Evaluation of Phytochemical and Anti-Bacterial Activity of *Nigella Sativa* L.

M.Asrar Sheriff*, N.Md.Azmathullah and A.K.Sultan Mohideen


Abstract

Plants have been documented for their medicinal potential which is in use by the traditional healers and in Indian system of medicine namely, Ayurveda, Unani and Siddha. *Nigella sativa* L belonging to the family Ranunculaceae is a popular plant in Indigenous medicine all over the world used for treating various diseases. Phytochemical screening of *Nigella sativa* seed was carried out following the standard procedures. Aqueous seed extract of *Nigella sativa* showed the occurrence of alkaloids, phenols, phytosterols, saponins, sterols, tannins, flavonoids and terpenoids. *Nigella sativa* oil was assessed for the anti bacterial activity against *S.pyogenes, S.aureus, E.coli* and *P.aeruginosa* by modified disc diffusion method. Out of the four species of bacteria tested for antibacterial activity only three species were inhibited by oil. *E.coli* was found to be resistant. The medical significance of various phytochemical constituents identified and the potential anti bacterial activity against medically significant human bacterial strains of *N. sativa* are discussed.

Key words: *Nigella sativa*, seed, phytochemicals, oil, antibacterial activity, *S.aureus, S.pyogenes, P.aeruginosa, E.coli*.

Introduction

Plants have been documented for their medicinal potential which is in use by the traditional healers and in Indian system of medicine namely, Ayurveda, Unani and Siddha. These plant species play major role in health care of the human population. Different national and international pharmaceutical companies are utilizing such plant based formulations in the treatment of various diseases and disorders around the world. The use of higher plants and their extracts to treat infections is an age old practice. World Health Organization has also suggested that medicinal plants would be the best source to obtain variety of drugs. Since the use of medicinal plant based drugs contain least or no side effects they are considered to be great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite, physiological action on the human body. The most important of these bio active constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). These substances are usually found in several of plants like root, leaves, shoot, bark and seeds. Many plants have therefore become source to important drugs and the pharmaceutical industries have come to consider traditional medicine as a source of bioactive agents that can be used in the preparation of synthetic medicines. 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 an herbaceous plant, used for centuries for the treatment of various ailments, including infectious diseases. *Nigella sativa* L. (Family: Ranunculaceae) is an annual flowering plant with the height of 20-30 cm. The fruits are large and inflated capsule composed of 3-7 follicles each containing numerous seeds. The seeds are tiny (1-2mm) and black in colour. The flowers are delicate. The seeds are used as a spice. *N.sativa* L. is native to Southwest Asia and cultivated in India. It is used in folk medicine all over the world for treatment and prevention of a number of diseases including allergies, asthma and treating immune disorders, lung complaints, cough, jaundice, hydrophobia, rheumatism and related inflammatory diseases (Chevallier, 1996). In Islam, it is regarded as one of the greatest forms of healing medicine available. The Islamic prophet Muhammad once stated that the black seed can heal every disease except death (http://en.wikipedia.org/wiki/Nigella_sativa, 2009).

Materials and methods

Collection of materials

The seeds of *Nigella sativa* were procured from an Indigenous medicine store in Chennai (Fig. 1). The seeds were ground into uniform powder using a grinder. The powder was used for phytochemical screening. *N.sativa* oil was bought from an Indigenous medicine store, Chennai (Fig. 2). The oil was originally manufactured using steam distillation method from Greenish (India) Trade Pvt. Ltd, Chennai.

Phytochemical screening

Extraction method

Seeds of *Nigella sativa* were powdered and stored in air tight container. The powdered seeds were dissolved in 100ml of distilled water left for 24 hours. Then the extracts were filtered using Whatman filter paper (125mm). Chemical tests were carried out on the aqueous extract and on the powdered specimens, using standard procedures to identify the phytochemical constituents as described by Sofowara (1993), Trease and Evans (1998) and Harborne (1998). Test for Anti bacterial activity of *N.sativa* seed oil:

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Inoculation of plates

This was done by the modified method of Acar and Golstein using flood-inoculation technique (Acar and Goldstein, 1996). Bacterial suspension having turbidity equivalent to 0.5 McFarland was freshly prepared and 2ml of this was transferred onto the Muellarr Hinton Agar plate and distributed gently over surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate was kept in incubator at 37 degree Celsius for 30 minutes for drying before application of discs.

The anti-bacterial activity of the N.sativa seed oil was determined using agar well diffusion method with slight modification (Perez, 1990). Nutrient agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells (8mm diameter) were punched in the agar and filled with seed oil diluted with Ethylene glycol of 10µl concentration. Control wells containing negative control and standard antibiotic solution positive control viz., Ampicillin (10µg/ml) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter for the zone of inhibition. The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the standard drug Ampicillin.

The resultant clear zones around the discs were measured in mm using a standard scale. The antibacterial activity of Nigella sativa seed oil was indicated by clear zones of growth inhibition.

Results and Discussion

Phytochemical screening of the aqueous extracts of N.sativa seed extract revealed the presence of various medically active constituents. The phytochemical compounds present in the plant extract were identified as alkaloids, phenols, phytoesters, saponins, sterols, tannins, flavonoids, terpenoids (Table-1). The presence of these chemical constituents in the seeds of N.sativa demonstrates to their antimicrobial activity. These phytochemicals are known to show medicinal as well as physiological activity (Sofowara, 1993). The occurrence of alkaloids, phenols, phytoesters, saponins, sterols, tannins, flavonoids, terpenoids in the aqueous root extract of N.sativa was also reported earlier by (Ali and Blunden, 2003).

Terpenoids are essentially lipids, known for their aromatic qualities. Different functions have been described to terpenoids including growth regulating, colour, odour and microbiological activity. The presence of terpenoids has been reported from many plants and is said to be responsible for anti-bacterial and anti-fungal activity (Harborne, 1998).

Tannins are used medicinally in anti-diarrhoeal, haemostatic and anti-haemorrhoidal compounds. Tea, wine and fruits like pomegranates, berries and persimmons in cinnamon, eucalyptus, oak, lemon balm, thyme, willow and neem may be responsible for its broad spectrum anti-microbial against bacteria, fungi and viruses (Nascimento et al 2000).

Saponins may be either triterpenoid or steroid in nature and are supposed to have immunological and pharmacological properties. Saponins are known to be anti-microbial and to inhibit moulds and to protect plant attacks (Francis et al 2002).

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 (Agarwal et al., 1979; Ali et al., 2001). A number of compounds derived from plants often show considerable activity against Gram +ve bacteria but not against Gram –ve species (Tegos et al., 2002).

Out of the four species of bacteria tested for antibacterial activity only three species were inhibited by oil. E.coli was resistant to oil (Table-2). The oil was also found active against multi drug resistant strains of S.aureus, Paeruginosa, S.pyogenes (Fig 3 a, b and c) . Earlier studies have demonstrated the effect of oil against sensitive strains of S.aureus, Paeruginosa El-Fatatry 1975 ; Agarwal et al 1979). The oil showed pronounced dose dependent activity against S.aureus followed by Pygogenes and finally S.aeruginosa, whereas Paeruginosa was least sensitive, as it is evident from
Table-2. This study demonstrates that *Nigella* seed oil has more potent antibacterial activity compared to commonly antibiotic ampicillin. Hence, there is potential for the use of *Nigella* seed oil to combat drug resistant strains of bacteria thereby having medicinal value.

**Table 1.** Qualitative analysis of phytochemicals in the aqueous extracts of *Nigella sativa* seeds.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table 2.** Antibacterial activity- Disc diffusion method

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>N. sativa Seed oil</th>
<th>Ampicillin</th>
<th>Ethylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>30mm</td>
<td>20mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>27mm</td>
<td>22mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>10mm</td>
<td>27mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

**Conclusion**

Hence, this study shows that the phytochemicals from *N. sativa* seeds oil possess potent anti-bacterial activity due to the presence of effective active constituents responsible for eliminating microbial pathogens. Further studies are required in this direction to optimize the varied potential applications of *N. sativa* seed oil.

On the basis of various studies it can be concluded that *N. sativa* seed oil should be studied more extensively to explore and exploit its potential in the treatment of many infectious diseases and for other promising applications like radiation protection efficacy, bio-
indicator studies etc. This study heralds an interesting promise of designing potentially active anti-microbial synergized agents of plant origin.

References