Inhibitory Effects of Habbat Al-Sauda Seed's (*Nigella Sativa)* Extract Against Some Pathogenic Bacteria Isolated from Sulaimani Teaching Hospital

Salih A. Hama
Department of Biology, College of Science, Sulaimani University. Sulaimani-Iraq.

Abstract

To investigate the antibacterial effects of ***Nigella sativa*** seed extracts, three different concentrations of this extract (100 mg/ml , 0.1mg/ml , and 0.0 mg/ml –control-) were prepared in, and tested against (12) ***S. aureus*** isolates, (6) ***S. epidermidis***, (15) ***P. aeruginosa*** and (14) ***E. coli*** isolates, which were isolated from Suleiman Teaching Hospital. For each bacterium three nutrient broth culture tubes were prepared and (0.25ml) of the pre-mentioned concentrations (tested) and distilled water (control) were added to the three cultivated tubes respectively. The inhibitory effects of seed extracts for the first and second cultivated tubes were determined turbidometrically at (640 nm). The effects were proportional with the extract concentration, the higher the concentration, the higher the inhibitory effects were. T-test was depended for statistically analyzing the obtained results from this study, significant differences were observed in regard to ***S. aureus*** (p= 0.00000474), ***S. epidermidis*** (p= 0.0327), ***P. aeruginosa*** (p= 0.00963) and ***E. coli*** (p= 0.03897) when the data from (100mg/ml) were compared with that of controls. Although the effects become limited (non-significant) when (0.1mg/ml) was used, but still within significant differences in regard to ***S. aureus*** (p= 0.000343) and ***E. coli*** (p= 0.0228).

الخلاصة

لغرض التحقق من الفعالية ضد البكتيرية لمستخلص بذور الحبة السوداء (***Nigella sativa***), تم تحضير ثلاثة تراكيز من المستخلص و كالاتى (100ملغم/مل) و (0.1 ملغم/مل) و 0.0 (ملغم/مل) – السيطرة–. تم اختبار التراكيز المذكورة ضد (12) عزلة من ***S. aureus*** (6) عزلات من ***S. epidermidis*** , (15) عزلة من ***P. aeruginosa*** , و (14) عزلة من ***E. coli*** من نماذج مرضية متباينة المؤخوذة من الراقدين فى مستشفى السليمانية التعليمى. تم تحضير ثلاث انابيب من المغذى السائل لكل بكتيريا و اضيفت (0.25 مل) من التراكيز المذكورة (تحت التجربة) و الماء المقطر (السيطرة) الى الانابيب المحضرة على التوالى. اعتمدت على الطريقة الطيفية (قياس العكورة) لغرض قياس الفعالية التثبيطية للمستخلص فى الانبوبتين الاولى و الثانية فى طول موجى (640 نانوميتر). كانت التأثيرات طردية مع التراكيز, كلما كانت التراكيز عالية كان التأثير اعلى و العكس صحيح. عند تحليل النتائج احصائياً بالاعتماد على (t-test) تبين وجود فرق معنوى بين نسبة النمو عنداستعمال التركيز الاولى (100ملغم/مل) بالمقارنة مع السيطرة لكل من ***S. aureus*** (p= 0.00000474), ***S. epidermidis*** (p= 0.0327) , ***P. aeruginosa*** (p= 0.00963), و ***E. coli*** (p=0.03897), بينما كانت التاثيرات محدودة (غير معنوية) عند استعمال التركيز الواطى (0.1 ملغم/مل) الا انها ظهرت معنوية للبكتيريا ***S. aureus*** (p= 000343) و ***E. coli*** (p=0.0228).

Introduction

Nigella sativa (black cumin) is an annual herbaceous plant belonging to the plant family Ranunculaceae [1] and have been used traditionally for centuries for treatment of various diseases[2, 3]. The plant extract and its essential oil showed a broad range of pharmacological effects and used as an anti-inflammatory agent[4]. Also other investigators showed the antibacterial property of the black cumin's extract when it used against different bacterial isolates[5]. In the past the antibacterial activity of *N. sativa* was reported by others including Topozada *et al.* (1965), who have been noted the antibacterial effects of the phenolic fraction of *N. sativa* oil [6]. In 1975 El-Fatary found that the thymohydroquinone obtained from *N. sativa* have antibacterial effects against different bacterial isolates from different sources, due to the chemical components[7], whereas others showed that the black cumin extract was more effective than some antibiotics[8].

In another study Hanafi and Hatem, found that diethyl ether obtained from *N. sativa* oil acts effectively against both Gram-positive and Gram-negative bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* [5]. Also the antibacterial activity of *N. sativa* seeds have been studied by Mawlood in 1995 when she tried to examine the effects of different concentrations of *N. sativa* extract on Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) [9]. The crude extract of *N. sativa* seeds has a broad antibacterial effect especially on multi-antibiotic resistant bacteria including both Gram-positive and Gram-negative bacteria, the highest activity was found against *Staphylococci*, α and β- *Streptococci* [10]. More recently, the antibacterial effects of the *N. sativa* seeds were investigated by other researchers when they tried to compare the effects of the *N. sativa* and other drugs against some bacteria [11].

Black cumin was known to be very important medically among people as GOD Profit Muhammad said (*N. sativa* –Habbat Al-sauda- are the treatment for all illness except death) [12].

The aim of this study is to investigate the effects of *N. sativa* seed extracts on some gram positive and gram negative bacteria isolated from different samples of hospitalized patients from Suleiman Teaching Hospital.

Materials and Methods

This work was achieved between (March 1and July 1. 2004). Forty seven different bacteria were isolated from different clinical samples of patients in Sulaimani Teaching Hospital included (12) isolates of ***S. aureus***, (6) ***S. epidermidis***, (15) ***P. aeruginosa*** and (14) isolates of ***E.coli*** as shown below. Each tested for their susceptibility to all concentrations of (***N. sativa***) black cumin extract. The (100 mg/ml) and (0.1 mg/ml) were chosen to show the effects of high and low concentrations of Habbat-Al Sauda (not different concentrations) on the growth rates of different bacterial isolates.

Sources, numbers, and symbols of bacterial isolates

Bacterial isolate	Source	No. of isolates	Symbol
Staphylococcus aureus	Burn	2	SAB
Staphylococcus aureus	Wound	2	SAW
Staphylococcus ureus	Urine	2	SAU
Staphylococcus aureus	Stool	2	SAS
Staphylococcus aureus	Throat	2	SAT
Staphylococcus aureus	Skin	2	SASK
Staphylococcus epedermidis	Burn	2	SEB
Staphylococcus epedermidis	Wound	2	SEW
Staphylococcus epedermid	Skin	2	SES
Pseudomonas aeruginosa	Burn	6	PAB
Pseudomonas aeruginosa	Urine	9	PAU
Escherichia coli	Urine	10	ECU
Escherichia coli	Wound	4	ECW
Total isolate number		47	

Bacterial Isolation

All the pre-mentioned bacteria which obtained from Sulaimani Teaching Hospital were re-identified in bacteriology Laboratory of Biology department through inoculation to selective media and performing necessary biochemical tests.

Preparation *of N. sativa* extract

The method of Mawlood (1996)[9] was followed to prepare the seed extract. ***N. sativa*** seeds (100 g) were grinded and dissolved in distilled water (200 ml) and placed in the water bath for boiling, and then filtrated. The filtrated suspension was then placed in an incubator at (37^0C) for drying. To obtain the first concentration (100 mg/ml), one gram of collected dried powder was redissolved in 5 ml of distilled water and the volume was completed to 10 ml. From this concentration the second concentration (0.1mg/ml) was prepared by dilution technique.

The blank (third concentration) was prepared by mixing (0.25 ml) seed's extract and (2.5 ml) of nutrient broth.

For each bacterial isolate three nutrient broth culture tubes (each contained 2.5 ml) were prepared. 0.25 ml of the seed extract (100 mg/ml) to the first tube, and the same amount of the extracts (0.1 mg/ml) to the second tube were added and both inoculated

with the tested bacterial isolate. For the third tube (0.0 mg/ml) 0.25ml distilled water was added and then inoculated with the tested bacterial isolate.

All the three cultivated tubes were incubated at 37°C overnight and the absorbency of each was recorded at 640 nm. As clarified previously the bacterial isolates from different sources were unequal and different and there were at least two replicates fore each although there were more than those for others. t-test design was depended for analyzing the results statistically.

Results

All Bacterial isolates were reidentified in Biology department bacteriological Laboratory depending on biochemical tests as shown below:

Bacteria	Performed positive biochemical tests
S. aureus	Coagulase, Catalase, Manitol fermentation, 7-10% NaCl tolerance
S.epidermidis	Same as ***S. aureus*** but no manitol fermented
P. aeruginosa	Cetrimide tolerance, Oxidase, citrate utilization, motile, no H_2S production.
E.coli	fermentation of lactose, manitole, and glucose, no oxidase production, no H_2S production, indole production.

The measurement of the absorbency (Turbidometry) was determined to express the growth rates of the bacterial isolates for comparing the effects of the concentrations of ***N. sativa*** seed extracts.

The first concentration (100 mg/ml) showed high inhibitory effect against both gram-positive and gram negative bacterial isolates, when the growth rates compared with that of controls (Figs.1, 2, 3, and 4).

Among Gram-positive bacteria the highest effect of the first concentration was against *S. aureus* from urine (absorbency was 0.276) (Table, 1 & Fig. 1), and the lowest effect was against ***S. aureus*** from throat –absorbency was 0.356- (Table, 1), whereas foe ***S. epidermidis*** the highest effect was against ***S. epidermidis*** from skin and the lowest effect was against isolates from burn (table-2-).

Among the Gram negative bacterial isolates the highest effect was against ***P. aeruginosa*** from buen with the absorbency (0.394) for the first concentration (table, 3 &Fig. 3), whereas the effects were lower against *E. coli* from wounds (0.288) (Table, 4 and fig. 4).

As shown in (Figurs, 1, 2, 3, and 4) there were different inhibitory effects of *N. sativa* seed extracts against the same bacterial isolates of different clinical sources. Also it was obvious that the concentrated ***N. sativa*** seed extract (100 mg/ml) was more effective than the lower concentration (0.1 mg/ml).

The statistical analysis (t- test was depended) showed significant differences between the growth rates of controls and ***S. aureus*** (p= 0.00000474), ***S. epidermidis*** (p= 0.0327), ***P. aeruginosa*** (p= 0.00963), and ***E. coli*** (p= 0.03897) when the (100 mg/ml) was tested, while the differences were significant only for ***S. aureus*** (p= 0.000343) and ***E. coli*** (p= 0.0228) when the (0.1 mg/ml) was used.

Table (1) Comparison of Growth rates of *S. aureus* isolates from different sources

Bacterial Isolate	Source	Growth rate (mean absorbency) 100mg/ml	0.1mg/ml	Cotrols	Number of isolates
SAB.	Burn	0.324	0.451	0.548	2
SAW	Wound	0.285	0.388	0.488	2
SAU.	Urine	0.297	0.501	0.572	2
SAS.	Stool	0.342	0.492	0.601	2
SAT.	Throat	0.356	0.485	0.612	2
SASK.	Skin	0.276	0.379	0.477	2

Table (2) Comparison of Growth rates of *S. epedermidis* isolates from different sources

Bacterial Isolate	Source	Growth rate (mean absorbency) 100mg/ml	0.1mg/ml	Controls	Number of isolates
SEB.	Burn	0.402	0.641	0.675	2
SEW	Wound	0.354	0.498	0.559	2
SES	Skin	0.287	0.403	. 0.468	2

Table (3) Comparison of Growth rates of *Pseudomonas aeruginosa* from different sources

Bacterial Isolate	Source	Growth rate (mean absorbency) 100mg/ml	0.1mg/ml	Controls	Number of isolates
PAB	Burn	0.394	0.498	0.791	6
PAU	Urine	0.409	0.770	0.875	9

Table (4) Comparison of Growth rates of *Escherichia coli* from different sources

Bacterial Isolate	Source	Growth rate (mean absorbency) 100mg/ml	0.1mg/ml	Controls	Number of isolates
ECU	Urine	0.294	0.588	0.667	10
ECW	wound	0.288	0.509	0.540	4

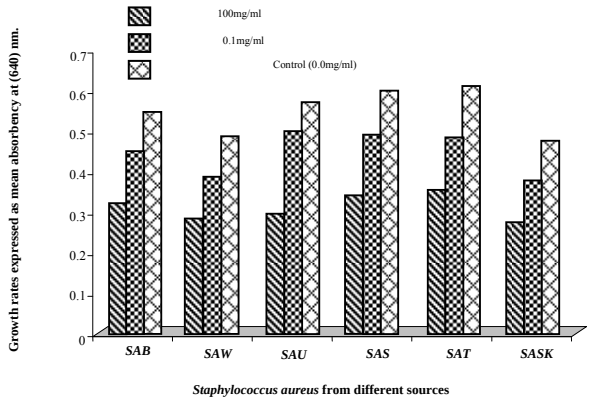

Figure -1- The comparison between the growth rates of *Staphylococcus aureus* from different sources after dealing with 100mg/ml and 0.1mg/ml *N. sativa*

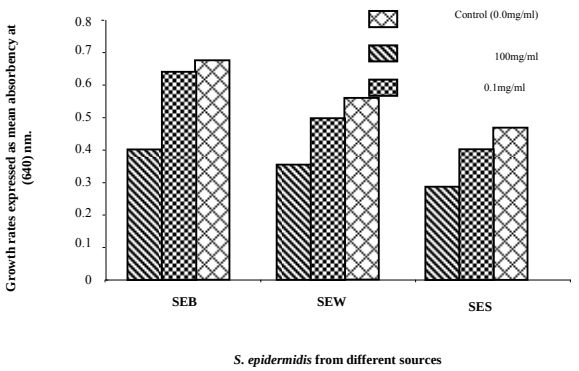

Figure -2- The comparison between the growth rates of *S. epidermidis* from different sources after dealing with 100mg/ml and 0.1mg/ml *N. sativa*

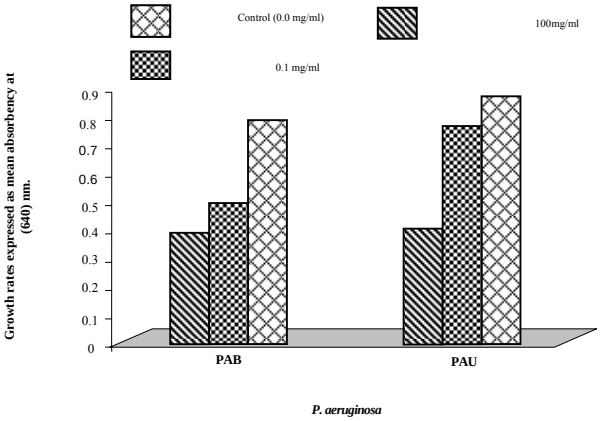

Figure -3- The comparison between the growth rates of *P. aeruginosa* from different sources after dealing with 100mg/ml and 0.1mg/ml *N. sativa*

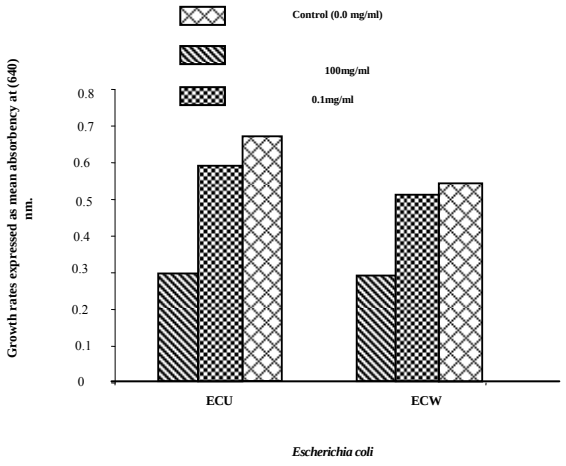

Figure -4- The comparison between the growth rates of *Escherichia coli* from different sources after dealing with 100mg/ml and 0.1mg/ml *N. sativa*

Discussion

In this study the antibacterial property of ***N. sativa*** seed extract was tested against different isolates of multi-resistant ***S. aureus***, ***S. epidermidis***, ***P. aeruginosa***, and ***E. coli*** isolates. The obtained results confirm that seed extract of ***N. sativa*** can inhibit the growth rates of the tested bacteria within different degrees, especially the concentration (100mg/ml), although the concentration (0.1mg/ml) showed lower effects as shown in (Figs.1, 2, 3, and 4). The results revealed that there is a correlation between the concentration of seed extracts of ***N .sativa*** and their effects on the bacterial growth (Figs. 1, 2, 3 & 4), the higher the concentration, the higher the effects, although the antibacterial activities sometimes were different for the same bacterial species from different sources.

The inhibitory effects of *N. sativa* seeds against different bacterial isolates are due to their chemical components[8]. As investigated by some researches the components are responsible for their effects especially they contain diethyl ether[9]. Some of the seed components act on the bacterial cell wall lipid, which may explain the significant effects of the concentration of (100 mg/ml) ***N. sativa*** seed extract used against different clinical bacterial isolates. Recent workers also confirmed the antibacterial activity of the seeds of ***N. sativa*** when tested against different bacterial isolates of different sources [6, 8, 9].

In the related works different results were obtained. The results obtained in this work are in agreement with some of them, where as some notable differences occur within the others. Mawlood in (1996) noted that the extracts of ***N. sativa*** seeds have no effects against ***E. coli*** [9] which do not agree with results of this work. Other workers reported that low concentrations of N. *sativa* extracts have valuable effects on some Gram-positive and Gram-negative bacteria including *E. coli* [9,10]. Results obtained in this work are related and confirm those recorded by Morsi (2000) when he examined the antibacterial activity of ***N. sativa*** seed extract against a group of bacteria known for their multi-resistant characteristics[10]. Finally our results are in agreement with results obtained by Mashhadian and rakhshandeh[11] as well as with that obtained by Tariq et al. who studied the effects of ***N. sativa*** seed extract on different bacteria [13].

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