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THIN-LAYER CHROMATOGRAPHY ANALYSIS OF *NIGELLA SATIVA* L. ESSENTIAL OIL

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Abstract

The seeds and oil of *Nigella sativa* L. (black cumin) are used in food and pharmacology industry possessing antioxidative, antimicrobial, antihypertensive and antidiabetic properties. The aim of this work was to introduce a simple, inexpensive method for determination of the main compounds in essential oil of black cumin.

The isolation of the essential oil of *Nigella sativa* L. seeds was done with steam distillation followed by extraction with diethyl ether (3 x 10 mL) and dried over sodium sulphate. The identification of the main compounds was performed with thin-layer chromatography (TLC). For chromatographic determination, 2.5 µL of sample standard solutions were spotted on 20 x 20 cm Merck pre-coated TLC plates (60 F254, 250 µm). A comparison study with two mobile phase solvent systems were applied to the analysis, (A) consisted of benzene : glacial acetic acid (1 : 1) and (B) consisted of carbon tetrachloride/acetone/glacial acetic acid (15.2 : 3 : 1).

The retention factor (Rf) less than 0.3 obtained with the (A) mobile phase was not satisfying in the separation of the compounds from the mixture. The identification of one spot-one compound was achieved with (B) mobile phase where the Rf values for two main compounds thymoquinone and dithymoquinone were 0.6 and 0.5, respectively. The high quality of essential oil from black cumin is as a result of the presence of thymoquinone in a quantity > 50%.

The oil with nutritive value and pungent bitter taste can be used as a food flavoring additive and as a functional food. The health-promoting bioactive compounds present in *Nigella sativa* L. essential oil could be detected with TLC as easy-to handle and rapid method for screening of oil quality.

***Key words**: TLC, Black cumin, Essential oil, Thymoquinone.*

1. Introduction

Plant material as a potential source of phytochemical and/or bioactive substance could be utilized widely in many fields and industries including food industry [1]. In traditional medicine plants have been used in prevention and treatment of many diseases [1 - 5]. Past years many research have been focused on natural products as a source of substances with health beneficial effects or as raw material for obtaining new pharmacologically active compounds. In the World Health Organization Traditional Medicine strategy 2014 - 2023 was stated that in many developing countries people still use the traditional medicine for health maintenance and in disease prevention and treatment especially for chronic diseases [6 - 12].

There are many substances in the food industry that increase the pleasant appearance of the products such as texture, colour, aroma and taste. The usage of spices as food additives, flavouring agents and also digestive stimulant have been practiced through the years [1, 2]. *Nigella sativa* L. (fam. *Ranunculaceae*) best known as black cumin is an annual fennel bushy flower plant (40 - 50 cm height) with white, pale to dark blue flowers and crunchy seeds [1, 10]. Phytochemical and pharmacological effects of black cumin were studied in many research due to its medical importance [13 - 15]. The taste can be recognized from slightly bitter to peppery. Cultivation of this herbal plant is mainly in the Mediterranean and the Middle East countries, Southern Europe, North Africa, Southwest Asia but the spread of its utilization is known worldwide as a miracle cure against many diseases [2].

From the whole plant of black cumin, seeds are extensively used in food products. They are characterized as angular, small size (1 - 5 mg), dark grey or black colour and a pungent bitter taste and smell [1]. The seeds are usually roasted and the obtained flavour is similar to coffee. It is reported that the black cumin can be added

to coffee, tea or casseroles used in canning or extracted in wine and vinegar [1]. Honey is usually mixed with the black cumin or the seeds are added to the salad. Food industry exploits black cumin as uncrushed in several types of bread, muffins and biscuits but also giving aroma to cheese products (cottage cheese, brynza) or in pickles [1].

The oil originated from black seeds is important in nutritional, food and pharmaceutical industry directly linked to the chemical composition of the herbal plant. Seeds were shown to contain fixed oil (> 30% w/w) and a volatile oil (0.4 - 0.45%). The essential oil is rich in nutrient and antioxidant compounds, phenols and flavonoids. Oil can be considered as essential which is characterized with strong odour, volatile and lower density than water. Studies show that the isolated oil from *Nigella sativa* L. has low level of toxicity. The oil possesses potent *in vitro* free radical scavenging effect which is correlated to the: total content of polyunsaturated fatty acids, phospholipids, tannins, alkaloids, phenolic compounds, amino acids, proteins, carbohydrates, crude fibres, terpenoids, and saponins [6, 7]. Now days the oil has tendency to be used in the process of cooking [7]. It is pungent to note that the oil of *Nigella sativa* L. can be used in increasing the shelf life of fresh fish fillets as a food preservative, stored at 2 ^{0}C [8]. The plant is also used as natural phototoxic potential on lettuce. Natural preservatives classified as natural antioxidants avoiding the potential synthetic toxic substances [10].

Active compounds in black cumin classified as phenolic compounds such as p-cymene, thymoquinone, and carvacrol, have beneficial effects thus the interest of the research is to determine the presence of the bioactive compounds [16, 17]. Phytochemicals are essential agents in reducing the toxicity and balancing the antioxidant levels. Focus of the black cumin use as a cure is primarily for treatments related to the body malfunctions, diseases and disorders such as: diabetes, headache, eczema, dizziness, fever and influence, high level of cholesterol and cardiovascular diseases, gastro-intestinal and respiratory disorders, and takes part in improvement of the immune and endocrine systems. The herbal plant also shows anti-inflammatory and antioxidant effects. Various studies have shown that the oil possess: anticancer, antimicrobial, antifungal, antihypertensive, antihistaminic, diuretic, renal-protective and hepatoprotective properties [5, 6, 9, 11 - 14]. Commercial available forms of these seeds use include shampoos, oils, and soaps. The oil is reported to be used also in the cosmetics showing good effects on the skin but also for dermatological disorders such as psoriasis and eczema [2].

Main ingredient in the essential oil, thymoquinone, 2-isopropyl-5-methyl-1,4-benzoquinone (Figure 1a) is a bioactive compound. It is available in tautomeric forms, where the keto form is important in showing anti-oxidative effect which directly affects the immune system [15, 16]. Role in the immune system is described as a mechanism where the substance directly influences on cell signalying and antioxidant molecular moderators involved in the process of inflammation. It is reported that if the quantity of thymoquinone is higher than 50%, the essential oil will possess an anti-inflammatory properties, and have a broad antimicrobial spectrum including Gram-positive and Gram-negative bacteria, viruses, parasites, and fungi [17]. It reacts as a potential radical and superoxide radical scavenger at range from nanomolar to micromolar scale. The dimer of thymoquinone, dithymoquinone (Figure 1b) is also isolated from the *Nigella sativa* L. oil.

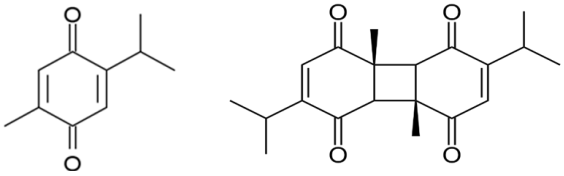


Figure 1. Chemical structures of the main compounds in black cumin: thymoquinone (a) and dithymoquinone (b)

Unfortunately, the infections nowadays are extended and the presence of the therapeutic problems are worldwide. Use of the natural products is increased due to the chemical properties of the compounds which are present in the essential oil of the herbal plants [1, 2]. Unique mechanism of plants action plays an important role in the treatment of many diseases. It is of great interest to carry out a screening of the plant material in order to validate its usage in alternative medicine [17]. On the other hand, the control of the oil enables the quality of the product with the isolation and characterization of the components consisted in the essential oil. In a literature survey a number of methods have been reported for the analysis of biological active compounds including spectroscopic and chromatography methods such as: high performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), gas chromatography-mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and ultraviolet-visible spectroscopy (UV/Vis) [18-27]. These methods are either time-consuming, have long sample preparation procedures, and/or high toxic reagents and expensive equipment.

The lack of literature data for the routine analysis of the black cumin herbal material rises the interest to develop a simple method for screening the main plant components. Successful determination of biologically active compounds from plant material depends on the nature of the solvent used in the extraction process and on temperature. The greater the temperature the more likely is to obtain compounds which are result from degradation of the analysed material.

The aim of this study was to introduce an easy to handle method for the control and ensure the quality of *Nigella sativa* L. oil. A simple, rapid and less laborious thin-layer chromatography (TLC) method was used in the analysis to determine the main components of *Nigella sativa* L.

2. Materials and Methods

2.1 Materials

Oil and seed of *Nigella sativa* L. were purchased from the local market (Figure 2). The seeds were crushed in order to obtain more homogeneous mixture and the extraction to be performed without obstacles. Merck (Germany) supplied analytical grade carbon tetrachloride, acetone, glacial acetic acid, benzene, diethyl ether and methanol. Visualization of the spots was done on 20 x 20 cm Merck pre-coated TLC plates (60 F_{254}, 250 µm). Utilized UV lamp was from Analytikjena (Germany).

Figure 2. Black cumin (oil and seed)

2.2 Methods

2.2.1 Steam distillation

Steam distillation was performed in a low temperature to prevent side reactions of degradation. According to a lab procedure 10 g of seed were mixed with 100 mL distilled water. The distillate was transferred quantitatively into a separatory funnel. The extraction was repeated three times using 10 mL of diethyl ether at each step, vigorously shaking the funnel several time (Figure 3). This method is proper to retain the quality of the substances which can be decomposed at higher temperature.

Figure 3. Extraction technique in obtaining black cumin extract

The goal of this type of technique is based on the principle of substance mass transfer into the solvent component where the movement began to occur at the interface layer following with diffusion into the solvent. Organic layer was dried with sodium sulphate and the residue of the organic solvent was evaporated in a water bath.

2.2.2 Preparation for TLC analysis

Small amount of seed powder (64 mg) were dissolved in 1 mL methanol. A preliminary study was performed using mobile phase consisted of benzene and glacial acetic acid. A comparison study with two mobile phase solvent systems were applied to the analysis, (A) consisted of benzene : glacial acetic acid (1 : 1) and (B) consisted of carbon tetrachloride/acetone/glacial acetic acid (15.2 : 3 : 1). Analysed samples were methanolic and water extract as well as commercially available black cumin oil (Figure 4).

a) b) c)

Figure 4. Development of the TLC plate: preparation of the plate (a), the chamber (b), UV lamp (c)

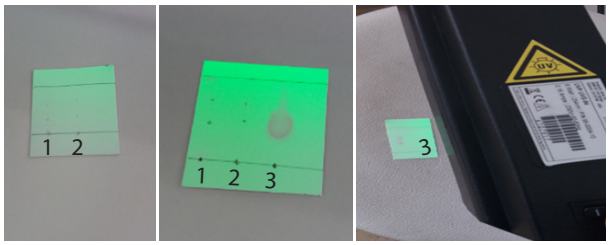
Start line was drawn with pencil on the pre-coated TLC plate (Figure 4a). Analysed samples were dropped as spots on the start line. The TLC plate was developed with a suitable mobile phase in the chamber (Figure 4b). After the plate development the finish line was drawn with pencil. Visualization of the spots in order to determine retention factors (R_f) was done under UV lamp (Figure 4c).

3. Results and Discussion

The chromatogram was examined with an UV lamp and the identified spots were compared with the literature data of the standards with the obtained oil. Preliminary analysis using the mixture of the mobile phase (A) benzene : glacial acetic acid (1 : 1) showed not satisfying separation due to the polar properties of the glacial acetic acid. The TLC analysis of the methanolic (1) and water extract (2) of the black cumin seed extract with the mobile phase (B) carbon tetrachloride/acetone/glacial acetic acid (15.2 : 3 : 1) (Figures 5a, 5b) had better separatory effect, but the polarity characteristics of the commercial oil was not yet satisfying in the separation. Using chloroform in the procedure (Figure 5c), the spots were successfully separated.

Table 1. Retention factor (R_f) values of the main compounds in *Nigella sativa* L. using different mobile phases

No	Sample	Type of mobile phase (v/v)	R_f (thymoquinone)	R_f (dithymoquinone)
1	methanolic extract	benzene : glacial acetic acid (1 : 1)	< 0.3	< 0.3
2	water extract	carbon tetrachloride/acetone glacial acetic acid (15.2 : 3 : 1)	0.6	0.5
3	oil	chloroform	0.62	0.45



a) b) c)

Figure 5. TLC chromatograms of extracted black cumin seed in methanol (1), water (2), and oil (3)

The retention factor (R_f) values of different types of samples of the analysis of black cumin either the extract or the commercial oil using different mobile phases is given in Table 1.

The choice of the solvent for the development process depends on the behaviour of the components which should be separated. The best separation is achieved when the value of the retention factors (R_f) is between 0.3 and 0.6 due to the stain development is within the progression to the end point of the development process.

4. Conclusions

- Developed TLC method was successfully applied in the analysis of *Nigella sativa* L. oil and gave clear evidence of bioactive compounds, thymoquinone and dithymoquinone. It can be used for phytochemical screening of black cumin seed oil as a need for standardization of plant products used in food industry.

- Method is simple, easy to handle and can be effectively used for the routine quality analysis of commercially available black cumin seed and oil.

5. References

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