

the increase of the spherosomes and the filling of the vacuoles by the osmiumphilic substance like in the case of the rose.

4. The fungal secretion of the fragrant substances to the environment is supposed to be one of the regulatory functions of their synthesis (“mechanism of overfill”).

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Semyonova Ye.F., Shpichka A.I., Presnyakova Ye.V., Mezhennaya N.A. Processes of essential oil accumulation in petals of *Rosa* (Rosaceae) and Mycelium *Eremothecium* (Eremotheciaceae). // Bull. of the State Nikit. Botan. Gard. – 2016. – № 118. – P. 25-33.

The plantation cultivation of an oil-bearing rose is not able to cover the increasing demand of the industry. Therefore, the interest to fungi strains *Eremothecium ashbyi* Guilliermond and *E. gossypii* Kurtzman, is rising. The features of secretory structures of the *Rosa* and *Eremothecium* species were found out. The investigation of biosynthesis, accumulation, and secretion of essential oils with a rose scent is crucial either for development of new ways to produce them or for rating the biological role of *Rosa* and *Eremothecium* secondary metabolites.

Key words: *essential oil; oil accumulation; spherosomes; secretory structures; Rosa; Eremothecium*

PLANT BIOCHEMISTRY

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BIOLOGICALLY ACTIVE SUBSTANCES OF NEPETA CATARIA L.

**Anfisa Yevgenyevna Paly, Ivan Nikolayevich Paly, Natalya Vladimirovna Marko,
Valery Dmitriyevich Rabotyagov**

Nikita Botanical Gardens – National Scientific Centre
298648, the Republic of Crimea, the city of Yalta, urb.vil.Nikita
onlabor@yandex.ru

Introduction

Nepeta Cataria L. is a perennial plant that belongs to Lamiaceae family. Overground mass of the cultivar possesses pretty lemon fragrance, pungent taste and takes a great interest in the field of food, perfume and soil boiling industries.

Various preparations that include *Nepeta Cataria* are used in folk medicine as spasmolytic, carminative, tonic and stimulative remedy. Besides, extracts of its overground part can be used to treat gastrointestinal and respiratory diseases, stagnations of gallbladder and bile passages, hysterical and depressive attacks [6, 12].

Complex of biologically active substances, such as volatile compounds, phenolic substances and vitamins in plant material of *Nepeta cataria* specifies its medicinal properties [5, 8, 14].

Essential oil (EO) has got a fine herbaceous and citrus fragrance and presents high antimicrobial action. Content and component composition of *Nepeta cataria* EO ranges a lot what depends upon ecological and genetical factors. Essential oil includes geranial, geraniol, camphora, carvacrol, caryophyllene, nepetalactone, nerol, citral, citronellal, citronellol and eugenol [9, 15]. Phenolic compounds of *Nepeta cataria* are presented by hydroxicoric acids – rosemary, caffeic, n-cumarin, ferulic acids; and flavonoids – flavones (apigenin, luteoline) and flavonols (quercetin, kaempferol, myricetin) [2, 7, 8].

Long-term research in *Nepeta cataria* introduction and selection has been kept on within NBG-NSC, as a result there is a new sort – Peremozhets-3, which is characterized by high crop capacity and EO output [1].

In terms of introduction 2010, new *Nepeta cataria* specimen was involved, it possesses lemon fragrance and can be a source of high crop capacity under conditions of the Steppe Crimea.

Purpose of this work was to investigate qualitative and quantitative composition of biologically active substance in water-ethanol extract of new promising *Nepeta cataria* specimen.

Objects and methods of the research

Overground part of *Nepeta cataria*, yielded during mass blooming became the study research.

Essential oil was extracted out of raw material applying method of hydrodistillation by Ginsberg, with further conversion into dry mass. Distillation time of EO – 1 hour.

Concentration of volatile substances was determined in water-ethanol extract (hereinafter extract), prepared from air-dry plant material. Raw material was dried in the aired room getting constant mass. Extraction was conducted by 50% ethyl alcohol having ratio of raw material and leach – 1:20 (preparation took 10 days at room temperature).

Component composition of volatile substances was determined applying chromatograph Agilent Technology 6890 with mass-spectrum detector 5973. Chromatographic heater – capillary HP-1 (30 m long); inside diameter – 0,25 mm. Thermostat temperature was programmed in a range 50-250 °C with velocity of 4°C/min. Injector temperature - 250°C. Carrier gas is helium, stream velocity – 1,0 sm³/min. Transfer from gas chromatograph to mass spectrometric detector was heated till 230°C. Source temperature was kept up at 200°C. Electronic ionization was carried out at 70 eV with mass range m/z from 29 till 450. Identification of essential oil components was guided by comparison results obtained in terms of chemical substances mass spectra with publishing data of mass spectra NIST05-WILEY2007 (about 500000 of mass spectra).

Component composition of phenol substances was determined applying chromatograph “Agilent Technologies” (model 1100), completed with running vacuum degasifier G1379A, 4-channeled pump of low pressure gradient G13111A, automotive injector G1313A, thermostat of heaters G13116A, diode-matrix detector G1316A, fluorescent detector G1315B. Chromatograph heater 2,1mm x 150 mm, filled with octadecilsilil sorbent “ZORBAX” SB-C18 and 3,5 mkm graining was applied during analysis. Gradient regime of chromatography that foresees changes of component A (0,1% orthophosphoric acid, 0,3%-tetrahydrofuran, 0,018%- triethylamine) and B (methanol) components in eluting mixture. Feed rate of mobile phase made 0,25 sm³/min; working pressure of eluent – 240-300 kPa; sample volume – 2 mkl; scanning time 0,5 sec, scale of measurements – 1,0.

Identification of phenol substances was carried out according to time of standard keeping and spectrum characteristics (parameters of spectrum reading - each peak 190-600 nm; wave length – 280, 313, 350, 371 nm).

Results and discussion

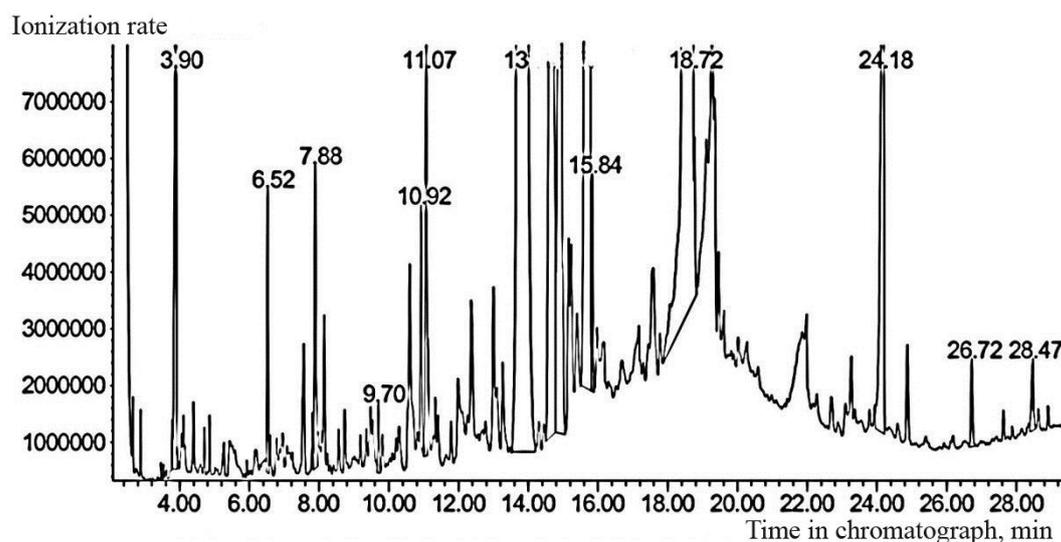
As a result of introduction work there is a new specimen of *Nepeta cataria*. Plants were cultivated in the steppe zone of the Republic of Crimea, where they are able to reach 179-190 sm and present a high crop capacity up to 70 center/ha. New specimen was marked out due to organoleptic method, as one with bright lemon fragrance. Mass fraction of essential oil inhere made 0,11% from raw material and 0,51 from the absolutely dry material (2,4% of dry substances).

Investigating component composition and concentration of biologically active substances in *Nepeta cataria* water-ethanol extract it was found out that concentration of volatile substances made 233 per 100 g of dry plant material (table 1, fig.1). Among volatile compounds 15 components were identified. The following volatile compounds are typical for extract of this specimen: monoterpene alcohols, aldehydes, lactones, as well as sesquiterpenoids. The dominant components of the extract are nepetalactone (34%), citronellol (33%) and geraniol (10%). At the same time considerable content of geraniol (7,96%) and caryophyllenoxide (6,19%). Findings coordinate with publishing data [3, 4, 8].

Table 1

Component composition of volatile compounds in *N.cataria* water-ethanol extract

Component	Mass fraction, %
acetic acid	2,70
6-methyl-5-hepten-2-one	0,70
phenylacetaldehyde	1,10
6-methyl-3,5-heptadien-2-one	0,23
phocitral A	0,84
citronellal	1,60
citronellol	33,13
geraniol	7,96
geranial	10,45
geranylformiat	0,56
nepetalactone	33,81
caryophyllene	0,50
caaryophyllenoxide	6,19
myristinic acid	0,35

Fig.1 Chromatogram of volatile compounds in *N.cataria* water-ethanol extract

The principal components that specify qualitative parameters of *N.cataria* essential oil are neral, geranial and geraniol. At the same time nepetalactone that also enters this composition causes high antimicrobial, repellent and insecticidal effect of the EO. It's a well-

known fact, that nepetalactone can act as pheromones for aphides and protect from orthopterans [6, 12]. Typical fragrance of nepetalactone attracts animals of cat family [13].

It was determined that *N.cataria* extract contains high concentration of phenolic compounds, carotenoids and ascorbic acid (table 2).

Table 2

Concentration of biologically active substances in *N.cataria* extract

Biologically active substances	Concentration, mg/100 g
Phenolic compounds	2218,0 ± 64,0
Ascorbic acid	308,0 ± 12,0
Carotenoids	2,8 ± 0,07

Phenolic compounds of *N.cataria* are presented by hydroxicoric acids and flavonoids. Concentration of hydroxicoric acids made 1,4 g/100 g of air-dry plant material, flavonoids – 0,8 g/100 g.

Studying component composition of the extract phenolic substances 12 components were revealed, while 8 of them were identified (table 3, fig.2).

Table

Component composition of *N.cataria* phenolic compounds

Component	Output time	concentration, mg/100 g
4- caffeoylquinic acid	19.91	84,94
5- caffeoylquinic acid	20.42	100,58
chlorogenic acid	26.09	1113,00
luteoline-7-diglycoside	28.82	133,81
<i>n</i> -cumaroil glycoside	30.62	31,34
<i>n</i> -coumaric acid	31.91	55,00
apigenin-7-diglycoside	32.79	499,88
luteoline-7-glycoside	33.67	43,43
apigenin-7-glycoside	34.42	33,08
acacitine-7-glycoside	35.7	65,33
apigenin	37.54	27,92
Unidentified	38.46	29,31

Dominant components are chlorogenic acid and diglycoside of apigenin flavone, their concentration makes 50,0% and 22,5% respectively, from the total content of phenolic substances; our findings coordinate with data of other scientists [9, 10]. Chlorogenic acid possesses strong antioxidant, antiviral, antibacterial antifungal and probiotic properties, presents hypoglycemic, hypocholesteremic, anticancer and hepatoprotective action. Inhibiting oxidation of lipoprotein with low concentration, what causes artery damages, chlorogenic acid makes it possible to prevent cardiovascular diseases [5, 10]. High concentration of chlorogenic acid, apigenin glycosides and luteoline isomers in *N.cataria* new specimen extract explains its biological value.

Flavones, luteoline and apigenin reveal a wide range of biological activity as well. They possess anti-inflammatory, spasmolytic, antitumoral, immunoregulatory and antiallergic action, high antioxidant and weak antibacterial activity [3, 11].

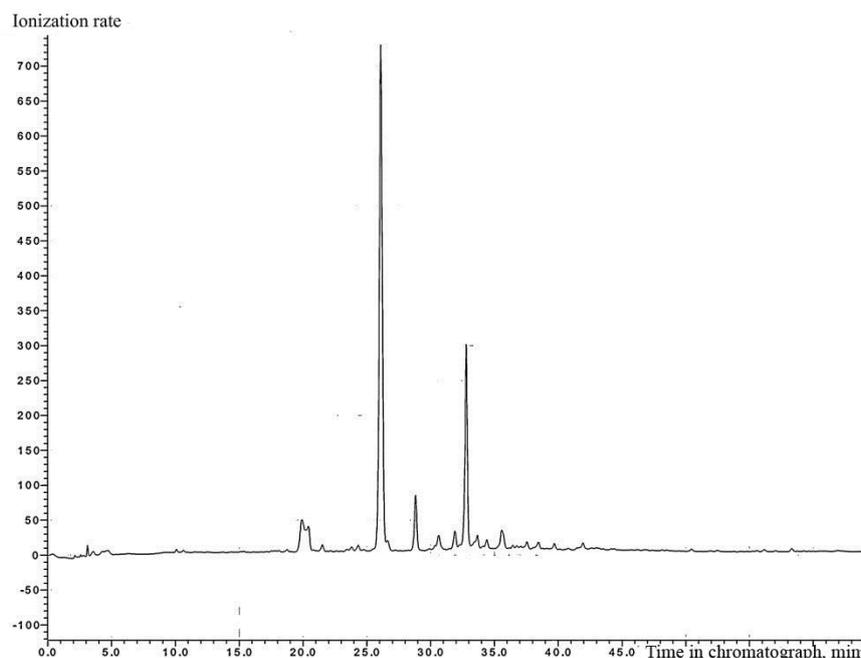


Fig.2 Chromatogram of *N.cataria* phenolic compounds

In this way overground part of study case (*N.cataria* specimen) is of great biological value due to monoterpenic aldehydes (nepetalactone, citronellol and geranial), phenolcarboxylic acids (mainly chlorogenic acid), ascorbic acid and carotenoids and could be used in the field of food, cosmetic and medioprophyllactic production, enriched with biologically active substances.

Conclusions

Qualitative and quantitative composition of biologically active substances (phenolic substances, volatile compounds, vitamins) in water-ethanol extract of the *N.cataria* promising specimen was determined in terms of the research.

It was found out that *N.cataria* extract contains the main volatile compounds, typical for essential oil – monoterpenoids: nepetalactone, citronellol, geranial and geraniol and sesquiterpenoid caryophyllenoxide. Dominant components are nepetalactone (34%), citronellol (33%) and geranial (10%).

Phenolic substances of this extract are presented by hydroxicoric acids and flavonoids. The principal components are chlorogenic acid (50%) and dilicoside apigenin (22,5%).

This water-ethanol extract of *N.cataria* possesses high biological value and fine minty-lemon fragrance. This extract can be a ground for food, medioprophyllactic and perfume-cosmetic production.

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Qualitative and quantitative composition of biologically active substances in aqueous-ethanol extracts of *Nepeta cataria* L. was investigated in terms of the research.

Volatile compound composition in *N. cataria* water-ethanol extract was determined as well. The main components of extract are nepetalactone, citronellol and geraniol.

Content of phenolic compounds was also revealed in aqueous-ethanol extract of *N. cataria*. Apigenin glycosides, luteolin and hydroxycinnamic acids – chlorogenic acid isomers were identified in the extract.

New specimen of *N. cataria* is possible to use as a source of biologically active substances for production of medicine, cosmetics and foods.

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