African Journal of Microbiology Research Vol. 5(26), pp. 4562-4568, 16 November, 2011
Available online at http://www.academicjournals.org/AJMR
ISSN 1996-0808 ©2011 Academic Journals
DOI: 10.5897/AJMR11.597

Full Length Research Paper

Studies on the antibacterial activity and chemical constituents of *Khaya senegalensis* and *Ximenia americana* leaf extracts

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Accepted 13 September, 2011

Studies on the antibacterial activity and chemical constituents of the aqueous and methonolic leaf extracts of *Khaya senegalensis* and *Ximenia americana* were carried out using standard methods. Disc concentrations of 125, 250, 500 and 1000 µg/ml of the extracts were used against five bacterial isolates obtained from post-surgical wounds at the National Orthopaedic Hospital, Kano, Nigeria. Both extracts showed no activity against all the test bacteria. The minimum inhibitory concentration for both extracts was above 1000 µg/ml. Phytochemical screening showed the presence of saponins and absence of flavonoids in both the aqueous and methonolic extracts of *K. senegalensis*. Steroids and reducing sugars were found only in the methonolic extract while alkaloids and tannins were present only in the aqueous extract. In *X. americana*, flavonoids, steroids, tannins and reducing sugars were found in the methonolic extract while the aqueous extract revealed the presence of alkaloids, saponins and tannins. Although there was no activity of both extracts against the test bacteria, however, the presence of these chemical constituents signifies the potential of these plants as sources of therapeutic agents. This supports the traditional use of these plants in curing wound infections. It is therefore suggested that further studies be carried out using higher extract concentrations as well as to isolate, purify and identify the active compounds present in these extracts with a view to justifying these claims.

Key words: Phytochemistry, antibacterial activity, *Khaya senegalensis*, *Ximenia americana*, leaf extracts.

INTRODUCTION

The use of plants as source of remedies for the treatment of diseases dates back to prehistory and people of all continents have this old tradition. Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries are derived directly or indirectly from plants (Mathias et al., 2000; Newman et al., 2000). Human disease management in Nigerian history also provides evidence of the relationship of plants and medicine (Ayandele and Adebiyi, 2007). Sofowora (1984) projected the importance of medicinal plants and traditional medicine. However, research and development of medicinal plants have not advanced to the stage of impacting positively on the health system in Nigeria like other African countries (Odugbemi, 2008). Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites (Fatope and Adoum, 1993). The medicinal value of these secondary metabolites is due to the chemical substances that produce a definite pharmacological action on the human body (Chidambaram et al., 2003). According to Duke (1992), Evans (1996), Lawal et al. (2005), Magaji and Yaro (2006) as well as Kawo et al. (2009), phytochemical components are responsible for both pharmacological and toxic activities in plants. These metabolites are said to be useful to the plant itself but can be toxic to animals including man.

In developing countries, new drugs are not often affordable. Thus, up to 80% of the population use medicinal plants as remedies. WHO (1978) notes that of 119 plant-derived pharmaceutical medicines, about 74%

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Plate I. Leaves of *K. senegalensis.*

Plate II. Leaves of *X. americana.*

are used in modern medicines in ways that correlate directly with their traditional uses as plant medicines by native cultures. In Northern Nigeria, the Hausa and the Fulani tribes utilize *Khaya senegalensis* (Madachi in Hausa, Oganwa in Yoruba and Oni in Igbo) ethnomedicinally as a remedy for several human and animal ailments (Deeni and Sadiq, 2002). *Ximenia americana* (known as Tsada in Hausa) is extensively used among the Hausa/Fulani as herbal remedies for the treatment of malaria, leproutic ulcers and skin infections of mixed origin (Ogunleye and Ibitoye, 2003). The leaves have been reported to have antibacterial activity (Ogunleye and Ibitoye, 2003) and have also been reported by Arbonnier (2004) to be used in the treatment of fever, tuberculosis, stiffness, onchocerciasis tooth decay and wounds. It was due to these claims by traditional healers that this study was carried out in order to corroborate the use of these plants in treating wound infections.

MATERIALS AND METHODS

Collection and identification of plant materials

Samples of the two plants were collected from two different States in northern Nigeria. Leaves of *K. senegalensis* (Plate I) were collected from Gidan Dan-Hausa, Nassarawa local government area of Kano State while the leaves of *X. americana* (Plate II) were collected from Doko, Garki local government area of Jigawa State. The leaves were first identified by a traditional healer, after which they were further identified, confirmed and authenticated at the Department of Biological Sciences, Bayero University, Kano using standard keys (Sofowora, 1984). Voucher specimens (ZAS-2010A and ZAS-2010B) were deposited at the Departmental Herbarium for future reference.

Preparation of the treatment samples

The leaves were first washed thoroughly with water and then air dried at room temperature in the herbarium. The dried leaves were made into powder form using a clean mortar and pestle (Fatope and Adoum, 1993).

Extraction protocols

This was carried out in accordance with the method of Fatope and Adoum (1993). Fifty grams of the fine powder of the leaves of *K. senegalensis* was percolated with 500 ml of absolute methanol, while another 50 g was percolated separately with 500 ml of distilled water. The same procedure was carried out for the fine powdered leaves of *X. americana*. Each was allowed to stand at room temperature (25℃) for two weeks with intermitte nt shaking using the hands. The percolates were filtered using Whatman No.1 filter paper and the residue was discarded. The filtrates (methanol and water) were finally evaporated to dryness for the aqueous extracts while the methanolic extracts were left to air dry at room temperature (25℃) for seven days.

Phytochemical screening of the extracts

Two grams of each extract of the two plants were dissolved in 20 ml of their own mother solvents to obtain a stock concentration of 10% (v/v) (Kumar et al., 2009). The extracts thus obtained were subjected to phytochemical screening to determine the secondary metabolites present in accordance with the methods of Kumar et al. (2009), Brain and Turner (1975) as well as Ciulel (1994). The phytochemicals screened included the flavonoids, alkaloids, steroids, saponins, reducing sugars and tannins.

Collection of samples and study population/subjects

Wound swabs were collected from the patients presenting with post-operative wound infections at the National Orthopaedic Hospital, Dala, Kano, Nigeria. The patients included both adult males and females at inward level and a total of thirty eight patients were sampled and examined. The samples were collected using sterile cotton-tipped swab sticks using aseptic techniques to avoid any possible cross contamination. The Levine's technique of wound swabbing was followed by rotating the swab stick over one centimetre square with sufficient pressure to extract fluid from within the tissues (Levine et al., 1976). The samples were transported to the laboratory for processing with minimum delay. The samples were cultured aseptically on Blood agar, Chocolate agar and MacConkey agar media. The plates were incubated at 35 to 37℃ aerobically and anaerobically for 18 to 24 h. Plates were observed for growth and results recorded. Typical colonies were subjected to morphological characterization using Gram's staining after which biochemical tests were carried out for further identification (Chesebrough, 2000).

Cultural, morphological and biochemical characterization of the bacterial isolates

Five different species of bacteria namely: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* sp., *Streptococcus pneumoniae* and *Escherichia coli* were isolated. The isolates were cultured on Nutrient agar slants and stored in a refrigerator at a low temperature of 4 to 8℃ and sub-cultured every three ot her days in order to have fresh and young colonies until required for use (Chesebrough, 2000). The identities of the isolates were confirmed by subjecting them to Gram's staining and biochemical tests (catalase, coagulase, indole, oxidase and urease) using standard methods (Chesebrough, 2000).

Bioassay studies

Preparation of sensitivity discs

Whatman No. 1 filter paper discs of 6.0 mm were punched appropriately using a paper puncher. Fifty discs were each dispensed into 16 clean Bijou bottles and were then sterilized by autoclaving at 121℃ for 15 min (Chesebrough, 2000).

Preparation of extract concentrations

One gram each of the plant extracts was dissolved in 1 ml each of their diluents in Bijou bottles (distilled water for the aqueous extracts and dimethylsulfoxide (DMSO) for the methanolic extracts). This yielded a concentration of 1.0×10^6 µg/ml, which served as the stock solution. From the stock solution, four different extract concentrations were prepared (1000, 500, 250 and 125 µg/ml) using serial double dilution technique. From each of the four varied concentrations of the two fractions of each plant, 0.5 ml was dispensed into respective bijou bottles containing 50 discs each, such that each disc absorbed 0.01 ml of the extract, resulting in 1000, 500, 250 and 125 µg/disc (Bukar et al., 2009).

Standardization of inoculum

Using a sterile wire loop, a loopful of the test organism from an overnight culture on Mueller-Hinton agar was removed and emulsified in a test tube containing 3 ml of normal saline to prepare a standard inoculum that is equivalent to McFarland 0.5. One percent (1% v/v) of sulphuric acid was prepared by adding 1 ml of concentrated (H_2SO_4) into 99 ml of water. One percent (1% w/v) solution of barium chloride was also prepared by dissolving 0.5 g of dehydrated barium chloride in 50 ml distilled water. Barium chloride solution (0.6 ml) was added to 99.4 ml of sulphuric acid solution to yield 1.0% w/v barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison (Chesebrough, 2000).

Susceptibility testing

Disc diffusion method was used in accordance with the technique described by Kirby et al. (1966) to test for the susceptibility of the test organisms to the two plant extracts. Mueller-Hinton agar plates were prepared and then dried in the hot air oven to remove the excess surface moisture. The standardized bacterial inoculum was then used to inoculate the plates by streaking method under aseptic conditions. Impregnated filter paper discs containing the extracts of *K. senegalensis* and *X. americana* were evenly distributed in a radial manner on the inoculated plates using sterilized needle mounted in a holder. The discs were pressed firmly to ensure even contact with the inoculated surface and were sufficiently spaced out from the edge of plate and from disc to disc to avoid overlapping of zones. Standard antibiotic (Gentamicin: 10 mcg) was used as positive control and was also placed on the surface of the plates. The plates were allowed a pre-diffusion time of about 15 min before being incubated at 37℃ for 18 to 24 h. Using a pla stic ruler, zones of inhibition were then measured in mm and recorded.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined using the broth dilution technique described by Chesebrough (2000). Four varied extract concentrations (1000, 500, 250 and 125 µg/ml) were prepared from the stock solution with distilled water using serial double dilution. Sterile test tubes in batches were dispensed with the extract concentrations and Mueller-Hinton broth respectively and 0.1 ml of the suspensions of the standardized inocula of the test organisms were added and the tubes were incubated aerobically at 37℃ for 18 h together with the extract s controls and organism controls.

RESULTS AND DISCUSSION

Five species of bacteria were isolated from the 38 samples collected from patients presenting with post-surgical wound infections. The cultural, morphological and biochemical characteristics of the isolates are presented in Table 1. Table 2 presents the physical characteristics of the extracts. A yield of 7 and 5 g were recovered from the methanolic and aqueous extracts of *K. senegalensis* while 6 and 4 g were recovered from the methanolic and aqueous extracts of *X. americana*. The methanolic extract of *K. senegalensis* was green while the aqueous extract was dark brown and both extracts had a gummy texture. World Health Organization (1991) consultative group defined a medicinal plant as 'any plant which, in one or more of its organs, contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. Results of the phytochemical analysis of *K. senegalensis* (Table 3) indicated the presence of alkaloid, saponin, tannins, reducing sugars and steroid in one or both of the extracts. This was in agreement with a similar study done by Kubmarawa et al. (2008), which reported the presence of alkaloid, saponin and tannin. Olmo et al. (1997) also reported the presence of various phytochemicals in the leaf extracts of *K. senegalensis*. Some of the metabolites have been reported to be responsible for antimicrobial activity (Kumar et al., 2009).

Specifically, saponins have been reported to have antimicrobial effect (Mahato et al., 1988) and could serve as precursors of steroidal substances with a wide range of physiological activities (Madusolomuo et al., 1999). Phytochemical screening of *X. americana* (Table 3) indicated the presence of flavonoid, alkaloid, saponin, tannins, steroid and reducing sugars in one or both of the extracts. This was in accordance with similar work reported by Ogunleye and Ibitoye, (2003), which showed the presence of the previously mentioned phytochemical compounds. Malairajan et al. (2006) also reported the presence of tannin, flavonoid, saponin and alkaloid in the leaf extracts of *X. americana*. The variation in type of phytochemicals present in different solvents as shown in the result of phytochemical screening might be attributed to the ability of the solvent to dissolve into solution specific type of phytochemicals as reported by Kawo (2007), Yusha'u et al. (2008) and Kawo et al. (2009). Table 4 presents the results of antibacterial susceptibility test of the extract of *K. senegalensis*. It showed no activity against all the test organisms at all concentrations, which was a contradiction to similar works done by Kubmarawa et al. (2008) and Makut et al. (2008) who reported the extracts of *K. senegalensis* as having antimicrobial properties. Results of the antibacterial activity of the methanolic extracts (Table 5) of *X. americana* showed no activity at all concentrations. This was in contradiction with a similar work done by Gronhaug et al. (2008) in which extracts of different polarity and from different parts of the plant (*X. Americana*) were tested and found the methanolic and aqueous extracts to have antimicrobial effects. However, a zone of inhibition of 9 mm was observed with the aqueous extract against *S. aureus* at the highest concentration of 1000 µg/disc (Table 5). This was in conformity with the work done by Gronhaug et al. (2008), which further supports the report by Bibitha et al. (2002) that variations exist in the antibacterial activities of different plant extracts.

The apparent resistance of the test bacteria against these extracts at all the concentrations might be as a result of transfer of resistance plasmids or indiscriminate and sub-therapeutic use of the extracts (Kawo et al., 2009). Lack of sensitivity in the Gram-negative bacteria could be due to the outer phospholipids membrane with the structural lipo-polysaccharide components, which make their cell walls impenetrable to antimicrobial agents (Nikaido and Va'ara, 1985; Madigan and Martinko, 2006). Seasonal variations can affect the chemical composition of the plants and thus biological activity (WHO, 1991; 2003). The geographical location of a plant can affect its active constituents, which may be induced by many factors like climate, soil, propagation method, etc. Time of collection of plant parts also affect its effectiveness (Adoum et al., 1997; Odugbemi, 2008). All or a combination of the above mentioned factors could have contributed in the lack of antimicrobial activity observed in this study. Results of the minimum inhibitory concentrations (Tables 6 and 7) showed that the lowest extract required to inhibit bacterial growth was above 1000 µg/ml. The non-activity and high MICs of the plant extracts could also be due to high resistance rate of the test isolates.

CONCLUSIONS AND RECOMMENDATIONS

The preponderance of the phytochemicals screened in *K. senegalensis* and *X. americana* leaf extracts indicates their pharmacological importance and could justify the use of these plants in the treatment of some microbial infections as claimed by traditional herbalists. The results obtained in this study suggest that the extracts of these plants probably contain active agents and this explains

Table 1. Cultural, morphological and biochemical characteristics of the bacterial isolates.

Cultural characteristics	Gram	Cell type	Catalase	Coagulase	Indole	Oxidase	Urease	Possible organism
BA,CA: Small slightly raised cream colonies, MC: Tiny lactose ferm-enting colonies	+	Cocci	+	+	-	-	-	*S. aureus*
BA: Large flat spreading colonies with a zone of partial haemolysis, green pigmentation and distinctive smell. MA: Pale coloured colonies and discolouration.	-	Bacilli	-	-	-	+	-	*P. aeruginosa*
BA: Small translucent colonies with slightly raised edges. CA: Small whitish colonies with a zone of α-haemolysis	+	Diplococci	-	-	-	-	-	*S. pneumoniae*
BA: Swarming colonies with a distinctive 'fishy' odour, MA: Pale coloured colonies (non lactose fermenting).	-	Bacilli	-	-	-	-	+	*Proteus* sp.
BA: Large mucoid colonies, CA: Large smooth pink (lactose fermenting) Colonies	-	Bacilli	-	-	-	+	-	*E. coli*

BA = Blood agar; CA = Chocolate agar; MA = MacConkey agar.

Table 2. Physical characteristics of the leaf extracts of *K. senegalensis* and *X. americana*

S/No	Extracts	Initial weight (g)	Final weight (g)	Colour	Texture
1	KSME	50	7	Green	Gummy
2	KSAE	50	5	Dark-brown	Gummy
3	XAME	50	6	Green	Gummy
4	XAAE	50	4	Dark-brown	Gummy

KSME = *K. senegalensis m*ethanolic extract, KSAE = *K. senegalensis a*queous extract, XAME = *X. americana m*ethanolic extract, XAAE = *X. americana* aqueous extract.

Table 3. Phytochemical characteristics of the leaf extracts of *K. Senegalensis* and *X. americana* (values in brackets are for *X. americana*).

Chemical component	Methanolic extract	Aqueous extract
Flavonoids	- (+)	- (-)
Alkaloids	- (-)	+ (+)
Saponins	+ (+)	+ (+)
Tannins	- (+)	+ (+)
Reducing sugars	+ (+)	+ (+)
Steroids	+ (+)	- (-)

- = Absent; + = Present

Table 4. Antibacterial activity of the aqueous and methanolic leaf extracts of *K. senegalensis* (values in brackets are for methanolic extract).

Isolates	Extract concentration (µg/disc) / Diameter of zone of inhibition (mm)				
	125	250	500	1000	Gentamicin (10 mcg)
S. aureus	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
P. aeruginosa	00 (00)	00 (00)	00 (00)	00 (00)	22 (25)
S. pneumonia	00 (00)	00 (00)	00 (00)	00 (00)	40 (40)
Proteus spp.	00 (00)	00 (00)	00 (00)	00 (00)	13 (15)
E. coli	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)

Table 5. Antibacterial activity of the aqueous and methanolic leaf extracts of *X. americana* (values in brackets are for methanolic extract).

Isolates	Extract concentration (µg/disc) / Diameter of zone of inhibition (mm)				
	125	250	500	1000	Gentamicin (10 mcg)
S. aureus	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)
P. aeruginosa	00 (00)	00 (00)	00 (00)	00 (00)	28 (25)
S. pneumonia	00 (00)	00 (00)	00 (00)	00 (00)	45 (40)
Proteus spp.	00 (00)	00 (00)	00 (00)	00 (00)	13 (14)
E. coli	00 (00)	00 (00)	00 (00)	00 (00)	00 (00)

Table 6. Minimum inhibitory concentrations of the aqueous and methanolic leaf extracts of *K. senegalensis* (values in brackets are for methanolic extract).

Isolates	Concentration of extract (µg/ml)			
	125	250	500	1000
S. aureus	+ (+)	+ (+)	+ (+)	+ (+)
P. aeruginosa	+ (+)	+ (+)	+ (+)	+ (+)
S. pneumonia	+ (+)	+ (+)	+ (+)	+ (+)
Proteus spp.	+ (+)	+ (+)	+ (+)	+ (+)
E. coli	+ (+)	+ (+)	+ (+)	+ (+)

Table 7. Minimum inhibitory concentrations of the aqueous and methanolic leaf extracts of *X. americana* (values in brackets are for methanolic extract).

Isolates	Concentration of extract (µg/ml)			
	125	250	500	1000
S. aureus	+ (+)	+ (+)	+ (+)	+ (+)
P. aeruginosa	+ (+)	+ (+)	+ (+)	+ (+)
S. pneumonia	+ (+)	+ (+)	+ (+)	+ (+)
Proteus spp.	+ (+)	+ (+)	+ (+)	+ (+)
E. coli	+ (+)	+ (+)	+ (+)	+ (+)

the rationale for the use of the plant in treating wound infections in traditional medicine. However, it is recommended that further research be carried out in order to isolate and purify the bioactive constituents using various extraction solvents as isolation of pure compounds increases the microbial activity (Cowan, 1999). On the other hand, indiscriminate and non-medical use of antibiotics, plant decoctions and concoctions should be

minimized as this could contribute to the emergence of resistant bacterial strains.

ACKNOWLEDGEMENTS

Authors are grateful to the Management of the National Orthopaedic Hospital, Kano, Nigeria for granting us the permission to obtain the swab samples from the patients. Special thanks to all the staff whose cooperation has helped greatly in the success of this research.

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