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In-vitro studies on the effect of *Nigella sativa* Linn., seed oil extract on Multidrug resistant Gram positive and Gram negative bacteria

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Abstract
Multi-drug resistance bacterial are a major public health concern in this post antibiotic era. The rate at which these bacteria are evolving is not synonymous with the rate at which new antibiotics are produce. In recent years, researches are being directed towards the use of herbal products in the treatment of various infections. One of such is the use of the Black seed cumin (*Nigella sativa*) in the treatment of ailments. In this study, different concentrations of *Nigella sativa* oil were tested for their antibacterial activity against different strains of Gram positive and Gram negative multi-drug resistant bacteria (MRSA, *Acinetobacter baumannii, Escherichia coli,* and *Pseudomonas aeruginosa*) by using well diffusion method. For all the different strains of *Acinetobacter baumannii* and *E. coli* that were tested against 100% of *N. sativa* oil, there was no recorded zone of inhibition. However, for the different strains of MRSA and *Pseudomonas aeruginosa,* different zones of inhibition where obtained for all the different oil dilutions used. Bacterial growth was inhibited at 100%, 80%, 50%, 40%, 30% and 20% N. sativa oil dilutions.

Keywords: *Nigella sativa*, Black cumin, MRSA, *Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa,* Antibacterial activity.

1. Introduction
The use of medicinal plants as medicine has increased world-wide due to factors such as drug failure, adverse reactions, cost of medications as well as resistance to antimicrobials by bacteria. About three-quarters of the world population residing in the developing countries still use medicinal plant, this is despite the availability of pharmaceutical antibiotics [1]. This use of herbal plants as alternative medicine over centuries remains a popular choice for primary health care [1]. However post antibiotic era, with the emergence and re-emergence of resistant strains of microorganisms, coupled with the side effects of the most conventional drugs, there is a renewed interest in the use of plants and plant products in the management of ailments [2]. One of such plants is the *Nigella sativa*, belonging to the family: Ranunculaceae. It is also commonly known as Black Seed or Black Cumin. The use of its plant produce appears to cut across a large list of ailments. *N. sativa* products have been reported to be used in the treatment of diseases such as asthma, bronchitis [3], inflammatory diseases [4] and antifungal [5]. The traditional uses of *Nigella sativa* seems to transcend time. Originating from ancient Egypt, Greeks and the Romans according to Amin and Hosseinzadeh [6]. The seed and oil have been recommended for use in a wide range of ailment by researchers such as Woo *et al*., [7]. Heiss *et al*., [8] and earlier on by Junemann *et al*., [9]. A comprehensive list for the uses of this herbal plant has been discussed in a review article by Amin and Hosseinzadeh [6]. For all the researched uses of *Nigella sativa* seed and oil products, Aftab *et al*., [10] commented that this has earned *N. sativa* the Arabic approbation "Habbatul barakah" translated to mean the seed of blessing. The antibacterial activities against multi-drug resistant bacteria has also been reported by researchers [11, 12].
The active ingredients of the black cumin have been demonstrated by researchers [13, 14, 15]. Also the chemical composition of the *N. sativa* seeds has been describe by Ramadan [16] Al-Jassir [17]. While Kamal *et al*., [18] and Cheikh-Rouham [19] described how these chemical composition of the seed vary geographically. .As the world seeks for a solution to multi-antibiotic resistant bacteria, it might need to probe further into herbal alternatives in tackling these bacteria. That *N. sativa* is available as an oil extract in herbal shops as well as capsules in

Pharmacies in Saudi Arabia, places this product in the class of herbal products available in direct consumable form. The present research therefore seeks to investigate the effect of the black seed oil extract against multi-drug resistant clinical isolates of Gram positive and Gram negative bacteria.

2. Materials and Methods

2.1. Sample collection

Samples were obtained from the stock at the microbiology laboratory of the College of Medicine at King Faisal University. The *Acinetobacter baumannii* isolates were those collected from diabetic and non-diabetic patients while the Methicillin Resistant *Staphylococcus aureus, Pseudomonas aeruginosa* were from routine hospital laboratory isolates.

2.2. Bacteria Isolates

Bacteria isolates consisted of Multidrug resistant Gram negative isolates of *Acinetobacter baumannii, Escherichia coli* (ATCC 25922), non-coded clinical *E. coli, Pseudomonas aeruginosa* (ATCC 27853) as well as *P. aeruginosa* obtained from routine laboratory isolates. Gram positive bacteria consisting of Methicillin resistant *Staphylococcus aureus* were also used for the study. *Pseudomonas aeruginosa* and MRSA were sub-cultured on blood agar while E. coli and *A. baumannii* were sub-cultured on MacConkey agar.

2.3. Antibiotic susceptibility

Pseudomonas aeruginosa (PsA) was tested against 16 different antibiotics. The list of the antibiotics used is presented in table 1. Also, Methicillin resistant *S. aureus* isolates were tested against 20 isolates and the pattern of susceptibility for the isolates is shown in table 1.

The isolates and the Antibiogram are presented in Tables 1. The *A. baumannii* isolates used were resistant to Imipenem, Meropenem and Tigecycline. *E. coli* which had shown resistance to between 10 and 11 antibiotics against which they had been tested as shown in table 1.

2.4. *Nigella sativa* oil

The black seed oil extract used for the investigation is a product of Al-Hussan food products factory in Riyadh, Saudi Arabia. It was bought from an herbal shop in Al-Ahsa. According to the manufacturer's information, it is 100% pure organic oil, devoid of cholesterol. Six serial dilutions of different concentrations were prepared by diluting the oil in Phosphate-buffered saline (PBS). Thus concentrations of 100%, 80%, 50%, 30%, 20%, and 10% of Nigella Sativa oil were obtained and used for the investigation.

2.5. Well diffusion susceptibility method

The Well diffusion susceptibility method described by Emeka *et al*., (2015) but with some modifications was used to determine the antibacterial activity of *N. Sativa* oil on the multi-drug resistant Gram positive and Gram negative bacteria. Muller Hilton agar was first seeded with the bacteria using moistened sterile cotton swabs. Three wells were cut in each agar plate using the open end of a sterilized cork borer. Three different oil concentration were introduced into the wells and the set up was incubated at 37 °C for 24 hours. Zones of inhibitions were measured in cm.

3. Results

3.1. Antibiogram

Isolates 1(PsA 1), 2(PsA 2) and 3 (PsA 3) were resistant to 56.3%, 62% and 81.3% of the tested antibiotics respectively as shown in Table 2. All isolates of Methicillin resistant *Staphylococcus aureus* had shown a 75% resistance to the antibiotics against which they had been tested as show in Table 3. Only *Acinetobacter baumannii* resistant to the Carbapenems were used for the study. For the *Escherichia coli*, antimicrobial resistance is as show in 1.

3.2. Effect of *N. sativa* oil extracts on Gram Negative

Of the different strains of *Acinetobacter baumannii* that were tested against 100% of *N. sativa*, there was complete resistance with no observed zones of inhibition for all the isolates. A similar pattern of results were obtained with the different strains of *E.coli*. However, for *P. aeruginosa* zones of inhibitions to the oil concentrations were seen with all the different strains *Pseudomonas aeruginosa* and for all the *N. sativa* oil dilutions. The result on the effect of *N. sativa* oil extract and dilutions, on *Pseudomonas aeruginosa* is shown in figure 1. The figure shows that all the oil dilutions used (100%, 80%, 50%, 40%, 30%, and 20%) had inhibitory effects on the different *P. aeruginosa* isolates including the coded *P. aeruginosa*. With the ATTC *P. aeruginosa* (ATTC 27853) there was a decrease in zones of inhibition as the oil concentration decreased. A similar pattern was exhibited by *P. aeruginosa* isolates (PsA 1) and 3 (PsA 3). However, observations for *P. aeruginosa* isolate 2 (PsA 2) was slightly different. For the 50% and 40% oil dilution, the recorded zones of inhibition remained the same. Zones of inhibition among the ATTC *P. aeruginosa* isolates is seen to have responded better to the different oil concentration as shown in figure 1.

3.3. Effect of *N. sativa* oil extracts on Gram Positive isolates

For the Gram positive bacteria isolates, zones of inhibitions to the oil concentrations were seen with all the strains of MRSA and with the different *N. sativa* oil dilutions. The result are presented in figure 2. The inhibitory pattern to the different oil concentrations exhibited by these group of isolates is similar to those of the Gram Negative *P. aeruginosa* isolates. Figure 2 shows that for all the oil concentrations (100%, 80%, 50%, 40%, 30%, and 20%) there was inhibitory effects on the four different *MRSA* isolates. With *MRSA* 2 and 3, a decrease in zone of inhibition was seen with a decrease in oil concentration. The observations for *MRSA* 1 and 4 isolates were slightly different. For oil dilutions of 40 and 50%, the zone of inhibition remained constant. Zones of inhibition among *MRSA* 3 isolates is seen to have responded better than different oil concentration as shown in figure 2.

Table 1: Showing antibiotic Susceptibility of P. aeruginosa, E.coli, MRSA and Acinetobacter baumannii.

	ANTIBIOTICS																			
Isolates	**Rifampicin**	**Tigecycline**	**Amikacin**	**Vancomycin**	**Gentamicin**	**Piperacillin/Tazobactam**	**Amoxicillin /Clav.Acid**	**Azithromycin**	**Cefipime-Protec**	**Cefoxitin**	**Ceftazidime**	**Ceftizoxime**	**Ceftriaxone**	**Cefuroxime**	**Ciprofloxacin**	**Imipinem**	**Levofloxacin**	**Meropenem**	**Oxacillin**	**Tetracycline**	**Trimeth/Sulphametho**	**Polymyxin B**
PsA 1	R	**	S	**	I	S	R	R	**	**	**	R	R	R	R	**	R	**	**	I	**	S
PsA2	R	**	S	**	S	S	R	R	**	**	**	R	R	R	R	**	R	**	**	R	**	S
PsA3	R	**	R	**	S	R	R	R	**	**	**	R	R	R	R	**	R	**	**	S	**	R
MRSA 1	S	S	**	S	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	**
MRSA 2	S	S	**	S	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	**
MRSA 3	S	S	**	S	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	**
MRSA 4	S	S	**	S	I	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	**
E. coli 1	**	**	R	**	R	R	**	**	R	**	R	R	R	R	R	S	R	S	**	S	R	**
E. coli 2	**	**	R	**	R	R	**	**	-	**	R	R	R	R	R	S	-	S	**	R	R	**
E. coli 3	**	**	R	**	R	R	**	**	-	**	R	R	R	R	R	S	-	S	**	R	R	**
E. coli 4	**	**	R	**	R	R	**	**	-	**	R	R	R	R	R	S	-	S	**	R	R	**
AB 1	**	S	**	**	**	**	**	**	**	**	**	**	**	**	**	R	**	R	**	**	**	**
AB 2	**	S	**	**	**	**	**	**	**	**	**	**	**	**	**	R	**	R	**	**	**	**
AB 3	**	S	**	**	**	**	**	**	**	**	**	**	**	**	**	R	**	R	**	**	**	**
AB. 3	**	S	**	**	**	**	**	**	**	**	**	**	**	**	**	R	**	R	**	**	**	**

Table 2: Showing antibiotic Susceptibility of *Pseudomonas aeruginosa* isolates

Isolates	Antibiotic sensitive	Antibiotic Intermediate	Resistance	Total anti. Tested	% Resistance
PsA 1	5	2	9	16	56.3
PsA 2	6	0	10	16	62
PsA 3	3	0	13	16	81.3

PsA = *Pseudomonas aeruginosa*

Table 3: Showing antibiotic Susceptibility of Multidrug Resistant *S. aureus*

Isolates	Antibiotic sensitive	Antibiotic Intermediate	Resistance	Total Anti. Tested	% Resistance
1MRSA	3	2	15	20	75
2MRSA	4	1	15	20	75
3MRSA	4	1	15	20	75
4MRSA	3	2	15	20	75

MRSA = Methicillin Resistant *Staphylococcus aureus*

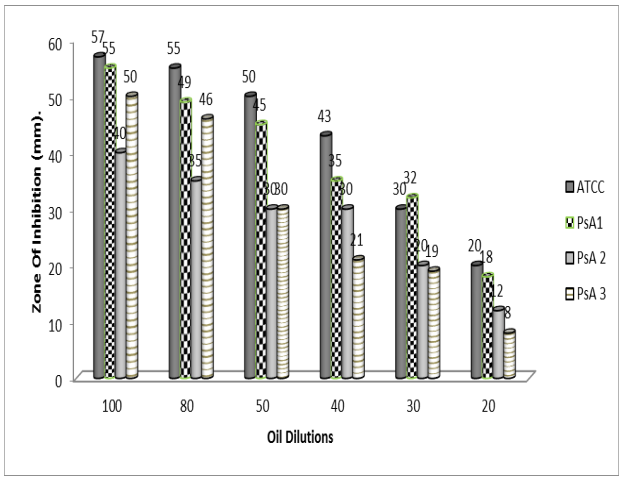

Fig 1: Showing a comparison of the Zones of inhibition by different *P. aeruginosa* isolates to *N. sativa* oil extracts.

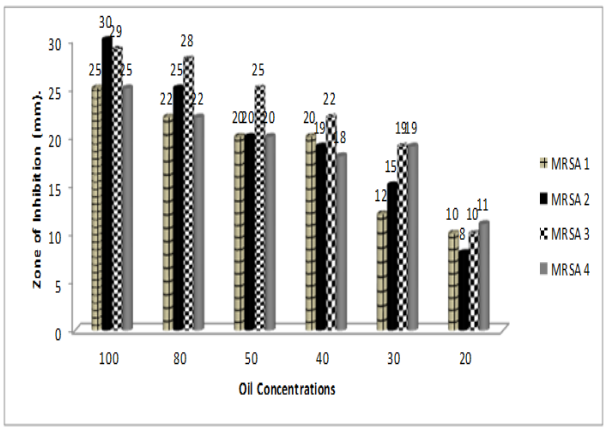

Fig 2: Showing a comparison of the Zones of inhibition by different MRSA isolates to *N. sativa* oil extracts.

4. Discussion

The results obtained showed that all the 4 strains of MRSA, which were resistant to 75% of antibiotics used, were sensitive to all *N. sativa* oil dilutions. These findings are similar to those of Emeka *et al*., [12] and Salman *et al*., [20] who reported that the growth of multidrug resistant *Staphylococcus aureus* was inhibited by different concentrations of *N. sativa* oil dilutions. It can therefore be optimistic to say that irrespective of differences in Staphylococcal strains, either based on the sites of isolation or geographical location, the oil extract under consideration is capable of inhibiting the growth of MRSA. This is due to the fact that the isolates in the Emeka *et al*., [12] research were from diabetic wounds while those Salman *et al*, [20] had been collected from different specimens. The isolates tested in the present study had been those from different specimens. Also, from the present investigation MDR *Acinetobacter baumannii* strains and the different *Escherichia coli* strains tested against *N. sativa oil* extract, showed a 100% resistance of the isolates to different concentrations of the oil extract. This is contrary to the findings of Mohammed [21] who showed MDR *E. coli* and *A. baumannii* to be sensitive to different concentrations of *N. sativa* produce, indicating the possibility that the Gram negative cell wall was not a hindrance to the mode of action of *N. sativa*. In the present investigation, *P. aeruginosa* was the only Gram negative isolate shown to be sensitive to the different *N. sativa* oil dilutions used in the present study. This is similar to the findings of Salman *et al*., [20]. It has been thought [20] that the apparent ineffectiveness of *Nigella sativa* oil extract against some multi-drug resistant Gram negative bacteria is due to its outer membrane, which acts as a selective barrier. However, in the present investigation, zones of inhibitions to the oil concentrations were seen with 4 different strains of multi-drug resistant Gram negative *Pseudomonas aeruginosa*, thus indicating that the effectiveness of *Nigella sativa* oil is variable among different species of Gram negative bacteria isolates. There is the probability that the nature of antimicrobial resistant in terms of the susceptibility of antibiotics might be a determining factor in the susceptibility of the isolate to *N. sativa* oil extract. Also it might be pertinent to know if the resistance shown by MDR bacteria isolates is plasmid based or not. Badger-Emeka *et al*., [22] showed MDR *S. aureus* contained plasmids and that the resistance shown by these bacteria, could be plasmid based. It was not ascertained whether the resistance to antibiotics by all the isolates considered in the present study was plasmid or chromosomal based. There is the possibility that this could be a determining factor in the microbial susceptibility by bacteria to *Nigella sativa* herbal produce. Patwardhan *et al*., [23] showed that root extracts of *Plumbago auriculata*, cured plasmids in MDR nosocomial isolate of *P. aeruginosa*, *E. coli* and *K. pneumoniae* with curing efficacies of 13%, 15% and 30% respectively. Plasmid elimination might therefore play a role in making non-susceptible MDR bacterial sensitive. In this study, the oil was found to be more effective on multi-drug resistant Gram negative *Pseudomonas aeruginosa* than Gram positive MRSA bacteria. This is contrary to the findings of Salman *et al*., [20] who reported that the zone of inhibition for *S. aureus* was greater than that of *Pseudomonas aeruginosa.* These differences can be attributed to differences in bacterial strains, differences in oil extract preparation, geographical differences, amongst other factors.

With the results from the present findings, it can be suggested that *Nigella sativa* oil extract having shown such levels of inhibitory effects on multidrug resistant bacteria, can be used in either the treatment of bacterial infections as topical applications [20] or as an adjuvant [24] or for the prevention of various bacterial infections. Researchers [24] suggested that *N. sativa* oil should be used as adjuvant in the treatment of various infectious diseases. Its antibacterial activity might be due to the presence of thymoquinone, Hannan *et al*., [11] which is a major active chemical component of the oil that possesses antibacterial activity. As there is an urgent search for solution to therapeutic and the economic burden in the treatment of MDR microbial infections, pharmaceutics might need to look into the possibility of using phyto-therapeutic plasmid curing agents [23]. The effect of *N. sativa* on MDR isolates as seen in the present study could simply be as a result of plasmid curing. There is however need for further investigations into the mode of action of this wonder herbal plant in microbial treatment.

5. Conclusion

Nigella sativa has been shown to vary on its antibacterial activity against multidrug resistant Gram positive and Gram Negative bacteria. There is need however to ascertain the mode of action of the plant extract of *N. sativa*. The question as to why it is capable of acting on some Gram negative and not on other remains unanswered. There is need for further investigations.

Conflict of Interest

The authors declare that there is none.

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