turbidity standard (for bacteria and *C. albicans*) or 10^6 spores/ml of fungi were seeded onto sterile Mueller Hinton agar (MHA) plates (for bacteria) and Potato Dextrose agar (PDA) plates (for fungi) and spread out using sterile glass rod in order to achieve confluent growth. The plates were left at ambient temperature for 5 min to dry. The impregnated discs were then placed on the MHA or PDA plates earlier seeded with the different test organisms and then incubated at 37ºC for 24 h (for bacteria and *C. albicans*) and 48 h at ambient temperature (for fungi). Antimicrobial activity was determined by measurement the zone diameter of inhibition against the test organism.

Effect of pH and temperature on activity of extracts

This was carried out as previously described (Doughari and Sunday, 2008). Briefly, 20 mg/ml of extracts were re-dissolved in 1 ml distilled water in different sets of test tubes. After that, they were treated at 4ºC (by refrigeration) and heated at 30, 60, 80 and 100ºC in a water bath and then allowed to cool to room temperature. The various extracts were then impregnated onto filter paper discs (20 mg/ml per disc) and antimicrobial susceptibility against the test organisms was determined as described earlier. Filter paper discs impregnated with untreated extracts were used as control. For the effect of pH, 2 ml 20 mg/ml of extracts were soaked in 2 ml of dilute HCl or dilute NaOH as the case may be, adjusted at different pH ranges (2.5, 6.0, 8.5 and 10.0) for 30 min. The extracts were then neutralized (pH 7) using either dilute HCl or dilute NaOH as the case may be and later impregnated onto filter paper discs (20 mg/ml per disc) which were then used for determination of antimicrobial activity as described earlier. Paper discs impregnated with untreated extracts (pH 4.5) were used as control.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs and MBCs of the extracts were carried out in triplicates against the test organisms. This was determined at varying concentrations of 10, 20, 30, and 40 mg/ml. To obtain these concentrations, varying concentrations (1 ml) of the extracts containing double strength of the concentrations (20, 30, 60, and 80 mg/ml) were constituted in different sets of test tubes, and 1 ml of NB was added. After that, a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced. The procedure was repeated on the test organisms using the standard antibiotic ciprofloxacin (for bacteria) and griseofulvin (for fungi) . A set of test tubes containing NB only were seeded with the test organisms as described above to serve as control. The test tubes were then incubated at 37ºC for 24 h (for bacteria and *C. albicans*) and 48 h at ambient temperature (for fungi). The concentration that showed no visible growth of the test organism was taken as the minimum inhibitory concentration (MIC) (Doughari and Sunday, 2008). For MBC determination, a loopful of broth was collected from each set of test tubes in the MIC determination which did not show any visible growth and subcultured onto sterile MHA plates and PDA plates and incubated at 37ºC for 24 h (for bacteria and *C. albicans*) and 48 h at ambient temperature (for fungi). After incubation, the concentration at which no visible growth was seen was noted as the minimum bactericidal concentration.

Table 1. Phytochemical analysis of root extracts of *Cochlospermum planchoni*

Phytochemical components	Stem bark extracts
Saponins	+
Tannins	+
Glycosides	+
Phenols	-
Alkaloids	+
Cardiac glycosides	-

Key: + = Positive; - = Negative.

RESULTS

Phytochemical analysis of root extracts of *C* . *planchoni* showed the presence of saponins, tannins, glucosides and alkaloids as phytoconstituents (Table 1). Results of antibacterial activity, minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) are shown in Table 2. Results showed that methanol extracts (40 mg/ml) exhibited the highest activity (16 - 30 mm zone diameter of inhibition, MIC and MMC values 2.5 - 22.5 mg/ml) against the test organisms. Results also showed that *E*. *coli* (30 mm zone diameter of inhibition; MIC 5 mg/ml, MMC 7.5 mg/ml), *S*. *typhi* (28 mm zone diameter of inhibition; MIC and MMC 7.5 mg/ml), and *C*. *albicans* (26 mm zone diameter of inhibition; MIC 12.5 mg/ml, MMC 15 mg/ml) were the most susceptible. While, *S*. *pyogenes* (18 mm zone diameter of inhibition, MIC 20 mg/ml, MMC 22.5 mg/ml), *A*. *flavus* (16 mm zone diameter of inhibition, MIC and MMC values of 15 mg/ml) and *Penicillium notatum* MIC 17.5 mg/ml, MMC 20 mg/ml, 14 mm zone diameter of inhibition) were the least susceptible to the methanol extracts. Results of antimicrobial activity were also concentration dependent. Results also showed that the activity of the standard antibiotic chloramphenicol (for the bacteria) and gresiofulvin for the fungi were slightly higher than that of the extracts at 40 mg/ml. Ciprofloxacin exhibited remarkable activity on all the test bacteria with the highest activity against *S*. *typhi* (32 mm zone diameter of inhibition), *E. coli* (MIC and MMC 2.5 mg/ml) and *P*. *aeruginosa* (MIC and MMC 5 mg/ml). Gresiofulvin was only effective against *A*. *flavus* (20 mm zone diameter of inhibition, MIC and MMC 7.5 mg/ml) and *P*. *notatum* (14 mm zone diameter of inhibition, MIC 20 mg/ml and MMC 22.5 mg/ml). Table 3 shows the effect of pH and on the antimicrobial activity of methanol extracts of *C*. *planchoni*. Results showed that the activity of the extracts increased as the pH was adjusted towards acidity but there was no significant change at alkaline pH. For example at pH 4.5 (non treated) the activity of the extracts against *S*. *aureus* increased from 13 mm (zone diameter of inhibition) to 16 mm as the pH was adjusted to 2.5, and 6.0 and remained the same at pH 8.5, but slightly reduced to 15 mm at pH 10. A similar trend was observed for the fungal isolates. For the effect of temperature on the activity of the extracts (Table 4), results showed that the activity increased with increase in temperature. For instance at ambient temperature (30ºC), the activity of methanol extracts (40 mg/ml) against *S*. *aureus* was 13 mm (zone diameter of inhibition), but this increased to 16 mm as the temperature was increased to 60ºC and to 20 mm at 100ºC. A similar result was recorded for all other test organisms.

DISCUSSION

Phytochemical screening of the extracts in this study revealed that *C* . *planchoni* contained some active chemical compounds - saponins, tannins, glycosides, phenols and alkaloids. These chemical compound have antibacterial effects on some gram-positive and gram-negative bacteria (Parekh and Chanda, 2007; Okemo et al., 2001). Notably, both tannins and phenolics have been reported to posses antibacterial activity (Ahanjan et al., 2008). A review of the literature revealed that information on the antimicrobial potential of *C*. *planchoni* is lacking, thus varying concentrations of aqueous and organic extracts of stem bark of the plant was evaluated for antimicrobial potential. Results of this study revealed very significant antimicrobial activity with the extracts demonstrating broad spectrum of activity against both bacteria (*S*. *pyogenes*, *E*. *coli*, *S*. *typhi* and *S. aureus*) and fungi (*A*. *fumigatus*, *A*. *flavus*, *P*. *notatum* and *C*. *albicans*). The organisms used in this study are associated with various forms of infections. The bacteria are associated with infections including upper respiratory tract infections (*S*. *pyogenes*), gastrointestinal infections, dysentery and urinary tract infections (*E*. *coli*), typhoid fever (*S*. *typhi*) and urinary tract infections (*S*. *aureus*), while the fungi are associated with systemic mycosis and aflatoxin production (*A*. *flavus* and *A. fumigatus*) and candidiasis (*C*. *albicans*) (Prescott et al., 2002). The demonstration of activity against all these organisms had shown that *C*. *planchoni* can be used to source antibiotic substances for the development of antibiotics with broad spectrum of activity. Methanol extracts demonstrated the highest activity against some of the test organisms compared to chloroform and aqueous extracts. Different solvents have different polarities, hence different degrees of solubility for the various phytoconstituents (Majorie, 1999). Based on the limited spectrum of activity of the other extracts compared with the methanol extracts, it suggests that the active principle is more soluble in methanol than in the other solvents. Results showed that the activity of the plants increased with increase in temperature. The stability of root extracts of *Carica papaya* against some pathogenic bacteria was earlier reported (Doughari et al., 2007). The activity of the extracts increased at acidic pH (pH 2.5) but remained unaffected at alkaline pH (pH 10). Acid and alkaline treatment was carried out in order to

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Table 2. Antimicrobial activity of stem bark extracts (50 mg/ml) of *Cochlospermum planchoni*.

Test organisms	Zone of inhibition (mm)					Extracts (ME) (mg/ml)		Ciproxin		Grisiofulvin	
	AQ	CF	ME	Cip	Gris	MIC	MMC	MIC	MMC	MIC	MMC
Staphylococcus aureus (SA12MBFTY)	12.0	8.0	26.0	23.0	x	2.5	2.5	1.25	1.25	x	x
Streptococcus pyogenes (SP006MBFTY)	10.0	7.0	18.0	22.0	x	20.0	22.5	0.625	1.25	x	x
Escherichia coli (EC22MBFTY)	15.0	6.0	30.0	30.0	x	5.0	7.5	2.5	2.5	x	x
Salmonella typhi (ST007MBFTY)	18.0	18.0	28.0	32.0	x	7.5	7.5	2.5	5.0	x	x
Pseudomonas aeruginosa (PA 008 MBFTY)	18.0	10.0	26.0	30.0	x	10.0	10.0	5.0	5.0	x	x
Aspergillus flavus (KB03SC)	8.0	10.0	24.0	x	20.0	15.0	17.5	x	x	7.5	7.5
Aspergillus fumigatus (KB04SC)	10.0	8.0	16.0	x	-	15.0	15.0	x	x	40.0	40.0
Candida albicans (CA 006 MBFTY)	8.0	12.0	26.0	x	-	12.5	15.0	x	x	40.0	40.0
Penicillium notatum (PN 004 MBFTY)	10.0	8.0	14.0	x	8.0	17.5	20.0	x	x	20.0	22.5

AQ = aqueous extracts; CF = chloroform extracts; ME= methanol extracts; Cip = ciprofloxacin; Gris = griseofulvin; x = not measured; - = no measurable zone of inhibition; methanol extracts; MIC = minimum inhibitory concentration; MFC = minimum fungicidal concentration.

Table 3. Effect of pH on antimicrobial activity of methanol extracts of *Cochlospermum planchoni*

Test organisms	Zone of inhibition (mm)/pH				
	4.5*	2.5	6.0	8.5	10.0
Staphylococcus aureus (SA12MBFTY)	13.0	16.0	16.0	16.0	15.0
Streptococcus pyogenes (SP006MBFTY)	9.0	12.0	11.0	11.0	12.0
Escherichia coli (EC22MBFTY)	14.0	14.0	15.0	15.0	15.0
Salmonella typhi (ST007MBFTY)	13.0	14.0	14.0	14.0	14.0
Pseudomonas aeruginosa (PA 008 MBFTY)	13.0	14.0	14.0	14.0	14.0
Aspergillus flavus (KB03SC)	12.0	14.0	13.0	13.0	13.0
Aspergillus fumigatus (KB04SC)	8.0	10.0	10.0	10.0	10.0
Candida albicans (CA 006 MBFTY)	11.0	11.0	12.0	12.0	12.0
Penicillium notatum (PN 004 MBFTY)	7.0	9.0	9.0	9.0	9.0

slightly simulate the stomach and duodenal conditions and also to predict the condition under which the drug would be more effective, if it is to be formulated for commercial purposes and since the plant is also used for the treatment of stomach disorders traditionally. Application of antibiotics sourced from this plant was possible on all parts of the body since pH variation did not in any way affect the activity negatively. As the temperature treatment was increased, activity of the extract was increased. It is assumed that the increase in temperature increases the activity of the bioactive components of the extracts probably due to increased solubility. This explains why traditionnally, the extracts are still effective in remedying the ailment they are being used against; besides the extracts are taken while still warm ensuring effectiveness and consequently their continued

Table 4. Effect of temperature on the antimicrobial activity of methanol extracts of *Cochlospermum planchoni.*

Test organisms	Zone of inhibition (mm)/Temperature (ºC)				
	30*	4	60	80	100
Staphylococcus aureus (SA12MBFTY)	13.0	13.0	16.0	18.0	20.0
Streptococcus pyogenes (SP006MBFTY)	9.0	9.0	12.0	14.0	16
Escherichia coli (EC22MBFTY)	14.0	14.0	14.0	15.0	17.0
Salmonella typhi (ST007MBFTY)	13.0	13.0	14.0	15.0	16.0
Pseudomonas aeruginosa (PA 008 MBFTY)	13.0	13.0	14.0	17.0	18.0
Aspergillus flavus (KB03SC)	12.0	12.0	14.0	16.0	20.0
Aspergillus fumigatus (KB04SC)	8.0	8.0	10.0	12.0	20.0
Candida albicans (CA 006 MBFTY)	11.0	11.0	11.0	14.0	16.0
Penicillium notatum (PN 004 MBFTY)	7.0	7.0	9.0	11.0	12.0

*ambient temperature

usage in traditional medicine. Though results of MIC and MMC investigation showed that the plants demonstrated slightly low values (MIC and MMC values 2.5 - 22.5 mg/ml), but the values of ciproxin were consistently lower than those of the methanol extracts of the plant. This is not surprising since ciproxin is in a refined state and is a choice drug in the treatment of many bacterial infections including gastroenteritis, typhoid and urinary tract infections (Daniyan and Muhammad, 2008; Prescott et al., 2002). Demonstration of low MIC and MMC values by especially the ethanol extracts is an indication that the phytoconstituens of the plant have therapeutic properties and therefore justifies its traditional medicinal uses. The MIC of gresiofulvin was however consistently higher than those of the plant extracts and indica-tion that the test organisms might have started developing resistance mechanisms to this antifun-gal agent.

Demonstration of lower MIC values than those of the antifungal agent by the plant extracts is an indication that when refined, very effective anti-fungal agents can be developed from *C*. *plan- choni*. This showed high efficacy of the plant against the test organisms since the values were used to determine the efficacy of an antimicrobial agent (Prescott et al., 2002).

Conclusion

Demonstration of antimicrobial activity by *C. plan-choni* in this study gives the basis for its traditional application as health remedy against diarrhea, wound and unitary tract infections and confirms the potential for sourcing antimicrobial substances for drug development against these infections and others such as typhoid fever and mycotic infections. Further work should be carried out to determine the antimicrobial activity of these plants against a wider range of bacteria and fungi. In addition, purification and toxicological studies should be carried out to determine its safety for h-man consumption.

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