

Full Length Research Paper

Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria

D. Kubmarawa^{1*}, M. E. Khan², A. M. Punah¹ and M. Hassan¹

¹Department of Chemistry, Federal University of Technology, P.M.B. 2076, Yola, Nigeria.

²Department of Chemistry, Gombe State University, Gombe, Nigeria.

Accepted 17 July, 2008

***Khaya senegalensis* has medicinal properties for the effective management of several ailments including diarrhea. To establish the pharmacological rationale for its traditional use, the powdered root, stem-bark and leaves were extracted with water and ethanol. All fractions were subjected to phytochemical screening and antimicrobial activity against gram-positive and gram-negative bacteria using the disc-diffusion method. The extracts contained saponins, tannins, phenols but no flavanoids, glycosides and resin. The stem-bark of the water fractions and the roots, stem-bark and leaves fractions of the ethanol fractions were active on *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Bacillus subtilis*. These findings support the claim for its treatment of bacterial and fungal infections.**

Keywords: *Khaya senegalensis*, antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Finding healing power in plants is an old idea. People world-over long applied poultices and imbibed infusions of thousands of indigenous plants dating to prehistory. Human disease management in Nigerian history also provides evidence of the relationship of plants and medicine ((Bannerman et al., 1986; Hammer, 1999; Raghavendra et al., 2006; Ayandele and Adebisi, 2007). The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites (Fatope et al., 2001). The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, glucosides, glycosides, steroids, flavanoids, fatty oils, phenols, resins, phosphorus and calcium for cell growth, replacement, and body building (Chidambara

et al., 2003).

In northern Nigeria, the Hausa and the Fulani tribes utilize *Khaya senegalensis* ethnomedicinally as a remedy for several human and animal ailments (Deeniand and Sadiq, 2002). According to Nacoulma/Ouédraogo (1996), the aqueous extract is taken against diarrhea, gynecologic disturbances, digestives disorders and nervous confusions. Biomolecules of plant origin appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis (Mathias et al., 2000; Ganguly et al., 2001; Martino et al., 2002).

The plant, *K. senegalensis* (Madachi in Hausa, Oganwa in Yoruba, and Ono in Igbo), is a savanna tree, easily recognized by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules. A tree of 30 m high and 3 m girth, with dense crown and short bole covered with dark grey scaly bark. Slash dark pink, bark bitter yielding gum when wounded. Leaves with 3 - 4 (exceptionally 5) pairs of leaflets, 5 - 10 cm long by 2.5 - 5 cm broad, more or less elliptic, round, obtuse or shortly acuminate at apex; stalks of leaflets 4 mm long (Irvine, 1961; Keay et al., 1989). It has attracted world-wide attention for its high quality timber production.

*Corresponding author. E-mail: dkubmarawa@yahoo.com.

It is highly used in the tropics (Arnold et al., 2004).

The stem-bark and leaves of *K. senegalensis* have been used in Adamawa State in Northern Nigeria in forms of decoction and concoctions for the cure of mucous diarrhea, syphilis, pyrexia, malarial fever (Olayinka et al., 1992). Dried stem-bark is used externally for the treatment of skin affections and blennorrhagia, and the fixed oil is used for the treatment of leprosy, syphilis, dermatomes, sores and ulcer in adults (Grand, 1989). The bark of the tree is very bitter and is extensively used for the treatment of fever and dressing ulcers on the backs of sheep, camels and horses. The flowers are used for treating stomach diseases, and as an ingredient in antisiphylitic prescriptions. The oil from the seeds is used as emmenagogue (Dalziel, 1956; Watt and Breyer-Brandwijk, 1962). Some limonoids have been isolated from the stems, barks, leaves and flowers of *K. senegalensis*. They include phragmalin limonoids named khayanolides D and E, khayanosides, 2,6-dihydrofissinolide and two mexicanolides named khayanone and 2-hydroxyseneganolide (Olmo et al., 1997; Khalid et al., 2002; Nakatani et al., 2001). Abdelgaleil et al. (2004) also reported the isolation of three other mexicanolide limonoids named seneganolide A, 2-hydroxyseneganolide A and 2-acetoxyseneganolide A. These limonoids have a wide range of biological activities, including insect antifeeding and growth-regulating properties, and medicinal activities in humans and animals. They also possess antiviral, antifungal and bactericidal properties (Abdelgaleil et al., 2001; Abdelgaleil and Nakatani, 2003; Ademola et al., 2004).

In most African cultures, it is believed that children suffer from severe diarrhea during teething. This does not have any scientific justification. But it could be inferred that this is a time when the children are exposed to several contaminated things as they crawl around and imbibe virtually everything. In Michika, Mubi North, and Konduga Local Government Areas of Adamawa and Borno States respectively, the stem-bark and roots of *K. senegalensis* are used (oral decoction) for the treatment of diarrhea in children (personal observation). This paper reports the anti-microbial activity of *K. senegalensis* especially against diarrhea.

MATERIALS AND METHODS

Sampling

Fresh sample of the roots, stem-bark and leaves of *K. Senegalensis* were collected in May 2006 from the North eastern States (Adamawa and Gombe) Nigeria. Identification was by the Forestry Department of Federal University of Technology Yola. The samples were air dried in the laboratory before powdering.

Extraction

180 g each of the powdered roots, stem-bark and leaves of the plant were percolated with 2 L of distilled ethanol for two-weeks.

After which there was decantation, filtration, and concentration on rotary evaporator (R110) at 40°C to obtain ethanol soluble fractions. The above procedure was repeated on 250 g each of the powdered roots, stem-bark and leaves of the plant with the use of 2 L of water.

Phytochemical screening

Phytochemical analysis of all the evaporated solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992). Tests for saponins, tannins, flavanoids, volatile oils, glycoside, alkaloids, phenols and resin were carried out in all the fractions.

Antibacterial assay

Six bacteria species: *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Bacillus subtilis* stock cultures were collected from the Specialist Hospital Yola. These organisms were identified in the Microbiology Department Federal University of Technology Yola. The stock were maintained on nutrient agar slant and subcultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing. Inoculation of the test organisms on nutrient agar-prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The discs were prepared using a what-man filter paper. 100 discs were obtained by punching and putting in vials-bottles and sterilizing in an oven at 150°C for 15 min. Prepared discs containing the various fractions were carefully placed on the inoculated plates using a sterilized forceps in each case. (Fatope and Adoum, 1993) The plates were then turned upside-down and incubated at 37°C for 24 h in an incubator.

The results was taken by considering the zone of growth and inhibition of the organisms by the test fractions (Mackie and McCartney, 1989). Activity and inactivity were observed in accordance with the standard and acceptable method (Table 2).

RESULTS AND DISCUSSION

Phytochemical screening shows that some of the natural products tested for were not present in the plant material, and these include flavanoids, glycosides and resin (Table 1). Volatile oils and alkaloids were only present in leaves and roots. Saponins, tannins and phenols were present in both water and ethanol extracts of all the parts. This shows the generality of the components in medicinal plants. Biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Mixtures of such chemicals show a broad spectrum of biological effects and pharmacological properties. To a large extent, the phonological age of the plant, percentage humidity of the harvested material, place and time of harvest, and the method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982).

Table 2 shows the zones of inhibition (mm) of the various plant parts against the microorganisms. From the table, the water fraction of the stem-bark, as well as the ethanol fractions of the roots, stem-bark and leaves are very efficacious, covering nearly the entire spectrum of

Table 1. Results of phytochemical analysis of extracts from roots, stem-bark and leaves of *K. Senegalensis*.

Chemical compounds	Water Extract			Ethanol Extract		
	Root	Stem-bark	Leaves	Root	Stem-bark	Leaves
Saponnins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavanoids	-	-	-	-	-	-
Volatile oils	-	-	+	-	-	-
Glycoside	-	-	-	-	-	-
Alkaloids	-	-	-	+	-	-
Phenols	+	+	+	+	+	+
Resin	-	-	-	-	-	-

- = Absent; + = present.

Table 2. Antimicrobial efficacy of different extracts of roots, stem-bark and leaves of *Khaya Senegalensis* against human pathogenic bacteria (zones of inhibition in mm).

Drug	Fraction		Microorganism					
			<i>S. aureus</i>	<i>Streptococcus spp.</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Salmonella spp.</i>	<i>B. subtilis</i>
<i>Khaya Senegalensis</i>	Water fraction	Roots	R	R	R	R	R	R
		S. Bark	14.2±0.2	12.2±0.2	14.3±0.4	16.5±0.1	15.5±0.3	10.4±0.1
		Leaves	R	R	R	R	R	R
	Ethanol fraction	Roots	06.2±0.4	14.4±0.4	16.3±0.5	16.2±0.0	12.2±0.1	16.4±0.2
		S. Bark	08.4±0.3	12.3±0.0	16.3±0.7	18.4±0.3	16.2±0.2	10.6±0.1
		Leaves	12.1±0.2	16.2±0.3	12.6±0.8	13.6±0.2	R	12.3±0.2
Gentamicin (control)			21.6±0.1	13.8±0.3	14.6±0.1	9.5±0.10	17.0±0.2	11.5±0.2

Values are mean of three trials ± standard error.
R = Resistance.

organisms. The organisms are resistant to the water extracts of the roots and leaves. The morphogenetic and phenotogenical variations of plants harvested at the vegetative, floral budding, full flowering, fresh fruiting and mature fruiting stages are factors that contribute the difference in activity (Cuneyt and Jolita, 2007).

This analysis suggests that, the water and the ethanol extracts of the roots, stem- bark and leaves of *K. Senegalensis* probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments. This assertion is also confirmed, as their extracts indicate a relatively moderate number of phytochemicals present. It is suggested that more research be conducted that will further elucidate and characterized the active components and their mechanism of action.

REFERENCES

- Abdelgaleil SA, Iwagawa T, Doe M, Nakatani M (2004). Antifungal limonoids from the fruits of *Khaya senegalensis*. *Fitoterapia* 77: 566-572.
- Abdelgaleil SA, Nakatani M (2003). Antifeeding activity of limonoids from *Khaya senegalensis*. *J. Appl. Entomol.* 127: 236-239.
- Abdelgaleil SA, Okamura H, Iwagawa T, Sato A, Miyahara I, Doe M, Nakatani M (2001). Khayanolides rearranged phragmalin limonoid antifeedants from *Khaya senegalensis*. *Tetrahedron* 57: 119-126.
- Ademola IO, Fagbemi BO, Idowu SO (2004). Evaluation of the antihelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.* 122: 151-164.
- Arnold R, Reilly D, Dickinson G, Jovanovic T (2004). Determining the climatic suitability of *K. senegalensis* for plantations in Australia. Working Paper presented at a Workshop on "Prospects for high-value hardwood timber plantations in the 'dry' tropics of northern Australia (Mareeba, Queensland, 19-21 October, 2004.) Published as CD-Rom by Private Forestry North Queensland Association Inc., Kairi, Queensland.
- Ayandele AA, Adebisi AO (2007). The phytochemical analysis and antimicrobial screening of extracts of *Olox subscorpioidea* Afr. *J. Biotechnol.* 6(7): 868-870
- Bannerman PC, Mirsky R, Jessen KR, Timpl R, Duance VC (1986). Light microscopic immunolocalization of laminin, type IV collagen, nidogen, heparin sulfate proteoglycan and fibronectin in the enteric nervous system of rat and guinea pig. *J. Neurocytol.* 15: 432-443.
- Chidambara K, Vanitha A, Mahadeva M, Ravishankar G (2003). Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. *J. Med. Food.* 6: 2.
- Cuneyt Cm, Jolita R (2007). Hypericins in *Hypericum montbreti* Variation among parts and phenological stages. *Med. Aromatic Plant sci. Biotechnol.* 1: 253-256.
- Dalziel JM (1956). *Useful Plants of West Tropical Africa*. Crown Agents for the Colonies, London, pp. 179-183.
- Deeniand YY, Sadiq NM (2002). Antimicrobial properties and phytoche-

- mical constituents of the leaves of African mistletoe (*Tapinanthus dodoneifolius* (DC) Danser) (Loranthaceae): an ethnomedicinal plant of Hausaland, Northern Nigeria. *J. Ethnopharmacol.* 83(3): 235-240.
- Fatope MO, Adoum OA (1993). Bioactivity of some savanna plants in the brine shrimp lethality test and in-vitro anti-microbial assay. *Int. J. pharmacog.* 35(5): 334-337.
- Fatope MO, Adoum OA, Takerda Y (2001). Palmitate Ximena Americana. *Pharmaceut. Biol.* 38(5): PP391-393.
- Felix MT (1982). "Medical Microbiology" Churchill Livingstone (Publishers), London. pp. 445-459.
- Ganguly R, Mishra P, Sharma A (2001). *Microbes infect. Ind. J. Microbiol.* 41: 211-213.
- Hammer K (1999). Antimicrobial activity of essential oils and other plant extracts *J. Appl. Microbiol.* 86: 985-990.
- Harborne JB (1992). *Phytochemical methods.* Chapman and Hall publications, London. pp. 7-8.
- Irvine FR (1961). *Woody Plants of Ghana (With Special Reference to Their Uses).* Oxford University, London, pp. 523-524.
- Keay RW, Onochie CF, Stanfield J (1989). *A Revised Version of Trees of Nigeria (1964).* Clarendon Press, New York. pp. 339-340.
- Khalid SA, Friedrichsen GM, Kharazmi A Theander TG, Olsen CE, Nakatani M, Abdelgaleil SA, Kassem SM, Takezaki K, Okamura H, Iwagawa T, Doe M (2002). Three new modified limonoids from *Khaya senegalensis*. *J. Nat. Prod.* 65: 196-216.
- Mackie R, McCartney C (1989). *Practical Medicinal Microbiology 3rd edition, Vol 2* Churchill Livingstone (Publishers), London and New York, pp. 100-106 121,141,163-167,303,432-491.
- Martino PD, Gagniere H, Berry H, Bret L (2002). Antimicrobial activity of extracts of some medicinal plants. *Microbes infect.* 4: 613-620.
- Mathias AJ, Somashekar RK, Sumithra S, Subramanya S (2000). Antimicrobial Agents, *Indian J. Microbiol.* 40: 183-190.
- Mshui EN (1976). Traditional Medicine research Unit Report for 1976. Muhimbili Medical centre, *Dar-Es-Salaam Nacoulma/Ouédraogo OG* (1996). *Plantes médicinales et pratiques médicinales traditionnelles au Burkina Faso : cas du plateau central. Thèse de doctorat ès-sciences. Université de Ouagadougou,* tomes II: 508-515.
- Nakatani M, Abdelgaleil SA, Kurawaki J, Okamura H, Iwagawa T, Doe M (2001). Antifeedant rings B and D opened limonoids from *Khaya senegalensis*. *J. Nat. Prod.* 64: 1261-1265.
- Olmo LR, Da Silva MF, Das GF, Fo ER, Vieira PC, Fernandes JB, Pinheiro AL, Vilela EF (1997). Limonoids from leaves of *Khaya senegalensis*. *Phytochemistry*, 44: 1157-1165.
- Raghavendra MP, Satish S, Raveesha KA (2006). Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. *J. Sci.* 1(1): 72-78.
- Watt JM, Breyer-Brandwijk MG (1962). *Medical and Poisonous Plants of Southern and Eastern Africa.* EXS Livingstone, London. pp. 1463-1469.