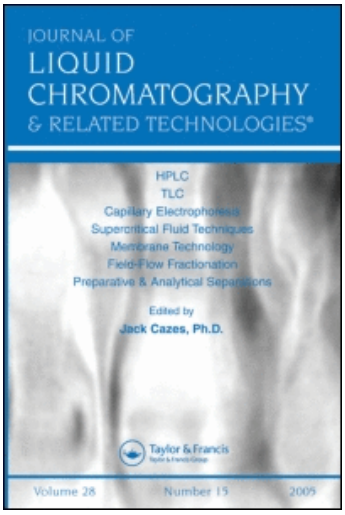

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TLC ASSAY OF THYMOQUINONE IN BLACK SEED OIL *(NIGELLA SATIVA LINN)* AND IDENTIFICATION OF DITHYMOQUINONE AND THYMOL

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ABSTRACT

A simple quantitative TLC method for the determination of thymoquinone in commercially available black seed oil obtained from *Nigella Sativa L.* (Ranunculaceae) using a scanning densitometer is described. Also the identification of thymol, dithymoquinone in this sample was established. The R_f values for thymoquinone, thymol and dithymoquinone are 0.77, 0.37 and 0.52 respectively. The identification of the thymoquinone spot, obtained from the methanol extract of oil is confirmed by GC/MS which is essentially identical to thymoquinone standard. The solvent system consisted of benzene: isopropyl ether (1:1). All the spots were visualised and quantitated at 254 nm. The method proposed is simple, reproducible with a lower limit of detection of 100 nmoles/ml and can be used in routine analysis of thymoquinone in black seed oil for quality control purposes.

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INTRODUCTION

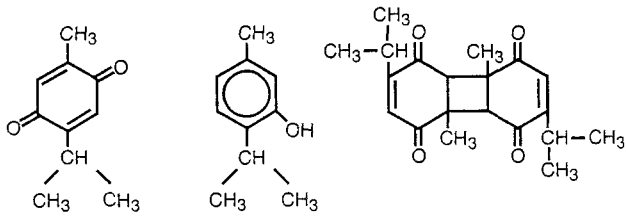
Nigella Sativa L. (Ranunculaceae), it was first identified and described by Linnaneus, 1753.

Nigella Sativa L. grows in Mediterranean countries and is cultivated in others. The black seed oil has a long history of folklore medicine in Arabian and other countries for the treatment of various diseases (1,2). Its main constituents are fixed oil, volatile oil and alkaloids.

Tapozada et al (3) and Rathee et al (4) demonstrated that the volatile oil content of black seed oil is equivalent to 1.4-1.9% based on the weight of total oil extracted.

The percentage of thymoquinone isolated from *Nigella sativa L.* volatile oil was only 24% w/w of the volatile oil weight (5). Thymoquinone was isolated in a yield of 18.4 w/w from the volatile oil (6). Other constituents detected in the volatile oil were thymol and thymoquinone dimer (dithymoquinone) (7). The chemical structure of thymoquinone, thymol and dithymoquinone are shown in Figure 1. Aboutabl et al, (8) reported on the presence of monoterpenes, phenols and some esters in black seed oil.

It has been shown that the volatile oil revealed some pharmacological activities such as bronchodilators (9,10,11), increases bile flow and concentration of bile salts (12), decreases blood pressure in dogs (13) and rats (14). Accordingly, it was considered worthwhile to establish an analytical method for the determination of thymoquinone to which the pharmacological activities of black seed oil are attributed.



Thymoquinone Thymol Dithymoquinone

Figure 1. The chemical structure of thymoquinone, thymol and dithymoquinone.

MATERIALS AND METHODS

Chemicals

Methanol, benzene, isopropyl ether were HPLC grade (Springfield, New Jersey, USA). TLC, Alumina GF plates (20 cm X 20 cm, 250 microns, Analtech, Inc. Newark, USA) were used. Authentic thymoquinone obtained from Aldrich Chemical Co., Milwaukee with 99% purity was used without further purification. The three black seed oil samples obtained from the local market. Thymol was obtained from BDH Pool, UK. Dithymoquinone was prepared according to the method described by Smith & Tess (15).

Uniscan video densitometer (Analtec. Newark. DE, USA) was used in the analysis of thymoquinone and it consist of viewbox fitted with the appropriate light sources, video camera and an IBM compatible Central Processing Unit (CPU) with appropriate computer boards and densitometer software.

The solvent system used for TLC runs was benzene: isopropylether 1:1. A saturation time of 30 min is allowed before each run (Chamber dimensions are:

30 cm, 10 cm, 25 cm length, width and height respectively). All sample spots were quantitated using the Uniscan integration software at wavelength 254 nm.

Extraction procedure for oil:

One ml of methanol was added to 1 ml of oil (commercial black seed oil) in a glass centrifuge tube with cover. Vortex mix for 2 mins, the methanol top layer was transferred to a small glass vial and 5 μl was spotted on TLC plate.

The methanol extract of black seed oil and the spots corresponding to thymoquinone scrabed from the plates extracted with methanol, for further identification by GC/MS.

Gas chromatography mass spectrometry (GC/MS):

Electron impact (EI)-GC/MS analysis of the samples were carried out using a Hewlett-Packard (Palo Alto, CA, USA) 5988A GC/MS system equipped with a Hewlett-Packard 5890 GC and a 7673A autosampler. An Ultra-1 crosslinked methylsilicone capillary column (Hewlett-Packard, 25mx0.2mm i.d. x 0.33 μm film thickness) was used for analysis. The column oven was programmed as follows: 1 min at 80^{o}C, followed by an increase of 6^{o}C/min up to 290^{o}C followed by 5 min at 290^{o}C and injections were made in the splitless mode. The mass spectrometer interface was maintained at 280^{o}C and the mass spectrometer was scanned after a 4 min delay from m/z to 550.

Variability and Percentage Recovery:

The black seed oil sample 3 (1 ml) spiked with 500 nmol internal thymoquinone standard. The spiked sample extracted and 5 μl was spotted on TLC plate. The spiked sample assayed 6 times during two weeks period to evaluate the precision of the assay.

RESULTS AND DISCUSSION

The TLC solvent described above gave optimum separation of thymoquinone, its dimer dithymoquinone and thymol. Identification of thymoquinone, dithymoquinone and thymol was achieved by using their corresponding R_f values, as shown in Table 1.

GC/MS

Both the oil extract and the TLC purified sample showed a peak at 11.8 min. This peak had the same retention time as that obtained by injecting a solution of standard thymoquinone. The spectra obtained from the extract and the purified sample were essentially identical with the thymoquinone standard and also matched with the NIST library spectrum. The spectrum showed a strong molecular ion at m/z 164 and a strong M-CH_3 at m/z 149 as well as a strong ion corresponding to M-CO at m/z 136 and also showed M-C_3H_7 at m/z 121 and also a strong ion at m/z 93, possibly corresponding to M-C_3H_7-CO. A typical spectrum of the scrabed thymoquinone spot is shown in Figure 2.

Table 1. R_f values of Thymoquinone, Dithymoquinone and Thymol

	$R_f$values*
Thymoquinone	0.77
Dithymoquinone	0.52
Thymol	0.37

* Average of 6 determinations.

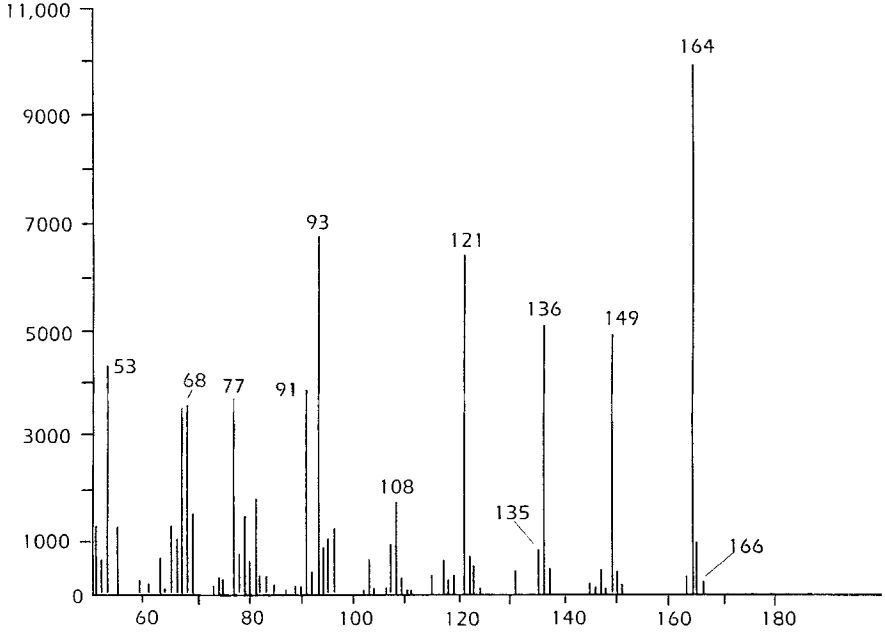


Figure 2. A mass spectrum of scraped thymoquinone TLC spot.

Linearity

Linear regression curves were constructed over the range of 1 - 30 nmoles with the correlation coefficient 0.979, (n=6) (Figure 3). Each determination (n=6) for the thymoquinone contents in black seed oil consisting of calibration curve and extracts of interest, was on one TLC plate. The lower limit detection of the method was 100 nmoles/ml as shown in Figure 4.

Percentage Recovery and Variability

The percentage recovery of spiked oil sample 3 was 90, with a coefficient of variation 5.6. Table 2 summarizes the results for the quantitative assay of thymoquinone in black seed oil.

Thorough literature review revealed that thymoquinone, was constantly the main active constituent of volatile oil contents of black seed oil (5,6,8).

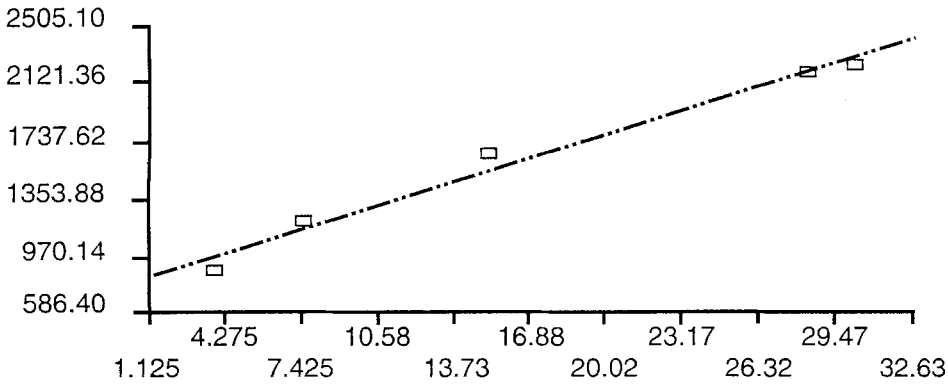


Figure 3. Linear regression curve for the quantitative determination of thymoquinone.

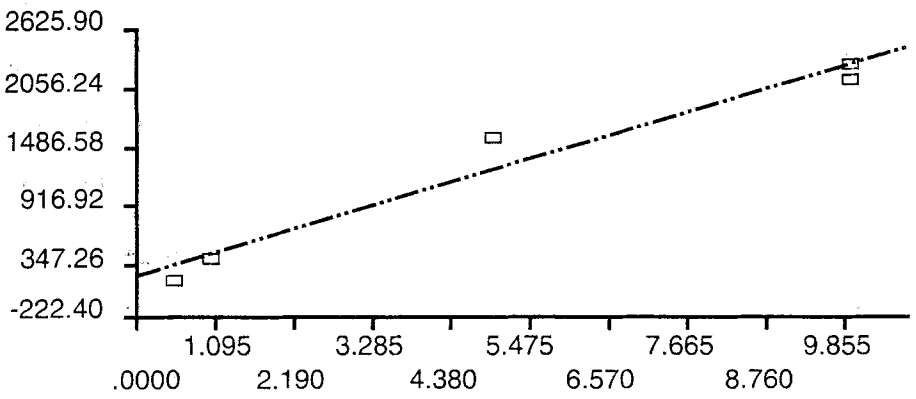


Figure 4. Linear regression curve for the quantitative determination of the lower limit of thymoquinone.

Table 2: Thymoquinone content in black seed oils (3 commercial samples) and sample 3 spiked with thymoquinone internal standard.

Black seed oil sample	Mean* ± S.D (nmoles/5μl)	C.V.	Thymoquinone nmoles/ml
1	14.42 ± 1.07	7.4	2.9×10^3
2	4.46 ± 0.6	6.0	0.9×10^3
3	undetected	-	< 100
3 spiked	2.25 ± 0.13	5.7	0.45×10^3

*Average of 6 determinations.

Marozzi et al. (16) claimed that the pharmacological activities of the volatile oil is due to its thymoquinone contents. The previous statement suggest that the quality of black seed oil was related to its thymoquinone contents. This content may vary according to the method of processing.

CONCLUSION

The quantitative TLC determination for thymoquinone described in this study provides a simple, rapid, reproducible method of analysis which can be used to establish the criteria required for the quality control of this constituent.

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