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Studies on the antibacterial activity of *Khaya senegalensis* [(Desr.) A. Juss)] stem bark extract on *Salmonella enterica* subsp. *enterica* serovar Typhi [(ex Kauffmann and Edwards) Le Minor and Popoff]

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PEER REVIEW

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Comments

This is a valuable research work in which the authors have demonstrated the antibacterial activity of *K. senegalensis* stem bark extracts on *S. enterica* subsp. *enterica* serovar Typhi. The activity was assessed based on the MIC and the MLC of the stem bark extract. *K. senegalensis* was found to be a promising antibacterial agent against *S. enterica* subsp. *enterica* serovar Typhi.
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ABSTRACT

Objective: To study the phytochemical screening and antibacterial activity of the stem bark extracts of *Khaya senegalensis* (*K. senegalensis*) against *Salmonella enterica* subsp. *enterica* serovar Typhi.

Methods: The plant components were extracted using methanol, ethanol and water. The phytochemical screening of the stem bark extracts were carried out using a standard method. The antibacterial assay of the stem bark extracts against *Salmonella* Typhi (*S.* Typhi) using the agar well diffusion method with different concentrations of 50, 100, 200, 400 and 500 mg/mL and the corresponding concentrations of the control was carried out and the result compared with a standard antibiotic, amoxicillin as the control.

Results: The results obtained from the phytochemical screening of the three plant bark extracts of *K. senegalensis* showed 10 plant secondary metabolites including saponins, tannins, reducing sugars, aldehyde, phlobatannins, flavonoids, terpenoids, alkaloids, cardiac glycoside and anthroquinones. The ethanol and aqueous extracts showed antibacterial activities against *S.* Typhi at concentration of 50 mg/mL with the zone diameter of inhibition (ZDI) of 14 mm and 15 mm respectively. The ethanol and aqueous extracts also showed zone diameter of inhibition of 23 mm and 25 mm respectively at 250 mg/mL and 27 mm each at 500 mg/mL. The ethanol and aqueous stem bark extracts gave the highest ZDI at 500 mg/mL while 100 mg/mL gave the least ZDI for ethanol extract and 50 mg/mL for the aqueous extract. This was followed by 400 mg/mL that gave 24 mm ZDI of the aqueous extract and 27 mm of the ethanol extract. The methanol extract showed intermediate susceptibility evidenced by ZDI of 10 mm at 100 mg/mL concentration. The methanol extract also showed antibacterial activity of 24 mm ZDI against the test organism at a higher concentration of 250 mg/mL and 26 mm at 500 mg/mL concentration. The methanol, ethanol and aqueous extracts displayed antibacterial activities against *S.* Typhi with a statistical significant difference at ($P \leqslant 0.05$). The extracts compared favourably with the standard antibiotic, the control. The minimum inhibitory concentration of the extracts was 250, 200, 200 and 100 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively. The minimum lethal concentration of the extracts was 250, 250, 400 and 200 mg/mL for methanol, ethanol, aqueous extracts and control respectively.

Conclusions: The antibacterial properties of *K. senegalensis* stem bark extract can be harnessed for the production of new antibiotics or the enhancement of already existing antibiotics.

KEYWORDS

Khaya senegalensis, Antibacterial, Zone diameter of inhibition, Minimal inhibitory concentration

1. Introduction

The increase of life threatening infections that are resistant to commonly used antibiotics has become a worldwide problem. It is becoming an important cause of morbidity in immune compromised patients in developing countries[1]. The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with

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reduced susceptibility to antibiotics has raised the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[2,3].

Presently, there are global problems of emergence of multiple antibiotics resistance as well as emergence of new and resurrection of previously eradicated diseases. Reports on ethno botanical survey indicate a general consensus on the use of antimicrobial active medicinal plants to provide cheaper drugs[4]. There is a need to search for new and more potent antimicrobial compounds of natural origin to complement the existing synthetic antimicrobial drugs that are gradually becoming less potent against pathogenic microorganisms.

The continuous spread of multidrug-resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide[5]. In addition to increasing the cost of drug regimens, this scenario has paved way for the re-emergence of previously controlled diseases and has contributed substantially to the high frequency of opportunistic and chronic infection cases in developing countries[6,7].

For a long time, plants have been an important source of natural products for human health. The antimicrobial properties of plants have been investigated by a number of studies worldwide and many of them have been used as therapeutic alternatives because of their antimicrobial properties[8]. This study therefore focuses on the antibacterial potency of *Khaya senegalensis* (Desr.) A. Juss (*K. senegalensis*) stem bark extract on *Salmonella enterica* subsp. *enterica* serovar Typhi and its histopathological effects. *K. senegalensis* is highly priced in traditional medicine in West Africa. The main part used is the very bitter stem bark. Ethno-medically, the uses range from the treatment of fever, lumbago, cough, rheumatism and stomach/gastric pains[9]. In veterinary medicine, the bark is also used in the treatment of worm infestation, ulcer and mucous diarrhea in horses and camels[10].

Typhoid fever is a worldwide bacterial disease that is caused by the ingestion of food and/or water contaminated by the feces and urine of infected persons or animals. The causative agent of the disease is *Salmonella enterica* (*S. enterica*) serovar Typhi and sometimes *S. enterica* serovar Paratyphi A, B, and C[11]. Symptoms usually develop 1-3 weeks after exposure, and may be mild or severe. They include high fever, malaise, headache, constipation or diarrhea, rose-colored spots on the chest, and enlarged spleen and liver. Healthy carrier state may follow acute illness. The bacteria responsible for typhoid fever are deposited in water or food by a human carrier and are then spread to other people in the area. Typhoid fever is rare in developed countries of the world, but continues to be a significant public-health issue in the developing countries like Nigeria. The disease has been given various names at different times in history, such as gastric fever, abdominal typhus, infantile remittent fever, slow fever, nervous fever or pathogenic fever.

2. Materials and methods

2.1. Study area

The study area for this research is Federal Capital Territory (FCT) Abuja. The territory is located just north of the confluence of the River Niger and River Benue. It is bordered by the states of Niger to the west and north, Kaduna to the northeast and south and Kogi to the southwest[12]. The Federal Capital lies between latitude 8.25 and 9.20 north of the equator and longitude 6.45 and 7.39 east of Greenwich Meridian. Abuja is geographically located in the Centre of the country. The FCT has a landmass of approximately 7.315 km^2 of which the actual city occupies 275.3 sq. km. It is situated within the savannah region with moderate climatic conditions[12].

The population of FCT by the 2006 census is 1 405 201 and a population density of 192.1/km^2[12]. The territory is currently made of six local councils, comprising the city of Abuja and five local government areas namely Abuja, Gwagwalada, Kuje, Bwari, Kwali.

2.2. Preparation of stem bark extract

The stem bark of *K. senegalensis* was collected from within FCT Abuja, Nigeria. The plant material was air-dried to constant weight and milled into powder[13]. Twenty grams of powder was percolated in 200 mL of methanol, ethanol and distilled water[14,15]. The percolated mixture was filtered and evaporated at 45 °C using a water bath. An aqueous solution of the extract was made corresponding to different concentrations of 500, 250, 100 and 50 mg/mL.

2.3. Phytochemical analyses

Phytochemical analyses for qualitative detection of alkaloids, flavonoids, tannins and saponins were performed on the extracts[16-18].

2.4. Test organism

The test organism was obtained from the stock culture of the Microbiology laboratory of Federal University of Technology, Minna, Nigeria and authenticated using cultural and morphological identification, microscopy after Gram's staining as well as biochemical characterization of test organism[5], and maintained in a nutrient broth medium in a refrigerator for future use.

2.5. Standardization of inoculums

The density of suspension inoculated on to the media for susceptibility test was determined using the standard curve[19]. Cells corresponding to 1.0×10^8 CFU/mL were used[20].

2.6. Preparation and sterilization of media

All the media used in this study were obtained in powdered form and constituted in distilled water according to the manufacturers' instructions. The various quantities and volumes of water depended on the particular medium. A weighed quantity of each medium was dissolved in specific volume of de-ionized water in a chemical flask, which was stoppered properly. It was sterilized by autoclaving at 121 °C and 15 pounds per square inch for 15 min and cooled to 45-50 °C before dispensing into pre-sterilized Petri dishes. These were left to gel on the workbenches. Glass materials used in this work were also sterilized by autoclaving at 121 °C and 15 pounds per square for 15 min. They were then brought out and allowed to cool down properly before use.

2.7. Membrane filtration

Membrane filters were used to sterilize the plant stem bark extracts.

2.8. Antibacterial assay

The sensitivity of the plant extract was determined using agar well diffusion technique with modifications[21-23]. Wells were bored into the already gelled nutrient agar medium which has been previous seeded with the test organism using the spread plate method. The 6 mm diameter wells were bored using a sterile cork borer. The wells were then filled with 0.2 mL of each of the extracts (500, 400, 250, 100 and 50 mg/mL) and care was taken not to allow the solution to spill to the surface of the medium. The plates were allowed to stand on the laboratory bench undisturbed for 1 h to allow proper absorption into the medium before the plates were incubated at 37 °C for 18 h. The plates were later observed for the zone diameter of inhibition (ZDI). The effects of the extracts on the test organism were compared with that of a standard antibiotic, amoxicillin as a control, using 12 mm as indicative of sensitivity according the guidelines of the Clinical and Laboratory Standards Institute of 2013[24].

2.9. Minimum inhibitory concentration (MIC) of the extract on the bacterial test organism

The MIC of the extract was determined using methods of Bukar *et al.* with modification[25]. Plant extracts of 500, 400, 250, 200, 100 and 50 mg/mL concentrations were prepared. One milliliter of the different concentrations of each extract was added to 9 mL of the nutrient broth in test tubes and 1 mL of the standardized inoculum of the test organism was also added. The control was also set up, but amoxicillin was used instead of the plant extracts. The activity was determined by visual method and increase in turbidity of the test tubes using spectrophotometer.

2.10. Minimum lethal concentration (MLC) of the extracts on the test organism

The MLC of the extracts was determined using the method of Ebomoyi *et al.* with modifications[4]. Samples were taken from tubes with no change in turbidity in the MIC assay and sub cultured onto freshly prepared nutrient agar plates and incubated at 37 °C for 18 h. The lowest concentration of the extract that did not allow any increase in number of viable cells or bacterial growth on the surface of the agar plates was taken as the MLC.

2.11. Statistical analyses

The results were expressed as mean±SD. The one-way ANOVA test was used to compare results among and within groups for any significant difference in antibacterial activity of the extracts and the control.

3. Results

3.1. Phytochemical screening of extracts

The result of the phytochemical screening revealed the following metabolites as present in the methanol, ethanol and aqueous extracts of *K. senegalensis* (Table 1). The methanol extract contains the 10 secondary metabolites identified in the stem bark extracts of *K. senegalensis* in this work, which included saponnins, tannins, reducing sugar, aldehyde, phlobatannins, flavonoids, terpenoids, alkaloids, cardiac glycoside and anthroquinones. The ethanol extract had all the metabolites present in the methanol extract except the reducing sugar and the flavonoids. The aqueous extract lacked reducing sugar, aldehyde, phlobatanins, cardiac glycoside and anthroquinones but had flavonoids.

Table 1
Phytochemical analysis of the methanol, ethanol and aqueous extracts.

Metabolites	Methanol	Ethanol	Aqueous
Saponins	+	+	+
Tannins	+	+	+
Reducing sugars	+	−	−
Carbonyl or aldehyde	+	+	−
Phlobatannins	+	−	+
Steroids	−	−	−
Flavonoids	+	−	+
Terpenoids (Salkowski method)	+	+	+
Alkaloids	+	+	+
Cardiac glycoside	+	+	−
Anthroquinones	+	+	−

+: Present, −: Absent.

3.2. ZDI

The ZDI of the various extracts at different concentrations

are shown in Table 2. The highest ZDI of the extracts against *Salmonella* Typhi (*S.* Typhi) were 26 mm, 27 mm and 27 mm at 500 mg/mL for methanol, ethanol and aqueous extracts respectively and 29 mm for the control (amoxicillin). This was followed by the ZDI of 24 mm, 23 mm and 25 mm at 250 mg/mL for methanol, ethanol and aqueous extracts respectively and 25 mm for the control at 250 mg/mL.

Table 2

ZDI of the different concentrations of extracts against *S.* Typhi (mm).

Extract	Concentration (mg/mL)					
Methanol	10.0±1.0^a	10.0±2.0^a	15.0±2.0^a	24.0±1.0^a	24.0±2.0^b	26.0±1.5^a
Ethanol	14.0±1.0^a	13.0±2.0^a	17.0±1.5^a	23.0±1.0^b	24.0±1.0^a	27.0±2.0^b
Aqueous	15.0±2.0^b	17.0±1.5^a	21.0±1.0^a	25.0±1.5^a	27.0±1.0^a	27.0±1.5^a
Amoxicillin	20.0±1.5^a	20.0±1.5^a	23.0±1.0^a	25.0±1.0^b	28.0±2.0^b	29.0±2.0^a

Values are expressed as mean±SD. Values with the same alphabets are significantly different ($P \leqslant 0.05$).

3.3. MIC

The results of the MIC of the different extracts against *S.* Typhi were 250, 200, 200 and 100 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively.

3.4. MLC

The results of the MLC of the different extracts against *S.* Typhi were 250, 250, 400 and 200 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively.

4. Discussions

The results obtained from the phytochemical screening of the three plant bark extracts of *K. senegalensis* showed 10 plant secondary metabolites including saponins, tannins, reducing sugars, aldehyde, phlobatannins, flavonoids, terpenoids, alkaloids, cardiac glycoside and anthroquinones.

The methanol extract contains the 10 secondary metabolites identified in the stem bark extracts of *K. senegalensis* in this work. This was followed by the ethanol and aqueous extracts. It is believed that the presence of these plant secondary metabolites may be responsible for the antibacterial activity exhibited by these stem bark extracts.

It can be seen that at low concentrations (10-200 mg/mL), the ZDI of the control [($\geqslant$20.0±1.5) mm] is significantly higher ($P \leqslant 0.05$) than the ZDI of the extracts [(10.0±1.5) mm]. However, at higher concentrations, the ZDI of control did not differ from those of the extracts ($P \geqslant 0.05$). According to Baker and Silverton[26], an organism is considered sensitive to a chemical agent only when the ZDI is either equal to the control, more than or not more than 3 mm smaller than the control. The highest ZDI of the extracts against *S.* Typhi are 26 mm, 27 mm and 27 mm at 500 mg/mL for methanol, ethanol and aqueous extracts respectively and 29 mm for the control (amoxicillin). This is followed by the ZDI of 24 mm, 23 mm and 25 mm at 250 mg/mL for methanol, ethanol and aqueous extracts respectively and 25 mm for the control. These data are above the reference range of the Clinical Laboratory Standard Institute[24], as such are considered sensitive in this work.

The MIC of the extracts is 250, 200, 200 and 100 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively.

The MLC of the extracts is 250, 250, 400 and 200 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively. The MLC of methanol and ethanol extracts competes favorably with that of the control. The MLC of the aqueous extract is higher which may probably be as a result of some of the active principles lacking in the extract.

The antibacterial properties of *K. senegalensis* stem bark extract can be harnessed for the production of new antibiotics or the enhancement of already existing antibiotics that are fast developing resistance to combat the problem of multi-drug resistance of *S.* Typhi.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

This study focuses on the antibacterial potency of *K. senegalensis* stem bark extract on *S. enterica* subsp. *enterica* serovar Typhi.

Research frontiers

The present research work depicts the phytochemical screening and the antibacterial activity of aqueous and alcoholic extracts of *K. senegalensis* stem bark against *Salmonella* serovar Typhi.

Related reports

This report is related to some earlier report on the phytochemical screening and antibacterial activity of some other plant material extracts. It is related to the work of Ebomoyi and Okojie (2012) on the physiological mechanisms underlying the use of *Garcinia kola* Heckel in the treatment of asthma and Halilu *et al.* (2010) on the preliminary phytochemical screening, antibacterial activity

and elemental analysis of the leaves and the root bark of *Parinari curatellifolia*.

Innovations and breakthroughs

K. senegalensis is a medicinal plant that can be used in the treatment of typhoid fever. In the present study, authors have demonstrated the antibacterial activity of *K. senegalensis* stem bark extract on *S. enterica* subsp. *enterica* serovar Typhi.

Applications

From the literature survey, it has been found that *K. senegalensis* stem bark is safe to humans. This scientific study supports and suggests that this plant may be used as an antibacterial agent.

Peer review

This is a valuable research work in which the authors have demonstrated the antibacterial activity of *K. senegalensis* stem bark extracts on *S. enterica* subsp. *enterica* serovar Typhi. The activity was assessed based on the MIC and the MLC of the stem bark extract. *K. senegalensis* was found to be a promising antibacterial agent against *S. enterica* subsp. *enterica* serovar Typhi.

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