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Impact study of Nigella sativa Extract on Some Virulence Factor of *Staphylococcus aureus* Bacteria which Isolate from the Clinical Specimen

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ABSTRACT
In this study, the effect of Nigella sativa oil on some of the virulence factors of *staphylococcus* bacteria was considered. Study of the effect of Nigella sativa oil on two enzymes of *Staphylococcus aureus*, proteases, coagulase attended three concentrations of Nigella sativa oil 15, 30, and 45% were observed through the study. Great for reducing the effectiveness of this enzyme By comparison with control, the concentration of 45% was one of the best concentrations which gave a big difference in reducing the effectiveness of this enzyme, coagulase, for the second enzyme, significant differences were observed to decrease the effectiveness of this enzyme and the concentration was 45%. It was better to reduce the concentration of this enzyme.
Keywords: Nigella sativa extract, virulence factor
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INTRODUCTION

The Nigella sativa (scientific name: Nigella sativa Linn) belongs to the Ranunculaceae family. It is classified within the annual flowering plant, and it is known by several names, such as: black seed, and black cumin, and it originated in southeast Asia, used in ancient Egypt, Greece, the Middle East, and Africa. Ranging from 1 to 2 millimeters, these seeds are used for medicinal purposes. Mild asthma Eating a blessing pill can help relieve symptoms of asthma, and a small study published in the Fundamental Clinical Pharmacology in 2007 showed that people who took a blessing pill improved their severity of asthma symptoms like wheezing, as a pill could relieve Baraka is one of these conditions, but more research is needed to confirm this effect. Reducing blood pressure: The effect of the Nigella sativa extract was observed to lower the blood pressure of people with high blood pressure, but this decrease was very small, ranging from 1 to 3 mm Hg, and the Nigella sativa may have many effects such as antioxidants and channel blockers. Calcium, in addition to lowering blood pressure and diuresis that lowers blood pressure. Possibility to reduce cholesterol: Eating the nigella may contribute to reducing the level of cholesterol in the blood, and some research has indicated that eating one gram of whole nibbled grains.

MATERIAL AND METHOD

Collection of clinical specimens

Ninety clinical specimens were collected from different hospitals in Baghdad city, which had been from various sources including burns, wounds, infections. These samples were collected through sterile cotton swabs. While urinary tract infections (UTI) samples were collected by sterile container from Ramadi Teaching Hospital.

Isolation of Bacterial and identification

Bacterial isolates have been subjected to a number of cultural and biochemical tests. ()

Extraction of Nigella sativa oil

Nigella sativa oil was extracted by Clevenger-type apparatus as 50 g of these seeds weighed with 500 ml of distilled water and extracted for two hours after which the extract was filtered and kept in the refrigerator.()

Protease production

Protease activity was assayed by mixed 1.8 ml of casein substrate answer and 0.2 ml of supernatants from overnight cultures of bacterial growth and incubated in the water bath at 37°C for 20 min. The reaction used to be stopped by the addition of 3 ml of 5% Trichloroacetic acid (TCA) and then centrifuged at 2500 rpm for 20 min. The control was organized using the same steps except for the addition of TCA reagent before supernatants, then absorbance was once measured at 275 nm.()

$$Enzyme\ activity\ (U/ml) = \frac{Absorbance\ at\ 275\ nm}{(0.001)(0.2)(20)}$$

RESULT AND DISCUSSION

Table 1: Shows the value of nutrients in 100 grams of pond

The nutritional component	Nutritional value
Calories	400 calories

Protein	16.67 grams
Fat	33 grams
Carbs	50 grams

Table 1 shows the nutritional value of the seeds of the Nigella sativa, as 100 grams of these seeds contain 400 calories and also contain an amount of protein estimated at 16.67 grams, in addition to an amount of fat estimated at 33 grams and carbohydrates estimated at 50 grams. This nutritional value made of Nigella sativa A great antioxidant for many bacteria, as well as an antioxidant and many many functions, which made it a great nutritional value.

Table 2: Impact study of Nigella sativa extract on the activity of protease enzyme from *Staphylococcus aureus*

Con.	Protease activity U/ml				
	T1	T2	T3	T4	T5
15 % of Nigella sativa extract	77	73	72	73	77
30 % of Nigella sativa extract	66	62	61	63	63
45 % of Nigella sativa extract	44	40	44	43	43
Control	188	182	183	184	182

Table 2 shows the Impact study of Nigella sativa oil extract on the activity of protease enzyme from staph bacteria. This enzyme is considered one of the virulence factors in bacteria, which plays a fundamental role in the pathogenesis of these bacteria. Use three concentrations of Nigella sativa oil 15, 30, 45% on Respectively, all the concentration used gave significant differences to reduce the effectiveness of this enzyme compared to control. As for the ideal concentration that reduced the enzyme concentration to a very low level is the concentration of 45%, there were no significant differences for the concentrations of Nigella sativa extract together to reduce the effectiveness of the protease enzyme.

Table 3: ANOVA table Impact study of Nigella sativa extract on the activity of protease enzyme from *Staphylococcus aureus*

ANOVA
activity of protease U/ml

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	62493.750	3	20831.250	13439.516	.000
Within Groups	24.800	16	1.550		
Total	62518.550	19			

Test of Homogeneity of Variances
activity of protease U/ml

Levene Statistic	df1	df2	Sig.
.801	3	16	.511

It appears from the table of analysis of variance that there are significant differences between the transactions that represent the concentrations of the oil seed extract in reducing the effectiveness of the protease enzyme, and also appears through the homogeneity table that there is homogeneity between the variables used as the value of Sig reached 0.5 and this value is greater than the value of 0.05 and this indicates There is uniformity in the variables used.

activity of protease U/ml
Tukey HSD[a]

concetration of Nigella sativa oil extract	N	Subset for alpha = 0.05			
		1	2	3	4
45%	5	43.20			
30%	5		66.00		
15%	5			75.60	
control	5				187.80
Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 5.000.

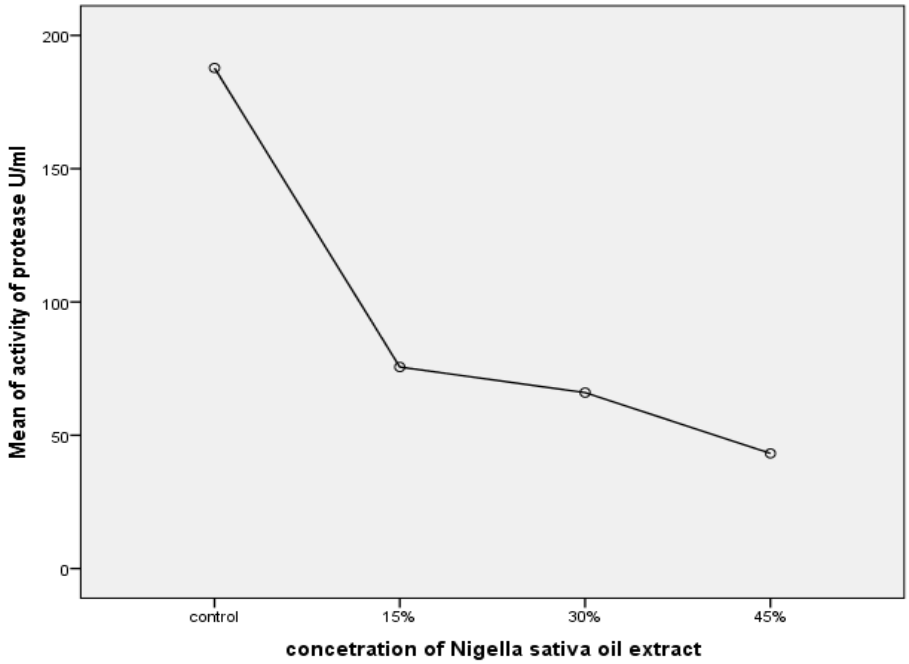

Figure 1: Impact study of Nigella sativa extract on the activity of protease enzyme from *Staphylococcus aureus*

Figure No. 1 shows the effect of Nigella sativa oil extract on reducing the activity of protease enzyme from staph bacteria. We notice through the figure that the more Nigella sativa oil extract concentration increases, the protease enzyme concentration decreases until it reaches 45%, the protease enzyme drop to a very low level.

Table 4: Impact study of Nigella sativa extract on the activity of coagulase enzyme from *Staphylococcus aureus*

Con.	Coagulase activity U/ml				
	T1	T2	T3	T4	T5
15 % of Nigella sativa extract	99	92	93	91	99
30 % of Nigella sativa extract	55	54	54	52	51
45 % of Nigella sativa extract	33	34	32	33	31
Control	199	192	190	192	191

Table (4) shows the Impact study of Nigella sativa oil extract on the activity of coagulase enzyme from staph bacteria. This enzyme is considered one of the virulence factors in bacteria, which plays a fundamental role in the pathogenesis of these bacteria. Use three concentrations of Nigella sativa oil 15, 30, 45% on Respectively, all the concentration used gave significant differences to reduce the effectiveness of this enzyme compared to control. As for the ideal concentration that reduced the enzyme concentration to a very low level is the concentration of 45%, there were no significant differences for the concentrations of Nigella sativa extract together to reduce the effectiveness of the coagulase enzyme.

Table 5: ANOVA table Impact study of Nigella sativa extract on the activity of coagulase enzyme from *Staphylococcus aureus*

ANOVA
activity of coagulase U/ml

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	79739.800	3	26579.933	6859.338	.000
Within Groups	62.000	16	3.875		
Total	79801.800	19			

Test of Homogeneity of Variances
activity of coagulase U/ml

Levene Statistic	df1	df2	Sig.

6.567	3	16	.004

It appears from the table of analysis of variance that there are significant differences between the transactions that represent the concentrations of the oil seed extract in reducing the effectiveness of the coagulase enzyme, and also appears through the homogeneity table that there is homogeneity between the variables used as the value of Sig reached 0.5 and this value is greater than the value of 0.05 and this indicates There is uniformity in the variables used.

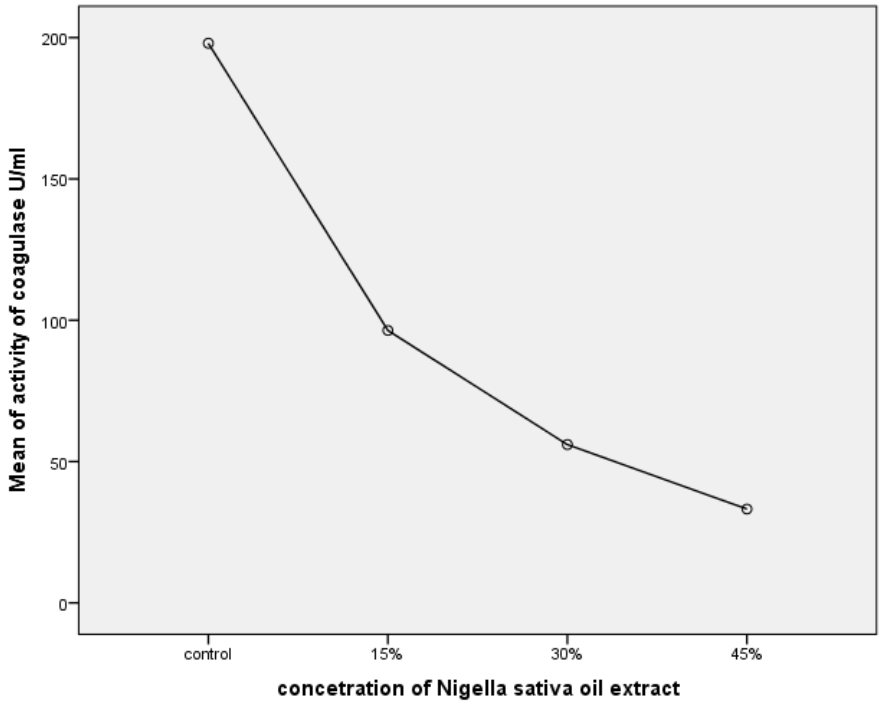

Figure 2: Impact study of Nigella sativa extract on the activity of coagulase enzyme from *Staphylococcus aureus*

Figure No. (2) shows the effect of Nigella sativa oil extract on reducing the activity of coagulase enzyme from staph bacteria. We notice through the figure that the more Nigella sativa oil extract concentration increases, the protease enzyme concentration decreases until it reaches 45%, the coagulase enzyme drop to a very low level.e

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