

Preliminary communication

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA* CRUDE AND ESSENTIAL OIL

A. MOUWAKEH^a, P. RADÁCSI^b, Zs. PLUHÁR^b, É. NÉMETH ZÁMBORINÉ^b, G. MURÁNSZKY^c,
Cs. MOHÁCSI-FARKAS^a and G. KISKÓ^{a*}

^aDepartment of Microbiology and Biotechnology, ^bDepartment of Medicinal and Aromatic Plants, ^cDepartment of Food Chemistry and Nutrition, Szent István University, H-1118 Budapest, Villányi út 39–43. Hungary

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Nigella sativa L. (black cumin) is well known for its benefits in the field of traditional medicine. The aim of this study was to determine the chemical composition and investigate the antimicrobial activity of cold pressed oil (CO) and essential oil (EO) of *Nigella sativa* L. on food-borne pathogenic and spoilage bacteria. The microdilution method was used to determine the minimal inhibitory concentration (MIC) of *Nigella sativa* crude oil (CO) and essential oil (EO) against 4 Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Listeria monocytogenes*) and 3 Gram-negative (*Salmonella* Hartford, *Pseudomonas aeruginosa*, and *Escherichia coli*) food-borne pathogenic and spoilage bacteria occurring in food products. Total fatty acid composition of CO was analysed by GLC, while the EO was analysed by GC-MS to detect its active compounds. The results showed that the major fatty acid of CO was palmitic acid (C16:0), as saturated fatty acid, however, linoleic acid (C18:2) was the main unsaturated fatty acid. The major compounds of the EO were *p*-cymene and thymoquinone. The inhibition on all tested bacteria of EO was 10 times higher than of CO, and the lowest concentration value was observed in case of *Bacillus subtilis* (0.003%). Hence, results reinforce the ambition to apply *Nigella sativa* oils in food as natural preservative.

Keywords: *Nigella sativa*, crude oil, essential oil, chemical composition, antimicrobial activity.

Nigella sativa L. is a spicy plant, commonly known as black seed or black cumin, which has been traditionally used in the Arabian countries (SAYED, 1980) as herb, pressed seed oil, and as spice. Many healing effects related to respiratory, stomach, and intestinal diseases, circulatory and immune system support, and for the general well-being are attributed to it. The seed oil of *Nigella sativa* has been reported to have antioxidant, cytostatic, antibacterial, and anti-inflammatory activities (TOMA et al., 2010; SHOKRI, 2016).

Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against different microbes, including food-borne pathogens (FRIEDMAN et al., 2002).

As a source of natural microbial agents, essential oils (EOs) are becoming more popular due to their wide applications as food preservative, complementary medicine, and therapeutic agents (ROWSNI et al., 2014). The main constituents of essential oils, mono- and sesquiterpenes, phenols, alcohols, ethers, aldehydes, and ketones are responsible for the biological activity of aromatic and medicinal plants as well as for their fragrance. Due to these properties, spices and herbs have been added to food since ancient times, not only as flavouring agents but also

* To whom correspondence should be addressed.

Phone: 06-1-305-7201; fax: 06-1-305-7340; e-mail: kisko.gabriella@etk.szie.hu

as preservatives (KALEMBA & KUNICKA, 2003). Most of the antimicrobial activity in essential oils derived from spices and culinary herbs is believed to be linked to their phenolic compounds. *Nigella sativa* essential oil contains significant amounts of phenolic compounds, such as *p*-cymene, thymoquinone, and carvacrol, which might be the reason of their potential antimicrobial activity. The strength of inhibition and the spectrum of antimicrobial activity of *Nigella sativa* essential oil suggest that interactions between individual components led to the overall activity (HASSANIEN et al., 2015). Different extracts of *N. sativa* have broad antimicrobial spectra, including Gram-negative, Gram-positive bacteria, viruses, parasites and fungi. The effectiveness of *N. sativa* seeds is variable and depends on species of target microorganisms (FOROUZANFAR et al., 2014).

Over the last few years, increased interest in cold-pressed oils has been observed, as these oils have better quality than those after refining (PARRY et al., 2006). Cold-pressing involves no heat or chemical treatments. Cold-pressed black cumin seed oil may contain a higher level of hydrophilic phytochemicals, including natural antioxidants. Furthermore, antimicrobial activity of natural extracts is closely linked with their phenolic content (AHN et al., 2004). Therefore, oils rich in phenolics and other bioactive compounds may serve as potential natural antimicrobial agents (RAMADAN, 2013).

The aim of this study was to determine the chemical composition and investigate the antimicrobial activity of cold pressed oil and essential oil of *Nigella sativa* L. on food-borne pathogenic and spoilage Gram-positive and Gram-negative bacteria. Hence, gas chromatography–mass spectrometry (GC-MS) and gas-liquid chromatography (GLC) were used to determine the chemical composition of oils studied, and the microdilution broth method was used to evaluate their antimicrobial activity.

1. Materials and methods

1.1. Chemicals

Iso-octane HPLC grade (Merck) was used as solvent, all the other chemicals were of analytical grade.

1.2. Oils preparation

Nigella sativa seeds were purchased from local Turkish market and were cold pressed to produce the crude oil (CO). This last was hydro-distilled at 100 °C in a Clevenger apparatus to extract the essential oil (EO). The EO was collected, dried over anhydrous sodium sulphate, and stored finally in a refrigerator for further analysis. Dimethyl sulphoxide (DMSO) was used with tween 80 (1% final concentration in stock solution) to dissolve the CO and EO and set the appropriate concentration.

1.3. GC-MS analysis of the essential oil

The composition of the EO was determined by the method described by RADÁCSI and co-workers (2016) with the following modification: during the analysis only HP-5MS capillary column (5% phenyl, 95% dimethyl polysiloxane, length: 30 m, film thickness: 0.25 µm, id. 0.25 mm) was used.

1.4. Fatty acids methylation and GLC analysis

The fatty acids methylation and GLC analysis were carried out according to TOMA and co-workers (2013) with some modifications as follows: A quantity of 0.1 g of CO was dissolved in 2 ml *iso*-octane and 0.2 ml methanolic KOH (2 M) was added. The mixture was shaken for two min and allowed to stand for 10 min. The upper layer was removed and washed with water. The organic phase containing methyl esters of the fatty acids was diluted with *iso*-octane, then analysed by gas-liquid chromatography (GLC) using a Thermo Finnigan Trace GC, with split/splitless injector and flame ionization detector, a capillary column: Supelco, SP 2340 (30 m × 0.32 mm × 0.25 μm), the carrier gas was nitrogen (N₂) with a flow rate 0.5 ml min⁻¹. The column temperature was programmed from 70–180 °C at a rate of 4 °C min⁻¹.

1.5. Test organisms

Seven food-borne pathogenic and spoilage bacteria were included in this study: *Bacillus cereus* T1, *Bacillus subtilis* T1 from the culture collection of the Department of Microbiology and Biotechnology, Faculty of Food Science, SZIE, and *Staphylococcus aureus* ATCC6538, *Listeria monocytogenes* CCM4699, *Pseudomonas aeruginosa* ATCC9027, *Salmonella* Hartford B1310, and *Escherichia coli* B01909 from NCAIM (National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary). Bacterial isolates were subcultured at least twice from the stock solution in Tryptic Soy Broth (Sigma Aldrich, Germany) to obtain a fresh culture before analysis.

1.6. Antimicrobial activity

The antimicrobial activity of CO and EO were determined using broth microdilution method. Briefly, the stock solutions of CO and EO were serially half diluted in TSB to get the appropriate concentration in the microtiter plates. Then the culture was added at a concentration of 10⁶ CFU ml⁻¹ to the final volume of 0.1 ml/well. After 24 h of incubation at 37 °C, 10 μl of resazurin reagent was added to each well. After 2 hours of incubation at 37 °C, the fluorescence intensity was measured at 550 nm and 959 nm, using a microplate reader (Tecan, Mannedorf/Zurich, Switzerland). The minimum inhibitory concentrations (MICs) were defined as the minimal concentration at which the fluorescence signal declined to the level of the blank (negative control). All MICs measurements were carried out in triplicate. The positive controls were obtained by preparing culture medium with the bacterial suspension and DMSO with the bacterial suspension corresponding to the highest concentration present in the preparation. The negative control was obtained by preparing the culture medium with the oils or the DMSO.

2. Results and discussion

2.1. Fatty acids composition

The results of fatty acids analysis of *Nigella sativa* crude oil (Table 1) showed ten saturated and unsaturated fatty acids. The major fatty acid of CO was linoleic acid (18:2n-6) with a level of 55.03 g/100 g total fatty acids (TFA), followed by oleic (18:1n-9) with a level of 22.53 g/100 g TFA, and palmitic acid (16:0) with a level of 14.39 g/100 g TFA. The ratio of linoleic acid to oleic was about 2:1. This ratio agreed with previously published data (ATTA, 2003; NICKAVAR et al., 2003; PIRAS et al., 2013).

Table 1. Fatty acids composition of *N. sativa* crude oil

Fatty acid	Retention time (min)	Percentage (w/w %)
Myristic acid (C14:0)	17.132	0.35
Palmitic acid (C16:0)	20.835	14.39
Stearic acid (C18:0)	24.283	4.77
Arachidic acid (C20:0)	27.507	0.24
Behenic acid (C22:0)	30.523	1.44
Lignoceric acid (C24:0)	32.668	0.3
Palmitoleic acid (C16:1)	20.835	0.45
Oleic acid (C18:1)	27.738	22.53
Linoleic acid (C18:2)	25.823	55.03
Linolenic acid (C18:3)	27.177	0.49
Total saturated fatty acids (TSFA)		21.49
Total unsaturated fatty acids (TUFA)		78.05

Chemical composition of the oils depends on many factors, such as the origin of the samples and climatic conditions. Therefore, different varieties of chemotypes have been described in the literature. Our results (55.0% linoleic acid and 22.5% oleic acid) are in agreement with those reported by ATTA (2003), while black cumin oils from other geographical area contained different main constituents (e.g. a chemotype from Morocco with 33.0% *p*-cymene and 26.8% thymol and a chemotype from Poland with 60.2% *p*-cymene and 12.9% γ -terpinene were reported by PIRAS and co-workers, 2013).

Seed oils are the main sources of dietary unsaturated fatty acids. Among these, linoleic acid is an essential fatty acid that cannot be synthesized by the human body, yet is necessary for health. It has an important role, e.g. in the prevention and treatment of cardiovascular diseases (PREEDY et al., 2011). It is abundant in fatty seeds and their oils (e.g. olive oil (7–13%), peanut oil (14–43%), sesame oil (35.5%), pumpkin oil (44%), and poppy seed oil (74.5%)) (BOZAN & TEMELLI, 2008; PREEDY et al., 2011). Our black cumin oil contains significant amount of linoleic acid compared to other seed oils. This suggests that cold-pressed black cumin oil may also serve as a potential dietary source of polyunsaturated fatty acids.

2.2. Active compounds

The identified active components, their percentages, their retention indices, and their class can be seen in Table 2.

The results showed that 10 components were identified in the EO (seven monoterpene hydrocarbons (MH), two oxygenated monoterpenes (MO), and one sesquiterpene hydrocarbon (SH)) representing 94.03% of the total amount. The oil consisted mainly of MH with 71.69% followed by lower contents of MO with 20.51% and very low amounts of SH (1.83%). Therefore, among the monoterpene hydrocarbons, *p*-cymene was the major constituent (52.24%), while thymoquinone was dominating the oxygenated monoterpenes (18.76%).

Table 2. Chemical composition of *N. sativa* essential oil

Component	Retention time (min)	Percentage (%)*	LRI	Class
α -Thujene	5.31	6.08±0.30	928	MH
α -Pinene	5.56	1.46±0.04	938	MH
Sabinene	6.52	0.96±0.02	976	MH
β -Pinene	6.64	2.07±0.06	981	MH
<i>p</i> -Cymene	8.09	52.24±1.04	1026	MH
Limonene	8.19	2.21±0.11	1029	MH
γ -Terpinene	9.78	6.67±0.33	1033	MH
Thymoquinone	17.93	18.76±0.38	10,6	MO
Carvacrol	19.71	1.75±0.11	1251	MO
Longifolene	24.92	1.83±0.07	1290	SH

*Minor components (<1%) were not evaluated. MH: Monoterpene hydrocarbons; MO: oxygenated monoterpenes; SH: sesquiterpene hydrocarbon

The amount of *p*-cymene, which was found as a major compound in our oil, was higher than those reported in the literature (NICKAVAR et al., 2003; PIRAS et al., 2013) and quite similar to those reported by TOMA and co-workers (2010) in the case of hydrodistillation of Tunisian *Nigella sativa* essential oil extracts. However, thymoquinone that was found as second major compound of the essential oil, was similar to those reported by BENKACI-ALI and co-workers (2006) in the case of hydrodistillation of Algerian *Nigella sativa* essential oil extracts, and was much higher than those reported for the Tunisian and Iranian *N. sativa* essential oils (NICKAVAR et al., 2003; TOMA et al., 2010). These differences could be attributed to several factors, such as origin of the samples, environmental, climatic conditions, and harvest year.

2.3. Antimicrobial activity

The results of minimal inhibitory concentration (MIC) of the *Nigella sativa* crude and essential oils evaluated by microdilution method against seven bacteria (spoilage and/or pathogenic) are shown in Table 3. The results of the antimicrobial activity showed that both oils of *Nigella sativa* had a good activity on the tested strains. DMSO, as a diluent of oils, did not show any inhibitory effect on the growth of the strains.

Table 3. Minimal inhibitory concentration (MIC) of *N. sativa* crude oil and essential oil

Strains	Crude oil (w/w%)	Essential oil (w/w%)
<i>Bacillus cereus</i>	0.06	0.006
<i>Bacillus subtilis</i>	0.06	0.003
<i>Staphylococcus aureus</i>	0.25	0.025
<i>Listeria monocytogenes</i>	0.125	0.025
<i>Pseudomonas aeruginosa</i>	0.25	0.025
<i>Salmonella</i> Hartford	no effect	0.46
<i>Escherichia coli</i>	no effect	0.23

The activity of essential oil was 10 times higher than the crude oil. In addition, the MIC of essential and crude oils varied between 0.003–0.46% and 0.06–0.25%, respectively, for all bacteria tested.

The mode of action of essential oils depends on the properties of the essential oil and on the type of microorganisms mainly relating to their cell wall structure. Generally, Gram-positive bacteria are more sensitive to essential oils than Gram-negative bacteria. Resistance of Gram-negative bacteria to a wide variety of essential oils is associated with the hydrophilic surface of their lipopolysaccharide rich outer membrane that serves as a permeability barrier. Small hydrophilic molecules can pass through the outer membrane, but hydrophobic macromolecules (e.g. essential oil constituents) are not able to penetrate this barrier (KALEMBA & KUNICKA, 2003). Our results were in accordance with these statements, as the order of sensitivity of microorganisms was Gram-positive (in decreasing order: *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*) followed by Gram-negative (in decreasing order: *Salmonella* Hartford, *Escherichia coli*, *Pseudomonas aeruginosa*) for both oils. However, no inhibitory activity was observed against *Salmonella* and *E. coli* with the crude oil at the maximum concentration tested, 100% (it was checked with disc diffusion method (10 µl of the undiluted crude oil/disk), results are not shown).

A previous study has shown that long chain fatty acids have antimicrobial effect against a few strains of *Staphylococcus aureus* (KABARA et al., 1972). KABARA and co-workers (1972) compared the antimicrobial effect of saturated and unsaturated fatty acids and found that as compared with their saturated counterparts, the C14:1, C16:1, and C18:2 derivatives were more active. They also stated that the second double bond further increased the bacteriostatic effect of the C18 derivatives. Therefore, the antibacterial activity of our crude oil may be attributed to the notable amount of oleic (C18:1 - 22.53%) and linoleic acids (C18:2 - 55.03%) as the main components in the oil composition of *N. sativa*.

Characterization of *N. sativa* essential oil composition by gas chromatography-mass spectrometry analysis has revealed the presence of a variety of compounds possessing antimicrobial properties, including *p*-cymene and γ -terpinene (GÜLLÜCE et al., 2003), thymoquinone (HALAWANI, 2009), carvacrol (SUNTRES et al., 2015), and limonene (OUMZIL et al., 2002). Oxygenated monoterpenes (e.g. thymoquinone and carvacrol) exhibit stronger antimicrobial activity than hydrocarbon derivatives (e.g. *p*-cymene, limonene, γ -terpinene). The relative low inactivity of hydrocarbons can be attributed to their limited hydrogen bond capacity and water solubility (KNOBLOCH et al., 1988) that limit their penetration through the cell wall (OUMZIL et al., 2002). Although *p*-cymene (52.24%) formed a significant percentage of our essential oil, its antimicrobial effect was more likely caused by TQ (22.53%), which has greater antimicrobial potential as an oxygenated monoterpene. Crude oil also contained these compounds (as the essential oil was extracted from it), but the concentrations of these compounds were much higher in the essential oil due to the chemical extraction step, resulting much stronger antimicrobial activity.

3. Conclusions

In our study, the major fatty acids of the Turkish *Nigella sativa* crude oil were linoleic (18:2n-6), oleic (18:1n-9), and palmitic (16:0) acids, while the major compounds of its essential oil were *p*-cymene and thymoquinone. The essential oil of the Turkish *Nigella sativa* showed 10

times higher antimicrobial activity than the crude oil, however, the crude oil still showed good inhibitory effect against Gram-positive bacteria.

The present study showed that *Nigella sativa* essential oil was a source of biological active compounds, which contributed to its antimicrobial properties. Our findings enhance further study to use this oil as a potential food preservative.

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