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

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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 (Sati *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 (Jamal, 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 (Ojueromi *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 ^{o}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 ^{o}C for 24 h and stored at 4 ^{o}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 3-5 colonies of each bacterial strain into 5 mL of nutrient broth. The inoculated broths were then incubated at 35 ^{o}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 undisturbed for 30 min to facilitate proper adhesion of the organisms to the agar surface. Subsequently, four wells were aseptically bored into the agar using a sterile cork borer with a diameter of 6 mm. Subsequently, the wells were filled with 0.2 ml of *N. sativa* extracts at concentrations of 100 mg/mL, 50mg/mL, 25mg/mL, 12.5mg/mL, and 6.25 mg/mL, respectively. Positive control wells were filled with 0.2 ml of a 20 mg/mL ciprofloxacin solution, while negative control wells were filled with 0.1 ml of DMSO. The plates were left to dry and then incubated at 37 ^{o}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 ^{o}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.

S/N	Phytochemical	Presence (+) and absence (-) in different extracts	
		Methanol extract	Aqueous extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Phenols	+	+
4	Tannins	+	+
5	Cardiac Glycosides	+	-
6	Steroids	+	+
7	Saponins	-	+
8	Terpenoids	+	+

Key: + Positive -Negative

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

Concentration (mg/mL)		Zone of Inhibition (mm)					
		E. coli		*S. aureus*		*P. aeruginosa*	
Cipro	**Extract**	**AQET**	**MTET**	**AQE**	**MTE**	**AQE**	**MTE**
	100	8.93 ±0.15^{a}	12.10 ±0.38	19.30 ±0.61^{b}	16.03 ±0.37	12.00 ±0.29^{a}	13.80 ±0.40
	50	8.43 ±0.35^{a}	10.80 ±0.65	12.43 ±0.23^{bc}	14.03 ±0.12^{c}	9.83 ±0.20^{ac}	12.03 ±0.18^{c}
	25	7.03 ±0.09^{c}	8.07 ±0.32^{cd}	7.40 ±0.27^{acd}	11.10 ±0.40^{cd}	6.50 ±0.17^{acd}	10.20 ±0.12^{cde}
	12.5	6.43 ±0.23^{cd}	7.30 ±0.42^{cd}	6.23 ±0.15^{acd}	7.97 ±0.12^{cde}	5.90 ±0.38^{cd}	6.97 ±0.12^{cde}
20		35.01 ±0.01		30.02 ±0.01		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 ($p < 0.05$) 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 ($p < 0.05$) 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.*

Organisms	Extract	Concentration (mg/mL)					
		100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	3.125 mg/mL
S. aureus.	AE	-	-	- *	-	+	+
	ME	-	-*	+	+	+	+
E. coli	AE	-	-	-*	-	+	+
	ME	-	-	-*	+	+	+
P. aeruginosa	AE	-	-	-*	+	+	+
	ME	-	-*	+	+	+	+

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

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

Test organisms	Aqueous extract (mg/mL)	Methanol extract (mg/mL)
S. aureus	25	25
E. coli	25	25
P. aeruginosa	25	50

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 (Lefebvre *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 amphipathic 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*. Similarly, 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.

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

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REFERENCES

Abdallah, E. M., Alhatlani, B. Y., de Paula Menezes, R., & Martins, C. H. G. (2023). Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, *12*(17), 3077.

Abraham, A. O., Abdulazeez, A. K., Seun, O. O., & Ogonna, D. W. (2019). Antimicrobial activity of N-hexane extract of Nigella sativa against some pathogenic bacteria. *Am. J. Biomed. Sci. Res*, *6*, 430-434.

Abraham, A. O., Abdulazeez, A. K., Seun, O. O., & Ogonna, D. W. (2019). Antimicrobial activity of N-hexane extract of Nigella sativa against some pathogenic bacteria. *Am. J. Biomed. Sci. Res*, *6*, 430-434.

Ahmed, S., Ahmed, M. Z., Rafique, S., Almasoudi, S. E., Shah, M., Jalil, N. A. C., & Ojha, S. C. (2023). Recent approaches for downplaying antibiotic resistance: molecular mechanisms. *BioMed Research International*, *2023*.

Akram, F., Imtiaz, M., & ul Haq, I. (2023). Emergent crisis of antibiotic resistance: A silent pandemic threat to 21st century. *Microbial Pathogenesis*, *174*, 105923.

Al-Garadi, M. A., Qaid, M. M., Alqhtani, A. H., Pokoo-Aikins, A., & Al-Mufarrej, S. I. (2022). In vitro phytochemical analysis and antibacterial and antifungal efficacy assessment of ethanolic and aqueous extracts of Rumex nervosus leaves against selected bacteria and fungi. *Veterinary World*, *15*(11), 2725.

Al-Khayri, J. M., Rashmi, R., Toppo, V., Chole, P. B., Banadka, A., Sudheer, W. N., ... & Rezk, A. A. S. (2023). Plant secondary metabolites: The weapons for biotic stress management. *Metabolites*, *13*(6), 716.

Arora, P., Garg, M., Gera, T., Vaid, L., Sood, P., Kaur, L., ... & Sahu, S. K. (2024). Antimicrobial Activity of Secondary Metabolites in Medicinal Plants: An Update. In *BIO Web of Conferences* (Vol. 86, p. 01040). EDP Sciences.

Balogun, S. T., Umar, A. I., Oluwasoji, A. A., Gulani, I., Adebola, O. S., (2019) Antibacterial activity of the aqueous and methanolic seed extract of Nigella sativa LINN. *World Journal of Pharmaceutical and Life Science*, 2-4.

Chandran, G. R., Dailin, D. J., Manas, N. H. A., El-Ensashy, H. A., Man, M., Edis, Z., ... & Azelee, N. I. W. (2023). Antimicrobial properties of deep-sea water towards escherichia coli and staphylococcus aureus. *Journal of Bioprocessing and Biomass Technology*, *2*(1), 13-17.

Dahiru, M. M., Abaka, A. M., & Artimas, S. P. (2023). Phytochemical Analysis and Antibacterial Activity of Methanol and Ethyl Acetate Extracts of Detarium microcarpum Guill. & Perr. *Biology, Medicine, & Natural Product Chemistry*, *12*(1), 281-288.

Dalli, M., Bekkouch, O., Azizi, S. E., Azghar, A., Gseyra, N., & Kim, B. (2021). Nigella sativa L. phytochemistry and pharmacological activities: A review (2019–2021). *Biomolecules*, *12*(1), 20.

Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. (2023). Exploring the diverse bioactive compounds from medicinal plants: a review. *J. Phytopharm*, *12*, 189-195.

Ezzaky, Y., Elmoslih, A., Silva, B. N., Bonilla-Luque, O. M., Possas, A., Valero, A., ... & Achemchem, F. (2023). In vitro antimicrobial activity of extracts and essential oils of Cinnamomum, Salvia, and Mentha spp. against foodborne pathogens: A meta-analysis study. *Comprehensive Reviews in Food Science and Food Safety*, *22*(6), 4516-4536.

Hamidi, S. (2023). Assessment of undeclared synthetic drugs in dietary supplements in an analytical view: A comprehensive review. *Critical Reviews in Analytical Chemistry*, *53*(5), 986-996.

Jamal, A. (2023). Embracing nature's therapeutic potential: Herbal medicine. *International Journal of Multidisciplinary Sciences and Arts*, *2*(1), 117-126.

Lefebvre, T., Destandau, E., & Lesellier, E. (2021). Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A*, *1635*, 461770.

Lila, A. S. A., Rajab, A. A., Abdallah, M. H., Rizvi, S. M. D., Moin, A., Khafagy, E. S., ... & Hegazy, W. A. (2023). Biofilm lifestyle in recurrent urinary tract infections. *Life*, *13*(1), 148.

Loh, T. P., Cooke, B. R., Markus, C., Zakaria, R., Tran, M. T. C., Ho, C. S., ... & IFCC Working Group on Method Evaluation Protocols. (2023). Method evaluation in the clinical laboratory. *Clinical Chemistry and Laboratory Medicine (CCLM)*, *61*(5), 751-758.

Lu, L., Zhao, Y., Li, M., Wang, X., Zhu, J., Liao, L., & Wang, J. (2023). Contemporary strategies and approaches for characterizing composition and enhancing biofilm penetration targeting bacterial extracellular polymeric substances. *Journal of Pharmaceutical Analysis*.

Maione, A., Galdiero, E., Cirillo, L., Gambino, E., Gallo, M. A., Sasso, F. P., ... & Galdiero, M. (2023). Prevalence, resistance patterns and biofilm production ability of bacterial uropathogens from cases of community-acquired urinary tract infections in South Italy. *Pathogens*, *12*(4), 537.

Moiketsi, B. N., Makale, K. P., Rantong, G., Rahube, T. O., & Makhzoum, A. (2023). Potential of selected African medicinal plants as alternative therapeutics against multi-drug-resistant bacteria. *Biomedicines*, *11*(10), 2605.

Nisar, F., Rasool, S., Khan, M. K., Khan, W., Qaisar, Z., & Akhter, S. (2023). Health Care System of Islam: Physical, Mental and Spiritual Remedies from Islamic Perspective and Public Policy for Administration. *Research*, *8*(2), 83-113.

Ojueromi, O. O., Oboh, G., & Ademosun, A. O. (2022). Black seed (Nigella sativa): a favourable alternative therapy for inflammatory and immune system disorders. *Inflammopharmacology*, *30*(5), 1623-1643.

Pammi, S. S., Suresh, B., & Giri, A. (2023). Antioxidant potential of medicinal plants. *Journal of Crop Science and Biotechnology*, *26*(1), 13-26.

Rauniyar, M. (2023). *ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF Escherichia coli ISOLATES OF URINARY TRACT INFECTION FROM PATIENTS VISITING A TERTIARY CARE HOSPITAL OF MORANG, NEPAL* (Doctoral dissertation, Department of Microbiology Central Campus of Technology, Dharan, Nepal TU Registration Number: 5-2-0003-0451-2013 2023 Tribhuvan University).

Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023, July). Antimicrobial resistance: a growing serious threat for global public health. In *Healthcare* (Vol. 11, No. 13, p. 1946). MDPI.

Sati, P., Sharma, E., Dhyani, P., Attri, D. C., Rana, R., Kiyekbayeva, L., ... & Sharifi-Rad, J. (2024). Paclitaxel and its semi-synthetic derivatives: comprehensive insights into chemical structure, mechanisms of action, and anticancer properties. *European Journal of Medical Research*, *29*(1), 90.

Saxena, D., Maitra, R., Bormon, R., Czekanska, M., Meiers, J., Titz, A., ... & Chopra, S. (2023). Tackling the outer membrane: facilitating compound entry into Gram-negative bacterial pathogens. *npj Antimicrobials and Resistance*, *1*(1), 17.

Shafodino, F. S., Lusilao, J. M., & Mwapagha, L. M. (2022). Phytochemical characterization and antimicrobial activity of Nigella sativa seeds. *PloS one*, *17*(8), e0272457.

Sharma, R., Bhattu, M., Tripathi, A., Verma, M., Acevedo, R., Kumar, P., ... & Singh, J. (2023). Potential medicinal plants to combat viral infections: A way forward to environmental biotechnology. *Environmental Research*, 115725.

Sulaiman, A. N., & Muhammad, H. M. (2023). Phytochemical constituents and antibacterial activity of Nigella sativa seeds against some pathogenic bacteria. *Microbes and Infectious Diseases*.

Usman, M., Kabiru, M., Manga, S., Opaluwa, S., Nataala, S., Garba, M., ... & Bukar, A. (2017). Evaluation of antibacterial activity and phytochemical screening of the crude extract of nigella sativa seeds on the bacterial isolates of wound.

Zhou, Y., Zhou, Z., Zheng, L., Gong, Z., Li, Y., Jin, Y., ... & Chi, M. (2023). Urinary tract infections caused by uropathogenic Escherichia coli: Mechanisms of infection and treatment options. *International journal of molecular sciences*, *24*(13), 10537.