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Anti-Biofilm Activity of *Nigella sativa* Extracts against Pathogenic Bacteria

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Abstract

Antimicrobial resistance (AMR) is still a serious problem for global health; however, new antibiotic research is lagging behind AMR. There are dangers associated with bacterial biofilms in hospitals, restaurants, and water treatment plants. Several medicinal herbs are utilized ethnomedically in India to cure infectious diseases. However, apart from the conventional inhibitory effects on cell development, little is known about the possible anti-biofilm action of medicinal herbs, which may help in the prevention of bacterial infection. Here, we investigated the *in vitro* anti-biofilm activity of plant extracts of *Nigella sativa* (black cumin) seeds. The minimum inhibitory concentration (MIC) test was performed using the two-fold serial dilution technique. The establishment and growth of biofilms were evaluated using crystal violet (CV) tests. Methanol extracts showed antibacterial action against all bacterial strains, with *S. aureus* showing the most activity. *N. sativa* seed extract had good activity against bacterial biofilms. Further research into the isolation of antimicrobial compounds and the mechanism of action of *N. sativa* seed methanol extract may provide promising leads.

Keywords: drug-resistant bacteria, biofilm, plant extracts, *Nigella sativa*

Резюме

Антимикробната резистентност (АМР) все още е сериозен проблем за световното здравеопазване, но изследванията на нови антибиотици изостават от неговото развитие. Съществуват опасности, свързани с бактериалните биофилми в болници, ресторанти и пречиствателни станции. Няколко лечебни билки се използват етномедицината в Индия за лечение на инфекциозни заболявания. Въпреки това, освен конвенционалните инхибиторни ефекти върху развитието на клетките, малко се знае за възможното антибиофилмово действие на лечебните билки, което може да помогне за предотвратяване на бактериални инфекции. Тук изследвахме *in vitro* антибиофилмовото действие на растителни екстракти от семена на *Nigella sativa* (черен кимион). Тестът за минимална инхибиторна концентрация (MIC) е извършен чрез техниката на двукратно серийно разреждане. Растежът на биофилмите е оценен с помощта на тестове с кристал-виолет (CV). Метаноловите екстракти показват антибактериално действие срещу всички бактериални щамове, като най-голяма активност показват срещу *S. aureus*. Екстрактът от семената на *N. sativa* има добра активност срещу бактериални биофилми. По-нататъшните изследвания за изолиране на антимикробни съединения и механизма на действие на метаноловия екстракт от семена на *N. sativa* могат да осигурят обещаващи резултати.

Introduction

The emergence of multidrug resistance in bacterial pathogens has presented a unique challenge for antibiotic therapy (MacPherson *et al*., 2009). Several factors contribute to the emergence of antimicrobial-resistant bacterial species, including the widespread and inappropriate use of antibiotics sometimes, the widespread use of these substances as growth promoters in animal feed, and a rise in the transboundary transmission of bacteria resistant to antibiotics (Lowy, 2003). The World Health Organization (WHO) published a list of priority pathogens in 2017, including bacteria *Acineto-*

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bacter baumannii, Escherichia coli, *Enterobacter* spp., *Klebsiella* spp., *Streptococcus pneumoniae, Staphylococcus aureus* and others (Tacconelli *et al.*, 2018). Most current antibiotics, including ampicillin, carbapenem, penicillin, vancomycin, and the third-generation antibiotic cephalosporin, are highly resistant to these bacteria.

Many bacteria use biofilm development as a resistance strategy, making them more difficult to treat than planktonic cells (De La Fuente-Núñez *et al*., 2012). Biofilms are microbial colonies of microorganisms enclosed in a polymeric matrix of proteins, polysaccharides, and other organic compounds in which cells adhere to surfaces (Bazargani and Rohloff, 2016). Biofilms are an important virulence factor that contributes to the emergence of chronic infection; they are resistant to antibiotics and as well as the body's immune system (Grant and Hung, 2013). Biofilms may be involved in more than 75% of microbial infections (Musk *et al*., 2005), and biofilms are responsible for two-thirds of all human bacterial infections (De La Fuente-Núñez *et al*., 2016). The ability to produce biofilms makes it difficult to treat infections caused by many human and veterinary bacteria, including *Escherichia, Staphylococcus, Pseudomonas, Pasteurella, Bacillus, Salmonella*, etc. (Chakraborty *et al*., 2018).

As a result, new pharmacological targets must be identified, and novel therapies developed to combat bacterial infection. One of the most active areas of study to reduce the risk of infectious diseases caused by bacteria, fungi, viruses, and parasites has been the search for compounds with strong antimicrobial activity (Cos *et al*., 2006).

Medicinal plants remain one of the most important sources of many therapeutic agents, including antimicrobial compounds used to treat infectious diseases (Cos *et al*., 2006). Medicinal plants are becoming more popular as a therapeutic option in medicine and food preservation. Their potent actions and low toxicity have led to a surge in their popularity. Up to 80% of the population in certain affluent nations has utilized medicinal plants in their basic health care, according to WHO data (Zhang, 2002). Eighty percent of people in China and India rely on these treatments. Medicinal plants are used to develop antibacterial medications to cure infectious diseases. Medicinal plants are often more appealing to customers, and if these alternative ways work, they may reduce dependency on synthetic compounds (Ahmad and Aqil, 2009). Medicinal plants have long been the primary natural producers of bioactive phytochemicals, such as alkaloids, flavonoids, phenols, steroids, tannins, and terpenoids (Mengesha-Yessuf, 2015). Barks, fruits, flowers, leaves, roots, and seeds of medicinal plants are all employed in the production of phytomedicines, and many bioactive plant constituents have been isolated and characterized (Thilakarathna *et al*., 2018).

N. sativa Linn. (*Ranunculaceae*), also known as black seed or black cumin, has been used for years in Asia, the Middle East, and Africa as a herb and pressed into oil (Zohary and Hopf, 1988). It is considered one of the most powerful types of curative medication accessible in Islam and is included in the medicine of the Prophet Mohammed. In the Unani system of medicine, black cumin has been recognized as an effective treatment for various diseases. Black cumin has been used historically for various health issues, including the lungs, digestive system, kidneys, liver, blood circulation, immunity, and overall health. Indians utilized this plant as a food preservative as well as a preventive and curative therapy for a range of infections. Black cumin seeds are abundant in phytoconstituents i.e. alkaloids, polyphenols, flavonoids, steroids, and terpenes with a wide range of pharmacological activity (Jin, 2019). *N. sativa* seeds have previously been studied for their anti-cancer (Majdalawieh *et al.*, 2017), anti-diabetic (El Rabey *et al.*, 2017), anti-inflammatory (Houghton *et al*., 1995), antimicrobial (Abdurrezagh Elfahem, 2013), antioxidant (Tiji *et al*., 2021), and anti-tumor (Majdalawieh and Fayyad, 2016) properties. In addition, black cumin was shown to be effective in treating diseased bones, accelerating the healing process, and reducing the risk of infection(Al-Mutheffer, 2014; Arslan *et al*., 2017).

This study tested black cumin for its antibacterial and anti-biofilm potential against bacteria species, *Bacillus cereus, E. coli*, *K. pneumoniae*, and *S. aureus*, which are well-known for causing multidrug-resistant infections in healthcare.

Materials and Methods

Bacterial isolates

The following bacterial isolates: *Bacillus subtilis* (str. 168, FRA), *E. coli* (str. O157:H7, US), *K. pneumoniae* (str. MGH 75858, UK), and *S. aureus* (str. MSSA, JAP) were used in this study. All the bacterial isolates were maintained in Nutrient agar.

Extraction of N. sativa seeds

N. sativa seeds were bought from the herbal market and authenticated in the Department of Bot-

any, Aligarh Muslim University. The seeds were rinsed with distilled water, shade-dried, and then ground in a blender. 10gm of powder was soaked for 48 hours at room temperature in 100 mL volumes of distilled water, methanol, ethyl acetate, or hexane, with regular stirring. The extracts were filtered using the Whatman filter paper-1. All filtrates were concentrated using the rotatory evaporator and stored at 4°C in an airtight container for future use. Antibacterial activity was tested on all of the extracts.

Calculation of percentage yield

The various extracts were weighed, and the following equation was used to determine the % yield of each extract.

Percentage yield (%Y) =

= (Extract weight / Powder weight) × 100.

Antibacterial activity test

The agar well diffusion method was utilized for the antibacterial activity of seed extracts. On Muller Hinton Agar (MHA) plates, 100µL of diluted inoculum of bacterial strain (10^5 CFU/ml) was spread. Plant extract and solvent blank (DMSO) were each added to separate wells in the agar medium. The plates were incubated overnight at 37°C. Each well has a bacterial growth inhibition zone surrounding it.

Determination of Minimum Inhibitory Concentration (MIC)

MIC was performed for those extracts that indicated a zone of inhibition against bacteria. For MIC determination of seed extracts broth microdilution method was used, with minor modification. The plant extract, including Muller-Hinton broth, was serially diluted two-fold to achieve varying concentrations and tested in a 96-well ELISA plate. Bacteria were cultured in various concentrations of plant extract and incubated for 18 hours at 37°C. Following incubation, 20 microliters of Triphenlytetrazolium chloride (3mg/mL) were added to each well and left for 30 minutes at room temperature. Following incubation at room temperature, the pink color indicated bacterial growth. The MIC was calculated as the lowest concentration at which no color change was visible, indicating that no metabolically active cells were present.

Antibiofilm activity of plant extracts

The effect of *N. sativa* extracts on bacterial biofilm formation was assessed by the crystal violet (CV) staining assay (Fig. 1).

In brief, 20 µL of overnight bacterial culture

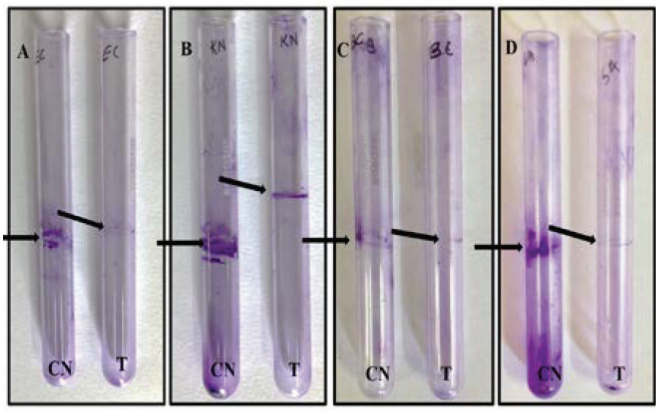

Fig. 1. Anti-biofilm activity of *N. sativa* seed extracts against pathogenic bacteria. (A) *E. coli,* (B) *K. pneumoniae,* (C) *B. cereus,* (D) *S. aureus.* CN= control and T= treated

and *N. sativa* seed extracts were loaded in a sub-MIC concentration (MIC/2-MIC/16) into 180 µL of Luria-broth (LB) medium in the 96-well microtiter plate and incubated for 24 hours at 37°C. After incubation, the planktonic cells were rinsed away with sterile water, and the well-adherent biofilm was stained with a crystal violet solution (0.1%v/v). After 15 min room temperature incubation, unbound crystal violet was rinsed with sterile water. Finally, the adhering biofilm-bound crystal violet was solubilized in 200 µL of ethanol (95%) and measured the absorbance at OD_{620} (Khan *et al*., 2022).

Visualization of biofilm

The antibiofilm activity of extracts was evaluated qualitatively using the tube method (Kalishwaralal *et al*., 2010). The tested bacteria were cultured in LB broth, and the turbidity was adjusted to 0.5 McFarland Standards. After adding plant extracts (MIC/2), the suspension was transferred to 2 ml of sterile LB broth and incubated for 24 hours at 37°C. The experiment included plant extract-free as a control. After incubation, the broth culture was removed, and the tube was washed with PBS. The tubes were stained with 0.1% crystal violet dye for 30 minutes, and then the excess dye was removed with distilled water. The capacity to form biofilms was measured by seeing a thin blue film on the tube walls.

Statistical Analysis

Experiments were performed in triplicate. The data were presented as the mean with standard deviation.

Results

Yield extraction

Table 1 displays the extraction yields of the various extracts that were studied. During extrac-

tion, various extracts had diverse results. The *N. sativa* seed aqueous extract had the highest yield (30.14%), whereas the hexane extract had the lowest yield (2.1%).

Table 1. Percentage yield of seed extracts

Extract	Yield (%)
Water	16.45
Methanol	28.36
Ethyl Acetate	8.16
Hexane	4.54

Antimicrobial activity

N. sativa seed extract was assessed *in vitro* for antibacterial activity against pathogenic bacterial strains. Consequently, antibacterial activity against various tested bacteria was measured at various values in plant extract samples, with inhibition zones ranging in diameter from 9.15 mm to 15.80 mm (Table 2, Fig. 2).

Table 2. *N. sativa* extracts antibacterial activity (zones of inhibition)

Bacteria	Zone of inhibition (mm ± SD)			
	Water extract	Methanol extract	Ethyl acetate extract	Hexane extract
E. coli	-ve	15.50 ± 0.25	-ve	-ve
K. pneumoniae	-ve	15.80 ± 0.25	-ve	-ve
S. aureus	-ve	13.50 ± 0.25	-ve	-ve
B. cereus	-ve	9.15 ± 0.50	-ve	-ve

"-ve" = no activity

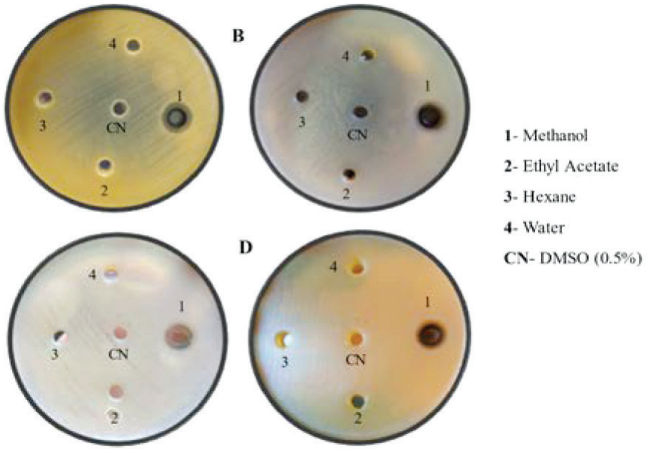

Fig. 2. Antimicrobial activity of *N. sativa* seed extracts against pathogenic bacteria. (A) *S. aureus,* (B) *B. cereus,* (C) *K. pneumoniae,* (D) *E. coli*

MIC determination

To quantify the activity of extracts, the MIC was calculated against the chosen bacteria. The extracts MIC varied from Gram-negative and Gram-positive bacteria (Table 3).

Table 3. Minimal inhibitory concentration of methanol extract of *N. sativa* seed

Bacteria	MIC (mg/mL)
E. coli	1.1
K. pneumoniae	1.4
S. aureus	0.5
B. cereus	0.7

Antibiofilm activity

In this study, biofilm quantification using the CV method demonstrated a concentration-dependent reduction in the biofilm formation when treated with the *N. sativa* seed extract. The extract inhibited the biofilm production of *E. coli*, *K. pneumoniae*, *S. aureus*, and *B. cereus* by 7.03-71.55%, 8.92-6591%, 10.06-76.76%, and 9.46-67.5% at increasing concentrations corresponding to MIC/16-MIC/2 (Fig. 3).

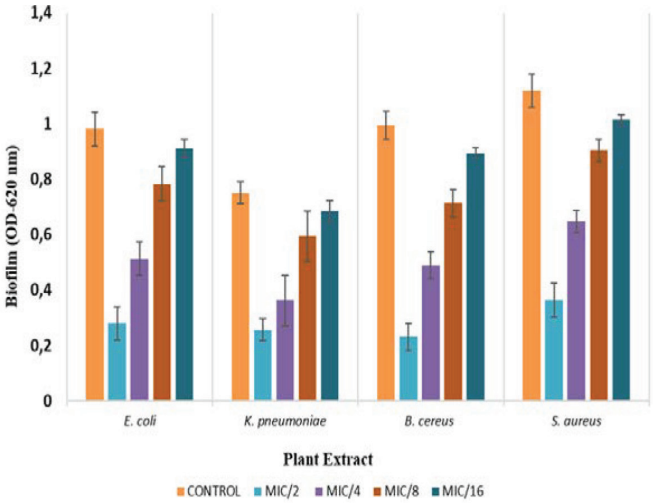

Fig. 3. Anti-biofilm activity of *N. sativa* seed extracts against pathogenic bacteria.

Discussion

The yield of a plant extract is important in determining overall activity when comparing plants for bioprospecting (Eloff, 2000).

The search for novel antimicrobial drugs has increased in recent decades due to the simultaneous rise of antibiotic-resistant bacterial strains (Wigmore *et al*., 2016). Using compounds extracted from medicinal plants may be advantageous for developing antibiotics, according to studies done in many nations (Abdurrezagh Elfahem, 2013). In the present investigation, plant extract showed antibacterial activity against pathogenic bacteria.

The lower the MIC, the more effective the drug (Eloff, 2004). Antimicrobial activity of plant extracts has been categorized as excellent when the minimum inhibitory concentration (MIC) is less than 0.1 mg/mL, moderate when the MIC is

between 0.1 and 0.625 mg/mL, and weak when the MIC is more than 0.625 mg/mL (Kuete, 2010). A high-activity extract was selected for further experiments.

Bacterial biofilm continues to be a worldwide health problem due to its resistance to treatment and tendency to cause nosocomial infections. As a result, finding innovative, effective compounds to combat this problem is a prime objective (Nostro *et al*., 2016).

The bacterial strains *E. coli*, *K. pneumoniae*, *S. aureus*, *B. cereus* significantly reduced their biofilm biomass after being treated with *N. sativa* seed extract at MIC/2 concentration. The effect of the extract on the development of biofilms was determined using the tube method, which involved cultivating different bacteria in the presence of the extract. The growth of a thin layer of biofilms following dye staining confirmed the results.

In the case of the treated (T) tube, there is no or very little visible layer of biofilm found which confirms that extract inhibits the formation of biofilm. A significant reduction in forming an aggerate-like structure in bacterial cells was observed after treatment with the sub-MICs. The ability of *N. sativa* seed extracts to inhibit the development of biofilms by bacterial pathogens may be related to interference with forces (such as Brownian, sedimentation, Lifshitz–Van der Waals, and electrostatic interaction forces) that promote the deposition and adhesion of bacteria to surfaces (Roy *et al*., 2018). Certain inorganic and organic compounds and nutrients are important for cell development and adhesion (Sandasi *et al*., 2010). Plant extracts may restrict nutrition availability. Active plant extracts may reduce surface colonization and epithelial infections. In a study, ethanol and acetone extracts of *Psidium guajava* (Myrtaceae) reduced *S. mutans* adhesion to oral surfaces (Gomashe *et al*., 2014).

Conclusion

Considering the results of this study, it is concluded that *N. sativa* seed extract is a potential source of antibacterial and antibiofilm agents against pathogenic bacteria biofilm and may be used in natural remedies for infectious diseases. However, this is one of the few studies examining the antibiofilm activity of *N. sativa* seed. Thus, the inhibitory effect of the extracts observed in this investigation on biofilm development is significant. Additional research will be required to isolate and identify the bioactive molecules responsible for extracting antibiofilm action. Furthermore, assessing antibiofilm efficacy *in vivo* will be crucial in determining the mechanism by which plant extract compounds influence biofilm development.

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References

Abouzeed, Y. M., E. Abdurrezagh, F. Zgheel, M. O. Ahmed (2013). Antibacterial *in vitro* activities of selected medicinal plants against methicillin resistant S*taphylococcus aureus* from Libyan environment. *J. Environ. Anal. Toxicol.* **3**: 1-3.

Ahmad, I., F. Aqil (2009). New Strategies Combating Bacterial Infection. In: Ahmed, I., F. Aqil (Eds.), New Strategies Combating Bacterial Infection, Wiley-Blackwell, London. pp. 177-182.

Al-Mutheffer, E. A. (2014). The percutaneous effect of black seed (*Nigella sativa*) oil as external topical treatment on bone healing in rabbits. *Al-Qadisiyah J. Vet. Med. Sci.* **13**: 133–138.

Arslan, A. H., C. O. Tomruk, E. G. Meydanl, I. Özdemir, G. Duygu Çapar, E. Kütan, A. Yılmaz, G. M. Yalçın Ülker (2017). Histopathological evaluation of the effect of systemic thymoquinone administration on healing of bone defects in rat tibia. *Biotechnol. Biotech. Eq.* **31**: 175-181.

Bazargani, M. M., J. Rohloff (2016). Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and *Escherichia coli* biofilms. *Food Control* **61**: 156–164.

Chakraborty, S., T. K. Dutta, A. De, M. Das, S. Ghosh (2018). Impact of bacterial biofilm in veterinary medicine: An overview. *Int. J. Cur. Mobiol. Appl. Sci.* **7**: 3228–3239.

Cos, P., A. J. Vlietinck, D. Berghe, B. Vanden, L. Maes (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* "proof-of-concept." *J. Ethnopharmacol*. **106**: 290–302.

De La Fuente-Núñez, C., M. H. Cardoso, E. De Souza Cândido, O. L. Franco, R. E. W. Hancock (2016). Synthetic antibiofilm peptides. *Biochim. Biophys. Acta-Biomembranes* **1858**: 1061–1069.

De La Fuente-Núñez, C., V. Korolik, M. Bains, U. Nguyen, E. B. M. Breidenstein, S. Horsman, S. Lewenza, L. Burrows, R. E. W. Hancock (2012). Inhibition of bacterial biofilm formation and swarming motility by a small synthetic cationic peptide. *Antimicrob. Agents Chemother.* **56**: 2696–2704.

El Rabey, H. A., M. N. Al-Seeni, A. S. Bakhashwain (2017). The antidiabetic activity of nigella sativa and propolis on streptozotocin-induced diabetes and diabetic nephropathy in male rats. *Evid.-Based Complement. Altern. Med.* **2017**: 5439645.

Eloff, J. N. (2000). On expressing the antibacterial activity of plant extracts - A small first step in applying scientific knowledge to rural primary health care. *S. Afr. J. Sci*. **96**: 116–118.

Eloff, J. N. (2004). Quantification the bioactivity of plant extracts during screening and bioassay guided fractionation. *Phytomedicine* **11**: 370–371.

Gomashe, A. V, A. A. Sharma, A. Kasulkar (2014). Original Research article investigation of biofilm inhibition activity and antibacterial activity of *Psidium guajava* plant extracts against *Streptococcus* mutans causing dental plaque. *Int. J. Cur. Microbiol. Appl. Sci.* **3**: 335–351.

Grant, S. S., D. T. Hung (2013). Persistent bacterial infections, antibiotic tolerance, and the oxidative stress response. *Virulence* **4**: 273–283.

Houghton, P. J., R. Zarka, B. De Las Heras, J. R. S. Hoult (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.* **61**: 33–36.

Jin, Y. S. (2019). Recent advances in natural antifungal flavonoids and their derivatives. *Bioorg. Med. Chem. Lett*. **29**: 126589.

Kalishwaralal, K., S. BarathMani Kanth, S. R. K. Pandian, V. Deepak, S. Gurunathan (2010). Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. *Colloids Surf B*. **79**: 340–344.

Khan, M. A., H. M. Khan, I. B. Ganie, S. Kumar, A. Shahzad, I. Celik, M. Shahid (2022). Anti-quorum sensing, antibiofilm, and antibacterial activities of extracts of *Centella asiatica* L. leaves, and *in vitro* derived leaves-calli through tissue culture: a potential for biofouling-prevention. *Biofouling* **38**: 715-728.

Kuete, V. (2010). Potential of *Cameroonian* plants and derived products against microbial infections: A review. *Planta Med*. **76**: 1479–1491.

Lowy, F. D. (2003). Antimicrobial resistance: The example of *Staphylococcus aureus*. *J. Clin. Invest*. **111**: 1265–1273.

MacPherson, D. W., B. D. Gushulak, W. B. Baine, S. Bala, P. O. Gubbins, P. Holtom, M. Segarra-Newnham (2009). Population mobility, globalization, and antimicrobial drug resistance. *Emerg. Infect. Dis.* **15**: 1727–1732.

Majdalawieh, A. F., M. W. Fayyad (2016). Recent advances on the anti-cancer properties of Nigella sativa, a widely used food additive. *J. Ayurveda Integr. Med.* **7**:173–180.

Majdalawieh, A. F., M. W. Fayyad, G. K. Nasrallah (2017). Anti-cancer properties and mechanisms of action of thymoquinone, the major active ingredient of *Nigella sativa*. *Crit. Rev. Food Sci. Nut.* **57**: 3911–3928.

Mengesha-Yessuf, A. (2015). Phytochemical extraction and screening of bio active compounds from black cumin (*Nigella sativa*) seeds extract. *Amer. J. Life Sci*. **3**: 358-364.

Musk, D. J., D. A. Banko, P. J. Hergenrother (2005). Iron salts perturb biofilm formation and disrupt existing biofilms of *Pseudomonas aeruginosa*. *Chem. Biol.* **12**: 789–796.

Nostro, A., A. Guerrini, A. Marino, M. Tacchini, M. Di Giulio, A. Grandini, M. Akin, L. Cellini, G. Bisignano, H. T. Saraçoğlu (2016). *In vitro* activity of plant extracts against biofilm-producing food-related bacteria. *Int. J. Food Microbiol.* **238**: 33–39.

Thilakarathna, R. C. N., G. D. M. P. Madhusankha, S. B. Navaratne (2018). Phytochemical analysis of Indian and Ethiopian black cumin seeds (*Nigella sativa*). *Agricult. Res. Technol.* **17**: 12-17.

Roy, R., M. Tiwari, G. Donelli, V. Tiwari (2018). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence* **9**: 522–554.

Sandasi, M., C. M. Leonard, A. M. Viljoen (2010). The *in vitro* antibiofilm activity of selected culinary herbs and medicinal plants against *Listeria monocytogenes*. *Lett. Appl. Microbiol.* **50**: 30–35.

Tacconelli, E., E. Carrara, A. Savoldi, S. Harbarth, M. Mendelson, D. L. Monnet, C. Pulcini, et al (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* **18**: 318–327.

Tiji, S., O. Benayad, M. Berrabah, I. El Mounsi, M. Mimouni (2021). Phytochemical profile and antioxidant activity of *Nigella sativa* L growing in Morocco. *Sci. World J.* **2021**: 6623609.

Wigmore, S. M., M. Naiker, D. Bean (2016). Antimicrobial activity of extracts from native plants of temperate Australia. *Phacog. Commn*. **6**: 80–84.

Zhang, X. E. A. (2002). Traditional medicine strategy 2002-2005. *World Health Organisation* **1**: 1–74.

Zohary, D., M. Hopf (1988). Origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. In: Hopf, M., D. Zohary (Eds.), Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley. Oxford University Press, NY. pp: 202-208.