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Phytochemical Screening and Antimicrobial Evaluation of Stem Bark Extract of *Pseudocedrela kotschyi* (Schweinf.) Herms

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMA carried out the extraction and partition of the plant material, phytochemical analysis and drafted the manuscript. Author IM participated in the phytochemical analysis. Author MIA performed antimicrobial evaluation. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study was design to screen the stem bark extract of *Pseudocedrela kotschyi* for the presence of phytochemical constituents and evaluate the extract for antimicrobial activity on wide range of pathogenic bacteria and fungi species.
Place and Duration of Study: Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University Sokoto and Laboratory of Pharmaceutical Microbiology, Ahmadu Bello University Zaria, between April 2013 and Oct 2013.
Methodology: Plant material was extracted with methanol and phytochemical screening carried out. Sequentially, the methanol extract was partitioned against chloroform, ethyl acetate and *n*-butanol to afford chloroform, ethyl acetate and *n*-butanol soluble fractions respectively. All fractions were evaluated against panel of pathogenic bacteria and fungi to include Methicillin Resistant *Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Candida albicans, Candida krusei and Candida tropicalis.*
Results: Phytochemical screening of the extracts revealed the presence of saponins,

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flavonoids, tannins, glycosides, anthraquinones, steroids/terpenes as well as alkaloids. The susceptibility test of the fractions at 30mg/ml have displayed activity against *S. aureus*, *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa, C. albicans, C. krusei* and *C. tropicalis* at zone of inhibition ranges between 20-28mm while the MIC and MBC/MFC results showed spectrum of antimicrobial activity ranges between 2.5-10mg/ml and 5-30 mg/ml respectively.
Conclusion: The activity of the extracts against *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa and C. albicans,* justify the traditional use of stem bark of *Pseudocedrela kotschyi* in the treatment of diarrhoea, dysentery and oral infection which are diseases commonly caused by these organisms.

Keywords: Pseudocedrela kotschyi; antimicrobial; phytochemical; Staphylococcus aurous; Escherichia coli; Candica albicans.

1. INTRODUCTION

The widespread use of antimicrobial agents in human and veterinary medicine have led to the emergence of antibiotic resistant pathogens, which result in an increasing need for new effective drugs. Herbal remedies used in traditional medicine provide a rich but yet an unexplored source of potentially new chemotherapeutic agents which might help to combat the growing problem of drug resistance and also the toxicity of currently available commercial antibiotics [1].

Pseudocedrela kotschyi (Schweinf.) Harms belong to the family Meliaceae. It is a common tree found in the savannah region of West Africa [2]. The roots, leaves and stem bark of *P. kotschyi* are used for various medicinal purposes in Nigeria. The leaves are used for the treatment of rheumatism and dysentery [3]. The root and stem bark are used for the treatment of malaria, dysentery, diarrhoea, worm infestation and oral infection [4-6]. *P. kotschyi* wood is also used as a chewing stick for dental cleaning [7].

Pseudocedrela kotschyi root extracts have been shown to inhibit the *in vitro* growth and development of the schizont stage of *Plasmodium falciparum* [8]. The leaves have been reported to have some antibacterial and antifungal activity [9]. The Aqueous stem bark extract was also investigated to have antiulcer activity [10]. Some of the chemical constituent of *P. kotschyi* reported includes limonoids, 7-desacetoxy-7- oxogedunin and pseudrelones A, B and C which displayed good antiprotozoal activity [11]. However, the widespread use of the stem bark extract for the management of bacterial and fungal infections suggests its broad spectrum of antimicrobial activity. Therefore, the aim of the present study is to investigate the antimicrobial activity of the stem bark extract of *P. kotschyi* on wide range of pathogenic bacteria and fungi species.

2. MATERIALS AND METHODS

2.1 Plant Materials

The stem bark of *P. kotschyi* was collected from Nasarawa area of Nasarawa state, North central Nigeria in June, 2012. The plant was identified with Voucher Number 900243 by Mallam Musa Muhammad at the Herbarium, Department of Biological Sciences, Ahmadu

Bello University, Zaria, Nigeria. The stem bark was air-dried, powdered and stored in polythene bags before use.

2.2 Extraction Procedure

Powdered stem bark (500g) was continuously extracted with 1.5L of methanol (75%) by maceration for 4 days; the extract was filtered and the filtrate dried *in vacuo* to afford a gummy dark brownish product. The procedure was repeated and the 2 extracts combined (39.76g). The crude methanol extract (30g) was suspended in 200ml of water and transferred into a 500ml separating funnel. It was then successively partitioned with chloroform (1:1), ethyl acetate (1:1) and *n*-butanol (1:1) to afford chloroform, ethyl acetate and *n*-butanol soluble fractions respectively. Each fraction was dried *in vacuo* and refrigerated at 4ºC prior to use.

2.3 Preliminary Phytochemical Analysis

The crude methanol extract was subjected to phytochemical screening for the presence of alkaloids, flavonoids, saponins, tannins and steroids/triterpenes, according to standard procedures [12].

2.4 Bioassay Studies

2.4.1 Test organisms

The organisms used were clinical isolates obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital, Zaria-Nigeria. All bacterial cultures were checked for purity and maintained in a blood agar slant while the fungus was maintained on a slant of Sabraud dextrose agar (SDA). The organisms tested include; Methicillin Resistant *staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium ulcerans, Bacillus subtilis, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Candida albicans, Candida krusei, Candida tropicalis.*

2.4.2 Susceptibility testing

The disc diffusion method [13] was used for the test. Filter paper discs (7mm in diameter) impregnated with sample solutions were placed on blood agar plates which had been inoculated with test organisms according to standard protocol described by National Committee of Clinical laboratory standards [14]. The extracts dissolved in their respective extraction solvents were tested at a concentration of 30mg/ml. The plates were incubated at 37ºC for 24h for the bacteria and at 25ºC for 48 h for the fungi, after which the diameters of the inhibition zones were measured and recorded in millimetres using a transparent ruler. Filter paper discs containing extraction solvents without any test extract served as control and no inhibition was observed. The reference antibacterial and antifungal drugs used as positive controls were sparfloxacin and fluconazole respectively. All experiments were carried out in duplicate and the results were consistent with zero standard deviation.

2.4.3 Determination of minimum inhibitory concentration (MIC)

Broth dilution method was used to determine the MIC values of the extracts that showed inhibitory effect on the test microorganism [15]. Nutrient broth was prepared according to the manufacturer's instructions. 2mls of the medium was dispensed in screw-capped test tubes and sterilized at 121ºC for 15 min. Mc-Farland's turbidity standard scale (0.5) was prepared by adding 9.95 ml of 1% H_2SO_4 and 0.05ml of 1% $BaCl_2$ to give a turbid solution. Ten ml sterile normal solution was used to make a turbid suspension of the micro-organism. Dilution of the organism suspension was done continuously using normal saline until the turbidity marched that of Mc-Farland's scale by visual comparison.

At that point, the concentration of the micro-organisms was about $1.5x10^8$ Cfu/ml. Two-fold serial dilution of the extract in the sterile broth was performed to obtain concentrations of 30, 15, 7.5, 3.75 and 1.875mg/ml of the crude methanolic extract and 20, 10, 5, 2.5 and 1.25mg/ml of the various fractions. 0.2ml of the micro-organism suspension was inoculated into the different concentration of the extract in test tubes. The tubes were incubated at 37ºC for 24h and at 25ºC for 48 h for bacteria and fungi respectively after which the plates were observed for growth. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each microorganism. All experiments were carried out in duplicate and the results were consistent with zero standard deviation.

2.4.4 Determination of minimum bactericidal concentration (MBC)

Blood agar plates were prepared according to the manufacturer's instructions. The contents of the MIC tubes and the following tubes in the serial dilution were sub-cultured into appropriately labelled blood agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the labelled blood agar plates. The plates were then incubated at 37ºC for 24h after which they were observed for growth. The MBC was the plate with the lowest concentration of the extract in serial dilution without growth.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The result of the preliminary phytochemical screening of the extracts revealed the presence of saponins, flavonoids, tannins, glycosides, anthraquinones, steroids/terpenes as well as alkaloids as shown in Table 1.

Table 1. Preliminary phytochemical tests on the crude methanol extract of stem bark extract of *P. kotschyi*

Phytoconstituents	Results
Tannins	+
Alkaloids	+
Anthraquinones	+
Saponins	+
Glycosides	+
Flavonoids	+
Triterpenoids	+

+present

3.2 Antimicrobial Activity

The results of susceptibility testing were as shown in Table 2 while that of MIC and the MBC/MFC have been summarized in Tables 3 and 4, respectively. The crude methanolic extracts and the three fractions at 30mg/ml were shown to have activity against *S. aureus*, *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa, C. albicans, C. krusei,* and *C. tropicalis* at zone of inhibition ranges between 20-28mm while the MIC and MBC/MFC results showed spectrum of antimicrobial activity ranges between 2.5-10mg/ml and 5-30mg/ml respectively.

Table 2. Susceptibility test of the crude methanol stem bark extract and different fractions of *P. kotschyi* against the test organisms (mm)

	Zone of inhibition (mm)					
Test organism	**MPK (30mg/ml)**	**CPK (30mg/ml)**	**EPK (30mg/ml)**	**NPK (30mg/ml)**	**SFN (0.05mg/ml)**	**FNL (0.05mg/ml)**
MRSA	-	-	-	-	27	-
S. aureus	24(0.00)	24(0.00)	26(0.50)	20(0.00)	26(0.00)	-
S. pyogenes	27(1.00)	23(0.00)	25(0.00)	21(0.00)	31(0.50)	-
C. ulcerans	-	-	-	-	29(0.00)	-
B. subtilis	-	-	-	-	27(0.00)	-
E. coli	24(0.00)	21(0.00)	24(0.50)	20(0.00)	26(0.50)	-
S. typhi	-	-	-	-	27(0.00)	-
S. dysenteriae	25(0.00)	27(0.00)	27(0.50)	22(0.00)	32(1.00)	-
S. pneumoniae	-	-	-	-	30(0.00)	-
P. aeruginosa	20(0.00)	25(0.00)	28(0.50)	20(0.50)	26(0.00)	-
N. gonorrhoeae	-	-	-	-	27(0.00)	-
C. albicans	(0.57)	24(0.00)	27(0.50)	21(0.00)	-	22(0.00)
C. krusei	21(0.00)	22(0.50)	24(0.50)	20(0.00)	-	27(0.00)
C. tropicalis	23(1.00)	24(0.00)	24(0.00)	20(0.00)	-	30(1.73)

- activity not detected, MRSA: Methicillin resistance Staphylococcus aureus, MPK: Crude methanol, CPK: Chloroform fraction, EPK: Ethylacetate fraction, BPK: n-Butanol fraction, SFN: Sparsfloxacin, FNL: Fluconazole

Table 3. Minimum inhibitory concentration (MIC) of crude methanol stem bark extract and different fractions of *P. kotschyi* against the test organisms (mg/ml)

	MIC (mg/ml)			
Test organism	**MPK**	**CPK**	**EPK**	**NPK**
S. aureus	7.5	5.0	2.5	5.0
S. pyogenes	3.75	5.0	2.5	5.0
E. coli	7.5	5.0	5.0	10.0
S. dysenteriae	7.5	2.5	2.5	5.0
P. aeruginosa	7.5	2.5	2.5	10.0
C. albicans	7.5	5.0	2.5	5.0
C. krusei	7.5	5.0	5.0	5.0
C. tropicalis	7.5	5.0	5.0	10.0

MIC: Minimum inhibitory concentration in mg/ml, MPK: Crude methanol, CPK: Chloroform fraction, EPK: Ethylacetate fraction, BPK: n-Butanol fraction

Table 4. Minimum bactericidal/fungicidal concentration of stem bark extract and different fractions of *P. kotschyi* the test organisms (mg/ml)

	MBC/MFC (mg/ml)			
Test organism	**MPK**	**CPK**	**EPK**	**NPK**
S. aureus	30.0	10.0	10.0	20.0
S. pyogenes	15.0	5.0	5.0	10.0
E. coli	15.0	20.0	10.0	20.0
S. dysenteriae	15.0	5.0	5.0	10.0
P. aeruginosa	30.0	10.0	5.0	20.0
C. albicans	15.0	10.0	5.0	20.0
C. krusei	30.0	10.0	10 .0	20.0
C. tropicalis	30.0	10.0	10.0	20.0

MBC/MFC: Minimum bactericidal concentration/ Minimum fungicidal concentration in mg/ml, MPK: Crude methanol, CPK: Chloroform fraction, EPK: Ethylacetate fraction, BPK: n-Butanol fraction

Similar phytochemical constituents have also been reported to be present in the leaves [16], roots [17] as well as the stem [18] extract of *P. kotschyi*. Both the crude methanol and the partitioned fractions showed broad spectrum activity against the tested bacteria and fungi. The ethyl acetate fraction showed the highest activity, particularly on *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa and C. albicans,* as indicated by the by susceptibility, MIC and MBC/MFC tests. This suggests that more of the bioactive chemical constituents were extracted by ethyl acetate during the partition separation. These compounds could probably be moderately polar compounds such as flavonoids. The extracts do not inhibit the growth of Methicillin-resistant *S. aureus*, *N. gonorrhoeae*, *C. ulcerans*, *B. subtilis*, *S. typhi* and *K. pneumonia* at the concentration tested; suggesting that this these organisms are resistant to the test extracts.

The activity of the extracts on *S. aureus* and *C. albicans* provides scientific explanation for the use of the stem bark *P. kotschyi* in the treatment of oral infections. *S. aureus* and *C. albicans* are associated with oral infectious diseases such as cheilitis [19], parotitis [20-21], staphylococcal mucositis [22] and oral candidiasis [23]. Furthermore, the activity displayed by the extract against *S. dysenteriaei* and *E. coli* justify the ethnomedicinal use of the stem bark of *P. kotschyi* in the treatment of dysentery and diarrhoea. Adeniyi et al. [18] have reported a similar investigation where he tested the stems of *P. kotschyi* against 7 clinical strains *Streptococcus mutants*, *S. aureus*, *C. albicans*, *C. tropicalis* as well as *C. krusei*. The stems extract was reported to show no activity on all the tested isolates except on *C. krusei* with MIC of 6.25mg/ml. The apparent differences may be as a result of different plant material used for the investigations. Similar antimicrobial activity have also been reported in the leaves extract of *P. kotschyi* against *S. aureus*, *S. typhi*, *S. pyogenes*, *C. albicans* as well as *E. coli* [9]. The results of the antimicrobial activity reported showed that ethyl acetate fraction was more effective against all the test microorganisms having MIC of 10mg/ml. These results were however consistent with our present finding. In related report, the antimicrobial activity of chewing sticks of *P. kotschyi* was tested against *Streptococcus mutants*, *S. aureus*, *S. pyrogenes*, and *C. albicans* [24]. The test extract therefore show no activity on the entire tested microorganism which is consistent with the report by Adeniyi et al. [18] since both of them uses the same plant material.

Some major plant constituents known to have antimicrobial activities include phenolics, flavonoids, tannins, and terpenoids [25] also, are found to be present in the stem bark of *P.*

kotschyi. The presence of these plant secondary metabolites, however, might be responsible for the antimicrobial activity in the present investigation.

4. CONCLUSION

In conclusion, both the crude methanol and the partitioned fractions of *P. kotschyi* showed broad spectrum activity against the tested bacteria and fungi with ethyl acetate fraction having the highest activity, particularly on *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa and C. albicans*. The activity of the extracts against *S. pyogenes, E. coli, S. dysenteriae, P. aeruginosa and C. albicans,* justify the traditional use of stem bark of *Pseudocedrela kotschyi* in the treatment of diarrhoea, dysentery and oral infection which are diseases commonly caused by these organisms.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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