Antimicrobial Activity of *Acacia nilotica* and *Ziziphus Mauritiana* against Clinical Isolates of *Escherichia coli* and *Klebsiella aerogenes*

*Usman, A.,* 1 *Ahmad, M.,* 1 *Hamza, M. M.,* 1 *Hussaini I. M.,* 1 *Sanusi, S. B. and* 1 *Innocent, A. A.

1 Department of Microbiology, Faculty of Science, Kaduna State University, Kaduna.

2 Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria *

Corresponding author: Usmanaiisha1104@gmail.com

**Abstract**

*Klebsiella aerogenes* is an opportunistic pathogen linked to several nosocomial illnesses, including pneumonia, sepsis, and infection of the urinary tract. *Escherichia coli* is frequently the source of numerous common bacterial illnesses, including bacteremia, cholecystitis, cholangitis, urinary tract infections (UTI), traveler’s diarrhoea, and other medical conditions such as meningitis in babies and pneumonia. This study aimed at determining the antimicrobial activity of *Acacia nilotica* and *Ziziphus mauritiana* on clinical isolate of *E. coli* and *K. aerogenes*. The phytochemical constituents of *Z. mauritiana* and *A. nilotica* were determined. *E. coli* and *K. aerogenes* isolates were obtained from the Barau Dikko Teaching Hospital, Kaduna and reconfirmed using standard microbiological techniques. The antibacterial activity of *Z. mauritiana* and *A. nilotica* extracts against the isolates was determined using Agar well diffusion assay, the MIC and MBC was also determined and recorded. The findings of this study revealed that tannins, quinone, phenols, terpenoids, and steroids are present in *Z. mauritiana* extract while tannins, saponin, quinones, and terpenoids in *A. nilotica* extract. There was no activity of *Z. mauritiana* against any of the test isolates at all concentrations used in this study. Whereas *A. nilotica* exhibited an antibacterial activity against both *E. coli* and *K. aerogenes* recording respectively a zone of inhibition of 24 mm and 25 mm with MIC and MBC value of 600 mg/ml. This research displayed an antibacterial activity of *A. nilotica* and no activity of *Z. mauritiana* against *E. coli* and *K. aerogenes*.

**Keywords:** Klebsiella aerogenes, Escherichia coli, Acacia nilotica, Ziziphus mauritiana, antibacterial activity

**INTRODUCTION**

The emergence and spread of antibiotic resistance among pathogenic bacteria have become a critical global health challenge. Traditional antibiotics are losing their effectiveness, leading to limited treatment options for infectious diseases caused by drug-resistant strains. In response to this alarming situation, researchers are increasingly exploring alternative sources of antimicrobial agents, including natural products derived from medicinal plants. Many years ago, medicinal plants were considered well-established natural sources for the treatment of various diseases with or without scientific bases. Research has mostly focused on using ethnomedical knowledge of biosciences for the exploration of novel bioactive chemicals and the polypharmacological formulation of plant extracts for use in primary healthcare (*Adeeyo et al.*, 2018). Over the years, medicinal plants have been found to be useful in the medical field. Studies have been conducted all around the world to support the effectiveness of medicinal plants, and some of these pieces of evidence have shed light on the synthesis of plant-based chemicals with therapeutic uses (*Dham et al.*, 2014).

*Acacia nilotica* commonly known as Bagaruwa in Hausa (Nigeria) is widely distributed in Egypt, South Africa, India, Australia, and Central America (*Alduraihem et al.*, 2023) and it is a valuable plant with medicinal properties and a wide range of therapeutic applications (*Ali et al.*, 2012). The plant is known to be rich in antioxidant substances. The whole plant is enriched with biomolecules (including proteins, amino acids, sugars, and antioxidants), gallic acid, ellagic acid, isoquercetin, leucocanidol, and glucopyranoside, which is used in the treatment of many diseases due to its antimicrobial, anti-plasmodial, and antioxidant properties (*Alduraihem et al.*, 2023). Compounds such as phenols present in this plant can block or suppress carcinogens and significantly inhibit have the capacity to block or suppress carcinogens and significantly inhibits the development of tumours.
It has been discovered that A. nilotica can inhibit the growth of bacteria by precipitating its proteins causing the depletion of nutritional proteins. It can also stop the production of proteins by forming irreversible complexes with proline-rich proteins in the bacterial cells (Ali et al., 2012). It is useful for the treatment of venereal diseases, nausea, burns and wounds, stomachache, and diarrhoea (Ali et al., 2012; Farzana et al., 2014). Farzana et al. (2014) added that A. nilotica is beneficial in preventing premature ejaculation, relieving irritation in acute gonorrhoea and leucorrhoea, and urine-genital such as pelvic prolapsed.

Ziziphus mauritiana popularly known as Magary in Hausa is a domestically grown species and widely distributed in nature. Pakistan, India, and China are the major cultivators of this plant (Alsayari and Wahab, 2021). There are several bioactive compounds found in this plant such as the alkaloids protopine and berberine; sterols; flavonoids; oses and holosides; mucilage and terpenes (Cubero et al., 2023). Parveen et al. (2023) in their study reported that Z. mauritiana has been widely used in the treatment of many diseases including urinary problems, depression, allergies, liver diseases, insomnia, and chronic bronchitis. It has been discovered that secondary metabolites derived from Z. mauritiana are effective as antidiarrheal, antibacterial, poison antidote for carbon tetrachloride damage in the liver, antioxidant, anti-hemorrhagic, anti-diabetic, sleep booster and anticancer (Cubero et al., 2023).

Escherichia coli is frequently the source of numerous common bacterial illnesses, including bacteraemia, cholecystitis, cholangitis, urinary tract infections (UTI), traveler’s diarrhoea, and other medical conditions such as meningitis in babies and pneumonia (Omololu et al., 2017). E. coli, according to Haris et al. (2017), belongs to the family of facultative, gram-negative, non-spore-producing bacilli bacteria called Enterobacteriaceae. Most of its strains are motile and typically have both sex pili and adhere fimbriae. Among the normal intestinal flora, E. coli is one of a typical aetiological agent of opportunistic infections.

Formerly known as Enterobacter aerogenes, the enterobacterial Klebsiella aerogenes is a facultative Gram-negative anaerobe (Wesevich et al., 2020). It is a prevalent opportunistic infection in hospitals, is widely spread in the environment, and can be detected in the human gastrointestinal tract. Infection of the pulmonary, circulatory, or urogenital systems may result from intestinal mucosa injury or a weakened host immune system (Gu et al., 2022). The aim of this study is to determine the antimicrobial activity of A. nilotica and Z. mauritiana against clinical isolate of E. coli and K. aerogenes.

MATERIALS AND METHODS

Collection of plant samples and processing

Dried fruits of A. nilotica and seeds of Z. mauritiana were obtained from Central Market, Kaduna-Kaduna State in a clean container. The samples were taken to the Botany laboratory at the Department of Biological Sciences at Kaduna State University for further processing. Dried seeds of Z. mauritiana were removed from the skin, while A. nilotica seeds were removed from their pods, both seeds were then pulverised into a powder using a mortar and pestle and 50 g of each of the powdered form of the seeds were macerated in 500 ml of a 95% ethanol solution. The mixtures were left to stand for 72 hours with periodic stirring and then filtered using Whatman No. 1 filter paper. The extracts were heated to 400°C and evaporated to dryness (Garg et al., 2016).

Phytochemical Screening of Z. mauritiana and A. nilotica extracts

The extracts of Z. mauritiana and A. nilotica were screened for the presence of the following phytochemicals: anthraquinones, tannins, saponins, flavonoids, terpenoids, steroids, glycosides, quinones, phenols, alkaloids, and cardiac glycosides as described below.

i. Test for anthraquinones:

To 1 ml of each of the extracts, a few drops of 10% ammonia solution were added and the appearance of a pink colour precipitate indicates the presence of anthraquinones (Mengesha, 2015)

ii. Test for tannins:

To 1 ml of each of the extracts, 2 ml of 5% ferric chloride was added and a formation of dark blue or greenish black colour indicates the presence of tannins (Mengesha, 2015).

iii. Test for saponins

Each of the extracts was diluted with an equal amount of distilled water and was shaken in a graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponins (Mengesha, 2015).

iv. Test for flavonoids

To 2ml of each of the extracts, one ml of 2N sodium hydroxide was added and the presence of yellow colour indicates the presence of flavonoid (Mengesha, 2015).
v. Test for terpenoids
The extracts were treated with chloroform and a few drops of concentrated sulfuric acid were added, appropriately agitated, and let to stand for a while; the appearance of a lower layer that was yellow-coloured showed the presence of terpenoids (Mengesha, 2015).

vi. Test for Steroids
After adding concentrated H₂SO₄ to 2 mL of gel, 5 mL of chloroform, 2 mL of acetic anhydride, and 5 mL of chloroform were added. The presence of steroids was detected by a reddish-brown colouring at the contact (Mengesha, 2015).

vii. Test for glycosides
Three millilitres of chloroform and a 10% ammonia solution were added to 2 mL each of the extracts. The presence of glycosides is indicated by the formation of pink colour (Mengesha, 2015).

viii. Test for quinones
One mL of concentrated sulfuric acid was added to 1 mL of each of the extracts. The appearance of red suggests the existence of quinones (Mengesha, 2015).

ix. Test for phenols
To 1 mL of each of the extracts, 2 mL of distilled water was added, followed by a few drops of 10% ferric chloride. The presence of blue-green colour indicates the presence of phenols (Mengesha, 2015).

x. Test for alkaloids
Concentrated hydrochloric acid in the amount of 2 mL was added to each of the extracts. Mayer’s reagent was then added in a few drops. The appearance of green colour indicated the presence of alkaloids (Mengesha, 2015).

xi. Test for cardiac glycosides
In a test tube, 3 mL of glacial acetic acid, 1 drop of 5% ferric chloride, and 2 mL of the gel were combined. Half mL of concentrated sulfuric acid was carefully added to the test tube’s side. The development of blue colour in the acetic acid layer showed the presence of cardiac glycosides (Mengesha, 2015).

Collection and reconfirmation of test bacterial isolates
Escherichia coli and Klebsiella aerogenes isolates were obtained from the Microbiology laboratory of the Barau Dikko Teaching Hospital, Kaduna, and transported to the Department of Microbiology laboratory at Kaduna State University, Kaduna, for further analysis.

The isolates were reconfirmed based on the colony morphology, Gram reaction, and biochemical reactions of the isolates (including indole, citrate utilisation, Methyl-Red, Voges-Proskauer and Triple Sugar Iron, and urease) as stated by Cheesbrough (2012).

Antibacterial activity using Agar diffusion assay
With minor modifications, the antibacterial activity of ethanol extracts of the seeds prepared above were assessed against each of the test isolates using the agar well diffusion method according to Daudi et al. (2017). Fresh overnight-grown cultures were suspended in 5 mL of normal saline and the inoculum density of the liquid culture was adjusted to 0.5 McFarland concentration. Each liquid culture was inoculated onto Mueller-Hinton agar medium using a sterile swap stick, and 5 mm-diameter wells were created concentrically in the agar using a sterile hollow punch. Each extract was poured into each well in 50 uL at concentrations of 800, 600, 400, and 200 mg/ml. These concentrations were prepared by diluting the respectively 800 mg, 600 mg, 400 mg and 200 mg of the extracts in 1 ml distilled water. The positive control was a standard infusion of ciprofloxacin (30µg), the extracts and the control were allowed to be pre-diffused at laboratory temperature for 30 minutes before being incubated for 24 hours at a temperature of 37°C. The inhibition zones’ diameters were measured in millimetres following incubation.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
A modification of the dilution method for the determination of MIC and MBC was used as described by Chikezie (2017). Five test tubes containing 5ml of Mueller-Hinton broth for each organism were inoculated with a loopful of each culture using a standard wire loop. Each test tube then received 0.5 ml of each plant extract at different concentrations of 200, 400, 600, and 800 mg/ml. After 24 hours of incubation at 37°C, the tubes were examined to see if growth or turbidity had occurred. A Mueller Hinton plate was then inoculated with a loopful of broth from each test tube that did not exhibit obvious growth, and it was cultured for 24 hours at 37°C. The agar plates were then checked visually for growth. (CLSI, 2012).

RESULTS
The phytochemical screening of the ethanolic extracts showed the presence of Tannins, Quinone, Phenols, Terpenoids, and Steroids with the absence of Saponins, Alkaloids, Glycosides, Cardio glycosides, Anthraquinones, and Phlobatannins for Phlobatannins Z. mauritiana while that of A. nilotica displayed...
the presence of Tannins, Saponin, Quinones, and Terpenoids and absence of Alkaloids, Glycosides, Phenols, Cardio glycosides, Anthraquinones, Steroids and Phlobatannins (Table 1).

Table 1: Phytochemical constituent of ethanolic seeds extract of *Z. mauritiana* and *A. nilotica*.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Ziziphus mauritiana</em></td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key:** + = present; - = absent

The result of the antibiotic susceptibility testing of the extracts using the agar well diffusion method revealed no zone of inhibition against both *E. coli* and *K. aerogenes* for all *Z. mauritiana* concentrations used in this study (Table 2), while that *A. nilotica* revealed zones of inhibition for all concentrations used with highest activity on *K. aerogenes* at 25mm zone at a concentration of 800 mg/ml (Table 3).

Table 2 Antibacterial activity of ethanolic seeds extract of *Z. mauritiana*

<table>
<thead>
<tr>
<th>Extract concentration (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>200</td>
<td>-</td>
</tr>
<tr>
<td>400</td>
<td>-</td>
</tr>
<tr>
<td>600</td>
<td>-</td>
</tr>
<tr>
<td>800</td>
<td>-</td>
</tr>
<tr>
<td>Control (ciprofloxacin 30µg)</td>
<td>14</td>
</tr>
</tbody>
</table>

**Key:** - = no zone of inhibition

Table 3 Antibacterial activity of ethanolic seeds extract of *A. nilotica*

<table>
<thead>
<tr>
<th>Extract concentration (mg/ml)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>200</td>
<td>16</td>
</tr>
<tr>
<td>400</td>
<td>19</td>
</tr>
<tr>
<td>600</td>
<td>22</td>
</tr>
<tr>
<td>800</td>
<td>24</td>
</tr>
<tr>
<td>Control (ciprofloxacin 30µg)</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 4 shows the MIC of the ethanolic extract of *Z. mauritiana* against both *E. coli* and *K. aerogenes* with visible growth in all concentrations while Table 5 shows the MIC of the ethanolic extract of *A. nilotica* against the test isolates starting from 600 mg/ml for both *E. coli* and *K. aerogenes*. The MBC of the ethanolic extract of *A. nilotica* against both organisms was recorded at 600mg/ml (Table 6).

Table 4: Minimum Inhibitory Concentration of ethanolic seed extract of *Z. mauritiana*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>+</td>
</tr>
<tr>
<td><em>K. aerogenes</em></td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:** + = presence of visible growth
The ethanolic seed extract of *A. nilotica* in this study revealed secondary metabolites like tannins, saponins, quinones, and terpenoids, but alkaloids, glycosides, phenols, cardio glycosides, anthraquinones, steroids, and phlobatannins were not found. This is in accordance with the research conducted by Srivastava et al. (2014) and Abd’quadri-Abojukoro et al. (2022) with the absence of tannins in the earlier and presence of alkaloids and steroids in the latter. The ethanolic seed extract of *Z. mauritiana* extracts in this study contained Phenols, Quinones, Terpenoids, Steroids, and Tannin while Alkaloid, Glycoside, Saponin, Cardioglycoside, Anthraquinones, Phlobatannins were absent. Khamsatul et al. (2019) showed that saponin, tannin and steroids were present while alkaloids and terpenoids were absent while Hussain et al. (2021) and Yusof and Saat, (2017) revealed the presence of all these chemicals except for Cardiac glycoside, Anthraquinones, and Phlobatannins which were not evaluated in their research. Abd’Quadri-Abojukoro et al. (2022) reported that these variations were caused by several varied factors which are significant determinants of the phytochemical composition of different plant species, these variables include each plant’s physiological stage, exposure to herbivory, geographical location, harvest season, and extraction process (including solvent).

*Acacia nilotica* showed strong inhibitory effects against all test organisms at varying concentrations. *E. coli* and *K. aerogenes* were inhibited by the extract with the largest zone measuring 24 mm and 25 mm, respectively. The results of this study agree with those of Abeer et al. (2007), in their study of the antimicrobial activity of *A. nilotica* extracts against *E. coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* although they used different solvents for the extraction. Shukla and Sharma (2023) also reported the activity of *A nilotica’s* seed ethanol extract against *E. coli* and *Bacillus subtilis* while Magnini et al. (2020) reported the activity of *A. nilotica* against Enterobacteriaceae.

*A. nilotica* showed more substantial action against *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in the study conducted by Srivastava et al. (2014) utilizing different solvents of *A. nilotica* seed extracts, with its highest zone of inhibition of 10mm at 30mg/ml. N-hexane and chloroform were less efficient than acetone, ethanol, and water extracts. The diameter of the zones of inhibition showed that the activity of the extracts increased with concentration, and it was noted that because of organisms’ cell walls contents, they may require higher quantities to limit their growth. The ethanol extract was reported to have higher antimicrobial effect than other solvents, this may be due to the ability of the ethanol to extract a wide range of chemical constituents of the plant fruit (Abeer et al., 2007). Because there was no noticeable growth of the isolate at 600 mg and 800 mg of the extract, it is likely that the high Minimum Inhibitory Concentration obtained from the results of *A. nilotica* was caused by the high level of resistances of the organisms to the extract bioactive chemicals present in the plant extract. The lowest inhibitory concentration for *K. aerogenes* and *E. coli* was determined to be 600 mg.

*Z. Mauritiana* failed to exhibit any apparent zone of inhibition against test organisms of this research. Khamsatul et al. (2019) tested the antibacterial activity *Z. mauritiana* leaves extract against *E. coli* and *S. aureus*, and the results revealed that there was no antibacterial activity against *E. coli* but was effective against *S. aureus*. According to research by Saroinsong et al. (2014), this could have been caused by a low concentration of *Z. mauritiana* extract that was unable to harm *E. coli’s* cell wall. The lack of saponins in the plant extract may be the cause of *Z. mauritiana’s* impotence. As a result of saponins’ capacity to lower surface tension, cells become more permeable or are damaged, which allows intracellular chemicals to escape (Nuria et al., 2009).
The American Society of Microbiology Journal expounded that this substance diffuses through cell walls and the outer membrane before attaching to the cytoplasmic membrane and lowering stability. The type of solvent employed can sometimes affect the efficacy of an extract. There was no antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *S. aureus* when *Ziziphus* seed extracts were tested by Ali et al. (2012) using different solvents (ether, chloroform, methanol, and water), except for methanol which showed zone of inhibition against *E. coli* to be 16mm. It is assumed from this study that the type of solvent employed for extraction may affect the antibacterial activity of plant extracts.

**CONCLUSION**

The results of this investigation showed that *A. nilotica* extract had antibacterial activity against both *E. coli* and *K. aerogenes*, whereas *Z. mauritiana* had no antibacterial activity at any concentration against the test isolates. The outcome raises the possibility of using *A. nilotica* as a different antibacterial agent to treat illnesses brought on by *E. coli* and *K. aerogenes*.

**REFERENCES**


Adeeyo, A., Odiyo, O.J. and Odelade, K. (2018). Chemical profiling and antimicrobial properties of phyto-active extracts from Terminalia glaucescens stem against water microbial contaminants. The *Open Biotechnology Journal*, 12(1) [Crossref]


Klebsiella aerogenes-caused lumbar spine infection identified by metagenome next-generation sequencing. *BMC Infectious Diseases*, 22(1). [Crossref]


Mengesha Y. A. (2015). Phytochemical extraction and screening of bio active compounds from black cumin (*Nigella sativa*) seeds extract. *American Journal of Life Sciences*, 3(5): 358. [Crossref]


Shukla, R. S., & Sharma, V. (2023). Validating the antimicrobial potentiality of peptides from pods of *Acacia nilotica* willd.ex delile: A spotlight on bacterial fauna. *Toxicology International*: 111-119. [Crossref]

