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CONTENT

INVESTIGATION OF THE PHENOLIC COMPOUNDS CONTENT OF THE RAW MATERIAL OF MELISSA OFFICINALIS L.

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Abstract. *The studies on the phytochemical and pharmacological study of M. officinalis raw materials are being conducted in different countries of the world. There are reports that M. officinalis raw material is rich in flavonoid, terpenoid, phenolic acids, tannins and essential oils. The purpose of the work is to study the phenol content of M. officinalis raw material from the flora of Azerbaijan. Qualitative reactions and chromatographic studies confirm the presence of phenolic compounds in raw materials. Thus, as a result of the study of the composition of phenolic compounds of M. officinalis L. raw material from the flora of Azerbaijan by the high-performance liquid chromatography method, 18 substances were determined and identified. The indicated substances were identified for the first time in M. officinalis raw materials distributed in Azerbaijan.*

Keywords. *Melissa officinalis, phenolic compounds, HPLC, flavonoids*

INTRODUCTION

Identification of raw materials rich in phenol compounds from the flora of Azerbaijan and creation of new medicines based on them is one of the important problems facing pharmaceutical science [Garibli et all., 2021]. One such source of raw materials is M. officinalis L., a plant of the Lamiaceae family.

Currently the studies on the phytochemical and pharmacological study of M. officinalis raw materials are being conducted in different countries of the world. There are reports that M. officinalis raw material is rich in flavonoid, terpenoid, phenolic acids, tannins and essential oils [Gabriela Petrisor, et all., 2022]. Rosmarinic acid, caffeic acid, protocatechinic acid, quercetin, rhamnocitrin and luteolin substances were obtained from polyphenol compounds from plant raw materials and identified with the help of chromatographic and spectroscopic methods. In addition to polyphenol compounds, monoterpenes¸ sesquiterpenes and triterpene terpenoids, as well as essential oils, were identified in the raw material [Nevena Draginic et all., 2021].

There is a lot of information about the medicinal lemon balm plant having high antioxidant activity. It is shown that the antioxidant activity of the plant is related to the flavonoids, phenolic compounds, and organic acids in the raw materials. The fact that the plant has high antioxidant activity creates a basis for its effectiveness in the treatment of diseases related to oxidative stress [Sepide Miraj et al ., 2017].

In general, M. officinalis raw materials are widely used in traditional medicine in European countries, Mediterranean coast and Middle Eastern countries in the treatment and prevention of various diseases. The extract of the plant and the complex of biologically active

substances have anxiolytic, antiviral and antispasmodic activity, as well as cognitive functions and memory strengthening properties [Shakeri et all., 2016].

According to clinical studies, it was found that depression, stress and sleep disorders decrease in patients who take M. officinalis for 8 weeks [Haybar et al ., 2018]. As a result of the study of the antiviral properties of M. officinalis raw materials, it was found that the plant has activity against Herpes simplex virus, Severe acute respiratory syndrome coronavirus 2 and Herman immunodeficiency virus components [Behzadi et al., 2023]. It was determined that M. officinalis raw material plays an imperative role in acute menopausal syndrome diseases [Kargozar et all., 2017]. M. officinalis raw material has been found to have an effective effect on anxiety and depression [Ghazizadeh et all ., 2021].

Thus, M. officinalis raw materials are used in different countries of the world as antiviral, antidepressant, anxiolytic, anti-stress, in diseases accompanied by acute menopausal syndrome, etc. it is applied. It should be noted that all medicinal preparations and food supplements with biological activity produced on the basis of M. officinalis are brought to our Republic from foreign countries. Therefore, the pharmacochemical study of M. officinalis raw material, which is widespread in the flora of the country, is relevant.

MATERIALS AND METHODS

The goal of this research is to study the phenol content of M. officinalis raw material from the flora of Azerbaijan.

Raw material of M. officinalis used for research was supplied in 2022 from the territory of Astara region during the flowering phase of the plant.

The methods of extraction and alternate fractionation were used to obtain the aggregate of phenolic compounds from raw materials. 80% ethyl alcohol and methanol solutions were used for extraction.

Characteristic qualitative reactions and chromatographic methods were used to determine phenolic compounds, including coumarins, flavonoids, oxycinnamic acids in raw materials. Benzene-ethyl acetate-acetic acid (5:5:1), 15% acetic acid, chloroform-methanol (9:1-1:9) systems were used as the chromatographic system.

The constituent components of phenolic compounds were studied by high-performance chromatography method [Suleymanov & Pashayeva, 2018]. For this purpose, a chromatograph with Agilent Technologies 1200 brand, G-1316 A column and Agilent chemistation software was used. HPLC grade methanol, acetonitrile, orthophosphoric acid and water solvents were used for chromatography.

Chromatographic conditions: C-18 brand (Discovery), 4.6x250 nm size chromatographic column;

Mobile phase: acetonitrile-water-orthophosphoric acid (40:60:0.5);

Speed of mobile phase - 0.9 ml/minute;

The temperature of the thermostat is 25°C;

The injection volume of the studied sample is 3 µl;

Analysis time - 50 minutes

RESULTS AND DİSCUSSİON

Specific qualitative reactions (10% sodium hydroxide solution, cyanide test, boron-lime reaction, 2% solution of aluminum chloride, etc.) to determine the presence of phenol compounds, including oxycinnamic acids, coumarins and flavonoids in M. officinalis raw materials has been used. Qualitative reactions and chromatographic studies confirm the presence of phenolic compounds in raw materials.

The total amount of phenolic compounds from M. officinalis raw material was obtained by extraction method. Grade brand methanol solvent was used for this. The obtained methanol extract was combined and turned into a dry residue. The sample solutions prepared from the obtained dry residue were injected into a high-performance liquid chromatograph in a volume of 3 µl.

Gallic acid, pyrohallol, cyanidin-3-glucoside,

chlorogenic acid, benzoic acid, catechin, epicatechin, caffeic acid, vitexin, hesperidin, naringenin, taxifolin, rosmarinic acid, myricetin, luteolin, quercetin, apigenin, isoramnetin and etc. standard samples were used.

The peak areas of the phenolic compounds in the analyzed raw material sample and their retention periods were studied in a comparative manner with the calibration indicators of the standard samples and the data of the spectra library, and the substances were identified.

The method of performing measurements in a high-performance liquid chromatograph reflects the separation of the substances contained in the studied sample solution into individual components, and then determining their concentration.

The high-performance liquid chromatography method is based on the chromatographic separation of phenolic compounds into their individual constituents on a C-18 column and the recording of chromatograms at the appropriate wavelength on a diode-matrix ultraviolet detector. Coumarins and flavonoids, as well as oxycinnamic acids, are identified both by the retention time of the peaks and by the characteristic of the spectrum of the investigated substance. In order to identify the substances separated from the phenolic compounds, the relative retention time of the sample peak in the chromatogram was compared with the relative retention time of the standard sample solution. The results of the chromatographic analysis are given in figure 1 and table 1.

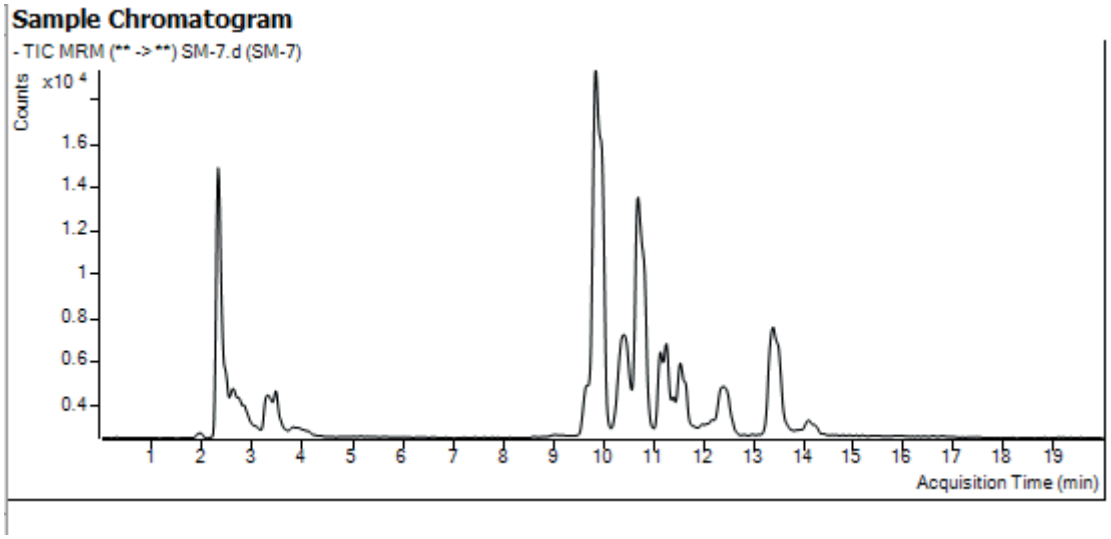

Figure 1. *Chromatogram of methanol extract of M. officinalis L. raw material.*

As a result of research using high-performance liquid chromatography, 18 substances belonging to the group of phenolic compounds were found in lemon balm. As a result of comparing the peak areas of the detected substances with the peak areas of the standard samples, the substances were identified as quercetin, sinic acid, rosmarinic acid, ferulic acid, taxifolin, coumaric acid, naringin, syringic acid, vanillic acid, epicatechin, catechin, benzoic acid, peonidin-3-glucoside, cerasiamine chloride, fumaric acid, and quinic acid.

The amount of substances is calculated and determined based on the calibration characteristics, as well as the dependence of the peak area in the chromatogram on the concentration of the corresponding substances in the sample solution. The amount of individual substances in the total of phenolic compounds was calculated based on the weight of raw materials and the final volume of the sample (Table 1).

Table 1.

Results on quantification of phenolic compounds in M. officinalis L. raw material.

Quantitation Results

Compound	RT	Response	Conc
Quinic Acid	2,332	80714	31049,1190
Fumaric Acid	3,858	8761	5231,9451
Keracyanin Chloride	10,400	27	8,1450
Cyanidin-3-o-glucoside	10,401	52073	5254,2023
Chlorogenic Acid	10,708	133493	5070,7861
Peonidin-3-o-glucoside	10,923	1899	379,7892
4-OH-Benzoic Acid	11,138	3447	114,7593
Catechin	11,305	5656	606,3201
Epicatechin	11,305	6688	543,7056
Vanillic Acid	11,499	51	41,6823
Syringic Acid	11,555	87	88,8841
Naringin	11,938	961	53,2220
p-Coumaric Acid	12,211	4953	60,8696
Taxifolin	12,377	15148	202,6475
Ferulic Acid	12,397	639	106,1832
Rosmarinic Acid	12,462	5886	1461,1454
Sinapic Acid	12,469	178	31,0028
Quercetin	13,393	50605	1519,9404

CONCLUSION

Thus, as a result of the study of the composition of phenolic compounds of M. officinalis L. raw material from the flora of Azerbaijan by the high-performance liquid chromatography method, 18 substances were determined and identified. The indicated substances were identified for the first time in M. officinalis raw materials distributed in Azerbaijan. The fact that the composition of the raw material is rich in phenolic compounds creates a basis for extensive pharmacochemical research.

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