Full Length Research Paper

Antibacterial activity of *Nigella sativa* seed in various germination phases on clinical bacterial strains isolated from human patients

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*Nigella sativa* is an important spice and flavoring agent which is widely used in various European and Asian cuisines. It harbors an array of medicinal properties as shown by various researches. Germination is a phenomenon during which rapid changes in metabolic activities and the interconversions of metabolites take place. The objective of present study was to evaluate the antibacterial activity of *N. sativa* seed that are on various germination phases against clinical bacterial strains isolated from pus, urine, ascitic fluid and cerebrospinal fluid of various patients. The minimum inhibitory concentration (MIC) values were determined by using a modified macro-broth dilution technique. The agar well diffusion method was used to test the antimicrobial effects of *N. sativa* extracts. Some broad spectrum antibiotics were used as positive control. The phytochemical constituents of *N. sativa* seed were also studied in germination phases. The distilled methanolic extracts of *N. sativa* showed significant antimicrobial activity against tested clinical strains of Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* bacteria. Results showed day-dependent and dose-dependent activity and a significant antimicrobial effect was observed as germination proceeded.

Keywords: *Nigella sativa* seed, germination phases, phytochemicals, clinical isolates, antibacterial activity.

INTRODUCTION

*Nigella sativa* Linn. is commonly known as black seed which belongs to the botanical family of Ranunculaceae. *N. sativa* seeds have been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world (El-Dakhakhny et al., 2000; Al-Ghamdi, 2001). *N. sativa* plant is commonly referred as “Love in a Mist” in English. Seeds of *N. sativa* are frequently used in folk medicine in the Middle East and some Asian countries for acquiring good health and treatment of many ailments including fever, common cold, headache, asthma, rheumatic diseases and various microbial infections and to expel worms from the intestine (Akhtar and Riffat, 1991; Al-Jassir, 1992; Al-Ghamdi, 2001).

The present study was conducted on the evaluation of antibacterial properties of *Nigella sativa* during different phases of germination on some clinical pathogenic bacteria. Germination of seeds has been used for centuries to soften the kernel structure, to increase the anti-nutritive compounds. During the recent years interest has arisen also in the secondary metabolites produced during germination which can have valuable health promoting properties and act as bioactive or functional compounds in foods. All this requires knowledge and know-how of the germination process and the biochemistry behind it (Kaukovirta-Norja et al., 2004). The seeds of *N. sativa* in different germination stages have revealed the presence
of alkaloids, tannins and flavonoids (Kamal et al., 2010). Bacterial infection is responsible for up to a quarter of the deaths of patients with liver disease (Sloth et al., 1970; Correia et al., 1971; Powell et al., 1971; Rimola et al., 1984). 58% of Gram-positive and 42% of Gram-negative bacteria responsible for chronic liver diseases (Jones et al., 1967; Wyke et al., 1982). Few prospective surveys of the incidence of bacteraemia among patients with liver disease are present. Patients with chronic active hepatitis or primary biliary cirrhosis are rarely affected by bacteraemia (Mistilis and Blackburn, 1970; Crowe et al., 1980). In immunocompromised patients P. aeruginosa frequently causes septicemia and a high incidence of P. aeruginosa bacteremia was observed in patients with impaired barrier function of the liver (Pollack, 1990; Korvik et al., 1991).

Clinical isolates of multi-drug resistant Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Proteus mirabilis bacteria were used in present study. They may be responsible for many types of infections including bacteraemia and liver infections or diseases.

The production of new and potent antibacterial agent is urgently needed, especially for hospitals and health centers, keeping in mind that study on strains of clinical microbes are scarce. So, the present study was taken to investigate the antimicrobial effects of N. sativa crude methanolic extracts of successive germination phases against five clinical bacterial strains.

**MATERIALS AND METHODS**

**Collection of Nigella sativa**

Seeds of N. sativa were procured in the month of March, 2012 from a grocery shop in Lucknow and authenticated by Dr. Shanthy Sundaram, Centre for Biotechnology, University of Allahabad, Allahabad (U.P.) India.

**Germination of seeds**

Seeds of Nigella sativa were grown in glass petri plates. Seeds were placed on four folds of damp filter paper at 25°C and incubated in the dark till the initiation of sprouting 3rd day after which they were placed at a light intensity of 100 μmol m⁻² s⁻¹ measured by LI-190SA quantumSensor (Li-COR Co., USA) and a 14/10 h (day/night) photoperiod till the complete plantlet with two leaves was obtained.

**Preparation of distilled extracts**

The samples of seed and germinated phases 3rd, 5th, 7th, 9th and 11th day were shade-dried and ground to a fine powder. The powder (20gm) was extracted by using soxhlet apparatus with 200 ml methanol solvent for 48 h in order to extract bioactive compounds (AOAC method 1980). The extracts were filtered using Whatman filter paper and evaporated using rotary distillation apparatus. Oily fraction of extracts (12g) stored at 4°C until use.

**Qualitative study of phytochemicals of Nigella sativa during germination**

The phytochemical properties (saponin, tannin, alkaloids, polyphenols, sterols, flavonoids) were determined by the methods of Sofowora (1993), Trease and Evans (1983) and Evans and Brightman (1980).

**Clinical bacterial strains used for the study**

The clinical bacterial isolates used in study are listed in Table 1. These isolates were collected from Era’s Lucknow Medical College and Hospital U.P. (India) from various patients. They were authenticated by Dr. Vineeta Mittal, MD, Department of Microbiology, Era’s Lucknow Medical College, Lucknow.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Organism</th>
<th>Patient code</th>
<th>Date</th>
<th>Disease</th>
<th>Age/Gender</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>U/397</td>
<td>22-02-2012</td>
<td>Intestine infection</td>
<td>17 yr/M</td>
<td>Urine</td>
</tr>
<tr>
<td>2</td>
<td>S. aureus</td>
<td>P/312</td>
<td>13-02-2012</td>
<td>Stomach ulcer/ infection</td>
<td>26yr/M</td>
<td>Ascitic Fluid</td>
</tr>
<tr>
<td>3</td>
<td>K. pneumoniae</td>
<td>P/299</td>
<td>11-02-2012</td>
<td>wound at surgical sites</td>
<td>50yr/F</td>
<td>Pus</td>
</tr>
<tr>
<td>4</td>
<td>P. aeruginosa</td>
<td>P/322</td>
<td>14-02-2012</td>
<td>Jaundice</td>
<td>1day/F</td>
<td>CBF</td>
</tr>
<tr>
<td>5</td>
<td>P. mirabilis</td>
<td>U/401</td>
<td>25-02-2012</td>
<td>Cystitis</td>
<td>51yr/M</td>
<td>Urine</td>
</tr>
</tbody>
</table>

**Inoculum preparation**

The test microorganisms were maintained at 4°C on nutrient agar slants. Active cultures for each bacterial species were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth. The inoculated tubes were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh nutrient broth to achieve optical densities corresponding to 10⁶ cfu mL⁻¹ (Duraipandiyan et al., 2006).

**Determination of in vitro anti-microbial effect**

**Broth dilution assay**

The minimum inhibitory concentration (MIC) values were
Table 2. Screening of phytochemicals in methanol extracts of *N. sativa* during germination.

<table>
<thead>
<tr>
<th>Days</th>
<th>Sterols</th>
<th>Alkaloids</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Phenols</th>
<th>Flavonoids</th>
<th>Terpenoids</th>
<th>Cardiac glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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</tr>
<tr>
<td>5</td>
<td>+++</td>
<td>+++</td>
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<td>+++</td>
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<td>+++</td>
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<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>9</td>
<td>+++</td>
<td>++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>11</td>
<td>+++</td>
<td>+</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

*Data is a mean of three replications.*

determined by using a modified macro-broth dilution technique (Ibrahim et al., 1997). Overnight culture of bacteria grown in nutrient broth cultures were diluted 100 folds in NB (100μl bacterial cultures in 10ml NB which contained 10^5 cfu of bacteria). Gradually increasing volumes of the extracts were added to test tubes containing the bacterial cultures to know the inhibitory concentration in a particular tube inhabiting the bacterial growth. The tubes were incubated at 37°C for 18-24 hours. The tubes were examined for visible turbidity and optical density of cultures was determined at 620nm using NB as control. The lowest concentration that inhibited visible growth of the tested organisms was recorded as the MIC.

**Agar well diffusion assay**

The agar well diffusion method was used to test the antimicrobial effect of *N. sativa* methanolic extracts in different stages of germination. (Perez et al., 1990; Okeke et al., 2001). All media plates (9 cm in diameter) were prepared with nutrient agar. After agar solidification, the well (7 mm in diameter) was cut from the agar to produce a total of four wells per agar plate. For test, three doses of extract (25, 50, 75 μg/well) were use. Standard antibiotics (HIMEDIA) streptomycin (30 μg), ciprofloxacin (10 μg), doxycycline (30 μg), ampicillin (10 μg) and ofloxacin (5 μg) were used as positive control. 100 μl (10^6 cfu) of each diluted microbial suspension were inoculated on nutrient agar plate and allowed to diffuse at room temperature for 15-20 min. After incubation at 37°C for 24h, all plates were examined for zones of growth inhibition and the diameter of these zones was measured. The assay was repeated three times for each extract. The antimicrobial effects were recorded as the mean diameter of the resulting inhibition zones of growth in millimeter.

**RESULTS**

The qualitative analyses of phytochemicals present in the methanolic extract of *N. sativa* seed showed the presence of sterols, alkaloids, saponins, phenols, flavonoids, terpenoids and cardiac glycosides. No effect of germination was observed on the presence of sterols, phenols and cardiac glycosides. There was a slight increase in the alkaloid content from 3rd to 7th day of germination. A slight decrease was observed in tannins, saponins, phenols and terpenoids from 5th to 7th day, from 7th day, from 3rd to 7th day and from 9th to 11th day respectively (Table 2).

A clear increase in sterol, tannins, phenols, and in cardiac glycosides was observed as the germination proceed. The presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. remedial properties in medicinal plants. Thus the preliminary screening tests for bioactive compounds may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

In this study, we investigated the antibacterial effects of methanol extracts of successive stages of the germinating seed on Gram-positive and Gram-negative clinical bacterial isolates collected from different pathological samples. The results of antibacterial test is presented in Table 3 and 4 which indicated that different germination extracts of *N. sativa* showed different degrees of growth inhibition depending on the day of germination and bacterial strains.

The general trend of the inhibition of bacterial strains by the extracts was the increase in the activity from 0 day reaching maximum on 5th day and then showed decline again. On 11th day, the inhibitory activity was equal to that of 5th day extract in case of *S. aureus*, *P. aeruginosa* and *P. mirabilis* (Figure 1). The 9th and 11th day extracts of germinating seeds did not show the inhibition of *E. coli*. Maximum sensitivity was shown by *S. aureus* (5th and 11th day inhibition zone, 34mm) followed by *P. aeruginosa* (5th and 11th day inhibition zone, 30mm) and *P. mirabilis* (5th and 11th day inhibition zone, 30mm). K. *Pneumonia* showed moderate sensitivity (5th day inhibition zone, 28mm) and *E. coli* was the least sensitive organism towards these extracts (5th day inhibition zone, 13mm). From the above results, it could be said that the extracts showed day-dependent activity and the 5th day extract of germinating seed was most effective in inhibiting the growth of bacterial pathogens isolated from human patients (Table 4).

The minimum inhibitory concentration of the germinating seed extract was moderate (0.80 μg ml⁻¹) for *E. coli*, *S. aureus* and *K. pneumoniae* whereas it was
**Table 3.** Minimum Inhibitory Concentration of methanol extracts of *N. sativa* seed in different germination phases on clinical bacterial isolates.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Organism</th>
<th>Minimum Inhibitory Concentration (µg ml⁻¹)</th>
<th>Day of germination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td></td>
<td>1.6±0.09</td>
</tr>
<tr>
<td>2</td>
<td><em>S. aureus</em></td>
<td></td>
<td>1.5±0.11</td>
</tr>
<tr>
<td>3</td>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>1.3±0.10</td>
</tr>
<tr>
<td>4</td>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>1.3±0.12</td>
</tr>
<tr>
<td>5</td>
<td><em>P. mirabilis</em></td>
<td></td>
<td>1.0±0.05</td>
</tr>
</tbody>
</table>

*Data is a mean ± SD of three replications.** Technique used was broth dilution assay.

**Figure 1.** The zones of inhibition shown by 5th day germinating seed extract of *N. sativa on S. aureus, P. aeruginosa* and *P. mirabilis.*

maximum for *P. aeruginosa* (1.0 µg ml⁻¹) and least for *P. mirabilis* (0.60 µg ml⁻¹). From these data, it could be said that the extracts of *N. sativa* during various germination phases were effective in inhibiting *P. mirabilis* at very low concentration (MIC, 0.60 µg ml⁻¹) (Table 3) but they were most effective against *S. aureus* (5th and 11th day inhibition zone, 34mm) as it showed maximum zone of inhibition (Table 4).

All the pathogenic organisms were resistant towards ciprofloxacin (10 µg), doxycycline (30 µg), ampicillin (10 µg) and ofloxacin (5 µg) and sensitive towards streptomycin (30 µg). The discs of these standard antibiotics (HIMEDIA) served as positive controls in the experiment. The extracts were more effective on Gram-positive *S. aureus* (5th and 11th day inhibition zone, 34mm) as compared to Gram-negative *E. coli* (5th day inhibition zone, 13mm), *K. Pneumonia* (5th day inhibition zone, 28mm), *P. aeruginosa* (5th and 11th day inhibition zone, 30mm) and *P. mirabilis* (5th and 11th day inhibition zone, 30mm) (Table 4).

**DISCUSSION**

Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. The extracts of seeds of *N. sativa* in different germination stages have exposed the presence of sterols, alkaloids, tannins, saponins, phenols, flavonoids, terpenoids and cardiac glycosides in most of the samples (Table 2). An interesting consequence is that these compounds are potent bioactive compounds that could be used for therapeutic purpose or which are precursors for the synthetic of useful drugs (Sofowora, 1982).

Level of antimicrobial activities of the methanolic extracts on clinical bacterial strains was compared with the chemical composition of extract to determine the chemical composition-activity relationship of extract. The alkaloid and saponins content showed a decrease while the phenol, tannin and flavonoid contents have showed an increase with germination. High tannin and flavonoid contents might also be responsible for the antibacterial activity in later stages of germination (Table 2).

Recent studies have shown that the secondary metabolite content varies during germination of seeds. A significant antibacterial effect of *Allium roseum* L (bulb, leaf, seed and flower) extracts on *S. aureus, B. subtilis, B. cereus, E. faecalis, E. coli, P. aeruginosa, S. typhimurium* and *C. albicans* strains has been s hown...
Nigella sativa oil (Abou Basha et al., 1995) and thus will be extracted in methanolic extract of seed also.

Second, the extracts of germination phase revealed good inhibitory effect when compared with the standard antibiotics (Table 4). These antibiotics are the inhibitors of cell wall synthesis, the cross-linking of different peptidoglycan strands etc. The extracts of N. sativa showed the activity against standard bacterial isolates from human patients. N. sativa seed extracts during various germination phases possesses potential antimicrobial activity against several multidrug resistant clinical bacterial isolates. High metabolic activity and higher contents of secondary metabolites during germination might be responsible for the antibacterial activity.

**REFERENCES**


