Biologically active substances of new marjoram varieties and prospects for their use

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Abstract. Biologically active substances, essential oils, and plant extracts were studied obtained from new high-yield varieties of *Origanum vulgare* L. (Raduga, Belaya Ptitsa, Krymchanka) and *Origanum tyttanthum* Contsch. (Alcina) of the GSBSI "NBG – NSC RAS" breeding. It was found that the *O. vulgare* variety Belaya Ptitsa was distinguished by the content of essential oil, the main component of which was carvacrol. The maximum total content of phenolic substances and flavonoids was distinguished by an alcoholic extract from the *O. tyttanthum* variety. Neochlorogenic and rosemary acids, apigenin-6,8-di-C-glucoside, carvacrol, and thymol were identified in extracts of all the studied marjoram varieties. It is shown that the vegetable raw material *O. vulgare* cv "Belaya Ptica" is promising for the production of essential oil with a high content of carvacrol, and *O. tyttanthum* cv " can serve as a raw source of rosemary acid.

1 Introduction

The genus marjoram (*Origanum* L.), belonging to the Lamiaceae family (Lamiaceae Lindl.), includes about 55 species [1], the most common species is common origanum (*Origanum vulgare* L.). It is traditionally used as a spicy-aromatic, medicinal, and essential oil culture. *O. vulgare* is a pharmacopoeial plant and is widely used in the production of combined medicines with anti-inflammatory, antimicrobial, expectorant, diuretic, choleretic, sedative, and antispasmodic effects [2]. Marjoram preparations have a calming effect for the central nervous system, enhance the secretion of digestive and bronchial glands, intestinal peristalsis, contributing to the restoration of impaired metabolic processes. [3] Another representative of the genus *Origanum* – small-flowered marjoram (O. *tyttanthum* Gontsch.) has long been used in Persian-Tajik folk medicine in the treatment of acute and chronic bronchitis, pneumonia, bronchial asthma, urolithiasis, diabetes mellitus, as well as as a sedative, anticonvulsant, diaphoretic, and diuretic. This species is not a pharmacopoeia plant, but is comprehensively studied as a promising medicinal plant with a high content of phenolic substances and antioxidant activity [4].

In the collection of the Nikitsky Botanical Garden – the National Scientific Center, there are 6 species of the genus *Origanum*, such as: *O. onites* L., *O. virens* Hoffmanns. Link., *O. vulgare* L., *O. heraclioticum* L., *O. laevigatum* Boiss., *O. tyttanthum* Gontsch. [5]. As a

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result of breeding work, new highly productive varieties of common marjoram and white-flowered marjoram were obtained. A comprehensive study of biologically active substances of new marjoram varieties is relevant. In this regard, the **purpose of the work** was a comparative analysis of biologically active compounds of plant raw materials of *O. vulgare* and *O. tyttanthum varieties* to determine the directions of their further use.

2 Materials and Methods

Objects of study were 3 varieties of *Origanum vulgare* L.: Raduga cv, cv Belaya Ptitsa, cv Krymchanka, and 1st grade *Origanum tyttanthum* Contsch.: cv Alciona of the breeding of FSBSI "NBG – NSC RAS". Plant raw materials (the aboveground part) for research were selected from the collection sites of the Nikitsky Botanical Garden in the phase of mass flowering.

The mass fraction of essential oil was determined by the method of Clevenger hydrodistillation from dried raw materials [6]. The component composition of the essential oil was studied on a chromatograph Chromatek-Crystal 5000.2 (Chromatek, RF) with a mass spectrometric detector. The following parameters were used during the research: capillary column CR –5ms, length 30 m, inner diameter 0.25 mm. Phase 5% phenyl 95% polysilphenylenesiloxane, film thickness 0.25 microns. The thermostat temperature was programmed from 75 °C to 240 °C at a speed of 4 °C/min. The evaporator temperature is 250 °C. The carrier gas is helium, the flow rate is 1 ml/min. The temperature of the transition line is 250 °C. The temperature of the ion source is 200 °C. Electronic ionization of 70 eV. The scanning range is 20-450 Da. The scan duration is 0.2 s. The sample volume is 0.2 µl. The flow division is 1:200. Identification was performed on the basis of comparison of the obtained mass spectra with data from the NIST 14 library (National Institute of Standards and Technology, USA). The EM components were identified by comparison with the data of the NIST14 MS Search mass spectrum library [7, 8].

Extracts for the determination of phenolic compounds were prepared from vegetable raw materials by infusing in 80% aqueous ethanol (raw material: extractant ratio - 1: 50) for 7 days with regular stirring. The flasks with extracts were placed in an ultrasonic bath for 30 minutes at a temperature of no more than 40 °C. The resulting extract was filtered through a teflon membrane filter with a pore size of 0.45 microns and used in further studies. The content of the sum of phenolic substances was determined by the Folin-Chocalteu method, in terms of Gallic acid [9], flavonoids by the Murri method in terms of rutin [10] using the Evolution 220 UV/VIS spectrophotometer of Thermo Scientifc company.

The component composition of polyphenolic compounds was determined using a liquid chromatograph of Agilent Technologies, model 1100, (Germany); equipped with a flow vacuum degasser G1379A, a 4-channel low pressure gradient pump G13111A, an automatic injector G1313A, a column thermostat G13116A, a diode matrix detector G1316A, a fluorimetric detector G1321A. A chromatographic column with a size of 2.1-150 mm filled with an octadecylsilyl sorbent with a grain size of 3.5 microns, "ZORBAX-XDB C-18" was used for the analysis. Chromatography was performed in gradient mode. The composition of the eluent: eluent A 0.1% aqueous solution of phosphoric acid – solution B – acetonitrile: water (ratio 9: 1). The composition of the eluent changed according to the scheme (according to the content of the comp. B): 0 min 5%; 0-6.5 min 5-10%; 6.5-14 min 10-25%; 14.0-21 min 25-55%; 21.0-29 min 55-100%; 29.1-34.0 min 5%. The volume of the injected sample is 1 ml. The analysis was carried out at a thermostat temperature of 40 °C. The feed rate of the eluent is 0.3 ml/min. The analysis duration is 34 minutes. Detection was carried out using an Agilent Technologies diode matrix detector model G1316A, at wavelengths: 280 nm for thymol and carvacrol; 350 nm for apigenin

glycoside; 313 nm for neochlorogenic and rosemary acids [11]. Identification of phenolic compounds was carried out according to the retention times of standards and spectral characteristics. The content of phenolic substances was calculated for dry weight. The experiments were carried out in 3-fold repetition.

3 Results and Discussion

An analysis of previous studies [12] showed that the highest essential oil content is 1.51% (of the crude mass) and the main component of carvacrol accumulates in the *O. vulgare* raw material of the Belaya Ptitsa variety, which significantly exceeds the same pharmacopoeial indicators (Table 1) [13]. The rest of the studied varieties are inferior in the content of essential oil, including the mass fraction of carvacrol. Carvacrol is a monoterpene phenol, recently this compound has attracted a lot of attention for its numerous biological properties (antioxidant, anti-inflammatory, neuroprotective, antitumor, antibacterial and a number of others [14].

We have established that in the essential oil obtained from the raw material *O. tyttanthum* cv Alciona the component with the maximum mass fraction is thymol. Thymol has high bactericidal, anthelmintic, antibiotic, fungicidal activity, immunomodulatory, and antispasmodic effects [15].

The presence of monoterpene phenols carvacrol and thymol in the essential oils of *O. vulgare* and *O. tyttanthum* determines a wide range of their biological action.

Table 1. The content of essential oil and its main components in the varieties *Origanum vulgare* and O. *tyttanthum.*

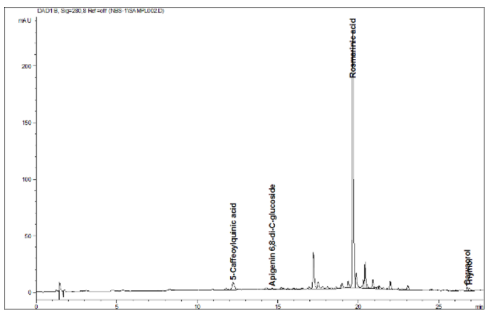
Indicator	*O. vulgare*			*O. tyttanthum*
	cv ′Raduga′	cv ′Belaya Ptitsa'	cv ′Krymchanka′	cv ′Alciona'
Essential oil content, %	0.31	1.51	0.75	0.35
Carvacrol, mass fraction of the sum of components, %	-	78.3	51.0	2.1
Thymol, mass fraction of the sum of the components, %	-	-	-	49.1

The next stage of research was devoted to the study of the component composition and content of phenolic compounds in alcohol extracts from marjoram plant raw materials. The maximum concentrations of flavonoids and amounts of phenolic compounds were detected in extract of marjoram O. *tyttanthum* cv ′Alciona' (Table 2). The following neochlorogenic acid, apigenin-6,8-di-C-glucoside, rosemary acid, carvacrol, and thymol were detected in the studied marjoram varieties by high-performance liquid chromatography (Fig. 1).

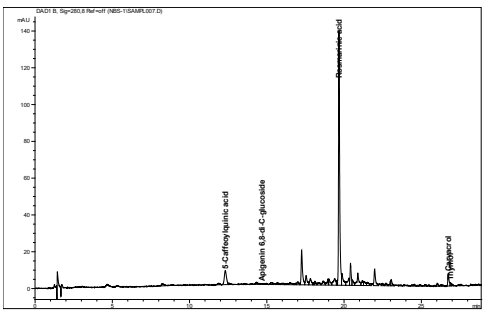
Table 2. Phenolic compounds of *Origanum vulgare* and O. tyttanthum *varieties.*

Indicator	*O. vulgare*			*O. tyttanthum*
	cv 'Raduga'	cv 'Belaya Ptitsa'	cv 'Krymchanka'	cv 'Alciona'
Neochlorogenic acid, content mg/kg	676±29	867±41	915±70	567±20
Apigenin-6,8-di-C-glucoside, content mg/kg	63±10	26±10	181±30	1574±50
Rosemary acid, content mg/kg	10111±150	6581±712	10577±56	13468±354
Carvacrol, content mg/kg	324±23	593±202	461±34	17920±471
Thymol, content mg/kg	273±12	256±63	217±10	225±15
Phenolic compounds, content mg/dm^3	3054±35	2068±159	2909±40	4726±54
Flavonoids, content mg/dm^3	3055±6	2287±145	3170±16	5656±141

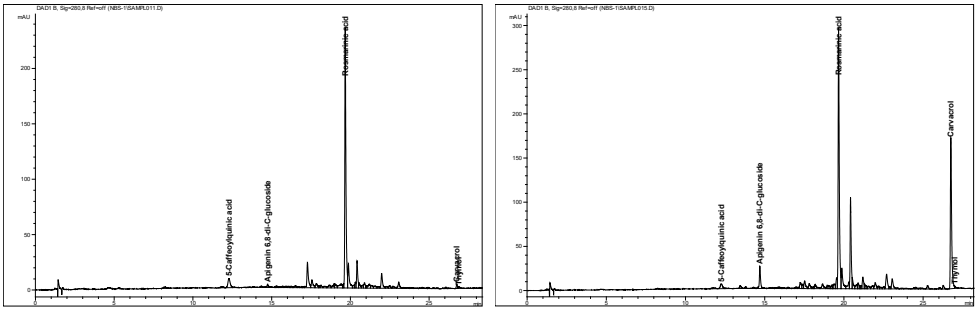
According to the content of neochlorogenic acid, the varieties O. *vulgare* 'Belaya Ptitsa' and 'Krymchanka' were distinguished. Neochlorogenic acid is a strong antioxidant, can act as an antitumor agent, and also plays an important role in the prevention of chronic diseases when eaten [16]. The maximum content of apigenin-6,8-di-C-glucoside and rosemary acid was distinguished by the *O. tyttanthum* cv variety 'Alciona'. It is known that apigenin-6,8-di-C-glucoside has anti-carcinogenic properties [17, 18], rosemary acid exhibits very high antioxidant activity, has an anti-inflammatory effect, antimicrobial effect on *Staphylococcus aureus* strains, inhibitory effect on the growth of *Escherichia coli* [19, 20]. The highest content of carvacrol was found in the extract of *O. tyttanthum* cv 'Alciona'. There were no noticeable differences in thymol content in the extracts of all the studied varieties. Thymol and carvacrol are isomers, their formation proceeds by hydroxylation of γ-terpinene either in the 3- (to thymol) or in the 6- (to carvacrol) position, which is catalyzed by enzymes monooxygenase, short-chain dehydrogenase. At the same time, through intermediate products, it is possible to change the position of the hydroxyl group from the 3rd to the 6th position [21]. A similar spontaneous conversion of thymol to carvacrol probably occurred in the alcohol extract of *O. tyttanthum* cv 'Alciona'.



O. vulgare cv 'Raduga'



O. vulgare cv 'Belaya Ptitsa'



O. vulgare cv ′Krymchanka′ *O. tyttanthum* cv ′Alciona′

Fig. 1. Chromatograms of alcohol extracts from vegetable raw materials of *Origanum vulgare* and *O. tyttanthum varieties*

Thus, the vegetable raw material *O. vulgare* cv ′Belaya Ptitsa/ can be used to obtain high-quality essential oil with a high content of carvacrol, and the vegetable raw material *O. tyttanthum* cv 'Alciona' is a valuable source of rosemary acid and other polyphenolic compounds.

4 Conclusions

A comparative analysis of biologically active substances of essential oils and plant extracts from 4 new varieties of *Origanum vulgare* and O. *tyttanthum* was carried out. It has been established that their main components are phenolic compounds: monoterpene phenols, hydroxycinnamic acids, and flavonoids. It was revealed that the *O. vulgare* 'Belaya Ptitsa' variety has a high content of essential oil with a mass fraction of carvacrol of more than 78%, and the alcoholic extract of the *O. tyttanthum* variety 'Alciona' contains maximum concentrations of rosemary acid, apigenin-6,8-di-C-glucoside, and carvacrol. It has been shown that *O. vulgare* cv 'Belaya Ptitsa' and *O. tyttanthum* cv 'Alciona' varieties are promising plant sources of phenolic compounds with a wide spectrum of biological action.

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