

# ANTIBACTERIAL EFFECTS OF *KHAYA SENEGALENSIS* (AFRICAN MAHOGANY) METHANOLIC LEAF EXTRACT ON *SALMONELLA* SPECIES

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## ABSTRACT

*Khaya senegalensis* is a deciduous evergreen tree belonging to the *Meliaceae* family that is native to the sub-Saharan savannah. It is one of the most popular traditional medicines in Africa. This study aimed to evaluate the antibacterial effects of *Khaya senegalensis* methanolic leaf extract on *Salmonella* species. The presence of phytochemicals in the methanolic extract of *Khaya senegalensis* leaf was tested using both qualitative and quantitative screening methods. The cultural and biochemical characteristics of clinical isolates of *Salmonella* spp. were determined. Susceptibility of bacteria to the extract was assessed by gauging the diameter of the inhibition zones. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extract were determined against the isolate. The results of quantitative and qualitative phytochemical analysis of the leaf extract of *Khaya senegalensis* showed the presence of alkaloids (0.234±0.10), flavonoids (0.019±0.30), saponins (0.563±0.20), phenols (0.044±0.30), tannins (0.023±0.12) and glycosides (0.067±0.20). At the same time, terpenoids were absent in the extract. The results of cultural and biochemical characteristics of clinical isolates of the bacteria *Salmonella* spp. showed colourless, smooth and transparent properties on MacConkey Agar. The biochemical test confirmed that the bacterium was methyl red, motility and catalase positive but Voges-Proskauer, citrate, coagulase, indole and oxidase negative. The results of the MIC and MBC revealed a significant zone of inhibition against *Salmonella* species by the plant extract at different concentrations. The highest zone of inhibition activity was recorded by the extract at 500mg/mL (38.00mm), at 400mg/mL (25.00mm), at 300mg/mL (21.00mm), at 200mg/mL (16.00mm), at 100mg/mL (11.00mm) and the standard (22.00mm). In conclusion, the results obtained indicated that methanolic leaf extract of *Khaya senegalensis* has an effect on preventing *Salmonella* infection and opens new perspectives in the strategies of controlling the prevalence of *Salmonella* by plant secondary metabolites.

**Keywords:** *Khaya senegalensis*, *Salmonella* species, phytochemical analysis, clinical isolates.

## INTRODUCTION

A Gram-negative intracellular intestinal bacterium with significant public health implications is *Salmonella* spp. (Li *et al.*, 2018). Salmonellosis is a significant zoonotic disease, which means it may infect its hosts and cause salmonellosis in both humans and animals. In both industrialized and developing nations, salmonellosis is the most typical bacterial foodborne infection

(Wibisono *et al.*, 2020). Millions of cases of both typhoidal and non-typhoidal salmonellosis were reported each year due to *Salmonella*, causing significant monetary losses as well as possible fatalities (Luvsansharav *et al.*, 2020). Non-typhoidal *Salmonella* (NTS) disease is most commonly associated with contaminated animal-based foods, particularly poultry meat and, in certain cases, vegetables (Pandey & Goud, 2021). According to the report of the European Food Safety Authority (EFSA), *Salmonella* is responsible for approximately 90,000 cases of zoonotic food-borne illnesses in the European Union (EU) each year (EFSA, 2017). *Salmonella* is frequently harmful to humans and animals if it is obtained orally. They spread from animals and their products to people, causing systemic infection, enteritis, and enteric fever (Carroll *et al.*, 2015). Infected humans and animals are frequently subjected to acute and chronic diarrhea, as well as death (Barbour *et al.*, 2015; Eng *et al.*, 2015).

*Salmonella* can infect a wide range of animals, but only humans have the *S. typhi* and *S. paratyphi* serotypes linked to typhoid fever. Therefore, cattle, domestic pets, and other animals are neither typhoid fever carriers nor vectors (Chakraborty *et al.*, 2023). Humans spread the illness to one another orally through feces, most frequently in environments with contaminated food or water. It is not unexpected that the poorest nations in the world, where water and sanitation systems are the weakest, have the highest illness burden. Salmonellosis that is not typhoidal (NTS) can infect animals besides people and causes a milder form of gastroenteritis (Mahindroo *et al.*, 2024).

There are several plants utilized in herbalism that are considered to have medicinal characteristics. According to DEBTEC (2003), medicinal plants are those that have healing qualities or have positive pharmacological effects on animal bodies. One or more of their components contain chemicals that have potential as therapeutic agents or as building blocks for the production of effective medicines (Ahmed *et al.*, 2024). Medicinal plants have been used in ancient times in almost all cultures as sources of medicine. The medicinal importance of certain plants has been almost as important as their food uses. Historically, plants have been found to be the principal source of drugs used in preventing and curing diseases (Abdallah *et al.*, 2023). The medicinal plants that thrive there may not have any obvious morphological traits, but they do have some unique qualities or virtues that make them valuable in medicine (DEBTEC, 2003). It is now known that plants that naturally produce and accumulate secondary metabolites, such as alkaloids, glycosides, and tannins, as well as those that contain minerals and vitamins, have therapeutic effects (Siddiqui *et al.*, 2022).

Numerous phytochemicals produced by plants have been proven to have positive impacts on human health over the long term when ingested and can be used to successfully cure human ailments (Yang & Ling, 2024). Global health services greatly benefit from the use of traditional and folk medicines. About 75 percent of people worldwide rely on plant extracts and other plant products for their health. Our populace relies heavily on herbal cures, especially those who live in the villages. For the treatment of many different ailments, including allergies, metabolic, cardiovascular, and others, the majority of these herbal medicines have shown effectiveness throughout time (Wong *et al.*, 2025).

The extraction and development of medicine have led to an increased reliance on the use of medicinal plants in industrialized nations (UNESCO, 1999). In several developing countries, using local traditions and beliefs is still the mainstay of healthcare. Traditional medicine is widely used in nations like China, India, Thailand, and Africa. Traditional medicines account for around 40% of all medical usage in China (Oyebode *et al.*, 2016). In Japan, herbal medicinal preparations are more sought-after than conventional pharmaceuticals (Inoue *et al.*, 2017). Both herbal traditional medicine and orthodox medicine are practiced in Nigeria, while doctors with conventional training are considering the former. In locations with poor sanitation and hygiene, the illness burden is frequently unevenly distributed (Majumdar *et al.*, 2025). A major concern is the need to reduce the use of antibiotics and other allopathic medicinal products, largely due to concerns over bacterial resistance (Bassetti *et al.*, 2022). Despite the widespread use of the traditional method of healthcare delivery and the accessibility of medicinal plants in our local market, there are very few, if any, of them in public health facilities. There is a need to investigate the different phytochemical compounds in plant extracts with minimal side effects and greater potential as antibacterial compounds against microorganisms for the possible development of new drugs for the prevention and treatment of infectious diseases caused by bacterial pathogens. Different secondary metabolites possess various biological properties, and there is little knowledge about the mechanisms by which these compounds can help in the treatment of infectious diseases caused by *Salmonella*. This study was aimed at evaluating the antibacterial effects of methanolic leaf extract of *Khaya senegalensis* on *Salmonella* species.

## MATERIALS AND METHODS

### Plant Material Collection, Identification and Processing of Plant Sample

Fresh leaves of the plant *Khaya senegalensis* were collected from Kaduna North Local Government Area of Kaduna State in Nigeria. The plant was identified at the herbarium unit of the Department of Biological Sciences, Nigerian Defence Academy. The leaves of the plant *Khaya senegalensis* used for the study were plucked from the stem, washed with distilled water and then dried at room temperature under shade. The dried sample was then ground into powder in the laboratory using a mortar and pestle. The powdered sample was kept in a closed plastic container for later use during the experiment.

### Extract Preparation

About 250 grams of the plant sample were cold-extracted by maceration with 1.5 litres of methanol. The extract was filtered

using Whatman No. 1 paper. The methanolic extract filtrate was then evaporated initially in a hot-air oven at 40°C for 72 hours before being lyophilised under vacuum at -80°C and a pressure of 0.06 mbar, after which the dried/powdered extract was weighed, and the percentage yield was calculated, stored at -20°C for further analysis (Kong *et al.*, 2014).

## QUALITATIVE PHYTOCHEMICAL ANALYSIS

The phytochemical analysis was carried out following the procedures described by Harborne (1992). The secondary metabolites screened include:

### Alkaloids

To test for alkaloids, 0.1 mg of each plant extract was measured into 6 mL of diluted HCl. The mixture was boiled at 85°C, allowed to cool at 40°C and filtered using a small 1inch sieve. The presence of alkaloids in filtrates was tested using Dragendorff's reagent, Meyer's reagent and Wagner's reagent, each in a separate container.

### Terpenoids

In order to test for terpenoids, 1 mL of each extract was dispensed into 10 mL of deionized water before adding 3 drops of ferric chloride to the solution.

### Flavonoids

The flavonoid composition in the samples was determined by boiling 0.2 mg of each extract for 3 minutes at 40°C in 10 mL of ethyl acetate, allowing it to cool at room temperature and filtering it using a small 1inch sieve before introducing 4 mL of the filtrate into 1ml of dilute ammonia solution and shaking vigorously for about 1 minute.

### Saponins

The presence of saponin was determined by adding 5 mL of each plant extract into 20 mL of deionized water and shaking vigorously.

### Phenols

To test for the presence of phenols, 0.1 g of each sample was added to 10 mL of distilled water. The solution was heated in a water bath for 3 minutes, then filtered using a small sieve.

### Tannins

About 0.5g of the powdered samples were mixed in 20 mL of distilled water in a test tube and boiled for 5min at 40°C, then filtered using a 2inch sieve before adding 0.1% FeCl<sub>3</sub>.

### Glycosides

The presence of glycosides in the samples was tested by adding 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to 5 ml of aqueous plant extract mixed with 2 ml of glacial CH<sub>3</sub>CO<sub>2</sub>H containing 1 drop of FeCl<sub>3</sub>.

## QUANTITATIVE PHYTOCHEMICAL ANALYSIS

The phytochemicals that are present in the methanolic extract were determined and quantified by standard procedures.

### Determination of total phenols

One hundred milligrams (100 mg) of the extract of the sample was weighed accurately and dissolved in 100 mL of triple distilled water (TDW). One milliliter (1 mL) of this solution was transferred to a test tube, then 0.5 ml 2N of 2 N Folin-Ciocalteu reagent and 1.5 mL of

20%  $\text{Na}_2\text{CO}_3$  solution were added. Ultimately, the volume was made up to 8 mL with TDW, followed by vigorous shaking and finally allowed to stand for 2 hours, after which the absorbance was taken at 765nm. The data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Hagerman *et al.*, 2000).

#### Determination of total flavonoids

The method is based on the formation of the flavonoids-aluminium complex, which has an absorptivity maximum at 415nm. One hundred microliters (100  $\mu\text{l}$ ) of the sample extracts in methanol (10 mg/mL) were mixed with 100  $\mu\text{l}$  of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 mL. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 mL of sample extracts and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorption of standard rutin solution (0.5 mg/mL) in methanol was measured under the same conditions. All determinations were carried out in triplicate (Kumaran & Karunakaran, 2006).

#### Determination of total alkaloids

Five grams (5 g) of the sample was weighed into a 250 mL empty beaker, and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 h. This was filtered using Whatman filter paper, and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborne, 1992).

#### Determination of total tannins

Five hundred milligrams (500 mg) of the sample was weighed into a 50 mL plastic bottle. Fifty milligrams (50 ml) of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M  $\text{FeCl}_3$  in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 mins (Van-Burden & Robinson, 1981).

#### Determination of total saponins

The samples were ground, and 20 g of each was put into a conical flask, and 100 cm<sup>3</sup> of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered using Whatman filter paper, and the residue was re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel, and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, and 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight, and the saponin content was calculated (Obdoni & Ochuko, 2001).

#### Determination of Glycosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy (1994). One gram (1g) of the fine powder of *Khaya senegalensis* leaves was soaked in 10mL of 70% alcohol for 2 h and then filtered using Whatman filter paper. The extract obtained was then purified using lead acetate and  $\text{Na}_2\text{HPO}_4$  solution before the addition of freshly prepared Buljet's reagent (containing 95mL aqueous picric acid + 5mL 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

#### Collection of Test Bacteria

The clinical isolate of *Salmonella* species used in this study was collected from Yusuf Dan Tsoho Memorial Hospital, Tudun Wada, Kaduna. *Salmonella* type strain of a similar isolate was collected from the National Veterinary Research Institute (NVRI), VOM, Jos, Plateau State, to serve as a positive control. All samples were collected in nutrient agar slants, labelled, placed in a cold box and transported to the postgraduate laboratory of the Biochemistry Department, Kaduna State University. Samples were incubated at 37°C for 24 hours.

#### Bacterial Culture

An inoculum from an overnight growth culture of *Salmonella* species was streaked on a freshly prepared plate of MacConkey Agar (MA). The plates were incubated for 24 hours at 37 °C to obtain a pure culture of the test isolates. The medium for the isolates was prepared according to the manufacturer's instructions. Following incubation, *Salmonella* species were expected to produce pale, colourless or translucent colonies on MacConkey Agar, indicating non-lactose fermentation, since *Salmonella* does not ferment lactose. The media for the isolate were prepared according to the manufacturer's instructions.

#### IDENTIFICATION OF SALMONELLA SPECIES

##### Gram Staining and Microscopy

Gram's staining was used to identify the bacterial species by their Gram's reaction and morphological characteristics. An inoculum from a pure bacterial isolate was emulsified into a drop of distilled water on a glass slide using a sterile wire loop. It was smear-fixed by gentle heating and then flooded with crystal violet stain for 60 seconds. The stain was washed and flooded with lugol's iodine for 60 seconds. It was thoroughly washed again with water. The smear was further decolorized with alcohol and immediately washed again with water. It was then stained with safranin for 60 seconds and then washed with water, rinsed and air-dried. The slide was finally observed under the microscope at x100 oil immersion objective lens (Chees-brough, 2016).

#### BIOCHEMICAL TESTS OF SALMONELLA SPECIES

##### Indole Test

Using a sterile wire loop, a colony of the isolate was aseptically inoculated into 5 mL peptone water and incubated at 37°C for 24 hours. After the incubation period, three drops of Kovac's indole reagent were added. It was shaken gently, and the colour changed from yellowish to pinkish, indicating a positive result (Salwan *et al.*, 2023).

#### Methyl Red Test

Using a sterile wire loop, a colony of the isolate was inoculated into 5 mL MR-VP broth and incubated at 35°C for 48 hours. After the incubation period, 1 mL of the broth was transferred into a small bottle, and 5 drops of methyl red were added. A change of colour was noted on addition of the methyl red from yellow to red colour, hence confirming a positive result (Ikram *et al.*, 2022).

#### Voges-Proskauer Test

To the aliquots of each broth culture separated during the methyl red test, 10 drops of Barritt's reagents were added and shaken every 3 minutes; the absence of deep rose colour 15 minutes after the addition of Barritt's reagent indicated a positive or negative test (AL-Joda *et al.*, 2021).

#### Citrate Utilization Test

The isolates were inoculated into 5 mL of Simon's citrate agar slants and incubated for 24 hours at 37°C. The colour changed from greenish to deep blue, indicating a positive reaction. Meanwhile, no colour change indicated a negative reaction (Jannesari *et al.*, 2023).

#### Coagulase Test

One colony of the test organism was emulsified on a clean slide and placed into two drops of normal saline. To one, a drop of citrate human plasma was added and mixed. Clumping within 2 seconds indicated a positive reaction (Mahmood *et al.*, 2022).

#### Catalase Test

A colony was emulsified in a drop of 3% hydrogen peroxide and was placed on a clean glass slide. Frothing or bubbling confirmed a positive reaction (Jannesari *et al.*, 2023).

#### Motility Test

A sterile straight wire loop was used to inoculate a test bacterium down the center of motility medium by means of stab inoculation. Cultures were inoculated for 24 hours at 37°C. Growth of non-motile organisms was confined to the line of inoculation. Motility was recognized when culture growth spread through the medium and was not restricted to the line of inoculation, showing slightly opaque coloration (Palma *et al.*, 2022).

#### Oxidase Test

A few drops of oxidase reagent were added to a colony of bacteria grown on plate medium. No colour change, or light pink colouration on the colony, was indicative of the absence of oxidase activity and was a negative test. Development of dark purple colouration indicated a positive test.

The results of biochemical tests were compared with the standard biochemical tables provided by Chees-brough (2000) and Cappuccino and Sherman (2011).

### SUSCEPTIBILITY STUDIES OF SALMONELLA SPECIES

#### Preparation of Extract Concentration

About 1 gram each of the methanolic crude extracts of *Khaya senegalensis* was weighed accurately and dissolved in 10 mL each of 10% dimethyl sulfoxide (DMSO) to give a concentration of 100mg/mL stock solutions of each concentration. Using two-fold serial dilution, concentrations of 50mg/mL, 25mg/mL and 12.5mg/mL were prepared and used for treatment.

#### Preparation of Turbidity Standard and Standardization of Bacterial Inoculum

MacFarland standard was prepared by diluting 1 mL of concentrated sulphuric acid with 99 mL of sterile distilled water (1% v/v). Another 1% (w/v) Solution of Barium chloride was also prepared by dissolving 0.5g of dehydrated Barium chloride in 50 mL distilled water. About 0.6 mL of the Barium chloride solution was mixed with 99.4 mL of H<sub>2</sub>SO<sub>4</sub> solution to yield a 1% w/v Barium sulphate suspension. The turbid solution (McFarland scale No. 1) was used as a reference to adjust the turbidity of the bacterial suspension. Some quantity of test bacteria from a freshly grown overnight culture was added to 2 mL of sterile physiological saline as a suspension medium. The bacterial suspension was compared to the 0.5 McFarland standard (1.5×10<sup>8</sup> CFU/mL) under a white background with contrasting black lines (Chees-brough, 2016).

### ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF *KHAYA SENEGALENSIS* ON CLINICAL AND STANDARD STRAINS OF *SALMONELLA* SPECIES.

#### Preparation of Culture Media

Mueller Hinton agar was prepared according to the manufacturer's specifications. The Mueller-Hinton agar was prepared by weighing 38 grams of the powdered agar into 1000ml of distilled water in a clean conical flask. It was soaked for 20 minutes and then covered with cotton wool and foil paper, autoclaved at 121°C and 115 psi for 15 minutes. The medium was cooled to 50°C, and 4ml of the medium was poured into a sterile glass Petri dish and allowed to solidify. The sterility of the medium was tested by allowing it to stay overnight and checking for contamination. A flaming wire loop was used to inoculate the organism by streaking.

#### Agar Well Diffusion Method

The agar well diffusion method of Doughari *et al.* (2007) was adopted for the determination of the antibacterial activity of methanolic extracts of *Khaya senegalensis* against the clinical and reference isolates of *Salmonella* species. Using a micropipette, about 100 µl of standardized inocula of a bacterial suspension was inoculated into a Mueller-Hinton agar plate (in triplicate) and spread evenly over the entire surface of the plates using a sterile cotton swab stick. The plates were left for 10 minutes before wells of 9mm were dug in the agar using a sterile cork borer. About 100 µl volumes of the various concentrations of extracts (100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL) were each filled into the wells. Additional wells were filled with 10% DMSO to serve as negative controls. The plates were then left for 10 minutes at 25 °C for diffusion of extracts into the agar to take place and then incubated at 37°C for 24 hours. For each bacterium tested, the zone of inhibition of growth was examined, and the diameter of each zone was recorded in millimetres with a meter rule. The means were calculated to the nearest whole number. The experiment was performed in replicates.

#### Determination of Minimum Inhibitory Concentration (MIC)

The MICs of crude extract against the clinical and reference isolates of *Salmonella* spp. were determined using the broth dilution method. The following concentrations of extracts: 100 mg/ml, 50 mg/ml, 25 mg/mL and 12.5 mg/mL were prepared by two-fold serial dilutions. One milliliter (1 mL) of each of the various extract concentrations was added to a test tube containing 9 mL of

Mueller-Hinton broth. About 100µl each of a standardized inoculum of the test bacterium was added to mixtures of different concentrations of extracts with Mueller-Hinton broth. The test tube was incubated at 37°C for 24 hours. The growth of bacteria in the broth examined was indicated by the turbidity of the broth. However, the lowest concentration of extract that inhibited the growth of a test organism was recorded as the Minimum Inhibitory Concentration (MIC) (Andrew, 2011). Negative control was set up as follows: Mueller-Hinton broth only and Mueller-Hinton broth with extract. The positive control comprised Mueller-Hinton broth with a typed strain and Mueller-Hinton broth with an antibiotic (Ciprofloxacin).

#### Determination of Minimum Bactericidal Concentration (MBC)

The positive MIC tubes were sub-cultured on nutrient agar plates with proper labels, followed by incubation at 37°C for 24 h. They were examined for bacterial growth. The tube with the minimum concentration of extract in which growth was completely stopped was noted as the minimum bactericidal concentration. The negative controls were nutrient agar only and nutrient agar with extracts only (Andrew, 2011).

#### Determination of Diameter Zone of Inhibition

The diameter of the zone of inhibition for each test organism was determined by the paper disc diffusion method. Plates of Mueller-Hinton agar were prepared and inoculated in triplicate with the test organisms by the spread plate method. Paper discs of 6 mm diameter were oven-sterilized and impregnated with each extract at 15.63 mg mL<sup>-1</sup>, 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 measured as the diameter of the zones of inhibition.

## RESULTS

#### The Percentage Yield of *Khaya senegalensis* Leaf Extract

The percentage yield of *Khaya senegalensis* leaf extract is approximately 13%, as shown in Table 1. About 250 g of the leaf powder prepared from *Khaya senegalensis* was used for the extraction. With 70% methanol, 32.78 g of extract was obtained as concentrate and a percentage yield of 13.11 % was obtained (Table 1).

**Table 1:** Percentage yield of *Khaya senegalensis* Crude Leaf Extract

| Plant Material            | Initial Weight of Sample (g) | Weight of Extract (g) | Yield of Extract (%) | Extract Characteristic     |
|---------------------------|------------------------------|-----------------------|----------------------|----------------------------|
| <i>Khaya senegalensis</i> | 250.00                       | 32.78                 | 13.11                | Greenish and gummy texture |

#### Qualitative and Quantitative Phytochemical Analysis

The results of qualitative and quantitative phytochemical analysis of the leaf extract of *Khaya senegalensis* are presented in Tables 2 and 3. The qualitative analysis of the extract showed the presence of alkaloids, flavonoids, saponins, phenols, tannins and glycosides, while terpenoids were absent in the extract. The quantitative analysis of the extract revealed total phenols (134.77±3.10 µgGAE/mL), total flavonoids (46.19±0.30 µgRE/mL), total alkaloids (163.66±0.20 µg/mL), total tannins (188.56±0.19 µgTAE/mL), total saponins (231.44±0.52 µgDE/mL) and total glycosides (367.33±2.20 µgCNE/mL).

**Table 2:** Qualitative Phytochemical Screening of Methanolic Extract of Leaf of *Khaya senegalensis*.

| Phytochemical Compounds | Leaf Extract | Absorbance (nm) |
|-------------------------|--------------|-----------------|
| Alkaloids               | +            | 0.234±0.10      |
| Terpenoids              | -            | -               |
| Flavonoids              | +            | 0.019±0.30      |
| Saponins                | +            | 0.563±0.20      |
| Phenols                 | +            | 0.044±0.30      |
| Tannins                 | +            | 0.023±0.12      |
| Glycosides              | +            | 0.067±0.20      |

- = Absent; + = Present. Each value is a mean of three determinations ± SEM

**Table 3:** Quantitative Phytochemical Screening of Methanolic Extract of Leaf of *Khaya senegalensis*.

| Phytochemical Compounds | Absorbance (nm)      |
|-------------------------|----------------------|
| Total Phenols           | 134.77±3.10 µgGAE/mL |
| Total Flavonoids        | 46.19±0.30 µgRE/mL   |
| Total Alkaloids         | 163.66±0.20 µg/mL    |
| Total Tannins           | 188.56±0.19 µgTAE/mL |
| Total Saponins          | 231.44±0.52 µgDE/mL  |
| Total Glycosides        | 367.33±2.20 µgCNE/mL |

The values are the means of three replicates ± standard error of the mean SEM. GAE = Gallic acid equivalents, RE = Rutin equivalents, TAE = Tannic acid equivalents, DE = Diosgenin equivalents and CNE = Cyanogen equivalents.

#### Biochemical Identification of Clinical Isolates

From the results of cultural and biochemical characteristics of the clinical isolates. *Salmonella* spp. showed colourless, smooth and transparent properties on MacConkey Agar. The biochemical tests confirmed that the bacterium was methyl red, motility and catalase positive, but Voges-Proskauer, citrate, coagulase, indole and oxidase negative, as shown in Table 4.).

**Table 4:** Cultural and Biochemical Characteristics of *Salmonella* species

| Bacteria               | Colony morphology               | Gram reaction | Cell morphology | Biochemical test |    |    |    |    |    |    |    |
|------------------------|---------------------------------|---------------|-----------------|------------------|----|----|----|----|----|----|----|
|                        |                                 |               |                 | IN               | MR | VP | CI | MO | CA | CO | Ox |
| <i>Salmonella</i> spp. | Colourless, smooth, transparent | Negative      | Rod shaped      | -                | +  | -  | -  | +  | +  | -  | -  |

**Key:** IN= Indole, MR= Methyl red, Mo= Motility, Ox= Oxidase, CI= Citrate, VP= Voges-Proskauer, CO= Coagulase and CA= Catalase.

#### Antimicrobial Susceptibility Tests

The results of the antimicrobial susceptibility tests were expressed in terms of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and diameter of zones of inhibition of the test organism with respect to the methanolic extract and ciprofloxacin as standard control, as shown in Tables 4, 5, 6 and 7, respectively. A zone of inhibition measuring more than 8mm signifies that bacteria are susceptible to the tested antibiotics. The results of the MIC and MBC revealed a significant zone of inhibition against *Salmonella* species by the plant extract at different concentrations. The extract showed strong MIC activity of  $23.47 \pm$

$0.033$  at 500mg/ml,  $18.50 \pm 0.320$  at 400mg/mL,  $13.80 \pm 0.000$  at 300mg/ mL,  $11.00 \pm 0.010$  at 200mg/ mL,  $9.07 \pm 0.067$  at 100mg/ mL and the standard  $15.07 \pm 0.067$  at 500mg/ mL. The MBC analysis showed the activity of the extract at 500mg/ mL ( $33.13 \pm 0.186$ ), at 400mg/ mL ( $22.07 \pm 0.067$ ), at 300mg/ mL ( $18.00 \pm 0.000$ ), at 200mg/ mL ( $16.10 \pm 0.058$ ), at 100mg/ mL ( $13.33 \pm 0.043$ ) and standard ( $27.53 \pm 0.033$ ). Highest zone of inhibition activity was also recorded at 500mg/ mL (38.00mm), at 400mg/ mL (25.00mm), at 300mg/ mL, (21.00mm), at 200mg/ mL (16.00mm), at 100mg/ mL (11.00mm) and standard (22mm) as shown in Table 7.

**Table 5:** Minimum Inhibitory Concentration (MIC) of Methanolic Extract of the leaf of *Khaya senegalensis*.

| Bacteria               | MIC $\pm$ Standard error of the mean |                   |                   |                   |                  |                   |
|------------------------|--------------------------------------|-------------------|-------------------|-------------------|------------------|-------------------|
|                        | Concentration (mg/mL)                |                   |                   |                   |                  |                   |
|                        | 500                                  | 400               | 300               | 200               | 100              | CX                |
| <i>Salmonella</i> spp. | $23.47 \pm 0.033$                    | $18.50 \pm 0.320$ | $13.80 \pm 0.000$ | $11.00 \pm 0.010$ | $9.07 \pm 0.067$ | $15.07 \pm 0.067$ |

Values are expressed as mean  $\pm$  standard error of the mean, which are significantly different ( $P < 0.05$ ) using the Bonferroni correction; N = 3.  
Key: CX = Ciprofloxacin.

**Table 6:** Minimum bactericidal concentration (MBC) of Methanolic Extract of the leaves of *Khaya senegalensis*.

| Bacteria               | MBC $\pm$ Standard error of the mean |                   |                   |                   |                   |                   |
|------------------------|--------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                        | Concentration (mg/mL)                |                   |                   |                   |                   |                   |
|                        | 500                                  | 400               | 300               | 200               | 100               | CX                |
| <i>Salmonella</i> spp. | $33.13 \pm 0.186$                    | $22.07 \pm 0.067$ | $18.00 \pm 0.000$ | $16.10 \pm 0.058$ | $13.33 \pm 0.043$ | $27.53 \pm 0.033$ |

Values are expressed as mean  $\pm$  standard error of the mean, which are significantly different ( $P < 0.05$ ) using the Bonferroni correction; N = 3.  
Key: CX = Ciprofloxacin.

**Table 7:** Diameter (mm) of the Zone of Inhibition of Methanolic Extract of *Khaya senegalensis*.

| Bacteria               | Mean zone of inhibition (mm) |     |     |     |     |               |
|------------------------|------------------------------|-----|-----|-----|-----|---------------|
|                        | Concentration (mg/ mL)       |     |     |     |     |               |
|                        | 500                          | 400 | 300 | 200 | 100 | Ciprofloxacin |
| <i>Salmonella</i> spp. | 38                           | 25  | 21  | 16  | 11  | 22            |

#### DISCUSSION

Given the rising levels of antibiotic resistance among harmful bacteria, the quest for novel antibacterial medicines has grown significantly in importance recently. The utilization of medicinal herbs, which are widely accessible, have no negative side effects, are less expensive, and have demonstrated antibacterial qualities, is one of the research efforts (Rubeus *et al.*, 2015). Additionally, the therapeutic benefits of medicinal plants are well known on a

global scale, particularly for the creation of antibiotics. As a result, research on complementary and potent plant-based medications to combat these resistant bacteria has gained significant global attention (Angelini, 2024). Misuse of antibiotics is thought to be a major factor in the development of antibiotic-resistant microorganisms. As a result, bacteria develop resistance to antibiotics, which, when used over extended periods of time, become less effective. Pharmaceutical firms that specialize in the

development and production of antibiotics work hard to create new generations of antibiotics that can effectively treat these antibiotic-resistant bacterial strains (Terreni *et al.*, 2021; Brüssow, 2024).

*Khaya senegalensis*' phytochemical screening revealed the presence of flavonoids, saponins, tannins, alkaloids, glycosides, and phenols but revealed the absence of terpenoids. This concurs with the conclusions made by Sani *et al.* (2012). These chemical compounds may have been the cause of the plant extract's antibacterial activity (Alibi *et al.*, 2021). Tannins have also been used in the leather processing industry and have been proven to have antibacterial and antiviral potential (Villanueva *et al.*, 2023). The ability of tannins to create proline-rich proteins, which inhibits the creation of cell walls, has been discovered through recent research on the benefits of tannins against cancer through several methods. This finding may help to explain how these extracts work. Alkaloid is one of a sizable number of nitrogen-containing compounds produced by plants. Alkaloids frequently have strong pharmacological effects. Cocaine, nicotine, strychnine, caffeine, morphine, pilocarpine, atropine, methamphetamine, mescaline, ephedrine, and tryptamine are only a few of the alkaloids that have been identified (Heinrich *et al.*, 2021). According to Donatus and Vitus (2018), alkaloids found in the stem-barks and a leaf of *Mangifera indica* promotes its medicinal relevance especially for malaria and fever treatment in Nigeria. Saponins are substances that act as natural antibiotics, aiding the body's defense against illnesses and microbial invasion (Okwu and Emenike, 2016). These chemicals also appear to improve the efficacy of some vaccines significantly. They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting potential for saponins is not how they inhibit or kill cancer cells, but rather that they could do so without harming healthy cells, as some cancer-fighting drugs do (Okwu and Emenike, 2016). According to Danladi *et al.* (2008), *K. senegalensis* extract contained the aforementioned phytochemicals which are responsible for the observed antibacterial activities. This corroborates the findings in the present study. According to Clinical and laboratory standard institute CLSI (2005), any plant material should be considered an effective therapeutic agent if it extracts produces zones of inhibition of between 15-22mm on the target pathogenic organism. Activity of plant extracts to test bacteria is normally expressed in-vitro as zones of inhibition in millimeter (greater than or equals to 7mm) around the organism on test (Gislene *et al.*, 2000).

The present study has provided evidence that methanolic extract of *K. senegalensis* exhibit antibacterial properties against *Salmonella* species, which suggests that they may be clinically useful. Preliminary phytochemical analyses revealed that *K. senegalensis* consisted of flavonoids, alkaloids, saponins, tannins, glycosides and phenols. These bioactive compounds have been reported to be used by plants for protection against bacteria and are responsible for antimicrobial activity (Álvarez-Martínez *et al.*, 2021; Victory *et al.*, 2022). *K. senegalensis* was shown to be potentially developed as antibacterial agents for *Salmonella* species. Antimicrobial effect of the plant extract was ascertained or determined against the activity of microbes (*Salmonella* spp.). Different concentrations of the extract (500mg/mL to 100mg/mL serial dilutions), and ciprofloxacin (standard drug) were tested against the growth or activity of *Salmonella* spp.

The results of the maximum inhibitory concentration (MIC) and maximum bactericidal concentration of the extract showed antimicrobial activity against the bacteria. All the concentrations have effects on the bacteria in dose-dependent manner with a

concentration of 500mg/mL producing the best MIC and MBC against the bacteria, followed by 400mg/mL, 300mg/mL, 200mg/mL and 100mg/mL was the lowest as observed. Clear zones of inhibition were observed for all the varied concentrations of the extract against the growth of the bacteria, with 500mg/mL of the extract showing much more effect on the bacteria with the concentration of 100mg/mL having the lowest zone of inhibition. On the other hand, the standard drug inhibited the growth or action of the microbe more compared to the extracts 300mg/mL, 200mg/mL and 100mg/mL concentrations. This shows how the extract can be considered as an effective antimicrobial agent at a higher concentration. Therefore, the higher the concentration of the extract, the higher the zone of inhibition. According to Baker and Silverton (1985), an organism is considered sensitive to a chemical agent only when the zone of inhibition is either equal to the control, more than or not more than 3mm smaller than the control. The highest zone of inhibition of the extract against bacteria are 38.00 mm, 25.00 mm, 21.00 mm, 16.00 mm and 11.00 mm at 500 mg/mL, 400mg/mL, 300mg/mL, 200mg/mL and 100mg/mL for methanolic extract respectively and 27.00 mm for the control (ciprofloxacin). These data are above the reference range of the Clinical Laboratory Standard Institute (Bagchi *et al.*, 2002), as such are considered sensitive in this research work. All active extracts exhibited antibacterial activity that varied with concentration. A classification method based on MIC findings for plant materials was proposed by Aligiannis *et al.* (2001), and as a result, it was explained and put into practice by Duarte *et al.* (2005). According to Fabry *et al.* (1998), all plant species with MIC values up to 8 mg/mL are thought to have at least some inhibitory effect, and any dose below this should not be regarded effective.

## Conclusions

The findings of this study revealed the antibacterial properties of *K. senegalensis* leaf extract. The observed antibacterial activity might be attributed to the presence of flavonoids, alkaloids, saponins, tannins, steroids/terpenoids and anthraquinones which are known to possess efficient antibacterial activity that can be harnessed for the production of new antibiotics or the enhancement of already existing antibiotics for *Salmonella* species.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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