Antibacterial effect of Turkish black cumin (Nigella sativa L.) oils

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1. INTRODUCTION

Nigella sativa L. (family Ranunculaceae) is commonly known as black cumin or black seed. The seed or its oil is used as a carminative, diuretic, lactagogue and vermifuge (Akgul, 1989; Ali and Blunden, 2003). The dried seeds from black cumin are also used for sprinkling on bread or flavouring foods, especially bakery products and cheese (Ustum et al., 1990; Takruri and Daneh, 1998). Nigella sativa seeds contain 36-38% fixed oils, proteins, alkaloids, saponin and 0.4-2.5% essential oil (Ali and Blunden, 2003).

The antioxidant, antibacterial and antifungal activities of spices and their derivatives have been investigated by some researchers (De et al., 1999; Sagdic et al., 2002; Sagdic, 2003). Many bioactive properties have been attributed to black cumin seed, fixed oil and/or essential oil, including antibacterial (Akgul, 1989; Hanafy and Hatem, 1991; Farrag et al., 2000), antifungal (Akgul, 1989; Khan et al., 2003) and antioxidant activities (Burits and Bucar, 2000).

In the present study, an attempt has been made to investigate the antibacterial characteristics of five different Turkish black cumin samples. The present communication deals with the investigation of seventeen spoilage and/or pathogenic bacteria, and seven lactic acid bacteria (LAB). This study will help to establish a traditional role for black cumin as preservative, antiseptic and disinfectant.

2. MATERIALS AND METHODS

2.1. Black cumin samples

Five samples of black cumin (Nigella sativa L.) seeds which were grown in five different regions (A: Antalya region; B: Erzurum region; C: Kayseri region; D: Konya region; E: Tekirdag region) of Turkey were used in the study and purchased from different retail groceries in Istanbul, Turkey.

2.2. Bacterial strains

In this study, the seventeen spoilage and/or pathogenic bacteria from a total of twenty-four bacteria used for testing antibacterial activity were Aeromonas hydrophila ATCC 7965, Bacillus cereus FMC 19, Bacillus subtilis IMG 22, Corynebacterium...
xerosis UC 9165, Enterobacter aerogenes CCM 2531, Enterococcus faecalis ATCC 15753, Escherichia coli DM, Escherichia coli O157:H7, Klebsiella pneumoniae FMC 5, Listeria monocytogenes Scott A, Mycobacterium smegmatis RUT, Proteus vulgaris FMC 1, Pseudomonas aeruginosa ATCC 27853, Pseudomonas fluorescens EU, Salmonella typhimurium, Staphylococcus aureus Cowan 1 and Yersinia enterocolitica EU. These bacteria were supplied by the Department of Biology, Sutcu Imam University, Kahramanmaras-Turkey. Additionally, seven LAB including Streptococcus salivarius ssp. thermophilus S51, Lactobacillus delbrueckii ssp. bulgaricus A42, Lb. casei ssp. casei K64, Lb. paracasei ssp. paracasei A27, Leu. pseudomesenteroides E83, Leu. gelidum E26 and Weissella paramesenteroides E95 were obtained from Dr. O. Sagdic (Department of Food Engineering, Erciyes University).

2.3. Preparation of black cumin oils

One hundred g of each black cumin was ground in an ominixer and extracted for 10 h in a Soxhlet extractor with 500 ml n-hexan (Merck-Darmstadt, Germany) at 70°C. The fixed oils were pooled and concentrated in a rotary evaporator (Buchi Rotavapor-RE 111), and then kept in small (10 ml) sterile bottles under refrigerated conditions until use (Ozcan, 1998).

2.4. Determination of antibacterial activity

All test bacteria in nutrient or MRS broths (Merck-Darmstadt, Germany) were enumerated using the serial dilution method. Final cell concentrations were 10^5-10^6 cfu/ml 200 µL (1%) of the bacterial suspensions was seeded on 20 mL of nutrient or MRS agars at 43-45 °C. The prepared bacterial cultures were poured onto petri plates (9 cm diameter), and then agars were allowed to solidify. The agar diffusion method was used to detect the antibacterial activity of the fixed oils. The wells at 4 mm diameter were cut in nutrient or MRS agars. 50 µl of a solution with 0.5%, 1.0% and 2.0% concentrations of the oils in absolute methanol (Merck-Darmstadt, Germany) were added into the wells on nutrient or MRS agars. Absolute methanol was also used as control. The plates were incubated at a suitable temperature for 18-24 h (Aureli et al., 1992; Ozkan et al., 2003; Baydar et al., 2004). The diameter (mm) of inhibition zones of the oils was measured by compass. All tests were carried out in triplicate.

3. RESULTS AND DISCUSSION

The antibacterial activities of black cumin samples against a total of twenty four bacteria (spoilage and/or pathogenic and LAB) are shown in Table 1.

When the agar diffusion method was used, the black cumin oils caused the different inhibition zones on the tested bacteria. Antibacterial effects of the tested black cumin oil concentrations showed variations against the bacterial strains. Additionally, the control treatment (absolute methanol) was inactive against all the bacteria.

The antibacterial effects of the oils were similar to each other. The most active oil was sample A at 1.0% and 2.0% concentrations completely inhibiting the growth of all bacteria. Also, other black cumin oils had some inhibitive effects against all bacteria at 1.0% and 2.0%, while samples of D and E had the lowest activity (Table 1).

The lowest active concentration was 0.5% in all of the cumin samples. In general, this concentration was ineffective against E. coli, E. coli O157:H7, K. pneumoniae, P. aeruginosa, Y. enterocolitica, Lb. casei ssp. casei, Leu. pseudomesenteroides and W. paramesenteroides. In 0.5% concentrations of B, C, D and E samples also had no inhibitory effect against E. aerogenes, S. typhimurium, S. salivarius ssp. thermophilus and Lb. paracasei ssp. paracasei (Table 1).

Of all the bacteria, A. hydrophila was the most sensitive bacteria against all of the concentrations of black cumin oils, while Y. enterocolitica was the most resistant bacteria. Generally, the fixed oils of the black cumin samples had higher antibacterial activity against spoilage and pathogenic bacteria than LAB.

The inhibitory effects of black cumin were previously determined against bacteria, yeasts and moulds by some researchers. Akgul (1989) reported that 0.05% and 1% concentrations of black cumin essential oil had antibacterial and antifungal effects. Hanafy and Hatem (1991) announced that the diethyl ether extract of Nigella sativa seeds (25-400 µg extract/disc) caused concentration dependent inhibition (but not S. thyhimurium) of E. coli, P. aeruginosa, S. aureus and Candida albicans. Farrag et al. (2000) found that the fixed oil of black cumin had an inhibitory effect against gram positive such as S. aureus and B. cereus and Gram negative bacteria. Ozcan (1998), De et al. (1999) and Khan et al. (2003) reported that the extract from Nigella sativa seeds had antifungal activity against Aspergillus parasiticus, Candida albicans and Saccharomyces cerevisiae, respectively.

Many components of black cumin were characterised by Burits and Bucar (2000) using GC-MS, but the major ones were thymoquinone, p-cymene and carvacrol. All of these compounds had antibacterial effect (Ali and Blunden, 2003). Hence, the antibacterial effects of our samples may be closely related to their high percentage of these compounds.
Table 1

Inhibition zones (measured as mm) of the essential oils from various black cumin at different concentrations against bacteria*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Different concentrations of the black cumin samples (%)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Spoilage and/or pathogenic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. hydrophila</td>
<td>22</td>
<td>27</td>
<td>37.5</td>
<td>21</td>
<td>26</td>
<td>36.5</td>
</tr>
<tr>
<td>B. cereus</td>
<td>12.5</td>
<td>19.5</td>
<td>29.5</td>
<td>11.6</td>
<td>19.2</td>
<td>28.5</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>14</td>
<td>21</td>
<td>27</td>
<td>13.8</td>
<td>20.1</td>
<td>27</td>
</tr>
<tr>
<td>C. xerosis</td>
<td>17.5</td>
<td>22.5</td>
<td>32.5</td>
<td>17.5</td>
<td>22.1</td>
<td>32.4</td>
</tr>
<tr>
<td>E. aerogenes</td>
<td>7</td>
<td>16.5</td>
<td>23.5</td>
<td>16.2</td>
<td>23.6</td>
<td>16.5</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>16</td>
<td>19.5</td>
<td>-</td>
<td>15.8</td>
<td>19.5</td>
</tr>
<tr>
<td>E. coli O157 H7</td>
<td>-</td>
<td>15</td>
<td>19</td>
<td>-</td>
<td>15.2</td>
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<td>E. faecalis</td>
<td>8.5</td>
<td>17.5</td>
<td>24</td>
<td>8.5</td>
<td>18.8</td>
<td>23.8</td>
</tr>
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<td>-</td>
<td>13.5</td>
<td>18.5</td>
<td>-</td>
<td>12.8</td>
<td>18.8</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>7</td>
<td>15.5</td>
<td>21.5</td>
<td>-</td>
<td>14.6</td>
<td>21</td>
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<tr>
<td>M. smegmatis</td>
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<td>22</td>
<td>32</td>
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<td>21.5</td>
<td>32.2</td>
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<td>18.5</td>
<td>23.8</td>
<td>34.8</td>
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<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>16</td>
<td>21</td>
<td>-</td>
<td>16.4</td>
<td>20.6</td>
</tr>
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<td>P. fluorescens</td>
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<td>13.5</td>
<td>21.5</td>
<td>8</td>
<td>13</td>
<td>21.4</td>
</tr>
<tr>
<td>S. aureus</td>
<td>16.5</td>
<td>21.5</td>
<td>29.5</td>
<td>16.5</td>
<td>21.5</td>
<td>29.2</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>7</td>
<td>16.5</td>
<td>24.5</td>
<td>16.8</td>
<td>24.4</td>
<td>16.2</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>-</td>
<td>11</td>
<td>16.5</td>
<td>-</td>
<td>11</td>
<td>16.4</td>
</tr>
</tbody>
</table>

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LAB

S. salivarius ssp. thermophilus 10.5 | 16.5 | 24 | - | 13.5 | 19 | - | 13 | 18.8 | - | 13.2 | 19.2 | - | 13 | 19

Lb. casei ssp. casei 13.5 | 19.5 | - | 14.3 | 20.8 | - | 14 | 20.5 | - | 14.2 | 20.2 | - | 13.8 | 20.5

Lb. paracasei ssp. paracasei 7 | 14.5 | 21 | - | 12.2 | 18.8 | - | 12.5 | 19 | - | 12 | 18.6 | - | 12 | 18.4

W. paramesenteroides 12.5 | 19 | 10.4 | 16.2 | 23.3 | 10 | 16.5 | 23 | 10.2 | 16 | 22.8 | 10.2 | 15.8 | 22.8

Lb. delbrueckii ssp. bulgaricus 11 | 18.5 | 24.5 | 11 | 18.3 | 23.8 | 10.8 | 18.1 | 24.4 | 11 | 18.1 | 24 | 10.6 | 17.6 | 23.6

Leu. pseudomesenteroides 14 | 19 | - | 14.5 | 19.3 | - | 14 | 19.1 | - | 14.2 | 18.6 | - | 13.8 | 19

Leu. gelidum 8.5 | 14 | 19.5 | 8 | 14.4 | 19.6 | 8.4 | 13.5 | 19.2 | 8.1 | 13.8 | 19 | 8.3 | 13.5 | 19

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*: The diameter of the well (4 mm) is included in the final zone measure.

A: Antalya region in Turkey; B: Erzurum region in Turkey; C: Kayseri region in Turkey; D: Konya region in Turkey; E: Tekirdag region in Turkey.
As a result, antibacterial activities of the black cumin fixed oils against food spoilage and/or pathogenic, and lactic acid bacteria are an important finding. Therefore, the oil of black cumin may be used in food as a preservative.

REFERENCES


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