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CHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF THE ESSENTIAL OIL OF *NIGELLA SATIVA* L.

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Abstract

The chemical composition of the essential oil obtained from *N. sativa* was analyzed by GC/MS and the components identified were: *p*-cymene (22.05%) followed by *α*-thujene (6%), *α*-pinene (1.11%), camphene (11%), sabinene (1%), *β*-pinene (7%), *β*-myrcene (0.21%), *α*-phellandrene (0.45%), limonene (0.13), γ-terpinene (5.12%), terpinolene (0.23%), camphor (1%), carvone (0.32%), thymoquinone (20.32%), thymol (10.12%), carvacrol (10%), longicyclene (0.9%) and borneol (0.43). The oils were also subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Thymol (13.0 ± 0.8 µg/ml), thymoquinone (12.6 ± 0.0 µg/ml) and carvacrol (12.03 ± 0.0 µg/ml) showed appreciable antioxidant activity in DPPH test. Antioxidant activity guided fractionation of the oil was carried out by the TLC-bioautography screening and fractionation resulted in the separation of the main antioxidant compound which were identified as thymoquinone (51%), thymol (25%) and carvacrol (8%).

Introduction

Essential oils are aromatic oily liquids, volatile, characterized by a strong odour, rarely coloured, and generally with a lower density than that of water. They can be synthesized by all plant organs and therefore extracted from these parts, where they are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Burt 2004; Bakkali *et al.* 2008). In nature, essential oils play an important role in as antibacterials, antivirals, antifungals, insecticides, herbicides, or have feeding deterrent effects against herbivores by reducing their appetite for such plants. The antioxidant activity of essential oils is another biological property of great interest because they may preserve foods from the toxic effects of oxidants (Maestri *et al.* 2006). Black cumin (*Nigella sativa* L.) belongs to Ranunculaceae family. As an aromatic plant, *N.sativa* is widely grown in different parts of the world and the seeds of black cumin have been used to promote health for countries especially in the Middle East and Southeast Asia (Hedrick 1972). *N. sativa* seeds yield esters of fatty acids, free sterols and steryl esters (Menounos *et al.* 1986). The seeds also contain lipase, phytosterols and sitosterol (Duke 1992). During the past few decades, many phytochemical and pharmacological studies have been conducted on *N. sativa* seeds because of its marked biological activities, antioxidant, anti-inflammatory and antiulcer activity (Ali and Blunden 2003). The biological properties of the essential oils have been found to be directly linked to their chemical compositions, which are influenced by the origin of the plants (Celiktas *et al.* 2007). An Iranian *N. sativa* essential oil was found to be dominated by phenylpropanoid components and displayed a trans-anethole chemotype (Nickavar *et al.* 2003). The main purpose of this study was to investigate the chemical composition of *N.sativa* essential oil and to determine its antioxidant activity by scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) test.

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Materials and Methods

The plant materials were collected from the mountains in the city of Ilam-Iran in 2012- 2013. The *N.sativa* seeds were ground and the resulting powder was subjected to hydrodistillation for 3 hours in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia (1975). The obtained essential oil was dried over anhydrous sodium sulphate and after filtration, stored at +4ºC until tested and analysed.

The GC/MS analyses were executed on a Hewlett–Packard 5973N gas chromatograph equipped with a column HP-5MS (30 m length × 0.25 mm i.d., film thickness 0.25 l m) coupled with a Hewlett-Packard 5973N mass spectrometer. The column temperature was programmed at 50ºC as an initial one, holding for 6 min, with 3ºC increases per minute to the 240ºC, followed by a temperature enhancement of 15ºC per minute up to 300ºC, holding at the mentioned temperature for 3 min. Injector port temperature was 290ºC and helium used as carrier gas at a flow rate 1.5 ml/min. Ionization voltage of mass spectrometer in the EI-mode was equal to 70 eV and ionization source temperature was 250ºC. Linear retention indices for all components were determined by coinjection of the samples with a solution containing homologous series of C8-C22 *n*-alkanes and comparing them and their mass spectra with those of authentic samples or with available library data of the GC/MS system (WILEY 2001 data software) and Adams libraries spectra (2001).

The efficacy of the essential oils to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method (Cuendet *et al.* 1997, Kirby and Schmidt 1997). On basis of bleaching of the bluish-red or purple colour of DPPH solution as a reagent. Briefly, a 50 ll volume of various dilutions of each samples was mixed with 5 ml of 0.004% methanol solutions of DPPH followed by 30 min incubation at ambient temperature. Thereafter, the sample absorbance was recorded against control at 517 nm. The inhibition percentages were measured using Eq. (1). The antioxidants activity of the test samples in concentration providing 50% inhibition, were considered as IC50 (lg/ml).

$$\text{Inhibition per cent} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \times 100 \quad (1)$$

Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls. All experiments were repeated three times and the average results and standard deviations calculated.

For screening of antioxidant compounds in *N.sativa* essential oil, the TLC-bioautography method was carried out (Burits and Bucar 2000, Guleria *et al.* 2012). The diluted oil (1 : 20 in methanol) was spotted on silica gel sheets (silica gel 60 F254 TLC plates) and developed in *n*-hexane-ethyl acetate (9 : 1). Plates were sprayed with the methanolic solution of DPPH• (0.2%). The active constituents were detected as yellow spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

For the isolation and identification of the active compounds in the essential oil, PTLC was performed using the conditions previously described (Guleria *et al.* 2012). The regions showing DPPH• scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC/MS and also tested for their antioxidant activities.

The quantitative data of major components of oil were statistically examined by one-way analysis of variance (ANOVA), and significant differences among groups were subsequently analyzed by DMRT ($p < 0.05$). Correlation and regression coefficients were performed using Statistical Package for the Social Sciences (SPSS).

Results and Discussion

The essential oil of *N. sativa* seeds obtained using hydrodistillation was isolated in high yield (0.84%). Results of GC/MS analysis of the essential oil (Table 1) indicate that the essential oil was characterized mainly by monoterpenes. The major constituent of the oil was the hydrocarbon monoterpene *p*-cymene, with a relative concentration of 22.05%. The GC/MS analysis of *N. sativa* oil showed 18 compounds representing 98.39 % of the total oil; *p*-cymene was the main constituent (32.05%) followed by *α*-thujene (6%), *α*-pinene (1.11%), camphene (11%), sabinene (1%), *β*-pinene (7%), *β*-myrcene (0.21%), *α*-phellandrene (0.45%), limonene (0.13), γ-terpinene (5.12%), terpinolene (0.23%), camphor (1%), carvone (0.32%), thymoquinone (20.32%), thymol (10.12%), carvacrol (10%), longicyclene (0.9) and borneol (0.43). Previous studies have shown monoterpenes, including *p*-cymene, α-thujene, γ-terpinene, carvacrol, α-pinene and *b*-pinene, to be the main components of the essential oil from black cumin (Burits and Bucar, 2000, Rchid *et al.* 2004). Our results reinforce previous data on the variability seed volatile oils, depending on the origin of the samples, environmental and climatic conditions. A variety of chemotypes have been described in the literature. In Iranian *N. sativa* essential oil was found to be dominated by phenylpropanoid components and displayed a trans-anethole chemotype (Nickavar *et al.* 2003), other *N. sativa* from Iran (Hajhashemi *et al.* 2004), Algeria (Benkaci-Ali *et al.* 2007) and India (Singh *et al.* 2005) was found *p*-cymene/thymoquinone chemotype. It has been reported that the chemical compositions of the essential oil are highly influenced by climatic conditions and geographical factors (Fatima *et al.* 2002, Shareef 2011). The high level of *p*-cymene, thymoquinone and thymol in the essential oil could contribute to the valorization of Iranian *N. sativa* species, since this monoterpene is of great importance in industry as intermediate for synthesis of fragrances, pharmaceuticals and herbicides.

Table 1. Chemical composition of *Nigella sativa* volatile oil constituents.

Compound	%	RI	Compound	%	RI
α-Thujene	6	916	γ-Terpinene	5.12	1068
α-Pinene	1.11	920	Terpinolene	0.23	1080
Camphene	11	928	Camphor	1	1120
Sabinene	1	956	Borneol	0.43	1168
β-pinene	7	960	Carvone	0.32	1240
β-myrcene	0.21	968	Thymoquinone	20.32	1252
α-phellandrene	0.45	1000	Thymol	10.12	1290
limonene	0.13	1020	Carvacrol	10	1301
p-Cymene	22.05	1022	Longicyclene	0.9	1387
Total			98.39		

Reactive oxygen species (ROS), including oxygen radicals and their reaction products, are known to react with biological molecules, leading to cell and tissue damage. *In vitro* methods have been widely used to assess antioxidant properties of medicinal plant extracts, however, these assays are often very specific for a particular mode of action and do not necessarily reflect the normal biological context in which they react (Girard-Lalancette *et al.* 2009). Antioxidant activity is a complex process usually occurring through several mechanisms. Due to its complexity, the evaluation of the antioxidant activity for pure compounds or extracts should be carried out by more than one test methods (Aruoma 2003). Recently, DPPH radical scavenging activity, a useful indicator of reactive oxygen species (ROS), has been developed as a new sensitive test which allows detection of both the anti- and pro-oxidant properties. The lower IC_{50} value indicates a

stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. The results presented in Table 2 revealed that *N.sativa* essential oil and its main constituents exhibited a remarkable activity. In particular, Thymoquinone showed clearly a higher activity (IC50 = 12.6 ± 0.0 µg/ml) followed by *N.sativa* essential oil (14.56 ± 0.4 µg/ml), thymol (13.0 ± 0.8 µg/ml) and carvacrol (12.03 ± 0.0 µg/ml). α-Thujene (22 ± 0.1 µg/ml), camphene (23 ± 0.9 µg/ml), b-pinene (19.4 ± 0.0 µg/ml), *p*-cymene (20 ± 0.4 µg/ml) and γ-terpinene (20.03 ± 0.6 µg/ml) were inactive (Table 2), despite previous reports of their *in vitro* antioxidant activities (Ruberto and Baratta 2000). BHT and ascorbic acid as positive controls were

Table 2. DPPH radical scavenging activity of *Nigella sativa* seeds essential oil and its main constituents. Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls.

Tested compounds	IC50 (µg/ml)
Nigella sativa essential oil	14.56 ± 0.4
α-Thujene	22 ± 0.1
Camphene	23 ± 0.9
β-pinene	19.4 ± 0.0
P-Cymene	19.7 ± 0.1
γ-Terpinene	20.03 ± 0.6
Thymoquinone	12.6 ± 0.0
Thymol	13.0 ± 0.8
Carvacrol	12.03 ± 0.0
BHA	13.09 ± 0.0
AA	14.00 ± 0.4

Table 3. Components identified and their antioxidant activity relative percentages constituents.

compounds	Percentage
α-Thujene	trace
Camphene	trace
β-pinene	4
P-Cymene	5
γ-Terpinene	1
Thymoquinone	51
Thymol	25
Carvacrol	8

exhibited IC_{50} values equal to 13.09± 0.0µg/ml and 14.00± 0.4 µg/ml, respectively. The observed antioxidant potential should be addressed to the phenolic oil constituents (Hazzit *et al.* 2009), while the oil chemo-protective efficacy against oxidative stress-mediated disorders is mainly due to its free radical scavenging and metal chelating properties. Thymoquinone, carvacrol and thymol have been reported to contribute to the in vitro antioxidant activity of essential oil (Piccaglia *et al.* 1993, Burits and Bucar 2000). Therefore, a preliminary screening was initially carried out using the dot-blot DPPH• staining method on TLC. As the essential oil presented a significant antioxidant activity in the assays and bioautography test, it was subjected to the PTLC for isolation of the active compounds. Components identified and their antioxidant activity relative

percentages are shown in Table 3. The major compound found in the active band were thymoquinone (51%), thymol (25%) and carvacrol (8%). Woo *et al.* (2012) have noted that thymoquinone could act as a free radical and superoxide radical scavenger, as well as preserving the activity of various antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione-S-transferase.

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