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BIOLOGICALLY ACTIVE SUBSTANCES OF SPICES, AROMATIC AND MEDICINAL PLANTS FROM NIKITSKY BOTANICAL GARDENS

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Introduction

Since long ago spices and aromatic plants are widely used in different spheres of human life and the most often in cookery, alcohol and cool drinks production, preparation of cosmetics and parfume output, medical treatments and medicinal forms [19]. They have great popularity in home and traditional medicine for treatment of various diseases and prophylaxis [17].

Biological value of spices and aromatic plant raw materials is determined by wide range of biologically-active substances: essential oils, glycosides, vitamins, mineral substances and phenolic compounds including flavonoids [18]. These substances improve culinary qualities of products, stimulate taste and digestive organs, cause appetite, increase digestability of foods and have beneficial effect on metabolism, work of cardiovascular system and general condition of the person. Many of spices and aromatic plants have preserve, anticeptic and bactericide qualities [4].

It has been known that qualitative and quantitative composition of biologically active substances in plant is highly depends from its genetic origin and growth conditions, besides, the processes extract them from plant raw material are important. Thus, native extracts, that do not undego therm reaction and processes of separation and purification, as a rule have higher biological activity due to the whole complex of physiologically active plant substances [5].

There is large collection of spices, aromatic and medicinal plants in Nikitsky Botanical Gardens (NBG). For many years, works on their introduction, breeding and selection have been carrying out with the researchers of this institution. As the result of those investigations perspective varieties and forms have been selected [6, 7].

On the base of all above-mentioned it should be noticed that complex investigations of biologically active substances compositions in perspective varieties and forms of spices, aromatic and medicinal plants grown in the conditions of the Southern Coast of the Crimea is of great actuality.

The aim of our work was to make scrining investigations of biologically active substances composition in some spices, aromatic and medicinal plants from NBG collection for giving the ground of their further use.

Objects and methods of the research

Objects of our investigation were: *Achillea millefolium* L. var. *rosea, Achillea collina* Becker ex. Rchb., *Artemisia annua* L. 'Novichok', *Artemisia scoparia* Waldst. et Kit. 'Vetvistyi', *Artemisia absinthium* L., *Artemisia dracunculus* L. 'Travnevyi', *Echinacea angustifolia* DC., *Echinacea purpurea* (L.) Moench., *Helichrysum italicum* (Roth.) Guss. 'VIM', *Hyssopus officinalis* L. 'Nikitsky Belyi', *Levisticum officinale* W.D.J. Koch., *Melissa officinalis* L., *Mentha spicata* L., *Mentha longifolia* L., *Nepeta cataria* L. *f. citriodora* Dum. 'Peremozhets-3', *Ocimum basilicum* L., *Origanum vulgare* L., *Salvia officinalis* L., *Satureja taurica* Velen. 'Krymsky Smaragd', *Satureja hortensis* L. 'Martian', *Scutellaria baicalensis* Georgi., *Thymus vulgaris* L., *Thymus vulgaris* L. 'Yalos'. Studied plants were grown in NBG

on the collection plots by the researchers from Technical and Medical Plants Laboratory. Vegetative material for analyses has been collected in 2011-2013 during the period of mass blossom.

Content of biologically active substances was determined in water-ethanol extracts prepared from the air-dry plant raw material. It has been dried in the aired and dark place up to the constant mass. Extraction was made by 50% ethanol with the proportion of plant raw material to the extragen – 1:10 and 10 days saturation under the room temperature was applied.

Component composition of volatile substances was determined by gas-liquid partition chromatography on Agilent Technology 6890 with mass spectrometer 5973. Column HP-1 height 30 m; inner diameter – 0.25 mm. Thermostat temperature rised from 50 to 250 ^{o}C with the step 4 ^{o}C/min. The temperature of injector was 250 ^{o}C. Carrier-gas – helium, the streem speed 1 sm^3/min. Transfer from gas chromotograph to mass spectrometer was warmed up to 230 ^{o}C. Temperature of the source was kept on 200 ^{o}C. Electron ionization was carried out under 70 eV in the range of masses m/z from 29 to 450. Identification was made on the base of comparison of the obtained mass-specters with the data from combined library NIST05-WILEY2007 (nearly 500000 mass-specters).

Content of phenolic substances was determined according to Folin-Ciocalteu (Gerzhikova, 2002) and flavons – by spectrophotometric method (Chemesova et al., 2000). All meanings were calculated for the air-dry mass.

Component composition of phenolic substances was determined by the method of highly effective liquid chromatography on chromatograph Agilent Technologies 1100. Hromatographycal column 2.1 × 150 mm filled with octadescillic sorbent «ZORBAX-SB C-18» granules 3.5 μm was used. Gradient regime of chromatography, that provides changes of correlation between the components A (0.1% ortophosphore acid, 0.3% tetrahydrofuran, 0.018% threethilamine) and B (methanol), was used for analyses. Speed of movable fraction giving was 0.25 sm^3/min; working eluent pressure – 240-300 kPa; volume of one sample - 2μl; time of scanning – 0.5 sec; measurements` scale was 1.0. Idefication of phenolic substances was made according to the time of standards` keeping and spectral characteristics (parameters for taking spectre – each top 190-600 nm; wavelength - 280, 313, 350, 371 nm (Murrough et al., 1982)).

Results and discussion

Qualitative composition and consentration of volatile compounds and phenolic substances in water-ethanol extracts for 22 species of spices, aromatic and medicinal plants from the families Lamiaceae, Asteraceae и Apiaceae have been determined with chromatogrphical methods.

Concentrations of volatile compounds in vegetative extracts were from 0.01 g per 100 g of the air-dried raw material to 3.37 g/100 g (tab. 1). Maximum concentrations of volatile compounds were noticed in the extracts of *Thymus vulgaris* and *Ocimum basilicum*, minimum – in the extracts of *Echinacea angustifolia*, *Echinacea purpurea* and *Scutellaria baicalensis*.

Component composition of volatile compounds in the extracts of studied plants is characterized with great diversity and mostly represented by terpene and aromatic substances (phenolic compounds and phenilpropanoids). The exeption was extract of *Echinacea angustifolia* mostly composed from aliphatic compounds (monocarboxylic acids).

Thus high concentrations of monoterphens are typical for *Achillea collina* and *Helichrysum italicum* 'VIM'. *Mentha arvensis* is also characterized with high enough consentration of limonene (9.7%). Monoterpene compounds have anticeptic (especially to bacterias presented in the air), bactericidal, stimulated and expectorant influence [2]. Though

some researchers consider that antimicrobial and antioxidant features of spices and medicinal plants are due to the presence of monoterpenes, particularly limonene, in their parts [28].

Maximum concentrations of monoterpene alcohols were noticed for *Thymus vulgaris* 'Yalos', *Ocimum basilicum*, *Nepeta cataria*, *Achillea millefolium* and *Artemisia absinthium*. Such monoterpene alcohols as linalool, terpinene-4-ol, α-terpeneol and borneol have antimicrobial activity [13, 24]. Borneol, terpinene-4-ol, α-terpeneol demonstrate antifungal activity [23]. Besides, for some alcohols, particularly geraniol and terpeneol, high antifungal activity has been determined and anti-inflammatory activity was also noticed for cytronellol and virucidal activity – for linalool [13].

Extract of *Levisticum officinale* is characterized with high concentration of α-terpene-acetate (69.8%). Compound alcohol ethers demonstrate different kinds of biological activity: anti-inflammatory, wounder-healing and antimicrobial influence [13]. α-terpene-acetate has pleasant odour of bergamot and is widely used for aromatization parfume production [3].

High content of monoterpene aldegids is a feature of *Melissa officinalis* and *Nepeta cataria* extracts. Monoterpene aldegids demonstrate antifungal activity and also have anticeptic, analgesic, antihistamine and anti-hypertensive effect, stimulate the gastrointestinal tract [13]. These compounds provide sedation [10]. Monoterpene aldegids have pleasant odor and could be used in parfume industry [3, 11].

Significant concentrations of monoterpene ketones were found in the extracts of *Artemisia annua*, *Hissopus officinalis*, *Salvia officinalis*, *Mentha arvensis* and *Mentha longifolia*. Properties of monoterpene ketones haven`t been fully studied yet. It has been known that some ketones have unfavourable influence on human organism [20]. At the same time ketones demonstrate immunostimulated, antispasmodic and sedative effect [22]. Particularly, camphor affects the central nervous system and soothes the heat in stress [13]. Besides, high content of momoterpene ketones give plants their antibacterial and antifungal features [15, 28, 29]. Some researchers consider that presence of ketones, particularly carvone and menthon guaranty antioxidant features of plants [25, 27]. But it should be taken in attention that thujone in high concentrations could be the reason of spasms, so plant extracts with this substance should be used only after carefully determined tujone concentration [16].

Table 1

Volatile compounds in spices, aromatic and medicinal plants

Species	Concentration, g/100 g	Number of identified components	Main components
Achillea millefolium	0.43	35	borneol (18.0%), β-pinen (5,9%), terpenen-4-ol (6.1%)
A. collina	0.12	20	β-pinen (19.2%), 1.8-cinneol (7,5%), γ-kadinol (9.0%)
Artemisia absinthium	0.04	20	sabinol (23.5 %), β-tujone (13.8 %)
A. annua	1.54	43	artemisia-ketone (40.9%), camphor (19.9%)
A. dracunculus	0.32	21	trans-isoelemicin (62.7%), methileugenol (17,1%), cys-methilisoeugenol (4.2%)
A. scoparia	0.43	33	hexa-5-en-1,3-diinyl-benzene (27.4%), scoparon (18.1%), eugenol (12.9%)
Echinacea angustifolia	0.03	22	linoleic acid (31.6%), palmitic acid (31.0 %)

E. purpurea	0.03	22	p-cymene (27.0%), γ-cadinene (9.4%), spathulenol (8.1%)
Helichrysum italicum	0.33	35	α-pinen (43.3%), limonene (12.2%), rozifoliol (4.5%), penduletin (4.3%)
Hissopus officinalis	0.63	33	isopinocamphon (3.2%), β-pinen (4.2%), pinocamphon (78.2%)
Levisticum officinale	0.38	14	α-terpene-acetate (69.8%), *butilidendigidroftalid* (12.5%)
Melissa officinalis	0.22	38	citronellal 4.0%, neral (6.0%), geranial (8.2%)
Mentha spicata	0.37	43	cys-dihydrocarvone (15.1%), carvone 29.3%, ethil linolenat (11.0%)
M. longifolia	0.97	20	methone (53.2%), isomethone (27.7%), trans-sabinene hydrate (8.2%)
Nepeta cataria	0.23	14	citronellol (33,1%), nepetalactone (33.8 %), geranial (10.5%), geraniol (8.0%)
Ocimum basilicum	2.31	43	linalool (46.2%), methyl chavikol (24.0%), 1,8-cineole (9.1%)
Origanum vulgare	0.62	32	p-cymene (10.0%), thymol (10.3%), γ-terpinen (15.4%), carvacrol (37.7%)
Salvia officinalis	1.80	26	α-tuion (33.3%), camphor (30.5%), 1,8-cineole (6.4%)
Satureja taurica	0,32	18	carvacrol (81.6%), p-cymene (9.7%)
S. hortensis	0,24	15	carvacrol (75.1%), γ-terpenen (7.5%)
Scutellaria baicalensis	0,01	26	phenylacetaldehyde (14.7 %), 4-vinylphenol (9.1%)
Thymus vulgaris	2.07	29	thymol (74.2%), p-cymene (4.1%), carvacrol (2.3%)
Th. vulgaris ‘Yalos’	3.73	41	linalool (84.5%), thymol 3.4%, linalyl acetate (3.4%)

The greatest amount of sesquiterpenes was noticed in the extract of *Echinacea purpurea*. Sesquiterpenes are among the strongest anti-inflammatory components of essential oils. Some of them have anasthetic features and others demonstrate antispasm activity [2].

High enough concentrations of sesquiterpene alcohols were determined for *Achillea collina* and *Echinacea purpurea*. Sesquiterpene alcohols are valuable compounds that have relax and stimulative effect, they stimulate heart work and regenerate liver, have antiallergene and anti-inflammatory effect [2].

In other studied species volatile aromatic compounds of nonterpene origin predominate. High concentrations of simple phenolic compounds were determined in the extracts of *Artemisia scoparia*, *Origanum vulgare*, *Satureja taurica*, *Satureja hortensis*, *Scutellaria baicalensis*, *Thymus vulgaris* and phenilpropanoids – in the extracts of *Artemisia dracunculus* and *Ocimum basilicum*. Both simple phenols (thymol, carvacrol and others) and phenilpropanoids (eugenol, methyl chavikol and others) demonstrate significant anti-inflammatory,expectorate, untifungal, protistotsidшc, antiseptic, antispasmodic and antioxidant activity [13, 14, 17].

Thus extracts of *Thymus vulgaris* ‘Yalos’, *Ocimum basilicum* and *Achillea millefolium* due to their high concentrations of monoterpene alcohols and phenilpropanoids could be used for treatment and prevention. Extracts from *Melissa officinalis* and *H. italicum* ‘VIM’ are rich in aromatic substances that let us to recommend them as aromatizes for parfume-cosmetics and food production. More than 85% of aromatic substances in *Thymus*

vulgaris variety ‘Yalos’ bred in NBG are monoterpene alcohol linalool that has delicate odor of lily of the valley. Unusual ordor of this *Thymu*s variety makes it attractive for parfume industry.

Content of phenolic compounds in vegetative extracts is from 0.91 g per 100 g of the air-dried raw material up to 2.48 g/100g (tab. 2). Their maximum concentrations were found in the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Levisticum officinale*, minimum – in the extracts of *Achillea collina* and *Hissopus officinalis*. Component composition of phenolic compounds in studied species is mostly represented with flavonoids and hydroxycinnamic acids. In the species of *Artemisia* L. coumarins were found.

At the present time many sides of flavonoid substances` biological effect became clear and their P-vitamin activity has been known long ago [1]. Flavonoids stimulate heart activity and decrease blood pressure for short period of time due to dilatation of the abdominal cavity. They demonstrate hepartoprotective, anti-tumour and antimicrobial action [8, 21]. High flavonoids` content is typical for the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Salvia officinalis*.

Flavonoids in the studied species are represented by flavons` glycosides: luteolin, apigenin, acacetin, and also by quercetin flavonol glycosides. *S. baicslensis* that has unequal set of flavonoids originated from scutellarin and baicalin should be highlighted.

Hydroxycinnamic acids are biogenetic predecessors for most of other phenolic compounds. Their imunostimulative, antivirus and anti-inflammatory effects are well studied. It has been determined that ferulic, caffeic, chlorogenic acids and especially cinarin (1,4-caffeoylquinic acid) have bile-pursue effect. Sum of ferulic, caffeic, chlorogenic, coumaric and other caffeoylquinic acids have hypoazotemic effect enhances the function of the kidneys, stimulates the antitoxic function of the liver, has antimicrobial and antineoplastic action [30].

Table 2

Phenolic compounds in spice-aromatic and medicinal plants

Species	Concentrations, g/ 100 g			Number of identified components	Main components
	Phenolic compounds	Flavonoids	Hydroxycinnamic acids		
Achillea millefolium	2.36±0.12	1.44	0.92	11	luteolin-7-O-glycoside (28.6%), 4- caffeoylquinic acid (18.5%)
A. collina	2.34±0.10	1.32	1.02	11	luteolin-7-O-glycoside (24.2%), caffeoylquinic acid (20.5%)
Artemisia absinthium	0.83±0.03	0.10	0,.0	12	chlorogenic acid (27.0%), kaempferrol (8.7%)
A. annua	3.99±0.13	1.28	2.72	16	rosmarinic acid (18.9%), chlorogenic acid (11.5%)
A. dracunculus	1.49±0.10	0.11±	0.32	14	rosmarinic acid (21.4%), coumarin (15.2%)
A. scoparia	2.75±0.12	0.48±	1.07	16	coumarin (36.8%), chlorogenic acid (16,8%)
Echinacea angustifolia	2.41±0.10	0.05	2.29	17	rosmarinic acid (68.5%), caffeoylquinic acid (5.6%)
E. purpurea	3.27±0.16	0.06	2.70	9	caffeoylquinic acid (71.3 %), p-coumaroylquinic acid (2.3%)
Helichrysum italicum	1.69±0.06	0.93	0.76	6	4- caffeoylquinic acid (40.0%), chlorogenic acid (19.6%)
Hissopus officinalis	0.59±0.01	0.16	0.43	17	rosmarinic acid (28.6%), luteolin-7-O-glucoside (18.9%)
Levisticum officinale	4.18±0.18	3.41	0.77	9	quercetin biglucoside (79.3%), caffeic acid (7.9%)

Melissa officinalis	0.91±0.02	0.20	0.72	8	rosmarinic acid (41.8%), glucoside of apigenin (21.5%)
M. longifolia	3.00±0.14	1.99	1.61	13	rosmarinic acid (50.2%), luteolin-7-O-glucoside (13.2%)
Mentha spicata	2.26±0.12	0.85	1.41	17	rosmarinic acid (57.8%), luteolin-7-O-glucoside (5.7 %)
Nepeta cataria	2.21±0.16	0.84	1.41	12	chlorogenic acid (50.2%), apigenin-7-diglucoside (22.5%)
Ocimum basilicum	1.04±0.03	0.16	0.88	11	rosmarinic acid (20.9%)
Origanum vulgare	5.57±0.18	3.01	2.56	12	rosmarinic acid (40.1%), luteolin-7-O-glucoside (1.5%)
Salvia officinalis	2.49±0.08	2.03	0.46	8	luteolin-7-O-glucoside (42.0%), apegenin-7-O-glucoside (26.8%)
Satureja taurica	1.86±0.15	0.68	0.74	16	rosmarinic acid (32.7%), apigenin (11.3%)
S. hortensis	1.33±0.03	0.59	0.74	16	rosmarinic acid (46.0 %), luteolin-7-O-glucoside (18.2 %),
Scutellaria baicalensis	18.51±0.50	18.51	-----	15	dihydroscutellarin (41.3%), scutellarin-7-O-glucoside (11.7%)
Thymus vulgaris	2.84±	0.92	1.62	15	rosmarinic acid (54.5%), luteolin-7-O-glucoside (20.0%),
Th. vulgaris 'Yalos'	1.02±	0.92	0.10	11	luteolin (22.7%), luteolin-7-O-glucoside (24.1%)

From the group of hydroxycinnamic acids caffeic, chlorogenic acids and some isomers of the last were found in all studied species. In some plants isomers of rosmarinic acid were found.

Maximum concentration of hydroxycinnamic acids is characteristic for *Echinacea angustifolia* and *Origanum vulgare* extracts.

Thus on the base of our investigations such species with high conten of biological active substances have been marked out: *Thymus vulgaris*, *Ocimum basilicum*, *Levisticum officinale*, *Scutellaria baicalensis*, *Origanum vulgare*, *Salvia officinalis*.

Conclusions

Qualitive and quantative composition of biological active substances in some spices, aromatic and medicinal plants from collection of Nikitsky Botanical Gardens has been studied.

It has been determined that amount of volatile substances in vegetative extracts was from 0.01 to 3.37 g/100g. Their maximum concentrations were noticed in the extracts of *Thymus vulgaris* and *Ocimum basilicum*.

Amount of phenolic compounds was 0.59 – 18.51 g/100 g. There maximum concentrations were found in the extracts of *Scutellaria baicalensis*, *Origanum vulgare* and *Levisticum officinale*.

On the base of the investigations such species with high conten of biological active substances have been marked out: *Thymus vulgaris*, *Ocimum basilicum*, *Levisticum officinale*, *Scutellaria baicalensis*, *Origanum vulgare*, *Salvia officinalis*.

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Qualitative and quantitative composition of volatile compounds and phenolic substances from the extracts of some spices, aromatic and medicinal plants of Nikitsky Botanical Gardens collection has been determined. Species with a high content of biologically active substances have been marked out. The concentration of volatile compounds in vegetative extracts was from 0.01 to 3.37 g/100 g. and its composition was mainly represented by terpenic and aromatic substances. The content of phenolic substances in the vegetative extracts mostly presented by flavonoids and hydroxycinnamic acids, is in the range of 0.91 – 2.48 g/100 g.

Key words: *spices, aromatic and medicinal plants, water-ethanol extracts, volatile compounds, phenolic substances.*