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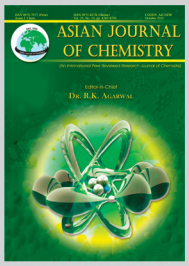

# Extraction of Volatile Oil of *Nigella sativa* L. Using Multivariate Analysis

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The present study investigate multivariate analysis to extract volatile oil of *Nigella sativa* L. cultivated in Middle East and East of Asia. Each cultivated seed used to extract volatile oil by specific method. The used method affect both the quantity and compound of the oil. To determine the best method factor analysis was applied and out of five different methods, three methods are recommended.

**Key Words: *Nigella sativa* L., Chemical composition of volatile oil, Best technology, Factor analysis.**

## INTRODUCTION

*Nigella sativa* has been used for medicinal purposes for centuries, both as an herb and pressed into oil, in Asia, Middle East and Africa. It has been traditionally used for a variety of conditions and treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support and for general well-being. Alternative medicine practices are as diverse in their foundations as in their methodologies. Practices may incorporate or base themselves on traditional medicine, folk knowledge, spiritual beliefs or newly conceived approaches to healing[1]. Recently scientists around the world have confirmed the antibacterial and antimycotic effects of black seed oil. Health practitioners in various countries around the world are using the oil against inflammation of all sorts as well as fungi infections. Even a remarkable reduction of blood sugar levels has been found by scientists. Black seed extracts have been found help to stimulate bone marrow and immune cells, so the scientists of the cancer Immuno-Biology laboratory and adding that it raises the interferon production, protects normal cells against cell destroying effects of viruses, destroys tumor cells and raises the number of antibodies producing B cells[2]. There are many methods of extracting essential oils such as hydrodistillation, distillation, steam distillation, water and steam distillation, solvent extraction, carbon dioxide extraction, cold pressing, florasols/phytols[3]. Laboratories should focus their attention on evaluating and improving the quality and performance of the methods currently employed rather than attempting to institute a wide range of methods using new, untried, methods or losing confidence because of their lack of sophisticated equipment. In many cases, implementing a data quality assurance system and training staff are often better ways to produce good-quality compositional data[4]. In nutrient analysis for a food composition database, the requirements for accuracy and precision may be orientated more towards the recommended intake of a nutrient and the relative importance of the food being analyzed in the diet[5].

## EXPERIMENTAL

Table-1 shows the method of extraction volatile oil from five different literatures. **Method 1:** Seeds of *N. sativa* were purchased from the local market in Tehran, Iran[6]. **Method 2:** Black cumin seeds of indigenous variety were obtained from Barani Agricultural Research Institute (BARI), Chakwal, Pakistan[7]. **Method 3:** The seeds of black cumin were purchased from the local market of Gorakhpur, Uttar Pradesh, India. A voucher specimen (herbarium no.123) was deposited at the herbarium of the faculty of science, Deendayal Upadhyay University, Gorakhpur University[8]. **Method 4:** The seeds of *N. sativa* were procured from local market, Anantapura-A.P.-India[9]. **Method 5:** Mature seeds of *N. sativa* were collected in April and May 2005 from cultivated plants from the Nabeul and Menzel Temime regions (Northeast of Tunisia). Samples of these seeds were brought to the Pharmacognosy Department of the Faculty of Pharmacy, Vasile Goldis, West University of Arad. They were soaked in water, washed, air-dried and stored in hermetic bags and deep frozen until used[10].

**Method 1:** Petroleum ether → reduced pressure → hydrodistilled (4 h) → extracted with *n*-hexane → reduced pressure→dried over anhydrous sodium sulfate.

**Method 2:** Extracted through solvent extraction technique; hexane.

TABLE-1
CHEMICAL COMPOSITION OF THE VOLATILE CONSTITUENTS OF *NIGELLA SATIVA* L. FROM DIFFERENT LITERATURES

| Compound of volatile oils | Petroleum ether[6] (%) | Hexane[7] (%) | Soxhlet (acetone)[8] (%) | Hydro-distilled at 100 $^0$C[9] (%) | Hexane[10] (%) |
|---|---|---|---|---|---|
| α-Thujene | 2.4 | 2.4 | 10.03 | 2.4 | 13.75 |
| α-Pinene | 1.2 | 1.48 | 3.33 | 1.2 | |
| Sabinene | 1.4 | | 1.34 | 1.4 | 1.66 |
| β-Pinene | 1.3 | | 3.78 | 1.3 | 3.00 |
| Myrcene | 0.4 | | | 0.6 | 0.94 |
| α-Phellandrene | 0.6 | | | 0.6 | |
| p-Cymene | 14.8 | | 36.2 | 9.0 | 43.58 |
| Limonene | 4.3 | 1.72 | 1.76 | 4.3 | |
| γ-Terpinene | 0.5 | | 0.16 | 0.5 | 1.40 |
| Monoterpenoid hydrocarbons | 26.9 | 5.6 | 56.6 | 21.3 | 64.33 |
| Fenchone | 1.1 | | | 1.1 | |
| Dihydrocarvone | 0.3 | | | 0.3 | |
| Carvone | 4.0 | | 0.16 | 2.0 | |
| Thymoquinone | 0.6 | 23.25 | 11.27 | 11.8 | 1.65 |
| Monoterpenoid ketones | 6.0 | 23.25 | 11.43 | 15.2 | 1.65 |
| Terpinen-4-ol | 0.7 | | 2.37 | 0.7 | 4.25 |
| p-Cymene-8-ol | 0.4 | 32.02 | 0.09 | 0.4 | |
| Carvacrol | 1.6 | 10.38 | 2.12 | 3.7 | 2.53 |
| Monoterpenoid alcohols | 2.7 | 42.4 | 4.58 | 4.8 | 6.78 |
| α-Longipinene | 0.3 | | 1.54 | 0.3 | 0.95 |
| Longifolene | 0.7 | | 6.32 | 5.7 | |
| Sesquiterpenoid hydrocarbons | 1.0 | 0 | 7.86 | 6.0 | 0.95 |
| Estragole | 1.9 | | | 1.9 | 0.91 |
| Anisaldehyde | 1.7 | | | 1.7 | |
| Trans-anethole | 38.3 | 2.1 | 0.61 | 27.1 | |
| Myristicin | 1.4 | | | 1.4 | |
| Dill apiole | 1.8 | | | | |
| Apiole | 1.0 | | | 1.0 | |
| Phenyl propanoid compounds | 46.1 | 2.1 | 0.61 | 33.1 | 0.91 |
| *n*-Nonane | 1.7 | | | | |
| 3-Methyl nonane | 0.3 | | | 0.6 | |
| 1,3,5-Trimethyl benzene | 0.5 | | | | |
| *n*-Decane | 0.4 | | | 0.4 | |
| 1-Methyl-3-propyl benzene | 0.5 | | | 0.7 | |
| 1-Ethyl-2,3-dimethyl benzene | 0.2 | | | 0.2 | |
| *n*-Tetradecane | 0.2 | | | 0.2 | |
| *n*-Hexadecane | 0.2 | | | 0.2 | |
| Nonterpenoid hydrocarbons | 4.0 | 0 | 0 | 2.3 | 0 |
| Total compounds | 86.7 | 73.35 | 81.08 | 82.7 | 74.62 |

**Method 3:** Cold pressed → hydrodistilled (3 h) → dried over anhydrous sodium sulfate.

**Method 4:** Plants are fully submerged in water → soup is heated at 100 ºC → hydrodistilled → dried over anhydrous sodium sulfate.

**Method 5:** Seeds are soaked in water, washed, airdried and stored in hermetic bags and deep frozen until used → cold pressed → hydrodistilled (4 h) → extracted with *n*-hexane → dried over anhydrous sodium sulfate.

**Extraction and analysis of volatile oil:** Nickavar *et al.*[6] used 25 mL of concentrated extracted oil were hydrodistilled for 4 h. The distillate was extracted with *n*-hexane. The organic layer was separated, concentrated to 1 mL under reduced pressure and dried over anhydrous sodium sulfate to produce volatile oil[6]. The oil from the black cumin seed was extracted through solvent extraction technique. Black cumin seeds were ground (750 mesh) with a domestic electronic grinder and hydrodistilled in a clevenger-type apparatus for 3 h according to the method recommended by the European pharmacopoeia. Fine powered and hydro-distilled at 100 ºC in a Clevenger apparatus. Air-dried and finely ground *N. sativa* seeds samples (100 g) were hydrodistilled for 4 h. The distillate was extracted with *n*-hexane. The organic layer was separated, concentrated under reduced pressure and dried over anhydrous sodium sulfate.

## RESULTS AND DISCUSSION

To find out the best method to extract volatile oil, five methods were examined and factor analysis is used to achieve the objective. Table-2, shows the eigenvalues for each component along with the percentage of the total variance explained by that component. First component accounts for nearly half of the variability in the set of eight predictor variables, meaning that this single component by itself carries about half of the information in all eight predictors. Second component and third one account together for nearly half of the variability in the set of eight predictor variables. The remaining component is small enough to be ignored.

TABLE-2
TOTAL VARIANCE EXPLAINED

| Component | Extraction sums of squared loadings | | | Rotation sums of squared loadings | | |
|---|---|---|---|---|---|---|
| | Total | Variance (%) | Cumulative (%) | Total | Variance (%) | Cumulative (%) |
| **1** | 8.436 | 49.625 | 49.625 | 6.682 | 39.305 | 39.305 |
| **2** | 4.462 | 26.245 | 75.870 | 5.858 | 34.460 | 73.765 |
| **3** | 3.141 | 18.479 | 94.349 | 3.499 | 20.584 | 94.349 |
| **4** | 0.961 | 5.651 | 100.000 | | | |

Table-3 shows the three components that account for as much of the variability as possible while retaining a relatively small number of components (only three methods were suggested). Method 3 is the best to extract (β-pinene, α-longipinene, α-pinene, carvone, limonene, longifolene and *trans*-anethole) with variance 49.625 %. **Method 5** is the best to extract (α-thujene, estragole, myrcene, *p*-cymene, sabinene, terpinen-4-ol and γ-terpinene). **Method 2** is the best to extract (carvacrol, *p*-cymene-8-ol and thymoquinone).

TABLE-3
ROTATED COMPONENT MATRIX

| | Best method to extract volatile oil | | |
|---|---|---|---|
| | Method 3 | Method 5 | Method 2 |
| β-Pinene | 0.901 | | |
| α-Longipinene | 0.950 | | |
| α-Pinene | 0.961 | | |
| α-Thujene | | 0.745 | |
| Carvacrol | | | 0.999 |
| Carvone | -0.943 | | |
| Estragole | | -0.957 | |
| Limonene | -0.777 | | |
| Longifolene | 0.740 | | |
| Myrcene | | 0.871 | |
| *p*-Cymene-8-ol | | | 0.952 |
| *p*-Cymene | | 0.751 | |
| Sabinene | | 0.967 | |
| Terpinen-4-ol | | 0.907 | |
| Thymoquinone | | | 0.882 |
| *trans*-Anethole | -0.829 | | |
| γ-Terpinene | | 0.935 | |

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