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Antibacterial activity of honey alone and in combination with *Nigella sativa* seeds against *Pseudomonas aeruginosa* infection

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ABSTRACT

Objective: To evaluate the *in vitro* activities, of three honeys sample, and *Nigella sativa* (*N. sativa*) against *Pseudomonas aeruginosa* (*P. aeruginosa*) alone and in combination. Methods: The antibacterial test and minimum inhibition concentration (MIC) was determined by using agar well diffusion and dilution methods respectively against *P. aeruginosa*. Results: The MIC for the three varieties of honey without *N. sativa* against *P. aeruginosa* ranged between 46% and 50% (v/v). Addition of *N. sativa* (8%) resulted in synergistic bactericidal activity. An MIC drop was noticed with each variety and it ranged between 77.77% and 84.21%. Conclusions: These antibacterial properties would warrant further studies on the clinical applications of *N. sativa* and honey against *P. aeruginosa*.

1. Introduction

Infectious diseases still represent an important cause of morbidity and mortality among humans, especially in developing countries[1]. In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases[2]. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant–based products, including honey and *Nigella sativa*[3]. The application of honey in medicine has recently been rediscovered and is gaining acceptance as an antibacterial agent for the treatment of ulcers, wounds, and other surface infections. Honey has also been shown to be effective in rapidly responding to standard antiseptic and antibiotic therapy and as a method of accelerating wound healing[4]. Honey is such a complex and variable natural product that the search for specific inhibitors has been extensive[5]. The antibacterial activity of honey has been confirmed by numerous scientific studies[6–8]. Antibacterial activity has been demonstrated against both Gram–positive and Gram–positive, both aerobic and anaerobic types.

*Nigella sativa* L. (*N. sativa*) also known as black cumin is an annual herbaceous plant belonging to the Ranunculaceae family[9]. The plant is indigenous to Mediterranean areas, though it is grown in other parts of the world as well[10]. Its seeds have played an important role over the years in ancient Islamic system of herbal medicine and in Algeria where they have been traditionally used in folk medicine. Traditionally, it is used as a natural remedy for a number of illnesses that include asthma, cough, hypertension, bronchitis, diabetes, headache, eczema, fever, inflammations, and other diseases[11]. Different crude extracts of *N. sativa* have shown effectiveness against multiantibiotic resistance bacterial isolates[12]. The antimicrobial effects of *N. sativa* seeds against different pathogenic microbes were investigated. The importance of *N. sativa* and honey cannot be over emphasized as regards their rule in health remedy. Therefore this study detailed the antibacterial activities of honey and *N. sativa* on selected pathogenic bacteria.

2. Material and methods

2.1. Honey samples
During the 2009 flowering seasons, three honey samples were gathered and provided by various bee-keepers from two area different from the Algeria west. These honey samples were aseptically collected in sterile screwed cups and kept in a cool and dry place (at room temperature) overnight before they were finally transported to the laboratory.

2.2. Plant materials and preparation

*N. sativa* seeds were obtained from the local seed supplier. The seeds were crushed manually in a mortar with a pestle. A volume of 100 mL of distilled water was added to 20 g of dry powder. It was vortexed continuously until there was no further change in color of the solution. This solution was centrifuged at 800 g for 15 min. The supernatant (brownish–orange in colour) was filtered through Whatman filter No. 4 and stored at 4 °C in sterile tubes until use.

2.3. Test organism

Micro-organism was obtained from the Department of Biomedicine, Institute of Veterinary Science University Ibn-Khaldun, Algeria.

2.4. Preparation of bacterial inoculum

Stock cultures were maintained at 4 °C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from each stock culture of *Pseudomonas aeruginosa* (*P. aeruginosa*) to test tubes of nutrient agar medium and incubating without agitation for 24 h at 37 °C. The cultures were diluted with fresh nutrient agar broth to achieve optical densities corresponding to 2.0 × 10^6 colony forming units (CFU/mL)[13].

2.5. Antibacterial assay

### Table 1

<table>
<thead>
<tr>
<th>Honeysample</th>
<th>Honey only % (vol/vol)</th>
<th>MIC of the mixture of honey and <em>N. sativa</em> % (vol/vol) (H:NS)</th>
<th>[Honey:NS] DZ (mm)</th>
<th>Honey MIC drop %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>&gt;4 mm 84.21%</td>
</tr>
<tr>
<td>H2</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>&gt;6 mm 80.00%</td>
</tr>
<tr>
<td>H3</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>&gt;6 mm 77.77%</td>
</tr>
<tr>
<td>Control (Water)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 mm 0.00%</td>
</tr>
</tbody>
</table>

NS: *N. sativa*.

4. Discussion

*P. aeruginosa* is a major nosocomial pathogen, particularly dangerous to cystic fibrosis patients and populations having weak immune system. In wounds, *P. aeruginosa* has emerged as a multidrug–resistant organism that gives rise to persistent infections in burns patients and chronic venous leg ulcers[14-16]. Novel antimicrobial interventions are needed. Natural medicinal products have been used for millennia to treat multiple ailments. Although many have been superseded by conventional pharmaceutical approaches, there is currently resurgence of interest by physicians in natural products. *N. sativa* seeds play an important role in folk medicine and some of its major constituents are reported to be pharmacologically active[17]. Phytochemical studies of the seeds have revealed the presence of volatile oil (1.5%), fixed oil (37.5%), nigellicin, melanthin, arabin acid, carvene, carvone, cymene[18], thymohydroquinone and thymoquinone[19]. It has been reported that crude extracts and essential oil possess antibacterial activity against several bacteria[20-22].

Several bioactive compounds have been identified in honey which contributed to its antibacterial action. The commonly accepted list of contributors include osmolarity[23,24], hydrogen peroxide[25], polyphenols[26], antioxidants[27] antibiotic peptides[28], and recently, Maillard reaction products[27].
Honey and *N. sativa* has been found to possess antibacterial activity and this has been attributed to specific chemicals in the honey and *N. sativa*. The results of this study show that adding honey to *N. sativa* increases the antibacterial effect against *P. aeruginosa* (Table 1).

Its combination with *N. sativa* displayed valued potency on the test organisms than when used in single form. This emphasised that combination of two or more substances with medicinal values could be better if their components will not cause a reaction that could cause health disaster than healing. The exact mechanism of combination between medicinal plants and honey requires further investigation. It is therefore concluded that honey and *N. sativa* combinations due to their synergistic effect have potential to be used in the treatment of *P. aeruginosa* infections.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgements**

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**References**


