



## ***Study of the antibacterial activity of Nigella sativa ethanol extract on the growth of Staphylococcus aureus in culture media***

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### **Summary:**

Bacterial infections become a great health problems. The new antimicrobial agents are will reduce this problem. In this study, antimicrobial of activity of extract of *Nigella sativa* seed that used against *Staphylococcus aureus* isolates was evaluated. Started with an objective to appraisal the antibacterial potentials of ethanol extract of *Nigella sativa* prepared in different concentrations (50, 100,200& 400 mg/ml) against *Staphylococcus aureus* and associated its activity with antibiotic concentration value Ciprofloxacin(5µg), Amoxicillin(25µg) & Penicillin(6µg) by determining the inhibition zone produced around the holes after growth on Muller-Hinton agar .

This results showed activity of antibacterial of the *Nigella sativa* at different concentrations and standard antibiotics exhibited various degrees of zones of inhibition in the culture media depending mainly upon the type of plant extract, concentration of extract in addition to the type of tested bacterial. Nearly all the *Nigella sativa* extracts were found to have significant activity ( $p<0.05$ ) against all tested bacteria related with negative control. In the same time the current study was recorded that inhibition zones diameter against examined bacteria raised significantly difference ( $p<0.05$ ) at extract concentration raised (400 mg / ml ). The MIC values of ethanolic extracts of *Nigella sativa* extended from 0.156 to 0.312 mg/ml against tested bacteria . Whereas the MBC values ranged 1.248 mg/ml .

**Key words:** *Nigella sativa*, antibacterial activity, *Staphylococcus aureus* .

### **دراسة الفاعلية المضادة للجراثيم للمستخلص الأيثانولي للحبة السوداء على نمو بكتريا المكورات العنقودية الذهبية في الاطباق الزرعية**

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### **الخلاصة:**

أصبحت العدوى البكتيرية المسببة للأمراض مشكلة صحية رئيسية في جميع أنحاء العالم. وهناك حاجة ماسة إلى عوامل مضادات الميكروبات الجديدة للتغلب على هذه المشكلة. في هذه الدراسة، تم تقييم النشاط المضاد للميكروبات لمستخلص بذور الحبة السوداء ضد المكورات العنقودية الذهبية. بدأت مع هدف لتقييم إمكانات مضادة للجراثيم من عدة مقتطفات من الحبة السوداء والتي أعدت في تراكيز مختلفة (50، 100، 200 و 400 ملغ / مل) ضد المكورات العنقودية الذهبية والتي يرتبط نشاطها مع قيمة المضادات الحيوية القياسية (سيبروفلوكساسين) (5 ميكروغرام) ، (اموكسيسيلين) (25 ميكروغرام) ،

البنسلين (6 ميكروغرام) من خلال تحديد نطاق تثبيط نمو الجراثيم والتي تنتج حول الثقوب بعد النمو على وسط مولر هينتون .

اظهرت نتائج الفعالية المضادة للبكتريا لمختلف مستخلصات الحبة السوداء المستخدمة في الدراسة والمضادات القياسية درجات متفاوتة من نطاق تثبيط النمو حول حفر الوسط الزراعي , كما وجد ان جميع مستخلصات الحبة السوداء لها فعالية مضادة للجراثيم وبصورة معنوية ضد جميع البكتريا المختبرة مقارنة مع مادة السيطرة السالبة , كما لوحظ ان اعلى فعالية سجلت لمستخلص الحبة السوداء الايثانولي , في حين اظهرت بقية المستخلصات فعالية متقاربة على الاعم . في الوقت نفسه اظهرت الدراسة الحالية ان زيادة التركيز المستخدم ادى الى زيادة معنوية في نطاق تثبيط النمو حول الحفر المعمولة بالوسط الزراعي .

**الكلمات الافتتاحية :** الحبة السوداء ، الفعالية المضادة للجراثيم ، المكورات العنقودية الذهبية .

## Introduction:

The microbial resistance to antibiotics increase overtime and form threatens against public health , and it reduces efficiency of antibiotic treatment and it will increase mortality , morbidity, in addition to care costs . the search about new antimicrobial agent become important demand [1,2] *Staphylococcus spp* are distributed in nature, it is part of the normal flora and microbiota in the skin and mucosa layer in all the animals such as birds. Some *Staphylococcus spp* are opportunistic and causes infections in human and animals. *Staphylococcus aureus*, also causes series of types of intoxications, *S. aureus* is associated bacteria commonly in purulent infections (such as pneumonia, abscess meningitis, carbuncle, endocarditis and furuncle) [3,4]. Since ancient peoples, plant natural sources are used as medicinal therapeutic because it consists from many substances that are think it has great role in the cure to many of infectious diseases. The plants supply a lot of chemical compounds, which have many of therapeutic uses such as antibacterial, antiviral, anticancer, antifungal effecting [5]. *Nigella sativa* is spread in Asia Minor, northern Africa and southern Europe. The seeds are black and small and have aromatic taste and odor. This seeds sometime called black seed and it used in popular medicine commonly against many of diseases such as diabetes, cough, asthma, hypertension, eczema, inflammation and fever. Furthermore Seed oil has antineoplastic, analgesic and antipyretic,

effect [6]. also consist from saponins, alkaloids and proteins and antioxidant [7,8]. the seed oil has antimicrobial effecting [9], antitumor [10] and a activated effect in immune status [11]. the seed oil showed wide spectrum effective against bacteria like *B. pumilus*, *S. abony*, *E. coli*, *Bacillus cereus*, *S. aureus* and *B. subtilis* [12,13]. *Nigella sativa* seeds contain thymoquinone, it is an active constituent of, and contain quinone, that it has many of pharmacological uses including anti-inflammatory and analgesic [14]. the 4-terpineol (2.0%–6.6%), t-anethole (0.25%–2.3%), p-cymene (7.1%–15.5%), carvacrol (5.8%–11.6%) and thymoquinone (27.8%– 57.0%) of *N. sativa* composition [15]. In addition, thymoquinone and its reduced product thymohydroquinone have been reported to have an antibacterial activity and beneficial interaction with some antibiotics [16] also Thymol is reported as antibacterial activity [17,18]. Thymoquinone is the methanol soluble compound present in *N. sativa* oil [19]. The tannins is compound extracted from the seeds by use methanol [20] and many of studies found the tannins has antimicrobial effecting [21] Antibacterial characteristic of *N. sativa* can possible because the ability of the oil to enter over the membranes and destroy bacteria cell leading to cell death [22].

## Material and methods:

### \* Plant materials:

*Nigella sativa* were taken from the local markets in Al-Diwanyia province, Iraq.

Department of Clinical Laboratory Science, collage of pharmacy has recognized the premeditated plant.

**\* Preparation and plant extracts:**

Seeds was keep at (45°C) under lower light, then mashed by use mechanical blender a gruff powder, A weighed quantity (100 gm) of the powdered plant was then undergo to continuous hot extraction in soxhlet apparatus with ethanol . the extracts were purring by filter paper and intensified to dryness at evaporator in (25°C) till completely solvent had been removed to give pure Concentration of *Nigella sativa* extract with a yield of (50mg/ml), (100mg/ml), (200mg/ml) and (400mg/ml) respectively in relationship to the dried starting plant material [23].

**\* Test microorganisms:**

The organisms of *staphylococcus aureus*, was gotten from bacteriological laboratory of the Diwanayah Teaching Hospital in Al- Diwanayah province. They was sub- cultured on Nutrient agar slants and used throughout the studies. it was keep at 40C for next steps [24].

**\* Assay of antibacterial activity:**

The Agar well diffusion method is used to extraction of *Nigella sativa* to evaluate antibacterial activities. it was placed 1.0 ml of broth cultures of the bacteria ( $1.0 \times 10^7$  cfu/ml) In a Petri dish with 15 ml of molten nutrient agar ,and by inserting the broth cultures into sterile Petri dishes, combining the molten agar , quietly circular motion to ensure spreading of the microorganisms. after that, allowed to harden on a flat surface. On the center of growth, three wells (6 mm) as diameters using a sterile pasture pipette and complete with 100 µl of each concentration of plant extracts. The distribution of the material extract for one hour then put in incubator at 37 C ° for overnight. In the end of incubation period the plates were collected and examined for

any zones inhibition [25]. Negative Control plates (diluted solvent) and standard reference antibiotics plates (Ciprofloxacin (5µg) , Penicillin(6µg) , Amoxicillin ( 25µg) , and ready for testing antibacterial effecting. each experiment repeated again three times and calculate diameter of zone inhibition .

**\* Determination of MIC & MBC values**

Minimum inhibitory concentration (MIC) was detected by the micro-dilution method using serially diluted (2-fold) plant extracts according to the NCCLS (7) . A serial dilution concentration was carried out to give final lie between 6.25 to 0.781 mg/ml from each plant extract. Bacteria were adjusted to contain approximately  $10^5$  CFU/ml. The test plates were incubated for 18 hour at 37 ¼C . least concentration of MIC was taken which inhibited by bacteria. Then sub-culturing the test dilutions onto fresh solid medium and incubated at 37C (24) hour. the Highest dilution was chosen the MBC [26].

**\* Statistical analysis :**

the reading is done by use the mean  $\pm$  S.E.M. Statistical , and was done the comparison among the groups. (ANOVA) test are used , in SPSS software program (V18) and submitted to (LSD) test P value  $\leq$  0.05 .

**Results:**

The antibacterial screening of different extracts prepared from seeds of *Nigella sativa* plant compared with standard antibiotics as positive control were done by agar well diffusion method and serial microdilute against *Staphylococcus aureus*.

The control positive ciprofloxacin (5µg) showed respective mean of inhibition zones of  $34 \pm 1.2$  mm against the growth of *staphylococcus aureus*. Penicillin antibiotic disc (6µg) showed respective mean of

inhibition zone of  $14.2 \pm 2.2$  mm against the growth of *staphylococcus aureus* . Amoxicillin ( 25 $\mu$ g) showed respective mean of inhibition zone of  $32 \pm 1$  mm against the growth of *staphylococcus aureus* (table 1 )

The negative control (diluted ethanol ) show inhibition zone on tested growth bacteria of *S.aureus* that gave inhibition zone of  $8.2 \pm 0.8$  mm (table 1)

The comparison of inhibitory zones that caused by *Nigella sativa* extract with those caused by positive control antibiotics there results were and there was no deference (table 2).

The extract at concentration 200 mg/ml caused zone of inhibition more than concentration 50 and 100 mg/ml and more than penicillin

But the most effective concentration was 400 mg/ml of *Nigella sativa* extract it was more than other concentrations and ethanol and antibiotics even ciprofloxacin.

The MIC & MBC of the *Nigella sativa* extract was shown in the tables (3). The ethanolic seeds extract showed MIC & MBC values at 0.156 - 1.248 mg/ml against *S.aureus* .

**Table 1** illustrate the zone of inhibition to *S. aureus* caused Positive control (reference antibiotics)

Positive control (reference antibiotics)	The inhibition zone of <i>S. aureus</i> growth in plate (mm)
Ciprofloxacin	$34 \pm 1.2^{(b)}$
Amoxicillin	$32 \pm 1^{(c)}$
Penicillin	$14.2 \pm 2.2^{(f)}$
<b>Negative control</b>	
Ethanol 40%	$8.2 \pm 0.8^{(g)}$

Each value represent means  $\pm$  standard error , each well contain volume 100  $\mu$ l the values of inhibition zone measured by mm in the diameter where . Same letters (no significant difference) and vesa versa at ( $p < 0.05$ ).

**Table (2) :** The antibacterial effects of different extracts of *Nigella sativa* compared with some antibiotics against *Staphylococcus aureus* growth in culture media .

Concentration of <i>Nigella sativa</i> extract (mg/ml)	The inhibition zone of <i>S. aureus</i> growth in plate (mm)
50	$26 \pm 1.2^{(e)}$
100	$28.5 \pm 1.4^{(d)}$
200	$29.8 \pm 0.9^{(d)}$
400	$37.4 \pm 1.6^{(a)}$

Each value represent means  $\pm$  standard error , each well contain volume 100  $\mu$ l the values of inhibition zone measured by mm in the diameter where . Same letters (no significant difference) and vesa versa at ( $p < 0.05$ )

**Table (3) :** the values of MIC and MBC (mg/ml) for different extracts of *Nigella sativa* against *Staphylococcus aureus* pathogen.

Concentration of <i>Nigella sativa</i> extract												MIC	MBC
0.03 9	0.078	0.156	0.312	0.624	1.248	2.5	5	10	20	40			
-	-	+	+	+	++	++	+	+	+	+	+	0.156	1.248

- Represent no effect
- + Represent the value of MIC
- ++ Represent the value of MBC

### Discussion:

The plants in general contain many bioactive ingredients that can be interest in therapeutic as well as their low toxicity equivalent with chemical drugs , Thus the present work aimed to study the possibility the use of certain preparation made from *N.sativa* extract as active components for pathogenic bacteria through the testing its activity in the culture media [27 ] .

By comparing ethanolic extracts of *N.sativa* with control positive antibiotic against *S.aureus* bacteria, ethanolic extract at concentration 400 mg/ml showed higher inhibition zone than control antibiotic.

The results were in similarity with those informed by[28] screening singnificant antibacterial activity of *Nigella sativa* against *microorganism* .Hence, *Nigella sativa* formed from compounds have antibacterial effecting [29].

it's contains a many of substances like dithymoquinone and thymoquinone, these compounds have effect against organisms, there are four component reported in seed of *N. sativa* seeds, nigellidine, nigellicine, isoquinolines and nigellimine [30].

sterols and phenolic constituents were extracted from *N. sativa*. thymohydroquinone and Thymoquinone are active substances that have different pharmacological effecting such as antihypertensive, antioxidant and anti-inflammatory [31]. So the antibacterial activity of *Nigella sativa* is associated with thymoquinone dithymoquinone content.

On the other hand the concentration of thymoquinone when increased, the antibacterial activity of *Nigella sativa* will be increased also, therefore antibacterial

activity caused by concentration 400 mg/ml was more than other concentrations due to high content of thymoquinone and the zone of inhibition was more than other zones even the control antibiotics [32].

The Gram positive bacterial resistes for many of antibiotics because outer membrane prevent antibiotics getin [33]. Effect of the antibacterial of the extracts could be suggested by disturbance across the permeability barrier of the bacterial membrane [34].

In the present study the MIC values of the plant extracts components was taken in this study were lower than the MBC values this belived that *Nigella sativa* extracts where become bactericidal in higher concentration and bacteriostatic in lower concentration[35].

*Nigella sativa* has antimicrobial activity against the bacteria that used in our study [36] may be present non-enough amount of active gradient compound in the crude extracts to show complete activity with the dose levels employed , the conclusion is advice to use of *Nigella sativa* to treatment infectious diseases.

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