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Full Length Research Paper

Antimicrobial activity of extracts of leaves of *Pseudocedrela kotschyi* (Schweinf.) Harms

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The aim of the experiment was to investigate the phytochemical composition and antimicrobial activity of extracts of *Pseudocedrela kotschyi* (Schweinf.) Harms used in folklore medicine in order to authenticate some of its therapeutic claims. The antimicrobial activity of petroleum ether, ethyl acetate and methanol extracts of the leaves of *P. kotschyi* was investigated against *Staphylococcus aureus, Salmonella typhi, Streptococcus* pyogenes, *Candida albicans* and *Escherichia coli*, using agar diffusion technique. Carbohydrates, reducing sugars, glycosides, cardiac glycosides, saponins, flavonoids, alkaloids, steroids and tannins were present in the leaves of *P. kotschyi*. The results of the antimicrobial activity showed that the ethyl acetate extract was more effective against all the test microorganisms than the methanol extract. The petroleum ether extract was resistant to all the test microorganisms. The minimum inhibitory concentration (MIC) exhibited by the ethyl acetate extract against the microorganisms was 10 mg/ml, except for *S. typhi* and *C. albicans* that had the value of 20 mg/ml. The methanol extract was active only against *S aureus, S. typhi and E coli*, and the MIC value for the microorganisms was 20 mg/ml. The lowest value of minimum bactericidal concentration exhibited by the ethyl acetate extract was 20 mg/ml, and the value was obtained for *S. aureus S. pyogenes* and *E. coli*. In conclusion, the leaves of *P. kotschyi* are a potential source of novel antimicrobial agents.

Key words: *Pseudocedrela kotschyi,* leaves, extracts, phytochemical composition, antimicrobial activity.

INTRODUCTION

Pseudocedrela kotschyi (Schweinf.) Harms belongs to the Meliaceae family. It is widespread in savannah woodland (Hutchinson and Dalziel, 1958; Shahina, 1989). *P. kotschyi* is a tree of up to 20 metres high with a wide crown, fissured bark and fragrant white flowers (Shahina, 1989). The bark is bitter and exudes a dark-coloured gum. The root bark of *P. kotschyi* is used in Togo as a febrifuge and in the treatment of gastro-intestinal diseases and rheumatism (Hutchinson and Dalziel, 1958). In Ghana, the twigs and leaves are of value in the treatment of malaria and stomach aches (Asase et al., 2005). The decoction is used as a wash for ulcers (Hutchinson and Dalziel, 1958; Oliver-Bever, 1986). In Nigeria, the roots and leaves are used to treat rheumatism and dysentery. In Northern Nigeria, the plant serves as an occasional ingredient for use in arrow poison (Oliver-Bever, 1986). In West Africa, it has been established that the root of *P. kotschyi* is widely used as chewing sticks for dental cleaning (Akande and Hayashi, 1998; Tapsoba and Deschamps, 2006; Okunade et al., 2007; Kassim et al., 2009); and in North Côte d'Ivoire, it is of value in the treatment of toothache and internal wound. The root of the plant, which is also used to treat intestinal helminthosis, has been found to be a potential source of antibacterial agents (Koné et al., 2004). The stem and root barks of *P. kotschyi* contain essential oils, comprising exclusively sesquiterpenoids with very low antiradical and antioxidant activities (Boyom et al., 2004).

P. kotschyi root extracts have been shown to inhibit the *in vitro* growth and development of the schizont stage of *Plasmodium falciparium*, and may provide affordable means of treating malaria (Kassim et al., 2009). The bark of *P. kotschyi* contains a bitter non-nitrogenous principle,

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Abbreviations: MICs, Minimum inhibitory concentrations; **MBC,** minimum bactericidal concentration.

Table 1. Phytochemical composition of the extract of the leaves of *P. kotschyi*.

Phytochemical	Availability
Carbohydrates	+
Glycosides	+
Cardiac glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Anthracene derivatives	-
Alkaloids	+
Tannins	+

+ = Present; - = absent.

pseudocedrelin, demonstrated to possess piscidal activity (Oliver-Bever, 1986). Limonoids, 7-desacetoxy-7-oxogedunin and pseudrelones A, B and C were isolated from the wood oil (Ekong and Olagbemi, 1967; Taylor, 1979; Niven and Taylor, 1988). The n-butanol soluble portion of the ethanolic extract of the leaves of *P. kotschyi* has been shown to possess anti-nociceptive and anti-inflammatory activities in mice and rats, respectively (Musa et al., 2005). Aqueous leaf extract of the plant reduced the onset and the duration of the sleeping time, induced by pentobarbitone in rats. It increased the depression or sedation time followed by sleep (Anuka et al., 2005). The leaves of *P. kotschyi* contain 3-*O*-rhamnosides of myricetin and quercetin, and 3-*O*-glucosides (or galactosides) of these aglycones (Asase et al., 2008). There is paucity of information in the available literature on the antimicrobial activities of extracts of the leaves of *P. kotschyi*, used in traditional medicine to treat various infectious diseases (Asase et al., 2005).

The aim of the present study was to investigate the phytochemical composition and antimicrobial activity of the leaves of *P. kotschyi* which have been claimed to possess some ethno-medicinal uses.

MATERIALS AND METHODS

Plant materials

The leaves of *P. kotschyi* were collected from Samaru, Zaria (11° 10′N, 7° 38′E), located in the Northern Guinea Savannah zone of Nigeria, in September, 2009. The plant was identified with Voucher Number 900243 by Mallam Musa Abdullahi at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The leaves were air-dried, powdered and stored in polythene bags before use.

Extraction procedure

The powdered *P. kotschyi* leaves (250 g) were exhaustively extracted by Soxhlet apparatus using each of the following solvents: Petroleum ether (60 - 80°C), ethyl acetate and methanol. Each extract was concentrated and evaporated to dryness on a rotary evaporator.

Preliminary phytochemical analysis

The preliminary phytochemical screening of the powdered leaves of *P. kotschyi* for carbohydrates, glycosides, flavonoids, tannins, saponins, steroids and alkaloids was carried out according to standard laboratory procedures (Harbone, 1984; Silva et al., 1998).

Test microorganisms

Pure isolates of antimicrobial-resistant *Staphylococcus aureus, Salmonella typhi, Streptococcus pyogenes*, *Candida albicans* and *Escherichia coli* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Shika – Zaria, Nigeria.

Antimicrobial activity screening

The antimicrobial activity of different concentrations of the petroleum ether, ethyl acetate and methanol extracts was determined by modified agar-well diffusion method of Bauer et al. (1966) and Perez et al. (1990). Briefly, solutions of varying concentrations ranging from 2.5 - 40 mg/ml were prepared for each of the three extracts. Nutrient agar was prepared, sterilised and used as the growth medium for the microorganisms. Normal saline was used to prepare a turbid suspension of the microorganisms and the solution was diluted to the McFarlands scale (1.5×10^8 cfu/ml). The medium was seeded with the test microorganisms and the inoculum was spread evenly over the surface of the medium on the plates using sterile swabs. A standard cork borer of 8 mm diameter was used to cut the wells on the surface of the medium and the extracts were introduced into the wells. The plates were incubated at 37°C for 24h and observed for zones of inhibition of growth, produced by the minimum inhibitory concentrations (MICs). The diameters of the zones of inhibition were measured and recorded in mm.

The minimum bactericidal concentration (MBC) test was carried out as described by Kirven and Thornsberry (1978) in order to determine whether the extracts induced the death or only the inhibition of growth of the microorganisms. Briefly, nutrient agar plates were prepared according to the manufacturer's instructions, and sterilised at 121°C for 15 min. Thereafter, the medium was poured into plates, cooled and allowed to solidify. The contents of the MIC test-tubes in the serial dilution were sub-cultured into each labelled blood agar plate using sterile wire loop, and spread on the blood agar. The plates were incubated at 37°C for 24 h, after which they were observed for growth. The MBC was the value obtained in the plate with the lowest concentration of extract without growth. The MBC values were determined and recorded. A control experiment was also set up using only the extruding solvent for each of the test microorganisms.

RESULTS

The phytochemical analysis revealed the presence of carbohydrates, reducing sugars, glycosides, flavonoids, steroids, saponins, tannins and alkaloids (Table 1). The ethyl acetate extract was sensitive to all the test microorganisms, and it exhibited the highest activity against all the microorganisms tested (Table 2). Methanol extract was sensitive to *S. aureus, E. coli* and *S. typhi*, but

Table 2. Sensitivity screening of leaf extracts of *P. kotschyi* against some common pathogenic microorganisms.

Test organism	Sensitivity of leaf extracts to pathogens		
	Methanol extract	Petroleum ether extract	Ethyl acetate extract
S. aureus	S	R	S
S. typhi	S	R	S
S. pyogenes	R	R	S
E. *coli*	S	R	S
C. albicans	R	R	S

R, Resistant; S, sensitive.

Table 3. Zones of inhibition (mm) of the leaf extracts of *P. kotschyi* against some pathogenic microbes.

Test microorganism	Zone of inhibition (mm)	
	Methanol extract	Ethyl acetate extract
S. aureus	18	28
S. typhi	20	26
S. pyogenes	0	30
E. *coli*	15	27
C. albicans	0	25

resistant to *C. albicans* and *S. pyogenes*. The petroleum ether extract was completely resistant to all the test microorganisms (Table 2). The diameters of zones of inhibition, manifested by the methanol and ethyl acetate extracts ranged from 15 - 30 mm (Table 3). All the test microorganisms were sensitive to ethyl acetate extract of *P. kotschyi*. The ethyl acetate extract had the highest value of zone of inhibition of 30 mm, induced against *S. pyogenes*, while the lowest value of 25 mm was exhibited by the extract against *C. albicans*. The highest zone of inhibition induced by the methanol extract was 20 mm and it was against *S. typhi*, but the methanol extract did not manifest any zone of inhibition against *S. pyogenes* and *C. albicans* (Table 3). The MIC of methanol extract was found to be 20 mg/ml for *S. aureus, S. typhi* and *E. coli*, while that of ethyl acetate extract was 10 mg/ml for *S. aureus*, *S. pyogenes* and *E. coli* (Table 4). The MBC values of the extracts are shown in Table 5. Although the MBC of methanol extract was 40 mg/ml for *S. aureus* and *S. typhi,* that of E. coli was 20 mg/ml. For ethyl acetate extract, the MBC value obtained for *S. aureus, S. pyogenes* and *E. coli* was 20 mg/ml, while that of *S. typhi* and *C. albicans* was found to be 40 mg/ml.

DISCUSSION

The phytochemical constituents identified in the leaf extracts of *P. kotschyi* such as alkaloids, tannins, terpenoids and flavonoids, which are the plant secondary metabolites, have been established to be frequently responsible for the antimicrobial properties of most medicinal plants (Cowan, 1999; Esimone et al., 2003; Adejumobi et al., 2008). The result of the phytochemical composition was similar to that obtained from the extracts of the root and leaves of *P. kotschyi* (Otimenyin et al., 2004; Asase et al., 2008; Musa et al., 2008). Thus, the antimicrobial activity of the leaf extracts against the test microorganisms may be due to the presence of the above phytochemical components.

The results demonstrated that the petroleum ether extract was not sensitive to the pathogenic microorganisms. The methanol extract at the concentration of 20 mg/ml contained active principles that were sensitive to *S. aureus, S. typhi* and *E. coli*, and at this concentration did not exert any bactericidal effect. The findings demonstrated that the methanol extract was not active against these microorganisms, and may not be of any prophylactic and therapeutic value against *S. pyogenes* and *C. albicans*. The ethyl acetate extract was sensitive to all the test microorganisms, and thus showed that the extract contained potential antimicrobial agents, especially those that may be active against *S. aureus, S. pyogenes* and *E. coli*. On the overall, the results of the sensitivity test showed that the methanol extract contained less potent antimicrobial agents against *S. typhi* and *C. albicans*, when compared with the ethyl acetate extract.

The results of the MIC showed that the methanol extracts possessed active constituents with antibacterial effects against *S. typhi*, *S. aureus* and *E. coli*. However, the ethyl acetate extract with lower MIC value of 10 mg/ ml

Table 4. The minimum inhibitory concentration of extracts of the leaves of *P. kotschyi* against some common pathogenic microorganisms.

Test organism	Minimum inhibitory concentration of extract (mg/ml)									
	Methanol extract					Ethyl acetate extract				
	40	20	10	5	2.5	40	20	10	5	2.5
S. aureus	-	0*	+	++	+++	-	-	0*	+	++
S. typhi	-	0*	+	++	+++	-	0*	+	++	+++
S. pyogenes	-	-	-	-	-	-	-	0*	+	++
E. coli	-	0*	+	++	+++	-	-	0*	+	++
C. albicans	-	-	-	-	-	-	0*	+	++	+++

0* = Minimum inhibitory concentration; + = scanty growth; ++ = moderate growth; +++ = dense growth; - = no growth.

Table 5. The minimum bactericidal concentration of extracts of the leaves of *P. kotschyi* against some pathogenic microorganisms.

Test organism	Minimum bactericidal concentration of extract (mg/ml)									
	Methanol extract					Ethyl acetate extract				
	40	20	10	5	2.5	40	20	10	5	2.5
S. aureus	0*	+	++	+++	+++	-	0*	+	++	+++
S. typhi	0*	+	++	+++	+++	0*	+	++	+++	+++
S. pyogenes	-	-	-	-	-	-	0*	+	++	+++
E. coli	-	0*	+	++	+++	-	0*	+	++	+++
C. albicans	-	-	-	-	-	0*	+	++	+++	+++

0* = Minimum bactericidal concentration; + = scanty growth; ++ = moderate growth; +++ = dense growth; - = no growth.

for *S. aureus*, *S. pyogenes* and *E. coli* may possess active principles with a more potent antibacterial effects than the methanol extract of *P. kotschyi*. This finding requires further investigations, involving bioassay-guided isolation, identification and characterisation of the active constituents contained in the extracts, especially in the ethyl acetate extracts of the leaves of *P. kotschyi*.

The results of the antimicrobial activity of the extracts of the leaves of *P. kotschyi* obtained in the present study agreed with the previous reports that the root of the plant is often used as chewing sticks for oral cleaning and is active against tooth-decaying bacteria, *S. aureus* and *S. auricularis* (Akande and Hayashi, 1998). Since the leaves, which are more readily accessible than the roots of *P. kotschyi,* also contain active agents against the tested pathogenic microorganisms, they could be used to treat some microbial diseases in the absence of the root of this plant.

The results obtained in the present study justify for the first time the use of the extracts of *P. kotschyi* leaves in traditional medicine for the treatment of microbial diseases, especially those caused by *S. aureus, S. pyogenes, E. coli*, *S. typhi* and *C. albicans*. Further studies are required to isolate and characterise the active constituents contained in the leaf extracts of *P. kotschyi*, which are involved in the antimicrobial activity.

In conclusion, the ethyl acetate and methanol extracts of the leaves of *P. kotschyi* possess antimicrobial activities, and the use of the leaves of the plant in ethnomedicine is thus authenticated.

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