

# Antibacterial Activity of *Nigella Sativa* Linn. Seeds Against Multiple Antibiotics Resistant Clinical Strains of *Staphylococcus aureus*

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## ABSTRACT

**Objective:** *Staphylococcus aureus* is one of the major resistant pathogens extremely adaptable to antibiotic pressure. *Nigella sativa* (black cumin) seed extracts and essential oil have been shown to possess antimicrobial activity against several bacteria but little work has been done on their effect against multidrug resistant *S. aureus* strains isolated from patients. So, we studied antibacterial activity of *Nigella sativa* against multidrug resistant clinical strains of *Staphylococcus aureus*. It was an experimental, *in vitro* study.

**Materials and methods:** *Nigella sativa* (black cumin) seed essential oil and extracts were tested in varying dilutions against 40 clinical strains of *Staphylococcus aureus* which were isolated from patients attending a tertiary care teaching hospital in North India using disc agar diffusion technique on inoculated Mueller Hinton agar plates under standard laboratory conditions. The tested strains were resistant to 4 or more clinically used antibiotics belonging to at least 3 different classes.

**Results:** The Methanolic extract and oil of *Nigella sativa* were found active against 38 and 35 multi-drug resistant strains respectively. Both the oil and Methanolic extract showed remarkable dose dependant antibacterial activity against the tested strains up to a dilution of 1:50 as evident from the zones of inhibition.

**Conclusion:** *Nigella sativa* possesses antibacterial activity against multidrug resistant clinical strains of *Staphylococcus aureus*

**Key words:** *Nigella sativa*, Black seed, Black cumin, *Staphylococcus aureus*, Antimicrobial activity, Antibiotic resistance.

## INTRODUCTION

*Staphylococcus aureus* is one of the major resistant pathogens and a common organism isolated from clinical specimens in tertiary care hospitals.<sup>[1]</sup> Found on the mucous membranes and the skin of around a third of the population, it is extremely adaptable to antibiotic pressure. This resistance problem demands that a renewed effort be made

to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals.

*Nigella sativa* L. (Ranunculaceae) is an herbaceous plant whose seeds and essential oil derived from them have been used for centuries for treatment of various ailments, including infectious diseases. It has been recommended for use on a regular basis in Tibbe Nabwi (Prophetic Medicine) as is evident from the following tradition: Narrated Abu Hurairah, "I heard Allah's Apostle saying, 'There is healing in black cumin for all diseases except death<sup>[2]</sup>.'" In the Unani system of medicine, black cumin has been regarded as a valuable remedy in a number of diseases. Ibn Sina (Avicenna, 980-1037 A.D.), most famous for his volumes called 'The Canon of medicine' regarded as one of the most famous book in the history of medicine, refers to black cumin as the seed that stimulates the body's energy and helps recovery from fatigue and dispiritedness and several therapeutic effects on digestive disorders, gynaecological diseases and respiratory system have been ascribed to it.<sup>[3]</sup>

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The seeds have been thoroughly studied scientifically in the last 3-4 decades and reported to possess a number of medicinal properties like immunomodulatory<sup>[4]</sup> and antioxidant<sup>[5]</sup> activities. Their crude extracts<sup>[6]</sup> and essential oil<sup>[7]</sup> have been shown to possess activity against several bacteria. However, little work has been done on their effect against multidrug resistant *S. aureus* strains isolated from patients. Hence this study was undertaken.

## MATERIALS AND METHODS

**Acquisition of seeds and oil of *Nigella sativa*:** Seeds of *N. sativa* (locally known as Kalonji) were procured from a local dealer at Aligarh and authenticated by a botanist at Department of Botany, Aligarh Muslim University, Aligarh. *N. sativa* essential oil (Kalonji oil) was procured from Mohammedia products, Red Hills, Nampally, Hyderabad, Andhra Pradesh, India. As per manufacturer's information, it was prepared by steam distillation at Hyderabad, India.

### Preparation of extracts

- (a) **By Soxhlet extraction:** Methanolic extract was prepared using HPLC grade methanol. Seeds were crushed and extracted with methanol in Soxhlet apparatus by heating at its boiling point (50 °C) till it cleared.
- (b) **By maceration:** Methanolic extract was prepared using HPLC grade methanol as described previously.<sup>[8]</sup> Briefly, 150 grams of ground seeds were soaked in 150 ml of methanol for 7 days at room temperature, followed by filtration and removal of solvent under aseptic conditions. The extract thus prepared was transferred as aliquots of 1 ml each into sterile vials and stored at -20 °C till further use.

### Preparation of drug impregnated filter paper discs:

This was done by the method of Morley, 1945<sup>[9]</sup> with slight modification.

Methanolic extracts and oil were tested as previously described.<sup>[10]</sup> Briefly, the extracts and oil were diluted using methanol and Ethylene glycol respectively upto a dilution of 1:100. During sensitivity testing, 4 µl of extract or oil in pure or diluted form was impregnated on filter paper disc of 6 mm diameter, placed on Mueller Hinton Agar plate previously inoculated with bacteria.

**Inoculation of plates:** This was done using flood-inoculation technique.<sup>[11]</sup> Bacterial suspension in Nutrient Broth having turbidity equivalent to 0.5 McFarland was freshly prepared and 2 ml of this was transferred onto the Mueller Hinton Agar plate and distributed gently over the surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate was kept in incubator at 37 °C for 30 minutes for drying before application of discs.

**Disc susceptibility testing:** This was carried out by placing discs impregnated with test material on surface of inoculated agar plates.<sup>[12]</sup> For sensitivity testing with standard antibiotics, commercial antimicrobial susceptibility testing discs obtained from HiMedia Laboratories Limited, Bombay were used. The plates were

then kept in incubator at 37 °C for 18 hours and diameters of zones of inhibition were measured.

*N. sativa* oil and methanolic extracts were tested in different dilutions against Oxford *S. aureus* (NCTC 6571) and *S. aureus* (ATCC 25923). All the experiments were repeated in triplicate. Ampicillin (10 µg/disc) and Amoxicillin (10 µg/disc) obtained from HiMedia Laboratories Limited, Bhaveshwar Plaza, LBS Marg, Mumbai, India were kept as standard. Discs soaked in respective diluents were also kept as negative control.

Since Methanolic extract prepared by maceration showed more pronounced activity as compared to that derived by Soxhlet, it was used for further studies on clinical isolates. *N. sativa* oil also showed pronounced antibacterial activity against standard strains and was studied further on clinical isolates.

Strains of *S. aureus* were isolated from pus, blood, cervical and conjunctival swabs, ear discharge, semen and CSF of various patients attending Jawaharlal Nehru Medical College Hospital, Aligarh and tested for their sensitivity to a number of clinically used antibiotics. The concentrations of antimicrobial sensitivity testing discs used and interpretation of sizes of zones of inhibition were in accordance to Performance Standards for Antimicrobial Susceptibility Tests, CLSI.<sup>[13]</sup> The antibiotics tested and their concentrations used were: Ampicillin (10 µg/disc), Amikacin (30 µg/disc), Cotrimoxazole (trimethoprim-1.25, sulphamethoxazole-23.75 µg/disc), Cefaclor (30 µg/disc), Ciprofloxacin (5 µg/disc), Ceftriaxone (30 µg/disc), Cefotaxime (30 µg/disc), Cefotaxime (30 µg/disc), Cefotaxime (30 µg/disc), Erythromycin (15 µg/disc), Gentamicin (10 µg/disc), Gatifloxacin (5 µg/disc), Ofloxacin (5 µg/disc), Roxithromycin (15 µg/disc), Sparfloxacin (5 µg/disc), Tetracycline (30 µg/disc) and Tobramycin (10 µg/disc). Out of these, 40 strains which were resistant to 4 or more antibiotics belonging to at least 3 different classes were tested for their sensitivity to Methanolic extract and oil of *N. sativa* in various dilutions.

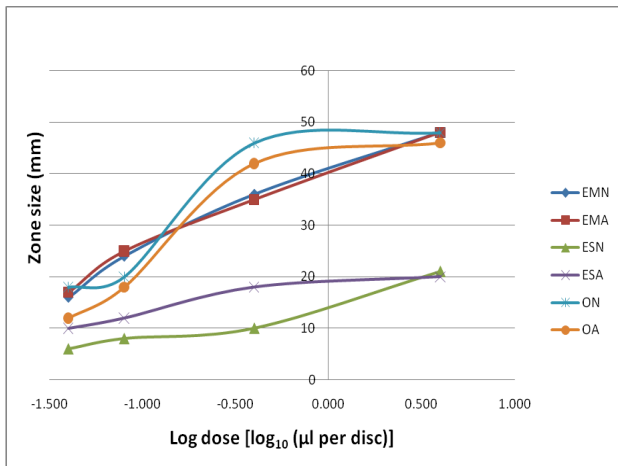
### Statistical Analysis:

Clinical strains which were sensitive to oil or extract and those which were resistant were compared statistically for their sensitivity to various antibiotics using Fisher's Exact Test. Clinical strains sensitive or resistant to oil/extract were also compared for number of antibiotics to which each of the strains was resistant and for number of groups of antibiotics to which each of the strains showed resistance using Mann Whitney U test. P value <0.05 was considered as significant. All analyses were done using SPSS 16.0 software.

## RESULTS

### The Effect of *Nigella sativa* against standard strains.

*N. sativa* oil as well as Methanolic extracts showed remarkable dose dependant antimicrobial activity against the standard strains (Fig. 1). The methanolic extract obtained by maceration showed highest antibacterial activity. The antibacterial activity was observed up to a dilution of 1:100, the least concentration tested.

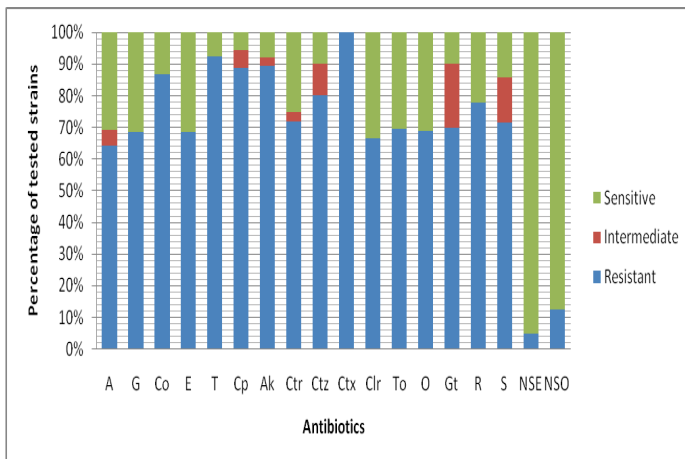


**Figure 1: Dose response curves showing effect of Methanolic extracts and oil of *Nigella sativa* against standard strains of *Staphylococcus aureus*.**

EMN- Extract (maceration) against Oxford *S. aureus* (NCTC 6571), EMA- Extract (maceration) against *S. aureus* (ATCC 25923), ESN- Extract (soxhlation) against Oxford *S. aureus* (NCTC 6571), ESA- Extract (soxhlation) against *S. aureus* (ATCC 25923), ON- Oil against Oxford *S. aureus* (NCTC 6571), OA- Oil against *S. aureus* (ATCC 25923),

#### Effect of *Nigella sativa* against Clinical isolates

Out of 40 strains tested, 16 were resistant to 4-6 antibiotics, 16 to 7-9 antibiotics, 3 to 10-12 antibiotics and 5 to 13-16 antibiotics. Resistance was highest to Aminoglycosides, Tetracycline, Cotrimoxazole, Ciprofloxacin, Ampicillin, 3rd generation Cephalosporins and Erythromycin (Fig 2).

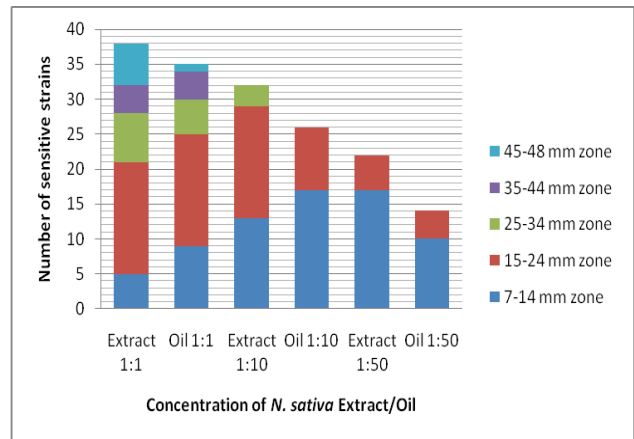


**Figure 2: Sensitivity pattern of Clinical isolates of *Staphylococcus aureus*.**

A- Ampicillin, Ak- Amikacin, Co- Cotrimoxazole, Clr- Cefaclor, Cp- Ciprofloxacin, Ctr- Ceftriaxone, Ctz- Cefotaxime, Ctz- Ceftazidime, E- Erythromycin, G- Gentamicin, Gt- Gatifloxacin, NSE- *N. sativa* extract, NSO- *N. sativa* oil, O- Ofloxacin, R- Roxithromycin, S- Sparfloxacin, T- Tetracycline, To- Tobramycin

The oil was active against 35 strains, up to a dilution of 1:50 against 14, up to 1:10 against 11 and only in undiluted state against 10 strains. The Methanolic extract was active against 38 strains, up to a dilution of 1:50 against 22 strains, up to 1:10 against 10 and only in undiluted state against 6 strains (Fig 3).

It showed zones of inhibition larger than oil at all concentrations. No significant correlation was found between the resistance to *N. sativa* oil/extract and resistance to any other tested antibiotics, number of antibiotics to which the isolate was resistant or the number of classes of antibiotics to which the isolate showed resistance.



**Figure 3: Effect of *Nigella sativa* Methanolic Extract and Oil on Clinical isolates of *Staphylococcus aureus*.**

## DISCUSSION

In *Nigella sativa* oil and Methanolic extracts derived by Soxhlet as well as by maceration, when tested in varying concentrations (1:1 to 1:100) showed dose dependent antibacterial activity against 2 standard strains of *S. aureus*. Out of 40 clinical strains of *S. aureus* resistant to four or more antibiotics belonging to at least 3 different classes, the extract was active against 38 strains and the oil against 35. This confirms earlier studies which reported antibacterial activity of alcoholic extract of seeds [6,14], volatile oil (obtained by steam distillation of fixed oil), [14] essential oil, [15] methanolic extract of *N. sativa* cell culture, [16] methanolic extract of germinating seeds [17] and essential oil (obtained from crude extract). [18] In a study by Sokmen et al, (1999) [19] methanolic extract derived by soxhlet extraction was not active against one strain of *S. aureus* derived from a clinical sample. This may be due to difference of strains tested.

An alarming increase in bacterial strains resistant to a number of antimicrobial agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit growth of pathogenic bacteria. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. [20] This hypothesis is further supported by our study in which no significant correlation was found between the resistance to *N. sativa* oil/extract and resistance to any other tested antibiotics showing absence of cross-resistance with the tested antibiotics.

Thymol is a phenolic alcohol present in the essential oil [3] that has been reported to possess antibacterial activity. [21] Since Thymol is present in the methanol soluble portion of oil [22], it will also be extracted in the Methanolic extract. Thymoquinone present in volatile oil obtained from the crude extract was shown to exhibit remarkable inhibition of the growth of various strains of bacteria. [18] Thymoquinone is present in the

methanol soluble portion of *N. sativa* oil<sup>[23]</sup> and thus will be extracted in Methanolic extract of seeds also. High antimicrobial activity in Thymoquinone and longifoline and weak antimicrobial activity in p-cymene present in *N. sativa* oil has also been reported.<sup>[24]</sup> P-cymene has been demonstrated to work synergistically with carvacrol, also present in *N. sativa*, and a mixture exhibited greater antibacterial activity than the terpenoids on their own.<sup>[25]</sup> The seeds also contain tannins, which can be extracted by methanol,<sup>[26]</sup> and number of studies have reported antimicrobial properties of tannins.<sup>[27]</sup> Antibacterial properties of *N. sativa* essential oil could be due to the ability of the oil to permeabilize membranes and to destroy cellular integrity of bacteria leading to cell death by necrosis and apoptosis.<sup>[28]</sup>

It may be concluded that *Nigella sativa* oil as well as Methanolic extract are active against multidrug resistant strains of *Staphylococcus aureus*, with absence of cross-resistance with commonly prescribed antibiotics, and may be used at least topically in susceptible cases. Further research is needed to advocate its use in systemic infections.

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