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Thymoquinone content in marketed black seed oil in Malaysia

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Abstract

Thymoquinone (TQ) is the major active compound in black seed oil (BSO). Many pharmacological effects of TQ, such as anti-inflammatory, hypoglycemic, antioxidant, immune stimulator, and anticancer, have been reported. TQ can be considered as a biomarker for BSO, but its content in the commercial products is rarely reported. TQ content varies based on the oil source and extraction method. This study aimed to quantify the TQ content in the commercial BSO products in Malaysia and to evaluate whether the products can be used as a source of TQ for therapeutic benefits. TQ was quantified using an established high-performance liquid chromatography (HPLC) method. TQ human equivalent dose (HED) was calculated based on reported animal studies from literature, and theoretical BSO amount containing the TQ dose was calculated based on the HPLC analysis. TQ content in the commercial BSO products ranged from 0.07% wt/wt to 1.88% wt/wt. The product with the highest TQ concentration is approximately 27-fold higher than the product with the lowest TQ concentration. Consequently, theoretical BSO amounts needed for specific diseases varied and some products cannot provide practical amount of TQ. This study recommends the regulation of TQ content in BSO and suggests that the BSO might be fortified with extra TQ to be effectively used in some diseases.

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Introduction



Thymoquinone (TQ) is the main active compound in black seed oil (BSO, *Nigella sativa* oil or black cumin oil), which constitutes approximately 30% of the seeds.^[1] Many pharmacological effects of TQ, such as anti-inflammatory and hypoglycemic, have been reported.^{[2],[3]} Badary *et al.*^[4] reported that the TQ can be used as an antioxidant. Many other medical and pharmacological uses of TQ, such as immune stimulator and anticancer, have also been reported.^{[5],[6]} Recent studies have attempted to use the standardized *N. sativa* extract based on the quantification of TQ.^{[7],[8]} Therefore, TQ can be used as a biomarker for *N. sativa* oil.

BSO is used in folk medicine to treat several illnesses, such as cough, fever, diarrhea, and abdominal disorders, but only a few clinical trials were conducted either on the oil or its purified compound, TQ.^[9] Most of the research on *N. sativa* was conducted on the purified TQ in animal models or in cultured cells. The amount of TQ in BSO was rarely reported, and it is not regulated in the commercial BSO.^{[10],[11]} In a recent study, Khonche *et al.*^[8] used standardized *N. sativa* seed fixed oil containing 0.987 ± 0.07 mg/mL of TQ to analyze hepatic steatosis, aminotransferase, and lipid levels in nonalcoholic fatty liver disease. As BSO can be administered as a source of TQ, the TQ content should be known to use it scientifically to treat some diseases. A phase I clinical trial on TQ found that TQ was well tolerated at a dose from 75 to 2600 mg/day with no toxicity to be reported.^[12] Considering that TQ content in BSO is generally less than 2% as stated in the reported studies, therefore no toxicity is expected from TQ in BSO when taken at the recommended doses.^{[7],[8]}

Therefore, this study aimed to quantify the TQ content in the commercial BSO available in Malaysia and to find the amount of commercial BSO needed to obtain a specific amount of TQ.

Materials and Methods

**Materials**

TQ standard was purchased from Sigma-Aldrich (St. Louis, Missouri). Acetonitrile (high-performance liquid chromatography [HPLC] grade) and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Samples

Commercially available BSO products were purchased from local markets and pharmacies in Peninsular Malaysia. The products were selected to contain mainly BSO in capsules and liquid dosage form only (the commercially available forms of BSO). The analysis was performed on the available products in the market, and 10 such products were selected.

Thymoquinone quantification

The HPLC method adopted from Salmani *et al.*^[13] was used to quantify the TQ in BSO. Briefly, BSO sample (50 mg directly from the liquid dosage form or from the capsule form to obtain the liquid BSO) was dissolved in 10 mL of methanol to extract the TQ. After that, it was mixed well by inverting the tube 10 times, then centrifuged at 4000rpm at room temperature for 5 min to separate the methanol layer. The upper methanol layer, well separated from the bottom BSO, was collected and filtered using a 0.45- μ m syringe filter. The HPLC analysis was applied using Shimadzu LC-20AT equipment (Shimadzu, Kyoto, Japan). A mixture of acetonitrile and water in the ratio of 60:40 was used as a mobile phase at a flow rate of 1 mL/min using Inspire C18 (4.6mm \times 250mm, 5 μ m) analytical column. The detection was performed at an ultraviolet wavelength of 254nm using a diode-array detector.

Calculation of theoretical amount of black seed oil for selected diseases

The doses of TQ used for specific diseases in animal models were converted to human doses by using the following equation:^[14]

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$$\text{HED (mg / kg)} = \frac{\text{Animal dose (mg / kg)} \times \text{Animal Km}}{\text{Human Km}}$$

where HED stands for human equivalent dose, Animal Km is a constant value for each animal (mouse: 3, rat: 6), and human Km is a constant value equates to 37.

According to the quantification results of TQ in the commercial BSO products, the theoretical amounts of the BSO needed to provide the TQ amount for different diseases were calculated.

Results

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HPLC analysis revealed well-separated peaks of TQ at retention time of approximately 6.1 min [Figure 1]. The method showed linearity in the range 0.01–150 µg/mL with $R^2 = 0.9943$.

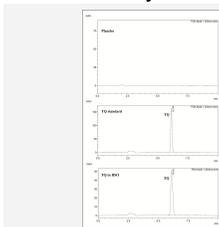


Figure 1: Chromatogram of placebo, TQ standard, and one BSO sample. For placebo, olive oil was used and it was processed exactly as BSO samples. BSO = black seed oil, TQ = thymoquinone

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Quantification of the percentage of TQ in the commercial BSO products revealed large differences as low as 0.07% wt/wt up to 1.88% wt/wt as shown in [Table 1]. TQ percentage in BSO packed in bottles ranged from 0.14% to 1.88%, whereas it ranged from 0.07% to 1.86% for capsule forms. The product with the highest TQ concentration is approximately 27-folds higher than the product with the lowest TQ concentration.

Product	Form	TQ Concentration (%)
P1	Bottle	0.14
P2	Bottle	0.15
P3	Bottle	0.16
P4	Bottle	0.17
P5	Bottle	0.18
P6	Bottle	0.19
P7	Bottle	0.20
P8	Bottle	0.21
P9	Bottle	0.22
P10	Bottle	0.23
P11	Bottle	0.24
P12	Bottle	0.25
P13	Bottle	0.26
P14	Bottle	0.27
P15	Bottle	0.28
P16	Bottle	0.29
P17	Bottle	0.30
P18	Bottle	0.31
P19	Bottle	0.32
P20	Bottle	0.33
P21	Bottle	0.34
P22	Bottle	0.35
P23	Bottle	0.36
P24	Bottle	0.37
P25	Bottle	0.38
P26	Bottle	0.39
P27	Bottle	0.40
P28	Bottle	0.41
P29	Bottle	0.42
P30	Bottle	0.43
P31	Bottle	0.44
P32	Bottle	0.45
P33	Bottle	0.46
P34	Bottle	0.47
P35	Bottle	0.48
P36	Bottle	0.49
P37	Bottle	0.50
P38	Bottle	0.51
P39	Bottle	0.52
P40	Bottle	0.53
P41	Bottle	0.54
P42	Bottle	0.55
P43	Bottle	0.56
P44	Bottle	0.57
P45	Bottle	0.58
P46	Bottle	0.59
P47	Bottle	0.60
P48	Bottle	0.61
P49	Bottle	0.62
P50	Bottle	0.63
P51	Bottle	0.64
P52	Bottle	0.65
P53	Bottle	0.66
P54	Bottle	0.67
P55	Bottle	0.68
P56	Bottle	0.69
P57	Bottle	0.70
P58	Bottle	0.71
P59	Bottle	0.72
P60	Bottle	0.73
P61	Bottle	0.74
P62	Bottle	0.75
P63	Bottle	0.76
P64	Bottle	0.77
P65	Bottle	0.78
P66	Bottle	0.79
P67	Bottle	0.80
P68	Bottle	0.81
P69	Bottle	0.82
P70	Bottle	0.83
P71	Bottle	0.84
P72	Bottle	0.85
P73	Bottle	0.86
P74	Bottle	0.87
P75	Bottle	0.88
P76	Bottle	0.89
P77	Bottle	0.90
P78	Bottle	0.91
P79	Bottle	0.92
P80	Bottle	0.93
P81	Bottle	0.94
P82	Bottle	0.95
P83	Bottle	0.96
P84	Bottle	0.97
P85	Bottle	0.98
P86	Bottle	0.99
P87	Bottle	1.00
P88	Bottle	1.01
P89	Bottle	1.02
P90	Bottle	1.03
P91	Bottle	1.04
P92	Bottle	1.05
P93	Bottle	1.06
P94	Bottle	1.07
P95	Bottle	1.08
P96	Bottle	1.09
P97	Bottle	1.10
P98	Bottle	1.11
P99	Bottle	1.12
P100	Bottle	1.13
P101	Bottle	1.14
P102	Bottle	1.15
P103	Bottle	1.16
P104	Bottle	1.17
P105	Bottle	1.18
P106	Bottle	1.19
P107	Bottle	1.20
P108	Bottle	1.21
P109	Bottle	1.22
P110	Bottle	1.23
P111	Bottle	1.24
P112	Bottle	1.25
P113	Bottle	1.26
P114	Bottle	1.27
P115	Bottle	1.28
P116	Bottle	1.29
P117	Bottle	1.30
P118	Bottle	1.31
P119	Bottle	1.32
P120	Bottle	1.33
P121	Bottle	1.34
P122	Bottle	1.35
P123	Bottle	1.36
P124	Bottle	1.37
P125	Bottle	1.38
P126	Bottle	1.39
P127	Bottle	1.40
P128	Bottle	1.41
P129	Bottle	1.42
P130	Bottle	1.43
P131	Bottle	1.44
P132	Bottle	1.45
P133	Bottle	1.46
P134	Bottle	1.47
P135	Bottle	1.48
P136	Bottle	1.49
P137	Bottle	1.50
P138	Bottle	1.51
P139	Bottle	1.52
P140	Bottle	1.53
P141	Bottle	1.54
P142	Bottle	1.55
P143	Bottle	1.56
P144	Bottle	1.57
P145	Bottle	1.58
P146	Bottle	1.59
P147	Bottle	1.60
P148	Bottle	1.61
P149	Bottle	1.62
P150	Bottle	1.63
P151	Bottle	1.64
P152	Bottle	1.65
P153	Bottle	1.66
P154	Bottle	1.67
P155	Bottle	1.68
P156	Bottle	1.69
P157	Bottle	1.70
P158	Bottle	1.71
P159	Bottle	1.72
P160	Bottle	1.73
P161	Bottle	1.74
P162	Bottle	1.75
P163	Bottle	1.76
P164	Bottle	1.77
P165	Bottle	1.78
P166	Bottle	1.79
P167	Bottle	1.80
P168	Bottle	1.81
P169	Bottle	1.82
P170	Bottle	1.83
P171	Bottle	1.84
P172	Bottle	1.85
P173	Bottle	1.86
P174	Bottle	1.87
P175	Bottle	1.88

Table 1: Thymoquinone concentration in commercial black seed oil products

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TQ was used in different diseases for treatment or protection. Selected TQ animal studies are summarized in [Table 2]. The calculated TQ doses in HED varied widely from 4.8 mg/day for hypertension and renal damage to 780 mg/day for diabetes mellitus. The amount of BSO required to provide the specific amount of TQ was further varied among different products [Table 2]. For example, compared with the low BSO amount needed for hypertension and renal damage (0.26–0.52g/day from P10), an extraordinarily large amount is needed for diabetes mellitus (277.71–555.42g/day from P9).

Product	Disease	TQ Dose (mg/day)	BSO Required (g/day)
P1	Hypertension	4.8	0.26
P2	Renal damage	4.8	0.52
P3	Diabetes mellitus	780	277.71
P4	Diabetes mellitus	780	555.42

Table 2: Thymoquinone doses in different animal studies and the calculated human equivalent dose per 60kg together with the theoretical black seed oil quantity needed from different products (g/day) to provide the calculated thymoquinone quantity

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Discussion

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TQ percentage in BSO was not directly related to packaging of the products (capsule and liquid in bottle). A large difference in the TQ content in the products could be attributed to the source of BSO as well as the extraction method. Different studies on the BSO showed that TQ content varied considerably in oils from different origins. For example, Houghto *et al.* reported 0.17% wt/vol TQ in the Ethiopian BSO, whereas Azad *et al.* reported 1.96% wt/wt (~2.06%

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wt/vol) TQ in BSO sourced from Malaysia and Bangladesh.^{[15],[16]} In addition, the TQ content in BSO varied based on the different extraction techniques. In one study, BSO was extracted from supercritical carbon dioxide and the TQ contents varied in the range of 3.04–4.09 mg/g oil (0.30%–0.41%) based on the extraction parameters.^[17] In another study, six different batches of cold-pressed black cumin seed oils were prepared, and TQ content was found in the range of 3.48–8.73 mg/g (0.35%–0.87%).^[18]

The therapeutic effects of black seed and its oils or extracts were investigated in several studies but without quantification of the TQ content. For example, Qidwai *et al.*^[10] conducted a clinical trial using the powdered black seed in a capsule at a dose of 1g twice daily (2g/day). However, there is a trend to use standardized *black seed* extracts in the more recent studies. The standardization was based on the quantification of TQ.^{[21],[8]} For example, a study investigated the efficacy and safety of standardized BSO in the treatment of patients with nonalcoholic fatty liver disease. Therefore, TQ can be used as a biomarker for *N. sativa* oil.

Since clinical studies of TQ or standardized BSO are lacking, we calculated the human TQ doses based on available animal studies. To obtain a specific dose of TQ from the commercial BSO products, we used the TQ analysis results for calculation. This method of estimation has some limitations like the lack of bioavailability data to know how much from the ingested dose will reach the general circulation. The estimated BSO amount that contains theoretical therapeutic TQ amount was found to be very large to be consumed. For example, at least 277.71g of the commercial product P9 is needed for diabetes mellitus. Such BSO amount clearly seems to be very large and even exceeds the 27g/day recommended daily intake of oils.^[19] Considering the low percentage of TQ in BSO (usually <2%) and the high safety margin of TQ (up to 2600 mg/day with no reported toxicity), consumption of large amount of BSO is expected not to result in toxicity from TQ; however, effects of overconsumption of lipids may occur as BSO consists mainly of fatty acids.^[20]

Conclusion



Using BSO in the treatment of a specific disease needs a clinical trial to evaluate the safety and efficacy. However, due to the lack of clinical trials using BSO, the data from TQ may be used as a starting guide. The patient may consider taking an amount of BSO that has the required amount of TQ, with limitations such as lack of bioavailability data. Calculating the BSO amount that contains specific TQ amount can only be accomplished by knowing the TQ content in the commercial BSO, which is now not available and not regulated. As a result, commercial BSO products have large variations in TQ content and some of them may not provide the TQ amount needed for some diseases. This study recommends the regulation of TQ content in the commercial BSO. It also suggests that BSO could be fortified with additional TQ to be beneficial in more diseases.

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Conflicts of interest

There are no conflicts of interest.

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Figures

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