Phytochemical Profile and Antibacterial Activity of Nigella Sativa against Biofilm-producing Bacteria Uropathogens

Abdulazeez Mumsiri Abaka1,*, Mubarak Muhammad Dahiru2, Keta Biman Abubakar1, Jordana Luka1, Aisha Abubakar1, Tukur Baba Abdullahi3, Saminu Hamman Barau2

1Science Laboratory Technology Department, School of Science and Technology, Adamawa State Polytechnic Yola, Nigeria.
2Pharmaceutical Technology Department, School of Science and Technology, Adamawa State Polytechnic Yola, Nigeria.
3Science Laboratory Technology Department, Modibbo Adama University Yola, Nigeria.

Corresponding author*
abdulazizelnino22@adamawapoly.edu.ng

Manuscript received: 01 April, 2024. Revision accepted: 20 May, 2024. Published: 27 May, 2024.

Abstract

This study explores the antibacterial effects of Nigella sativa seeds on bacteria obtained from clinical samples. The aim was to assess the antibacterial properties of both aqueous and methanolic extracts of Nigella sativa seeds against E. coli, S. aureus, and P. aeruginosa. The three samples were collected from the Microbiology Laboratory of Modibbo Adamawa Medical Centre and were reconfirmed using culture, microscopy, and some biochemical tests. The seed samples of N. sativa were procured from herbal point Yola, Adamawa State, Nigeria. The phytochemical assay of the extracts revealed the presence of flavonoids, alkaloids, tannins, phenols, cardiac glycosides, steroids, saponins, and terpenoids in both extracts. The highest antibacterial activity against S. aureus, E. coli, and P. aeruginosa was demonstrated by the aqueous extract of N. sativa seeds, with inhibition zone diameters of 19.30 ±0.61 mm, 8.10 ±2.17 mm, and 12.00 ±0.29 mm, respectively. However, the methanol extract exhibited slightly greater activity against E. coli and P. aeruginosa, with inhibition zone diameters of 12.10 ±0.38 mm and 13.80 ±0.40 mm, respectively. Both methanol and aqueous extracts showed minimum inhibitory concentrations (MICs) of 25 mg/mL against S. aureus and E. coli. Similarly, for P. aeruginosa, the MIC was 25 mg/mL for methanol extract and 50 mg/mL for aqueous extract. The minimum bactericidal concentration (MBC) for both extracts against S. aureus and E. coli was determined to be 25 mg/mL. However, for P. aeruginosa, the MBC was 25 mg/mL for the aqueous extract and 50 mg/mL for the methanol extract. The study indicates that N. sativa seed extract possesses antibacterial properties against S. aureus and P. aeruginosa, underscoring its potential as an effective medicinal antibacterial agent.

Keywords: Bacteria; Phytochemical; Biofilm; Uropathogens; Nigella sativa.

Abbreviations: UTI (Urinary Tract Infection), MIC (minimum inhibitory Concentration), MBC (Minimum Inhibitory Concentration), AE (Aqueous Extract), ME (Methanol Extract).

INTRODUCTION

Throughout history, natural remedies, particularly those derived from plants, have been employed for medicinal purposes due to their diverse array of components thought to combat various infectious ailments (Sharma et al., 2023). Plant biodiversity serves as a valuable reservoir of chemical compounds with therapeutic potential, including antiviral, antibacterial, antifungal, and anticancer properties (Dar et al., 2023). Medicinal plants serve as a valuable reservoir of bioactive compounds that are commonly employed in traditional medicine practices, nutraceuticals, dietary supplements, modern pharmaceuticals, and synthetic drug development (Pammi et al., 2023).

In recent times, there has been a notable increase in utilizing plants for therapeutic purposes for several reasons, including their easy accessibility without prescription, affordability, natural origins, and potential to cut reliance on synthetic drugs with severe side effects (Satū et al., 2024). Moreover, plants have a long-standing reputation as a valuable source of new drug compounds. Herbal combinations have significantly benefitted human health and overall well-being (Jamil, 2023). Various secondary metabolites found in plants, such as tannins, terpenoids, flavonoids, alkaloids, and quinines, possess antimicrobial properties (Arora et al., 2024).

Extensive research has been dedicated to examining the chemical composition and pharmacological effects of N. sativa seeds. These seeds, as well as the oils derived from them, are recognized for their diverse health-promoting properties, including antitumor, antioxidant, anti-inflammatory, antibacterial, and immune-stimulating effects (Ojieronmi et al., 2022). Consequently, they are frequently utilized as nutritional supplements. N. sativa seeds, known worldwide by various names such as "seed
of blessing” (habbat-ul baraka), "habbatussauda” (in Hausa), black caraway, and black cumin, among others, are purported to possess a wide range of actions, encompassing both antibacterial and anticestodal effects (Usman et al., 2017). In Islamic tradition, the black seed is believed to serve as a universal remedy for various ailments, except aging or death (Nisar et al., 2023).

The increasing resistance of microorganisms to numerous standard antibiotic therapies presents a global challenge and raises significant public health concerns (Akram et al., 2023). The efficacy of current drugs is diminishing due to the proliferation of multi-drug-resistant bacterial strains, including pneumococci resistant to penicillin and macrolides, methicillin-resistant staphylococci, vancomycin-resistant enterococci, and multidrug-resistant Gram-negative organisms (Moiketsi et al., 2023). Therefore, there is an urgent need to find alternatives for the treatment of various diseases caused by diverse microbial agents.

Synthetic drugs are not only expensive and inadequate but also often have issues with adulterations and side effects (Hamidi, 2023). With the current advancement of technology, scientists are challenged to come out with new ideas for alternative and novel drugs to dazed the usage of microbial-resistant drugs (Ahmed et al., 2023). Black seed extracts have also proven to be potent antibacterial agents against specific pathogenic Gram-positive and Gram-negative bacteria (Usman et al., 2017). The surge in antibiotic resistance represents a critical global health crisis, acknowledged by governments as one of the paramount challenges to public health (Salam et al., 2023). Resistance to antibiotics is increasingly becoming a serious global problem, reaching dangerous levels. This resistance poses significant challenges to effectively treating infectious diseases worldwide. Additionally, it undermines the effectiveness of many medical advancements (Salam et al., 2023).

Urinary tract infection (UTI) remains a prevalent issue globally, affecting both community and hospital settings, with approximately 150 million cases reported annually worldwide (Rauniyar, 2023). The rise of antibiotic resistance has become increasingly prominent and poses a significant challenge in UTI management, largely ascribed to the formation of biofilms (Maione et al., 2023). Within the epithelium lining of the bladder, certain uropathogenic bacteria have been observed to form intracellular bacterial groups with characteristics akin to biofilms. (Lila et al., 2023). These biofilm-producing bacteria undergo alterations in growth rate and genetic expression. Consequently, biofilms hinder the diffusion of substances and the binding of antimicrobial agents, creating an effective barrier against large molecules such as antimicrobial proteins lysozyme, and complement (Lu et al., 2023). Biofilm is structured with layers of cell clusters enveloped within a matrix of extracellular polysaccharides, referred to as polysaccharide intracellular adhesion (PIA) (Maione et al., 2023). Responsible for over 80% of microbial infections, biofilms contribute to dogged infections and recurrences (Lila et al., 2023). The formation of biofilms by uropathogenic bacteria is regarded as a pathogenic trait, facilitating colonization and resulting in elevated rates of UTI (Zhou et al., 2023). Consequently, these infections pose challenges in treatment due to the emergence of multiple drug resistance.

Nonetheless, there is limited research conducted on the antibacterial activity of N. sativa extracts against biofilm-producing bacterial uropathogens and pathogenic bacteria in Adamawa State, Nigeria. This study aimed to assess the antibacterial potential of N. sativa extracts against various bacterial uropathogenic isolates.

**MATERIALS AND METHODS**

**Collection of Plant Materials**

Seeds of N. sativa was obtained from a vendor located at Yola Market, Nigeria, and their authenticity was verified by a botanist from the Department of Plant Science at Modibbo Adama University in Yola. The specimen voucher number is MAU/PLS/0712. Subsequently, the seeds were finely ground into powder using a blender.

**Extraction**

Aqueous and methanolic extracts were prepared following the procedure outlined by Usman et al. (2017). In brief, 350 g of N. sativa powder was soaked separately in 500 mL of distilled water and methanol for three (3) days at 25 °C using percolation. Subsequently, the mixtures were filtered using Whatman's No. 1 filter paper and evaporated using a rotary evaporation apparatus. The resulting extracts were then further dried in a hot air oven at 50 °C for 24 h and stored at 4 °C until further analysis.

**Concentrations of Extracts:**

Each extract, weighing 1 gram (1g), was individually dissolved in 1 milliliter (1 mL) of 10% dimethyl sulfoxide (DMSO) to obtain a stock solution with a concentration of 1000 mg/mL. From this stock solution, four different concentrations were prepared by serial dilutions, resulting in concentrations of 100, 50, 25, and 12.5 mg/mL, achieved through dilutions ranging from 10^{-1} to 10^{-4}.

**Bacterial Strains:**

The biofilm-producing bacterial strains, including S. aureus, E. coli, and P. aeruginosa, were obtained from the Department of Microbiology at Modibbo Adama University in Yola. Inocula were prepared by transferring 2-3 colonies of each bacterial strain into 5 mL of nutrient broth. The inoculated broths were then incubated at 35 °C for 2-3 h until reaching the logarithmic growth phase.
Subsequently, the bacterial suspensions were adjusted to match the 0.5 McFarland Standard for susceptibility assay, following the guidelines outlined by the National Committee for Clinical Laboratory Standards (Loh et al., 2023).

**Antibacterial sensitivity assay**

The antibacterial assay of *N. sativa* extracts against the bacterial isolates was assessed using the agar well diffusion method, following the protocol outlined by Dahiru et al. (2023). Sterile nutrient agar was poured into sterile Petri dishes and left to solidify. A sterile swab stick was immersed into a standardized inoculum and used to spread the bacteria evenly on the agar surface in aseptic conditions, with proper labeling. The inoculated plates were left to dry and then incubated at 37°C for 24 h. After incubation, zones of inhibition around the wells were observed, measured, and recorded in millimeters.

**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)**

The broth dilution assay, as outlined by Dahiru et al. (2023) was employed for testing the extracts. The extracts were diluted to 10-fold concentrations in nutrient broth. To each dilution, 0.1 milliliters of standardized bacterial inoculum was added. Negative control tubes devoid of bacterial inoculation were prepared concurrently. The tubes were then aerobically incubated at 37°C for 24 h. The MIC was identified as the lowest concentration of the extract that hindered the growth of the test bacterium. To determine the MBC, a loopful from each tube with no visible growth in the MIC assay was transferred onto fresh nutrient agar plates (Oxoid). These plates were then incubated at 37°C for 24 h, followed by observation and recording of any growth.

**Statistical Analysis**

The values were analyzed via the Statistical Package for Social Sciences (SPSS) version 16 and were presented as means ± standard error of the mean (SE). Comparisons between different groups were revealed using a one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT). The significance level was set at P < 0.05.

### Results

**Table 1.** Phytochemical screening results for methanol and aqueous extracts of *N. sativa* seeds are as follows.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Phytochemical</th>
<th>Methanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + Positive -Negative

**Table 2.** Zone of inhibition (mm) of the organism caused by Aqueous and Methanol extracts of *N. sativa*.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cipro</td>
<td>AQET</td>
<td>MTET</td>
<td>AQE</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>8.93 ±0.15b</td>
<td>12.10 ±0.38</td>
<td>19.30 ±0.61b</td>
</tr>
<tr>
<td>25</td>
<td>8.43 ±0.35b</td>
<td>10.80 ±0.65</td>
<td>12.43 ±0.23bc</td>
</tr>
<tr>
<td>12.5</td>
<td>7.03 ±0.09b</td>
<td>8.07 ±0.32</td>
<td>7.40 ±0.27abcd</td>
</tr>
<tr>
<td>30</td>
<td>6.43 ±0.23cd</td>
<td>7.30 ±0.42</td>
<td>6.23 ±0.15abcd</td>
</tr>
</tbody>
</table>

| MTET                  | 35.01 ±0.01 | 30.02 ±0.00 | 30.02 ±0.02 |

Values are the mean of triplicate determinations (±SEM), AQET = Aqueous extract treatment, MTET = Methanol extract treatment, Cipro = Ciprofloxacin.

Values with *a* superscript in the same row are significantly lower (p < 0.05) than the MTET for the same organism.

Values with *b* superscript in the same row are significantly higher than the MTET of the same organism.

Values with *c* superscript in the same column are significantly (p < 0.05) lower than the 100 mg/mL concentration.

Values with *d* superscript in the same column are significantly (p < 0.05) lower than the 50 mg/mL concentration.

Values with *e* superscript in the same column are significantly lower than the 25 mg/mL concentration.

All values were significantly lower than Cipro at 20 mg/mL concentration.
Table 3. Minimum Inhibitory Concentration of *N. sativa* extracts against *S. aureus*, *E. coli*, and *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Extract</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100 mg/mL</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>AE</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>AE</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>AE</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>ME</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: AE= Aqueous extract ME= Methanol extract * = MIC value + = turbidity - = no turbidity

Table 4. Minimum Bactericidal Concentration of *N. sativa* extracts on test isolates.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Aqueous extract (mg/mL)</th>
<th>Methanol extract (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Natural products are renowned for their diverse array of secondary bioactive metabolites, which exhibit various pharmacological activities in living organisms, often serving as a form of defense (Al-Khayri et al., 2023). Crude methanol and aqueous extract of *N. sativa* crude contains alkaloids, phenols, flavonoids, tannins, cardiac glycosides, saponins, terpenoids, and steroids, as seen in Table 1. This observation aligns with the reports of Shafodino et al. (2022). These phytochemicals have different distinctive biological functions. Therefore, the qualities of these phytochemicals in *N. sativa* gave optimism that this plant, if screened properly, could eventually give a template for medicine (Dalli et al., 2021).

The antibacterial assay varied greatly in terms of inhibitory potential. Table 2 shows the antibacterial activity of four *N. sativa* doses against *S. aureus*, *E. coli*, and *P. aeruginosa*. Aqueous extract (AE) of *N. sativa* seeds had the highest activity against *S. aureus*, *E. coli*, and *P. aeruginosa*, with inhibition zones measuring 19.30 ±0.61 mm, 8.10 ±2.17 mm, and 12.00 ±0.29 mm, respectively. Nonetheless, the methanol extract (ME) demonstrated marginally higher effectiveness against *E. coli* and *P. aeruginosa*, showcasing inhibition zone diameters of 12.10 ±0.38 mm and 13.80 ±0.40 mm, respectively. The findings indicate that the methanol extract of *N. sativa* inhibited bacterial growth more effectively than the aqueous extract.

The type of solvent for extracting bioactive components from plant extracts depends on specific properties of the compounds being targeted and their solubility characteristics. Some common solvents used for this purpose include methanol, ethanol, acetone, chloroform, and water, among others. Each solvent comes with its own set of advantages and disadvantages, and the selection is typically influenced by factors such as the polarity of the compounds being extracted and the intended application of the extracted components (Lelebvre et al. 2021). Usman et al. (2017) obtained similar results using the same genus of plant in their study. However, a contrary result was obtained by Balogun et al. (2019), which was conducted in Maiduguri, Nigeria which showed that aqueous extract had no antibacterial activity on *S. aureus*. Overall, methanol extracts were more bioactive than aqueous extracts.

Several factors could influence the agar-well diffusion technique, with inoculum size being among them smaller inoculum sizes can lead to potentially exaggerated inhibition zones, whereas larger sizes might result in underestimated zones (Chandran et al. 2023). Hence, it's crucial to ensure the proper alignment of the inoculum suspension with the McFarland standard (0.5).

The extracts were more efficient against Gram-positive than Gram-negative bacteria. This may be ascribed to Gram-negative bacteria's excellent permeability barrier, such as the outer membrane, which limits amphiphatic compound penetration, as well as multi-drug resistant pumps that release toxins over the barrier (Saxena et al. 2023). This permeability barrier could be the fundamental explanation for the apparent ineffectiveness of plant antibacterial activity. This discovery is congruent with the findings of Abdallah et al. (2023), who investigated the same plant genus.

Both ME and AE showed MIC values of 25 mg/mL against *S. aureus* and *E. coli*. Similarily, for *P. aeruginosa*, the MIC was 25 mg/mL for methanol extract and 50 mg/mL for aqueous extract. The observations align with the outcomes documented by Balogun et al. (2019) and Sulaiman and Muhammad, (2023). However, Abraham et al. (2019) observed lower MIC values of 32, 1.28, and 1.28 mg/mL for *N. sativa* against *E. coli*, *S.
aureus, S. typhi, and S. pyogenes. The differences in MIC values across various bacteria suggest that the extract’s efficacy in inhibiting bacterial growth may be influenced by the specific characteristics and vulnerabilities of individual bacterial species (Ezzaky et al., 2023).

The MBC for both extracts against S. aureus and E. coli was determined to be 25 mg/mL. However, for P. aeruginosa, the MBC was 25 mg/mL for the AE and 50 mg/mL for the methanolic extract. These results contrast with those reported by Usman et al. (2017) while Sulaiman and Muhammad (2023) obtained comparable results, with an MBC value of 50 mg/mL for Salmonella species and 100 mg/mL for Escherichia coli. The different MBCs of a plant extract among diverse bacteria indicate that the extract’s efficacy in killing bacteria may vary due to the individual traits and vulnerabilities of each bacterial species (Al-Garadi et al., 2022).

A substantial association has been instituted by statistical analysis between the concentrations used and the zone of inhibition. According to both extracts, there was a robust and positive association value for every examined bacterium (n = 4). An expanded inhibitory zone diameter produced by bacteria suggests a positive correlation with concentration.

CONCLUSION

This study reveals that aqueous and methanol extracts of N. sativa seeds have antibacterial activity against S. aureus, E. coli, and P. aeruginosa. Consequently, it highlights the considerable potential of N. sativa as a valuable antibacterial agent for medicinal applications.

Acknowledgments: Special gratitude goes to the Department of Science Laboratory Technology, Adamawa State Polytechnic Yola.

Authors Contributions: For Example, Abdulazeez Mumsiri Abaka & Mubarak Muhammad Dahiru designed the study. Abdulazeez Mumsiri Abaka, Jordana Luka, Keta Biman Abubakar & Aisha Abubakar carried out data collection and laboratory work. Abdulazeez Mumsiri Abaka, Mubarak Muhammad Dahiru, Saminu Hamman Barau & Tukur Baba Abdullahi wrote the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are competing interests.

Funding: No funding.

REFERENCES


