

Antibacterial Activity of the Essential Oils of *Nigella sativa* L. against Pathogens Bacteria

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Abstract: The essential oils of *N. sativa* have been commonly employed as a crude drug by people in Asia, Middle East and Africa. Due the development of antimicrobial resistance by the using of antibiotics among pathogenic bacteria, a natural products extracted from the plants can be used as alternative strategies to reduce pathogenic bacteria from foods and patients. The aim of this study is the evaluation of the antibacterial properties of the essential oils of *N. sativa* L, which were evaluated against pathogens bacteria *E. coli*, *Ps. aeruginosa*, *S. aureus* and *B. cereus*, by the using agar well diffusion method and by the study of bacterial growth in the absence and the presence of essential oils. The obtained results showed that the antibacterial activity of the essential oils of *N. sativa* L at various concentrations has indicated an excellent antibacterial activity by *S. aureus*, *B. cereus*, *E. coli*, *S. typhi*, *E. aerogenes*, *Ps. aeruginosa* ATCC 27853, *P. vulgaris* with a maximal diameter of inhibition zone 32, 15, 14.14, 13 mm respectively. Furthermore, the study of *S. aureus* ATCC 25923 and *P. vulgaris* growth in the absence (Control) and in the presence the essential oils of *N. sativa* has manifested a considerable biomass reduction accompanied with unbalanced growth after adding of the crude extract.

Key words: *Nigella sativa* • Antibacterial Activity • Essential Oils • Minimum Inhibitory Concentration

INTRODUCTION

The widespread use of commercially available antimicrobials with prolonged use has negative effect on human health led to alarming increase in antibiotic resistance among microorganisms, thus necessitating the need for development of novel antimicrobials. Recent years have witnessed a renewed interest in exploring natural resources for developing such compounds [1-4]. Furthermore, since 1980 the introduction of new antimicrobials has declined due to the huge expense of developing and testing new drugs [5, 6].

N. sativa (Family Ranunculaceae) is a spice which is well known for its medicinal properties. It has been extensively in use for centuries in folk medicines, both as herb and for oil by people in Asia, Middle East and Africa for medicinal purposes. Seeds are used as a new source of

edible oils and food applications as spice and condiments in cakes, breads, pastries, curries, pickles and in seasoning etc. The seeds contain 40% fixed oil, a saponin (Melantin) and up to 1.4% volatile oil [7-9].

Black seed oil has been shown to be effective against a wide spectrum of organisms-bacteria like *Bacillus cereus*, *B. subtilis*, *B. pumilus*, *S. aureus*, *E. coli*, *S. abony* [10-14]. A few antimicrobial work of the volatile oil of the seeds of *N. sativa* has been reported [15, 16].

The main objective of this work is based on the study of the antibacterial properties of the essential oils of *N. sativa* L such pathogens bacteria: *E. coli*, *S. typhi*, *P. vulgaris*, *K. pneumonia*, *E. aerogenes*, *St. aureus*, *Listeria*, *B. cereus*, *Ps. aeruginosa* by the using agar well diffusion method and the growth of the tested bacterial growth in the presence and in the absence of the essential oils of *N. sativa* L.

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MATERIALS AND METHODS

Extraction and Preparation of Oil: *N. sativa* seeds were purchased from local market of Sidi Bel Abbès, Algeria and extraction of oil from *N. sativa* seeds was carried out by standard hydro distillation method from Cleavenger's apparatus and all operation were carried out at room temperature.

The crushed seed powder (200 g) was placed in a separate flask together with distilled water (1 L). After 5 to 6 h, the oil was collected from the apparatus and it was dehydrated by passing through anhydrous sodium sulphate for removal of water traces stored into dark bottle and at 4°C until use, yield was 0.35% (w/v). The essential oil was used for disc diffusion test and determination of minimum inhibitory concentration (MIC). Essential oils yield was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Weight of EO recovered}}{\text{Weight of spices}} \times 100$$

Each essential oil dilution (60 mg/mL) was prepared in (DMSO), followed by sterilization using a 0.45 µm membrane filter.

Microbial Strains: The used strains in this work were *E. coli*, *S. typhi*, *E. aerogenes*, *Ps. aeruginosa*, *P. vulgaris* (Gram negative) and *S. aureus*, *B. subtilis* (Gram positive) were provided from the bacteriological laboratory of the hospital CHU of Sidi Bel Abbès, Algeria. They were sub-cultured and used throughout the studies. Each of the bacterial specimens was incubated in liquid culture dilutions in Mueller Hinton Agar (MHA Oxoid- CM337) and incubated at 37°C for 20 min to reach the logarithmic phase, then measured to a 0.5 McFarland dilution (Standard concentrations), which delivered a final concentration of approximately 10⁵ CFU per ml. Then the agar plates were inoculated with the essential oils of *N. sativa L* and incubated over night at 37°C [17].

Determination of Antibacterial Activity: The disc diffusion method described by Modzelewska *et al.* [18] was used for the investigation of the antibacterial activity. For this purpose, the MHA plates were prepared by pouring 15 mL of molten media into sterile petri dishes. Different concentrations of the essential oils of *N. sativa L* were prepared by dissolving in DMSO to obtain the following concentrations 16, 8, 4, 2, 1 µl. After that a sterile paper disc (6 mm) was placed on the surface of medium to allow the diffusion of the compounds of the

extract residue diluted into corresponding extraction solvents, so that each disc was impregnated with 6 µL of residue and the plates were kept for incubation at 37°C for 24 hours. The sensitivity of different bacterial strains to the essential oils was calculated by measuring of the diameter (In millimeters) of inhibition zone. Readings were taken at the end of 24 hours and 48 hours. Bacteria showing a clear zone of inhibition >6 mm were considered to be sensitive. Experiments have been achieved in triplicates for each combination of extract and the tested bacterial strain. Discs containing water and ethanol were used as controls. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the discs.

Disc Diffusion Assay: Standardized inoculum of bacteria 0.1.10⁸ cells per mL was spread on the solid media and nutrient Agar (NA) plates respectively with the help of sterile spreader. The inoculated plates were allowed to dry and sterile cork borer of diameter 8.0 mm was used to bore wells in center of inoculated agar plates. Subsequently, a 60 iL volume of oil of test spices were introduced in wells. Sterile DMSO served as the control. The plates were allowed to stand for 1 hour to diffuse and then incubated at 37°C for 24 h for bacteria. The zone of inhibition was recorded to the nearest size in mm.

Determination of Minimum Inhibitory Concentration: The minimum bactericidal concentration was performed to test the antibacterial activity of active extract by the using tube dilution method. The MIC was defined as lowest concentration able to kill any microbe. The minimum inhibitory concentrations (MICs) of the essential oil are determined according to the method reported by Pinto and Savadoro *et al.* [19, 20].

Dilutions of the essential oil of *N. sativa L* were prepared in sterile nutrient broth to get a final concentration of 16, 8, 4, 2, 1, 0.5, 0.25 µL respectively. To each of these dilutions, a loop full of bacterial culture adjusted to 0.5 McFarland standards was inoculated in Mueller Hinton Agar (MHA) and all the tubes were incubated at 37°C for 24 hours. After incubation, loop full from each tube was inoculated onto nutrient agar plates. The plate without growth was recorded as MIC.

RESULTS AND DISCUSSION

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including bacteria and fungi [21].

Table 1: Illustration of the antibacterial activity by the produced zone inhibition (mm) in the presence of the several concentrations of the essential oils of *N. sativa L* (mg/mL) against pathogen bacterial

Tested bacteria	The produced zone inhibition (mm) by the using of increasing concentration of essentials oils of <i>N.sativa L</i> (mg/ml).						
	0	1	2	4	6	8	12
<i>E. coli</i>	0	1	7	8	12	12	12
<i>S. typhi</i>	0	0	8	10	10	12	14
<i>P. vulgaris</i>	0	6.5	9	10	14	14	14
<i>K. pneumonia</i>	0	8	7	8	10	10	13
<i>E. aerogenes</i>	0	0	9	9	11	13	15
<i>Listeria</i>	0	7	7	8	10	11	13
<i>Ps. aeruginosa</i>	0	0	0	0	8	10	13
<i>St. aureus</i>	0	0	24	26	26	29	32
<i>Ba. cereus</i>	0	22	7	10	11	11	13



Fig. 1: The produced zone inhibition (mm) of the antibacterial activity of the essential oils of *N. sativa L* against *P. vulgaris* (left), *S. aureus* (center), *Ps. aeruginosa* ATCC 27853 (right).

In the present study, the antibacterial activity of the essential oils of *N. sativa L* against various Gram positive and Gram negative bacteria has been investigated.

For the evaluation of the antibacterial activity of the essential oils of *N. sativa L*, the disc diffusion method, the determination of MIC and the bacterial growth in the presence and the absence of the essential oils have been studied.

The illustrated results in (Table 1 and Figure 1) revealed the essential oils of *N. sativa L* in the dilution of 1 to 16 μl.

The obtained results on the antibacterial activity of the essential oils of *N. sativa L* has manifested clear inhibition zone of at least 7 mm for all the tested strains.

The obtained activity of the essential oils of *N. sativa L* at various concentrations against *S. aureus*, *B. cereus* and *E. coli*, *S. typhi*, *E. aerogenes*, *Ps. aeruginosa* ATCC 27853, *P. vulgaris* has indicated an excellent antibacterial activity by *S. aureus* ATCC 25923, *E. aerogenes*, *S. typhi*, *P. vulgaris* and *B. cereus* ATCC 6633 with a maximal diameter of inhibition zone 32, 15, 14.14, 13 mm respectively.

The obtained results indicated that the essential oil of *N. sativa L* possesses a wide inhibition activity spectrum on pathogenic bacteria for humans. A more careful analysis should be performed *in vivo* in order to determine its real effects. In particular, the sesquiterpenes and their derivatives seem to be a promising class of natural compounds in the search for new antibacterial agent [14, 18].

Topozada *et al.* [12] has reported that *Salmonella* and *Pseudomonas* bacteria are very sensitive to the essential oil of *N. sativa L* and with the exception of *E. coli* ATCC 25922, other Gram negative strains are less sensitive than Gram positive strains.

Likewise, Singh *et al.* [22] has reported that the essential oils of *N. sativa* showed complete growth inhibition against *B. cereus*, *B. subtilis* and *S. aureus*, *Ps. aeruginosa* by the agar well diffusion method. This resistance is due to the chemical composition of the wall which is rich in lipopolysaccharides not allowing the penetration of hydrophilic molecules.

Table 2: Determination of MIC of essential oil of *N. sativa L* against pathogen bacterial

Strains	Dilution		
	10 μ l	50 μ l	100 μ l
<i>E. coli</i>	+	++	+++
<i>S. typhi</i>	+	++	+++
<i>P. vulgaris</i>	+	++	+++
<i>K. pneumonia</i>	+	+	+++
<i>E. aerogenes</i>	+	++	+++
<i>Listeria</i>	+	+	+++
<i>Ps. aeruginosa</i>	+	+	+++
<i>St. aureus</i>	+	++	+++
<i>Ba. cereus</i>	+	++	+++

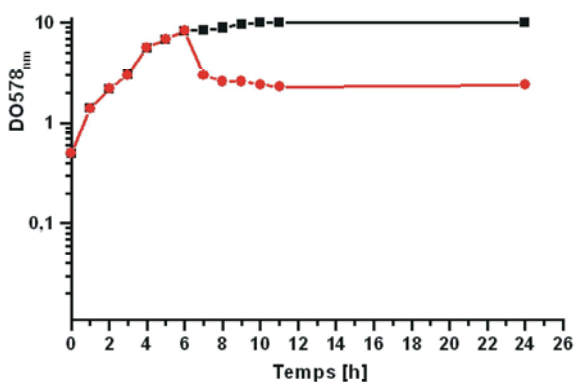


Fig. 2: The study of *St. aureus* growth, cultivated on seed medium, incubated at 30°C for 24 hours, in the presence (—■—) and in the absence (—●—) of the essential oils of *N. sativa L*.

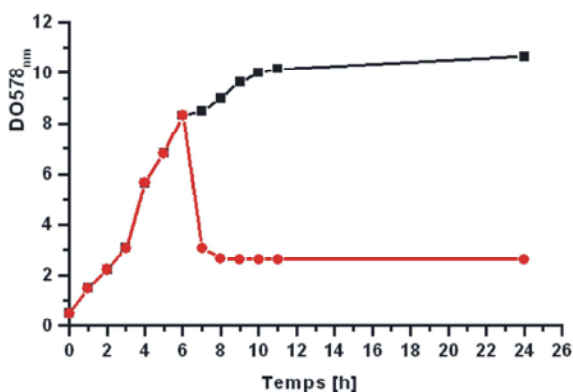


Fig. 3: The study of *P. vulgaris* growth, cultivated on seed medium, incubated at 30°C for 24 hours, in the presence (—■—) and in the absence (—●—) of the essential oils of *N. sativa L*.

For essential oils, the therapeutic dose is very close to the lethal dose, so that the calculation of CMI is very important. The obtained results are reported in Table 2. The MIC results depend on the strain tested and the dilution [23].

This antibacterial activity may be indicative the presence of metabolic toxins or broad spectrum antibacterial compounds. This is in agreement with previous reports by the several researchers [24]. Furthermore, Olila *et al.* [25] has reported that the essential oil of *N. sativa* showed high inhibitory activity against a range of bacteria resistant to antibiotics. It may be related to the fact that gram positive bacteria such as *S. aureus* ATCC 25923, *B. subtilis* are more sensitive against antibacterial agents compared to the tested gram negative bacteria because of the difference in their cell wall structures [26]. In order to explore the effect of the compounds present in of the essential oils of *N. sativa* *Lin* tested pathogens bacteria, the bacterial growth of *S. aureus* and *P. vulgaris* in the absence (control) and in the presence of the essential oils of *N. sativa* has been investigated. For this purpose, *S. aureus* and *P. vulgaris* were inoculated in seed media with initial optical density of 0.5 at 578nm according the protocol described by Abbouni *et al.* [4], in the absence and in the presence of the essential oils of *N. sativa*, which was added 6 hours after the onset of the bacterial growth. The obtained results manifested (Figure 2, 3) showed a considerable inhibition of the growth of *S. aureus* and *P. vulgaris*, after the adding of the essential oils of *N. sativa* *Lin* in the early exponential growth phase. In conclusion, the molecules present in the essential oils of *N. sativa L* were able to induce unbalanced growth and further the arrest of the cell cycle of *S. aureus* and *P. vulgaris* in comparison with the untreated biomass with the crude extract (Balanced growth) [27, 28].

CONCLUSION

A number of plants have been documented for their biological and antimicrobial properties. The most tested bacteria are characterized by the developing a resistance to commonly employed antibiotics and are a common cause of many enteric infections. Therefore, in the present study, the antibacterial activity of the essential oils of *N. sativa* has been investigated against a gram positive (*S. aureus*, *B. cereus*) and Gram negative bacteria such as *E. coli*, *S. typhi*, *E. aerogenes*, *Ps. aeruginosa* ATCC 27853, *P. vulgaris*.

The tested essential oils of *N. sativa L* have manifested an important antibacterial activity by *S. aureus* ATCC 25923, *E. aerogenes*, *S. typhi*, *P. vulgaris* and *B. cereus* ATCC 6633 with a maximal diameter of inhibition zone 32, 15, 14.14, 13 mm respectively.

Therefore, a further studies involving the purification of the chemical compounds of the essential oils of *N. sativa L* by the using a modern technique such as Gas chromatography, HPLC, IRM, required for determination of the active metabolites present in the essential oils of *N. sativa L*.

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