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Phytochemistry, Antibacterial Properties, and FT-IR Spectra of *Khaya senegalensis* Stem Bark Extracts against *Escherichia coli* 0157: H7 and *Salmonella typhimurium*

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Abstract

The increasing prevalence of multidrug-resistant bacterial pathogens has intensified the search for alternative antimicrobial agents derived from medicinal plants. This study investigated the phytochemical composition, antibacterial activity, and Fourier-transform infrared (FTIR) spectra of methanolic and n-hexane extracts of *Khaya senegalensis* stem bark against *Escherichia coli* O157:H7 and *Salmonella typhimurium*. The extracts were obtained by reflux extraction, and their phytochemical constituents were quantified. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations were determined using broth microdilution and subculture methods, respectively, while functional groups were characterized using a PerkinElmer 100-series FTIR spectrometer. The methanolic extract contained higher concentrations of phenols, flavonoids, alkaloids, and tannins, whereas the n-hexane extract contained higher concentrations of saponins and steroids. The methanolic extract exhibited greater antibacterial activity, with *E. coli* O157:H7 showing the highest susceptibility (MIC = 3.12 mg/mL), followed by *S. typhimurium* (MIC = 12.5 mg/mL). By comparison, the n-hexane extract produced an MIC of 25 mg/mL against both bacterial isolates. The FTIR spectrum of the methanolic extract displayed five absorption peaks, including bands at 3271.86 and 1604.91 cm^{-1} associated with terminal alkyne C–H and aromatic C=C stretching, respectively, and bands at 1031.28, 562.00, and

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552.94 cm^{-1} within the fingerprint region. The n-hexane extract exhibited bands corresponding to O–H stretching, aromatic C=C stretching, N–O stretching, alkane C–H bending, C–O–C stretching, and C–O stretching. These findings demonstrate that the antibacterial activity of *K. senegalensis* stem bark varies according to solvent polarity and phytochemical composition, with the methanolic extract showing superior efficacy against the tested organisms. The study provides preliminary scientific support for the traditional use of *K. senegalensis* stem bark in managing diarrhoea, dysentery, and other gastrointestinal ailments, while further toxicological and in vivo investigations are required to establish its therapeutic applicability.

Keywords: *Khaya senegalensis*; Antibacterial Activity; *Escherichia coli* O157:H7; *Salmonella typhimurium*; FTIR Spectroscopy

INTRODUCTION

The advancing challenge of microbial infections, increased by the rise of multidrug resistant bacterial pathogens, has emphasized the urgent need for alternative and more effective treatment options (Davis and Choisy, 2024). Traditional herbal medicine remains a critical alternative to conventional antibiotics in many developing nations, these bioactive compound-rich plants have become well-known for their antiviral, antifungal, and antibacterial qualities (Murtala *et al.,* 2026).

For millennia, plants have served as a natural source of medicine, offering a wealth of bioactive compounds with therapeutic properties (Lawal *et al.,* 2022). Investigating these plant-derived compounds has resulted in the discovery of new medications and treatments, establishing plants as an essential resource in the continuous effort to tackle global health challenges (Abdullahi *et al.,* 2024).

Khaya senegalensis (African mahogany), a member of the *Meliaceae* family, has long been used in African ethnomedicine for the treatment of malaria, gastrointestinal disturbances, jaundice, fever, and parasitic infections, traditional applications emphasize bark decoctions, leaf poultices, and seed preparations as essential remedies. Pharmacological studies have validated many of these practices by identifying limonoids, flavonoids, and tannins with proven antimicrobial, antioxidant, hepatoprotective, and antimalarial properties (Poonguzhali *et al.,* 2025)

Modern scientific methods, including advanced phytochemical analysis, plant tissue culture, and biotechnology, are now paving the way for the discovery and standardized production of valuable natural substances. By applying these rigorous *in vitro* and *in vivo* methodologies to traditionally revered plants like *Khaya senegalensis* (Dahiru *et al.,* 2025). It is estimated that humanity has discovered only a fraction of the bioactive compounds existing in diverse plant species, indicating that the prospects for research in natural medicine are exceptionally bright (Nnamani *et al.,* 2025).

Escherichia coli O157:H7 is the predominant and most virulent Serotype of *E. coli* associated with bloody and non-bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). Ingestion of E. *coli* O157:H7 with contaminated food products of animal origins and following contact with infected animals or the contaminated environment has led to human infection.

Additionally, antimicrobial resistance among enteric bacteria is an increasing global public health concern with *E. coli* O157:H7 causing 2.8 million acute illnesses annually.

Among bacterial pathogens, non-typhoidal serovars of *Salmonella enterica,* such as serovar Typhimurium are a primary cause of foodborne illnesses that lead to hospitalizations and deaths worldwide, *S. Typhimurium* causes acute inflammatory diarrhea that can progress to invasive systemic disease in susceptible patients (Anderson and Kendall, 2017).

S. Typhymurium is a Nationally notifiable disease with a wide range of hosts such as rodents, cattle and mammals (Gart *et al.,* 2016), Equipped with multiple virulence factors encoded in their genes; Pathogenicity Island (SpI) and many other factors that may enhance the fitness and Virulence (Lucas *et al.,* 2010).

Seeing the pathogenesis and the worrying global records of damages caused by *E. coli* 0157:H7 and *Salmonella enterica* serovar Typhimurium and the adverse effects of using synthetic antibiotics for their treatment, it is in this regard that this research work studied the therapeutic potency of stem bark extracts of *Khaya senegalensis* against *E.coli* 0157:H7 and *Salmonella enterica* serovar Typhimurium in the quest for discovering a natural, more effective and safer drug for their treatment.

MATERIALS AND METHODS

Collection of plant materials

Stem barks of *Khaya senegalensis* were collected within Vunoklang community, Girei local government area, Adamawa State, Nigeria between February and April, 2023. The plant was identified and authenticated using the Herbarium Specimens of the Department of Plant Science, Faculty of life Sciences, Modibbo Adama University, Yola. With the Voucher number: *Khaya senegalensis* -**02**



Fig1: Map of Vunoklang community, Girei Local government Area, Adamawa State (www.google.com/maps)

Test organisms

Escherichia coli 0157:H7 and *Salmonella enterica* serovar Typhimurium were obtained from the department of Microbiology, Central diagnostic, National institute of veterinary research VOM, Jos south, Plateau State Nigeria.

Preparation of Crude Plant Extracts

Preparation of plant materials

The plants materials (Stem barks) were shade-dried until a constant weight was obtained after which they were crushed with mortar and pestle and grinded into powder using a grinder (Ewansiha *et al.,* 2016), the powdered plant materials were sieved and stored in an air tight container until further use.

Extraction

The reflux extraction method was used as described by Ewansiha *et al.* (2017), to obtain the crude extract at a temperature of 30°C for 3 hours, this was achieved by dissolving 100 grams of the dried plant material in 400 ml of the extracting solvent (Methanol and n-hexane). After 3 hours of refluxing, the mixture was filtered through a

Whatman No 1 filter paper with pore size of 0.7 μm to obtain a clear filtrate which was concentrated to a semisolid substance with the use of a hot air oven.

Quantitative determination of phytochemical constituents

Quantification of Flavonoids

Ten grams (10 g) of plant extract was measured and 100 mL of 80% aqueous methanol was added repeatedly. After that, by use of Whatman filter paper, filtration of the solution was performed. Later, the filtered solution was vaporized under water bath until it become dried completely and was weighed to obtain constant weight (Hashmi *et al.,* 2021).

Quantification of Terpenoids

Powder form of 10 g of each extract was soaked in alcohol for a day. Later it was filtered and petroleum ether was used for purpose of extraction. The extracted material was calculated and considered as Terpenoids (Hashmi *et al.,* 2021).

Quantification of total Saponins

Twenty grams (20 g) of each extract was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml 20% ethanol. The combined extracts was reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator 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. 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 was dried in the oven to a constant weight which is the saponin content (Ajiboye *et al.,* 2013).

Quantification of total Alkaloids

Five grams (5g) of the extract was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitated was

collected and washed with dilute ammonium hydroxide and then filtered. The residue is the Alkaloid, which was dried and weighed (Ajiboye *et al.,* 2013).

Quantification of total Tannins

Five hundred milligram (500 mg) of the sample was weighed into a 50 ml plastic bottle. 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 out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Ajiboye *et al.,* 2013).

Quantification of total Phenolic compounds

One hundred grams (100 mg) of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was transferred to a test tube, then 0.5 ml 2N of the Folin-Ciocalteu reagent and 1.5 ml 20% of Na_2CO_3 solution was added and 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 765 nm. These data was used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of Gallic acid (Ajiboye *et al.,* 2013).

Minimum Inhibitory Concentration (M.I.C) of the plant extract against the test organisms

The Minimum Inhibitory Concentration (M.I.C) of the plant extract against the test organisms was carried out using broth micro dilution test as established in C.L.S.I standard methods as carried out by Bussmann et al. (2010) and Vanega et al. (2021).

Inoculum preparation

Twenty four hours culture of the *E. coli* 0157:H7 was emulsified in 3 mL Normal saline and adjusted to 0.5 MacFarland standard equivalent turbidity, the inoculum was further reduced to $5x10^5$ CFU/mL by mixing 0.1mL of the 0.5 MacFarland standard adjusted inoculum with 9.9mL of Normal saline.

Extract stock preparation

Stock concentration of the extract was prepared by dissolving 2 grams of the test extract in 10mL of Mueller-Hinton broth medium to obtain 200mg/mL.

Inoculation

A 1mL volume 96-well micro-plate (12x8) was used for the process, 100 µL of MH broth was dispensed in all the wells except those in the first column, 200 µL of the stock concentration (200mg/mL) was dispensed in the first well of the first column (A1), contents in A1 was mixed by repeated pipetting (3-5 times) after which 100 µL was taken and dispensed in the next well along the same row (A2) and be labeled '100mg/mL', same procedure was repeated progressively along the same row and stopedd at A10 where after pipetting to mix, 100 µL was be taken and discarded, this procedure yielded the following concentrations A1(200mg/mL.), A2(100mg/mL.), A3(50mg/mL.), A4(25mg/mL.), A5(12.5mg/mL.), A6(6.25mg/mL.), A7(3.12mg/mL.), A8(1.56mg/mL.), A9(0.78mg/mL.) and A10(0.39mg/mL.) all containing 100 ùL volume. 100 ùL of the $5x10^5$ CFU/mL adjusted *E. coli* 0157:H7 was added to the first eleven (11) wells in row A (A1-A11). 100 ùL of 0.1mg/mL of Amoxicillin was added to the last well in the row (A12) and labeled as 'Antibiotic control' while A11 (100 ùL MH broth +100 ùL $5x10^5$ CFU/mL *E. coli* 0157:H7) served as the positive control.

The above procedure was carried out separately with both Methanol and n-Hexane extracts and *S. Typhimurium* as the test organism. The micro-plate was covered with a sterile film cover and incubated for 18-24 hours at 37°c.

Subsequently, 14 ùL of 2% P-iodonitrotetrazolium salt (bacterial growth indicator dye) was added to all the wells and incubated for 30 minutes at 37°c.

Pink coloration (presence of viable bacteria) was looked out for in the test wells, where the least concentration well with no pink coloration was considered as the Minimum Inhibitory Concentration (M.I.C) of such stem bark extract against the test organisms.

Same procedure was repeated using *S. Typhimurium* as the test organism against n-Heaxane and Methanolic extracts

Minimum Bactericidal Concentration (M.B.C) of the plant extract against the test organisms

For Minimum Bactericidal Concentration (MBC) determination, a loopful from each tube showing no growth in the MIC assay was transferred to fresh nutrient agar plates

(Oxoid). These plates were then incubated at 37 °C for 24 h, and any subsequent growth was observed and recorded (Abdullahi *et al.,* 2024).

FTIR analysis

FTIR analysis followed the methodology carried out by Abdullahi et al. (2024). Approximately 500 mg of finely powdered fraction was placed directly on the diamond crystal or light path for analysis of functional groups. Spectra were recorded using a Perkin-Elmer FTIR (model spectrum 100 series, USA) at ambient temperature, spanning wavenumbers from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} . Each spectrum was an average of 4 scans with a scan speed of 0.2 cm^{-1} s^{-1}

RESULTS

Table 3: Quantitative phytochemical components of n-Hexane and Methanolic Stem bark crude extracts of *K. senegalensis*

		PHYTOCHEMICALS (mg/g)						
Plants	Solvents	TEP	TAN	SAP	ALK	FLA	STR	PHE
K. Senegalensis (Stem bark)	n-Hexane	17.1	58.1	70.3	47.1	35.1	13.4	98.3
	Methanolic	-	70.4	65.7	61.3	40.6	9.3	101.1

Keys: **TEP**: Terpenoids. **TAN**: Tannins. **SAP**: Saponins. **ALK**: Alkaloids. **FLA**: Flavonoids. **STR**: Steroids. **PHE**: Phenols.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Methanolic and n-Hexane Stem bark Extracts of *Khaya senegalensis* against *E. coli* 0157:H7 and *Salmonella Typhimurium*

Test organism	Solvents	Concentration (mg/mL)								MIC (mg/mL)	MBC (mg/mL)
		200	100	50	25	12.5	6.25	3.12	1.56		
E. coli 0157:H7	Methanol	-	-	-	-	-	-	-	+	3.12	25
	n-Hexane	-	-	-	-	+	+	+	+	25	100
S. Typhimurium	Methanol	-	-	-	-	-	+	+	+	12.5	50
	n-Hexane	-	-	-	-	+	+	+	+	25	200

Key: + = Growth, - = No growth

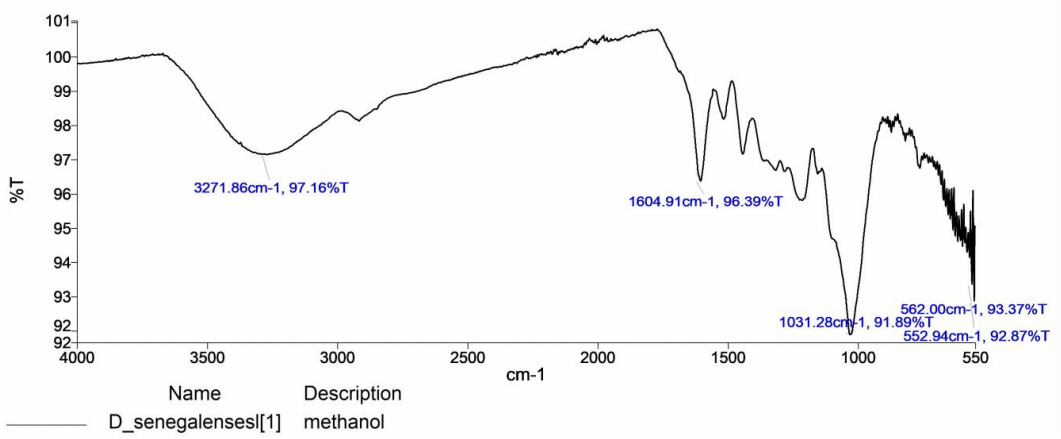

Fig 2: FTIR Spectrum of *D. senegalensis* methanolic stem bark extract

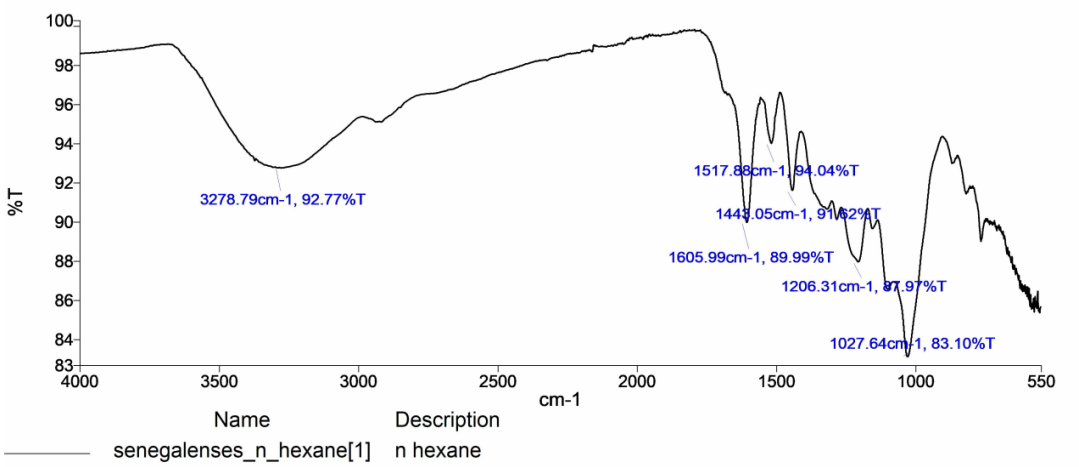

Fig 3: **FTIR Spectrum of *D. senegalensis* n-Hexane stem bark extract**

DISCUSSION

Biomedical Sciences have exploited plants as the potential sources of drugs to prevent and cure human diseases, (Kadebe *et al.,* 2021). Identification of the Antimicrobial action of Indigenous plants extracts and plants secondary metabolites is important in sampling their potential as Natural antimicrobial agent and alternative therapeutic agents which can be used alone or in combination with antibiotics (Chadha *et al.,* 2021).

In this study, Reflux Extraction was adopted using two different solvents n-Hexane and Methanol with different polarity indices of 0.1pand 5.1p respectively (Ewansiha, 2020) to obtain more composition of the plants extract as the percentage yield of extracts is a function of relationship between extraction solvent, solubility of the desired components,

solvents to solid ratio, the extraction temperature and the extraction duration (Zhang *et al.,* 2018).

The methanol was particularly effective in extracting Phenols, Flavonoids, Alkaloids and Tannins showing higher concentrations whereas the n-Hexane extract contained greater levels of Steroids and Saponins with Terpenoids found only in n-Hexane extract. The use of n-Hexane and Methanol as extraction solvents as employed in this study is recognized as a best practice for extracting broad spectrum antimicrobial compounds (Ramasamy *et al.,* 2022). This phytochemical profile provides scientific foundation for the plant's traditional medicinal application, particularly its Antibacterial effects. The Identification of known antimicrobial compounds such as flavonoids, Tannins, Alkaloids and phenols is in agreement with earlier studies on Nigerian *K. senegalensis* (Abalaka, *et al.,* 2016). The Antimicrobial action can be attributed to the documented properties of these metabolites; for example, Tannins can inhibit microbial enzymes (Dahiru *et al.,* 2025) and flavonoids are known to disrupt bacterial cell membranes (Okonkwo *et al.,* 2025). High levels of flavonoids, Tannins and phenols in the methanol extracts align with findings that alcoholic solvents more efficiently extracts these polar bioactive components from medicinal plants which implies stronger antimicrobial activity (Ezeonu *et al.,* 2024). Consequently, the Antibacterial action of Methanolic *K. senegalensis* stem bark extract can be associated to the synergistic combination of these antimicrobial compounds (Dahiru *et al.,* 2025) highlighting its potential in future research for novel antimicrobial agents.

From this study, The Minimum Inhibitory Concentration (MIC) values for n-Hexane and Methanol stem bark extracts of *K. senegalensis* are summarized in **Table 4**. The n-Hexane exhibited an MIC value of 25mg/mL against *E. coli* O157:H7 and *S. typhimurium.* The Methanol extract showed an MIC value of 3.12mg/mL against *E. coli* O157:H7 and 12.5mg/mL against *S. typhimurium.* Greater sensitivity against *E. coli* O157:H7 could be due to the structural difference in the outer membrane or more effective efflux mechanism in *S. typhimurium* that provides inherent resistance (Nnamani *et al.,* 2022; Dahiru *et al.,* 2025). The potent Antibacterial action is probably as a result of synergy among the inherent phytochemical of the *K. senegalensis* stem bark extract which includes Phenols, Steroids, Tannins, flavonoids and Terpenes (Olorundare *et al.,* 2024; Dahiru *et al.,* 2025). Higher MIC values of methanol in relation to n-Hexane indicates limited solubility of certain bioactive constituents in n-Hexane as emphasized by Adebayo et al. (2025) and Dahiru et al. (2025). Invariably, variations in MIC values among different bacterial reveals that the effectiveness

of an extract in inhibiting bacterial growth can be influenced by the unique characteristics and susceptibilities of the various bacterial species (Abaka *et al.,* 2024).

From this study, the Minimum Bactericidal Concentrations (MBC) of *K. senegalensis* Methanolic stem bark extracts against *E. coli* O157:H7 and *S. typhimurium* are 25 mg/mL and 50 mg/mL respectively, for n-Hexane stem bark extracts are 100 mg/mL and 200 mg/mL against *E. coli* O157:H7 and *S. typhimurium* respectively. This is in fair agreement with study on stem bark extracts of *K. senegalensis* reported against *S. typhi* recovered from slaughter slab surfaces and meat handler's aprons from Aleiro town, Kebbi state Northwestern Nigeria by Murtala et al. (2026). The variations in MBC values observed could be attributed to differences in the phytoconstituents of the extracts due to solvent variations, dictating their inhibitory and bactericidal effects compared to pure antibiotics (Abaka *et al.,* 2024).

From this study, FT-IR spectrum of *K. senegalensis* stem bark Methanol extract, **Fig 3**. depicting the various peaks. A total of 5 peaks were detected these includes 2 at the group frequency region and 3 at the finger print region. The first absorption peak was at 3271.86 cm^{-1} corresponds to the -C-H stretching frequency of terminal Alkynes. The second (1604.91 cm^{-1}) corresponds to C=C stretching frequency of Aromatic compounds. In the finger print region, the peaks at 1031.28 cm^{-1} (C-O), 562.00 cm^{-1} (C-H band) and 552.94 cm^{-1}(C-H band), the fingerprint regions cannot be used as the sole identifier, these peaks are characteristics of prominently bending and skeletal stretches (C-C, C-O, C-N) which may include alcohols, esters, alkanes, etc. This corresponds to the amine, alkane, aromatic amine, alkyl aryl ether, secondary alcohol, and ortho aromatics compounds.

From this study, FT-IR spectrum of *K. senegalensis* stem bark n-Heaxane extract, **Fig 2**, depicting the various peaks. The first absorption peak was at 3278.79 cm^{-1} corresponds to -OH stretching frequency of the alcohol compounds. The second (1605.99 cm^{-1}) and third (1517.88 cm^{-1}) peaks at the frequency regions correspond to C=C stretching frequency of Aromatic compounds and N-0 stretching frequency region respectively that correspond to Nitro compounds. while the 1443.05 cm^{-1} corresponds to the C-H bending vibration frequency of simple Alkanes. In the finger print region, the peak at 1206.31 cm^{-1} corresponds to (C-O-C) stretching and 1027.64 cm^{-1} (C-O) stretching corresponds to the Polysaccharide and carbohydrate respectively.

The FT-IR results depict the presence of different functional groups in the methanol and n-hexane extracts of *K. senegalensis,* these might contribute to the pharmacological activities of the plant (Abdullahi *et al.,* 2024). Amine group containing compounds were reported by Li et al. (2019) and Hashem et al. (2022) to exert Antibacterial, Antifungal, and Antiviral activities. Hu et al. (2022) reported sulfonamides functional groups as important pharmaceutical intermediates used in drug synthesis.

CONCLUSION

Quantitative phytochemical profiling of *K. senegalensis* n-Hexane and Methanolic stem bark extracts has identified varying quantities of bioactive compounds like terpenes, tannins, saponins, alkaloids, flavonoids, steroids and phenolic compounds. These results provide scientific basics for the traditional application of *K. senegalensis* stem bark to treat gastrointestinal disorders. Generally, this underscores *K. senegalensis* as potential source of natural antibacterial agent. Methanolic extract showed higher inhibition potential with MIC values of 3.12 mg/mL against *E. coli* O157:H7 and 12.5 mg/mL against *S. typhimurium* and MBC values of 20 mg/mL and 50 mg/mL respectively. the *E. coli* O157:H7 and *S. typhimurium* MIC and MBC values from the Methanolic extract suggest that the use of polar solvents for extraction is better for secondary metabolites in the stem bark. FTIR results depicts the presence of many groups of compounds that have been previously reported to have Antibacterial, Antifungal and Antiviral activities. The result of this study supports the traditional use of *K. senegalensis* stem bark in the treatment of diarrhea, dysentery and other gastrointestinal ailments owing to the antibacterial activity seen against the isolates tested. This study highlights the potential for further pharmacological exploration and drug development. Further work should be targeted on fractionation, and compound isolation in order to identify the mechanism of action and toxicity/safety profile of specific compounds, which could contribute toward to the development of plant-based therapeutics for safe and effective treatment of diseases and reduce human suffering due to these bacterial diseases.

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