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Original Scientific Article

Phenolic Compounds and Antioxidant Activity of Extracts of *Nigella sativa* L.*

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Abstract: *Nigella sativa* L. (Black cumin) is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Middle East, Eastern Europe and Western and Central Asia. This plant has been extensively investigated in recent years, due to its notable pharmacological properties. This work presents the investigation of phenolic content and antioxidant activity in extracts obtained from seeds of *N. sativa*, using Soxhlet and ultrasound extraction techniques. Total phenolics content was measured using the Folin-Ciocalteu method, and they varied from 11.867±0.338 to 31.148±0.293 mg/g GAE. Radical scavenging activity of the samples was examined using two methods, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), (ABTS) test, and reducing power of these samples was examined by reducing ferric, and molybdenum cations. All examined samples showed prominent antioxidant activity, except *p*-cymene. Thymoquinone and ethanolic extracts revealed the best results among six investigated samples.

INTRODUCTION

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines. Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions (Ghosheh, Houdi, and Crooks, 1999). There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds. Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Especially popular today is the concept of food that combines nutritional and medicinal benefits, especially antioxidant activity.

Reactive oxygen species (ROS) are often generated as by products of biological reactions or from exogenous factors. These reactive species exert oxidative damaging effects by reacting with nearly every molecules found in living cells including DNA, if excess ROS are not eliminated by antioxidant system (Krötz, Sohn, Gloe, *et al*., 2002).

Nigella sativa L. (Black cumin) is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Middle East, Eastern Europe and Western and Central Asia. This plant has been have been extensively investigated in recent years, due to its notable pharmacological properties (Dubick, 1986). It tastes slightly bitter and peppery with a crunchy texture. Seeds are angular, of generally small size (1–5 mg), dark grey of black colour.

Seed oil of *N. sativa* is considered as health beneficial one among newer sources of edible oils, thanks to its important role in human nutrition
and health. This seed oil has been reported to possess antitumor activity (Worthen, Ghosheh, and Crooks, 1998), antioxidant activity (Burits, and Bucar, 2000), anti-inflammatory activity (Houghton, Zarka, de la Heras, and Hoult, 1995), antibacterial activity (Morsi, 2000) and astimulatory effect on the immune system (Salem, and Hossain, 2000).

*Parts of this paper have been presented as poster presentations at the IX Meeting of Young Chemical Engineers, February 16-17 2012, Zagreb, Croatia.

This investigation was undertaken to obtain information about the phenolic composition of seeds of *Nigella sativa* L. from market in Sarajevo, Bosnia and Herzegovina, and to determine antioxidant activity of isolated extracts.

EXPERIMENTAL

Isolation

The seeds of *Nigella sativa* L. were grounded and weighted in two portions of 20.0 g. Each portion has been used for successive Soxhlet extraction, and ultrasound extraction, using *n*-hexane and 96% ethanol as solvents (Fig. 1). The solvents were evaporated using rotary evaporator and crude extracts were dissolved in dimethyl sulfoxide in concentrations 0.10-20.0 mg/mL.

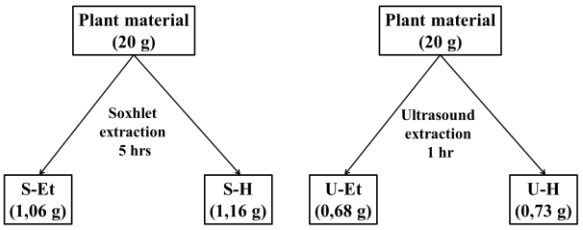

Figure 1: Extraction procedure.
S-Et, etanolic extraxt obtained by Soxhlet extraction; S-H, *n*-hexane extract obtained by Soxhlet extraction; U-Et, etanolic extraxt obtained by ultrasound extraction; U-H, *n*-hexane extract obtained by ultrasound extraction.

TLC preliminary investigation

Preliminary investigation of extracts composition were done by thin layer chromatography in toluene:ethyl acetate (93:7), and chloroform-acetone-formic acid (75:15:5) systems, for terpenoids and phenolics, respectively. Detection of sample components was done using vanillin-sulfuric acid, Folin-Ciocalteu and DPPH (1,1-diphenyl-2-picrylhydrazyl) reagents, and UV light. Thymoquinone, thymol and *p*-cymene were used as standards.

Determination of phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton, and Rossi, 1965), using gallic acid for calibration curve. Total flavone and flavonol content has been measured by spectrophotometric method using aluminum chloride as chromophore reagent (Woisky, and Salatino, 1998), using quercetine for calibration curve. Total flavonone content was measured using colorimetric method with 2,4-dinitrophenylhydrazine as specific chromophore for carbonyl compounds (Nagi, and Grancai, 1996), and naringenin was used for calibration curve.

Antioxidant activity

Radical scavenging activity of these samples was examined using two methods, 1,1-diphenyl-2-picrylhydrazyl (DPPH), (Ćavar, Maksimović, Šolić, *et. al.*, 2008), and 2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulphonic acid), (ABTS) test (Ćavar, Maksimović, Vidic, *et* al., 2012).

Reducing power of these samples was examined by reducing ferric (Ćavar, Maksimović, Vidic, *et* al., 2012), and molybdenum (Pisoschi, Cheregi, Danet, 2009) cations.

All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of radical, or transition metal. Thymoquinone and *p*-cymene were used as standard probes.

RESULTS AND DISCUSSION

Soxhlet and ultrasound extraction were employed for isolation of extracts *of N. sativa* seeds, with ethanol and n-hexane as solvents, and four extracts were obtained: S-Et (Soxhlet etanolic extract; yield: 5.30 %), S-H (Soxhlet *n*-hexane extract; yield: 5.38 %), U-Et (ultrasound ethanolic extract; yield: 3.38 %), and U-H (ultrasound *n*-hexane extract; yield: 3.64 %).

Preliminary investigation was done by thin layer chromatography (TLC) which has proved its worth as a simple, inexpensive method for the chemical and biological screening of plant extracts. Detection of natural products was done by spraying TLC plates with vanillin-sulfuric acid reagent, and for phenolic compounds using Folin-Ciocalteu reagent (Stahl, 1969). Positive detections were blue spots on white background (Fig. 2). The TLC plate with samples is developed with the elution solvent and dried. It is then sprayed with a DPPH solution. The plate is examined in daylight. Active (free-radical scavenging) compounds appear as yellow-white spots against a purple background (Marston, 2011).

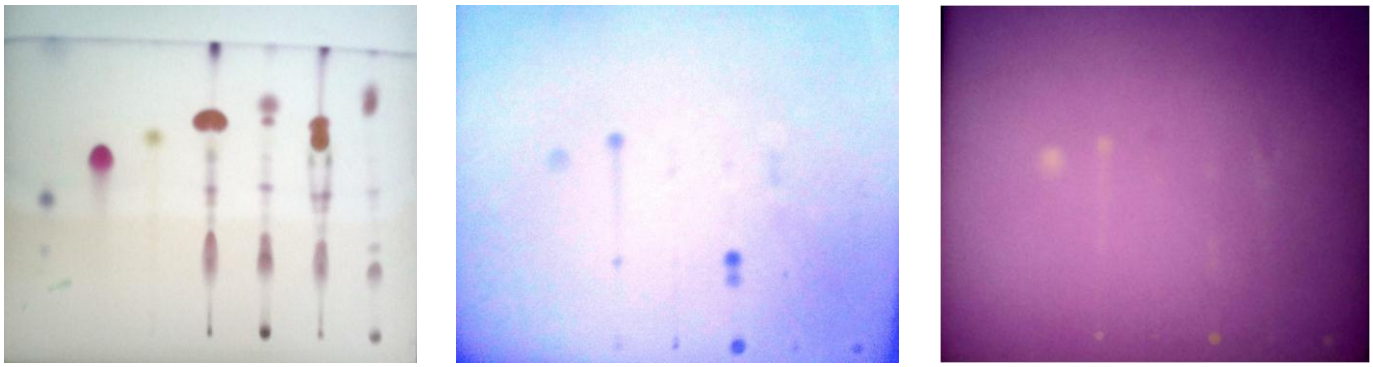

Figure 2: Thin-layer chromatograms of extracts of *N. sativa*.
(a) Vanilin-sulfuric acid reagent; (b) Folin-Ciocalteu reagent; (c) DPPH reagent; *p*-C, *p*-cymene; T, thymol; TQ, thymoquinone.

Results from the spectrophotometric determination of phenolic compounds were summarized in Table 1.

Table 1: The phenolic content of extracts of *N. sativa*.

Sample	TP mg GAE/g	TF-id mg GAE/g	TF-ol x 10^{-5} mg QE/g	TF-one x 10^{-3} mg NE/g
S-Et	**31.15±0.29**	**16.34±0.71**	6.86±2.34	3.64±0.46
S-H	5.58±0.31	6.35±1.40	**32.7±1.31**	1.52±0.16
U-Et	23.68±0.90	9.82±0.18	2.56±1.83	6.38±0.23
U-H	11.87±0.34	3.99±1.54	2.70±0.22	**8.09±0.65**

TP, total phenolics; TF-id, total flavonoids; TF-ol, total flavonols; TF-one, total flavonones.

Total phenolic content varied from 5.58 ± 0.31 to 31.15 ± 0.29 mg GAE/g (gallic acid equivalent), for Soxhlet etanolic and *n*-hexane extract, respectively, while total flavonoid content varied from 3.99 ± 1.54 to 16.34 ± 0.71 mg GAE/g, for ultrasound *n*-hexane and Soxhlet etanolic extract, respectively.

These results are expected due to the different polarity of used solvents. Ethanol is a polar solvent and extracts polar compounds. These results are consistent with the results Mariod *et al*. (2009), who also performed a determination of the total content of phenolic compounds in this plant. In comparison with results concerning *n*-hexane extracts, presented results are significantly lower than those published earlier (Martos, Mohamady, Fernández-Lòpez, *et al*., 2011). While, alcohol samples showed results comparable with those found in the literature (Tubesha, Iqbal, and Ismail, 2011).

Total flavone and flavonol content were ranged from (2.70 ± 0.22) x 10^{-5} to (32.7 ± 1.31) x 10^{-5} mg QE/g (quercetin equivalent). This result is consistent with these found in the literatue (Tubesha, Iqbal, and Ismail, 2011).

Total flavonone content varied from (1.52 ± 0.16) x 10^{-3} to (8.09 ± 0.65) x 10^{-3} mg NE/g (naringenin equivalent). To the best of our knowledge, there is no data concerning the the content of total flavanones in this plant species.

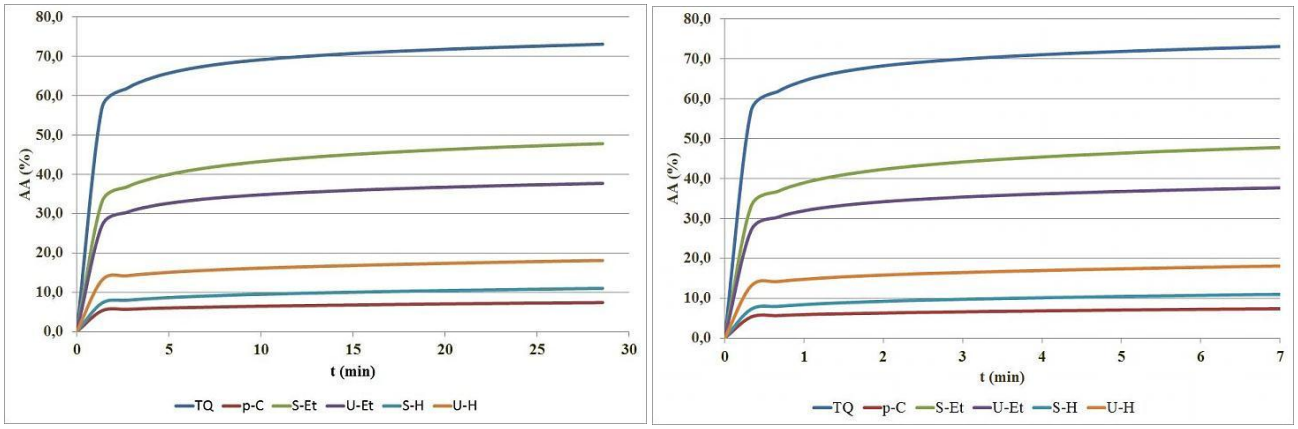

Figure 3: Progress of antioxidant activity of extracts of *N. sativa*.
(a) DPPH test; (b) ABTS test.

Antioxidant activity (Table 2) of isolated extracts as well as *p*-cymene and thymoquinone, as constituents of extracts, was examined by four different testing methods.

Figure 3 presents the progress of antioxidant activity of examined samples in concentration of 1.0 mg/mL tested by DPPH and ABTS methods.

The ability of samples to reduce stable DPPH radical, presented as IC_{50} values, were ranged from 0.70 ± 0.0.01 mg/mL, for thymoquinone, to 129.65 ± 1.67 mg/mL, for *p*-cymene. Moreover, thymoquionone showed the lowest IC_{50} values in the reduction of stable ABTS radical, while *p*-cymene showed the highest. Extracts of seeds of *N. sativa* revealed promient antioxidant activity in comparison with these two natural compounds.

However, the ability of samples to reduce molybdenum cations (Table 2), presented as IC_{50} values, were ranged from 12.90 ± 0.29 mg/mL, for U-Et, to 346.84 ± 8.57 mg/mL, for *p*-cymene. Moreover, thymoquionone showed the lowest IC_{50} value in reduction of ferric cations (20.23 ± 0.23 mg/mL), while again *p*-cymene revealed the lowest reduction potential (155.48 ± 9.48 mg/mL).

Table 2: Antioxidant activity of extracts of *N. sativa*.

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	RP-Fe IC_{50} (mg/mL)	RP-Mo IC_{50} (mg/mL)
S-Et	1.98±0.08	**14.02±0.62**	68.21±1.11	13.38 ±0.54
S-H	12.04±0.60	17.01±0.64	71.78±0.81	51.88±0.64
U-Et	**3.01±0.03**	16.79±1.05	**29.31±0.94**	**12.90±0.29**
U-H	8.177±0.11	18.67±1.54	73.32±3.91	25.45±0.59
p-C	129.65±1.67	165.65±16.43	155.48±9.48	346.84±8.57
TQ	0.70±0.01	6.07±0.57	20.23±0.23	39.98±2.12

In general, among examined estracts, ethanolic extracts revealed the lowest IC_{50} values that indicate the best antioxidant activity, compared with thymoquinone, an already known natural antioxidant (Milos, and Makota, 2012). This can be explained by the fact of high content of phenolic compounds found in these extracts.

Although DPPH and ABTS methods were based on the same principle; data obtained from ABTS assay are lower than those obtained from DPPH assay, but comparable. This is probably due to the steric factors that are one of the major factors for reducing of stable DPPH radical. Moreover, the IC_{50} values obtained from these two radical methods are much lower than IC_{50} values obtained from the methods of reduction of transition metals, iron and molybdenum.

The transition metal ions possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions even starting with relatively non-reactive radicals. The main strategy to avoid generation of reactive oxygen species is associated with redox active metal catalysis involves chelating of the metal ions.

However, presented results are in the agreement with those found in the literature (Khattak, Simpson, and Ihasnullah, 2008; Bourgou, Ksouri, Bellila, *et al*., 2008) and they suggest further analysis on chemical composition of the plant extracts in order to identify compounds with antioxidant properties.

CONCLUSIONS

To the best of our knowledge, this is the first study providing data on phenolic compounds and antioxidant activity of extracts of seeds of *Nigella sativa* L. found in the market in Sarajevo, Bosnia and Herzegovina. The samples obtained from investigated plant species are quite interesting from a pharmaceutical standpoint because of its prominent antioxidant properties.

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Summary/Sažetak

Nigella sativa L. je godišnja zeljasta biljka koja pripada obitelji Ranunculaceae. Biljka obično raste na Bliskom Istoku, Istočnoj Europi i zapadnoj i središnjoj Aziji. Zbog svojih značajnih farmakoloških svojstava ova biljna vrsta je intenzivno istraživana u posljednjih nekoliko godina. Ovaj rad predstavlja određivanje sadržaja fenolskih spojeva i antioksidacijske aktivnosti u ekstraktima dobivenih iz sjemenki *N. sativa*, koristeći Soxhlet i ultrazvučnu ekstrakciju. Ukupan sadržaj fenolskih spojeva određen je spektrofotometrijskom Folin-Ciocalteau metodom, i on varira od 11.867 ± 0.338 do 31.148 ± 0.293 mg GAE/g. Antioksidacijska aktivnost ekstrakata ispitana je pomoću četiri spektrofotometrijske metode. Dvije metode su bazirane na reduciranju slobodnih radikala, 1,1-difenil-2-pikrilhidrazil i 2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulfonska kiselina), a dvije na reduciranju prelaznih metala, željeza i molibdena. Svi ispitivani uzorci su pokazali značajnu antioksidativnu aktivnost, osim *p*-cimena. Timokinon i etanolni ekstrakti su pokazali najbolje rezultate.