Macedonian Pharmaceutical Bulletin, 66 (Suppl 2) 3 - 4 (2020)
Online ISSN 1857 - 8969
UDC: 615.322:582.675.1-148
DOI: 10.33320/maced.pharm.bull.2020.66.04.001
Short communication

A comparative study of extraction methods of bioactive compounds from *Nigella sativa* L. seed

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Introduction

Nigella sativa L. (black cumin) represents an annual herbaceous plant of the Ranunculaceae family (Palabıyık and Aytaç, 2018; Ramadan, 2007). The plant commonly grows in the Middle East, Western and Central Asia and Eastern Europe. The characteristics of the plant include a slightly bitter and peppery taste of the small-sized seeds with a crunchy texture and dark grey or black color. Plant research is of great interest due to the chemical compounds showing beneficial effects against diabetes, cancer, headache, eczema, dizziness, fever, hypercholesterolemia, cardiovascular diseases, gastrointestinal and respiratory disorders (Ramadan, 2007). Black cumin is also categorized as a plant with anti-inflammatory and antioxidant potential (Jerrine et al., 2017; Ramadan, 2007). Nutrients and antioxidant compounds such as phenolic compounds, flavonoids, polyunsaturated fatty acids, phospholipids, amino acids, proteins, carbohydrates, crude fibers, terpenoids and saponins are present in the essential oil isolated from black cumin (Samarakoon et al., 2010). The main bioactive compounds present in *Nigella sativa* L. seeds are thymoquinone, *p*-cymene and carvacrol (Palabıyık and Aytaç, 2018). The most common commercially forms of the plant include shampoos, oils, and soaps. In the literature, the described extractive methods include maceration, reflux, sonication (Kausar et al., 2017) or microwave extraction (Xue et al., 2013) in relation to relatively expensive methods like HPLC (Kausar et al., 2017). The aim of this study was to make a comparative study of extraction methods with an easy-to-handle and economic thin-layer chromatography (TLC) method for control analysis of the composition of *Nigella sativa* L.

Materials and methods

ThermoFisher (UK) supplied analytical grade methanol, hexane, diethyl ether, toluene and ethyl acetate, while Merck (Germany) supplied sodium sulfate. Commercially available black cumin seed and oil were used in the analysis.

Several extraction modes like liquid-liquid extraction followed by steam distillation and Soxhlet extraction, and reflux method were used in the analysis. Methanol and hexane were chosen for extraction of the most abundant compounds in the plant material (thymoquinone and dithymoquinone). The steam distillation and the process of reflux were carried out with 10 g of the black cumin and were placed in a 500 mL round-bottom flask with 150 mL of water. The distillate was collected until no further droplets of oil could be seen in the case of steam distillation, while the reflux extraction was performed for 2 hours. A separatory funnel was used for the liquid-liquid extraction with a hexane portion (3x5 mL). Anhydrous sodium sulfate was used as a drying agent. Evaporation of the organic solvent was performed in a moderate hot plate. A Soxhlet

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apparatus using 500 mL round-bottom flask, a 300 mm condenser and methanol as solvent was performed preliminarily for 2 cycles. The reflux material was filtered with Büchner funnel.

A preliminary thin-layer chromatography (TLC) method was developed for the qualitative determination of black seed content and fractions from various types of extracts. The TLC was carried out using Merck pre-coated plates (60 F_{254}, 250 µm) and visualized at 254 nm using an Analytikjena (Germany) ultraviolet lamp. A mixture of mobile phases like diethyl ether and toluene (1:1 *v*/*v*), mobile phase A and toluene and ethyl acetate (7:3 *v*/*v*), mobile phase B were used in the analysis.

Results and discussion

Methanol and hexane were chosen as proper solvents for black cumin not only for fewer hazard effects than organochloric substances (Kausar et al., 2017; Xue et al, 2013) but also for the high solubility of the plant in it. The main drawbacks of the use of Soxhlet extraction are time-consuming and decomposition of bioactive compounds (Kausar et al., 2017). The yield with hexane followed by the steam distillation was 1%. After the third cycle of the Soxhlet extraction, the result showed a relatively high yield (4%). The reflux method itself was achieved at hot with constant cooling. The yield was 3% using hexane as extraction solvent.

The TLC method was included as a control method for the process. The analytical purity was indicated with TLC (one spot) where the R_f values depended on the use of mobile phase. The R_f values for thymoquinone and dithymoquinone with the mobile phase A were 0.7 and 0.5 while when TLC was carried out with the mobile phase B the R_f values were 0.6 and 0.4, respectively, for both commercial oil and the oil obtained through different extraction mode.

Conclusion

The extraction mode and the used temperature are significant factors for the process. The methods which depend upon heat were carried out at a moderate temperature to protect the phytochemicals of the plant from degradation. The TLC method represents a choice to conduct analyses between the commercial oil and the oil obtained with several different extraction procedures including steam distillation, reflux and Soxhlet extraction. Both bioactive compounds of the oil, thymoquinone and dithymoquinone, could be successfully detected with TLC method.

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