Antibacterial Potential and Anti-Ulcer Activity of Methanol Extract of *Khaya anthotheca* on Indomethacin Induced Ulcer in Albino Wistar Rats

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ABSTRACT

**Background and Objective:** *Khaya anthotheca* is a member of the family Meliaceae, a native to Africa. It is a medicinal plant widely used for several therapeutic purposes. Herein, phytochemical study, anti-ulcerogenic and antibacterial investigations were carried out on the methanol extract of *Khaya anthotheca* root.

**Materials and Methods:** As 35 rats were distributed into seven groups to evaluate the anti-ulcer activity of *K. anthotheca* root methanol extract at the dosage range of 50-400 mg/kg/b.wt., while the antibacterial activity was determined at a concentration range of 100-400 µg/mL. The analysis was done using the software-SPSS one-way ANOVA at a significance level p < 0.05.

**Results:** The result of the antibacterial study reveals that extract elicited substantial (p < 0.05) growth inhibition of *Klebsiella pneumoniae* (18.34±0.16 and 18.22±0.26 mm) at the concentration of 300 and 400 µg/mL, respectively when compared with the standard drug (20.73±0.17 and 20.34±0.23 mm). The extract also showed mild inhibition of *Salmonella typhi* (15.54±0.13 mm) at the highest concentration employed, while displaying weak inhibitions of *Escherichia coli* and *Staphylococcus aureus* (13.54±0.14 and 12.23±0.12 mm). In addition, *Khaya anthotheca* root-bark methanol extract displayed profound (p < 0.05) anti-ulcerogenic action at the dosage of 300 mg/kg/b.wt. (50.42%) and 400 mg/kg/b.wt. (60.52%) against indomethacin-induced ulcerated rats.

**Conclusion:** This study therefore suggests the use of *Khaya anthotheca* as an antimicrobial agent and for treatment of ulcers. Further studies are needed to discover the bioactive constituent of the plant responsible for this anti-ulcer activity as well as other pharmacological activities in clinical trials.

**KEYWORDS**

*Khaya anthotheca*, methanolic extract, anti-ulcerogenic, antibacterial, albino rats, indomethacin induced ulcer

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INTRODUCTION

Peptic ulcer disease is a digestive tract condition characterized by mucosal damage brought on by the production of gastric acid and pepsin\(^1\). It typically affects the stomach and proximal duodenum; less frequently, it affects the lower esophagus, the distal duodenum, or the jejunum. Examples of these conditions include hiatal hernias (also known as Cameron ulcers), ectopic gastric mucosa (such as in Meckel’s diverticulum) and unopposed hypersecretory states like Zollinger-Ellison syndrome. Every year, about 500,000 Americans are diagnosed with peptic ulcer disease\(^1\). It happens in 70 percent of patients between the ages of 25 and 64\(^2\). An estimated $10 billion is spent each year on direct and indirect medical expenses related to the illness\(^1\). On the other hand, there appears to be a decrease in the prevalence of peptic ulcers, potentially due to rising proton pump inhibitor usage and falling *Helicobacter pylori* infection rates\(^3\). In the United States, the most common causes of peptic ulcer disease are *H. pylori* infection and nonsteroidal anti-inflammatory drug (NSAID) use, which account for 48 and 24 percent of cases, respectively\(^4\). A higher chance of developing peptic ulcer disease is linked to a number of other infections and comorbidities, such as sarcoidosis, myeloproliferative disorder, hepatic cirrhosis, cytomegalovirus, Crohn’s disease and chronic renal failure. Stress ulcers, also known as gastroduodenal erosions or ulcers, can arise after critical illness, surgery, hypovolemia, or splanchnic hypoperfusion. These ulcers can be asymptomatic or show signs of bleeding or perforation\(^5\).

There are several drugs used for the management of gastric ulcers and other diseases in humans. However, most synthetic drugs often elicit some side effects and severe complications as well as other disadvantages such as microbial resistance and unaffordability\(^6\). Over the years, medicinal plants have been in use especially in developing countries as a source of alternative medicine with little or no side-effect to combat several diseases. Consequently, extensive and concerted studies are being carried out by scientists across the globe in the search for plant’s bioactive compounds\(^7\).

*Khaya anthotheca* is a member of the family Meliaceae. Six species make up this little genus; two are found in Madagascar and the Comores and four are found in tropical Africa\(^8\). The paripinnate leaves and mostly spherical, 4-5 valved, dehiscent woody capsules make the genus easily identifiable\(^8\). In addition to being frequently used for window frames, paneling, doors and stair cases, the wood is highly prized for use in furniture, cabinetry, veneer and decorative boxes and cases. It works well for light flooring, automotive bodies, musical instruments, sporting goods, toys, plywood, pulpwood, shipbuilding and carving\(^9\).

The bitter bark is frequently utilized in African traditional medicine. It is used to treat coughs, while bark decoctions or infusions are applied topically to wounds, sores and ulcers and taken to treat fever, cold, pneumonia, stomach discomfort, vomiting and gonorrhea. Taking pulverized bark can help alleviate male impotence and act as an aphrodisiac. Root infusions are consumed in Tanzania as a remedy for diarrhea, anemia and rectal prolapse. The leaves are allegedly used to make arrow poison in the Democratic Republic of the Congo. The *K. anthotheca* is planted as a roadside tree and as an attractive shade tree quite frequently. In agroforestry systems, it is occasionally planted as a shade tree\(^9\).

Research also demonstrated that *Khaya anthotheca* possesses neuroprotective action\(^10\). Some important bioactive constituents such as gedunin and limonoids which are of great benefits have been reported to be present in this plant\(^11\). Yacine et al.\(^12\) also reported the presence of phytochemicals such as sterols and polyterpenes, flavonoids, polyphenols, saponins and tannins in the plant. Elegant research also reported that *K. anthotheca* possesses high antioxidant and antiradical properties due to the presence of bioactive constituents such as polyphenols, polyterpenes and in addition to tannins and saponins\(^13\). Studies also documented that *K. anthotheca* possesses antimalarial\(^7\), anti-protozoal\(^14\), anticancer\(^15\) and neuroprotective\(^16\) activities. However, reports on the anti-ulcerogenic and anti-bacterial efficacies of plants are rather rare or speculative. This study therefore evaluates the anti-ulcerogenic and anti-bacterial activities of methanolic crude extract of *Khaya anthotheca* root on indomethacin treated albino rats.

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MATERIALS AND METHODS

Study location: The research was conducted at the Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria between August 2023 and January 2024.

Chemicals: Methanol, Indomethacin and Omeprazole were purchased from Sigma Aldrich, Steinheim, Germany. Other chemicals used in this study were obtained from standard suppliers.

Plant collection and identification: The matured roots of *khaya anthotheca* were collected from a farm in Wukari Local Government Area of Taraba State, Nigeria. At the herbarium unit of the Department of Biological Sciences at Federal University Wukari in Taraba State, the roots were identified down to the species level. After washing the roots with tap water to get rid of any dirt particles, they were allowed to air dry in the shade to keep the chemical components from being inactivated by UV radiation. Using a mortar and pestle, the dried root was ground into a fine powder and kept in a dry container (Fig. 1).

Extraction of plant material: The powdered sample (150 g) was placed into a flask and methanol was used to extract the phyto-constituents for 7 days, while shaking at intervals and allowed to stand at room temperature. The mixture was filtered using Whatmann no. 4 filter paper and concentrated in a Buchi rotary evaporator (Gaithersburg, Maryland, United States). The dried extract was transferred to an airtight container, corked and preserved in a refrigerator.

Animal procurement: Wistar albino rats of weights between 100-200 g were purchased from an animal house, Yola, Taraba State, Nigeria. Rats were placed at room temperature of 22±2°C and 12/12 periods of light and dark with proper ventilation facility. Rats were acclimatized for one week and given the standard diet and water *ad libitum*. Standard laboratory protocols for animal studies were maintained as approved by the faculty of pure and applied sciences, Federal University Wukari, Taraba State, Nigeria.

Anti-ulcer study

Experimental design: Thirty-five albino Wistar rats were distributed into five groups, each group containing five animals (n = 5). After it was allowed to fast for 24 hrs, ulcer was induced by the administration of indomethacin (30 mg/kg) to all animals except normal control animals intragastrically. Treatment with the plant extract was administered daily for 14 days as follows:

- **Group 1**: Normal control (diet/water)
- **Group 2**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water)
- **Group 3**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water+Omeprazole)
- **Group 4**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water+100 mg/kg/b.wt., extracts)
- **Group 5**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water+200 mg/kg/b.wt., extracts)
- **Group 6**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water+300 mg/kg/b.wt., extracts)
- **Group 7**: Rats (induced ulcer indomethacin 25 mg/kg/b.wt.,+diet/water+400 mg/kg/b.wt., extracts)

Fig. 1: Powder and roots of *Khaya anthotheca*
Animals were sacrificed after 14 days under the influence of chloroform anesthesia, with the stomach removed and the larger curvature of the stomach opened. After fixing the tissue in saline with 10% formaldehyde, it was examined under a microscope and any lesions were scored using Das and Banerjee. Ulcer index and percentage of ulcer inhibition were determined as follows:

\[
\text{Ulcer index (UI)} = UN + US \times UP \times 10^{-1}
\]

Where:
- **UN** = Average number of ulcers per animal
- **US** = Average severity score
- **UP** = Percentage of animals with ulcers

\[
\text{Ulcer inhibition (\%)} = \frac{\text{UI control} - \text{UI test}}{\text{UI control}} \times 100
\]

**Gross examination of gastric ulcer:** After sacrificing the rats, the stomach was removed and washed with saline solution to clear and clean all the blood stains. This was followed by a macroscopic examination of the stomach for the detection of haemorrhagic lesions. The injured size of the lesion was measured in mm to determine the mean ulcer index (UI) and scored as follows: No ulcer score = 0 mm, small ulcer = 1-2 mm, medium ulcer = 3-4 mm and larger ulcer = 5-6 mm. The ulcer index was calculated by adding the sum of the total of the scores and dividing by the number of animals.

**Histopathology:** Histopathology of the stomachs of all the animals was fixed in 10% buffered formalin in labelled bottles and processed routinely for histology examination. The tissue embedded in paraffin wax was sectioned in 5 mm thick, stained with haematoxylin and eosin, mounted on glass slides and then examined under a standard microscope to observe for pathological changes.

**Antibacterial study**

**Preparation of test samples:** The crude extracts of *K. anthotheca* were used in antibacterial assay, of the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by Umaru et al. Exactly 3 mg of the crude sample was dissolved homogeneity in 3 mL of methanol giving a stock solution of 1000 µg/mL. Different volumes from the stock solution were taken, amounted to 50, 100, 250 and 500 ppm each and dissolved in 5 mL of methanol to make the final concentration, respectively.

**Preparation of agar plates:** Preparation of agar plates was performed based on the method described by Umaru et al. Nutrient agar was prepared according to manufacturer’s instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in an autoclave at 121°C. The agar solution was then poured into a sterile Petri plate and allowed to cool down and form a gel. Each test sample, specifically the 50, 100 and 250 ppm samples, as well as the positive control (tetracycline 30 µg) and negative control (methanol), were divided into eight portions. After the plate was inoculated with bacteria, it was sealed with parafilm and refrigerated at 4°C.

**Preparation of bacteria broth:** The antibacterial properties of *K. anthotheca* crude extracts derived from the stock culture provided by Federal University Wukari’s Microbiology Laboratory were assessed using a number of carefully chosen microorganisms. The nutrient broth was made in accordance with the manufacturer’s instructions, dissolving 2.6 g of the dried broth in 200 mL of distilled water and then autoclaving it at 121°C to sterilize it. The bacteria were subcultured for 16 hours at 37°C in an incubator with a shaker in 10 mL of broth per universal glass bottle. Using a UV microspectrophotometer (model 1240, Shimadzu brand), the turbidity (optical density/OD) of the bacterial broth was evaluated after...
16 hrs of incubation. The results were equivalent to those of a typical tube of nutritional broth for future usage. The bacterial broth was considered fit for use when its turbidity fell between 0.6 and 0.9 optical density (OD). The measurement was done at a wavelength of 575 nm. The turbidity was adjusted with nutrient broth until the target value was reached.

**Plate inoculation:** The process of inoculating the bacteria was based on the methodology outlined by Umaru *et al.*19 and was done in a biohazard cabinet. The prepared bacterial broth was poured into tiny centrifuge tubes in an amount of around 1 mL. The whole surface of the agar plate was streaked in four different directions using a sterile cotton swab dipped into the little centrifuge tube containing the bacterial broth. After that, the test samples were applied to the agar plate and left for 5-10 min. The utilized disc had a diameter of 6 mm. Using sterile forceps, insert a volume of 10 µL of each test sample (concentration 25, 50, 100, 250 ppm) into the discs, then gently press to establish contact with the agar plate. Subsequently, the disc was pupated with methanol as a negative control and 30 µg of tetracycline as a standard antibacterial drug was placed on the agar plate (positive control). To enable the standards and test samples to diffuse into the agar, the plates were kept at room temperature for ten minutes. For every bacterium that was utilized, each crude extract was examined three times. Following a 24 hrs incubation period at 37°C, the plate samples were analyzed to determine the inhibition zone surrounding each sample disc. Each sample’s antibacterial activity was determined by measuring the diameter of the inhibition zone in relation to the positive control.

**Statistical analysis:** Statistical analysis for biological activities was performed using SPSS version 20 programme. Values were expressed as Mean±Standard Deviation for three determinations of each experiment and were considered significant at p<0.05. The analysis was done using the software-SPSS one-way ANOVA.

**RESULTS**

**Effect of *K. anthotheca* methanol root-bark crude extract on bacterial growth:** Table 1 shows the effect of *K. anthotheca* methanol root-bark crude extract on gram positive and gram negative bacteria

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Organism</th>
<th>Tetracycline (30 µg/mL)</th>
<th>Extract (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td><em>Salmonella typhi</em></td>
<td>18.73±0.13</td>
<td>10.13±0.11</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>19.85±0.21</td>
<td>12.23±0.12</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>21.29±0.13</td>
<td>10.22±0.15</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20.52±0.15</td>
<td>12.31±0.12</td>
</tr>
<tr>
<td>100</td>
<td><em>Salmonella typhi</em></td>
<td>20.84±0.23</td>
<td>14.23±0.11</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>19.63±0.16</td>
<td>12.32±0.13</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>21.38±0.17</td>
<td>13.13±0.15</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20.25±0.11</td>
<td>16.34±0.16*</td>
</tr>
<tr>
<td>200</td>
<td><em>Salmonella typhi</em></td>
<td>20.74±0.16</td>
<td>13.33±0.11</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>19.75±0.16</td>
<td>12.13±0.13</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>21.13±0.17</td>
<td>11.13±0.14</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20.75±0.15</td>
<td>16.12±0.11*</td>
</tr>
<tr>
<td>300</td>
<td><em>Salmonella typhi</em></td>
<td>20.76±0.11</td>
<td>13.14±0.21</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>19.74±0.15</td>
<td>13.15±0.14</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>21.17±0.12</td>
<td>11.16±0.24</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20.73±0.17</td>
<td>18.34±0.16*</td>
</tr>
<tr>
<td>400</td>
<td><em>Salmonella typhi</em></td>
<td>20.76±0.21</td>
<td>15.54±0.13</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>19.55±0.13</td>
<td>13.54±0.14</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>21.25±0.18</td>
<td>12.23±0.12*</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20.34±0.23</td>
<td>18.22±0.26*</td>
</tr>
</tbody>
</table>

Result is Mean±SD, N = 3, *Significant activity when compared to the control (p<0.05), concentration of standard is 30 µg/mL of tetracycline and Conc.: Concentration.
Animals were treated orally with Omniprazole and *Khaya anthotheca* Root-Bark Methanol Crude Extract (KARB) at 400 mg/kg/b.wt., for 14 days. The blue arrow indicated the absence of epithelial layers (ulcer area internal) and the green arrow indicated epithelial layers remaining (ulcer edge). The red showed healing portion of the ulcerated area and the microphotographs depict the activity of the crude at 400 mg/kg/b.wt., in the groups’ magnification of 200 µg.

**Table 2: Effect of root-bark *Khaya anthotheca* methanol crude extract on indomethacin induced ulcer rats and percentage protection**

<table>
<thead>
<tr>
<th>Group</th>
<th>Ulcer index</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-ve control</td>
<td>2.93±0.70</td>
<td>0.00</td>
</tr>
<tr>
<td>+ve control</td>
<td>0.11±0.05</td>
<td>96.25</td>
</tr>
<tr>
<td>50 mg/kg/b.wt.,</td>
<td>2.19±0.24</td>
<td>7.98</td>
</tr>
<tr>
<td>100 mg/kg/b.wt.,</td>
<td>1.94±0.16</td>
<td>18.49</td>
</tr>
<tr>
<td>200 mg/kg/b.wt.,</td>
<td>1.49±0.23</td>
<td>37.39</td>
</tr>
<tr>
<td>300 mg/kg/b.wt.,</td>
<td>1.18±0.18*</td>
<td>50.42</td>
</tr>
<tr>
<td>400 mg/kg/b.wt.,</td>
<td>0.94±0.20*</td>
<td>60.52</td>
</tr>
</tbody>
</table>

Results are Mean±SD, N = 5. *Significant activity was observed and concentration of standard is 25 µg/mL of omniprazole.*

**DISCUSSION**

The result reveals that *K. anthotoca* methanol root-bark crude extract exhibited a consistent and substantial (p < 0.05) increase of growth inhibition of *Klebsiella pneumoniae* (18.34±0.16 and 18.22±0.26 mm) at the concentration of 300 and 400 µg/mL, respectively which compared with the control and these inhibitions are comparable to the standard drug (20.75±0.17 and 20.34±0.23 mm) (Table 1). In addition, the extract showed mild inhibition of *Salmonella typhi* (15.54±0.13 mm) and weak inhibitions of *Escherichia coli* and *Staphylococcus aureus* (13.54±0.14 and 12.23±0.12 mm) at the highest concentration employed.

**Effect of *K. anthotheca* methanol root-bark crude extract gastric ulcer:** The results of *K. anthotheca* methanol root-bark crude extract effect on gastric ulcer are displayed in Table 2 and Fig. 2. The administration of indomethacin caused gastric ulcer in group 2 animals (negative control) as demonstrated by their high ulcer index (2.93±0.70). However, *K. anthotheca* root-bark methanol crude extract displayed profound (p < 0.05) inhibition of gastric ulcer at the dosage of 300 mg/kg/b.wt. (50.42%) and 400 mg/kg/b.wt. (60.52%) in indomethacin-induced ulcerated rats.
The acute exposure of albino rats to indomethacin caused mucosal necrosis, edema and inflammation causing neutrophil infiltration as demonstrated by a high ulcer index (2.93±0.70) of the negative control. However, K. anthotheca root-bark methanol crude extract displayed profound (p<0.05) inhibition of gastric ulcer at the dosage of 300 mg/kg/b.wt. (50.42%) and 400 mg/kg/b.wt. (60.52%) in indomethacin-induced ulcerated rats (Table 2 and Fig. 2).

The K. anthotheca is a medicinal plant found in Africa, used in the treatment of several diseases including malaria, diarrhea and skin infections. Other pharmacological activities including anti-protozoa, anti-bacterial, and anti-ulcer activity in rat models were investigated. The result of the antibacterial study agreed with the report of Mota et al. that K. anthotheca extract exhibited significant activity against a variety of gram-positive and gram-negative bacteria.

This antiulcerogenic activity of K. anthotheca might be due to the presence of phytochemicals such as flavonoids and phenolic compounds which had been reported to possess gastroprotective activity, which is linked to their antisecretory and antioxidant mechanisms. It is therefore rational to suggest that the reported antibacterial and antiulcereric efficacies of Khaya anthotheca reported herein are consequent to these resident bioactive phytoconstituents.

This work has yielded important insights into the effectiveness of K. anthotheca in preventing bacterial infections and ulcer formation through meticulous experimentation and analysis. The results highlight how crucial it is to do additional research and obtain clinical validation to fully utilize this plant’s therapeutic potential. Finally, it is worth suggesting that K. anthotheca could be a viable option for the creation of cutting-edge remedies for the management of ulcers and bacterial infections. However, more research is needed to confirm these findings and to evaluate the potential clinical efficacy of K. anthotheca extracts for the treatment of ulcers.

CONCLUSION
This study concluded that Khaya anthotheca root-bark crude extract possesses substantial antibacterial and antiulcerogenic activities with an increase in concentration. This research will contribute to the discovery and development of bioactive compounds against bacteria and ulcers that have become a menace in less developed countries, as this will offer hope for healthcare improvement and well-being of people. However, more research is needed to confirm these findings and to evaluate the potential clinical efficacy of Khaya anthotheca extracts for the treatment of ulcers. Further studies are needed to discover the bioactive constituent of the plant responsible for the reported antibacterial and anti-ulcer activity as well as other pharmacological activities in clinical trials.

SIGNIFICANCE STATEMENT
Gastrointestinal diseases are becoming more common worldwide and antibiotic resistance is rising, it is imperative that the antibacterial and anti-ulcer properties of Khaya anthotheca are investigated. The goal of this research is to increase our knowledge of the effectiveness of natural cures, which may provide, cheaper, safer and more long-lasting substitutes for traditional therapies. This research may provide priceless insights into traditional medicine practices and pharmaceutical development from Khaya anthotheca. This could ultimately improve healthcare outcomes and promote the discovery of new drugs derived from natural sources. Findings reveal that the extract elicited considerable growth inhibition of Klebsiella pneumoniae and mild inhibition of Salmonella typhi and also displayed profound anti-ulcerogenic action against indomethacin-induced ulcerated rats.

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