# DETECTION OF THE ANTIBACTERIAL EFFECT OF NIGELLA SATIVA GROUND SEEDS WITH WATER

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## Abstract

*Nigella Sativa* (NS) seeds have been used for medicinal purposes for centuries both as herbs and its oil. In Islam it is regarded as one of the greatest forms of healing medicine included in the medicine of prophet Mohammed. Huge number of studies have been carried out in recent years on the pharmacological effects of these seeds and also the possible relationship with their constituents. A number of these investigations emphasized the antimicrobial effect of them by using different extracts. In our study we have tried to use the normal human mechanism in digestion by using the ground seeds. A modified paper disc diffusion method was used to test the antibacterial effect of NS seeds. Clear inhibition of the growth of *Staphylococcus aureus* was observed by concentration of 300mg/ml with distilled water (D.W.) as control , this inhibition was confirmed by using the positive control Azithromycin. The inhibition obtained was higher with *Nigella sativa* ground seeds from Hadramout (HNSGS) than with *Nigella sativa* ground seeds from Ethiopia (ENSGS). No inhibition was found in the growth of *E.Coli* and *Enterobacter*. This was emphasized by using the positive control Ciprofloxacin. The positive inhibition may be attributed to the two important active ingredients of NS, Thymoquinone and melanin .

**Key words:** *Nigella Sativa* , antibacterial effect , *Staphylococcus aureus*, paper disc-diffusion method.

## Introduction

The seeds of *Nigella Sativa* Linn. (Ranunculaceae ), commonly known as black seed or black cumin has been used for medicinal purposes for centuries both as herb and pressed into oil in Asia, Middle East, and Africa (Batheeb; Zohary and Hopf, 2000). It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support and general well-being (Khan et al., 2003). In Islam, it is regarded as one of the greatest forms of healing medicine available and included in the medicine of the Prophet Mohammed (Aljawezjjah, 2001). Its oil has been used in treatment of dermatological diseases e.g. eczema and boils (Zohary and Hopf, 2000). These many therapeutic uses earned *Nigella Sativa* the Arabic approbation (habbatul barakah the seed of blessing ).

Huge number of studies and researches have been carried out in recent years on the pharmacological effects of these seeds covered both fields of experimental and clinical pharmacology; not only in the use of these plants but also in the investigation of the constituents and active ingredients that may explain or interpret these pharmacological actions. One of these active ingredients is Thymoquinone (volatile oil of these seeds) and Melanine (fixed oil) (Roy et al. 2006; Adel, 2006). Some investigations performed to show the possibility of antimicrobial and antibacterial activities of these seeds using their extracts or oil (Ali and Blunden, 2003; El.Fatatry, 1975; Hanafy and Hatem, 1991; Morsi, 2000; Roy et al., 2006). Modern studies have been done to investigate immuno-modulatory, immunosuppressive and anticancer properties of these black seeds (Islam et al., 2004; Mbarek et al., 2007; Mohammed, 2005; Adel, 2006).

The antioxidant action of thymoquinone and its 5-lipoxygenase inhibition may explain the different anti-inflammatory effect of these seeds. Interestingly, it was found that the fixed oil of *N.Sativa* had both antioxidant and anti-eicosanoid effect greater than thymoquinone which is its active constituent (Ali and Blunden, 2003).

The aim of our study is to find out a method for testing the antibacterial effect of these seeds by using the ground seeds the way it used in the local human traditional diet.

## Materials and methods

### Method

A modified disc diffusion method was used for determination of the antibacterial activities of the *Nigella Sativa* ground seeds with water(NSGSW ), and the following steps were carried out:

1. The equipment used were cleaned and sterilized by immersing them in ethanol for 10 min then washed with distill water(D.W.) and dried adequately by using sterile bandage.
2. *Nigella sativa* HNS and ENS were ground by the grinding machine and then put in two separate bottles, one for HNS and the other for ENS.
3. 0.75gm of both type of *Nigella sativa* from the two separated bottles was measured by using sensitive balance, and transferred with aid of spatula into separate test tubes covered with foil. 2.5ml D.W. was added to each test tube in microbiology laboratory..
4. 2 agar plates were taken and divided to equal 4 regions or 3 regions as required.
5. The nutrient broth was inoculated with a loopful of the selected bacteria and the plain agar was also inoculated with the broth containing this bacteria.
6. The test tubes were shaken vigorously to form homogenous mixture and in first plate ENSGSW in region (1) 5 µl of mixture was added, in region (2) 10 µl mixture and in region (3)20 µl of mixture was added, in region (4) 10ml of D.W used as a negative control and in the second plate, by using 20 micropipette, 20 µl of HNSGSW was placed to region (1) and 20µl of ENSGSW to region (2) in region (3) positive control was added (Antimicrobial standard Azithromycin and Ciprofloxacin).
7. Finally, the tested plates were placed in the incubator at 37ºC for 24 hrs, and for the assessment of the results, pictures of the Petri plates were taken at the end of each experiment and the inhibition zones around each paper disc (5mm diameter, filter paper) were measured and recorded.

## Results and discussion

The identification of the antibacterial effect was achieved by taking different concentration of N.S. from the two preparations (HNSGSW and ENSGSW ) and applying it directly or by direct mixing with D.W. The concentration which produced best effect was 300mg/ml (Figures 1-3). Figure 3 showed the comparison of the inhibition caused by the different concentrations of both preparations. The largest inhibition was observed by the HNSGSW that reached 24 mm. No inhibition was observed by the negative control (D.W) (Figures 1 and 2). These positive effects of both preparations were emphasized by using the positive control antibiotic Azithromycin (Figure 4 and Table 3). These positive effects are in agreement with previous findings by other authors (El.Fatatry, 1975; Hanafy andHatem, 1991; Mashhadan and Rakhshandeh, 2005; Morsi, 2000; Roy et al., 2006; Adel, 2006).

Mashhadan found that the aqueous extract did not show any effect but other extracts (methanol, chloroform) showed high inhibitory effect against all the tested microorganisms including *Staphylococcus*, where concentration used ranged from 62.5mg/ml to 1g/ml (Mashhadan and Rakhshandeh,2005). Morsi had proven that both the crude alkaloid extract and the water extract of the seeds were effective against some tested microorganisms like *staphylococcus* despite their resistance to other antibiotics (Ali and Blunden, 2003; Morsi, 2000). Probably these variations could be explained by the different extraction methods, and also the amount of ingredients of the same plant can be affected by the area and season of collection. A combination of garlic with NS was tested individually and in combination for their antimicrobial activities against *Staphylococcus* where both showed modest antimicrobial effects (Roy et al., 2006). NS of both origins caused no inhibitory effect on the growth of *E.Coli* using D.W as control (Figures 5 and 6). The extract had no effect also on the growth of *Enterobacter* (Figure 7 and Table 4 ). Zuridah H. and et al found also negative effect of N.S. on the growth of *E. coli* by using methanolic extract and concentration 25mg/ml (Zuridah et al., 2008). Although the mechanism of the antimicrobial effect of these seeds has not been reported, its antimicrobial action could be attributed to the active ingredients especially thymoquinon and melanin (Roy et al., 2006; Adel, 2006).

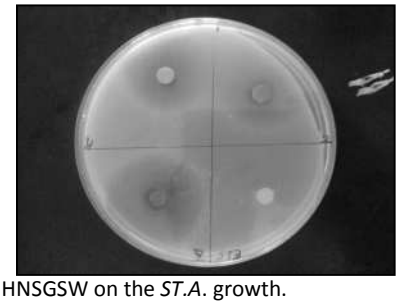

**Figure 1 :** inhibition effect of HNSGSW on the *ST.A*. growth.

**Table 1 :** The different volume of HNSGSW and the inhibition zone diameter

| Name | Volume in micro-liter | Inhibition zone diameter |
|---|---|---|
| HNSGSW1 | 5 | 10 mm |
| HNSGSW2 | 10 | 20 mm |
| HNSGSW3 | 20 | 24 mm |
| D.W. | 10 | 0 mm |

HNSGSW: Nigella sativa ground seeds in water (1,2,3: different volume).

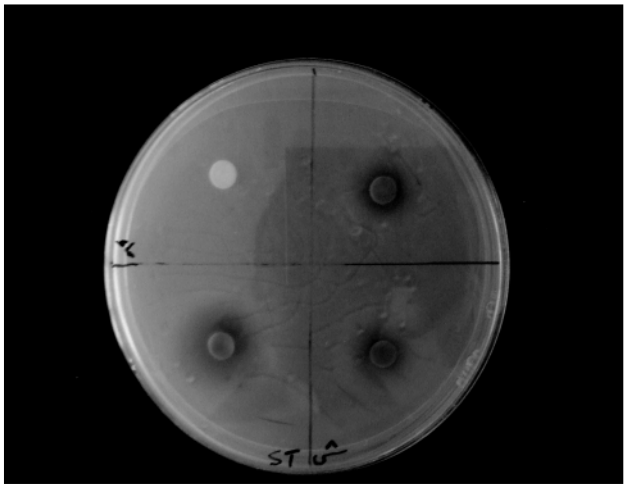

**Figure2** : picture showing inhibition effect of ENSGSW on the *Staphylococcus aureus*. growth.

**Table 2 :** The different volume of ENSGSW and the inhibition zone diameter

| Name | Volume in micro-liter | Inhibition zone diameter |
|---|---|---|
| ENSGSW1 | 5 | 10 mm |
| ENSGSW2 | 10 | 12 mm |
| ENSGSW3 | 20 | 20 mm |
| D.W. | 10 | 0 mm |

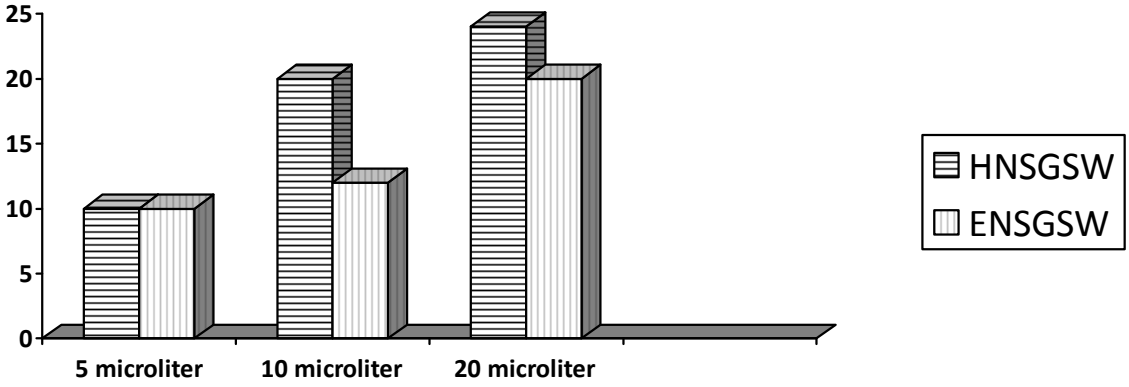


**Figure 3**: Comparison of the inhibition caused by the two preparations of N.S. in different volume concentration.

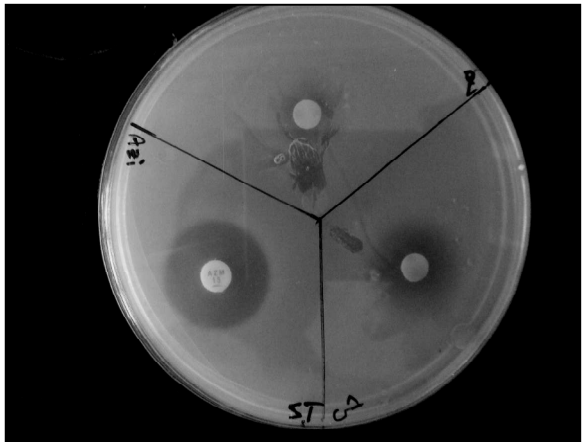

**Figure 4 :** inhibition effect of HNSGSW and ENSGSW on the ST.A. growth

with Azithromycin as positive control

**Table 3 :** The inhibition effect of HNSGSW and ENSGSW on the ST.A. growth with Azithromycin as positive control.

| Name | Volume in micro-liter | Inhibition zone diameter |
|---|---|---|
| **HNSGSW** | **20** | **15 mm** |
| **ENSGSW** | **20** | **20 mm** |
| **Azithromycin** | **15 micro gram** | **22 mm** |

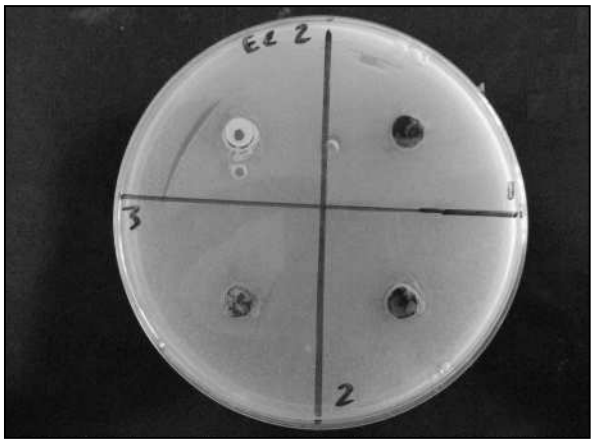


**Figure 5 :**Picture showing no inhibition effect of HNSGSW on the *E. coli* growth.

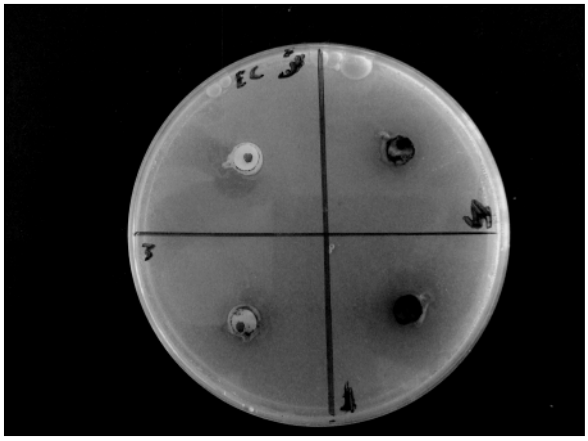


**Figure 6 :**Picture showing no inhibition effect of ENSGSW on the *E.Coli* growth.

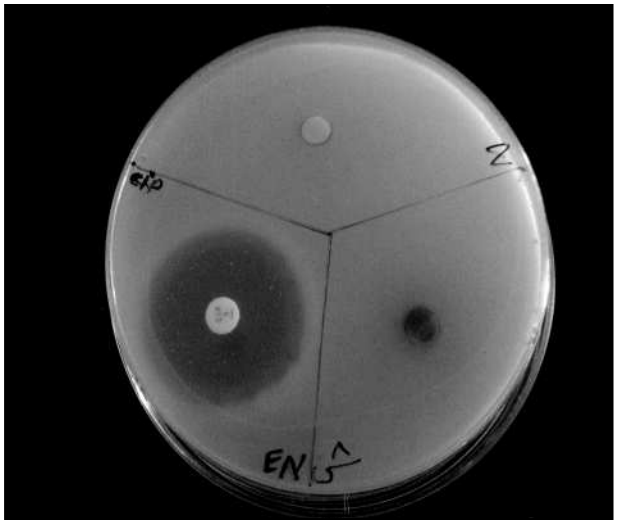


**Figure 7 :** Picture showing no inhibition caused by HNSGSW and ENSGSW on the *Enterobacter* spp. growth with Ciprofloxacin as positive control

**Table 4 :** Comparison between HNSGSW , ENSGSW and ciprofloxacin as positive control on the growth of *Enterobacter* Spp.

| Name | Volume in micro-liter | Inhibition zone diameter |
|---|---|---|
| ENSGSW | 20 | No inhibition |
| HNSGSW | 20 | No inhibition |
| Ciprofloxacin | 5 micro gram | 33 mm |

**Conclusion**

According to the method used, a clear and undeniable antibacterial effect caused by the N.S. ground seeds on the growth of *Staphylococcus* was obtained. The inhibition of the *Staphylococcus* growth is higher with NSGSW from Hadhramout than with NSGSW from Ethiopia.

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