

Quality Control of *Nepeta menthoides* Boiss & Buhse using Micromorphological Analysis and Phytochemical Screening

Kahkeshani N (Ph.D. student)¹, Khanavi M (Ph.D.)², Hadjiakhoondi A (Ph.D.)^{3*}

1- Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

2- Department of pharmacognosy and Traditional Iranian Medicine Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

3- Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

*Corresponding author: Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Tel: Tel/Fax: +98 21 66494996

Email: abbbadji@gmail.com

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Abstract

Background: *Nepeta menthoides* Boiss & Buhse is a species from Lamiaceae family. It is used as a herbal medicine with common name "Ostokhodus" in Iranian traditional and folk medicine but there is not enough information about its quality and quantity control methods.

Objective: In this study we use micromorphological and phytochemical evaluations for qualitative and quantitative control of *N. menthoides* powder.

Methods: Macroscopic and microscopic characteristics, phytochemical screening and flavonol quantification were done on *N. menthoides* powder.

Results: Results showed that all of these assessments can use as quality control parameters for authentication of *N. menthoides*, particularly micromorphological specifications.

Conclusion: Our study shows the importance of micromorphological evaluation in authentication of this herbal medicine but further investigations are needed to complete these data.

Keywords: *Nepeta menthoides*, Authentication, Microscopic evaluation, Ostokhodus, Phytochemical screening

Introduction

Nepeta L. (Lamiaceae) is a large genus with approximately 300 herbaceous species. Different species of this genus are widely distributed in southwestern Asia and western Himalayas especially Iran, Turkey and Hindu Kush. Various species have been used as sedative, diuretic and antispasmodic in traditional medicine of different countries [1, 2].

Different species of *Nepeta* genus particularly *Nepeta menthoides* are pointed as "Ostokhodus" in Iranian folk medicine and herbal shops. They usually use to treat nervous ailments, rheumatic pains and high blood pressure [1, 3-5].

Various phytochemical studies have shown the presence of flavonoids like cirsimaritin, isothymusin, genkwanin, apigenin, luteolin and quercetin and different phenolic acids and terpenoids in *Nepeta* species. Analysis of essential oils in different species of this genus demonstrated that the essential oil composition is related to different parameters like climate of growing site and analysis methods. According to previous studies, the essential oils of *Nepeta* species are rich in nepetalactone diastereoisomers or 1,8-cineole [2].

As we know, authentication of crushed and powdered medicinal plants in herbal markets is difficult because of morphological diagnostic characters disappearance. So, there is an increasing need to simple and reliable methods for authentication and quality control of medicinal plants purchased from herbal shops for the sake of consumer's safety [5]. In this investigation, we use some simple methods like macroscopic and microscopic analysis and phytochemical screening for quality and quantity control of *N. menthoides* powder.

Material and Methods

Plant materials

Aerial parts of *N. menthoides* were collected from Ardabil province during flowering stage. Voucher specimen is deposited at the Central Herbarium of the Institute of Medicinal Plants (ACECR), Karaj, Iran. Leaves and flowers were separated and air-dried at room temperature. Then, they crushed into fine powder for subsequent experiments.

Macroscopic and microscopic characters

Crushed plant was evaluated for organoleptic characters and macroscopic appearance. Prior to micromorphological analysis of the powder, it sieved and decolorized in boiled potassium hydroxide (10%) for 3 minutes and then rinsed with sodium hypochlorite and distilled water successively [6]. Microscopic slides were prepared with aqueous glycerin and stained with different coloring agents like methylene blue, carmine or alum carmine. Then, they observed under a laboratory microscope (Carl ZEISS Standard 14 Laboratory Microscope, Germany).

Phytochemical screening test

Powdered plant (50 g) was percolated with hydro alcoholic solution (methanol/water-80:20 v/v) for 3 consecutive 72 hours at room temperature. Screening tests for alkaloids, flavonoids, triterpenes, sterols and saponins were done based on previously mentioned standard methods [7-9].

Total flavonol quantification

Assay of total flavonol was done according to previous methods. Briefly, 0.5 ml of concentrated extract (0.4 mg/ml) was dissolved in 1.5 ml of methanol and then, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M

potassium acetate and 2.8 ml distilled water were added successively. The absorbance of final solution was measured at 415 nm after 30 minutes incubation at room temperature, using a UV spectrophotometer (Mecasys, Korea). Quercetin (5-50 $\mu\text{g/ml}$) was used as reference standard to draw the calibration curve [8].

Results

According to macroscopic analysis, *N. menthoides* powder had brown color, bitter

taste and aromatic odor. Phytochemical screening tests were positive for flavonoids, triterpenes and saponins and total flavonol content was $210 \pm 1.1 \mu\text{g/mg}$ based on quercetin equivalent.

In microscopic evaluation of *N. menthoides* powder, various tissue elements like leaf, petal and corolla epidermal layers and different types of covering and glandular trichomes were observed (Figure 1).

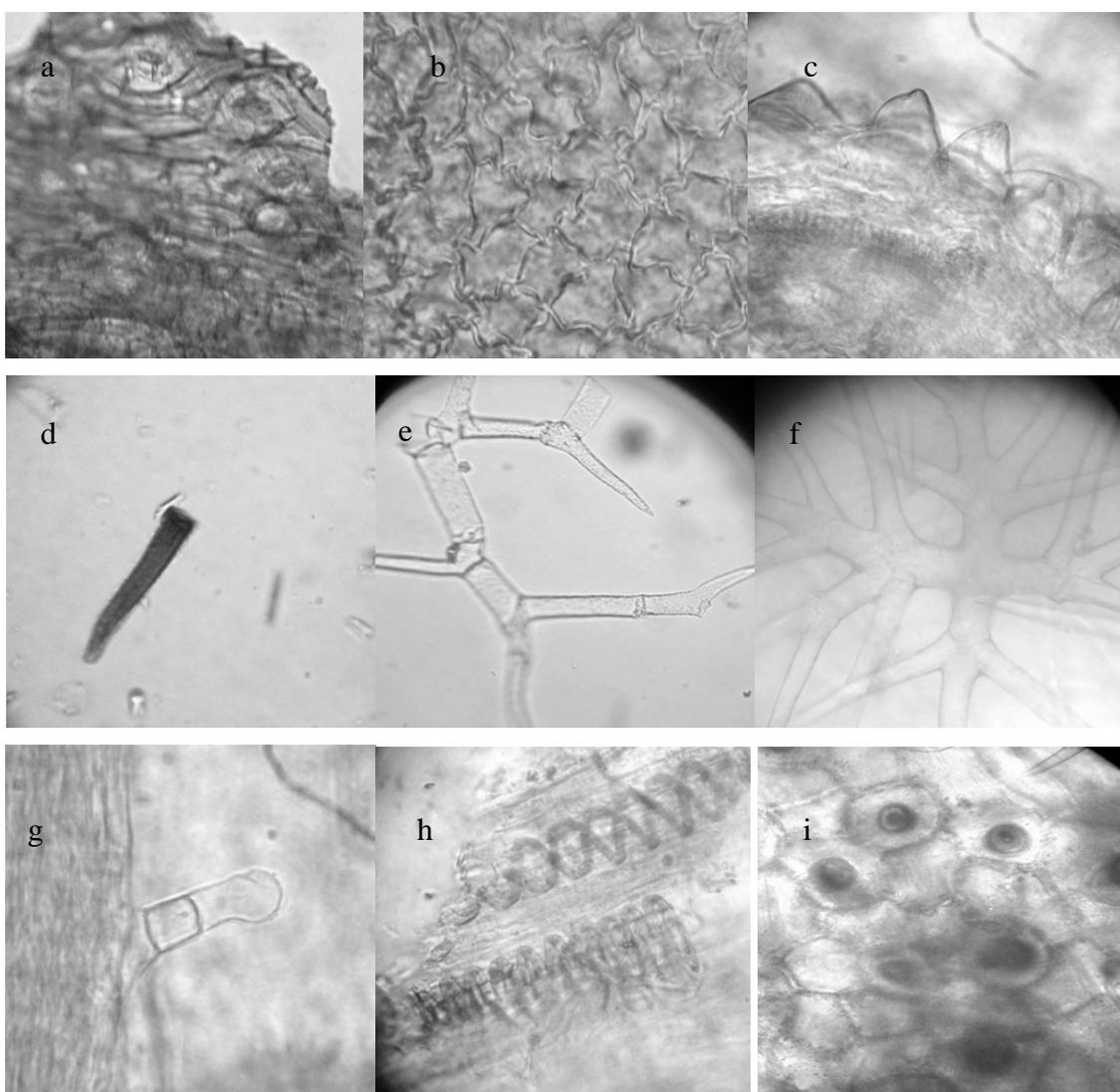


Figure 1- Micromorphological evaluation of *Nepeta menthoides* Boiss & Buhse. powder, a- epidermal layer of leaf with paracytic stomata, b- epidermal layer of petal with wavy cell wall, c- epidermal layer of corolla, d- unicellular covering trichome, e- multicellular covering trichome, f- multicellular branched covering trichome, g- capitate glandular trichome, h- spiral xylem, i- epidermis with cicatrix (microscopic figures $\times 40$)

Discussion

According to ethno botanical and folkloric assessments and analysis of samples in herbal shops, it is supposed that *Nepeta* L. especially *N. menthoides* is mainly used as Ostokhodus in Iran. As it mentioned in previous parts, authentication and quality control of herbal samples especially those purchased from market are important issues particularly because of efficiency and safety for consumers [3, 5].

In these cases, we can use simple tests like macroscopic and microscopic analysis, phytochemical screening and thin layer chromatography fingerprint evaluation for quality control of herbal medicines. [10 - 12].

According to our literature review, despite wide utilization of *N. menthoides* as a medicinal plant by Iranians, there was not enough information about its quality control parameters. In this study we tried to define some quality and quantity control markers for this important medicinal plant. Our phytochemical screening showed the presence of flavonoids and terpenoids in *N. menthoides* sample which is in accordance with previous phytochemical investigations in different general and species of Lamiaceae family [13]. Based on this phytochemical profile we used assay of total flavonol as a quantitative test which can be used for quality control of *N. menthoides* samples and standardization of

its medicinal products.

Previous studies have confirmed the importance of trichome types in taxonomy and phylogeny of various general in of Lamiaceae family [14 - 18]. In our microscopic study, we recorded and reported different micromorphological elements of *N. menthoides* powder as microscopic pictures for the first time. These pictures can be drawn as schematic shapes and added to the results of qualitative and quantitative phytochemical studies for preparation of a complete monograph for *N. menthoides* as a widely consumed medicinal plant in herbal pharmacopeia of Iran.

Conclusion

The results of macroscopic, microscopic and phytochemical analysis are helpful in quality and quantity control of *N. menthoides* powder but we have planned to do different complementary tests like preparation of TLC and HPTLC fingerprints of its extract or essential oil to bring our results perfect.

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