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FULL LENGTH ARTICLE

Chemical investigation of *Nigella sativa* L. seed oil produced in Morocco

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Abstract Seeds of *Nigella sativa* L. (black cumin or black seeds) are widely used in traditional Islamic medicine and for culinary purposes worldwide. Nigella seed oil is becoming popular in and out of the Islamic world. Composition of Nigella seed oil is known to be location-dependent. We investigated the composition of Nigella seed oil prepared by solvent- or cold press-extraction of Nigella seeds grown in Morocco. Oil extraction yield was 37% and 27% when solvent or cold press extraction methods were used, respectively. In terms of oil major components, composition of Nigella seed oil from Morocco is similar to that from other Mediterranean countries known for their Nigella seed-oil quality.

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1. Introduction

Nigella sativa L. (Ranunculaceae family) is an annual herbaceous plant whose growth area extends from the countries of the southern- and eastern-rim of the Mediterranean basin to Iran, Pakistan, and India. In the ethnopharmacology of those countries, *N. sativa* seeds are used to cure gastro-intestinal disorders as well as skin or respiratory ailments (Riaz et al., 1996). Several other pharmacological properties have been

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traditionally attributed to *N. sativa* seeds, simply as a crushed powder, or as an extract. Purified or as a mixture, metabolites of *N. sativa* seeds would present a potent and therapeutically interesting activity on the cardiovascular, respiratory, immune, and endocrine systems (Gilanie et al., 2004; El-Tahir et al., 2006; Ait Mbarek, 2007). Additional properties are frequently discovered (Al-Okbi et al., 2013). Most of these activities have already been attributed to thymoquinone, a major component of the essential oil of the seeds (Ali and Blunden, 2003). Additionally, in its native range and far beyond, *N. sativa* seeds are also frequently used as spice and condiment in various recipes due to their characteristic aroma and bitter and peppery taste (Hedrick and Sturtevant, 1972). Finally, *N. sativa* seeds are used to prepare a highly prized nutritive oil. Although on the world scale Nigella seed oil does not really have a significant economic market share, yet, it nevertheless constitutes a niche market whose size is constantly growing due to its alleged pharmacological properties, and to spirituous reasons resulting from its mention in sacred texts and reports of the presence of Nigella seeds in Tutankhamen tomb (Padhye et al., 2008).

In Morocco, large-scale production of Nigella seed oil is currently envisioned as a supplementary source of income for argan oil cooperatives. However, oil content of Nigella seed from Morocco has been reported to be low, compared to other regions (D'Antuono et al., 2002). Nevertheless, these results are questionable since for this study Nigella seeds were grown in Northern Italy. Because, previous investigations regarding *N. sativa* seed oil composition or yield have reported large variations (Cheikh-Rouhou et al., 2007), possibly resulting from a different plant geographical origin, we decided to investigate the chemical composition of *N. sativa* seed oil cultivated in Morocco. The oil was prepared by solvent- or cold press-extraction. This study is aimed at providing suitable tools for the resolution of two matters: (1) Could *N. sativa* seed oil from Morocco be used, alone or together with Nigella seed oil from a different origin, in the cosmetic, pharmacological or nutritious domains? and (2) Is Nigella seed oil from Morocco different from Nigella seed oil from a different origin and, if so, can these differences be characterized in order to possibly detect adulterations that, regretfully, are quite frequent (Aitzetmuller et al., 1997).

2. Material and methods

2.1. Plant material and chemicals

Cultivation of *N. sativa* was performed in the region of Gharb-Chrarda-Beni Hssen (34° 15′ 00″ North, 6° 35′ 00″ West) where the year average temperature reported is 17.4 °C (average low: 15.5 °C, average high: 19.3 °C) and average precipitation is 600 mm between October and May falls to nearly zero between May and October (Worldweatheronline, 2013).

Seeds were harvested in June 2012 in the agricultural province of Had-Kourt (Region of Gharb-Chrarda-Beni Hssen, North-West of Morocco). After harvest, the seeds were stored at 4 °C until processed.

All the reagents were of analytical or HPLC grade. Iso-octane and isopropanol used as HPLC mobile phase and cyclohexane used for extinction coefficient determination were purchased from Professional Labo (Casablanca, Morocco).

2.2. Seed analysis

Seed moisture content, expressed as percentage by mass, was determined using 5 g of seeds by adapting the AOAC method 934.06 (AOAC, 1990). A Jouan Quality Systems oven regulated at 105 °C was used. The difference between the results of two last determinations was 0.1 g of moisture per 100 g of sample. Oil yield was calculated following the DIN EN ISO 659 recommendation (ISO 659, 2009).

Nutritional composition of the Nigella seed oil cake was determined using the recommended methods of the association of official analytical chemists (AOAC, 2005). Ash content was determined by incinerating 5 g of oil cake at 550 °C in a muffle furnace. Crude protein content was calculated from the nitrogen content measured by the Kjeldahl procedure with Gerhardt model Vapodest 20 instrument, using a factor 6.25. Crude fiber was determined according to the gravimetric procedure on defatted samples. The difference between dry weight and ash content of the residue was taken as an estimation of the crude fiber content (AOAC, 2005). All analyses were performed in triplicate.

2.2.1. Nigella oil analysis

Oil extraction: Press-extraction was carried out using screwless cold presses (IBG Monforts Oekotec GmbH, Mönchengladbach, Germany). Solvent-extraction was performed using 20 g of ground seeds placed in a Soxhlet apparatus and extracted with hexane for 8 h. The organic phase was then concentrated under vacuum and dried for 5 min in an oven at 103 ± 2 °C. Oil samples were stored at 4 °C and protected from sunlight prior analysis.

Fatty acid composition: Fatty acid composition was determined following regulation EEC/2568/91 (EEC/2568, 2003). Before analysis, fatty acids (FAs) were converted to fatty acid methyl esters (FAMEs) by shaking for 25 min a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2 N methanolic potassium hydroxide. FAMEs were analyzed by gas chromatography using a Varian CP-3800 (Varian Inc.) chromatograph equipped with a FID. A split injector was used and the injected volume was 1 µl. The column used was a CP-Wax 52CB column (30 m × 0.25 mm i.d.; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium and the total gas flow rate was 1 mL/min. The initial and final column temperature was 170 and 230 °C, respectively, and the temperature was increased by steps of 4 °C/min. The injector and detector temperature was 230 °C. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Results were expressed as the relative percentage of each individual FA present in the sample.

Sterol composition: Sterol composition was determined using the NFT 60–254 method as previously described (Hilali et al., 2005). After trimethylsilylation of the crude sterol fraction, using a Varian 3800 instrument equipped with a VF-1 ms column (30 m; 9 0.25 mm i.d.) and helium (flow rate 1.6 mL/mn) as carrier gas a splitless injector was used and the volume injected set at 1 µl. Column temperature was isothermal at 270 °C, injector and detector temperature was 300 °C. Injected quantity was 1 µL for each analysis. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA).

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