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.

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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 then Gram-negative bacteria. Among Gram-positive bacteria tested, *Staphylococcus aureus* were more sensitive to oil then 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 plants1. 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 uses2. 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 properties3. Black cumin seed oil protects the liver, kidneys, and stomach/ digestive system and it is a powerful antioxidant4. 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). 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 Muellar 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 10⁶ CFU.

Antibacterial activity of N. sativa oil

The antibacterial activity of N. sativa oil was determined by modified method of Bauer et al, (1966). 0.1 ml of bacterial suspension of 10⁶ CFU was uniformly spread on Mueller- Hinton agar plate to form lawn cultures. The filter paper discs (6 mm in diameter) were individually impregnated with10L 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.

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

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

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against all extract of \textit{N. sativa} oil. While \textit{Pseudomonas aeruginosa} and \textit{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 \textit{N. sativa} oil against \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa} were reported by Toama \textit{et al.} (1974)\textsuperscript{7}.

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\textsuperscript{8}. The above results were consistent with the literature data reported by Agarwal \textit{et al.} (1979)\textsuperscript{1} and Alhaj \textit{et al.} (2008)\textsuperscript{9}.

**CONCLUSION**

It is concluded that the oil of \textit{N. sativa} extracted by petroleum ether were found more effective against \textit{Staphylococcus aureus}, \textit{Pseudomonas aeruginosa} and \textit{Escherichia coli} as compared to acetone extract and steam distillation. Among these bacteria \textit{Staphylococcus aureus} were more sensitive against the oil of \textit{N. sativa}. The antimicrobial activity of this oil may be attributed to the presence of thymoquinone, thymohydroquinone\textsuperscript{10}, and thymol in the oil all of which contributed antimicrobial activity\textsuperscript{11}. 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