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Original article

Alteration in Thymoquinone Content of *Nigella sativa* Seeds After Processing by a Traditional Method and Stability Assessment of Raw and Processed Seeds

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Abstract

Background and objectives: *Nigella sativa* L. is one of the most important species in Iranian traditional medicine (ITM). According to ITM, the plant seeds can cause bronchospasm in patients with hot temperament; therefore it is recommended to process the seeds with grape vinegar before usage. This process may influence some components of the seeds; thus, in the present investigation, the effect of processing on thymoquinone, which is one of the most important active ingredients of the seeds, has been studied. Moreover, the stability of Nigella capsules containing raw and processed seeds and *Nigella*-honey mixture, "Maajoon", were assessed. **Methods:** *Nigella sativa* seeds were processed by two methods: vinegar was added to the whole intact seeds, then the seeds were dried and crushed; the second method consisted of first crushing the seeds and then performing the vinegar adding and drying steps. The essential oil of the powders was obtained by using hydrodistillation method and thymoquinone content of the oils was measured by gas chromatography. In order to estimate the stability of the *Nigella* capsules and "Maajoon", thymoquinone content of the products was measured after one, two and three months at room temperature. **Results:** The results showed that thymoquinone was absent in the processed seeds which were powdered before processing; but thymoquinone percentage had decreased in processed seeds which were powdered after processing (maximum content: 64.1%). The reduction of thymoquinone after three months was 40.1 and 78.5% in raw and processed capsules, respectively. No thymoqinone was found in the "Maajoon". **Conclusion:** Since many effects of *Nigella* is due to thymoquinone, it seems that grinding before processing and making "Maajoon" are not suitable methods for *Nigella* preparation. Powdering after processing had decreased thymoquinone content which might result in decrease in bronchospasm as the side effect of thymoquinone; therefore, this method of processing seems to be suitable. Due to low stability of *Nigella* powder, powdering just before usage is recommended when necessary.

Keywords: Iranian traditional medicine; *Nigella sativa*; processing; thymoquinone; vinegar

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Introduction

Nigella sativa, (black cumin, Ranunculaceae family), is a popular treatment in Iranian traditional medicine (ITM). The seeds of the plant are believed to have hot and dry

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temperament. It has been used in the treatment of various disorders including coughs, vomiting, jaundice, dyspnea, kidney and bladder stones, arthritis, toothache and earaches [1,2]. Recent researches have reported the pharmacological activities of the plant including anti-inflammatory [3,4], analgesic [5] and antioxidant [6] effects. Black cumin seeds are rich in fixed and essential oils and the therapeutic effects of the species are known to be due to these components. The essential oil of the seeds contain thymoquinone which is thought to be the effective constituent;, the fixed oil comprises myristic, palmitic, stearic, oleic and linoleic fatty acids [7,8].

According to ITM, due to the hot and dry temperament of the species, it could result in adverse effects such as asphyxia in people with hot temperament; therefore, the seeds are recommended to be processed with grape vinegar prior to consumption by these patients in order to avoid such adverse effects [1]. Processing might affect the seed constituents and since thymoquinone is a characteristic compound of the plant, it was decided to evaluate the effect of processing on thymoquinone content of *N. sativa* seeds in the present study.

Processing could be carried out in two ways: adding vinegar to the whole intact seeds, drying and then crushing the seeds or first crushing the seeds and then performing the vinegar adding and drying steps. In the present study, the effect of both methods on thymoquinone content has been presented. Moreover, capsules containing seeds powder (raw and processed) and mixture of seeds powder and honey called "Maajoon" are used in traditional pharmacies; therefore, the stability of these forms has been evaluates as well.

Material and Methods

Plant material

Nigella sativa seeds were provided from three distinct local sources, Tehran province, Iran (2014). Their identity was confirmed using references [9].

Processing the seeds

Method 1: The seeds were ground and mixed with vinegar (two fold of the weight) and were kept for 24 h. They were dried in room temperature afterwards, ground again and screened (Mesh 20).

Method 2: Whole black cumin seeds received vinegar (two fold of the weight), the rest steps were performed according to the first method.

Nigella sativa capsules

Powdered raw and processed *N. sativa* seeds were passed through sieve (Mesh 20) and filled in opaque capsules (500 mg). The capsules were packed in bottles afterwards (30 capsules in each).

Nigella sativa "Maajoon"

Powdered raw and processed seeds were mixed with honey (1:3) and kept for further analysis.

Determination of thymoquinone content of the essential oil

The essential oils of raw and processed seeds as well as capsules and "Maajoon" were obtained by a Clevenger type apparatus for 4 h and the yield was recorded.

The thymoquinone content of the essential oils was identified by gas chromatography. The analysis was performed on a Shimadzu GC-17A gas chromatograph, equipped with a BPX5 capillary column (30 m×0.32 mm; film thickness 0.25 μm). The oven temperature programming was: 5 min at 50 °C, then the temperature was increased with the rate of 2.5 °C/min. After reaching 115 °C, the rate was increased to 265 °C with rate of 15 °C/min. Nitrogen was the carrier gas at flow rate of 1 mL/min. Injector and detector temperatures were 260 °C and 300 °C, respectively. Calibration curve of thymoquinone was prepared using different solutions (0.078-2.5 mg/mL). The thymoquinone content of each sample was measured using the calibration curve.

Results and Discussion

The GC chromatograms of thymoqionone and raw black cumin (as sample) have been presented in figures 1 and 2. Thymoquinone was found at 26.03 min. The amount of thymoquinone in each sample has been determined in table 1.

Table 1. Thymoquinone in raw and processed samples of *N. sativa* seeds

Sample		Thymoquinone (mg/100g)	Thymoquinone reduction (%)
Raw	1	16.84±0.71	-
	2	5.88±0.56	-
	3	13.37±1.13	-
Processed	1	6.04±0.35	64.1%
	2	2.38±0.12	59.2%
	3	8.86±0.15	34.05%

No thymoquinone was found in the seeds that were processed after grinding. Thymoquinone reduction was less in sample 3 compared to others; therefore it was selected for further stability studies.

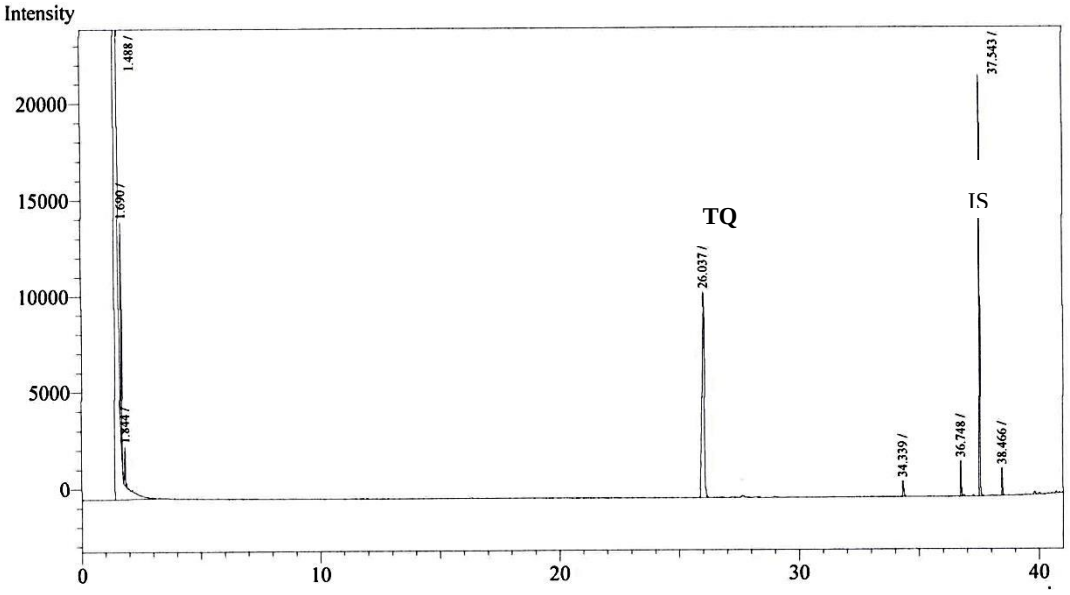

Figure 1. GC chromatogram for thymoquinone

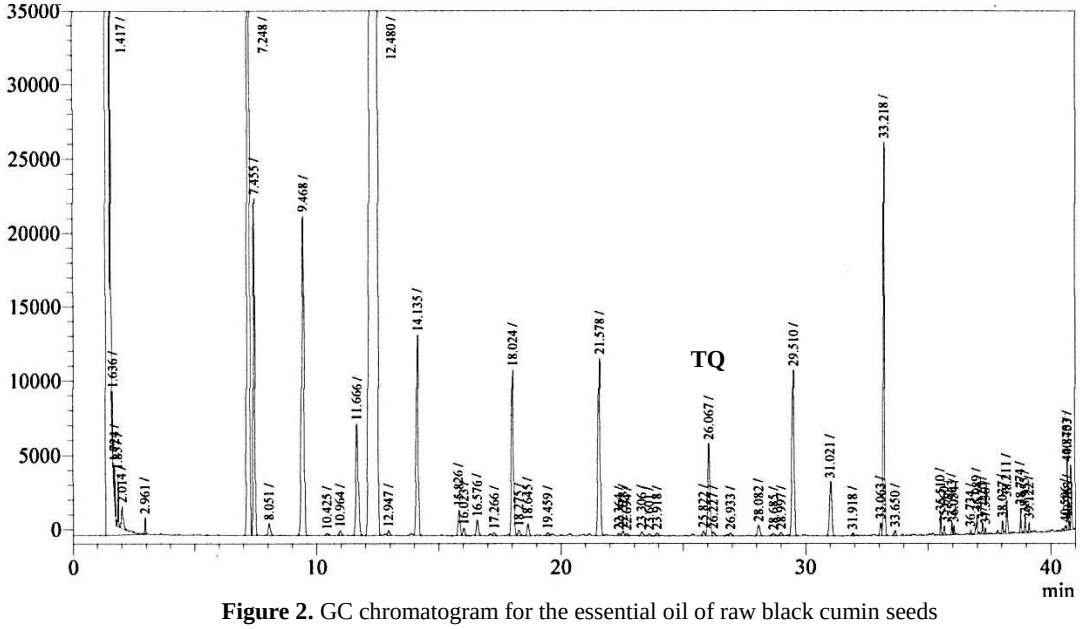

Figure 2. GC chromatogram for the essential oil of raw black cumin seeds

The amounts and alterations in thymoquinone content during three month storage have been presented in table 3 and figure 3. No thymoquinone was found in *"*Maajoon*"*.

Tables 3. Thymoquinone content of *N. sativa* seeds during storage time

Time (month)	Thymoquinone (mg/100g)		Thymoquinone reduction	
	Raw	Processed	Raw	Processed
0	13.37±1.13	8.86±0.15	-	-
1	11.80±1.47	2.54±0.56	11.7%	71.2%
2	8.80±0.67	2.31±0.21	34.2%	73.8%
3	8.01±1.01	1.90±0.23	40.1%	78.5%

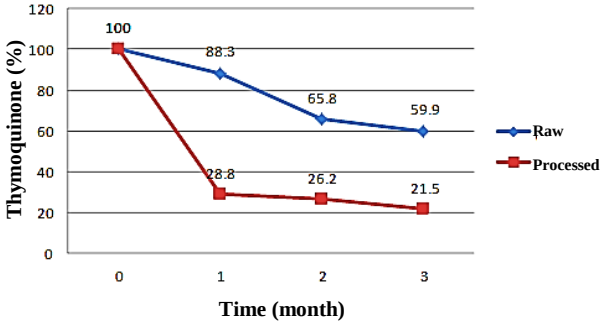

Figure 3. Thymoquinone content in raw and processed black cumin seeds in capsule during 3 month

N. sativa use as a medicinal plant goes back to history. It was known as the magic plant and due to its importance in traditional and folklore medicine, many researches have been conducted on this plant. The seeds contain thymoquinone (30-48%), thymohydroquinone, dithymoquinone, ρ-cymene, carvacrole, 4-terpineol, trans-anethol, langifolen, α-pinene and thymol. Some isoquinoline and pyrazole alkaloids as well as α-hydrine have also been isolated from the species [10-11]. The pharmacologic effects of the plant are attributed to the quinin compounds specially thymoquinone. Thymoquinone is converted to dithymoquinone and other oligomers during storage [10,12].

In a recent research, it was found that thymoqionone in the black cumin seed causes bronchospasm through releasing histamine [13]; this finding brings to the mind the emphasis of traditional physicians for processing the seeds to reduce the risk of side effects.

The results of the present study showed that thymoquinone was absent from samples that were treated after grinding; while it showed a reduction in the samples that were ground after processing. Thymoquinone content reduced during three month of storage and the rate of reduction was more for the processed samples. Thymoquinone and other major components of black cumin seeds are volatile. The results suggested that keeping the seeds either raw or processed in capsules is not a proper way for storage and results in losing many components of the ground drug.

"Maajoon" is a traditional formulation and black cumin "Maajoon" is prepared by mixing the ground black cumin seeds with honey. The results showed that thymoquinone could be found in none of samples that were prepared with honey suggesting that honey constituents might have broken down or made complexes with thymoquinone, making it impossible to be detected in the samples.

Elimination of thymoquinone could result in elimination or reduction of effects or side effects of *N. sativa* seeds. It has been reported that thymoquinone has shown cardiac effects and reduced blood pressure and cardiac rates [14]. It should be noted that these effects might be assigned to other components of the species as well; since α-pinene and ρ-cymene are also known to have cardiac effects; thus, the seeds that lack thymoquinone might not show the bronchospastic and gastroulcerogenic effects while still keeping the cardiac effects [15]. Researches have also shown that thymoquinone demonstrated hepatoprotective effects and increased the biliary salts and circulation [16-19]. Taking all into consideration, it seems that since various effects have been reported for thymoquinone, whole elimination of the compound might not be a good idea to be used for every disease and processing the ground seeds with vinegar or using it as "Maajoon" would not be a proper choice. On the other hand, processing the whole seed which results in reduction (not elimination) of thymoquinone would be a better choice of preparation. Finally, since grinding and storing *N. sativa* seeds results in degradation of compounds, it is suggested that the drug be prepared just prior to use.

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Author contributions

Homa Hajimehdipoor and Rasool Choopani designed and supervised the experiments; Leila Ara was involved in data collection; Homa Hajimehdipoor analyzed the data; Maryam Hamzeloo-Moghadam participated in data analysis and preparation of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

ITM: Iranian traditional medicine; GC: gas chromatography; TQ: thymoquinone