*Chemistry of Natural Compounds, Vol. 45, No. 2, 2009*

# COMPARISON OF EXTRACTS FROM SEEDS AND SPROUTS OF *Nigella sativa*

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UDC 547.96:577.15

Seeds of *Nigella* spp. have been used for millenia as spices and food preservatives. Oil and other seed components exhibit antioxidant properties [1]. Seeds of *N. sativa*, an annual herbaceous plant of the family Ranunculaceae, are used in the East to treat a broad spectrum of diseases including bronchial asthma, eczema, disentery, hypertonia, and gastro-intestinal tract problems [2, 3].

The chemical composition of *Nigella* is rather well studied. However, peptides contained in seeds have not yet been investigated.

Our goal was to isolate and characterize protein—peptide fractions from seeds and two-week sprouts, a part of which was infected beforehand with conidiae of *Verticillium dahliae* Kleb.

Acid extraction of seeds and sprouts was used to isolate the protein fraction. The resulting extracts were desalted and separated by analytical RP-HPLC. The resulting fractions were analyzed using MALDI TOF mass spectrometry (Table 1).

Seeds contained proteins and peptides of various molecular weights according to analytical RP-HPLC. The most interesting and promising were peaks 2 and 3, which contained a peptide of 5660 Da (with eight cysteines and classified as a γ-thionin according to the sequence of the *N*-terminus by Edman stepwise degradation), and peak 4, which contained peptides of 5699 Da (classified as a d-defensin according to the sequence of the *N*-terminus) and of 9602 Da (with eight cysteines and classified as a lipid-transport protein according to the amino-acid sequence).

Furthermore, seeds contained a broad family of antimicrobial peptides with molecular weights of the order of 3-4 kDa (from four to six cysteines in each peptide) and protective proteins of type 11 and 2S-albumins with molecular weights 11,284 and 16,296 Da.

Separation of the extract of intact sprouts detected nine peaks, one of which contained a lipid-transport protein of 9086 Da and a whole family of antimicrobial peptides with molecular weights of the order of 4 kDa. The extract of infected sprouts had seven peaks including defensins of 5075 and 5669 Da that were absent in untreated sprouts and seeds. Extracts of sprouts contained much smaller quantities of peptides than extracts of seeds. The appearance of defensins in infected sprouts were probably related to increased biosynthesis of protective proteins in response to the infection by spores of the pathogenic fungus.

**Isolation of Protein—Peptide Fraction from *Nigella* Seeds.** Seeds of *N. sativa* (50 g) growing in Uzbekistan were ground, defatted for 72 h in hexane in a Soxhlet apparatus, homogenized in acetic acid in de-ionized water (10%, 10 mL solution per 1 g of biological material in 10 mL) in the presence of protease inhibitors (serin proteases: trypsin, chemotrypsin, plasmin, callicrein, thrombin; cysteine proteases: calpain, papain, catepsin B, catepsin L; aminopeptidases: leucine aminopeptidase, alanyl aminopeptidase; serin and cysteine peptidases: plasmin, trypsin, papain, catepsin B; acidic proteases: pepsin, renin, catepsin D; metalloproteases). For this an acid solution (10%) was treated beforehand with a mixure of protease inhibitors (30 μL) calculated for inhibitors (1 mL) per extract (100 mL) from the material (30 g). The extraction was carried out at room temperature with constant stirring on a magnetic stirrer. The homogenate was centrifuged at 12,000 rpm. The precipitate was discarded. The supernatant was treated with acetone at a 1:5 ratio and left overnight at 4°C. The acetone was decanted. The precipitate was dried in air, dissolved in TFA (0.1%), and centrifuged to remove insoluble substances.

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0009-3130/09/4502-0293  

TABLE 1. Component Composition of Total Extracts from *Nigella sativa* from Mass Spectral Data, MW, Da

| Peak No. | Intact sprouts | Infected sprouts | Seeds |
|---|---|---|---|
| 1 | 2060, 2510, 2787 | 2161, 2318, 2745, 3208 | 2381, 3908, 4458 |
| 2 | 2060, 2480, 2593, 2813 | 2504 | **5660**, 8190, 10963, 16296 |
| 3 | 2141, 2530 | 1409 | **5660**, 8190, 9397, 11284, 16296 |
| 4 | 2360, 2492, 2839 | 5200, 5363 | 2195, 3056, 3555, **5699**, **9602** |
| 5 | 2510 | 6320, 6480 | 2148, 3263, 3789, 7100, 11475 |
| 6 | 2233, 3152, 4490, 6301 | 2483, **5075**, **5669**, 8289, 13203 | – |
| 7 | 2480 | 3588, 4924 | 4219 |
| 8 | 3565, 4222, 4930, 5980, **9086*** | – | 3577, 4308 |
| 9 | 3786, 4930 | – | 3979, 4072 |
| 10 | – | – | 3497 |

*Most interesting molecular weights.

**Isolation of Protein—Peptide Fraction from *Nigella* Sprouts.** A part of two-week sprouts was infected with conidiae of *V. dahliae* and stored at 4°C for 24 h. Sprouts (intact and infected) were homogenized in $H_2SO_4$ (0.05 N, 1:5 ratio, wt/vol). The protein—peptide fraction was extracted for 3 h with constant stirring at room temperature and centrifuged (6,000 rpm, 30 min, 4°C). The supernatant was filtered through filter paper, neutralized with NaOH solution (10 N), and stored at 4°C for 12 h. The resulting precipitate was separated by centrifugation (6,000 rpm, 30 min, 4°C). The supernatant was lyophilized and redissolved in TFA (10 mL, 0.1%).

**Desalting.** Dissolved precipitate was desalted over a column of Aquapore RP-300 C8 (10 × 100 mm, GE Healthcare, USA) by RP-HPLC.

Elution was performed at 1.5 mL/min at 38°C. The necessary concentration of eluent was created by solutions of TFA (0.1%, solvent A) and TFA (0.1%) containing $CH_3CN$ (80%, solvent B). The detector was set to 214 nm. The column was equilibrated with solvent A before placing the sample on it. Salts and other non-bound components of the extract were eluted from the column by this same solvent. The protein—peptide fraction was desorbed from the column by solvent B (70%). The collected fraction was evaporated to dryness in a Speedvac vacuum concentrator (Savant, USA) and redissolved in TFA (0.1%).

**RP-HPLC over $C_{18}$ Reprosile Pur ODS-300 Column (4.6 × 250 mm).** Components were separated analytically by RP-HPLC over a column equilibrated with TFA (0.1%) in $CH_3CN$ (5%). The elution took 60 min at flow rate 0.8 mL/min with a linear gradient from 5 to 50% $CH_3CN$ in TFA (0.1%). An additional gradient went from 50 to 70% $CH_3CN$ in 20 min. The detector for proteins and peptides was set at 214 nm.

**MALDI Mass Spectrometry.** Molecular weights of the isolated compounds were analyzed by MALDI TOF in a Micromass MALDI$^{TM}$ mass spectrometer using the MassLynks 4.0 program. The TOF mass spectra were obtained in direct flight using a reflector. The accuracy of the measured masses was 0.015%.

**Determination of *N*-terminus of Amino-Acid Sequence.** Previously reduced and alkylated protein was sequenced by Edman stepwise degradation on a Procise 492 automated sequencer (Applied Biosystems, USA) by the standard method. Homologies were sought using the BLAST algorithm.

## REFERENCES


1. M. L. Salem, *Int. Immunopharmacol.*, 5 (2005).
2. P. Schleicher and M. Saleh, *Healing Arts Press* (1998).
3. N. A. Al-Rowais, *Saudi Med. J.*, 23 (2002).