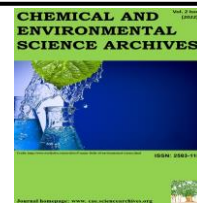




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Research Article

## Effect of aqueous and alcoholic extract of *Nigella sativa* seedson the *Staphylococcus aureus*, and *proteus mirabilis* in Shirqat Hospital wastewater, Iraq

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### Abstract

The study included the knowledge of the active chemical components present in the *Nigella sativa* seeds and the use of its water and alcohol extracts in a previously unused field, which is the treatment of hospital sewage, through the inhibition of some types of pathogenic bacteria. Where the study showed that the solutions of the aqueous and alcoholic extracts showed the presence of anthraquinone, alkaloids, amino acids, tannins, Saponins, flavonoids, protein, steroids, terpenoids, and the absence of carbohydrates and glycosides. The results showed the presence of functional groups such as amines, alkanes and acids, and the effect of aqueous and alcoholic extracts on different types of microorganisms was studied, and it was noted that the concentration (100) mg/ml of the aqueous and alcoholic extract had an effective effect towards inhibiting the microorganisms under study.

**Keywords:** Treatment, *Nigella sativa*, Aqueous and Alcoholic Extract, Wastewater

### Introduction

The benefits of medicinal plants to human and societal health cannot be overstated (Farnsworth, 1994). The treatment can be generated from the entire plant or from various parts, and it is based on compounds that cause a specific physiological impact (Motaleb, 2011). Secondary plant metabolites are the plant-based compounds that have been shown to have beneficial biological effects such as antioxidant activity, antibacterial action, enzyme modulation, and detoxification (Yeshi, 2022). amino acids, specifically amines, and amides, are the building blocks of proteins (Twaij, 2022). We make sure to highlight some of its most crucial applications in the realm of food. Because of its low toxicity, black cumin seeds are frequently employed as a flavoring component in baked goods and pickles (Amein, 2008). The nausea and vomiting can be stopped by ingesting roasted *Nigella sativa* seeds (Morsi, NM.2000 ; Yarnell, 2011).

The *Nigella sativa* plant, also known as nigella sativa seeds in Arabic and Middle Eastern nations, is a member of the Ranunculaceae family (also known as the sister plants) and is

a powerful anti-inflammatory, anti-hypertensive, and anti-oxidant (Zübeyde et al., 2018). This study was conducted to shed light on the effects of the extracts from the *Nigella sativa* plant, which had not previously been used in the treatment of biological pollutants, in light of the problems of environmental pollution represented by untreated sewage water from hospitals containing bio contaminants.

### Methodology

#### *Prepare the extracts*

The *Nigella sativa* grains (Figure 1) were prepared by drying them in the shade at room temperature (30-25 C) naturally for a week, considering the continuous stirring daily to prevent rotting.



Fig 1, *Nigella sativa* seeds

### Aqueous extract

The aqueous extract was prepared by dissolving (50) grams of *Nigella sativa* powder in 1 liter of distilled water, then leaving the mixture in the shaking incubator at a temperature of (35 °C) for 24 hours, after which the extract was filtered by filter paper, and then the filtrate was placed in a centrifuge. Duration (10) minutes at (2500) revolutions/min, then display the filtrate for evaporation in the incubator.

### Alcoholic extract

The exact steps for preparing the aqueous extract were followed, except for using ethyl alcohol (60%) instead of distilled water (Al-Sa'aidi et al., 2009).

### Estimation of pH

The aqueous and alcoholic extracts prepared in the aforementioned step (a) were taken to measure their pH (8 and 9).

### Estimating the quality and proportion of the elements present in the plant

3 gm of dry *Nigella sativa* powder was taken and placed in a glass flask, to which (8) ml of concentrated nitric acid and (2) ml of pyrochloric acid (60%) were added and left until the next day after covering it with an hour bottle, then put the mixture On a water bath at a temperature of (80°C) for approximately 6 hours until the color of the digested substance turns white, then it was supplemented with deionized water to a volume of (50) ml. absorption (György et al., 2013 ; Plummmmer, 1978).

### Chemical detection of some active ingredients in the plant

There has become an increasing awareness of linking phytochemical components and their biological activities (Laura, 2015). The following findings have been used in the chemical detection of some active chemical components and compounds (Asakawa, 2013) of *Nigella sativa*:

Molech and Benedict reagents, Iodine test for the detection of carbohydrates, glycosides, Bayuret for the detection of proteins, mercuric chloride, rapid shake for the detection of Saponins, Ninhydrin test for amino acids, aqueous ferric chloride for the detection of phenolic compounds, lead acetate with aqueous ferric chloride for the detection of tannins, alcoholic ethanol hydroxide for the detection of flavonoids, and Mayrowkner and picric acid reagents were used to detect alkaloids (Tulika, 2017). Terpenes and steroids were detected using chloroform with the addition of anhydrous acetic acid and concentrated sulfuric acid; the active substances were hexadecenoic, octadecatrienoic acid, Tetradecanoic acid, Stigma sterol by FTIR method (Ehoud,

2018). The tannins or tannins were detected by adding 0.1% ferric chloride (FeCl<sub>3</sub>), as reported in Nigam et al., 2014.

### Study of biological efficacy

The biological activity of the aqueous and alcoholic extracts was studied on the following bacterial models: *Staphylococcus aureus*, and *proteus mirabilis*.

### Results and discussion

The practical study of the qualitative chemical detections of the aqueous and alcoholic extracts of *Nigella sativa* seeds (Tables (1 and 2), respectively) proved that the plant contains effective compounds of phenolic compounds and flavonoids, and this follows what was done by some researchers (Majid, 1998).

**Table 1: Chemical analysis of the active compounds in the aqueous extract of *Nigella sativa* seeds**

Composite	Reagent used	Result
Glycosides	Iodin test	+Ve
	Mollisch test	-Ve
	Bendict test	-Ve
Proteins	Biuret reagent	+Ve
Saponins	Fast Stirring	-Ve
	Mercuric Chloride	-Ve
Phenolic compounds	Aqueous 1% Ferric chloride	+Ve
Tannins	Ferric chloride aqueous 1%	+Ve
	Lead acetate 1%	+Ve
Flavonoids	Aqueous Ferric chloride 1%	+Ve
	Ethanol hydroxide alcohol	+Ve
Alkaloids	Mayer reagent	+Ve
	Wagner reagent	+Ve
	Picric acid	+Ve

We also note that the plant gave a positive result in detecting Bayuret, indicating that the plant contains proteins and amino acids. Contains Saponins and a negative result of the presence of carbohydrates and glycosides.

The percentage of some mineral elements present in the plant's seeds was also estimated. The results shown in Table (3) indicate high calcium, sodium, and iron values. When measuring the pH of the aqueous and alcoholic extracts, the result was (pH = 8) and pH = 8.86, respectively, which means the basicity of the medium (the presence of alkaloids).

As for the antibacterial activity of the two extracts (aqueous and alcoholic), the results shown in Table (4) indicated that the concentration of 10 mg/ml and more for the alcoholic extract was inhibitory for both types of bacteria. In contrast, the aqueous extract had inhibitory activity at 50 mg/ml concentrations for each positive and harmful bacterium, respectively.

The reason for this effectiveness is that the *Nigella sativa* extract contains phenols, which have toxic properties that kill microorganisms to a specialized and robust degree. The formation of hydrogen bonds with these sites more than the base material and thus inhibits one or more of the metabolic reactions controlled by these enzymes, which may be necessary for the growth and reproduction of the organism or responsible for the construction of various cellular proteins (Muthulakshmi et al. 2012).

**Table 2. Chemical analysis of the active compounds in the Alcoholic extract of *Nigella sativa* seeds**

Composite	Reagent used	Result
Glycosides	Iodin test	+Ve
	Mollisch test	-Ve
	Bendict test	-Ve
Proteins	Biuret reagent	+Ve
Saponins	Fast Stirring	-Ve
	Mercuric Chloride	+Ve
Phenolic compounds	Aqueous 1%	+Ve
	Ferric chloride	+Ve
Tannins	Ferric chloride aqueous 1%	+Ve
	Lead acetate 1%	+Ve
Flavonoids	Aqueous Ferric chloride 1%	+Ve
	Ethanol hydroxide alcohol	+Ve
Alkaloids	Mayer reagent	+Ve
	Wagner reagent	+Ve
	Picric acid	+Ve

**Table 3. Mineral elements in the seeds of a *Nigella sativa***

Concentration in ppm	Metallic element
490	Na
110	Fe
1885	Ca

It is important to note that tannins work as an antibacterial agent by affecting microbial adhesions, enzymes, and cell envelope transport proteins through the creation of protein complexes via factors such as hydrogen bonding with the covalent bond formation and hydrophobic effects (Khalaf et al., 2007; Abdullahi, 2020).

According to Duke's Ethnobotanical Database of Plants and Chemistry, among the phytochemicals identified is hexadecanoic acid, an ester of fatty acids, and can be used as an antimicrobial (Falodun et al., 2009). octadecadienoic acid

is an unsaturated fatty acid with antimicrobial and antioxidant properties (Gross, 2009). At the same time, Stigmasterol is a steroid compound and can be used as an antimicrobial (Panda et al., 2009). Tetradecanoic acid is an alcoholic compound and may be used as an antimicrobial and antiviral agent (Saravana, 2013).

**Table 4: The inhibitory effect of aqueous and alcoholic extracts of *Nigella sativa* seeds on some bacterium types.**

Bacterial species	Alcoholic extract				Aqueous extract			
	1	10	50	100	1	10	50	100
Staphylococcus aureus gram(+ve)	-	+	++	+++	-	-	++	+++
Proteus mirabilis gram(-ve)	-	+	++	++	+	++	+++	++++

There is no damping area, + the diameter of the damping area is between 7-10 mm, ++ the diameter of the damping area is between 10-15 mm, +++ the diameter of the damping area, is between 15-20 mm, ++++ the diameter of the damping area, is 20 mm

### Conclusion

It can be concluded that *Nigella sativa* seeds contain many biologically active compounds and that isolation of individual phytochemical components and exposing them to biological activity will undoubtedly give fruitful results

### Conflict of Interest

The author hereby declares no conflict of interest.

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