

Original Article

Antibacterial and Antibiofilm Activities of *Nigella sativa* L. Seed Extracts

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The emergence of antibiotic-resistant bacteria is a major public health problem. Consequently, the development of new antimicrobials that act on novel bacterial targets and are less susceptible to resistance are of primary importance to researchers in academia and industry alike. The present study was aimed to determine the antimicrobial and antibiofilm activities of *Nigella sativa* seed extracts. For this, primary (qualitative) and quantitative antibacterial activities of *Nigella sativa* seed extracts were determined against ten human pathogenic bacteria including four biofilm producing bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The antibacterial activities as well as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were evaluated using disc diffusion and macro-broth dilution methods, respectively. The seed extracts of *N. sativa* exhibited the highest extract yields (w/v) 23.3% and 21.35% with ethanol and petroleum ether, respectively. The crude extract (essential oil) of petroleum ether exhibited superior antibacterial activity (36 mm in diameter zone of inhibition) against biofilm forming *E. coli* on a disc diffusion assay at a concentration of 1000 µg/disc when compared to that of ethanolic crude extract. Moreover, the lowest MIC and MBC values were determined as 500 µg/ml and 1500 µg/ml respectively with the same petroleum ether extract against *E. coli*. However, *N. sativa* essential oil obtained from petroleum ether extract of seed inhibited 94% biofilm formation of *E. coli* at 2×MIC concentration. Moreover, at the same concentration (2×MIC), the ethanol extract inhibited 56% biofilm formation of *P. aeruginosa*. These results consistently revealed that it is possible to isolate novel antimicrobial agents capable of completely eradicating microbial infections including antibiotic resistance. Therefore, bioactive natural products present in plant resources would represent a noteworthy alternative to commercial antibiotics helping treatment of human antibiotic resistant infections.

Keywords: Antibacterial activity, Antibiofilm activity, *Nigella sativa*, Seed extract.

Introduction

Microorganisms have developed resistance to many antibiotics, and this has created immense clinical problems in the treatment of infectious diseases¹. The biofilm mode of growth is the survival strategy of many environmental bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Burkholderia cepacia*². Although the above biofilm producing bacterial species are generally not virulent in healthy persons, these opportunists can cause severe and chronic respiratory tract infections in individuals with cystic fibrosis (CF)³. Unremitting infections and the associated inflammation result in progressive lung disease that culminates in pulmonary failure, the leading cause of death for CF patients. Effective therapy of CF pulmonary infection is severely limited by the broad-spectrum antimicrobial resistance exhibited by these species, which are among the most drug-resistant bacteria encountered in human infections. This situation forced scientists to search for new antimicrobial substances from various sources. These challenges have driven an increase in recent efforts to develop novel and efficient antimicrobial agents against these drug resistant pathogens.

Now-a-days, the natural products from plant extracts have been accepted as an important source of biologically active

(antimicrobial) substances⁴⁻⁶ and the major sources of which are still left undiscovered. It has now been discovered that secondary metabolites of some plants have surprisingly useful therapeutic properties and many of them are found to be active against multi-drug resistant pathogens⁷. The crude extracts from some dietary berries showed quorum sensing (QS) inhibitory activity against biofilm forming bacteria like *P. aeruginosa*. The effective phytochemicals inhibited the bacteria from recognizing a critical density of bacteria, i.e., quorum sensing (QS), and responding with the production of the biofilm-triggering chemical⁸. Even today, 85% of Indians use higher plants as effective antimicrobials for the treatment of various diseases⁸. A large number of antimicrobial agents derived from traditional medicinal plants are available for treating various diseases caused by microorganisms¹⁰⁻¹¹. Since these antimicrobial agents are derived from plant resources, they are generally recognized as safe and are rarely associated with any side-effects as seen in many antibiotic regimens¹².

Nigella sativa L., belonging to the family Ranunculaceae, is widely distributed in Eastern Europe, Middle East, and Western Asia¹³. The plant is a bushy small shrub with tapering green leaves and white or pale to dark blue flowers. Its tiny black seeds mainly contain biologically active 35% fixed and essential oils¹⁴, also

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contain 21% proteins, 29% carbohydrates, 6% crude fibers, 5% moisture, and 4% ash¹⁵. Therefore, three biological active compounds thymoquinone, carvacrol, and p-cymene have been reported in the essential oils of *N. sativa* seed, which have broad spectrum antimicrobial activity^{14,16}. The seeds of the *N. sativa* locally known as Kalonji or Kalojera which has been used in traditional and alternative medicines for the treatment of asthma, diarrhea, parasitic diseases, and allergy¹⁷⁻¹⁹. The present study was aimed to determine the antimicrobial and antibiofilm activities of *Nigella sativa* seed extracts.

Materials and methods

Seed extract for primary screening

Dried seeds of *N. sativa* were collected from local market, washed thoroughly using distilled water, dried in oven at 50°C and powdered mechanically. The powdered sample was extracted using five different organic solvents ethanol, chloroform, ethyl acetate, carbon tetrachloride and Petroleum ether. However, for extraction of primary screening, the sample was steeped separately into 95% ethanol and petroleum ether at a ratio of 1:3 (plant material: organic solvent) for 48 hours. The solvent extracts were collected separately by filtration. These crude extracts were then tested for primary screening of antibacterial activity against ten human pathogenic bacteria by disc diffusion method¹⁹.

Seed extract for quantitative antibacterial and antibiofilm activities

A measured amount (200g) of dried powder of *N. sativa* seeds were kept in dried 500 ml conical flask and steeped separately in 95% ethanol and petroleum ether at room temperature for five days. Each extract was then passed through the clean cheesecloth for removing plant materials and then filtered through Whatman filter paper No.-1. The filtrate was then concentrated under reduced pressure at 45-50°C using rotary vacuum evaporator, put into oven at 45°C until dry. The concentrated extract thus obtained is termed as dried crude extract. The dried crude extract obtained from petroleum ether extract was yellowish-pink essential oil. The crude extract was stored in sealed vials at 4°C.

Determination of extract yield

The crude extract was weighed and the percentage yield of each was calculated as follows:

$$\text{Extract yield \%} = W1 / W2 \times 100$$

Here, W1= Net weight of dried crude extract in grams after extraction.

And W2= Total weight of plant sample powder in grams taken for extraction.

Test organisms

Escherichia coli ATCC25923, *Pseudomonas aeruginosa* ATCC8027, *Salmonella abony* NCTC6017, *Klebsiella pneumoniae* ATCC13883, *Vibrio cholerae* AE14748, *Shigella dysenteriae* AE14612, *Acinetobacter baumannii* ATCC17978,

Bacillus cereus BTCC19, *Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC6538.

Crude extract solution for quantitative antibacterial and antibiofilm assay

A measured amount of dried crude extract or essential oil was dissolved in definite volumes of ethanol/DMSO to give solution (10%) of known concentration. From this solution desired concentrations were used for further test.

Treatment of paper disc by extracts

Solutions of known concentration of the crude extract or essential oil were prepared by dissolving measured amount of the sample in definite volume of solvent. Dried and sterilized filter paper discs were treated with known concentration of extract using micropipette. However, extracts for primary screening, 4mm in diameter paper discs were soaked with the respective extract.

Antibacterial activity assay

The antibacterial activities of the *N. sativa* seed extracts were done by disc diffusion method²⁰ and Muller-Hinton agar (17.5 g/L acid hydrolysate of casein, 2 g/L beef extract, 1.5 g/L starch and 15 g/L agar) medium was used. However, for seed culture of test bacteria, Luria-Bertani (LB) broth (10 g/L peptone, 5 g/L yeast extract and 10 g/L NaCl) medium was used. For *in vitro* sensitivity of the bacteria to the test materials, paper discs of 4 mm in diameter and petri dish of 100 or 150 mm in diameter were used throughout the experiment. All experiments were repeated three times.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of crude extracts or essential oils of *N. sativa* seed were determined by macro dilution broth technique²¹. Luria-Bertaini (LB) broth medium was used for bacterial culture. All experiments were performed in triplicate and the results are expressed as mean values of three replicates.

Anti-biofilm Activity

The effect of *N. sativa* seed extracts on biofilm formation was evaluated as described by Sandasi *et al.* (2010)²² with slightly modification mentioned in Christensen *et al.* (1985)²³. Briefly, the solutions of extract equivalent to 0.5× MIC, 1.0× MIC and 2.0× MIC were prepared. Each sample solution of 0.5mL was distributed into individual test tubes. Equal volumes of methanol or water were added as negative controls while tetracycline (0.00125 mg/ml) was added as a positive control. Then, 0.5 mL of each diluted overnight culture matched to 0.5 McFarland turbidity standard was added to the test tube to yield a final volume of 1.0 mL in each test tube and sterile 1.0 mL LB broth was also added as an additional nutrient, before plugging the tubes. The test tubes were then incubated for 48h at 37°C to allow cell attachment. Biofilm formation was assessed using the modified crystal violet assay described by Djordjevic *et al.* (2002)²⁴. After subsequent incubation, the content of each tube was carefully

removed with a pipette without disturbing the biofilm. Test tubes were washed five times with sterile distilled water to wash away any loosely attached bacterial cells. Then the test tubes were air dried and further oven dried at 60°C for few minutes. The cells in the biofilm were then stained with 1% crystal violet and incubated at room temperature for 15 minutes. The test tubes were then washed three times with sterile distilled water to remove the excess stain and 2 mL of 95% ethanol was added to the test tube to de-stain the tube. The de-staining solution from each test tube was then transferred to new test tube and absorbance was measured at 530 nm. The mean absorbance was used for determining the percentage of inhibition of biomass formation for each concentration using the following equation:

$$\text{Percentage of inhibition} = 100 - \left[\frac{(\text{OD}_{530} \text{ nm of experimental})}{(\text{OD}_{530} \text{ nm of control})} \times 100 \right]$$

Results and Discussion

Bacteria have the genetic ability to transmit and acquire resistance to drugs which are consumed as therapeutic agents. Therefore, resistant bacteria represent a challenge in the treatments of various well-known infections and requires the new substances with antimicrobial properties. Consequently, a great deal of attraction has been paid to the antimicrobial effects of plant extracts which show significant activity.

Yielding percentage of extract

Yielding percentages of crude extracts obtained using different solvents ethanol and petroleum ether were 21.35 and 23.3 respectively.

Primary screening for antibacterial activity

For primary screening, the ethanol, chloroform, ethyl acetate, carbon tetrachloride and petroleum ether extracts were screened primarily for their antibacterial activities against ten human

pathogenic bacteria including four biofilm producing bacterial strains *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus*. The results of the primary screening for antibacterial activities are presented in Table 1.

In this screening test, 4 mm in diameter paper discs were soaked with the crude extracts. From Table 1, it was appeared that both ethanol and petroleum ether extracts exhibited the largest zone of inhibitions (8 – 13 mm in diameter) against all the organisms. Among the pet ether and ethanol extracts of *N. sativa* seeds, the pet ether extract showed comparatively the better antibacterial activities compared to that of other extracts. The ethanol extract also showed moderate zone of inhibitions from 8 to 11 mm in diameter against all the bacterial pathogens tested herein (Table 1). The largest zone of inhibition 13 mm in diameter was exhibited by pet ether extract against *K. pneumoniae* and *P. aeruginosa* (Table 1). Therefore, the ethanol and petroleum ether extracts were selected for quantitative antibacterial and antibiofilm activities test. Similar antimicrobial activities of other plant extracts have been reported previously²⁵⁻²⁸.

Quantitative antibacterial activity of *N. sativa* seed extracts

After primary selection, the ethanol and petroleum ether extracts of *N. sativa* seed were tested for antibacterial activities against ten bacterial pathogens including biofilm producing *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. aureus*. Therefore, antimicrobial activities of ethanol and petroleum ether extracts of *N. sativa* seeds are presented in Table 2. From Table 2, it was appeared that crude extracts of 500 and 1000 µg/disc exhibited good inhibitions against all the ten bacterial pathogens. Moreover, 1000 µg/disc extract exhibited the better inhibitions compared to that of 500 µg/disc concentration. The largest zone of inhibitions 28 mm and 19 mm in diameters were exhibited by 1000 and 500 µg/disc methanol extracts, respectively against a biofilm producing bacterial pathogen *E. coli* (Table 2).

Table 1: Antibacterial activity of crude extracts of *Nigella sativa* seeds.

Test organisms	Zone of inhibition (mm in diameter)				
	Ethanol	Ethyl acetate	Petroleum ether	Carbon tetrachloride	Chloroform
<i>Escherichia coli</i>	10	08	11	08	08
<i>Klebsiella pneumoniae</i>	08	10	13	07	07
<i>Staphylococcus aureus</i>	08	08	12	07	-
<i>Salmonella abony</i>	09	07	08	-	-
<i>Acinetbacter baumannii</i>	11	07	10	08	09
<i>Bacillus cereus</i>	08	-	08	07	06
<i>Bacillus subtilis</i>	10	10	11	06	08
<i>Pseudomonas aeruginosa</i>	08	08	13	06	07
<i>Vibrio cholerae</i>	09	08	11	09	06
<i>Shigella dysenteriae</i>	08	07	10	07	-

Note: Bold indicates the largest zone of inhibition.

However, 1000 µg/disc petroleum ether extract (crude) of *N. sativa* seeds also showed the largest zone of inhibition 36 mm in diameter against *E. coli* (Table 2). Furthermore, the largest 25 mm in diameter zone of inhibition was obtained against biofilm producing bacterial pathogens *E. coli* and *P. aeruginosa* with 500 µg/disc crude extract of petroleum ether. Thus, the crude extract of pet ether exhibited comparatively the better antibacterial activities that of ethanol extract (Table 2). Similar antimicrobial activity of other plant extracts as reported previously^{25-26,29}. Hanafy and Hatem (1991)³⁰ showed the maximum zone of inhibition (16 mm) at 400 µg/disc of diethyl ether extract of *N. sativa* seeds against *S. aureus*.

Our results are in concurrence with the results reported previously by Chowdhury *et al.* (2007)³¹. They found highest zone of inhibition 44 mm in diameter at 1000 µg/disc and 27.5 mm in diameter at 500 µg/disc with the crude extract of *Mikania cordata* against *Vibrio cholerae* and *Salmonella paratyphi*, respectively. However, in this study, petroleum ether extract of *N. sativa* displayed 36 mm in diameter zone of inhibition against *E. coli* at 1000 µg/disc concentration. Furthermore, Pattanayak *et al.* (2015)³² evaluated the antimicrobial activity of succulent and methanol extracts of *Mikania scandens*. They found that the methanolic extract showed better effects against *S. aureus*. Soma

et al. (2018)²⁸ showed that ethanol extract had better antimicrobial activity than aqueous extract. However, the petroleum ether extract showed the better antibacterial activities than other extracts due to higher concentration of essential oils extracted from the *Nigella sativa* seeds.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Nigella sativa* seed extracts MIC and MBC values of the crude extracts were determined by macro-dilution broth technique²¹. MIC and MBC provide information whether antimicrobial agents are either bacteriostatic or bactericidal.

MIC and MBC values of the ethanol and pet ether extracts of *N. sativa* seeds are shown in Table 3A and Table 3B, respectively. The ethanol extract exhibited a wide range of MIC values from 1000µg/ml to 2000 µg/ml against the bacterial pathogens tested herein (Table 3A). Similarly, the same ethanol extract demonstrated the MBC values ranging from 2500 µg/ml to 4000 µg/ml against the bacterial pathogens. The lowest MIC value (1000 µg/ml) was recorded against *E. coli*, and *P. aeruginosa* with ethanolic extract of *N. sativa* seeds. On the other hand, the lowest MBC value (2500 µg/ml) was obtained against biofilm producing *P. aeruginosa* using the same ethanol extract (Table 3A).

Table 2: Quantitative antibacterial activities of ethanol and petroleum ether extracts of *Nigella sativa* seeds

Test organisms	Zone of inhibition (mm in diameter)			
	Ethanol extract		Petroleum ether extract	
	1000 µg/disc	500 µg/disc	1000 µg/disc	500 µg/disc
<i>Escherichia coli</i>	28	19	36	25
<i>Klebsiella pneumoniae</i>	25	16	32	22
<i>Staphylococcus aureus</i>	17	13	26	17
<i>Salmonella abony</i>	20	14	17	12
<i>Acinetobacter baumannii</i>	12	08	15	10
<i>Bacillus cereus</i>	18	12	18	11
<i>Bacillus subtilis</i>	16	11	17	11
<i>Pseudomonas aeruginosa</i>	20	13	35	25
<i>Vibrio cholerae</i>	19	11	21	14
<i>Shigella dysenteriae</i>	18	12	22	13

Note: Bolds indicate largest zone of inhibitions.

Table 3A: MIC and MBC of ethanol extract of *Nigella sativa* seeds against human pathogenic bacteria

Name of bacteria	Bacterial growth in LB broth (Extract concentration µg/ml)					MICµg/ml	MBCµg/ml
	500	1000	1500	2000	2500		
	<i>Escherichia coli</i>	+	-	-	-		
<i>Klebsiella pneumoniae</i>	+	+	-	-	-	1000	3500
<i>Staphylococcus aureus</i>	+	+	-	-	-	1500	3500
<i>Salmonella abony</i>	+	+	+	-	-	2000	3500
<i>Acinetobacter baumannii</i>	+	+	-	-	-	1500	4000
<i>Bacillus cereus</i>	+	+	+	-	-	2000	4000
<i>Bacillus subtilis</i>	+	+	-	-	-	1500	3500
<i>Pseudomonas aeruginosa</i>	+	-	-	-	-	1000	2500
<i>Vibrio cholerae</i>	+	+	-	-	-	1500	3500
<i>Shigella dysenteriae</i>	+	+	-	-	-	1500	3500

Note: (+) indicates presence of growth and (-) means absence of growth.

Table 3B: MIC and MBC of petroleum ether extract of *Nigella sativa* seeds against human pathogenic bacteria

Name of bacteria	Bacterial growth in LB broth (Extract concentration $\mu\text{g/ml}$)					MIC $\mu\text{g/ml}$	MBC $\mu\text{g/ml}$
	250	500	1000	1500	2000		
<i>Escherichia coli</i>	+	-	-	-	-	500	1500
<i>Klebsiella pneumoniae</i>	+	-	-	-	-	500	2000
<i>Staphylococcus aureus</i>	+	+	-	-	-	1000	2500
<i>Salmonella abony</i>	+	+	+	+	-	2000	3500
<i>Acinetobacter baumannii</i>	+	+	+	-	-	1500	3500
<i>Bacillus cereus</i>	+	+	+	-	-	1500	4000
<i>Bacillus subtilis</i>	+	+	+	-	-	1500	3500
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	1000	2500
<i>Vibrio cholerae</i>	+	+	+	-	-	1500	3500
<i>Shigella dysenteriae</i>	+	+	-	-	-	1000	3000

Note: (+) indicates presence of growth and (-) means absence of growth.

The MIC and MBC values of pet ether extract were presented in Table 3B. In case of pet ether extract, the MIC values were 500 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$, 1000 $\mu\text{g/ml}$, 2000 $\mu\text{g/ml}$, 1500 $\mu\text{g/ml}$, 1500 $\mu\text{g/ml}$, 1000 $\mu\text{g/ml}$, 1500 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ against *E. coli*, *K. pneumoniae*, *S. aureus*, *S. abony*, *A. baumannii*, *B. cereus*, *B. subtilis*, *P. aeruginosa*, *V. cholerae* and *S. dysenteriae*, respectively (Table 3B). Moreover, the MBC values of the same pet ether extract were 1500 $\mu\text{g/ml}$, 2000 $\mu\text{g/ml}$, 2500 $\mu\text{g/ml}$, 3500 $\mu\text{g/ml}$, 3500 $\mu\text{g/ml}$, 4000 $\mu\text{g/ml}$, 3500 $\mu\text{g/ml}$, 2500 $\mu\text{g/ml}$, 3500 $\mu\text{g/ml}$ and 3000 $\mu\text{g/ml}$ against *E. coli*, *K. pneumoniae*, *S. aureus*, *S. abony*, *A. baumannii*, *B. cereus*, *B. subtilis*, *P. aeruginosa*, *V. cholerae* and *S. dysenteriae* respectively. The pet ether extract exhibited the lowest MIC value 500 $\mu\text{g/ml}$ against *E. coli*, and *K. pneumoniae*. Moreover, the lowest MBC 1500 $\mu\text{g/ml}$ was obtained against *E. coli* using pet ether extract of *N. sativa* seed extract (Table 3B). Similar MIC and MBC of the other plant extracts have been reported previously by many workers^{28, 33-34}.

From the above results, it was found that the petroleum ether of *N. sativa* seed extract exhibited good antibacterial activity against the organisms tested herein. Therefore, the extract could be used as a potential antibacterial agent against selected pathogenic bacteria.

Anti-biofilm Activity

The antibiofilm activities of these ethanol and petroleum ether extracts of *Nigella sativa* seeds against four biofilm forming pathogens *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* were studied. Results of antibiofilm activities of ethanol and pet ether extracts are given in Figure 1 and Figure 2, respectively.

Ethanol extract of *N. sativa* seeds at a concentration of half of MIC (0.5 \times MIC) inhibited 27–30% biofilm formation, however, at MIC concentration it inhibited 35–49% biofilm formation of the four-biofilm forming bacterial pathogens tested herein (Fig. 1). Moreover, the same ethanol extract exhibited 48–56% biofilm inhibition at a concentration of double MIC (2 \times MIC) against the same four biofilm producing bacterial strains. In case of double concentration of MIC(2 \times MIC), the ethanol extract exhibited the highest 56% and the lowest 48% of biofilm inhibitions against *P. aeruginosa* and *S. aureus* respectively (Fig. 1). However, at the MIC concentration of ethanol extract, the highest inhibition 49% of biofilm was documented with the bacterial pathogen *P. aeruginosa*. In case of 0.5 MIC concentration, the ethanol extract exhibited the highest 30% and lowest 28% inhibitions of biofilms against *E. coli* and *S. aureus*, respectively (Fig. 1).

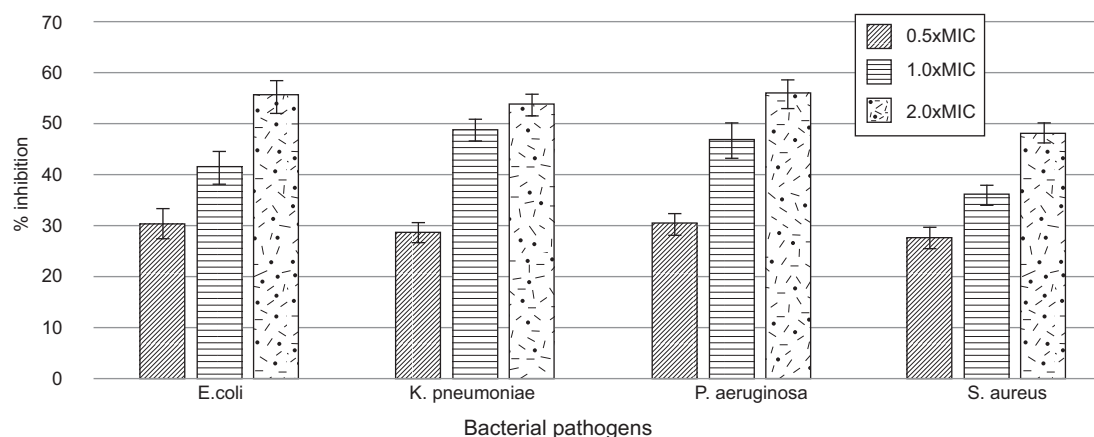


Fig. 1. Antibiofilm activity of ethanol extract of *Nigella sativa* seeds extract against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*.

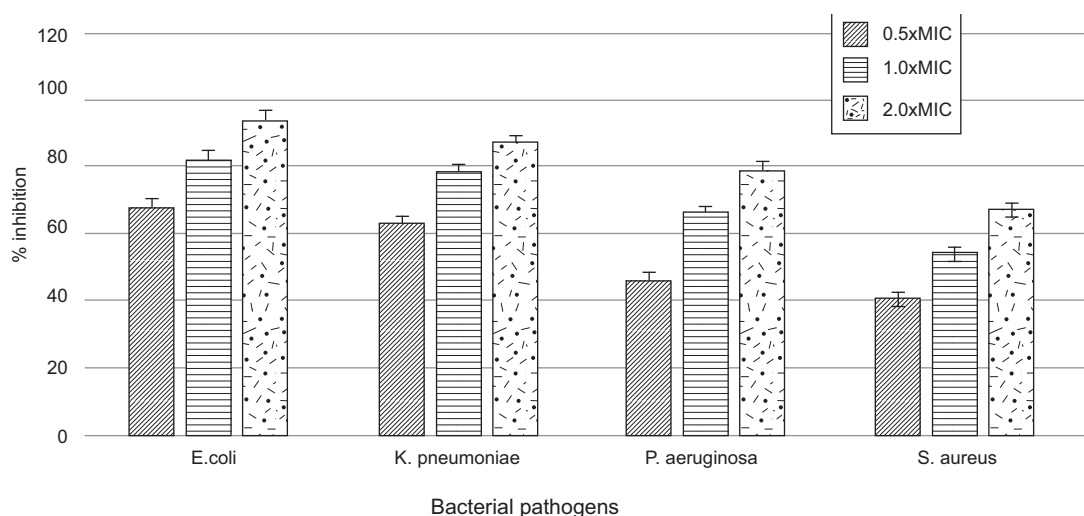


Fig. 2. Antibiofilm activity of petroleum ether extract of *N. sativa* seeds against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*.

The antibiofilm activities of petroleum ether extract (essential oil) of *N. sativa* seed against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* are presented in Figure 2. The crude extract (essential oil) obtained from petroleum ether extract of *N. sativa* seeds at half of MIC (0.5×MIC) concentration inhibited 40-67% biofilm formation of four biofilm forming bacterial strains, and the highest inhibition 67% was recorded against *E. coli* (Fig. 2). However, the MIC concentration of the essential oil inhibited 54-82% biofilm formation of the four-biofilm forming bacterial pathogens, and the highest 82% inhibition was noted against the same bacterial strain *E. coli*. Also, the same essential oil exhibited 67-94% inhibition of biofilm production at a concentration of double MIC (2×MIC) against the same four biofilm producing bacterial strains. Moreover, for double concentration of MIC(2×MIC), the essential oil (petroleum ether extract) exhibited the highest 94% and the lowest 67% of biofilm inhibitions against *E. coli* and *S. aureus*, respectively (Fig. 2). The essential oil extracted from petroleum ether extract of *N. sativa* seeds exhibited comparatively the better inhibition of *E. coli* biofilm formation compared to that of other bacterial strains (Fig. 2). Similar antibiofilm activities of other plants extracts were also done by other workers³⁵⁻³⁶.

Conclusion

The use of herbal drugs as complementary medicine is prevalent and gaining worldwide popularity. It is evident from this study that ethanol and petroleum ether extracts obtained from *Nigella sativa* seeds exhibited good antibacterial activity against a wide range of bacterial pathogens. Consequently, these research findings so far have confirmed the antibiofilm potentials of *Nigella sativa* seeds against biofilm producing bacterial pathogens *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. These results consistently revealed that it is possible to isolate novel antibacterial agents capable of eradicating antibiotic resistant biofilm producing bacterial pathogens from plant resources. Thus, the research work could bring about a novel therapeutic agent from plant extracts that can overcome the

antibiotic resistance problems and prevent biofilm formation of antibiotic resistant pathogens. Further investigations are required to isolate and characterize the pure compound(s) from *Nigella sativa* seed extracts which can then be tested for antimicrobial activity against antibiotic resistance as well as biofilm producing bacterial pathogens. The potent antibacterial agent of *Nigella sativa* seeds can be investigated further for *in vivo* studies including the mechanism of actions and its constituents by which they exert their therapeutic effects. Therefore, *Nigella sativa* seeds could be used as a potential antibacterial agent for the treatment of bacterial agents including biofilm producing strains.

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