Full Length Research Paper

Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of *Khaya senegalensis*

Makut, M. D.*, Gyar, S. D., Pennap, G. R. I. and Anthony, P.

Microbiology Unit, Department of Biological Sciences, Nasarawa State University, P.M.B. 1022, Keffi, Nigeria.

Accepted 13 August, 2007

Khaya senegalensis, a member of the family Meliaceae, is a plant commonly used by the local people of Nasarawa State of Nigeria for the treatment of dysentery, mucous diarrhoea and wound infections. The leaves and the bark of the plant were screened for their phytochemical properties and antimicrobial activity. Ethanol was used for the extraction of the active compounds. The test organisms were *Staphylococcus aureus, Steptococcus faecalis, Escherichia coli* and *Candida albicans*. Results of the phytochemical screening showed that saponins, tannins, alkaloids, glycosides, steriods, terpenoids and flavonoids were the active compounds present in the leaves and bark of the plant. The antimicrobial susceptibility test showed that *S. aureus, S. feacalis* and *C. albicans* were susceptible to both the leaf and bark extracts, while *E. coli* was not. The extracts were also found to be bactericidal to *S. aureus* and *S. feacalis*, and fungicidal to *C. albicans*. This study demonstrates the potentials of *K. senegalensis* as a source of antimicrobials that could be harness for use in the Health Care Delivery process.

Key words: Khaya senegalensis, antimicrobial activity, phytochemical.

INTRODUCTION

Plants have been used since antiquity for shelter, fire wood and food. One of the earliest records of herbal medicine is the use of chanlonoogra oil from species of Hydrocapus guartin, which was known to be effective for the treatment of leprosy in China between 2730 and 300 BC (Le Strange, 1977). Sofowora (1984) defined medicinal plant as any plant in which one or more of its organs contain substance(s) that can be used for therapeutic purpose or as precursors for pharmaceutical synthesis. The use of plant and animals parts in medicine have since been widely documented in the records of ancient China, India and Egypt, and practice was based on series of "trail and error", which could not be substantiated by proven scientific theories. However, these practices have produces results of proven efficacies compared to the conventional modern medicine (Chopra et al., 1956). In recent times, herbal medicines have become an integral part of the Primary Health Care system of many nations (Fajimi and Taiwo, 2000). Nevertheless, as far back as 300 B.C., man was fully aware of the medicinal value of plants, and probably their toxic effects (Le Strange, 1977).

Khaya senegalensi, a tree in the family Meliacea, is a native of West Africa (Senegal) and extends to Sudan and Uganda (Keay, 1989). The tree is commonly called the dry zone mahagony, and it is widely distributed in the Savannah regions. In Nigeria, the tree is called with many local names in different parts of the country; 'Madaci' in Hausa, 'Dalehi-Kahi' in Fulani. 'Oganwon' in Yoruba and 'Ono' in Igbo languages. In its natural habitat, the plant is a medium to large sized tree that grows up to 30 m. The bark and the leaves of the plant are used by the local people of Keffi for the treatment of diarrhoea, dysentery and wound infections. Medicinal plants are of great importance to the health of individuals and the local communities of Nigeria. The medicinal values of these plants rely in the presence of certain chemical substances that produce a definite physiological effect on the

^{*}Corresponding author. E-mail: makmakwin@yahoo.com.

	Ethanolic extracts		Methanolic extracts	
Phytochemical Compounds	Bark	Leaves	Bark	Leaves
Alkaloids	-	+	-	+
Flavonoids	+	+	+	+
Glycosides	+	+	-	-
Phlabatannins	-	-	-	-
Saponins	-	+	-	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Terpernoids	-	-	+	+

Table 1. Result of phytochemical screening of ethanolic extracts of leaves bark of *Khaya* senegalensis.

- = Absent; + = Present.

human body. The most important of these bioactive constituent of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952).

This investigation is aimed at screening for the compounds and the antimicrobial activity of the crude extracts of the leaves and bark of *K. senegalensis*.

MATERIALS AND METHODS

Plant collection and identification

The leaves and the bark of *K. senegalensis* were collected in the main campus of Nasarawa State University, Keffi. The plant was identified at the Botany Unit of the Department of Biological Sciences, Nasarawa State University, Keffi. The identity of the plant was subsequently confirmed at the Department of Botany, University of Jos and Federal College of Forestry, Jos, Nigeria. Fresh leaves and bark of the plant were collected and allowed to dry at room temperature in the laboratory for a period of 2 weeks.

Preparation of extraction and phytochemical analysis

Extraction from the ground dried leaves and bark of the plant was carried out using Soxhlet extractor. Ethanol was used as extractant. The method of Trease and Evans (1989) were employed to test for the presence of tannins, phlabatannins and alkaloids. The method of Harborne (1973) was used to test for the presence of steroids, saponins, glycosides and flavonoids. Terpenoids were tested using the method of Sofowora (1993).

Test organisms

The test organisms used for the study were *Streptococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. These were obtained from the stock cultures of the Medical Services Department, Nasarawa State University, Keffi. The organisms were subcultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques as described by Cowan and steel (1965). Stock cultures were maintained on nutrient agar slants at 4°C and then subcultures in nutrient broth at 37°C prior to each antimicrobial test. The inocula of the test organisms were standardized by methods of Baker and Thomsberry (1983). This was done by suspending 5 colonies of a

24 h culture in 5 ml of nutrient broth and comparing the turbidity with that of 0.5 Mac Farlan standards, after incubating at 35° C for 2 h 30 min.

Minimum inhibitory concentration (MIC)

A quantity of 0.5 g of each extract was dissolved in 4 ml sterile Mueller-Hinton broth which yield an initial concentration of 125 mgl⁻¹. Subsequently, two folds serial dilution were made from the stock of 4 ml containing 125 mgl⁻¹. Mueller-Hinton broth was used to obtain the following concentrations 125, 62.50, 31.250, 15.65, 7.83, 3.91, 1.95, 1.00, 0.50, 0.25 and 0.13 mgl⁻¹. One millilitre of a standardized inocolum of each test organisms was introduced into each extract-nutrient broth mixture and then incubated at 37°C for 24 h. The lowest concentration of the extract that inhibited the test organisms was recorded as the MIC.

Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration was determined by the method of Rotimi et al. (1988). All the tubes that showed no microbial growth (no turbidity) after 24 h of incubation were subcultured onto the surfaces of freshly prepared Mueller-Hinton agar and incubated at 37°C for another 24 h. The MBC was regarded as the lowest concentration of the extract that did not permit a visible bacterial growth on the Mueller Hinton agar after bacterial 24 h incubation.

Determination of diameter zone of inhibition

Diameter of zone of inhibition for each test organism was determined by paper disc diffusion method. Plates of Mueller Hinton agar were prepared and inoculated in triplicates with the test organisms by spread plate method. Paper discs of 6 mm diameter were oven-sterilized and impregnated with each extract at 15.63 mgml⁻¹ which was the average inhibitory concentration. The impregnated paper discs were subsequently placed on the agar and incubated at 37°C for 24 h. The potency of the crude extracts was determined by clear zones of inhibition around the paper discs and these were respectively measured as diameter zones of inhibition.

RESULTS AND DISCUSION

The results of the phytochemical screening of the leaves and bark of *K. senegalensis* are shown in Table 1. Tan-

	Ethanolic extr	acts (mg ml ⁻¹)	Methanolic extracts (mg ml ⁻¹)		
Test Organisms	Bark	Leaves	Bark	Leaves	
Staphylococcus aureus	7.81	31.25	7.81	31.25	
Escherichia coli	-	-	-	-	
Streptococcus sfeacalis	3.91	7.81	7.81	15.63	
Candida albicans	7.81	31.25	31.25	15.63	

 Table 2. Minimum inhibitory concentration (MIC) of the ethanolic extracts of the leaves and bark of

 Khaya senegalensis.

- = Not susceptible.

Table 3. Minimum bactericidal concentration (MBC) of ethanolic extracts of leaves and bark of Khaya senegalensis.

	Ethanolic extracts (mg ml ⁻¹)		Methanolic extracts (mg ml ⁻¹)		
Test Organisms	Bark	Leaves	Bark	Leaves	
Escherichia coli	-	-	-	-	
Streptococcus feacalis	31.25	31.25	15.63	31.25	
Candida albicans	-	-	65.25	65.25	

Table 4. Diameter (mm) zone of inhibition of ethanolic and methanolic extract of the leaves and the bark of *Khaya senegalensis*.

	Ethanolic extracts		Methanolic extracts	
Test organisms	Bark	Leaves	Bark	Leaves
Staphlococcus aureus	15.00	10.00	15.00	9.00
Streptococcus feacalis	15.00	15.00	15.00	10.00
Candida albicans	9.00	10.00	8.00	10.00

nins, steroids and flavonoids were found to be present in the ethanolic extracts of the bark and leaves of the plant. Saponinis and alkaloids were present in the methanolic and ethanolic extracts of the leaves, but absent in the barks extracts. Glycosides were however, found only in the ethanolic extracts of the two plant portions, while terpenoids and phlabatannins were absent in both the ethanolic and methanolic extracts of the two plant portions.

The results of the antimicrobial susceptibility tests were expressed in terms of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and diameter zones of inhibition of the test organisms with respect to the methanolic and ethanolic extracts of the two plant portions. These results obtained are shown in Tables 2, 3 and 4, respectively.

The results of the MIC and MBC showed that the extracts of *Khaya senegalensis* have bactericidal properties against *S. aureus* and *S. feacalis.* The extracts from both plant portions also had fungicidal effect against *C. albicans.* The leaf and the bark extracts of the plant revealed that the active compounds include tannins,

flavonoids, steroids, glycosides, and alkaloids. The extracts from both plant portions inhibited the growth of *S. aureus, C. albicans* and *S. feacalis.* The inhibitory effects of the extracts of *K. senegalensis* are most likely due to these active compounds.

The fact that the pathogenic strains of *S. aureus* can cause localized abscesses and septiceimia in humans implies that the ethanolic and methanolic extracts of *Khaya senegalensis* could be employed for the treatment of such infections. The sensitivity of *C. albicans* to the ethanolic and methanolic extracts of the plant portions has increased the chemotherapeutic potentials of using the active compounds of *K. senegalensis* in the treatment of diseases such as oral and viginal candidiasis. Moreso, the sensitivity of *S. faecalis* to the leaf and bark extracts of the plant could be employed for the treatment of unitary tract infections, bacteremia and bacterial endocarditis caused by *S. faecalis*.

The results obtained from the phytochemical analysis and the antimicrobial activity of this plant revealed that further investigations may lead to the development of antibiotic(s) from *K. senegalensis.*

ACKNOWLEDGEMENTS

The authors are grateful to the Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria, for providing the laboratory materials used in the investigation.

REFERENCES

- Baker CN, Thomsberry CH (1983). Inoculum standardization in antimicrobial susceptibility test: evaluation of the overnight agar cultures. J. Clin. Microbiol. 17: 450-457.
- Chopra RN, Naygar SI, Chopra IC (1956). Glossary of India Medical Plants. Council of Science Industrial Research, New Delhi, India, p. 160.
- Cowan M, Steel L (1965). Preparation of the test organisms. Niger. J. Microbiol. 22: 56-60.
- Fajimi AK, Taiwo AA (2000). Herbal remedies in animals parasitic diseases in Nigeria: a review. Afr. J. Biotechnol. 4(4): 303-307.
- Harborne JB (1973). Phytochemical Methods. Chapman and Hall limited, London. pp. 49-188.

- Hill AF (1952). Economic Botany: A Textbook of useful plants and plant products. McGraw-Hill Book Company, New York, p. 127.
- Keay RWJ (1989). Trees of Nigeria. Clarendon Press, Oxford. pp. 339-342.
- Le Strange JN (1977). The useful plants of West Tropical Africa. The Crown Agents, London, p. 325.
- Rotimi VO, Laughon BE, Bartlet JG, Mesodomi HA (1988). Activities of Nigeria chewing stick against *Bacteroides gingivalis* and *Bacteroides melaninogenicus*. Nig. J. Microbiol. 9: 13-16.
- Sofowora A (1984) Traditional Medicine: Practice in West Africa (2nd edn.). Ibadan University Press, Ibadan, Nigeria. p. 3-26.
- Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa (2nd edn.). Spectrum Books Ltd, Ibadan, Nigeria. p. 9-25.
- Trease GE, Evans WC (1989). *Pharmacognosy* (11th edn.). Braille Tirida Canada Macmillan Publishers, Canada, p. 257.