

UDC 615.32:582.681.71:582.929.4:615.28

**ANTIMICROBIAL ACTIVITY OF THE
SUBSTANCES RECEIVED FROM RAW
MATERIALS OF LAMIACEAE AND
CUCURBITACEAE FAMILY PLANTS**

Fedchenkova¹ Iu. A., Savinova² Y. M.,
Batyuchenko¹ I. I., Andrianov¹ K. V., Gamulya¹ O. V.

¹National university of pharmacy,
e-mail: fja_fja@ukr.net

²Kharkiv Medicine Academy of Post-Qualified
Education,

Introduction. The search of new sources of raw materials for medicine creation is one of the pharmacy priorities. In this, our attention was drawn by the plants which are widely cultivated in Ukraine and have a sufficient source of raw materials. It is summer squash (*Cucurbita pepo* L.) and sowing cucumber (*Cucumis sativus* L.) of *Cucurbitaceae* family and also peppermint (*Mentha piperita* L.) of two sorts “Chernolistnaya” and “Zgadka” of *Lamiaceae* family. Summer squash and sowing cucumber are sources of valuable food and dietary products and widely applied in cosmetology and traditional medicine [1,2]. Sowing cucumber is a neofitsinal plant which raw materials are used as antioxidant, antiinflammatory, antipyretic, antitussive, emollient and anesthetic drug [3].

Summer squash is applied at a metabolic disease, diseases of cardiovascular system, liver and kidneys, as general restorative, vitamin, antiinflammatory, hemostatic, diuretic drug [4,5]. As crude drug the scientific medicine uses the seeds of summer squash which have anthelmintic effect [6,7].

A plant as peppermint is a part of many medicinal remedy. It is irreplaceable at gastrointestinal diseases, meteorism, cholangitis, hepatitis, bile and stone illness, laryngitis, pharyngitis, tracheitis, tooth, headaches etc. [8].

However the drugs with antimicrobial action derived from the leaves of these plants in the market of Ukraine are absent. Spirit, lipophilic and polysaccharidic fractions were received from the leaves of sowing cucumber, summer squash and two sorts of peppermint by us. Antimicrobial activity studying of the received substances derived from the studied raw materials for expansion of the medicine range is promising.

The relevance of researches is that getting for the first time due to complex raw material processing the lipophilic, spirit and polysaccharidic fractions of leaves of a sowing cucumber, summer squash and two sorts of peppermint “Chernolistnaya” and “Zgadka” are systemically studied on existence of antimicrobial activity.

The purpose of our research is the studying of antimicrobial activity of getting substances derived from the leaves of sowing cucumber, summer squash and two sorts of peppermint “Chernolistnaya” and “Zgadka”.

Materials and methods. Raw materials for substances are the leaves of a sowing cucumber prepared in June, 2015 in the Kharkiv region, the village Selection, in the conditions of industrial cultivation (the selection station of

Institute of vegetable-growing and melon-growing of UAAN). The leaves of summer squash were being prepared also in June, 2015 in Valkovsky district of the Kharkiv region, the settlement Dobropolye.

The leaves of peppermint of “Chernolistnaya” and “Zgadka” sort were prepared on the industrial squares of Dnipropetrovsk region, Sofiyevskiy district, the settlement Ordo-Vasilyevka.

In a series of sowing cucumber leaf (in terms of absolutely dry raw materials) the quantitative content of the sum of phenolic substances (in terms of gallic acid) - $7,88 \pm 0,08\%$, the sums of oxycinnamic acids (in terms of chlorogenic acid) - $2,52 \pm 0,03\%$, the sums of flavonoids (in terms of cynaroside P) - $2,98 \pm 0,03\%$ is defined.

In a series of summer squash leaf (in terms of absolutely dry raw materials) the quantitative content of the sum of phenolic substances (in terms of gallic acid) - $7,87 \pm 0,11\%$, the sums of oxycinnamic acids (in terms of chlorogenic acid) - $3,51 \pm 0,02\%$, the sums of flavonoids (in terms of rutin) - $2,31 \pm 0,01\%$ is defined.

In series of peppermint leaf of “Chernolistnaya” and “Zgadka” sort (in terms of absolutely dry raw materials) is defined the quantitative content of the sum of phenolic substances (in terms of gallic acid) - $13,33 \pm 0,11\%$ and $5,28 \pm 0,01\%$ accordingly, the sums of oxycinnamic acids (in terms of chlorogenic acid) - $2,97 \pm 0,02$ and $2,59 \pm 0,01$ accordingly, the sums of flavonoids (in terms of rutin) - $0,45 \pm 0,01$ and $0,65 \pm 0,01$ accordingly.

Lipophilic fractions were received in Sokslet's device [9]. As an extractant, chloroform was used. From the received extraction the extractant was being deleted in vacuum. Lipophilic fraction represented viscous substances of black-green color with typical fragrant.

For receiving spirit fractions derived from solvent cake after receiving the lipophilic fractions it was used a method of fractional maceration at the general ratio raw materials-extractant 1:15, as an extractant was 50% ethyl alcohol. Extraction was carried out three times. The integrated extracts were concentrated in vacuum [10]. After a full removal of an extractant and drying, it had been received the hygroscopic powders of green-brown color with specific tastes and smells.

From the solvent cake which was remained after receiving lipophilic and spirit fractions polysaccharidic fractions representing water-soluble polysaccharides were evolved [11]. They were friable powders of light gray color.

The lipophilic fraction of sowing cucumber leaves (CL), lipophilic fraction of summer squash leaves (SSL), lipophilic fraction of peppermint leaves of “Chernolistnaya” sort (ChPL), lipophilic fraction of peppermint leaves of “Zgadka” sort (PZL), spirit fraction of cucumber leaves (SC), spirit fraction of summer squash leaves (SSS), spirit fraction of peppermint leaves of “Chernolistnaya” sort (SPCh), spirit fraction of peppermint leaves of Zgadka sort (SPZ), polysaccharidic fraction of cucumber leaves (PC), polysaccharidic fraction of summer squash leaves (PSS) were being studied.

Test of antimicrobial effect of substances was carried out by method of serial dilution concerning the following six reference test cultures: *Staphylococcus aureus* of ATCC 6538-P, *Candida albicans* of ATCC 885-653, *Escherichia coli* of ATCC 25922, *Bacillus subtilis* of ATCC

6833, *Bacillus cereus* of ATCC 10702, *Pseudomonas aeruginosa* of ATCC 9027. Test strains intended for studying of antimicrobial activity of medicines have been taken according to the State Pharmacopoeia of Ukraine, 2001. The researches were fulfilling in the Department of immunology and microbiology of Kharkiv Academy of Post-qualifying education [12,13].

For the determination of culture properties and the purity of test cultures nutrient mediums were used in which basis the agar-agar was taken, 04.2013 was produced, term of use 3 years (the producer is Spain), nutrient medium of cultivation of microorganisms (TUU 24.6 - 24367280-014:2011, batch No.64, 10.2014, use term 2 years) and Endo's nutrient medium, dry, is made for allocation and differentiation of enterobacteria 04.2013 (TUU 246-24367290-014:2011, batch No. 114, 10.2014, term of use 2 years (the producer is the State scientific-research enterprise of bacterial ferments, the Institute of milk and meat technology of National Academy of agrarian sciences of Ukraine, Kiev. Other nutrient mediums were made in laboratory of biochemistry and biotechnology of the State enterprise "Institute of Microbiology and Immunology of I. I. Mechnikov of NAMS of Ukraine".

For an experiment it was prepared tenfold dilution of substances (the tinctures had been used without dilution) using the meat-peptone broth 1:10 and 1:100 to which the reference cultures which were grown up on the corresponding differential and diagnostic medium depending on a type of cultures were added.

Concentration of suspensions of reference tests was prepared applying the method of Mc. Farland. (the order of Ukrainian Ministry of Health No. 167 dated 05.04.07).

As the solutions had been remained muddy after the incubation, for evaluation of antimicrobial action of the received fractions, the seeding was made on liquid nutrient medium taking into account the sort of test strain applied in experience. Lack of culture growth in liquid nutrient medium (transparency of liquid), testified to bactericidal activity of the studied fractions in the corresponding dilutions (in the presence of growth in control crops).

The results of minimum inhibiting concentration definition will be stated by us in the following report.

Results and discussion. The summaries of all the researches of antimicrobial activity of substances of sowing cucumber leaves, summer squash and two sorts of peppermint of "Chernolistnaya" and "Zgadka" are provided in tables 1-2.

The obtained data demonstrate that the widest range of antimicrobial activity was shown by SSL, ChPL and SPZ in the ratio of *E. coli*, *C. albicans* and *P. aeruginosa*.

Thus, SSL, ChPL, SC and SPZ had a bactericidal activity in dilution 1:10 and 1:100 concerning *P. aeruginosa* and *E. coli*. PZL - concerning *P. aeruginosa*. SSL, SC, SSS, ChPL, PZL, SPCh and SPZ have antimycotic activity concerning *C. albicans*. Concerning *E. coli* it was revealed that SSL, ChPL, SPZ had bactericidal activity, and concerning *P. aeruginosa* and *C. albicans* SC, ChPL, PZL, SPZ had it in dilution 1:10 and 1:100.

Among the polysaccharidic fractions the antimicrobial activity was revealed only for PC in both dilution concerning *B.cereus*.

As for *S. aureus* and *B. subtilis*, bacteria of these strains were resistant to all studied fractions.

Conclusions. Antimicrobial activity of a number of substances of sowing cucumber leaves, summer squash and two sorts of peppermint "Chernolistnaya" and "Zgadka" is studied.

Lipophilic fractions of raw materials (except CL) have an antimycotic activity concerning *C. albicans*.

Among all the polysaccharidic fractions only PC have antimicrobial action concerning *B. cereus*.

Strains of bacteria *S. aureus* and *B. subtilis* are resistant to both dilutions of all studied substances.

References:

1. Haemopoetic effect of Cucurbita pepo L. Diet preparation in albino rats / G. J. Esenowo, J. E. Okon, F. C. Udoukpo et al. // Continental J. Biological Sciences. – 2013. – Vol. 6. – P. 6.
2. Caili F. A review on pharmacological activities and utilization technologies of pumpkin. / F. Caili, S. Huan, L. Quanhong // Plant Foods Hum Nutr. – 2006. Vol.61 (2). – P.73-80.
3. Amri, E. The role of selected plant families with dietary Ethnomedicinal species used as anticancer / E. Amri // Journal of Medicinal Plants Studies. – 2014. – Vol. 2, № 1. – P. 28–39.
4. Lim, T. K. *Cucumis sativus*: Edible Medicinal and Non-Medicinal Plants / T.K. Lim. – London, New York: Springer, 2012. – Vol. 2. – P. 239–249
5. Traditional medicine. Cucurbita pepo "Gifts of Nature" [Electronic resource]. – Access to : <http://fito.kosiv.info/uk/narodna-medycyna/dykorostuchiroslyny/25-garbus-zvyhajnyj.html>
6. Caili F., Huan S., Quanhong L. // Plant Foods Hum. Nutr. – 2006. – № 61. – P. 73–80.
7. Harchuk O.A., Mitina T.F., Kirilov A.F. et al. // Buletinul IASM. Științevieți. Fiziologiasii Biochimia Plantelor. – 2013. - №.1 (319). - C. 71-78.
8. Peppermint (*Menta piperita*): an evidence-based systematic review by the Natural Standard Research Collaboration / D. Keifer, C. Ulbricht, T. R. Abrams et al. // Contact Dermatitis. – 2008. – № 6. – P. 364–365
9. Demeshko O.V. Chemical study of lipophilic fraction from the leaves of Robinia pseudoacacia / O.V. Demeshko, I. O. Zhuravel', A.M. Komisarenko // News of Pharmacy. — 2004 — V. - 38, №2. — P. 23-25.
10. Preliminary phytochemical study of flowers, leaves and stems of Verbascum phlomoïdes [Text] / A. A. Voloshyna, V. S. Kyslychenko, I. O. Zhuravel', N. Ye. Burda // Achievements and prospects of the pharmaceutical and healthcare industry in the world today : Mat. II All-Ukrainian scientific conference of young scientists and students, Lugansk, March 29, 2012 - Lugansk, 2012. – P. 80-81.
11. Bubenchikova, V. N. The investigation polysaccharide complexes isolated from above-ground part of Veronica austriaca L. / V. N. Bubenchikova, Yu. A. Kondratova // The journal "Belgorod State University Scientific Bulletin. – 2012. – № 10 (129). – P. 43 – 45.
12. State Pharmacopoeia of Ukraine / State Enterprise "Scientific and Expert Pharmacopoeial Center". – 1 st Ed., – Kharkiv, 2001. – 556 p.

13. Studying the specific activity of antimicrobial drugs: Methodical Recommendations [Text] : methodical recommendations / Yu. L. Volyans'kyy, I. S. Hrytsenko, V. P. Shyrobokov ta in. – K., 2004. – 38 p.

ANTIMICROBIAL ACTIVITY OF THE SUBSTANCES RECEIVED FROM RAW MATERIALS OF LAMIACEAE AND CUCURBITACEAE FAMILY PLANTS.

Fedchenkova Iu. A., Savinova Y. M., Batyuchenko I. I., Andrianov K. V., Gamulya O. V.

Introduction. The search of new sources of raw materials for medicine creation is one of the pharmacy priorities. In this, our attention was drawn by the plants which are widely cultivated in Ukraine and have a sufficient source of raw materials. It is summer squash (*Cucurbita pepo L.*) and sowing cucumber (*Cucumis sativus L.*) of *Cucurbitaceae* family and also peppermint (*Mentha piperita L.*) of two sorts "Chernolistaya" and "Zgadka" of *Lamiaceae* family. However the drugs with antimicrobial action derived from the leaves of these plants in the market of Ukraine are absent. Spirit, lipophilic and polysaccharidic fractions were received from the leaves of sowing cucumber, summer squash and two sorts of peppermint by us. Antimicrobial activity studying of the received substances derived from the studied raw materials for expansion of the medicine range is promising. The relevance of researches is that getting for the first time due to complex raw material processing the lipophilic, spirit and polysaccharidic fractions of leaves of a sowing cucumber, summer squash and two sorts of peppermint "Chernolistnaya" and "Zgadka" are systemically studied on existence of antimicrobial activity. **The purpose of our research** is the studying of antimicrobial activity of getting substances derived from the leaves of sowing cucumber, summer squash and two sorts of peppermint "Chernolistnaya" and "Zgadka". **Materials and methods.** Raw materials for substances are the leaves of a sowing cucumber prepared in June, 2015 in the Kharkiv region, the village Selection, in the conditions of industrial cultivation (the selection station of Institute of vegetable-growing and melon-growing of UAAN). The leaves of summer squash were being prepared also in June, 2015 in Valkovsky district of the Kharkiv region, the settlement Dobropolye. The leaves of peppermint of "Chernolistnaya" and "Zgadka" sort were prepared on the industrial squares of Dnipropetrovsk region, Sofiyevskiy district, the settlement Ordo-Vasilyevka. Lipophilic fractions were received in Sokslet's device. As an extractant, chloroform was used. From the received extraction the extractant was being deleted in vacuum. Lipophilic fraction represented viscous substances of black-green color with typical fragrant. For receiving spirit fractions derived from solvent cake after receiving the lipophilic fractions it was used a method of fractional maceration at the general ratio raw materials-extractant

1:15, as an extractant was 50% ethyl alcohol. Extraction was carried out three times. The integrated extracts were concentrated in vacuum. After a full removal of an extractant and drying, it had been received the hygroscopic powders of green-brown color with specific tastes and smells. From the solvent cake which was remained after receiving lipophilic and spirit fractions polysaccharidic fractions representing water-soluble polysaccharides were evolved. They were friable powders of light gray color. The lipophilic fraction of sowing cucumber leaves, lipophilic fraction of summer squash leaves, lipophilic fraction of peppermint leaves of "Chernolistnaya" sort, lipophilic fraction of peppermint leaves of "Zgadka" sort, spirit fraction of cucumber leaves, spirit fraction of summer squash leaves, spirit fraction of peppermint leaves of "Chernolistnaya" sort, spirit fraction of peppermint leaves of Zgadka sort, polysaccharidic fraction of cucumber leaves, polysaccharidic fraction of summer squash leaves were being studied. Test of antimicrobial effect of substances was carried out by method of serial dilution concerning the following six reference test cultures: *Staphylococcus aureus* of ATCC 6538-P, *Candida albicans* of ATCC 885-653, *Escherichia coli* of ATCC 25922, *Bacillus subtilis* of ATCC 6833, *Bacillus cereus* of ATCC 10702, *Pseudomonas aeruginosa* of ATCC 9027, according to the State Pharmacopoeia of Ukraine of 1 edition, in the Department of microbiology and immunology of KMAPQE.

Results and discussion. The obtained data demonstrate that the widest range of antimicrobial activity was shown by SSL, ChPL and SPZ in the ratio of *E. coli*, *C. albicans* and *P. aeruginosa*. Thus, SSL, ChPL, SC and SPZ had a bactericidal activity in dilution 1:10 and 1:100 concerning *P. aeruginosa* and *E. coli*. PZL - concerning *P. aeruginosa*. SSL, SC, SSS, ChPL, PZL, SPCh and SPZ have antimycotic activity concerning *C. albicans*. Concerning *E. coli* it was revealed that SSL, ChPL, SPZ had bactericidal activity, and concerning *P. aeruginosa* and *C. albicans* SC, ChPL, PZL, SPZ had it in dilution 1:10 and 1:100. Among the polysaccharidic fractions the antimicrobial activity was revealed only for PC in both dilution concerning *B. cereus*. As for *S. aureus* and *B. subtilis*, bacteria of these strains were resistant to all studied fractions. **Conclusions.** Antimicrobial activity of a number of substances of sowing cucumber leaves, summer squash and two sorts of peppermint "Chernolistnaya" and "Zgadka" is studied. Lipophilic fractions of raw materials (except CL) have an antimycotic activity concerning *C. albicans*. Among all the polysaccharidic fractions only PC have antimicrobial action concerning *B. cereus*. Strains of bacteria *S. aureus* and *B. subtilis* are resistant to both dilutions of all studied substances.






























Key words: antimicrobial activity, leaves, cucumber, summer squash, peppermint, substance.

Table 1. Antimicrobial activity of the substances made of sowing cucumber leaves and summer squash leaves.

Culture sample	CL		SSL		SC		SSS		PC		PSS		Culture
	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	
<i>E. coli</i>	●	●	○	○	●	●	●	●	●	●	●	●	●
<i>C. albicans</i>	●	●	○	○	○	○	○	○	●	●	●	●	●
<i>S. aureus</i>	●	●	●	●	●	●	●	●	●	●	●	●	●
<i>B. subtilis</i>	●	●	●	●	●	●	●	●	●	●	●	●	●
<i>B. cereus</i>	●	●	●	●	●	●	●	●	○	○	●	●	●
<i>P. aeruginosa</i>	●	●	○	○	○	○	●	●	●	●	●	●	●

Note : ● - growth of culture, ○ - absence of culture growth

Table 2. Antimicrobial activity of the substances made of peppermint leaves of “Chernolistnaya” and “Zgadka” sorts.

Culture sample	ChPL		PZL		SPCh		SPZ		PPCh		PPZ		Culture
	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	1:10	1:100	
<i>E. coli</i>													
<i>C. albicans</i>													
<i>S. aureus</i>													
<i>B. subtilis</i>													
<i>B. cereus</i>													
<i>P. aeruginosa</i>													

Note :  - growth of culture,  - absence of culture growth