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ORIGINAL ARTICLE / ÖZGÜN MAKALE

QUANTITATIVE ANALYSIS OF THYMOQUINONE IN *NIGELLA SATIVA* L. (BLACK CUMIN) SEEDS AND COMMERCIAL SEED OILS AND SEED OIL CAPSULES FROM TURKEY

TÜRKİYE'DE YETİŞEN *NIGELLA SATIVA* L. (ÇÖREK OTU) TOHUMLARI, TİCARİ TOHUM YAĞLARI VE TOHUM YAĞI KAPSÜLLERİNDEKİ TİMOKİNONUN KANTİTATİF ANALİZİ

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SUMMARY

Medicinally and economically important plant Nigella sativa L., popularly known as çörek otu in Turkey, is cultivated in many parts of Turkey. The most important component of its oil is thymoquinone. Nigella sativa is used in alternative medicine to treat cancer, rheumatism, headaches, and many diseases for thousands of years. In this study, 10 different seed and seed oil samples that have been supplied from local markets in Ankara, were investigated for their thymoquinone contents by HPLC.

Keywords: *black cumin; HPLC; Nigella sativa; thymoquinone*

ÖZET

Tıbbi ve ekonomik olarak oldukça büyük bir öneme sahip olan ve Türkiye'de çörek otu olarak bilinen Nigella sativa, Türkiye'nin çeşitli bölgelerinde yetiştirilmektedir. Yağının en önemli bileşiği timokinondur. Nigella sativa alternatif tıpta kanser, romatizma, başağrısı ve daha birçok hastalığa karşı binlerce yıldır kullanılmaktadır. Bu çalışmada; Ankara'da bulunan aktarlardan temin edilmiş 10 farklı çörekotu tohumu ve yağı, içerdikleri timokinon miktarlarına göre yüksek performanslı sıvı kromatografisi (HPLC) ile incelenmiştir.

Anahtar kelimeler: *çörek otu; HPLC; Nigella sativa, timokinon*

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INTRODUCTION

Medicinal plants have been an extensive source of therapeutic agents since ancient times for healing human diseases. *Nigella sativa* L., belonging to Ranunculaceae, is used as a natural remedy against to a number of illnesses and conditions such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza, for over 2000 years. It is a spicy plant and refined in various parts of the world. The seeds are known as "black cumin" or "black caraway", likewise "çörek otu" in Turkey and traditionally been used in the Indian sub-continent, Arabian countries, Middle Eastern, and Far Eastern Countries both as spice and natural remedy. In Turkey, it is cultivated in Afyon, Burdur and Isparta regions. It is an annual plant and grows up to 15–30 cm, branched, more or less furry with finely divided, linear (but not thread-like) leaves. The flowers are delicate, and usually white coloured, with five petals; the seeds are three-sided and black [1-3]. The seeds and the oil are believed to be carminative, diuretic, lactagoge and vermifuge [4]. Pharmacologically, active constituents of seed oils are thymoquinone, dithymoquinone, thymohydroquinone and thymol [5].

The seeds and/or oils of *Nigella* are also used in foods as spice and seasoning. According to the Islamic belief; the 'black seed', is accepted as a panacea (universal cure) i.e. a remedy for all ailments, but cannot prevent ageing or death in Arabic countries s well [6].

The chemical composition of *N. sativa* seeds was reported for the first time in 1880, and it is found to be composed of oils, proteins, carbohydrates, fibers, ashes, moisturizers, etc. The fixed oil component of *N. sativa* (36–38%) mostly consisted of linoleic (50–60%), oleic (20–23.4%), palmitic (12.5%), dihomolinoleic (10%), and eicosadienoic (3%) acids as well as arachidonic, stearic, and myristic acids; betasitosterol; cycloeucalenol; cycloartenol; sterolesters; and sterol glucosides with some other minor lipid constituents such as methylnonadeca-15,17-dienoate, pentylhexadec-12-enoate, and pentylpentadec-11-enoate. Their multifunctional preventive and relieving effects have been associated to significant constituents such as nigellicine, nigellidine, thymoquinone (TQ), dithymoquinone, thymol, and carvacrol [7].

Screening of vitamin content of the seeds indicated that *N. sativa* seeds contain significant amounts of vitamins B1, B6 and niacin, additionally vitamin B2 and folic acid relatively in smaller amounts. The results are summarized at Table 1 [8].

Table 1. Vitamin composition of *N. sativa* L. seeds [8]

Vitamin	Found (µg per 100 g)	RDA*(%)
B1 (Thiamin)	831 + 11.36	55.3
B2 (Riboflavin)	63 + 3.32	3-5
B6 (Pyridoxine)	789 + 8.89	35.9
PP (Niacin)	6311 + 16.52	33.2
Folic acid	42 + 4.58	10

*Recommended Dietary Allowances (Anon., 1980).

N. sativa seeds contain fixed oil, saponins, alkaloids, proteins and essential oil. Thymoquinone is the most prominent constituent in *N. sativa* and there has been, 406 posted research reports about TQ on the "PubMed" database since 1960. Beside the popularity of the Thymoquinone, it is also the main responsible constituent of the hepatoprotective, the anti-inflammatory and the anti-cancer and antitumor effects of *Nigella*'s seed essential oil [9].

The results of a study which investigate the hepatoprotective effects of TQ against acetaminophen-induced hepatotoxicity on Wistar albino rats shows that, the levels of aspartat aminotransferase (AST), oxidized glutathione (GSSG), serum alanine aminotransferase (ALT) and superoxide dismutase (SOD) activity were found to be lower compared to that of rats treated without thymoquinone [10].

According to Al Wafai [11], *N. sativa* and TQ treatment is responsible of the suppression of COX-2 enzyme's expression in the pancreatic tissue of streptozotocin (STZ)-induced diabetic rats.

It was shown that essential oil and methanolic extract of *N. sativa* and its active principle, thymoquinone, displays potent antileishmanial effects against *L. tropica* and *L. infantum* (genus of trypanosomes) species in the *in vitro* model [12].

In a recent study, immunohistochemical analysis confirmed that thymoquinone significantly decreased Cd-induced over expression of nuclear factor-κB in renal tissue. Moreover, thymoquinone treatment resulted decrease of the in apoptotic cell numbers. Thymoquinone considerably suppressed lipid peroxidation, atoned deficits in the antioxidant defenses (reduced superoxide dismutase, glutathione peroxidase and catalase activities) in renal tissue induced by Cd administration. These results suggest that thymoquinone's antioxidant and anti-apoptotic properties would be more advantageous for achieving maximum effects in nephrotoxicity induced by Cd [13].

In this study, all samples (seeds, seed oils and seed oil capsules) have different trademarks that supplied from local markets in Ankara and they were investigated for their thymoquinone contents by HPLC.

MATERIALS AND METHODS

Chemicals and Materials: HPLC grade methanol and 2-propanol (Fisher Scientific, Pittsburgh, PA) were used. Millipore filtered water was obtained by passing distilled water through a Milli-Q system (Millipore Corp., Milford, MA). Thymoquinone (Sigma, St. Louis, MO) was purchased and dissolved in HPLC grade methanol for the analysis.

Samples: Samples were supplied from local markets in Ankara. Each seeds, seed oils and seed oil capsules have a different brand. The seeds were coded as NS 1 to 10, the seed oils were coded as NO 1 to 10 and the seed oil capsules were coded as NOC 1 to 10.

Seed extraction: 5 g seeds were powdered and stirred with 200 ml methanol for 2 hours than filtered and evaporated by using rotavapor. The residue was solved gradually with methanol and completed to 50 ml with methanol.

Oil extraction: 20 µL black cummin oils of both oil products and capsules were filtered from Agilent ZORBAX SPE C18 (EC) cartridges (Agilent corporation, USA.) with 2x400µL methanol.

HPLC Quantification: For the quantitative analysis; standart thymoquinone solutions were prepared at 0.5, 1, 5, 15, 25, 50, 100, 250, 500 and 750 ppm concentrations. Areas of these concentrations were used for the calibration.

HPLC Conditions: Samples were analysed with Agilent Technologies 1200 series high pressure liquid chromatography (HPLC), including a binary pump, a vacuum degasser, an autosampler and a diode array dedector. Chromatographic separations were performed on Eclipse XDB-C18 column (150 mm x 4.6 mm, 5µm). Isocratic mobile phase was chosed for as mobile phase; water:methanol:2-propanol (50:45:5) solution was used for separation at a flow rate of 0.9 mL min^{-1}. Analysis time was 28 min and the detection wavelength was set at 254 nm for thymoquinone. Identification on thymoquinone was made by comparing the retention times and UV spectras of the peaks of pure standards. The injection volume was 10 µL for each sample and standard solutions. All the calculations concerning the quantitative analysis were performed with external standardization by measurement of peak areas. Limit of detection (LOD) and limit of quantification (LOQ) were established at a standard deviation over slope of 3.3 and 10, respectively.

RESULTS AND DISCUSSION

The HPLC chromotogram obtained from the analysis of the seeds and seed oils, described above showed well resolved peaks with no interference. Chromatograms of the standard and a sample examined are given in Figures 1 and 2 respectively; other chromatograms were not given since they are similar with the sample mentioned below. Thymoquinone was observed in all samples and the quantity

of the thymoquinone found in the seeds, seed oils and seed oil capsules are given in Table 2, Table 3 and Table 4 with both concentrations and percentages, respectively. Calibration equation, lOD and LOQ values are given in Table 5.

Interestingly, all the samples examined were found to contain only thymoquinone in significant amounts, and not the other derivatives such as dithymoquinone and thymohydroquinone. Although the percentages of thymoquinone vary for each sample, oils were found to contain higher amounts of thymoquinone compared to the seeds in general. According to the tables given below, sample NS1 has the highest and sample NS4 has the lowest amounts of thymoquinone. For oil samples; sample NO9 has the highest and sample NO4 has the lowest amounts of thymoquinone. Among the thymoquinone contents of oil capsules, while NOC3 sample has the highest amount of thymoquinone, NOC4 sample has the lowest. When we compare the results of oil samples with Ghoseh`s study [4], it clearly showes that we have similar results related to thymoquinone content.

The source of thymoquinone is not only black cumin. According to Tabosrky's research [14], there are many other plants which have high amount of thymoquinone, dithymoquinone and thymohyrdoquinone. For the identification of other sources of thymoquinone, dithymoquinone and thymohyrdoquinone; 7 plant families were investigated and it was revelaed that 11 out of 47 plant species also have these components. Results of the study shows that *Monarda didyma* L. (chemotype 1), *Monarda didyma* L. (chemotype 2), *Monarda media* Willd., *Monarda menthifolia* Graham and *Satureja montana* L. also contain thymoquinone, dithymoquinone and thymohyrdoquinone. Thymoquinone contents in that study found to be similar to to the findings of our study.

Table 2. Concentrations and amounts of thymoquinone in seeds (in mg)

Sample Name	Concentration (ppm)	% Amount of Thymoquinone
NS1	376.192	0.376
NS2	100.579	0.101
NS3	15.190	0.015
NS4	9.806	0.010
NS5	20.959	0.021
NS6	107.062	0.107
NS7	84.782	0.085
NS8	14.278	0.014
NS9	106.614	0.107
NS10	167.061	0.167

Table 3. Concentrations and amounts of thymoquinone in seed oils (in mg)

Sample Name	Concentration (ppm)	% Amount of Thymoquinone
NO1	84.217	0.230
NO2	126.521	0.346
NO3	96.467	0.264
NO4	18.121	0.050
NO5	226.276	0.619
NO6	177.964	0.486
NO7	69.931	0.191
NO8	55.848	0.153
NO9	175.454	0.480
NO10	51.837	0.142

Table 4. Concentrations and amounts of thymoquinone in seed oil capsules (in mg)

Sample Name	Concentration (ppm)	% Amount of Thymoquinone
NOC1	267.707	0.732
NOC2	185.426	0.507
NOC3	740.098	2.023
NOC4	13.459	0.037
NOC5	296.747	0.811
NOC6	37.363	0.102
NOC7	76.780	0.210
NOC8	15.681	0.043
NOC9	94.222	0.258
NOC10	105.649	0.289

Table 5. Calibration equation, limit of detection (LOD) and limit of quantification (LOQ) values

Compound	Standard curve	r2	LOD (ppm)	LOQ (ppm)
Thymoquinone	y = 57.518x + 168	0.9998	0.0303	0.0919

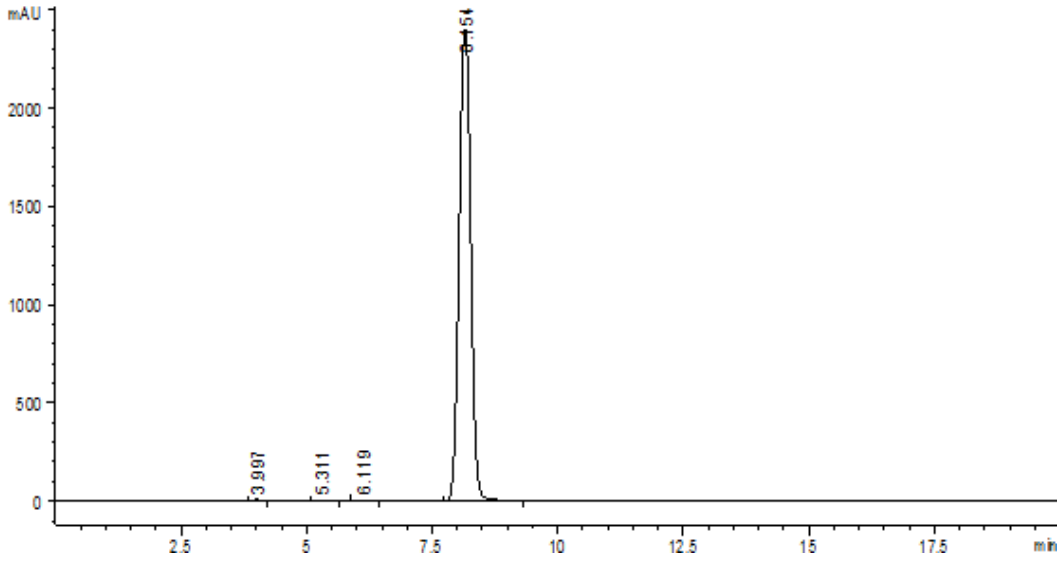

Figure 1. Chromatogram of Thymoquinone Standard

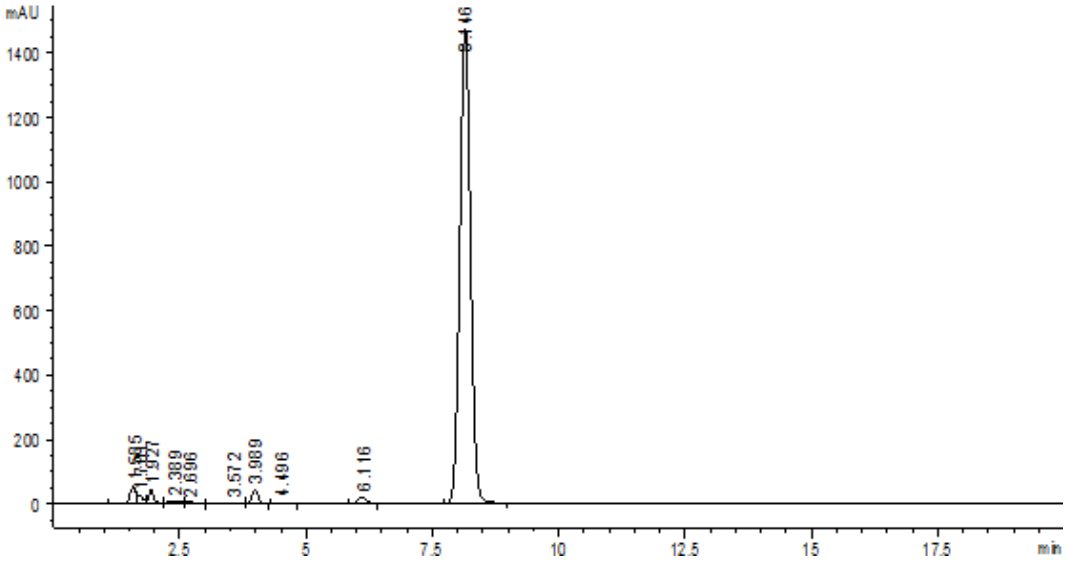

Figure 2. Chromatogram of *Nigella sativa* seed 1 (NS1)

CONCLUSION

In this study, the amount of thymoquinone is determined in black cumin seeds, seed oils and seed oil capsules quantitatively by HPLC. Thymoquinone determination in these three sample types containing *Nigella* (seeds, seed oils and oil capsules) is the main goal of this investigatation. As far as we were concerned, this is the first study examining and comparing thymoquinone contents of seeds of *Nigella sativa*, commercial seed oils and seed oil capsules in Turkey quantitatively. It can be concluded that commercial products sold in Turkey contain adequate thymoquinone.

REFERENCES

1. Gencler–Ozkan, A.M. (2011). *Nigella sativa* L. in FFD Monografları, Tedavide kullanılan Bitkiler. Demirezer, L.Ö. (Ed.) 2nd edition, MN Medikal & Nobel, pp. 423-431, Ankara.
2. Davis, P.H. (1965-1985). Flora of Turkey and the East Aegean Islands, Edinburg University Pres, 1-9, Edinburg.
3. Houghton, P.J., Zarka, R., Heras, B., Hoult, R.S. (1995). Fixed oil of *N. sativa* and derived thymoquinone inhibit eicosanoid generation in leucoytes and membrane lipid peroxidation. Planta Med., 61, 33-36.
4. Ghosheh, O.A., Houdi, A.A., Crooks, P.A. (1999). High performance liquid chromatography analysis of the pharmacologically active quinines and related compounds in the oil of the black seed (*Nigella sativa*). Journal of Pharmaceutical and Biomedical Analysis, 19, 757–762.
5. Bourgou S., Ksouri R., Bellia A., Skandrani I., Falleh H., Marzouk B. (2008). Phenolic composition and biological activities of Tunisian *Nigella sativa* L. shoots and roots. C. R. Biologies, 331(1), 48-55.
6. Ali B.H., Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res., 17, 299–305.
7. Gholamnezhad, Z., Havakhah, S., Boskabady, H.M. (2016). Preclinical and clinical effects of *Nigella sativa* and its constituent, thymoquinone: A review. Journal of Ethnopharmacology, 190, 372-386.
8. Nergiz C. (1993). Otles S. Chemical composition of *Nigella sativa* L. seeds. Food Chemistry, 48, 259-261.
9. Khander, M., Eckl, P.M. (2014). Thymoquinone: an emerging natural drug with a wide range of medical applications. Iran J Basic Med Sci, 17, 950-957.
10. Aycan, I.O., Tufek, A., Tokgoz, O., Evliyaoglu, O., Firat, U., Kavak, G.O., Turgut, H., Yuksel, M.U. (2014). Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. Int J Surg, 12, 213-218.
11. Al Wafai R.J. (2013). *Nigella sativa* and thymoquinone suppress cyclooxygenase-2 and oxidative stress in pancreatic tissue of streptozotocin-induced diabetic rats. Pancreas, 42, 841-849.
12. Mahmoudvand, H., Tavakoli, R., Sharififar, F., Minaie, K., Ezatpour, B., Jahanbakhsh, S., Sharifi, I. (2014). Leishmanicidal and cytotoxic activities of *Nigella sativa* and its active principle, thymoquinone. Pharmaceutical Biology., 53(7), 1052-1057.
13. Erboga, M., Kanter, M., Aktas, C., Sener, U., Fidanol Erboga, Z., Bozdemir Donmez, Y., Gurel, A. (2016). Thymoquinone Ameliorates Cadmium-Induced Nephrotoxicity, Apoptosis, and Oxidative Stress in Rats is Based on its Anti-Apoptotic and Anti-Oxidant Properties. Biological Trace Element Research, 170(1), 165-172.
14. Taborsky, J., Kunt, M., Kloucek, P., Lachman. J., Zeleny, V., Kokoska, L. (2012). Identification of potantial sources of thymoquinone and related compounds in Asteraceae, Cupressaceae, Lamiaceae and Ranunculaceae families. Central European Journal of Chemistry, 10(6), 1899-1906.