Research Paper

# Antimicrobial activity of *Nigella sativa* Linn. seed oil against multi-drug resistant bacteria from clinical isolates

**M T Salman[1]*, R A Khan[1] and I Shukla[2]**

Departments of Pharmacology[1] and Microbiology[2]

Jawaharlal Nehru Medical College

Aligarh Muslim University, Aligarh- 202 002, Uttar Pradesh, India

*Correspondent author, E-mail: mtariqsalman@gmail.com; Mob. 091-9358208397

*Received 9 January 2007; Accepted 28 July 2007*

## Abstract

An alarming increase in bacterial strains resistant to existing antimicrobial agents demands a renewed effort to seek agents effective against pathogenic bacteria resistant to current antimicrobials. ***Nigella sativa* Linn.** (Black cumin) essential oil was studied for antibacterial activity against various clinical isolates of bacteria resistant to a number of antibiotics, in varying concentrations by Disc Agar diffusion technique using impregnated filter paper discs on inoculated Muellar Hinton agar plates. The oil showed pronounced dose dependant antibacterial activity which was more against Gram positive than Gram negative bacteria. Among Gram positive bacteria tested, *Staphylococcus aureus, S. epidermidis*, other coagulase –ve Staphylococci and *Streptococcus pyogenes* were sensitive to the oil and *Enterococcus faecalis, Streptococcus agalactiae* were resistant. Among Gram –ve bacteria tested, only *Pseudomonas aeruginosa* was sensitive to oil and rest (*Acinetobacter baumannii, Citrobacter freundii, Klebsiella pneumoniae, Proteus mirabilis, P. vulgaris* and *Vibrio cholerae*) were insensitive. Out of 144 strains tested, most of which were resistant to a number of antibiotics, 97 were inhibited by the oil of black cumin. To the best of our knowledge, the activity of essential oil against coagulase negative Staphylococci (except *S. epidermidis*) and *Streptococcus pyogenes* is being reported for the first time.

**Keywords:** *Nigella sativa*, Black cumin, Antimicrobial activity, Essential oil, Antibiotic resistance, Clinical isolates.

**IPC code; Int. cl.[8]—** A61K 36/00, A61P 31/04

## Introduction

Even after introduction of new antimicrobial agents for clinical use an alarming increase in bacterial resistance to existing agents demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antimicrobials. ***Nigella sativa* Linn.** (Black cumin) is a herbaceous plant, used for centuries for the treatment of various ailments, including infectious diseases. The seeds, commonly used in recipes in Asian countries are reported to possess several medicinal properties[1-2]. Crude extracts and essential oil possess antibacterial activity against several bacteria[3-5]. However, the effect of oil against multidrug resistant pathogenic bacteria isolated from patients had not been studied. Hence, the objective of present study was to evaluate antibacterial activity of black cumin oil against clinical samples isolated from pus, blood, conjunctival swab, cervical swab, urine, ear discharge, CSF, etc. of various patients.

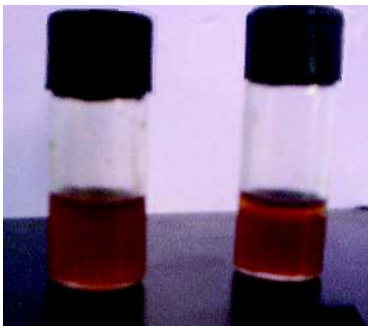
## Materials and Methods

***N. sativa*** (Hindi–*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.

### Inoculation of plates

This was done by the modified method of Acar and Goldstein using flood-inoculation technique[6]. Bacterial suspension having turbidity equivalent to 0.5 McFarland was freshly prepared and 2 ml of this was transferred onto the Muellar Hinton Agar plate and distributed

*Nigella sativa* seeds



*Nigella sativa* seed oil

gently over surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate was kept in incubator at 37ºC for 30 minutes for drying before application of discs.

**Disc susceptibility testing**

This was carried out using the modified method of Bauer *et al*[7]. The oil was diluted using ethylene glycol up to dilution of 1:200. During sensitivity testing, 4µl of oil in pure or diluted form was kept on filter paper disc of 6 mm diameter, placed on Muellar Hinton Agar plate inoculated previously with bacteria. For sensitivity testing with standard antibiotics, commercial antimicrobial susceptibility testing discs obtained from HiMedia Laboratories Limited, Mumbai were used. The plates were then kept in incubator at 37ºC for 18 hours and diameters of zones of inhibition were measured (Fig. 1).

The oil was tested in different dilutions against the following standard bacteria-Oxford: *Staphylococcus aureus* (NCTC 6571), *S. aureus* (ATCC 25923), *Escherichia coli* (NCTC 10418), *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *P. aeruginosa* (NCTC 10662). All the experiments were repeated in triplicate. Ampicillin disc (10 µg/disc), was kept as standard. A disc soaked in ethylene glycol was also kept as negative control.

Fig. 1 : Sensitivity testing showing zones of inhibition around oil impregnated discs kept on Muellar Hinton agar plate inoculated with bacteria

Strains of different bacteria were isolated from pus, blood, conjunctival swab, cervical swab, urine, ear discharge, CSF, etc. (Table 1) of various patients attending Jawaharlal Nehru Medical College Hospital, Aligarh, identified by standard microbiological techniques and tested for their sensitivity to oil as well as a number of other 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 Disk Susceptibility Tests, NCCLS, 2002[8]. The antibiotics tested and their concentrations used were: Ampicillin (10µg/disc), Amikacin (30 µg/disc), Amoxicillin (20µg/disc), Cotrimoxazole (trimethoprim-1.25, sulphamethoxazole-23.75 µg/disc), Cefaclor (30 µg/disc),

**Table1: Clinical isolates tested and their sources**

| S. No. | Name of bacteria (Bacterial isolates) | Source and number of strains tested | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Blood | Cervical swab | Conjunctival swab | CSF | Ear | Pus | Urine | Others# | Total |
| 1 | *Staphylococcus aureus* | 8 | 8 | 6 | 1 | 2 | 28 | - | 1[a] | 54 |
| 2 | *Staphylococcus epidermidis* | 9 | - | - | - | - | 4 | - | - | 13 |
| 3 | Other coagulase negative Staphylococci | - | - | 2 | - | - | 13 | 3 | - | 18 |
| 4 | *Enterococcus faecalis* | - | - | - | - | - | 1 | 2 | - | 3 |
| 5 | *Streptococcus agalactiae* | - | - | - | - | - | 3 | - | - | 3 |
| 6 | *Streptococcus pyogenes* | - | - | - | - | - | - | - | 1[b] | 1 |
| 7 | *Acinetobacter baumannii* | - | - | - | 1 | - | 3 | - | - | 4 |
| 8 | *Citrobacter freundii* | - | - | - | - | 1 | 2 | - | - | 3 |
| 9 | *Klebsiella pneumoniae* | 3 | - | - | - | 2 | 4 | - | 1[c] | 10 |
| 10 | *Proteus mirabilis* | - | - | - | - | - | 3 | - | - | 3 |
| 11 | *Proteus vulgaris* | - | - | - | - | - | 2 | - | - | 2 |
| 12 | *Pseudomonas aeruginosa* | 4 | 1 | - | - | 1 | 16 | - | - | 22 |
| 13 | *Vibrio cholerae* | - | - | - | - | - | - | - | 2[d] | 2 |

# Other sources: semen[a], sputum[b], skin[c] and stool[d].

Cephalexin (30 µg/disc), Chloramphenicol (30 µg/disc), Ciprofloxacin (5 µg/disc), Cephoperazone (75 µg/disc), Ceftriaxone (30 µg/disc), Cephotaxime (30 µg/disc), Ceftazidime (30 µg/disc), Erythromycin (15 µg/disc), Gentamicin (10 µg/disc), Gatifloxacin (5 µg/disc), Imipenem (10 µg/disc), Methicillin (5 µg/disc), Nalidixic acid (30 µg/disc), Ofloxacin (5 µg/disc), Roxithromicin (5 µg/disc), Sparfloxacin (5 µg/disc), Tetracycline (30 µg/disc) and Tobramycin (10 µg/disc).

### Statistical Analysis

Kruskal Wallis test was performed to test for difference in sizes of inhibitory zones formed by oil against different bacteria. Mann Whitney U test was performed to compare antimicrobial effects of Ampicillin and oil. Bivariate correlation analysis using Pearson's test was done to find relationship between dose of oil and inhibition zones. All tests were done using SPSS software 13.0 version.

## Results

### Effect on standard strains

The oil showed pronounced dose dependant activity against *S. aureus* (Oxford NCTC 6571, ATCC 25923) followed by *P. aeruginosa* (NCTC 10662, ATCC 27853) and no activity against *E. coli* (NCTC 10418, ATCC 25922). Oxford *S. aureus* (NCTC 6571) was the most sensitive, showing inhibition up to 1:200 dilution, whereas *P. aeruginosa* (ATCC 27853) was least sensitive, as is evident from Table 2.

### Effect on clinical isolates

*Staphylococcus epidermidis*: Thirteen strains were tested which showed variable resistance pattern to the tested antibacterials. Four were resistant to 0-1 antibiotics, 8 to 5-7 antibiotics and 1 was resistant to 10 antibiotics. Resistance was highest to second generation Cephalosporins and Amino glycosides. Five of these were Methicillin resistant (Table 3). Twelve out of 13 strains were inhibited by oil which showed activity upto a dilution of 1:100, 1:50, 1:10 and 1:1 against 1, 5, 2 and 2 strains, respectively in a dose dependant manner. One strain, which was resistant to 10 antibiotics, was also found resistant to oil (Table 4).

*Other coagulase negative Staphylococci*: Out of 18 strains tested, 4 were resistant to 1-3 antibiotics, 8 to 4-6 antibiotics, 3 to 8 antibiotics and 1 each to 13 and 15 antibiotics. Resistance was highest for Cotrimoxazole followed by Tetracycline, Amikacin, Ampicillin, Ciprofloxacin and Tobramycin (Table 3). The oil inhibited 17 of the above strains dose dependently. It was active against 11 strains upto a dilution of 1:50, against 2 strains upto 1:10 dilution and only in undiluted state against 4 strains (Table 4).

*Streptococcus pyogenes*: One strain, which was resistant to Erythromycin and

**Table 2 : Effect of *Nigella sativa* oil against sensitive strains of bacteria**

| Name of sensitive strains | Conc./disc (4 µl/disc) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1:1 | 1:10 | 1:50 | 1:100 | 1:200 | Ethylene glycol | Ampicillin (10 µg/disc) | Pearson's correlation** |
| Oxford *S. aureus* (NCTC 6571) | 48* | 46* | 20 | 18 | 09 | Nil | 27 | 0.935 |
| *S. aureus* (ATCC 25923) | 46* | 24 | 18 | 12 | Nil | Nil | 27 | 0.984 |
| *E. coli* (NCTC 10418) | Nil | Nil | Nil | Nil | Nil | Nil | 17 | |
| *E. coli* (ATCC 25922) | Nil | Nil | Nil | Nil | Nil | Nil | 16 | |
| *P. aeruginosa* (NCTC 10662) | 25* | 20* | 19* | 12 | Nil | Nil | 16 | 0.896 |
| *P. aeruginosa* (ATCC 27853) | 24 | 20 | Nil | Nil | Nil | Nil | Nil | 0.973 |
| Kruskal Wallis df | 3 | 3 | 2 | 2 | | | | |
| *P* | 0.018 | 0.020 | 0.046 | 0.038 | | | | |

*Significantly greater than Ampicillin ($P < 0.05$)

**Correlation between log dose and size of zones is significant it 0.01 level (2-tailed).

(Mean diameters of zones of inhibition in mm around 6 mm discs impregnated with *N. sativa* oil or commercial diagnostic antibiotic sensitivity testing discs)

**Table 3 : Antibiotics used for sensitivity testing and number of bacterial strains which were resistant to them**

| Name of bacteria | No. of isolates showing resistance to antibiotic (no. of resistant strains/ no. of strains tested) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Ampicillin | Gentamicin | Cotrimoxazole | Erythromycin | Tetracycline | Ciprofloxacin | Amikacin | Ceftriaxone | Ceftazidime | Cephotaxime | Cefaclor | Tobramycin | Ofloxacin | Gatifloxacin | Amoxicillin | Chloramphenicol | Cephoperazone | Others* |
| SA | 54 | 30 | 26 | 36 | 25 | 39 | 33 | 39 | 27 | 9 | 18 | 6 | 27 | 11 | 7 | - | - | - | 7[a],5[b] |
| SE | 13 | 4 | 7 | 4 | 3 | 3 | 3 | 8 | 8 | - | 9 | - | 4 | - | - | - | 1 | - | 5[c] |
| CN | 18 | 11 | 6 | 14` | 7 | 14 | 8 | 13 | 4 | 3 | 2 | 2 | 8 | 6 | 3 | - | - | - | - |
| PA | 22 | 16 | 16 | 9 | 4 | 11 | 14 | 14 | 13 | 14 | 13 | 5 | 8 | 13 | 3 | - | - | 12 | 7[d] |
| SP | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | - | - | - | - | - | - | - | - | - | - |
| SG | 3 | 3 | 0 | 3 | 3 | 3 | 3 | 3 | - | - | - | - | 0 | - | - | - | - | - | - |
| KP | 10 | 10 | 9 | 3 | - | 5 | 9 | 7 | 7 | - | 1 | - | 1 | 4 | - | 3 | - | - | - |
| AB | 4 | 3 | 3 | 2 | - | 3 | 1 | 2 | 3 | 1 | 2 | - | 0 | 2 | 2 | - | - | - | 1[e] |
| CF | 3 | 3 | 2 | 1 | - | 1 | 1 | 2 | 3 | 2 | - | - | - | 2 | 1 | - | - | - | - |
| EF | 3 | - | 2 | 2 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | - | 2 | 1 | - | 2 | - | - | - |
| PM | 3 | 3 | 3 | 3 | 2 | 3 | - | 2 | 1 | - | - | - | - | - | - | - | - | - | - |
| PV | 2 | 2 | 2 | 2 | 1 | 2 | - | 2 | 1 | - | - | - | - | - | - | - | - | - | - |
| VC | 2 | 1 | - | 2 | 2 | 2 | - | - | - | - | - | - | - | - | - | - | 2 | - | - |

SA- *S. aureus*, SE- *S. epidermidis*, CN- Other Coagulase negative Staphylococci, PA- *P. aeruginosa*, SP- *S. pyogenes*, SG- *S. agalactiae*, KP-*K. pneumoniae*, AB- *A. baumannii*, CF-*C. freundii*, EF-*E. faecalis*, PM- *P. mirabilis*, PV-*P. vulgaris*, VC- *V. cholerae*
*Other antimicrobials: Roxithromicin[a], Sparfloxacin[b], Methicillin[c], Imipenem[d], Cephalexin[e]

sensitive to Ampicillin, Gentamicin, Cotrimoxazole, Ciprofloxacin, Ceftriaxone and Bacitracin (Table 3), was inhibited by oil in undiluted state only (Table 4).

*S. aureus*: Out of 54 strains tested, which showed different resistance pattern to various antibacterials, the oil was found active against 50 strains (Table 2).

*P. aeruginosa*: Out of 22 resistant strains tested, the oil showed activity against 12 (Table 4).

Ten strains of *Klebsiella pneumoniae* and 2-4 strains each of *Acinetobacter baumannii, Citrobacter freundii, Enterococcus faecalis, Proteus mirabilis, P. vulgaris, Streptococcus agalactiae* and *Vibrio cholerae*, which were all resistant to 4-9

**Table 4 : Effect of *Nigella sativa* oil on various clinical isolates**

| Bacteria | Total | Conc./disc (4 µl/disc) | | | | | | Pearson's correlation |
|---|---|---|---|---|---|---|---|---|
| | | 1:1 | | 1:10 | | 1:50 | | |
| | | N | zone | N | zone | N | zone | |
| *S. epidermidis* | 13 | 12 | 23± 4 | 8 | 21± 5 | 5 | 14± 3 | 0.444* |
| Other Coagulase negative Staphylococci | 18 | 17 | 23± 2 | 13 | 18± 2 | 11 | 12± 1 | 0.615* |
| *P. aeruginosa* | 22 | 12 | 11± 1 | 6 | 10± 1 | 1 | 8± 1 | 0.692* |
| *S. pyogenes* | 1 | 1 | 19± 1 | 0 | - | 0 | - | 0.912 |
| Kruskal Wallis df<br>*P* | | | 4<br>0.000 | | 4<br>0.003 | | 4<br>0.004 | |

*Correlation between log dose and size of zones is significant at 0.01 level (2-tailed).
[Number of strains sensitive to different dilutions of oil (N) and average sizes of zones of inhibition (mm ±S.E.M) around 6 mm discs impregnated with oil

antibiotics (Table 3) were not inhibited by oil in any of the concentrations tested.

## Discussion

In this study, the oil was found to be more effective on Gram +ve than Gram –ve bacteria, which is in conformity with earlier studies[3, 9, 10]. A number of compounds derived from plants often show considerable activity against Gram +ve bacteria but not against Gram –ve species. Gram negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds and multidrug resistance pumps that extrude toxins across this barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier[11].

Out of 13 strains of *S. epidermidis* tested all except one were inhibited by oil. Seventeen out of 18 strains of other coagulase negative Staphylococci tested, resistant to a number of antibiotics, were inhibited by the oil. This is being reported for the first time as no activity against these has been reported in literature till now. One strain of *S. pyogenes*, which was resistant to Erythromycin and sensitive to 6 other antibiotics, was inhibited by oil. Such study has not been done earlier and this is the first report of antibacterial activity of ***N. sativa*** seed oil against *S. pyogenes*.

The oil was also found active against multidrug resistant strains of *S. aureus* and *P. aeruginosa*. Earlier studies have demonstrated the effect of oil against sensitive strains of *S. aureus* and *P. aeruginosa*[9,12].

Strains of *A. baumannii, C. freundii, E. faecalis, K. pneumoniae, P. mirabilis, P. vulgaris* and *S. agalactiae* tested, which were all resistant to 4-9 antibiotics were not inhibited by oil in any of the concentrations tested. Activity of the oil against these bacteria has not been studied earlier and literature is silent about these bacteria.

The antimicrobial activity of this oil may be attributed to the presence of thymoquinone[10], thymohydroquinone[13] and thymol[2] in the oil all of which possessed antimicrobial activity[10, 13, 14].

## Conclusion

***N. sativa*** seed oil possesses antimicrobial activity against several multidrug resistant pathogenic bacteria and may be used topically in susceptible cases. Further studies are required to advocate its systemic use in infectious diseases.

## References


1. Ali BH and Blunden G, Pharmacological and toxicological properties of *Nigella sativa*, *Phytother Res*, 2003, **17**(4), 299.
2. Randhawa MA and Al-Ghamdi MJ, A review of pharmacotherapeutic effects of *Nigella sativa*, *Pak J Med Res*, 2002, **41**(2), 77-83.
3. Ali NA, Julich WD, Kusnick C and Lindequist U, Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities, *J Ethnopharmacol*, 2001, **74**(2), 173-179.
4. Mouhajir F and Pedersen JA, Antimicrobial thymohydroquinones of Moroccan *Nigella sativa* seeds detected by electron spin resonance, *Pharm Biol*, 1999, **37**(5), 391-395.
5. Halwani R, Habbal MZ and Abdelnoor AM, The antibacterial effect of some constituents of *Nigella sativa* oil, *Arab J Pharm Sci*, 1999, **1**(1), 87-96.
6. Acar JF and Goldstein FW, Disk susceptibility test, *In*: Antibiotics in Laboratory Medicine, 4th edn, edited by L Victor, Williams and Wilkins Publishers, 1996, pp.1-51.
7. Bauer AW, Kirby WMM, Sherris JC and Turck M, Antibiotic susceptibility testing, by a standardized single disk method, *Am J Clin Pathol*, 1966, **45**, 493-496.
8. National Committee for Clinical Laboratory Standards, Performance standards for antimicrobial disc susceptibility testing, Twelfth information supplement (M100-S12), Wayne, PA: NCCLS, 2002.
9. Agarwal R, Kharya MD and Shrivastava R, Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn., *Indian J Exp Biol*, 1979, **17**(11), 1264-1265.
10. Kahsai AW, Isolation and Characterization of Active Ingredients from *Nigella sativa* for Antibacterial Screening; Master's Thesis, Department of Chemistry, East Tennessee State University, 2002. http://etd-submit.etsu.edu/etd/theses/available/etd-0715102-001118/; last accessed on 28/07/2007.
11. Tegos G, Stermitz SR, Lomovskaya O and Lewis K, Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials, *Antimicrob Agents Chemother*, 2002, **46**(10), 3133.
12. Toama MA, Taha S El-Alfy and El-Fatatry HM, Antimicrobial Activity of the Volatile Oil of *Nigella sativa* Linneaus Seeds, *Antimicrob Agents Chemother*, 1974, **6**(2), 225-226.
13. El-Fatatry HM, Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds, *Pharmazie*, 1975, **30**(2), 109-111.
14. Karapinar M and Aktug SE, Inhibition of food borne pathogens by thymol, eugenol, menthol and anethole, *Int J Food Microbiol*, 1987, **4**(2), 161-166.