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# BIOACTIVE PEPTIDES FROM SEVERAL PLANT SPECIES

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Bioactive peptides were observed in seeds of several plants. However, the number of peptides characterized in detail is currently small. Nevertheless, it was shown that cationic peptides containing cysteines in their structure exhibit strong protective properties against various types of infectious agents [1, 2]. Such peptides can provide alternatives to antibiotics to which several pathogens have developed resistance. We have shown previously that components of seeds from Apiaceae and Malvaceae plants (peptides and essential oils) exhibit pronounced antimicrobial activity [3–5].

Therefore we isolated peptides from seeds of pepper *Capsicum annuum*, *Nigella sativa*, *Sesamum indicum*, and *Cuminum cyminum*.

Peptides were extracted most completely from ground and defatted seeds using acetic acid (0.05 M). Using mineral acids caused partial destruction of the peptides. Extraction by acetone and buffers was incomplete.

The resulting extracts were neutralized with NaOH and centrifuged. The supernatant was lyophilized. Next, extracts were dialyzed twice against water and Tris-HCl buffer (pH 9.0). Then, extracts were separated successively by ion-exchange chromatography over columns of DEAE-servacel 23SN (2 × 10 cm, Reanal) and CM-TSK (2 × 5 cm, Tosoh Bioscience) using a stepwise NaCl gradient in ammonium acetate buffer (pH 6.0, 0–0.5 M).

Peptide content in the resulting fractions was analyzed by electrophoresis [6] (15% PAAG with 0.1% Na-SDS, pH 8.9). Peptides with molecular weight (MW) 2–10 kDa were found. According to the literature and our results [7, 8], it can be assumed that peptides with MW 2–5 kDa belong to the defensin class; with MW ~10 kDa, to the so-called lipid-transfer proteins.

The biological activity of the seed extracts and fractions obtained after separation over CM-TSK gel was checked in tests for fungitoxicity using a culture of pathogenic strains No. 108 *Verticillium dahliae* and a turbidimetric microplanchette method [9]; for cytotoxicity, KML murine melanoma cells in tissue culture and measurement of $^{3}$H-thymidine incorporation into tumor cell DNA [10] (Table 1).

According to the results, extract of *Nigella sativa* containing its total cationic peptides and its separate components obtained by ion-exchange chromatography had the highest biological activity. The pronounced cytotoxic activity of *Nigella saliva* peptides makes them promising for further in-depth studies of the antitumor activity for various tumor types in both *in vitro* and *in vivo* tests.

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TABLE 1. Biological Activity of *Capsicum annum*, *Nigella sativa*, *Sesamum indicum*, and *Cuminum cyminum* Seed Extracts and Their Most Active Fractions

| Extract and fraction | Fungitoxicity, $IC_{50}$ μg/mL | Cytotoxicity, % suppression of $^3H$-thymidine incorporation | Extract and fraction | Fungitoxicity, $IC_{50}$ μg/mL | Cytotoxicity, % suppression of $^3H$-thymidine incorporation |
|---|---|---|---|---|---|
| *Capsicum annuum* | 79 | 33.0 | *Cuminum cyminum* | 64 | 1.0 |
| Fraction P-1 | 70 | 10.9 | *Nigella sativa* | 65 | 95.0 |
| *Sesamum indicum* | 60 | 2.6 | Fraction C-1 | 41 | 39.5 |
| Fraction K-2 | 37 | 1.2 | Fraction C-2 | 58 | 96.3 |
| Fraction K-3 | 30 | 2.0 | Fraction C-3 | 18 | 42.9 |
| Fraction K-4 | 39 | 0 | | | |

$IC_{50}$, peptide concentration causing 50% suppression of fungus growth.

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