

## Antibacterial Activity of *Nigella sativa* L. Seed Oil Against Pathogenic Bacteria

Syed Sajeed Ali\*, D.T. Bornare and J.Y. Pathan

\*Department of Microbiology R.G.C.F.T. Parbhani,  
Department of Agricultural Engineering M.I.T. Aurangabad, India.

(Received: 11 August 2014; accepted: 20 October 2014)

Antibacterial activity of petroleum ether, acetone extracts and steam distillation oil of *N. sativa* seed were tested against pathogenic bacteria. The oil obtained by solvent extraction such as petroleum ether, acetone extract and steam distillation showed significant antibacterial activity which was more against Gram-positive than Gram-negative bacteria. Among Gram-positive bacteria tested, *Staphylococcus aureus* were more sensitive to oil than Gram-negative bacteria tested *Pseudomonas aeruginosa* and *Escherichia coli*. The oil obtained by petroleum extract has significant antibacterial activity compared to acetone extracts and steam distillation oil of *N. sativa* and related to Ampicillin, Tetracycline, and Ciprofloxacin.

**Key words:** *N. sativa* L. Seed oil, Antibacterial, Pathogenic Bacteria.

Multiple drugs resistance in bacteria has developed due to the indiscriminate use of antibiotics and antimicrobial agents. In addition to this antibiotic and antimicrobial produced various adverse reactions in host. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants<sup>1</sup>. Seeds of *Nigella sativa* L known commonly as “black cumin” have been employed for thousands years as a spice and food preservative. The plant belongs to the *Ranunculaceae* family of flowering plant and genus of about 14 species.

Among these, *Nigella sativa* is the species most exhaustively investigated for therapeutic purposes although other species have also been implicated for therapeutic uses<sup>2</sup>. Black cumin seeds and black cumin seed oil have been widely used for reducing blood pressure, reducing fluid retention, treating diarrhea, stimulating the appetite, supporting healthy digestion reducing pain, and treating skin disorders. Also studies have confirmed numerous pharmacological benefits that black cumin seeds have anti-diabetic and anti-cancer properties<sup>3</sup>. Black cumin seed oil protects the liver, kidneys, and stomach/ digestive system and it is a powerful antioxidant<sup>4</sup>. They can be used to regulate the immune system, reduce pain, reduce inflammation, inhibit spasmodic activity, and kill microorganisms. The objective of this study is to investigate the antibacterial activity of *Nigella sativa* seed extracts obtained by solvent extraction in soxhlet apparatus by using two different solvents against different bacterial pathogens.

---

\* To whom all correspondence should be addressed.  
E-mail: obaide2002@yahoo.com

## MATERIALS AND METHODS

### Black cumin (*Nigella sativa*) seeds

Cumin seed were procured from local market of Parbhani. The seeds were cleaned and grinded in to fine powder for extraction of oil.

### Cumin seeds oils

Cumin seeds oil was obtained by two different methods, one by solvent extraction and steam distillation.

### Solvent extraction

The *N. sativa* seeds oil was obtained by Soxhlet extraction method A.O.A.C. (1990)<sup>5</sup>. 25 g seeds were crushed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. The oil was extracted for 3h by using petroleum ether as a solvent. After extraction the solvent was removed by hot air oven. The same method was repeated by using acetone as extract agent.

### Steam distillation

Steam distillation oil of *N. sativa* 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.

### Bacterial inoculums preparation

The bacterial pathogen used for study was *Staphylococcus aureus* ATCC 9144, *Pseudomonas aeruginosa* ATCC 9027,

*Escherichia coli* ATCC 25922 were collected from NCL Pune. All bacterial strain cultures were activated on Mueller Hinton Agar. Subcultures of the bacterial strain were prepared from the stock for antibacterial activity testing. A loopful of culture was inoculated in 10 ml of sterile nutrient broth and incubated at 37°C for 3h turbidity to reach 10<sup>5</sup> CFU.

### Antibacterial activity of *N. sativa* oil

The antibacterial activity of *N. sativa* oil was determined by modified method of Bauer *et al*, (1966)<sup>6</sup>. 0.1 ml of bacterial suspension of 10<sup>5</sup> CFU was uniformly spread on Mueller- Hinton agar plate to form lawn cultures. The filter paper discs (6 mm in diameter) were individually impregnated with 10µL of petroleum extract (PE), acetone extract (AE) and steam distillation (SD) oil of *N. sativa* and placed to the surface of Mueller- Hinton agar plate seeded with 3h broth culture of test bacterium. For sensitivity testing standard antibiotics, disc such as Ampicillin (10µg/disc), Tetracycline (30µg/disc), Ciprofloxacin (10µg/disc) procured from Himedia Bioscience Laboratories Mumbai were used. The plates were incubated for 18h at 37°C. Antibiotics susceptibility discs, were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The antibacterial activity of oil and different antibiotic disc were determined by measuring diameter of zone of inhibition (ZOI) in millimeter.



*Nigella sativa* seed oil

## RESULTS AND DISCUSSION

The result of antibacterial activity reveals that the petroleum extracts (PE) showed an effective antibacterial activity against the tested bacteria than acetone extract (AE) and steam distillation (SD) shown in (Table. 1) and (Fig.1).

Among the test bacteria *Staphylococcus aureus* was shown greater zone of inhibition

**Table 1.** Antibacterial activity of different extract of *N. Sativa* oil

Test Bacteria	Petroleum Extract (PE) (ZOI)mm	Acetone extract (AE) (ZOI)mm	Steam Distillation (SD)(ZOI)mm
<i>Staphylococcus aureus</i>	24+ 0.2	23+ 0.1	20+0.4
<i>Pseudomonas aeruginosa</i>	18+ 0.5	16+0.3	17+0.5
<i>Escherichia coli</i>	22+ 0.4	21+ 0.3	18+ 0.2

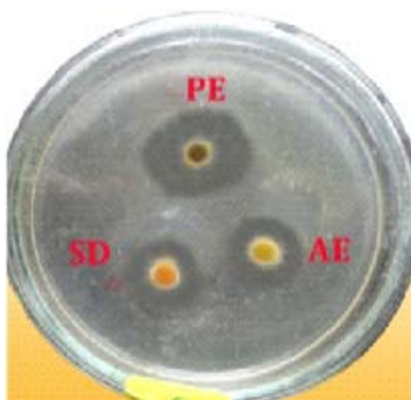


Fig. 1. Antibacterial activity of *N. sativa* oils

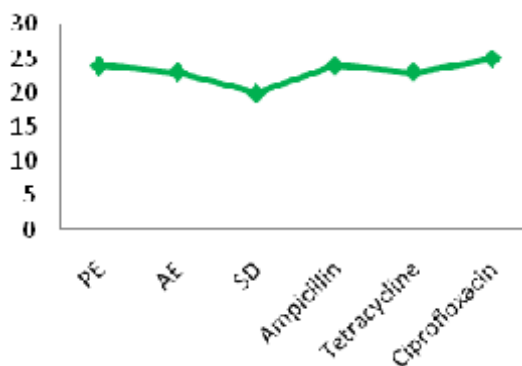


Fig. 2. ZOI of *N. Sativa* and Antibiotics against *Staphylococcus aureus*

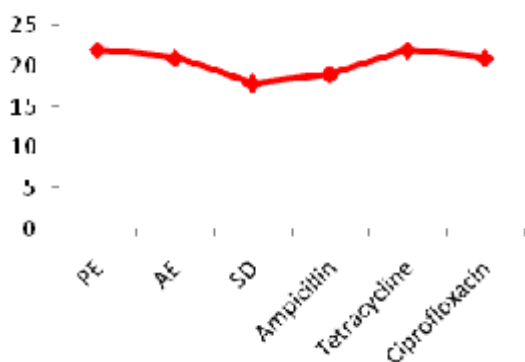


Fig. 4. ZOI of *N. Sativa* and Antibiotics against *Escherichia coli*

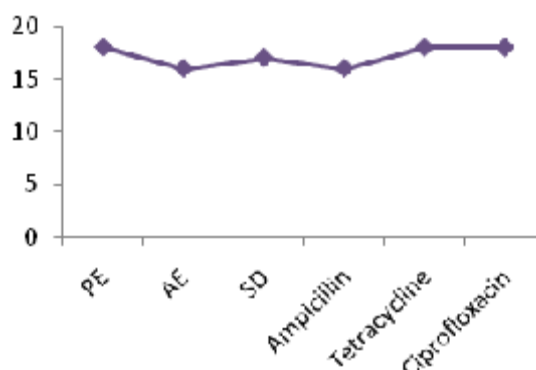


Fig. 3. ZOI of *N. Sativa* and Antibiotics against *Pseudomonas aeruginosa*

against all extract of *N. sativa* oil. While *Pseudomonas aeruginosa* and *Escherichia coli* were show moderate zone of inhibition with positive control of antibiotic such as ampicillin, tetracycline and ciprofloxacin shown in (Fig. 2,3,4). Similar finding of antibacterial activity of *N. sativa* oil against *Staphylococcus aureus* and *Pseudomonas aeruginosa* were reported by Toama *et al* (1974)<sup>7</sup>.

Further the extracts were found to be active against Gram-positive than Gram-negative bacteria. The higher resistance of Gram-negative bacteria to chemotherapeutic agents has been earlier documented, and it is attributed to the presence of lipopolysaccharides in their outer membranes, which make them inherently resistant to antibiotics, detergents and hydrophilic dyes<sup>8</sup>. The above results were consistent with the

literature data reported by Agarwal *et al.* (1979)<sup>1</sup> and Alhaj *et al.* (2008)<sup>9</sup>.

## CONCLUSION

It is concluded that the oil of *N. sativa* extracted by petroleum ether were found more effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* as compared to acetone extract and steam distillation. Among these bacteria *Staphylococcus aureus* were more sensitive against the oil of *N. sativa*. The antimicrobial activity of this oil may be attributed to the presence of thymoquinone, thymohydroquinone<sup>10</sup>, and thymol in the oil all of which contributed antimicrobial activity<sup>11</sup>. The oil extracted by petroleum ether may contain these compounds in high quantity. It is expected that

using oil of *N. sativa* as antimicrobial agents will significantly reduce growth and resistance of pathogenic microorganism in human and prevent several bacterial disease.

### REFERENCES

1. Agarwal, R., Kharya, M.D. and R. Shrivastava, R. Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. *Ind. J. Exp. Biol.* 1979; **17**: 1264-1265.
2. Nadkarni, K.M. *Indian Materia Medica*. Mumbai: Popular Prakashan. 1<sup>st</sup> (Ed). 1979.
3. Mohammad Yaheya, Mohammad Ismail. Therapeutic Role of Prophetic Medicine Habbat El Baraka (*Nigella sativa* L.) – A Review, *World Applied Sciences Journal*. 2009; **7**: 1203-1208.
4. Ahmad, A.L., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhour, Z.A., Anwar, F., Kishore, K. "A review on therapeutic potential of *Nigella sativa*: A miracle herb," *Asian Pac J Trop Biomed*. 2013 May.
5. A.O.A.C. Association of Official Analytical Chemists. Official Methods of Analysis of the AOAC, 15<sup>th</sup> Edn. 1990, Washington, D.C.
6. Bauer, A.W, Kirby, W.M.M, Sherris, J.C, and Turck, M. Antibiotic susceptibility testing, by a standardized single disk method, *Am J Clin Pathol*.1966; **45**: 493-496.
7. Toama, MA, Taha, S El-Alfy and El-Fatary, H.M. Antimicrobial activity of the volatile oil of *Nigella sativa* L. seeds, *Antimicrob Agent. Chemother*.1974; **6** : 225-226.
8. Nikaido, H. and Vaara, M. Molecular basis of bacterial outer membrane permeability. *Microbiol.Rev.* 1985; **49**: 1-32.
9. Alhaj, N.A., Shamsudin, M.N., Zamri, H.F. and R Abdullah, R. Extraction of essential oil from *Nigella sativa* using supercritical carbon dioxide: Study of antibacterial activity. *Am. J. Pharmacol. Toxicol.* 2008; **3**: 225-228.
10. Kahsai, A.W. 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.etu.edu/etd/these/available/etd-0715102-0011181>;last accessed on 28/07/2007.
11. El-Fatary, H.M. Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa*. L. seeds, *Pharmazie*. 1975; **30**: 109-111.