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Antibacterial activity of Nigella sativa against multi-drug resistant bacteria

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ABSTRACT

Background: Multidrug Resistant (MDR) bacteria are considered as the one of the most dangerous pathogens causing a high mortality rate globally. Medicines from the natural source might be the best therapeutic option in current emerging trends of resistance. **Methods**: We determined the antibacterial activity of *Nigella sativa* seeds extracts against the clinical isolates of MDR *Staphylococcus aureus* and *Escherichia coli.* The collection of different isolates was done from the different hospitals' settings in Faisalabad. Identifications of these isolates were done phenotypically and through the conventional PCR via genus and species-specific primers. The isolates were then tested for antimicrobial susceptibility using agar well diffusion technique. The activity of N. sativa seed oil was also evaluated in DMSO at concentrations (25%, 50% and 75%). **Results:** Against *S. aureus,* 50% oil concentration in DMSO exhibited highest inhibitory zone of 15 mm followed by 90% ethanol extract showed clear zone of 13 mm and 12 mm zone of inhibition showed by the methanol-based extract. Against *E. coli* 50% oil concentration in Dimethyl sulfoxide (DMSO) and 90% ethanol extract both showed clear zone of 12 mm and 11 mm zone of inhibition showed by the methanol-based extract. However, Minimum inhibitory concentration (MIC) of 50% oil concentration is 0.4 µl followed by 90% ethanol extract showed 0.4 mg and 0.8 mg showed by the methanol-based extract 90% on *S. aureus.* The oil concentration MIC value of 50% is 0.4 µl then followed by the ethanol extract at 90% and revealed 0.4mg and methanol was followed by 90% extract showed 0.4 mg to the E. coli. **Conclusion:** We concluded that N. sativa can be used as a pharmaceutical and new therapy for action against MDR bacteria.

Keywords: Multidrug Resistant Bacteria, Nigella sativa, Antibacterial Activity, Alternative Treatment

Introduction

In the past few years, several pathogens have developed rigorous virulent patterns and changed their mode of transmission, which led to a serious impact on the medical community. The increasing number of antibiotic resistant bacteria is a major health issue worldwide. Multidrug resistant (MDR) organisms causes serious health issues that are difficult to treat, and they possess serious health effects on the community and the environment (124). The MDR bacteria mainly the group of ESKAPE pathogens, including *Enterococcus faecium*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter* species have much importance in Clinical hospitals causing a number of infections in patients (125).

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These pathogens spread worldwide, and their increasing resistant pattern is a key concern. The MDR bacteria are now among the most crucial public health problems of the world (126, 127). The resistant may be due to misuse of antibiotics, sub-optimal dosage, lack of guidelines for selection of drugs, lack of education, lack of proper infection control program, and redundant use of antibiotics (128).

Literally MDR means, the resistance to more than one antibacterial agent, but the medical community still does not agree on one such standardized definition. Many others definition being used to characterize the MDR pattern in gram negative and gram positive bacteria (129). The data are hard to relate due to lack of specific definition for MDR bacteria in clinical protocols. The one other method is to characterize MDR by antimicrobial susceptibility and get the resistant to various antibacterial agents (130). The most frequent gram negative and gram-positive bacteria are resistant to three or almost all antimicrobial classes.

The problem of antibiotics resistance has become an albatross on the neck of clinicians, veterinarians and other infection control agents in their quest to treat and prevent infections that were once thought to be treated with antimicrobials. It is also becoming a major economic problem. The emergence of ESBL producing bacteria is becoming a greater concern as it increases the bacteria resistance to antibiotics (131). Carbapenemases emergence in many bacteria is responsible for resistance to almost all the β-lactams and carbapenems. Carbapenemases association with many bacteria has been reported from many geographic regions (132). This study aims to determine the antibacterial activity of Nigella sativa seeds extracts against the clinical isolates of MDR Staphylococcus aureus and Escherichia coli.

Methods

The study was conducted from December 2020 to august 2021 in the Postgraduate Research Laboratory at the Department of Microbiology, Government College University Faisalabad (GCUF), Pakistan; with ethical approval via letter no 177 of GCUF ethical committee and patient consent. Samples were collected from Aziz Fatima Hospital, Allied Hospital, and District Headquarters Hospital in Faisalabad, targeting MDR bacteria such as Staphylococcus aureus and Escherichia coli from various sources like blood, urine, pus, sputum, wounds, and surgical sites. Sputum specimens were collected in sterile containers, ensuring pathogen survival using Amies transport medium. Throat and mouth swabs were taken under proper lighting conditions, and effusions from infected sites were aspirated into sterile containers. Urine samples were collected mid-stream in leak-proof containers. Blood specimens wereobtained aseptically, and wound samples were collected using sterile techniques. All samples were gathered before administering empirical antibiotic treatment.

For the isolation of the *S. aureus* and *E. coli* from the fluids, sputum, fluids specimen and purulent greenish appearance sputum was initially transferred to glass slide by the help of a piece of stick and then the gram smear prepared. For culturing the *S. aureus* and *E. coli* the samples were washed in the 5 ml of the sterile saline (0.9 % of NaCl) and then inoculated on the Nutrient agar and MacConkey agar. The culture plates were then incubated for 24-48 hours at 37 °C. Then by use of the selective media for the isolation of *E. coli*, the enriched samples or colonies

from first culture of Nutrient or MacConkey agar was culture on the selective media i.e. Eosin Methylene Blue agar and incubated at the 37°C for the 24 hours (133). Morphologically typical colonies producing metallic sheen were seen on the plates. For the selective isolation of *S. aureus* used mannitol salt agar and streaked culture incubated at 37°C for the 24-48 hours.

After proper incubation colonies with the characteristics of *S. aureus* and *E. coli* were subjected to gram staining for the identification purpose.A smear of bacterial colony was prepared on different slides with sterile loop for each of *S. aureus* and *E. coli* staining and identification, smear was heating fix by passing the slide through the burner flame 2-3 times. Bacterial smears were then swamped with crystal violet (primary stain) for the time of one minute, then it was rinsed off with the distilled water, grams iodine was spread on the slide for one minute, and then it is washed off. The bacterial smear was decolorized by using 95% ethanol drop by the drop for 10-15 seconds. Then at once rinsed with the water and in last it was stained with safranin that is counter stain and left for one minute. In the end the slides were observed under the microscope 40x and 100x with oil immersion under lens (134). Shape, size and arrangement with staining of bacteria were observed for confirmation of required bacteria.

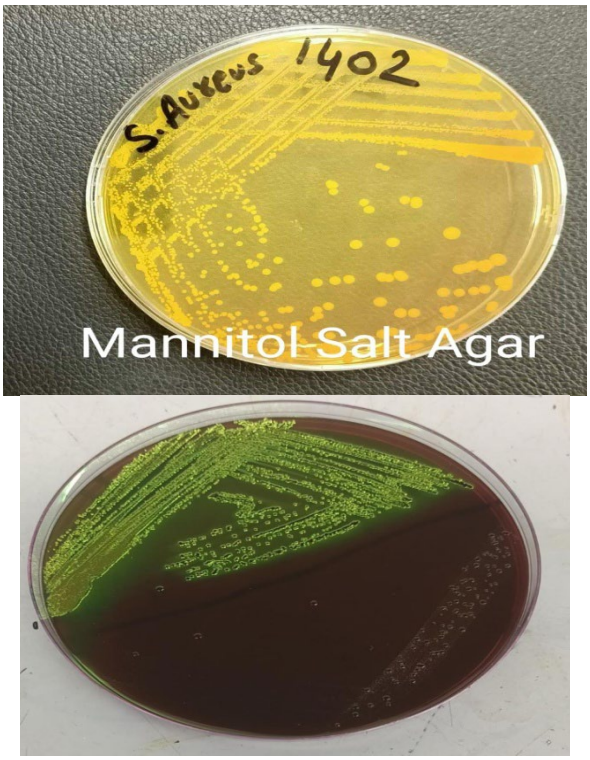


Figure 1: Bacterial growth (*S. aureus* on Mannitol Salt agar and *E. coli* on EMB agar)

Both E. coli and S. aureus were identified biochemically as per tests previously mentioned (135) as shown in table 1.

Table 1: Biochemical tests for *E. coli* and *S. aureus*

Biochemical test	*E. coli*	*S. aureus*
Catalase	Positive	Positive
Oxidase	Negative	Negative
Indole	Positive	Negative
Methyl red test	Positive	Positive
Voges Proskauer test	Negative	Positive
Urease test	Negative	Positive

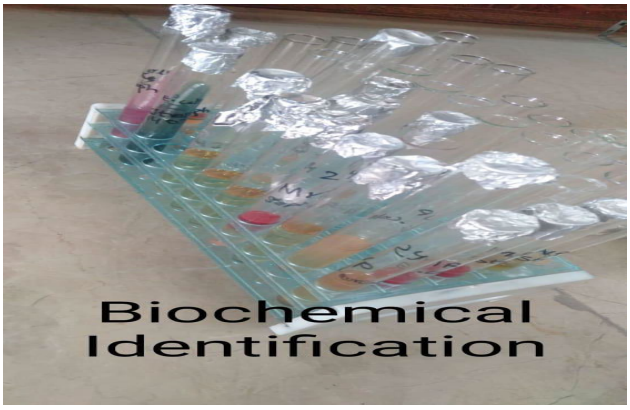


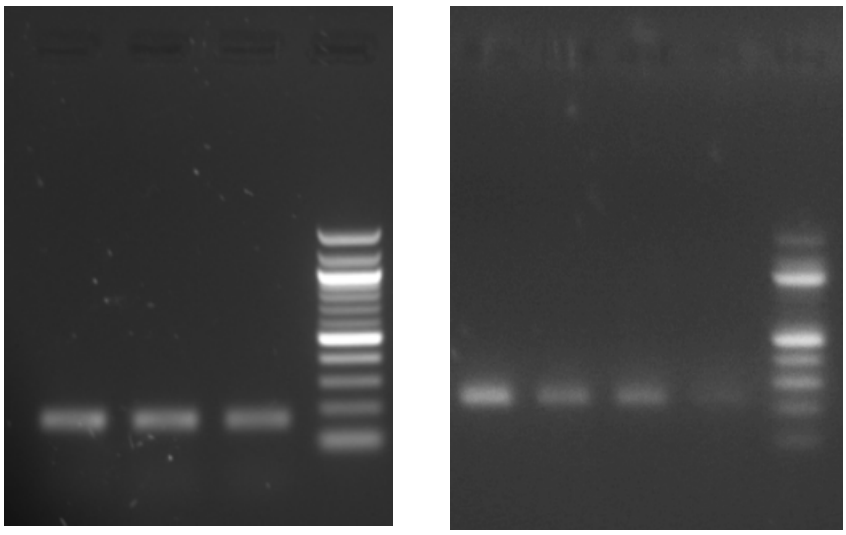
Figure 1: Biochemical identification

The boiling method was used to isolate bacterial DNA using methods as previously prescribed (136). Briefly, 200µl distill water was taken in pre-sterilized 2ml eppendorf tube and with the help of a sterile toothpick single bacterial colony was picked off from culture and thoroughly resuspended in the Eppendorf. Then, the tube was sealed with paraffin to prevent the accidental opening of the lid during boiling. The Eppendorf was placed in a water-bath at 95Ċ for 10 minutes. After boiling, we centrifuged it at 14000 rpm for 5 minutes, 100l of supernatant was collected by micropipette in another Eppendorf,and cell debris was discarded. This supernatant contains chromosomal DNA, which was then quantified using NanoDrop Spectrophotometer.

Specific confirmation genes and their respected primers for both E. coli and S. aureus are shown in table 2. PCR was carried out using total reaction volume of 25µl containing 5µl master mix (5x), 1µl of each primer (2mM), 2µl DNA and quantity sufficient distill water. The PCR was performed in thermal cycler with the following conditions; initial denaturation at 95^0C for 5minutes, followed by 35 cycles of 45 seconds at 95^0C, 55^0C annealing for 45 seconds and 72^0C extension for 45 seconds followed by final extension at 72^0C for 10 minutes. The PCR products were visualized under UV trans-illuminator after gel electrophoresis (1.5% gel stained with ethidium bromide). Figure 2 shows the PCR amplification of both genes.

Table 2: Primers details of both bacteria's

Bacterial specie	Gene of interest	Forward Primer	Reverse primer	Product length (bp)
E. coli	uidA	TGGTAATTACCGACGAAAACGGC	ACGCGTGGTTACAGTCTTGC	162
S. aureus	nuc	GCGATTGATGGTGATACGGTT	AGCCAAGCCTTGACGAACTAAAGC	287



(1) (2)

Figure 2: Amplification of uidA gene (1) of *E. coli* and nuc gene (2) of *S. aureus*

N. sativa seeds were produced from the Punjab seed cooperation, Faisalabad and the plant species was further confirmed and approved for research by botanical department of the Government College University, Faisalabad.Then the seeds were brought in the postgraduate microbiology lab of department of microbiology, GCUF.They were freed from dust, washed first with simple sterile distilled water, and then dehydrated at room temperature in a clean sterile place.The clean seeds were then used for the preparation of the extract. Commercially available extracted oil of *N. sativa* seeds also collected for use.

The collected *N. sativa* seeds were dried in an oven below 60 °C and then grinded in powder form with the help of an electric grinder. Different concentrations of alcoholic extract of *N. sativa* were prepared by using 70 %, 80% and 90% methanol and 70%, 80% and 90% ethanol. The powdered *N. sativa* seed was extracted with different concentrations of methanol and ethanol as 1g/10ml in culture bottle, placed in shaking incubator overnight. Then this collected extract was filtered through Whatman No. 1 filter paper and clean supernatant was obtained by the centrifugation. The different concentrations of extract were collected in the falcon tubes and stored at 4 °C until use.

Table 3: Different concentrations of methanol and ethanol extract

Concentration	Quantity
90%	90ml absolute methanol/ethanol + 10ml distilled water + 10g powdered *N. sativa*
80%	80ml absolute methanol/ethanol + 20 ml distilled water + 10g *N. sativa*
70%	70ml absolute methanol/ethanol +20ml distilled water + 10g powdered *N. sativa*

Commercially available *N. sativa* essential oil was taken from the local market and processed to evaluate its antimicrobial potential against MDR- bacterial species. Oil was diluted in the DMSO at different concentrations of 100, 75, 50 and 25. The different concentrations of oil and DMSO are shown in the table 4.

Table 4: Different concentrations of *N. sativa* essential oil

Concentrations	Quantity
50%	50µl oil + 50µl DMSO = 1ml
25%	25µl oil + 75µl DMSO = 1ml
12.5%	12.5µl oil + 87.5µl DMSO = 1ml

The extract prepared was standardized according to the standardized guidelines of the WHO and the other pharmacopoeia procedures (137). The physiochemical standardization that includes extractive values in the solvent, water-soluble ash value, acid insoluble ash value and total cash value, moisture content, pH value, loss on drying, heavy metals and pesticides residues in the extract were analyzed as per the standard methods.

The susceptibility test was conducted using agar well diffusion method as per recent Clinical and Laboratory standard Institute (CLSI) guidelines (138). Additionally, the susceptibility of the isolates to different antibiotics was assessed using the Modified Kirby Bauer Disc Diffusion method with antibiotics such as Gentamycin, Cefixime, Ampicillin, Tazobactam, Cefoxitin, Colistin sulfate, Cefepime, Sulfamethoxazole trimethoprim, Tetracycline, Sulbactam, Doxycycline, Chloramphenicol, Ciprofloxacin, Ceftaxidime, and Cefuroxime on Mueller-Hinton agar. Post-incubation, the

zones of inhibition around the antibiotic discs were measured and interpreted as resistant, susceptible, or intermediate according to CLSI criteria as shown in figure 3 and figure 4.

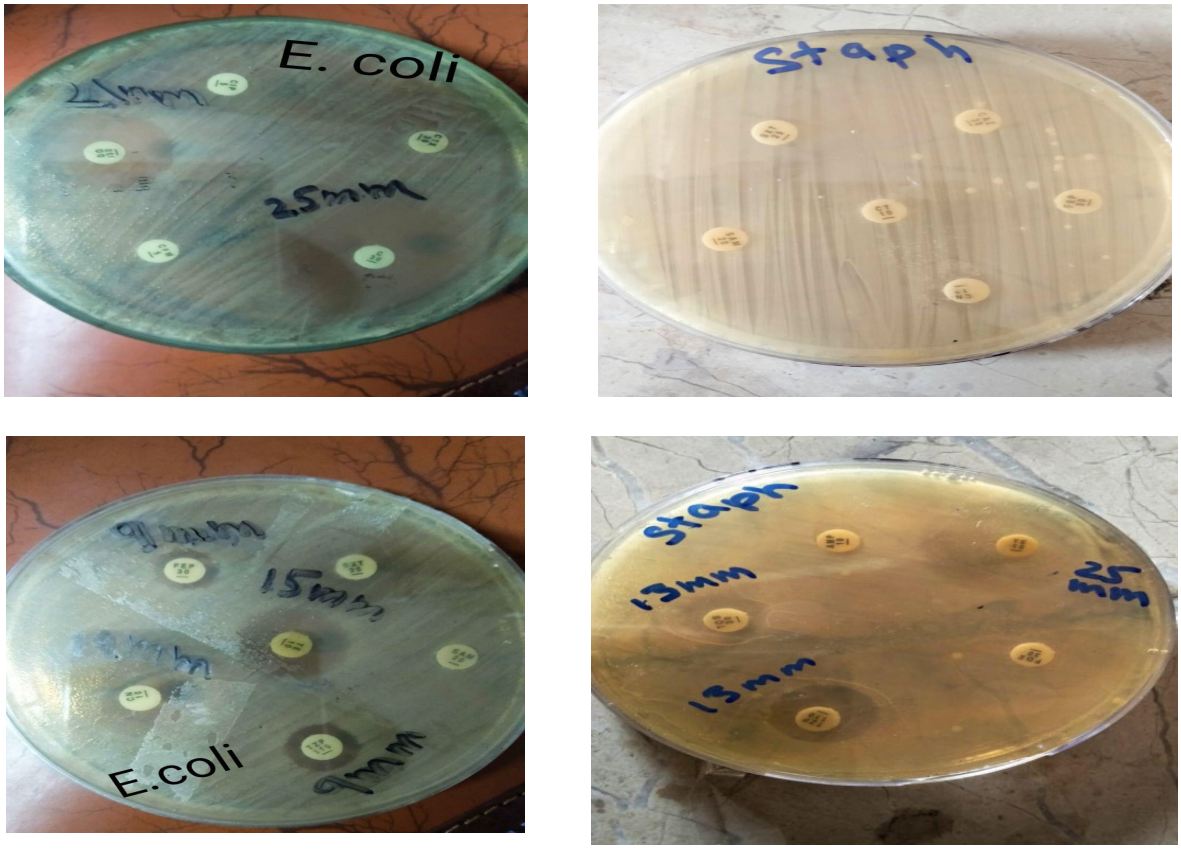


Figure 3: Determination of MDR E. coli and S. aureus

Antimicrobial activity of *N. sativa* extracts was evaluated by using agar well diffusion method. For this purpose, 500ml Mueller-Hinton agar (MHA) media was prepared. Autoclave the media at 121°C for 15-20 minutes and pour into sterile glass petri plate in laminar airflow cabinet. Spreading of the bacterial suspension containing 10^6 CFU/ml onto sterile glass petri plates done by using sterile glass spreader and leave to dry. After this, with the help of sterile borer on the surface of agar media wells of 6mm in diameter are punched on each plate and sealed the wells with molten media into the wells. 50-µl solution of the extracts was filled into the wells. To allow the diffusion of the extracts into the agar before the growth of organism started, the inoculated plates kept in refrigerator at 4°C for a single hour. Plates were kept in the incubator for 16-20 hours at 37°C. Next day the antibacterial activity was estimated by measuring the diameter of inhibition zone that was produced around the well

Minimum inhibitory concentration is the lowest quantity of material / antibiotic that is used to inhibit the growth of bacterial strain. Antimicrobial activity of alcoholic extract of the *N. sativa* and its essential oil was determined by measuring the MIC value using the two-fold broth micro micro-dilution method of sea et al., (2016) in 96 well

plates. The MIC of prepared methanolic, ethanolic and essential oil of *N. sativa* different concentrations against S. *aureus* and *E. coli* was found by this protocol. The different concentration of essential oil prepared in DMSO was used to determine MIC value against *S. aureus* and *E. coli*.

Briefly, 24 hours old bacterial strains were used to prepare inoculum of 0.5 McFarland containing 1.5 x 108 CFU. Then 100 µl of Mycorrhiza helper bacteria (MHB) was poured in each well of sterile micro titer plate and equal volume (100µl) of stock solution of alcohol extract / essential oil was poured in the well one and diluted by the two-fold serial dilution up to well 10. The 100-µl solution from well 10 was discarded to maintain equal amount in each well except well 11 that was used as a sterile control. Well 12 containing only MHB and bacterial inoculum was used as a positive control. Plates were sealed with para-film to prevent evaporation at 37 °C for 18-24 hours.

The minimum bactericidal concentration is defined as the concentration of the extract showing no bacterial visible growth on subculture. For MBC the culture of *S. aureus* & *E. coli* from the wells of micro titration plates showing MIC value i.e. 50µl streaked on Mueller-Hinton agar plates. After these plates were incubated over night at 37 °C, if no visible growth observed on the plates, then extract is bactericidal otherwise considered as bacteriostatic?

All the data were entered in SPSS version 22.0. Descriptive statistical model was used to describe variables in the data. Graphs were constructed through Microsoft Excel version 2013.

Results

Out of 50 samples, 25 samples were collected from puss exudates and 25 samples were collected from urine. Nine samples were detected as *E. coli* and 16 samples were detected as *S. aureus*. Other than clinical samples, 2 pure, well identifies and well characterized strains of both bacteria were used in this study. The results of susceptibility test confirming MDR bacteria are shown in table 6.

Table 5: Susceptibility results of E. coli and S. aureus against different drugs

Antibiotics	*S. aureus*	*E. coli*
Gentamycin (GN 10)	S	S
Cefixime (CFM 5)	R	R
Ampicillin (AMP 10)	R	R
Tazobactam (TZP 10)	S	R
Cefoxitin (CTX 30)	R	R
Colistin sulfate (CT 10)	R	R
Cefepime (FEP 30)	R	R
Sulfamethoxazole trimethoprim (SXT 25)	R	R
Tetracycline (TE 10)	R	S
Sulbactam (SAM 20)	R	R
Doxycycline (DO 30)	R	S
Chloramphenicol (C 30)	S	S
Ciprofloxacin (CIP 5)	S	R
Ceftaxidime (CAZ 30)	R	R
Cefuroxime (CXM 30)	R	R

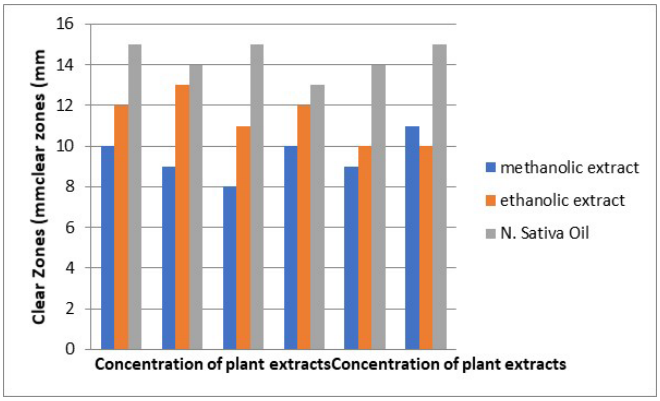


Figure 4: Clear zone agar well based diffusion of all extracts

The AST results for different extracts of *N. sativa* had showed antibacterial activity against the *E. coli* and *S. aureus*, which proved the presence of antimicrobial component in *N. sativa*. Among all the extracts, the essential oil extract shows highest antimicrobial activity followed by ethanolic extract.

Table 6: Clear zones data for all extract activity against *E. coli* and *S.aureus*

Extract	Clear Zone measurement Data for *E. coli*				Clear Zone measurement Data for *S. aureus*			
	Plate No.	70%	80%	90%	Plate No.	70%	80%	90%
Ethanolic	1	5	7	10	1	6	8	12
	2	4	8	12	2	4	9	13
	3	4	7	11	3	5	9	11
	4	3	7	12	4	4	9	12
Methanolic	1	3	6	9	1	3	7	11
	2	3	7	10	2	4	8	10
	3	4	7	11	3	5	9	12
	4	4	5	11	4	4	8	12
Oil	Plate No.	12.5 %	25 %	50%	Plate No.	12.5 %	25 %	50%
	1	0	8	12	1	0	11	15
	2	0	9	11	2	0.2	10	14
	3	0	8	10	3	0	9	14
	4	0.1	7	10	4	0.1	11	13

The MIC result of different extract of *N. sativa* were against *S. aureus* and *E. coli* in the micro titration plate and were placed in the Elisa reader machine the machine was set on the 620nm of wavelength in order to check the minimum or maximum of the absorbance. The different absorbance results of the different wells of titration plate are shown in the table below.

Table 7: MIC data for all extract activity against MDR

Bacterial strains	MIC of *Nigella sativa* extract and oil against MDR bacterial(mg/ml)		
	90%	90%	Oil
	methanol	ethanol	(50µl/ml)
Staphylococcus aureus	0.8	0.4	0.4
Escherichia coli	0.4	0.4	0.4

Minimal bactericidal of extract is defined as least concentration of extract that kills the bacteria. For the identification of MBC of seed extracts i.e. either bactericidal or bacteriostatic different concentration of extracts had tested. Took the solutions from the MIC well and streak on MH agar plates. There was no detectable growth of bacterial isolates so that was MBC as shown in figure 5 and 6.

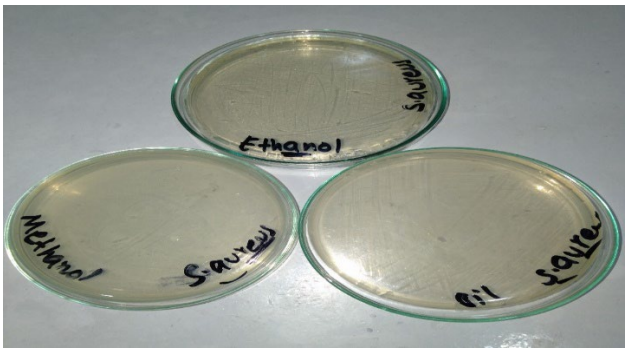


Figure 5: Showing MBC agar plates of *S. aureus*

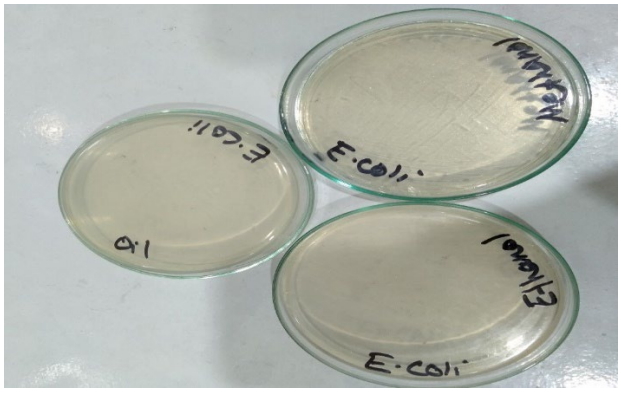


Figure 6: Showing MBC agar plates of *E. coli*

Discussion

The general goal of this study was to find a novel antimicrobial agent, which becomes a way to address this MDR problem. Present study demonstrated the antibacterial potential of the different extract of *N. sativa* as

by using methanolic, ethanolic and oil extract of *N. sativa* against multi drug resistant *S. aureus* and *E. coli*. The activity was tested for different concentrations of ethanolic and methanolic extract as 70, 80 and 90 % was for ethanolic and methanolic extract while 2.5, 25 and 50% was for oil extract of *N. sativa* against these selected bacteria. A remarkable antibacterial activity of *N. sativa* was observed during antibacterial susceptibility test for *N. sativa* against *S. aureus* and *E. coli*. This proves that *N. sativa* had components that are responsible for antibacterial actions. The MIC results had also proved the antibacterial potential of this plant. Similar to this study was performed by Halwani et al. (139) but this study was on some constituents of *N. sativa* that are responsible for antibacterial activity. In their study they observed the two components of the *N. sativa* oil, thymoquinone and linoleic acid that were found to show an antibacterial action against *S. aureus* and Enterococcus. The two components showed a synergistic impact when tested together. They calculated the MIC and the MBC of two compounds, and these concentrations were found in the plasma of seemingly healthy mice but were harmful to animals infected with an LD 50 of *S. aureus* systemically. Their study concluded that the Thymoquinone and the linoleic acid are the major constituents of the *N. sativa* oil that were found to have an antibacterial effect against the *S. aureus*. They also demonstrated that the toxicity of these compounds may limits the systemic use to some extent but in this time when the resistant to gram of negative and the gram of positive strains is rising day by day, the *N. sativa* is proving itself amoung the new alternatives as in search of treatment against these MDR pathogens (139)

The findings of current research are also parallel to the work of Foroughi et al (140), they studied to evaluate the chemical composition and the antibacterial activities of the oil of the *N. sativa* against the *S. aureus*, *B. subtilis* and *E. coli*. To specify their chemical composition they performed the mass spectrometry. They employed agar disk and agar well diffusion methods for the detection of antibacterial properties of the oil. MIC was determined by macro broth tube test. The presence of the longifolene and α-longipinene, α-pinene, p-cymene, β-pinene, cis-carveol, Tans-anethole, thymol, thymoquinone were identified in the composition of the oil of the *N. sativa*. According to their results, the MIC value for the oil was 0.031 gram per liter except the 0.015 gram per liter for the *B. subtilis*. Thus, their work represented the antimicrobial effects of the *N. sativa* on *E. coli*, *B. subtilis* and *S. aureus*. Their study had also provided support to the antibacterial properties of the *N. sativa*. Their results indicated that the oil of this plant could be useful as a medicinal or preservative composition. They also presented that the characterization of its active molecules will be the future work to investigate its further aspects.

Current study had also determined the antibiofilm activity of methanol, ethanol and oil extract of *N. sativa* by micro titer plate assay. It was observed that all extracts exhibited good antibiofilm activity and also treated the mature biofilms efficiently by targeting biofilms viability and biomass. The results of current studies indicated that the antibiofilm potential of methanol, ethanol and oil extract of *N. sativa* depended upon time of extract-bacterial surface interactions i.e. biofilms inhibition was greater at 24 hours and 48 and 72 hours. Sanchez et al also demonstrated that methanol and ethanol

extract of *N. sativa* has exhibited antibiofilm activity against bacteria (141). It was stated that these extracts can inhibit the formation of biofilm but have lower efficacy of inhibition on the preformed biofilms.

Many research works have demonstrated the potential of N. sativa extract against different bacteria. This plant should have the ability to treat MDR bacteria and may prove an effective alternative to antibiotics. Sharikh et al (142) also studied the antibacterial activity of *N. sativa* seed extracts on clinical separates of MRSA (Methicillin resistant *S. aureus*). Thus studies needs to be focused on other microorganisms to determine its therapeutic potential.

Conclusion

Based on the findings of this study, extracts from Nigella sativa seeds demonstrate significant potential as antibacterial agents against multidrug-resistant strains of S. aureus and E. coli. The research highlights that N. sativa extracts, particularly the oil extract, exhibit notable antibacterial and anti-biofilm activities, surpassing those of ethanol and methanol extracts. The observed increase in zones of inhibition with higher extract concentrations and the effective reduction in biofilm formation suggest promising applications in combating bacterial infections.

Conflict of Interest: Authors declare no conflict of interest.

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References

1. Cornejo-Juárez P, Vilar-Compte D, Pérez-Jiménez C, Ñamendys-Silva S, Sandoval-Hernández S, Volkow-Fernández P, et al. The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit. International Journal of Infectious Diseases. 2015;31:31-4.
2. Navidinia M. The clinical importance of emerging ESKAPE pathogens in nosocomial infections. 2016.
3. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed research international. 2016;2016(1):2475067.
4. Ahmad SS, Siddiqui MF, Maqbool F, Ullah I, Adnan F, Albutti A, et al. Combating Cariogenic Streptococcus mutans Biofilm Formation and Disruption with Coumaric Acid on Dentin Surface. Molecules. 2024;29(2):397.
5. Abu Taha A, Abu, Zaydeh A, Ardah R, Al-Jabi S, Sweileh W, Awang R, et al. Public knowledge and attitudes regarding the use of antibiotics and resistance: findings from a cross-sectional study among Palestinian adults. Zoonoses and public health. 2016;63(6):449-57.
6. Aminov R. History of antimicrobial drug discovery: Major classes and health impact. Biochemical pharmacology. 2017;133:4-19.
7. Navidinia M, Goudarzi M, Rameshe SM, Farajollahi Z, Asl PE, Mounesi MR, et al. Molecular characterization of resistance genes in MDR-ESKAPE pathogens. J Pure Appl Microbiol. 2017;11(2):779-92.
8. Khaertynov KS, Anokhin VA, Rizvanov AA, Davidyuk YN, Semyenova DR, Lubin SA, et al. Virulence factors and antibiotic resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis. Frontiers in medicine. 2018;5:225.
9. Burki TK. Superbugs: an arms race against bacteria. The Lancet Respiratory Medicine. 2018;6(9):668.

10. Sahibzada WA, Sahibzadi AG, Sana F, Tayba K, Adila S, Sahibzadi SG, et al. Detection of Escherichia coli and total microbial population in River Siran water of Pakistan using Emb and Tpc agar. African Journal of Microbiology Research. 2018;12(38):908-12.
11. Adetutu AA, Oritsewehinmi B, Ikhiwili OM, Moradeke AO, Odochi AS, Adeola OE, et al. Studies on Staphylococcus aureus Isolated from Pimples. Pakistan Journal of Biological Sciences: PJBS. 2017;20(7):350-4.
12. Kandil A, Elhadidy M, El-Gamal A, Al-Ashmawy M. Identification of S. aureus and E. coli from dairy products intended for human consumption. Adv Anim Vet Sci. 2018;6(11):509-13.
13. Dimitrakopoulou M-E, Stavrou V, Kotsalou C, Vantarakis A. Boiling extraction method vs commercial kits for bacterial DNA isolation from food samples. Journal of Food Science and Nutrition Research. 2020;3(4):311-9.
14. Organization WH. The international pharmacopoeia: World Health Organization; 2006.
15. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. Journal of clinical microbiology. 2020;58(3):10.1128/jcm. 01864-19.
16. Halwani R, I'labbal MZ, Abdelnoer AM. The anti-bacterial effect of some constituent nigella sativa oil. Arab journal of Pharmaceutical Sciences. 1999;1:89-96.
17. Foroughi A, Pournaghi P, Tahvilian R, Zangeneh MM, Zangeneh A, Moradi R. Ethnomedicinal plants: Study on the chemical composition and antibacterial activity of the Nigella sativa (Black seed) oil's. International Journal of Pharmaceutical and Clinical Research. 2016;8(11):1528-32.
18. Sánchez E, Rivas Morales C, Castillo S, Leos-Rivas C, García-Becerra L, Ortiz Martínez DM. Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. Evidence Based Complementary and Alternative Medicine. 2016;2016(1):1572697.

Sharikh SM, Rao JUP, Ackshaya M. A study of antibacterial effect of Nigella Sativa seed extract on clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA). Indian J Public Health Res Dev. 2020;11:1516-20.

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KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion