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Screening of Thymoquinone (Tq) Content in *Nigella sativa*-Based Herbal Medical Products

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ABSTRACT

Nigella sativa seed contains a bioactive compound known as thymoquinone (TQ) which is prominent for its pharmacological properties such as antioxidant; anti-microbial, antibacterial and anti-inflammatory hence, becomes an important ingredient in Herbal Medicinal Products (HMPs). However, adulteration of HMPs has raised concerns among consumers. This study aims to extract and characterize TQ from Nigella sativa based products. TQ was isolated from raw Nigella sativa seeds using methanolic and aqueous extraction methods. Extracts were subject to qualitative and quantitative analysis using High-Performance Liquid Chromatography (HPLC). Both extraction methods produced TQ extracts with methanol extraction yielded 63.4 µg/mL while aqueous extraction method produced 15.3 µg/mL. Quantitative analysis of 13 commercialized products containing Nigella sativa such as oil, capsule, facial foam, shower foam, and toothpaste showed presence of TQ at varying concentration. The effectiveness of TQ exhibits its pharmacological activity is questionable because the concentration of TQ in the product was not stated on the packaging.

Keywords: Herbal medicinal products (HMPs), *Nigella sativa*, Thymoquinone (TQ), HPLC, TLC.

INTRODUCTION

Herbal medicinal products (HMPs) are gaining popularity among consumers for its therapeutic value, attractive marketing technique, affordable and readily available in the market. Adulteration of HMPs has raised concerns among consumers which affects consumer's health, trigger allergy response and non-economical. The term adulteration refers to the fraudulent practices whereby the product is substituted partly or fully with impure, extraneous, improper or inferior products/substances [1]. Adulteration of food and medicinal products does not comply with Malaysia Food Act 1985 and Medicine Act 290.

Black seed or *Nigella sativa* is an example of medicinal herb consumes worldwide. Due to increasing production and consumption of HMPs in Malaysia, only a small number of HMPs were registered under the Ministry of Health. This has raised concern on the safety and efficacy of the HMPs especially no clinical studies were carried out to validate the pharmacological effects of these HMPs. To date, there are 37 HMPs containing *Nigella sativa* registered in National Pharmaceutical Regulatory Agency (NPRA) database, for example, Apotec Total Women Capsule, Saadi al habbatus cauda', Sinai Black Seed Oil Softgel 500 mg, Al-Ejib kapsul, Al-Habbah As Sawda Plus and i-Gift Capsule.

Research findings reported the association of *Nigella sativa* with various pharmacological effects such as anti-oxidant, anti-proliferation and anti-inflammatory activities [2]. In fact, *Nigella sativa* has been used as spice and food preservative in middle-east countries. In folk medicinal practices, they are ingested with food or mixed with honey [3]. Nowadays *Nigella sativa* is found in health supplement products [4]. Bioactive compound that derived from *Nigella sativa* is thymoquinone (TQ). This study aims to determine the presence of TQ in HMPs using qualitative and quantitative analysis.

METHODOLOGY

Sample preparation

Nigella sativa seeds were ground to obtain powder form and used in extraction methods. The leftover powder was stored at room temperature, 29°C and kept in dark prior extraction procedure. 13 samples from *Nigella sativa* based products involving shampoo, facial foam, toothpaste, oils, and capsules were collected and 5 g of the samples were mixed with methanol (HPLC grade ≥ 99.8%) to produce homogenous mixture before performing HPLC analysis.

Extractions

Extraction of crude *Nigella sativa* powder was carried out using distilled water and methanol (HPLC grade ≥ 99.8%). In methanol extraction, the mixture was stirred for 1 hour at room temperature (29°C) covered with aluminum foil while in aqueous extraction the mixture was heated at 80°C and stirred for 1 hour then cooled at room temperature.

Characterization of TQ

The composition of thymoquinone (TQ) in both extraction samples were characterized and quantified using high-performance layer chromatography (HPLC).

Quantitative analysis of TQ using high-performance liquid chromatography (HPLC): For HPLC, the quantification of TQ from extract samples was carried out using Column-ZORBAX Eclipse XDB-C18 (15 mm × 4.6 mm, 5µm) with mobile phase methanol: water (85:15) filtered using a 0.45 µm membrane. Analysis time was 6 min, and the detection wavelength was set at 274 nm with temperature 40°C. The flow rate was 1.0 ml/min with 2 µl injection volume. The standard curve was prepared by using TQ standard (analytical grade).

Screening of *Nigella sativa*-based products by HPLC

13 commercialized *Nigella sativa* products prepared from section 2.1 were characterized and quantified for TQ content using HPLC according to the condition as described in section 2.3.1.

RESULTS AND DISCUSSION

Extraction of thymoquinone from *Nigella sativa* seeds using aqueous and methanol

Extraction methods: Both extraction methods; aqueous and methanol extraction successfully yield dark brown residues following the drying process. The extraction yield does not represent the amount of TQ in the extracts. The amount of yield is dependent on the amount of time the solute and solvent were in contact with each other. Longer contact time favored the system to have a more mass transfer. Furthermore, TQ compounds in the extracts might be oxidized or degraded when exposed to air and light during the extraction process (Table 1).

Table 1: Time, Yield, Total bioactive compound, TQ (thymoquinone) values of the extracts of *Nigella sativa* seeds prepared using two different extraction methods.

Extraction Method	Yield (%)	Total TQ (%)
Aqueous extraction	5	15.3
Methanol extraction	2.7	63.4

Characterization and quantify the amount of TQ in the extraction sample

Figure 1 showed the reference peak for TQ appeared at retention time 2.28. Although both extract samples showed multiple peaks, peaks that appeared at 2.280 and 2.239 min represents TQ.

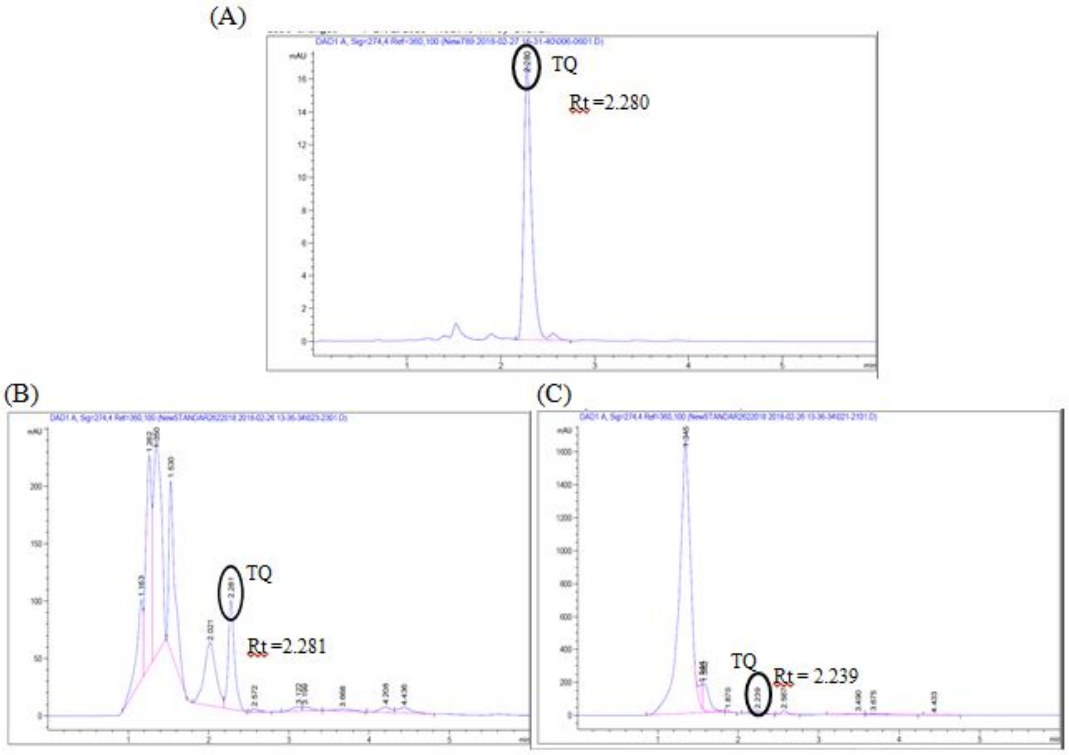

Figure 1: HPLC chromatogram of TQ (A) standard (Rt=2.280) as references and from (B) methanol extraction (Rt=2.281 min), (C) aqueous extraction (Rt=2.339 min), Rt=retention time.

Determination the presence of *Nigella sativa* in herbal medical products (HMPs)

Among the products selected in this study are health supplements, shampoo, facial foam, and toothpaste. Based on the description available on product packaging, *Nigella sativa* claimed for its antibacterial activity in shampoo, facial foam and toothpaste products while for health supplements, general description such as improve blood circulation, immune system, health maintenance, and anti-aging effects were mentioned hence we chose 'anti-oxidant' property to represent pharmacological activity of health supplement products.

According to in vitro antibacterial studies, TQ was reported to inhibit Staphylococcus aureus, a common pathogen on the skin at MIC=3-8 µg/ml [5,6]. Quantitative analysis performed on shampoo and facial foam products showed that the amount of TQ present in these products is more than the inhibitory concentration reported by Chaeib and Halawani hence these products are considered to have antibacterial property. However, the amount of TQ contains in two toothpaste products was recorded lower than in vitro inhibitory concentration for *Streptococcus mutans* and *Streptococcus mitis* (MIC=50 µg/ml) [7]. These bacterial strains are the most common causes of dental caries and bad breath [8,9].

The concentration of thymoquinone in Oil I is the highest followed by Capsule II and the lowest concentration of thymoquinone is in Toothpaste II. Table 2 shows the concentration of thymoquinone in *Nigella sativa* based products. These results show that Habatusauda' oil (Oil I) has the highest amount of thymoquinone which stated on the product packaging to contain 100% pure *Nigella sativa* compared to other samples. Overall, the amount of thymoquinone determined from HPLC verifies thymoquinone concentration as stated on the packaging.

Table 2: Concentration in samples of *Nigella sativa* based products.

No.	Samples	TQ concentration	Percentage TQ
1	Capsule I	53.4	4.62
2	Oil I	138.21	11.53
3	Milk	39.97	3.64

4	Shampoo I	30.4	2.82
5	Shampoo II	17.52	1.72
6	Shampoo III	4.14	0.38
7	Facial cleanser I	6.45	0.6
8	Facial cleanser II	5.18	0.51
9	Facial cleanser III	4.9	0.42
10	Toothpaste I	1.21	0.15
11	Toothpaste II	1.21	0.11
12	Capsule 1	118.15	11.3
13	Oil II	53.4	4.98

CONCLUSION

Investigation of the efficiency of two different extraction method which is aqueous and methanol extraction was successfully developed through thymoquinone screening, analysis, and characterization through HPLC analysis. Herein, the aqueous extraction had a high percentage yield of 5% compared to methanol extraction with percentage yield of 2.7%. *Nigella sativa* seed contains high amounts of phytochemical constituents which are medicinally important. Preliminary study on different extracts showed that the *Nigella sativa* seed has the most important essential oil which is thymoquinone, TQ. This study proposed a successful HPLC method in analyzing the thymoquinone, TQ in extracts of two different extraction methods correspond to the validated parameters of linearity, accuracy, LOD, and LOQ. The thymoquinone was present in high amount in methanol extraction compared to the aqueous extraction. The study revealed a concentration of thymoquinone in methanol (63.4 µg/mL) and aqueous (15.3 µg/mL). This proves that methanol extraction is best or better for thymoquinone compared to aqueous extraction. The 13 samples of *Nigella sativa* based product also be analyzed to study the presence of thymoquinone in this products and to aware people in adulteration by using Herbal Medicinal Product (HMPs) and this study shows that Oil I has the highest percentage of thymoquinone (138.21 µg/mL) followed by Capsule II (118.15 µg/mL) and the lowest concentration thymoquinone is in Toothpaste II (1.21 µg/mL). It can conclude that HPLC is a good method to characterize compound in products to detect the adulteration in the products. Adulteration is an addition of another substance to a food item in order to increase the quantity of the food item in raw form or prepared form, which may result in the loss of actual quality of food item. From the analysis of all 13 samples in this study, it shows there does not any adulteration and mislabelling occurred.

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