—Notes—

Effects of Essential Oil Extracted from *Nigella sativa* (L.) Seeds and Its Main Components on Human Neutrophil Elastase Activity

Rachid KACEM,*,[a] and Zahia MERAIHI[b]

[a]*Department of Biological Sciences, Faculty of Sciences, Ferhat Abbas University, 19000 Setif, Algeria, and* [b]*Department of Biological Sciences, Faculty of Sciences, University of Mentouri, 25000 Constantine, Algeria*

(Received November 18, 2005; Accepted January 25, 2006)

The effects of essential oil extracted from *Nigella sativa* (L.) seeds and its main components on human neutrophil elastase (HNE) activity were investigated. Essential oil was extracted from *N. sativa* (L.) seeds using hydrodistillation. The yield was equal to 0.4%. Inhibition of HNE activity by essential oil was found to be dose dependent. The highest inhibitory concentration (HIC) of essential oil which caused total inhibition of HNE activity was 5.8 mg/ml. Microassays carried out to evaluate the inhibitory effect of major components of essential oil on HNE activity revealed that carvacrol (5-isopropyl-2-methylphenol) showed marked HNE inhibitory activity with a very low IC_{50} value (12 μM). Based on these results, the inhibitory effects of essential oil on HNE activity are due to the presence of bioactive molecules, mainly carvacrol this compound is an inhibitor of HNE and could be considered as a natural antielastase agent and possible candidate for phytotherapy in the treatment of injuries that appear in some pathologic cases such as chronic obstructive pulmonary disease and emphysema.

Key words——*Nigella sativa*; essential oil; elastase; chronic obstructive pulmonary disease; emphysema

INTRODUCTION

Nigella sativa Linn (Ranunculaceae), commonly known as black seed, was used in herbal medicine, especially for the treatment of respiratory diseases such as chronic obstructive pulmonary disease (COPD).[1—3] Many research reports confirmed that the extract from *N. sativa* (L.) seeds have antiinflammatory activity.[4—6] This is mainly due to the enrichment of this extract with many bioactive molecules.[7] Essential oil produced from *N. sativa* (L.) seeds as secondary metabolites has been intensively investigated because of that enrichment, and the metabolites include many types of phenols.[8,9] Recent reports have indicated that the antiinflammatory effects of essential oil constituents extracted from *N. sativa* (L.) seeds are due to the presence of phenols, mainly thymoquinone.[4,6,10] Studies carried out by Al-Ghamdi[5] indicated that extract prepared from *N. sativa* (L.) seeds had antiinflammatory effects on edema induced by carrageanan in rats. In spite of these findings, the antiinflammatory mechanisms of action of this product at sites of inflammation are still not clear. Moreover, no research has been carried out to investigate the effects of bioactive molecules present in the extract of this medicinal plant on human neutrophil elastase (HNE) activity. This enzyme has been reported to play a crucial role in extracellular proteolytic processes at sites of inflammation[11—13] and capable of cleaving many proteins with important biologic functions, especially elastin, an important extracellular matrix protein that plays a mechanical function in the lungs. Thus it became evident that HNE is involved in the pathogenesis of different inflammatory diseases such as emphysema, cystic fibrosis (CF), and COPD. The indications of involvement of HNE in these different diseases prompted us to conduct this research, mainly to develop natural elastase inhibitors. Therefore this study aimed mainly to investigate the effects of essential oil extracted from *N. sativa* (L.) seeds and its main components on HNE activity and evaluate the bioactive molecules present in the extract of this medicinal plant that play an inhibitory effect against HNE activity. The possibility of using it as a natural antielastase agent for the treatment of injuries in emphysema and COPD was also examined.

MATERIALS

N. sativa (L.) seeds were collected south of Sétif, a region characterized by long hours of sunlight, and checked at the Department of Botany for its morpho-

*e-mail: Kacemrachid@yahoo.fr

logic characteristics. The seeds were dried at 50°C for 8 h before use, ground to powder, and placed in a desiccator prior to use. Pure HNE (EC 3.4.21.37), activity 20 U/mg protein, was purchased from ICN Biomedical Inc., Aurora, OH, USA. HNE substrate, N-methoxy-succinyl-Ala-Ala-Ala-Pro-Val p-nitroanilide was purchased from Sigma Chemical. Pure test compounds, i.e. thymoquinone (2-isopropyl-5-methylbenzo-1,4-quinone), FW 164.20, purity 99%; p-cymene (1-isopropyl-4-methylbenzene), FW 134.22, purity 99%. carvone (5-isopropenyl-2-methylcyclohexanone), FW 150.22 purity 98%; thymol (2-isopropyl-5-methylphenol), FW 150.22, purity 99% and carvacrol (5-isopropyl-2-methylphenol); FW 150.22, purity 99% were gifts from Dr. Belattar Noureedine, Laboratory of Chromatography, Faculty of Sciences, University of Sétif. All other compounds were purchased from Aldrich Brand, Sigma Chemical.

METHODS

1. Extraction of Essential Oil The essential oil used in the experiments was extracted by hydrodistillation.[14] *N. sativa* (L.) seeds were dried at 50°C for 8 h and powdered. The volatile fraction was isolated using the hydrodistillation method for 4 h with a Clevenger-type apparatus. Briefly, 250 ml of dH20 was added to 50 g of seed powder, and the temperature was set to boiling point. Condensation of vapor was achieved using a glass condensor. The rate of extraction was adjusted to 2—4 ml/min. Essential oil was collected in coated glass bottles. Diethyl ether 10 ml was added and the solution was left for 30 min to separate the water phase. Anhydrous sodium sulfate was added to the supernatant fraction with simple agitation to dry essential oil and filtered through 0.22 μm filters (Millipore, Bedford, MA, USA). Finally, rotatory evaporation under the same previous conditions was carried out to remove the remaining solvent. The final solution of essential oil was stored at 4 °C in coated glass bottles. The yield was 0.4%.

2. Elastase Assay HNE activity was determined using the method of Nakajima et al.[15] Briefly, 10 μl of pure HNE stock solution (prepared by dissolving pure HNE in sterile water) was dissolved in Tris-buffer solution (Tris 10 mM, NaCl 150 mM, 0.01 % Triton-X100), pH was adjusted to 7.5, a volume of 60 μl was added to each microassay tube (final concentration of HNE in each assay, 10 nM), assays were carried out in 96-well microplates at 37°C, and incubated with a range of concentrations of test compounds or controls [buffer containing dimethyl sulfoxide (DMSO) 10 μl] Test compounds were first diluted in DMSO and a range of concentrations were prepared in buffer solution (final concentration of DMSO in all assays was less than 5%). Incubation was carried out in a Heidolph incubator model 1000 with simple agitation (450 rpm, 37°C, 20 min). After incubation, 30 μl of substrate solution (165 μM) was added. All tubes were reincubated under the previous conditions. Triplicate control assays were performed simultaneously in the microplates. Absorbance was read immediately at 405 nm after stopping the reaction by adding 120 μl of soybean trypsin inhibitor solution (2 mg/ml) prepared in buffer solution and the mean ± S.E.M. of three different experiments was calculated.

3. Calculation of IC_{50} Inhibitory concentration (IC_{50}) values corresponding to the concentration of test compounds showing 50% inhibition of HNE activity were estimated based on the least-squares regression line of three plots of the logarithmic concentrations versus HNE activity.

4. Statistical Analysis Assays were performed at least three times with triplicate samples, and all inhibition rates were calculated as a percentage of controls (buffer containing DMSO) without inhibitors. All results are expressed as mean±S.E.M.

RESULTS

The yield of essential oil extracted from *N. sativa* (L.) seeds with hydrodistillation was equal to 0.4% (v/w). Many reports[6,16—19] demonstrated that the main components of essential oil extracted from *N. sativa* (L.) seeds are p-cymene (37.3%), thymoquinone (13.7%), carvone (0.9%), thymol (0.33%), and carvacrol (11.77%). Those findings confirmed that most bioactive molecules present in essential oil are phenols.[9] The chemical structures of these test compounds are shown in Fig. 1. These compounds comprise 65% of total essential oil and are the main components of this product. These compounds are monoterpenes. Our results (Table 1) indicate that thymoquinone inhibits HNE activity with an IC_{50} value of 30 μM. The IC_{50} value of carvacrol was the lowest (12 μM). Inhibition of HNE activity by total essential oil was found to be dose dependent (Fig. 2). The highest inhibitory concentration (HIC) of essen-

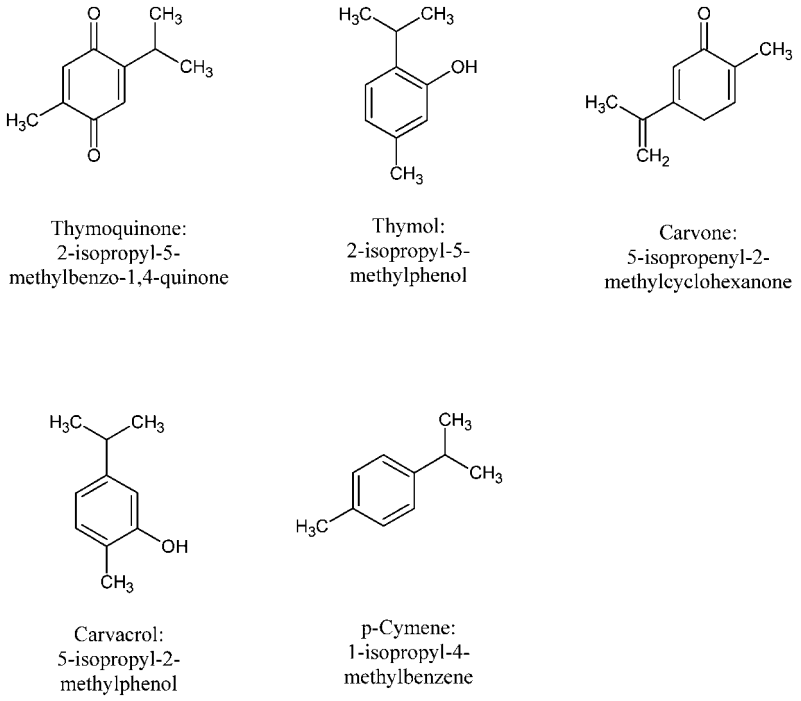

Fig. 1. Chemical Structure of Test Compounds Showing Inhibitory Activity against HNE

Table 1. Inhibition of HNE Activity by Essential Oil Components[a)]

Compound	Concentration (μM)	HNE inhibition (% of control)	IC_{50} (μM)
Thymoquinone	11	18	30
Thymol	15	7	104
Carvacrol	11	45	12
Carvone	7	25	14
p-Cymene	13	27	25

a) Performance of assays (*see* section 2).

tial oil (5.8 mg/ml) caused total inhibition of HNE activity. The lowest inhibitory concentration was 0.4 mg/ml. When the HIC of different test compounds was added to HNE simultaneously with the specific substrate N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitro-anilide (165 μM) a slow decrease in the rate of substrate hydrolysis was observed (Fig. 3). From our results, it was clear that the test compounds inhibited HNE activity with different IC_{50} values, but carvacrol inhibited HNE with a very low IC_{50} value (12 μM). The inhibitory concentration of thymol was the highest at 104 μM.

DISCUSSION

This study was carried out using pure test compounds representing major components of the essential oil of *N. sativa* (L.). These compounds are monoterpenes with low molecular weight, representing about 65% of the total essential oil. Although some studies indicated that thymoquinone is the most active molecule that could be responsible for the effects of essential oil from Nigella *Sativa*,[9)] the results of this study clearly indicate that this compound inhibits HNE activity with an IC_{50} value (12 μM) about 3-fold that of carvacrol. It is clear that carvacrol, which has a hydroxyl group in position 3 on the benzene ring, was the most bioactive molecule in inhibiting HNE activity. Because a specific substrate was used in this study, the inactivation of HNE could be explained by the competition of this compound and substrate for the same specific binding sites. We observed about 2 fold progressive inhibition of HNE by carvacrol compared with thymol. Inhibition of HNE activity by carvacrol was explained by its direct binding with the enzyme, forming an enzyme-inhibitor complex. The inhibitory effects of total essential oil on HNE activity can be explained by the presence of bioactive molecules, mainly carvacrol. Taking into consideration of the IC_{50} values of each compound, carvacrol is a potent inhibitor of HNE. In conclusion, pure essential oil extracted from *N. sativa* (L.) seeds inhibit the activity of HNE in a dose-

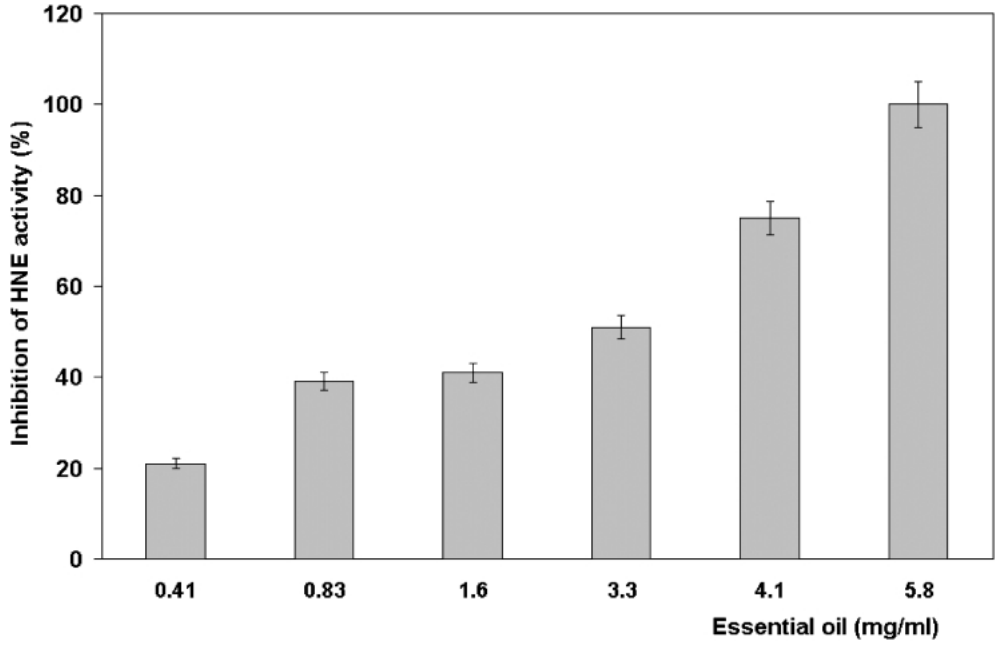

Fig. 2. Effects of Different Concentrations of Essential Oil Extracted from *N. Sativa* Seeds on HNE Activity, Performance of Assays is Illustrated in Section 2
Values±S.E.M. $p<0.01$.

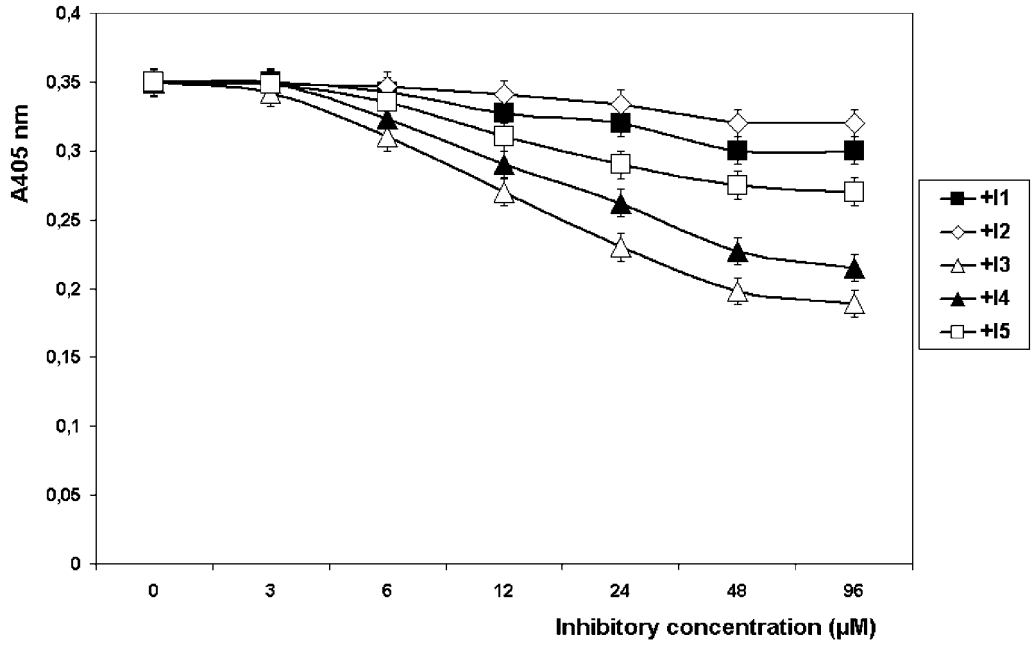

Fig. 3. Inhibition of HNE Activity by Test Compounds, Performance of Assays is Described in Section 2
Values±S.E.M. $p<0.01$. I1: thymoquinone, I2: thymol, I3: carvacrol, I4: carvone, I5: p-cymene.

dependent manner. The HIC observed in this study was 5.8 mg/ml. Carvacrol can be considered a natural antielastase agent in the treatment of injury in COPD and emphysema. Further *in vivo* studies are recommended.

Acknowledgments This work was supported in part by the Algerian government and Deutscher Akademischer Austansch Dienst (DAAD), Germany. The authors appreciate the helpful discussions and laboratory facilities offered by Professor Tobias Welte and Dr. Buhling Frank, Laboratory of Im-

munology, Institute of Medical Technology Magdeburg (ISTM) Otto Von Guericke Universität, Magdeburg, Germany.

REFERENCES

1) Ghazanfer S. A., ''Handbook of Arabian Medicinal Plants,'' CRC Press, 1994, pp. 180–181.
2) Belaiche P., ''Traité de Phytothérapie et D'Aromatherapie,'' Maloine, Paris, 1979, pp. 915–917.
3) Bruneton J., ''Plantes Médicinales, 3rd ed.,'' Lavoisier Tec., Paris, 1999, pp. 1095–1096.
4) Ali B. H., Blunden G., *Phytother. Res.*, 299–305 (2003).
5) Al-Ghamdi M. S., *J. Ethnopharmacol.*, **76**, 45–48 (2001).
6) Jafarabadi H., Hajhashmi V., Ghannadi H., *Phytother. Res.*, **18**, 195–199 (2004).
7) El-Sayed M., Hussiny A. H., Yassa A. I., *Deut. Lebensm. Rundsch.*, **93**, 149–152 (1997).
8) Ghosheh P., Houdi O. A., Crooks A., *J. Pharm. Biomed. Anal.*, **19**, 757–762 (1999).
9) Nickavar B., Mojab F., Javidnia K., Amoli M. A., *Naturforschung*, **58**, 629–631 (2003).
10) El-Dakhakhny M., Madi N. J., Lembert N., Ammon H. P., *J. Ethnopharmacol.*, **81**, 161–169 (2002).
11) Otlewski J., Krowarsch D., Apo-stoluk W., *Acta Biochim. Polon.*, **46**, 531–565 (1999).
12) Bank U., Engorge S., *J. Leukocyte*, **69**, 197–206 (2001).
13) Otlewski J., Jaskolski M., Buszek O., *Acta Biochim. Polon.*, **48**, 419–428 (2001).
14) Schefer J. C., *Phytother. Res.*, **10**, S6–S7 (1996).
15) Nakajima K., Powers J. C., Ashe B. M., *J. Biol. Chem.*, **254**, 4027–4031 (1979).
16) Ghosheh O. A., Houdi A. A., Crooks P. A., *J. Pharm. Biomed. Anal.*, **19**, 757–762 (1999).
17) El-Sayed A. M., Hussiny A. H., Yassa A. I., *Deut. Lebensm. Rundsch.*, **93**, 149–152 (1997).
18) Gad A. M., El-Dakhakhny M., Hassa M., *Planta Med.*, **11**, 134–138 (1963).
19) Rchid H., Namila R., Bessière J. M., Sauvaire Y., Chokaïri M., *JEOR*, **16**, 160–166 (2004).