Котов Андрій Георгійович, доктор фармацевтичних наук, старший науковий співробітник, Начальник відділу ДФУ, ДП «УНФЦЯЛЗ», вул. Астрономічна, 33, м. Харків, Україна, 61085
E-mail:fitex2@gmail.com

Котова Еліна Едуардівна, кандидат фармацевтичних наук, старший науковий співробітник, завідувач сектором, Сектор експериментальної підтримки розробки монографій на ЛРС, ДП «УНФЦЯЛЗ»
вул. Астрономічна, 33, м. Харків, Україна, 61085
E-mail: kotova-elina@mail.ru

Губарь Світлана Миколаївна, кандидат фармацевтичних наук, завідувач лабораторії, Державна науково-дослідна лабораторія з контролю якості лікарських засобів, Національний фармацевтичний університет, вул. Пушкінська, 53, м. Харків, Україна, 61002
E-mail: labcq@ukr.net

UDC 613.32:615.276:615.015.35:543.544.5.068.7

PHYTOCHEMICAL STUDY AND DETERMINATION OF PHARMACOLOGICAL ACTIVITIES OF CHERRY SHOOTS DRY EXTRACT

© L. Lenchyk, O. Shapoval, V. Kyslychenko

Aim. *The aim of our research was investigation of phenolic constituents, free and connected sugars of the cherry shoots dry extract by the HPLC method and determination of acute toxicity and anti-inflammatory activity of the extract.*
Methods. *Qualitative composition and content of phenolic compounds and sugars in the cherry shoots dry extract were studied by HPLC, with the Agilent Technologies chromatograph (model 1100). The acute toxicity was studied after single intragastric administration of cherry shoots dry extract to rats. Studying the influence of the cherry shoots dry extract during experimental inflammation exudative mechanism was carried out on a model of acute carrageenan-induced inflammation in white rats.*
Results. *As a result of the study 9 phenolic compounds as derivatives of hydroxycinnamic acids and flavonoids and 5 sugars were determined. it was established the absence of toxicity of the cherry shoots dry extract in a dose 5000 mg/kg and according to toxicological classification of substances by K.K.Sidorov the cherry shoots dry extract after intragastric administration belong to V toxicity class – practically harmless substances. Cherry shoots dry extract showed the best anti-inflammatory effect in a dose of 100 mg/kg; it was about 36 % on average for 5 hours.*
Conclusions. *Considering results of chemical analysis and determination safety and antiinflammatory activity we can assume that the cherry shoots dry extract can demonstrate analgesic and antiallergic effect, so it is promising for further study in order to create a new effective and safe drugs for use in medical practice*
Keywords: *cherry, extract, flavonoids, sugars, hydroxycinnamic acids, anti-inflammatory activity, acute toxicity*

Мета дослідження. *Метою нашого дослідження було вивчення фенольних сполук, вільних і зв'язкові цукрів сухого екстракту з пагонів вишні методом високоефективної рідинної хроматографії (ВЕРХ) і визначення гострої токсичності та протизапальну активності цього екстракту.*
Методи. *Якісний склад і вміст фенольних сполук і цукрів сухого екстракту з пагонів вишні було досліджено за допомогою ВЕРХ на хроматографі Agilent Technologies (модель 1100). Гостру токсичність вивчали на щурах після однократного введення внутрішньошлунково сухого екстракту пагонів вишні. Протизапальна дія сухого екстракту була досліджена на моделі гострого карагенан-індукованого запалення стопи білих щурів.*
Результати. *В результаті дослідження 9 фенольних сполук, серед яких гідроксикоричні кислоти і флавоноїди і 5 цукрів було ідентифіковано в сухому екстракті. Встановлено відсутність токсичності сухого екстракту пагонів вишні в дозі 5000 мг / кг, що відповідно до токсикологічної класифікації речовин К.К. Сидорова дозволяє віднести екстракт до класу V токсичності - практично нешкідливі речовини. Сухий екстракт пагонів вишні мав кращу протизапальну дію в дозі 100 мг / кг; що в середньому було близько 36 % за 5 годин.*
Висновки. *Беручи до уваги результати хімічного аналізу і визначення токсичності і протизапальної активності сухого екстракту пагонів вишні, ми можемо припустити, що цей екстракт може мати знеболюючу і протиалергічну дію, і є перспективним для подальшого дослідження з метою створення нових ефективних і безпечних лікарських засобів для застосування в медичній практиці*
Ключові слова: *вишня, екстракт, флавоноїди, цукри, гідроксикоричні кислоти, протизапальна активність, гостра токсичність*

1. Introduction

Search and development of medicines based on plant raw materials is an important task of our time. Preparations of natural origin, being quite effective, usually have fewer side effects. Among the medicinal plants sour cherry *Cerasus vulgaris*, family *Rosaceae*, is prospective for research. It is widely cultivated in Ukraine as horticultural crops. In folk medicine the

cherries fruits, fruitstalks, juice, shoots, leaves and gum are used as a medicinal raw material [1, 2].

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

At the Department of Chemistry of Natural Compounds of the National University of Pharmacy extracts of the cherry fruitstalks, shoots and leaves were obtained and their diuretic effect was studied on the rats in 2009. It was found that cherries shoots dry extract showed the highest diuretic activity in a dose100 mg/kg [3]. Further investigations are necessary to determine constituents, safety and efficacy of the extract.

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author

Qualitative composition and content of phenolic compounds in a cherry shoots dry extract have been determined by paper, thin-layer chromatography and spectrophotometry. Flavonoid rutin; chlorogenic and gallic acids were identified in the extract. The content of polyphenolic compounds, hydroxycinnamic acids and flavonoids were determined as 21,89±0,27 %, 5,42±0,11 % and 9,89±0,18 %, respectively by spectrophotometry. Obtained results are prospective for further study of the cherry dry extract [4]. It is known that the flavonoids provide antioxidant and antiinflammatory effects of herbal medicines and are components of cytoprotective action (eg hepatoprotective, cardioprotective, nephroprotective). So, study of anti-inflammatory action and safety of cherry shoots dry extract was actually, interesting and expedient.

4. Allocation of unsolved parts of the general problem, which is dedicated to the article

The method of high performance liquid chromatography (HPLC) is widely used for the study of different groups of phenolic compounds extracted from plants. This method has a high sensitivity and accuracy. Further depth study of biologically active substances of cherry shoots dry extract by HPLC is necessary to establish structure-activity relationships, and will be used in further standardization of the extract.

5. Formulation of goals (tasks) of article

The aim of our research was investigation of phenolic constituents, free and connected sugars of the cherry shoots dry extract by the HPLC and determination of anti-inflammatory activity and the safety of cherry shoots dry extract.

6. Statement of the basic material of the study (methods and objects) with the justification of the results

In February 2014 *Cerasus vulgaris* shoots were collected in the farm "The Garden", Sumy region, Ukraine, which is engaged in growing horticultural crops. We used varieties “Vasilisa”. Plant raw material was dried and grinded.

Cherry shoots dry extract was obtained by maceration that is the infusion of plant raw material with 30 % ethyl alcohol and heating. The process includes the following consecutive steps: loading plant material; filing extractant into extractor from the top on the layer of raw material; removing air from the extraction medium under the influence of a pressure-force and the formation of "mirrors"; soaking raw material (2 hrs.), extraction by heating (2 hrs.), filtration and evaporation of the extract under vacuum to dryness.

For hydrolysis, 300 µl of 4 % the extract alcohol solution was placed in a 2 ml vial, and 300 µl 6N solution of hydrochloric acid in ethanol (1:1 by volume) was added. The hermetically sealed vial was heated in an oven at 100 °C for 1 hour. After cooling, the content of the vial was centrifuged and transferred for analysis.

Qualitative composition and content of phenolic compounds in the cherry shoots dry extract were studied by HPLC, with the Agilent Technologies chromatograph (model 1100) completed with continuous-flow vacuum degasifier G1379A, 4-channel pump of low pressure gradient G13111A, automatic injector G1313A, column oven G13116A, diode array detector G1316A. The chromatographic column ZORBAX-SB C-18, 2.1 × 150 mm, filled with octadecyl silyl sorbent was used for analysis. Conditions of analysis was follow: temperature of the thermostat was 35 °C; mobile phase flow rate – 0.25 ml / min; gradient regime of chromatography was used with a mobile phase solution A (0,1 % H_3PO_4, in water) and solution B (MeOH) in a ratio of 90:10 (the first 8 min), 70:30 (8 to 24 min), 20:80 (25 min); from 26 to 29 min, only the solution B was used, and after again, solutions A and B in a ratio of 90:10 (30 to 35 min) were used. The operating pressure of the eluent was 240–300 kPa. Scale of measurement was 1.0; scan time – 0.5 seconds; options metering spectrum – each peak of 190–600 nm. Identification of phenolic compounds was performed by the retention time of standards of hydroxycinnamic acids and flavonoids and their spectral characteristics [3]. Each peak detected in the cherry extract was identified by comparing retention time and UV spectra given by the diode array detector with the standards. The flavonoids, hydroxycinnamic acids were quantified by calibration with the standards [5–7].

Investigation of the monosaccharide content in the extract before and after hydrolysis was carried out by HPLC with the chromatograph Agilent Technologies (model 1100) with refractometric detector. For this analysis, chromatographic column carbohydrate 7,8×300 mm «Supelcogel-C610H»was used. For the analysis, the following mode was set: mobile phase flow rate 0.5 ml/min; eluting with 0.1 % aqueous solution of H_3PO_4; operating pressure of the eluent – 33–36 kPa; column oven temperature – 30 °C; the sample volume was 5 µl. Identification of the sugars was carried out by the sugar standard samples retention time. These conditions refer to the known method and was performed with some modifications to the gradient [5–7]. The statistical processing of results was carried out using the package Statistica 6.0. The error did not exceed 5 %.

As a result of the study 9 phenolic compounds as derivatives of hydroxycinnamic acids and flavonoids and 5 sugars were determined (Tables 1–3). Graphical result of determination of phenolic compounds and sugars can be seen on figure 1. It was found nine phenolic compounds in the extract, among them: (+) D-catechin and

(-) – epicatechin, three hydroxycinnamic acids and four flavonoids (all quercetin glycosides). The total content of phenolic compounds in the extract was – 9,02 % and the predominant component was (–) – epicatechin – 3289.00 mg / 100g. Among the compounds of cherry extract, the share of catechins was accounted as 68.80 %.

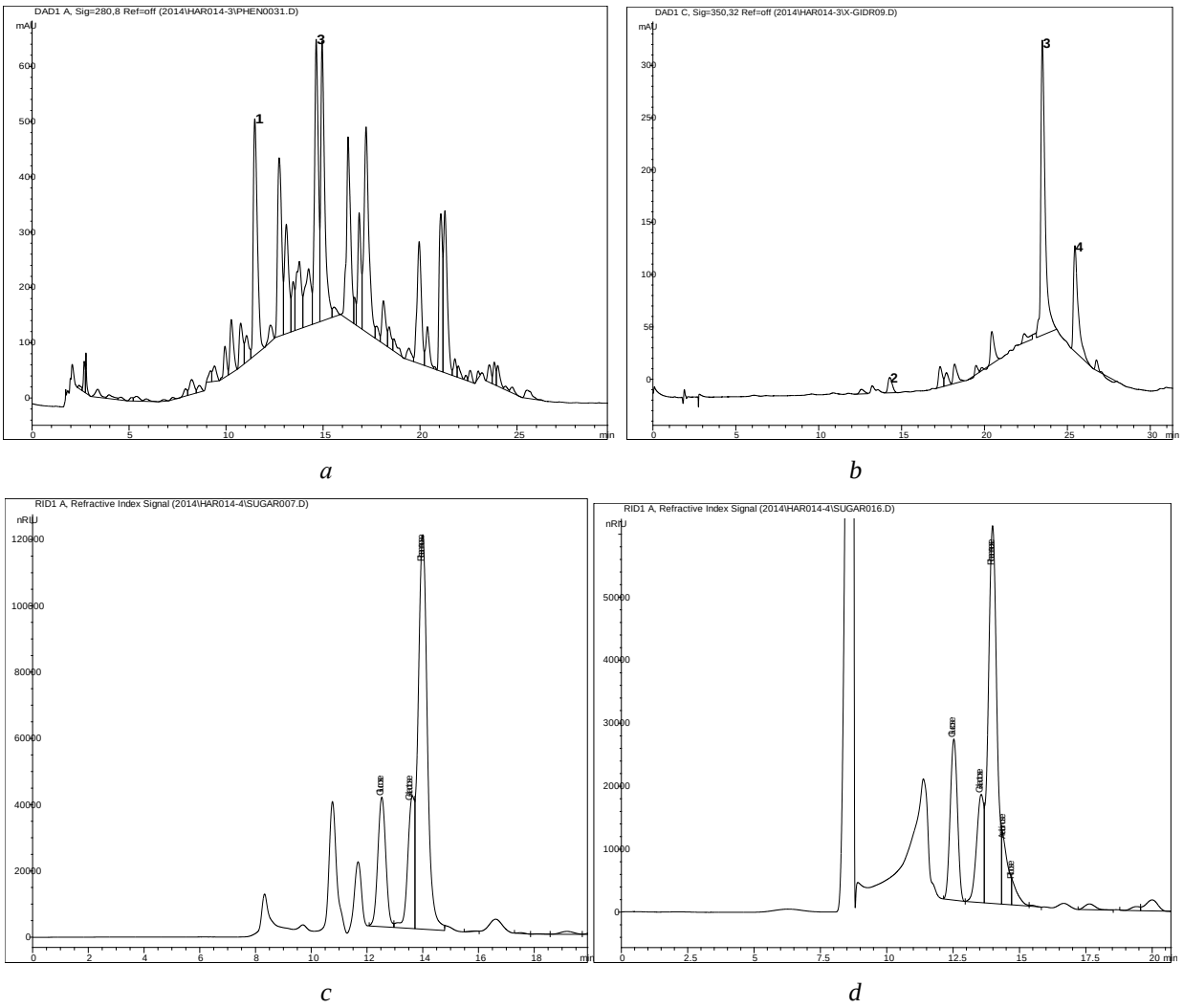

Fig. 1. Graphical results of HPLC investigation of cherry shoos dry extract: *a* – phenolic compounds before hydrolysis; *b* – phenolic compounds after hydrolysis; *c* – monosaccharides composition before hydrolysis; *d* – monosaccharides composition after hydrolysis

The content of hydroxycinnamic acids in the extract was significantly lower – 0.49 %; the content of flavonoids amounted to – 2.26 %. Among the hydroxycinnamic acids the most content was uncertain derivative of *p*-coumaric acid. Among the flavonoids content of quercetin-3-O-rhamnoside was the largest and was 746,76 mg/100g. It was in % of flavonoid sum – 33.10. The results of determination of phenolic compounds before hydrolysis are shown in the Table 1. After hydrolysis, free caffeic acid and aglycones of quercetin and kempferol were found in the extract. The total amount of free sugar before hydrolysis in the extract was found as 5.67±0.09 % and after hydrolysis it increased to 6.52±0.11 %. Free rhamnose, found mainly in free state, was the most abundant substance in the extract. Arabinose and ribose presented only as a part of glycosides.

Table 1

The phenolic composition of dry extract from cherry shoots Before hydrolysis (n=3)

№	Compound	Retention time, min	Content, (mg/100g)
1	(+)-D-Catechin	11.48	2980.46
2	p-coumaric acid derivative	12.73	178.15
3	(–)-epicatechin	14.65	3289.00
4	*p*-Coumaric acid	17.22	164.85
5	Ferulic acid	18.12	147.14
6	Rutin	19.96	629.72
7	Glycoside of quercetin	20.39	213.43
8	Quercetin-3-O-glycoside	21.06	666.26

9	Quercetin-3-O-rhamnoside	21.28	746.76

The results of determination of phenolic compounds after hydrolysis in cherry shoots dry extract are shown in the Table 2.

Table 2

The phenolic composition of cherry shoots dry extract after hydrolysis (n=3)

Compound	Retention time, min	Content, (mg/100g)
Caffeic acid	14.20	23.66±1.78
Quercetin	23.52	571.41±2.87
Kaempferol	25.46	153.37±2.55

The results of investigation of monosaccharide composition before and after hydrolysis in cherry shoots dry extract are shown in the Table 3.

Table 3

Monosaccharide composition of cherry shoots dry extract (n=3)

Monosaccharide	Content, %	
	Before hydrolysis	After hydrolysis
Glucose	0.89	1.10
Galactose	0.77	0.82
Rhamnose	4.01	4.04
Arabinose	–	0.41
Ribose	–	0.15

The acute toxicity was studied after single intragastric administration of cherry shoots dry extract to rats of both sex for determination the LD_{50} dose in accordance with the guidelines Ukraine DEC of MH [8]. The limiting measure in determination of the LD_{50} was the maximum dose of fourth-grade toxicity (low-toxic substances) – 5000 mg/kg. If the dose administered to animals is not their destruction, the introduction of high-dose is impractical [8]. Studies were performed in accordance with National guidelines, "The General Ethical Principles of Animal Experimentation" (Ukraine, 2001), which are consistent with the provisions of " The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [8–10]. Experimental animals were randomized into groups, which are presented in Table 4.

It was determined that after a single intragastric administration of cherry shoots dry extract in a dose of 5000 mg / kg signs of intoxication on the day of administration and for 14 days after is not revealed: animals were neat, active, responsive to auditory and visual stimuli, the process of urination and defecation were normal, respiratory failure and convulsions were not observed, reflex excitability has been saved. The rats of both experimental groups consumed food and water normally. Not registered the death of animals during the entire observation period (Table 5). Thus intragastric administration to rates maximum dose of cherry shoots dry extract 5000 mg/kg did not cause death of the animals and pathological changes of the functional state of the rats.

Therefore, the determination of the LD_{50} dose of the extract is impossible. So, it was established the absence of toxicity of the cherry shoots dry extract in a dose 5000 mg / kg and according to toxicological classification of substances by K. K. Sidorov the cherry shoots dry extract after intragastric administration belong to V toxicity class – practically harmless substances [9].

Table 4

Randomization of rats in an experiment in study the acute toxicity of cherry shoots dry extract

Conditions of the experiment	Dose, mg/kg	Number of rats	
		males	females
Intact control (IC)	–	6	6
Cherry shoots dry extract	5000,0	6	6

Table 5

The acute toxicity studies cherry shoots dry extract after the single intragastric administration to white rats of both genders

Conditions of the experiment	Dose, mg/kg	The observed effect, the number of dead animals/ total number of animals in the group	
		Males	Females
Intact control (IC)	–	0/6	0/6
Cherry shoots dry extract	5000,0	0/6	0/6

Studying the influence of the cherry shoots dry extract during experimental inflammation exudative mechanism was carried out on a model of acute carrageenan-induced inflammation in white rats 180–200 g weigh in doses of 10, 50 and 100 mg/kg, administered in maintenance mode for 7 days according to methodological recommendations [1]. The study was conducted in comparison with the reference drug Diclofenac sodium, due to the fact that it is recognized as the "gold standard" for the efficacy and safety of NSAIDs among modern mechanisms and its influence on the inflammatory process is well understood [1].

Study of antiinflammatort activity was carried out on the model of acute carrageenan-induced inflammation in the rat foot, characterized by the ability to study the dynamics of the process. It was determined that pathogenesis of inflammation involves serotonin and histamine in the first 30–90 minutes; in the range between 1.5–2.5 hours – kinins, and between 2,5–5,5 hours – prostaglandins, allowing indirectly suggest mechanisms for anti-inflammatory action of these substances [11]. Also this model demonstrated a direct correlation between effectiveness of the tested drug and its efficacy in the clinic.

Analysis of the results showed (Table 6, 7) that the comparator diclofenac sodium in dose 8 mg/kg expressed antiexudative activity about 50 %, indicating that the model of carrageenan inflammation in rat foot is adequate, reproducibility and reliability of the results. Cherry shoots dry extract showed the best anti-inflammatory effect in a dose of 100 mg/kg; it was about 36 % on average for 5 hours. It had mild effect on the inflammatory edema (on average – 16 %) in the dose 10 mg/kg and in the dose 50 mg/kg – about 27 %, which can also be characterized as weak.

Table 6

Cherry shoots dry extract anti-inflammatory activity in the model of carrageenan inflammation in rat foot, n=6

Conditions of experiment		The dynamics of inflammation				
		1 hours	2 hours	3 hours	4 hours	5 hours
Control	ΔV	21,2±1,6	21,4±0,8	24,7±0,6	34,0±0,9	37,7±1,6
Cherry shoots dry extract, 10 mg/kg	ΔV	16,4±1,6*	18,0±1,6	22,0±2,7	30,7±2,5	32,5±2,5
	AA, %	22,6	15,9	10,9	11,8	18,1
Cherry shoots dry extract , 50 mg/kg	ΔV	15,9±1,6 *	15,8±1,6 *	16,8±1,7 *	24,1±1,4 *	29,3±1,6 *
	AA, %	25,0	26,2	31,9	29,1	22,3
Cherry shoots dry extract, 100 mg/kg	ΔV	12,5±1,2 *	14,1±1,8*	15,5±2,2*	20,4±2,3*	27,6±1,5*
	AA, %	41,0	34,1	37,2	40,0	26,8
Diclofenac sodium	ΔV	12,1±1,7 *	11,0±1,9*	12,0±1,8*	14,6±1,6*	18,8±1,8*
	AA, %	42,9	48,6	51,4	57,1	50,1

*Notes: ΔV – difference between the original volume and volume of the foot at the time of measurement; AA – anti-inflammatory activity; * – Rate differences by false relative to the control group, p≤0,05 (Newman-Keyls criterion).*

Table 7

Cherry shoots dry extract anti-inflammatory action average for 5 hours

The name of the substance	Dose, mg/kg	Anti-inflammatory action, % (average for 5 hours)	Conventionally therapeutic dose (CTD), mg/kg
Cherry shoots dry extract	10,0	15,86±2,14	100,00
	50,0	26,90±1,66	
	100,0	35,82±2,55	
Diclofenac sodium	8,00	50,02±2,29	8,00*

*Notes: * – Diclofenac sodium ED_{50}, which was designed by us in other studies and confirmed by the literature dates [11].*

Analysis of the dynamics of anti-inflammatory activity of the cherry shoots dry extract in the dose of 100 mg/kg (Table 6, 7) allowed to make assumptions about the mechanisms of activity. cherry shoots dry extract showed antiinflammatory activity due to presence of flavonoids, hydroxycinnamic acids and sugars and it inhibited inflammatory mediators: serotonin, histamine, prostaglandins and kinins. It is known that flavonoids caused antiinflammatory action and polysaccharides – antiallergic. Considering the fact that serotonin and histamine are mediators of pain and allergies, kinins and prostaglandins – pain, it can be assumed that the cherry shoots dry extract can exhibit analgesic and antiallergic effect.

7. Conclusions

Investigation of chemical composition of the cherry shoots dry extract was carried out before and after hydrolysis by HPLC. Nine phenolic compounds, among them flavonoids and hydroxycinnamic acids, were established.

Considering results of determination safety and antiinflammatory activity the foregoing we can assume that the cherry shoots dry extract can demonstrate analgesic and antiallergic effect, so it is promising for further study in order to create a new effective and safe drugs for use in medical practice.

References

1. Upyr, L. V. Cherry [Text] / L. V. Upyr; V. P. Chernykh (Ed.). – 2-nd ed. – Kiev: «MORION», 2010. – P. 277–278.

2. Popovich, V. P. Effect of cherry fruit stalks and shoots dry extracts on diuresis and some indicators of nitrogen metabolism in rats [Text]: conference / V. P. Popovich,

L. V. Upyr, V. S. Kyslychenko // Current problems of theoretical, clinical, preventive medicine, dentistry and pharmacy. – Donetsk, 2010. – P. 280–281.

3. The State Pharmacopoeia of Ukraine. Suppl. 2 [Text] / State Enterprise "Ukrainian scientific Pharmacopoeial center". – 1-st ed. – Kharkiv: RIREG, 2008. – 620 p.

4. Gulieva, A. F. Phytochemical study of phenolic compounds cherry extract [Text]: scientific-practical conference / A. F. Gulieva, L. V. Lenchyk // Innovations in Medicine and Pharmacy. – Minsk: BSMU, 2015. – P. 836–838.

5. Court, W. A. High-performance reversed-phase liquid chromatography of naturally occurring phenolic compounds [Text] / W. A. Court // Journal of Chromatography A. – 1977. – Vol. 130 – P. 287–291. doi: 10.1016/s0021-9673(00)89807-6

6. Mabry, T. J. The Systematic Identification of Flavonoids [Text] / T. J. Mabry, K. R. Markham, M. B. Thomas. – New York: Springer-Verlag, 1970. doi: 10.1007/978-3-642-88458-0

7. McMurrough, I. Quantitative analysis of hop flavonols using high-performance liquid chromatography [Text] / I. McMurrough, G. P. Hennigan, M. J. Loughrey // Journal of Agricultural and Food Chemistry. – 1982. – Vol. 30, Issue 6. – P. 1102–1106. doi: 10.1021/jf00114a024

8. Zapadnyuk, I. P. Laboratory animals: breeding, keeping and using in experiment [Text] / I. P. Zapadnyuk, V. I. Zapadnyuk, S. A. Zechariah. – Kyiv: Vishcha shkola, 1983. – 552 p.

9. Kovalenko, V. M. Experimental study of toxic effects of potential drugs / Preclinical studies of drugs [Text]: method. rec. / V. M. Kovalenko, O. V. Stefanov, Y. M. Maximov, I. M. Trachtenberg; A. V. Stefanov (Ed.). – Kyiv: Avicenna, 2001. – P. 74–97.

10. European convention for the protection of vertebrate animals used for experimental and other scientific purposes [Text]. – Council of European. – Strasbourg, 1986. – Issue 123. – 51 p.

11. Preclinical studies of drugs (guidelines) [Text] / O. Stefanov (Ed.). – Kyiv: Avicenna, 2001. – P. 292–306.

References

1. Upyr, L. V.; Chernykh, V. P. (Ed.) (2010). Cherry. Kiev: «MORION», 277–278.

2. Popovich, V. P., Upyr, L. V., Kyslychenko, V. S. (2010). Effect of cherry fruit stalks and shoots dry extracts on diuresis and some indicators of nitrogen metabolism in rats. Current problems of theoretical, clinical, preventive medicine, dentistry and pharmacy. Donetsk, 280–281.

3. The State Pharmacopoeia of Ukraine. Suppl. 2 (2008). Kharkiv: RIREG, 620.

4. Gulieva, A. F., Lenchyk, L. V. (2015). Phytochemical study of phenolic compounds cherry extract. Innovations in Medicine and Pharmacy. Minsk: BSMU, 836–838.

5. Court, W. A. (1977). High-performance reversed-phase liquid chromatography of naturally occurring phenollic compounds. Journal of Chromatography A, 130, 287–291. doi: 10.1016/s0021-9673(00)89807-6

6. Mabry, T. J., Markham, K. R., Thomas, M. B. (1970). The Systematic Identification of Flavonoids. New York: Springer-Verlag. doi: 10.1007/978-3-642-88458-0

7. McMurrough, I., Hennigan, G. P., Loughrey, M. J. (1982). Quantitative analysis of hop flavonols using high-performance liquid chromatography. Journal of Agricultural and Food Chemistry, 30 (6), 1102–1106. doi: 10.1021/jf00114a024

8. Zapadnyuk, I. P., Zapadnyuk, V. I., Zechariah, S. A. (1983). Laboratory animals: breeding, keeping and using in experiment. Kyiv: Vishcha shkola, 552.

9. Kovalenko, V. M., Stefanov, O. V., Maximov, Y. M., Trachtenberg, I. M.; Stefanov, A. V. (Ed.) (2001). Experimental study of toxic effects of potential drugs / Preclinical studies of drugs. Kyiv: Avicenna, 74–97.

10. European convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986). Council of European. Strasbourg, 123, 51.

11. Stefanov, O. (Ed.) (2001). Preclinical studies of drugs (guidelines). Kyiv: Avicenna, 292–306.

Дата надходження рукопису 12.04.2016

Lenchyk Larysa, Candidate of pharmaceutical sciences, associate professor, Department of Chemistry of Natural Compounds, National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: larysa.lenchyk@gmail.com

Shapoval Olga, Candidate of pharmaceutical sciences, associate professor, Department of Pharmacology, National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: olana666@ukr.net

Kyslychenko Viktoriia, Doctor of pharmaceutical sciences, professor, Department of Chemistry of Natural Compounds, National University of Pharmacy, Pushkinska str., 53, Kharkov, Ukraine, 61002
E-mail: cncvc@mail.ru