Biological screening of *Nigella sativa* seeds crude extract towards food contaminating and spoilage pathogens

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Abstract

Food contamination and the consequences thereafter such as food borne infections, loss of food and emergence of different antibiotic resistant strains are of major concern globally. Hence, natural antimicrobial agents are being studied so as to provide an alternative solution. In this concern, seeds of *Nigella sativa*, a medicinal well known herbal drug, were analysed for their biological potential against selected five common food borne bacterial pathogenic strains (*S. aureus*, *L. monocytogenes*, *B. subtilis*, *P. aeruginosa* and *E. coli*). 100 mg/ml concentration of methanolic extract was found to be effective towards all the strains under study. *E. coli* was found to be most sensitive strain. The results of this study confer the antimicrobial potential of *Nigella sativa* and hence, must be exhaustively studied for its role in managing food borne diseases, by way of formulations.

Introduction

In estimation by WHO news release, contaminated food is the cause of sickness in one of every ten individuals globally per annum and consequently results to death of around 4,20,000 persons [1]. Hence, microbial contamination of food remains an issue of concern which even leads to deteriorated food quality and food loss of raw or ready to eat recipes. The presence of pathogenic microbes in the food such as *Bacillus cereus*, *E. coli*, *Salmonella species* and *Aspergillus species*, not only affects the quality of food but also are responsible for various food borne ailments such as diarrhoea, dysentery and typhoid [2]. To take care of such food-borne diseases, different strategies involving synthetic drugs such as antibiotics and natural drugs such as herbal formulations are adopted in different regions of the world. Emergences of antibiotic resistant strains and other adverse effects have resulted to failure of many drug efficacies and scientists are now looking forward for the alternative therapies [3]. The drugs of plant origin have been in applications since classical times, as reflected in Indian classical system of treatment called Ayurveda, and are in increasing demand nowadays. Different drugs such as *Curcuma longa* (haridra) and *Nigella sativa* (kalaunji) are not only used as therapeutic agents but also in certain preparations such as pickles, where they may contribute as taste enhancers or enhancing shelf life, which enables then to be used for long durations. Currently, the heightened demands of herbal drugs is recognised by certain factors such as being natural origin, easy access and availability, better efficacies, belief of lesser side effects and relatively cheaper therapy [4].
Of hundreds of herbal drugs used, the black seed producing herb with significant therapeutic value is *Nigella sativa* (NS), and is the member of Ranunculaceae family. The seeds also referred to be as ‘Healing power’ by Prophet Mohammad, ‘Curative black cumin’ by Holy Bible and ‘Glitch of Pliny” illustrates the potentials of this plant with religious background as well [5]. The dicot herb of NS inhabits North Africa, Southern Europe and Asia Minor. The bushy plant possesses white or blue flowers along with numerous black seeds (Figure 1), in the capsules.

![Figure 1: Seeds of Nigella sativa procured from the local market, Jalandhar](image)

The seeds are renowned as ‘black cumin’ and ‘Kalaunji’ and rich source of different phyto constituents. The different active ingredients are alkaloids which include isoquinoline (nigellimine-N-oxide) and pyrazole (nigelicine and nigellidine), unsaturated fatty acids, flavonoids, cardiac glycosides, saponins and other minerals such as iron, phosphorus, calcium and vitamins. The seeds are also rich source of volatile oils such as thymoquinone, limonene, thymol and carvacrol [4]. The bioactive ingredients of the seeds are responsible for multiple pharmacological actions such as anti-inflammatory, anti-tussive, anti-tumour, anti-oxidant and hypolipidaemic. Such potentials enable the seeds to be used as an anti-ageing agent along with in treating conditions such as toothache, anti-spasmodic, flatulence, asthma, rheumatism and uricosuria [3, 4]. Although a well-known food supplementing and therapeutic agent, this study aims to analyse the antimicrobial potentials of the seeds towards food pathogens.

**Materials and Methods**

Nearly 250 g of seeds of *Nigella sativa* were procured from the local market from Jalandhar, Punjab, India, powdered and were stored safely in the sterilized container. Thereafter, the glycerol stocks of three Gram positive food pathogens namely, *S. aureus*, *L. monocytogenes*, *B. subtilis*, and two Gram negative food pathogenic strains, *P. aeruginosa* and *E. coli* were procured from the microbiology lab from Department of Microbiology of our university and were revived using nutrient broth. Thereafter, the agar slants were prepared (on nutrient agar), inoculated by streaking and incubated at 37° C for 24 h to obtain the working cultures and plates stored at 4±2° C for further use. The refreshing was done periodically to ensure the purity of the indicator strains [6].

The various media and chemicals of analytical grade used were purchased from Himedia and Loba Chemicals, Mumbai.
Preparation of crude plant extracts

To 10 g of the *Nigella sativa* seeds powder, 100 ml of 95% methanol was added in a 250 ml conical flask, corked and covered with aluminium foil, and kept for 48 h including 6 h shaking (continuous) over the magnetic stirrer at 1000 rpm. This was followed by filtration, decantation and evaporation of the filtrate in a china dish over water bath at 45° C to obtain a thick syrupy mass. This extract was weighed and reconstituted with 100% DMSO to obtain a stock concentration of 100 mg/ml of methanolic extract of NS seeds. The stock solution was stored in the capped sterilised plastic bottle at 4±2° C for further use. From the stock, different working concentrations (50 mg/ml, 25 mg/ml, 20 mg/ml and 10 mg/ml) were prepared using 5% sterilised DMSO in the ependroff [6, 7].

Analysis of antimicrobial activity

To 90 mm petri plate, 25-30 ml sterilised Muller Hinton agar was added and cooled to solidification. The surface of each plate was swabbed with 18-24 h old culture of each indicator strain, followed by cutting of 8 mm wells with a sterilized borer. 100 μl of the test sample was added in the wells. Subsequently, the plates were incubated at 37° C for 24 h and the zone of inhibition observed thereafter. The tests were performed in triplicates [6].

Results and Discussion

The study aims to evaluate the biological potentials of methanolic extract of seeds of *Nigella sativa* towards well known food spoiling and contaminating pathogens, also responsible for causing diseases in the consumers. Five such pathogens were selected for the study and included *S. aureus*, *B. subtilis*, *L. monocytogenes*, *E. coli* and *P. aeruginosa*. The results exhibited the zones against all the indicator strains at 100 mg/ml concentration. 50 mg/ml was ineffective towards *L. monocytogenes*. *E. coli* was observed to be the most sensitive strain followed by *S. aureus*. Three test concentrations (mg/ml) viz. 25, 20 and 10 were not able to inhibit any of test strains except *E. coli*. Similar results have been illustrated in the former studies where methanolic extracts of seeds were found to be potential against the test pathogens [8, 9]. However, Benlafaya et al (2014) reported the insensitivity of NS methanolic extract against *E. coli* and *P. aeruginosa* [10]. This may be due to variations in the extent of phytochemicals which are actually responsible for such potentials in the herbal drugs.

Table1: Antimicrobial potential of methanolic extracts of *Nigella sativa* seeds against food borne indicator pathogens

<table>
<thead>
<tr>
<th>Extract Concentration (mg/ml)</th>
<th><em>E. coli</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>S. aureus</em></th>
<th><em>B. subtilis</em></th>
<th><em>L. monocytogenes</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>19.7±0.9</td>
<td>12.7±0.9</td>
<td>12.7±0.47</td>
<td>11.3±0.4</td>
<td>9.3±0.5</td>
</tr>
<tr>
<td>50</td>
<td>17.7±0.9</td>
<td>9.3±0.47</td>
<td>10±0</td>
<td>9.7±0.47</td>
<td>Nil</td>
</tr>
<tr>
<td>25</td>
<td>14±1.4</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>20</td>
<td>12±0.8</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>10</td>
<td>11.3±1.2</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>DMSO</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Conclusion

Biological contamination of food is an eminent source of food spoilage leading to food borne diseases along with loss of food from the food chains. Other concern related to emergence of antibiotic resistance bacterial species is well recognised in the scientific community. To tackle such issues, the herbal drugs and their phytoconstituents are being looked upon as the alternative solutions. Nigella sativa seeds, as selected in this study, seem to be a potential against some of the selected food pathogens and may be considered further as a member of newer herbal formulations which can provide solutions to the aforesaid problems.

References