

Hydrodistillation of *Nigella Sativa* Seed and Analysis of Thymoquinone with HPLC and GC-MS

Ümit Erdoğan*¹ , Mustafa Yilmazer² , Sabri Erbaş¹ 

Abstract: *N. sativa* seeds, commonly known as black seeds, are used for headaches in traditional medicine by many Asian, Middle Eastern and Far Eastern countries. It is used to treat cough, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been caused to be due to thymoquinone, the major component of the essential oil, but which is also present in the fixed oil. The essential oil of black cumin seeds, *Nigella sativa* L., was tested for a possible antioxidant activity. In our study, it was aimed to increase the yield of essential oil in hydrodistillation of black seed oil in the presence of surfactant (Tween 80). While traces of essential oil were obtained in hydrodistillation of black seed seeds under similar conditions, 2.1% essential oil was obtained in the presence of surfactant. In this study, we also measured the amount of thymoquinone compound in aromatic water. The proportion of thymoquinone passing into the aromatic water show that it is necessary to consume the correct amount of water and essential oil, which is significantly measurable by GC MS and HPLC. We used HPLC for the determination of the amount of thymoquinone in aromatic water, and GC-MS was used in the analysis of *Nigella* essential oil components. According to the results of analysis, thymoquinone was found to be 790 ± 12 ppm in aromatic water and 45.78% in essential oil. Sufficient analytic work was performed with this method and the results were reported in the study.

Keywords: *Nigella Sativa*, thymoquinone, hydrodistillation, GC-MS, HPLC.

¹**Address:** Isparta University of Applied Sciences, Faculty of Agriculture, 32260, Isparta, Turkey.

²**Address:** Süleyman Demirel University, Innovative Technologies Research Center, 32260, Isparta, Turkey.

***Corresponding author:** umiterdogan@isparta.edu.tr

Citation: Erdoğan, Ü., Yilmazer, M., Erbaş, S. (2020). Hydrodistillation of *Nigella Sativa* Seed and Analysis of Thymoquinone with HPLC and GC-MS. Bilge International Journal of Science and Technology Research, 4 (1): 27-30.

1. INTRODUCTION

Nigella sativa L., which is a member of the Ranunculaceae (Ranunculaceae) family, grows in South West Asia, Europe, North Africa and Turkey regions (Aljabre et al. 2015). It is a 20-30 cm long flowering plant. Sensitive flowers have 5-10 leaves and the colors are usually yellow, white, pink, pale blue or pale purple (Güzelsoy et al. 2018). Black colored seeds are flat, oblong, angular and juvenile. Seeds are 0.2 cm long and 0.1 cm wide (Forouzanfar et al. 2014). Black seed seeds, commonly known as black seeds, are headaches in traditional medicine by many Asian, Middle Eastern and Far Eastern countries (Salem 2005). It is used to treat cough, abdominal pain, diarrhea, asthma, rheumatism and other diseases (Al-Haj et al. 2010). Core seed has high nutritional value and contains various active chemical components. Mainly saturated / unsaturated fixed

oils (31.0-35.5 %), essential oils (0.40-0.45%), carbohydrates (33.0-34.0%), proteins (16.0-19.9%), amino acids, alkaloids, tannins, saponins, fibers, minerals (calcium, zinc, phosphate), vitamins (ascorbic acid, thiamine, niacin, pyridoxine and folic acid) (El-Tahir and Bakeet 2006). The main compounds of black seed are essential oil and fixed oil (Kiralan 2014). Black seed essential oil, anti-inflammatory, antimicrobial, anticancer (Bourgou et al. 2010; Harzallah et al. 2011) and has antioxidant activity (Burits and Bucar 2000). Thymoquinone, which is the main constituent of both essential oil and essential oil, inhibits nonenzymatic lipid peroxidation in liposomes (Houghton et al. 1995). According to GC-MS results of the main components of black seed essential oil; p-simene (32.02%), α -thujen (2.4%), α -pinene (1.48%), β -pinene (1.72%), carvacrol (10.38%), thymol (2.32%) and thymoquinone (23.25%)

reported (Sultan et al. 2009). Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) (Figure 1) is the main bioactive component of the essential oil of the medicinal plant *Nigella sativa* (Black seed) (Odeh et al. 2012).

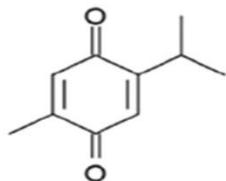


Figure 1. Chemical structure of Thymoquinone.

In the literature, it has been determined that thymoquinone (TQ) has promising antitumor properties against in vitro types such as human colorectal cancer cell (GaliMuhtasib et al. 2004), myeloblastic leukemia cells (El-Mahdy et al. 2005), prostate cancer (Kaseb et al. 2007), pancreatic adenocarcinoma (Worthen et al. 1998) and breast adenocarcinoma (Shoieb et al. 2003).

2. MATERIAL AND METHOD

2.1. Plant Material

N. sativa seeds were collected from Turkey Isparta region. After harvesting, all seeds were kept in the dark at +4 °C until extraction.

2.2. Chemicals

Thymoquinone (2-isopropyl-5-methyl-1,4-benzoquinone) (HPLC, 98%) and Tween 80 Sigma Aldrich (Sigma-Aldrich GmbH, Sternheim, Germany) were purchased. All solvents and reagents are of HPLC purity and were purchased from Sigma-Aldrich and Merck (MerckKGaA, Darmstadt, Germany).

2.3. Essential oil extraction

The extraction procedure of the essential oil is as follows; The extract (fixed oil) obtained from the *N. sativa* seeds by cold press method was applied hydrodistillation using Clevenger type apparatus (Isparta University of Applied Sciences-Industrial Crops Laboratory). 50 g of the fixed oil obtained by the cold press method was dissolved in 500 mL of water which contains 10% of Tween 80 (5g) according to oil weight and the final volume was completed to 1 L. 50 grams of fixed oil in 1 L of water, was hydrodistilled for 2.5 hours. The essential oil obtained with dark yellow color and pungent odor was dried with anhydrous sodium sulfate and kept in the dark at +4 °C until use. Aromatic water, along with essential oil from hydrodistillation products, was also collected for future analysis.

2.4. HPLC analysis of thymoquinone

The thymoquinone analysis of the black seed aromatic water was performed according to the modified HPLC method of developed by Selin et al. (2017).

For quantity analysis; standard thymoquinone solutions were prepared at concentrations of 62.5, 125, 250, 500 and 1000 ppm. The areas of all these concentrations were used for calibration ($r^2=0.9995$). Aromatic water was injected directly. Samples, a diode detector (DAD detector (Lambdamax = 278nm), an auto sampler (SIL – 10AD vp), a vacuum degasser (DGU-14A), system controlled (SCL-10Avp) and a pump (LC-10ADvp). Chromatographic separations were carried out on AgilentEclipse XDB-C18 column (250x4.60 mm, 5 μ m) The column temperature was 30 °C. 3% acetic acid, Methanol (50:50) was selected as mobile phase and the flow rate was 0.8. The injection volume is 20 μ L for each sample and standard The analysis time is 30 minutes and the detection wavelength is set to 278 nm for thymoquinone.

2.5. Essential oil components analysis with GC-MS

Essential oil analysis of volatile components were performed on a Shimadzu GCMS-QP2010 SE (Japan) model with Support Rx-5Sil MS capillary column (30 m x 0.25 mm, film thickness 0.25 μ m). GC analysis were performed under the following conditions. Carrier gas 1 mL / min. helium with flow. The split ratio is 1:10. After 1 minute at 60 °C, the temperature program reaches 250 °C with an increase of 4°C per minute and was kept at 250 °C for 15 minutes. The mass spectra were taken at 70 eV. 970 microlitre hexan is added over 30 microliters of pure essential oil. 1 microliter is injected from the capped vial. Identification of components with mass spectra data according to mass library was used NIST, WILEY also thymoquinone reference standart was used. % areas of essential oil showed that thymoquinone was the major volatile component.

3. RESULTS AND DISCUSSION

Fixed oil obtained from *N. sativa* seeds by cold press had a green color, pungent aromatic odor. From the fixed oil, dark yellow, pungent essential oil was obtained by hydrodistillation method according to the above method. In conventional hydrodistillation methods, plant materials such as leaves, flowers, seeds, roots and so on are used. In our study fixed oil obtained from *N. Sativa* seeds was dissolved in water with Tween 80 surfactant and then hydrodistillation was performed. The purpose of using Tween 80 is to minimize the problems in the fixed oil-water mixture during hydrodistillation and to ensure a homogeneous mixture of constant oil and water. In similar conditions, a trace of essential oil was obtained in the hydrodistillation of black seed oil without surfactant. On the other hand 2.1% essential oil was obtained from the fixed oil obtained from the *N. Sativa* seed and collected for analysis in aromatic water. In a similar study, yellow essential oil was obtained by hydrodistillation method from fixed oil obtained by hexane extraction (Nickavar et al. 2003).

The main pharmacologically active compound in the structure of *N. Sativa* essential oil is thymoquinone. Determination of thymoquinone content in aromatic water according to HPLC method was performed as above. Standard, aromatic water chromatograms are given in Figure 2, Figure 3 respectively. The amounts of

thymoquinone in the investigated sample are also given in Table 1.

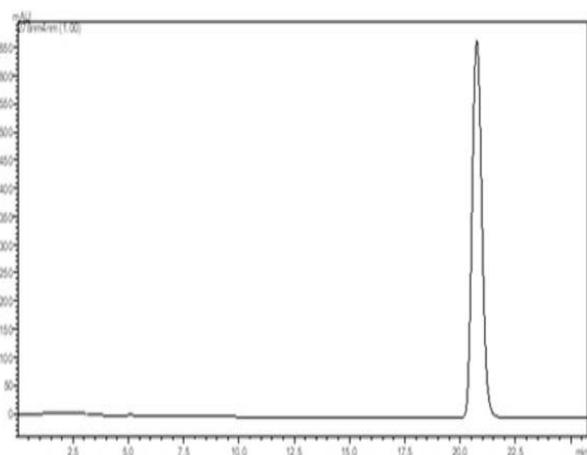


Figure 2. HPLC chromatogram of Thymoquinone standard.

Table 1. Concentration of thymoquinone in aromatic water

Sample	Amount (ppm)
Aromatic water	790 ±12

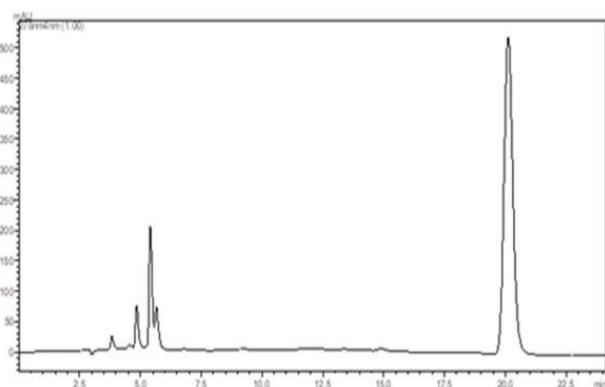


Figure 3. HPLC chromatogram of *N. Sativa* aromatic water.

Thymoquinone was determined by HPLC analysis of the aromatic water. In a similar study, 10 different black seed seeds and oil obtained from herbal extracts from Ankara were examined by HPLC according to the amount of thymoquinone they contain (Selin et al. 2017). Due to the presence of thymoquinone in aromatic water is likely. Solubility studies showed that thymoquinone had the same solubility in commonly used solvents such as 0.1N HCl and PBS (pH 5; 7.4 and 9.0) (Goyal et al. 2017) thought to be used.

The components of *N. sativa* essential oil obtained by hydrodistillation were investigated by GC-MS. 20 components were identified in essential oil (Table 2).

Table 2. GC MS % total percentage of *Nigella Sativa* L. seed essential oil results

Peak#	R.I	Compound Name	Area%
1	927.8	a-Thujene	6.50
2	936.1	a-Pinene	1.50
3	977.7	β- Pinene	1.94
4	1011.3	3-Carene	5.51
5	1024.3	p-Cymene	29.45
6	1029.5	Limonene	1.61
7	1030	β- Phellandrene	0.97
8	1031	1,8-Cineole	0.05
9	1059.7	γ-Terpinene	0.20
10	1086.9	Terpinolene	0.11
11	1098	Sabinene hydrate, trans-	0.96
12	1127.5	p-Mentha-2,8-dien-1-ol, trans-	0.51
13	1177.1	Terpinen-4-ol	0.82
14	1218.3	β- Cyclocitral	1.07
15	1249	Thymoquinone	45.78
16	1300.4	Carvacrol	0.44
17	1317	2,4-Decadienal, (2E,4E)-	0.05
18	1347	a-Terpinyl acetate	0.07
19	1352	a -Longipinene	0.34
20	1406.8	Longifolene	1.54

The main components were thymoquinone (45.78%), p-Cymene (29.45%), a- Thujene (6.5%), 3- Carene (5.51%), respectively. In a similar study, GC-MS analysis of 7 different black seed essential oils revealed that the main components were thymoquinone (30% - 48%), p- cymene (7% -15%), carvacrol (6% -12%), Terpinen-4-ol (2%-7%), t-anethol (1% -4%) and longifolene (1% -8%) (Burits and Bucar 2000).

4. CONCLUSIONS

In our study, 2.1% essential oil was obtained in hydrodistillation of black seed oil in the presence of surfactant (Tween 80). We used HPLC for the determination of the amount of thymoquinone in aromatic water, and GC-MS was used in the analysis of *Nigella* essential oil components. According to the analysis results thymoquinone was detected 790±12 ppm in aromatic water and 45.78% in essential oil.

In this original study, especially; It has been shown that Thymoquinone can be dissolved in aromatic water by distillation method. According to its benefits of Thymoquinone, this water can be used as drinking or other applications such as food and cosmetic. There are not much data for water solubility of Thymoquinone. With this study, we think that aromatic water can be contributed by the food, cosmetic and drug industry.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- Al-Haj, N.A., Shamsudin, M.N., Alipiah, N.M., Zamri, H.F., Ahmad, B., Siddig, I., Rasedee, A. (2010). Characterization of *Nigella sativa* L. Essential oil-loaded solid lipid nanoparticles. *American Journal of Pharmacology and Toxicology*. 5(1): 52-57.
- Aljabre, S.H., Alakloby, O.M., Randhawa, M.A. (2015). Dermatological effects of *Nigella sativa*. *Journal of Dermatology and Dermatologic Surgery*. 19(2): 92-98.
- Bourgou, S., Pichette, A., Marzouk, B., Legault, J. (2010). Bioactivities of black cumin essential oil and its main terpenes from Tunisia. *South African Journal of Botany*. 76(2) : 210-216.
- Burits, M., Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy research*. 14(5): 323-328.
- El-Mahdy, M.A., Zhu, Q., Wang, Q.E., Wani, G., Wani, A.A. (2005). Thymoquinone induce apoptosis through activation of caspase-8 and mitochondrial events in p53-null myeloblastic leukemia HL-60 cells. *International Journal of Cancer*. 117(3):409-417.
- El-Tahir, KEDH, Bakeet, D.M. (2006). The black seed *Nigella sativa* Linnaeus A mine formulticures a pleafor urgent clinical evaluation of its volatile oil. *Journal of Taibah University Medical Sciences*. 1(1): 1-19.
- Forouzanfar, F., Bazzaz, B.S.F., Hosseinzadeh, H. (2014). Black cumin (*Nigella sativa*) and its constituent (thymoquinone) a review on antimicrobial effects. *Iranian Journal of Basic Medical Sciences*. 17(12): 929.
- GaliMuhtasib, H., Diab Assaf, M., Boltze, C., Al-Hmaira, J., Hartig, R., Roessner, A., Schneider Stock, R. (2004). Thymoquinone extracted from blackseed triggers apoptotic cell death in human colorectal cancer cells via a p53-dependent mechanism. *International Journal of Oncology*. 25(4):857-866.
- Goyal, S.N., Prajapati, C.P., Gore, P.R., Patil, C.R., Mahajan, U.B., Sharma, C., Ojha, S.K. (2017). Therapeutic potential and pharmaceutical development of thymoquinone: a multitargeted molecule of natural origin. *Frontiers in Pharmacology*. 8: 656.
- Güzelsoy, P., Aydın, S., Başaran, N. (2018). Çörek Otunun (*NigellaSativa* L.) Aktif Bileşeni Timokinonun İnsan Sağlığı Üzerine Olası Etkileri. *Journal of Literature Pharmacy Sciences*. 7(2): 118-135.
- Harzallah, H.J., Kouidhi, B., Flamini, G., Bakhrouf, A., Mahjoub, T. (2011). Chemical composition, antimicrobial potential against cariogenic bacteria and cytotoxic activity of Tunisian *Nigella sativa* essential oil and thymoquinone. *Food Chemistry*. 129(4) : 1469-1474.
- Houghton, P.J., Zarka, R., de las Heras, B., Hoult, JRS. (1995). Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Plantamedica*. 61(01): 33-36.
- Kaseb, A.O., Chinnakannu, K., Chen, D., Sivanandam, A., Tejwani, S., Menon, M., Reddy, G. P V. (2007). Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone refractory prostate cancer. *Cancer Research*. 67(16): 7782-7788.
- Kiralan, M. (2014). Changes in volatile compounds of blackcumin (*Nigellasativa* L.) seed oil during thermal oxidation. *International journal of Food Properties*. 17(7): 1482-1489.
- Nickavar, B., Mojab, F., Javidnia, K., Amoli, M.A.R. (2003). Chemical composition of the fixed and volatile oils of *Nigella sativa* L. from Iran. *Zeitschriftfür Naturforschung C*. 58(9-10): 629-631.
- Odeh, F., Ismail, S.I., Abu Dahab, R., Mahmoud, I.S., Al Bawab, A. (2012). Thymoquinone in liposomes a study of loading efficiency and biological activity towards breast cancer. *Drug delivery*. 19(8): 371-377.
- Salem, M.L. (2005). Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. *International Immunopharmacology*. 5 (13-14): 1749-1770.
- Selin, I., Kartal, M., Erdem, S.A. (2017). Quantitative analysis of thymoquinone in *Nigella Sativa* L.(Black Cumin) seeds and commercial seed oils and seed oil capsules fromTurkey. *Ankara Üniversitesi Eczacılık Fakültesi Dergisi*. 41(1).
- Shoieb, A.M., Elgayyar, M., Dudrick, P.S., Bell, J.L., Tithof, P.K. (2003). In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. *International Journal of Oncology*. 22(1): 107-113.
- Sultan, M.T., Butt, M.S., Anjum, F.M., Jamil, A., Akhtar, S., Nasir, M. (2009). Nutritional profile of indigenous cultivar of black cumin seeds and antioxidant potential of its fixed and essential oil. *Pakistan Journal Botany*. 41(3): 1321-1330.
- Worthen, D.R., Ghosheh, O.A., Crooks, P.A. (1998). The in vitro anti-tumoractivity of some crude and purified components of blackseed, *Nigella sativa* L. *Anticancer Research*. 18(3A): 1527-1532.