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CONTENT OF PHENOLIC COMPOUNDS IN PLANT RAW OF *CICHORIUM INTYBUS* L., *LAMIUM PURPUREUM* L. AND *VISCUM ALBUM* L.

Objective *— to evaluate the antioxidant potential of ethanol extracts of wild selected plants in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine through the determination of phenolic compounds.*

Material and methods. *In this study used dried raw of Cichorium intybus L., Lamium purpureum L. and Viscum album L. Plants of C. intybus and L. purpureum harvested from natural flora of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine. V. album collected from crown trees of Tilia cordata Mill. 0.2 g of dried plant raw material was extracted with 20 mL of 80 % ethanol for 2 hours. The total polyphenol content of extracts was measured by the method described by Singleton and Rossi (1965) using Folin-Chiocalteu reagent and results were expressed in mg of gallic acid equivalent per one gram of dry matter (mg GAE/g). Determination of total flavonoids content was conducted using the modified method described in Shafii et al. (2017) and results expressed in mg quercetin equivalent per one gram of dry matter (mg QE/g). Detection of total phenolic acids content of extracts was carried out using the method described in Farmakopea Polska (1999) and results expressed in mg caffeic acid per one gram of dry matter (mg CAE/g). The antioxidant activity of samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH method) according to Sanchez-Moreno et al. (1998). Also, the antioxidant activity of extracts was determined by the phosphomolybdenum method described by Prieto et al. (1999) with slight modifications. Results of these parameters expressed in mg Trolox equivalent per one gram of dry matter (mg TE/g). Experimental data were evaluated by using Excel 2010. Correlation analysis performed using Pearson's criterion.*

Results. *The total content of polyphenol compounds for C. intybus, L. purpureum and V. album was 33.91, 34.61 and 31.28 mg GAE/g, respectively, the total content of flavonoids for C. intybus, L. purpureum and V. album — 26.29, 28.89 and 25.10 mg QE/g, the total content of phenolic acids — 4.56, 4.87 and 4.07 mg CAE/g, antioxidant activity of extracts by DPPH method was 8.35, 7.66 and 8.55 mg Trolox Equivalent/g, respectively, antioxidant activity by phosphomolybdenum method — 93.01, 142.62 and 9.31 mg Trolox Equivalent/g. Between the accumulation of polyphenol compounds and antioxidant activity of extracts found a strong positive correlation.*

Conclusions. *Wild plants of C. intybus, L. purpureum and V. album in M.M. Gryshko National Botanical Garden of the NAS of Ukraine accumulated polyphenol compounds with high antioxidant activity. Obtained data demonstrated that these plant species can be a potential source of natural antioxidants that can be used in the different pharmacological investigations. It is important to a branch of biological science to investigate biochemical properties of not cultivated plants only but wild plants also to identify new sources of biologically active compounds.*

Key words: *Cichorium intybus, Lamium purpureum, Viscum album*, polyphenols, flavonoids, phenolic acids, antioxidant activity.

Natural compounds isolated from plant raw material possess multiple biological activities such as antioxidant. Among biologically active compounds that cause the antioxidant activity can be highlighted polyphenol compounds with useful therapeutical properties [31]. Natural polyphenols are found in various plants including wild and weeds and play an important role in human life. The most investigated plant species of this direction related to traditional medicinal, fruits, aromatic, and food plants [29]. In present study considered results of a study of polyphenol compounds accumulation and antioxidant activity of three wild growing plant species, which are interesting objects in the relation of biochemical properties.

Cichorium intybus L. (chicory) belongs to Asteraceae Bercht. & J. Presl family and widely distributed in Asia, Europe, South Africa, etc. Plant raw material of this plant is an important source of biologically active compounds (Table 1). This plant used in the traditional medicine of many countries [17]. It is a perennial, a deep-rooting herb that usually described as wild plant [22]. In addition, chicory is known as a vegetable, fresh or cooked, while the ground and roasted roots are widely used for blending with coffee powder. Some varieties of this plant have cultivated in Italy [23].

Lamium purpureum L. (red dead-nettle) is a member of the Lamiaceae Martinov family and grows in Europe, Asia, Africa, etc. The last study showed that this plant can be used as control agents of stored food products due to inhibition action in relation to red flour beetle *Tribolium castaneum* Herbst [3].

Viscum album L. (mistletoe) belonging to Loranthaceae Juss. family and used for the treatment of many diseases as folk medicine in Europe and Northern Asian countries. This plant grows as a semi-parasitic on different trees and shrubs [38].

Biological activity and qualitative content of biologically active compounds in the raw of selected plans represented below (Table 1 and 2).

Material and methods

Plant materials

In this study used dried raw of *Cichorium intybus*, *Lamium purpureum* and *Viscum album*. Plants of *C. intybus* and *L. purpureum* harvested from natural flora of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine in the flowering stage. *V. album* collected from trees of *Tilia cordata Mill.*

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.2 g of dried plant raw material was extracted with 20 ml of 80 % ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids.

Total polyphenol content

Total polyphenol content (TPC) of extracts was measured by the method of Singleton and Rossi (1965) using Folin-Chiocalteu reagent [49]. 0.1 ml

Table 1. **Biologically active compounds in plant raw material of *Cichorium intybus* L., *Lamium purpureum* L. and *Viscum album* L.**

Species	Biologically active compounds in raw	References
Cichorium intybus	Alkaloids, inulin, sesquiterpene lactones, coumarins, vitamins, unsaturated sterols, flavonoids, tannins, cichoric acid, glycosides, anthocyanins, etc.	[1]; [7]; [9]; [13]; [33—36]
Lamium purpureum	Vitamins, phenylethanoid glycosides	[18]; [24]
Viscum album	Lectins, viscotoxins, alkaloids (doesn't contain typical alkaloids), amine alkaloids, triterpenes, flavanone glycosides, flavanones	[6]; [16]; [30]

of each sample extract was mixed with 0.1 ml of the Folin-Chiocalteu reagent, 1 ml of 20 % (w/v) sodium carbonate and 8.8 ml of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25—250 mg/L; $R^2 = 0.996$) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

Total flavonoid content

Determination of total flavonoids content (TFC) was conducted using the modified method described in Shafii et al. (2017) [47]. 0.5 ml of sample extract was mixed with 0.1 ml of 10 % (w/v) ethanolic solution of aluminum chloride, 0.1 ml of 1 M sodium acetate and 4.3 ml of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.01—0.5 mg/L; R^2=0.997) was used as the standard and the results were expressed in mg/g quercetin equivalents.

Total phenolic acid content

Determination total phenolic acids content (TPA) of extracts was carried out using the method described in Farmakopea Polska (1999) [19]. 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v) and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1—200 mg/L, $R^2 = 0.999$) was used as a standard and the results were expressed in mg/g caffeic acid equivalents.

Antioxidant activity by DPPH method

The radical scavenging activity of samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH) according to Sanchez-Moreno et al., 1998 [46]. The extracts (0.5 ml) were mixed with 3.6 ml of radical solution (0.025 g of DPPH in 100 ml ethanol). The absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10—100 mg/L; $R^2 = 0.988$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Antioxidant activity by phosphomolybdenum method

Reducing power of extracts was determined by the phosphomolybdenum (PhMo) method of Prieto et al. (1999) with slight modifications [44]. The mixture of sample (1 ml), monopotassium phosphate

Table 2. **Biological activity of plant raw material of *Cichorium intybus* L., *Lamium purpureum* L. and *Viscum album* L.**

Species	Biological activity of raw	Use in traditional and folk medicine	References
Cichorium intybus	Anti-hepatotoxic, anti-diabetic, anti--bacterial, anti-inflammatory, hyperglycaemic, antiulcerogenic, antioxidant, anti-allergic, anthelmintic	Fever, diarrhoea, jaundice, gallstones, AIDS, cancer, insomnia, splenitis, tachycardia, bruises	[1]; [2]; [4]; [8]; [14]; [26]; [28]; [36]; [41]; [42]; [45]
Lamium purpureum	Insecticidal, diuretic, anti-inflammatory, anti-diarrheal, astringent, expectorant, antirheumatic, haemostatic, antioxidant, antimicrobial	Disorders: trauma, fracture, paralysis, hypertension, uterine hemorrhage	[3]; [11]; [12]; [51]; [53]; [54]
Viscum album	Antioxidant, antitumor, anticancer, cytotoxic, antimicrobial, cytostatic, antihypertensive, antinociceptive, immunomodulatory, sedative, antipsychotic	Hypertension, diabetes, arthrosis, cancer, epilepsy, headache	[5]; [16]; [27]; [37—39]; [40]; [43]

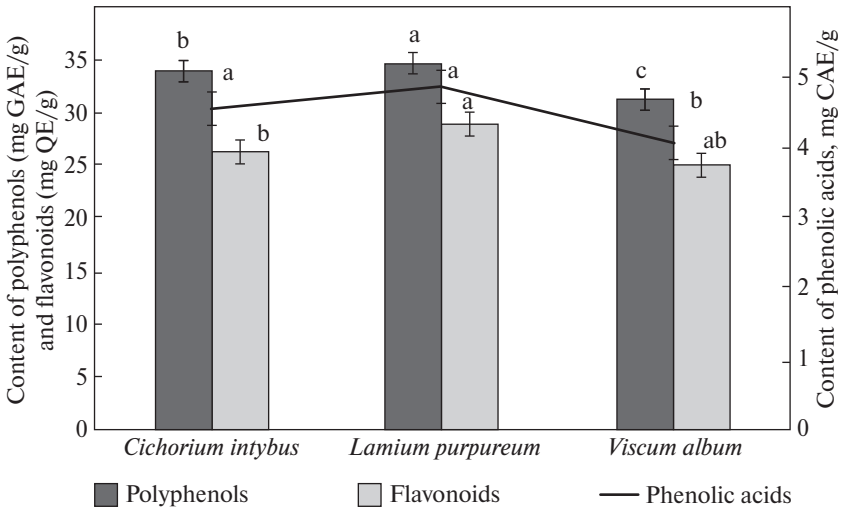

Fig. 1. The total content of polyphenolic compounds, flavonoids and phenolic acids in the above-ground part of investigated plants (means in columns followed by different letters are different at p = 0.05)

(2.8 ml, 0.1 M), sulfuric acid (6 ml, 1 M), ammonium heptamolybdate (0.4 ml, 0.1 M) and distilled water (0.8 ml) incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10—1000 mg/L; $R^2 = 0.998$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Experimental data were evaluated by using Excel 2010. Data were analyzed with ANOVA test and differences between means were compared through the Tukey—Kramer test ($\alpha = 0.05$); significance level at $p < 0.05$. Mean values of three replicates and standard deviation are given in Fig. 1, 2. Correlation analysis performed using Pearson's criterion.

Results and discussions

Study of antioxidant activity of plant raw and content of polyphenol compounds is a very popular branch of modern biology [29]. There are numerous investigations of antioxidant potential of different groups of plants such as medicinal, food, fruits, energetic, invasive, etc. [10; 15; 21; 48; 52]. Investigation of biological activity of wild plants that related to the weeds or even parasitic plants but can have useful properties can be an important direction of biology to find new sources of biologically active compounds.

In our study, we used ethanol extracts that are mostly used in traditional medicine along with water extracts [31]. The total content of polyphenol compounds for three investigated plants *C. intybus*, *L. purpureum,* and *V. album* was 33.91, 34.61 and 31.28 mg GAE/g respectively (Fig. 1). As reported Abbas et al. (2015), phenolic content in the *C. intybus* extracts was 85.0 mg GAE/g [1]. Study of Jancic et al. (2017) showed that TPC in extracts of wild and cultivated chicory were 1.05—3.73 and 0.65—0.82 mg GAE/g respectively [25]. Malik et al. (2017) demonstrated that TPC was 21.01 mg GAE/g for wild plants of chicory [32]. Innocenti et al. (2005) studied polyphenol complex of alcohol extracts of *C. intybus* and found that cichoric acid the most abundant compound among the total phenols [23].

In the study of Grujić et al. (2017) represented that the total content of polyphenols for ethanol extracts of *L. purpureum* was 89.23 mg GAE/g [20]. Our result was 2.5 times less comparing with this study. In the report of Kang (2016) indicated that the content of polyphenols was 60.46 mg CAE/g (catechin equivalents) [27]. Tahirović and Bašić (2017) determined the TPC for leaves and stems of

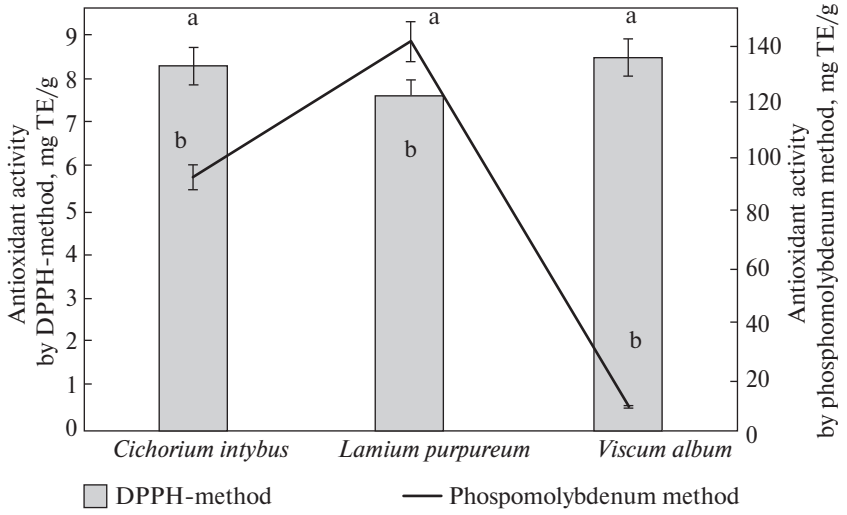

Fig. 2. Antioxidant activity of ethanol extracts of *Cichorium intybus* L., *Lamium purpureum* L. and *Viscum album* L. (means in columns followed by different letters are different at p = 0.05)

V. album that grew on *Tilia cordata* 10.34 and 11.32 mg GAE/g respectively [50]. In different extracts of *V. album,* TPC was from 10.92 to 37.66 mg GAE/g as reported Orhan et al. (2014) [38].

The total content of flavonoids (TFC) for *C. intybus*, *L. purpureum* and *V. album* was 26.29, 28.89 and 25.10 mg QE/g respectively. According to Abbas et al. (2015), flavonoid content of extracts of *C. intybus* was 6.82 mg/g of rutin equivalent [1]. Wild and cultivated chicory plants had TFC 2.29—4.42 and 1.57—2.01 μM of caffeic acid equivalent respectively [25]. Grujić et al. (2017) found that the concentration of flavonoids in ethanol extracts of *L. purpureum* was 32.8 mg QE/g [20]. As described by Kang (2016), the content of total flavonoids for *V. album* leaves extracts was 36.38 mg QE/g [27]. TFC of *V. album* extracts was from 1.76 to 9.11 mg QE/g, as reported by Orhan et al. (2014) [38].

The total content of phenolic acids (TPA) for *C. intybus*, *L. purpureum,* and *V. album* was 4.56, 4.87 and 4.07 mg CAE/g respectively. As described in Tahirović and Bašić (2017), TPA in stems and leaves was 1.31 and 1.59 mg CAE/g respectively [50].

Antioxidant activity of extracts in this study determined by two methods: DPPH-method and phosphomolybdenum (Fig. 2).

Antioxidant activity (AA) of extracts by DPPH method for *C. intybus*, *L. purpureum* and *V. album* was 8.35, 7.66 and 8.55 mg Trolox Equivalent/g. In some report, antioxidant activity by DPPH-method for *C. intybus* was 67.2 μg/ml [1].

AA by phosphomolybdenum method for *C. intybus*, *L. purpureum* and *V. album* was 93.01, 142.62 and 9.31 mg Trolox Equivalent per gram. Gruić et al. (2017) indicated that reducing power of different extracts of *L. purpureum* increased with increasing of concentration of extracts [20].

Conducted correlation analyze showed that found a strong correlation between antioxidant activity and polyphenol compounds of investigated plant species. Study of *C. intybus* extracts identified strong positive correlation between antioxidant activity by DPPH method and content of polyphenols (r = = 0.996), AA by DPPH method and flavonoids (r = 0.967) and AA by DPPH method and phenolic acids (r = 0.971); strong positive correlation between AA by PhMo and polyphenols (r = 0.971), AA by PhMo and flavonoids (r = 0.997), AA by PhMo and phenolic acids (r = 0.884). Study of *L. purpureum* demonstrated strong positive correlation between antioxidant activity by DPPH method and content of polyphenols (r = 0.977), AA by DPPH method and flavonoids (r = 0.976) and

AA by DPPH method and phenolic acids (r = = 0.929); strong positive correlation between AA by PhMo and polyphenols (r = 0.882), AA by PhMo and flavonoids (r = 0.881), AA by PhMo and phenolic acids (r = 0.792). Between accumulations investigated compounds and AA of *V. album* extracts found following relations: strong positive correlation between antioxidant activity by DPPH method and content of polyphenols (r = 0.927) and AA by DPPH method and flavonoids (r = 0.925); strong positive correlation between AA by PhMo and polyphenols (r = 0.997) and AA by PhMo and flavonoids (r = 0.998). Between AA and phenolic acids, accumulation found a negative correlation.

Conclusions

Thus, wild plants of *Cichorium intybus*, *Lamium purpureum* and *Viscum album* in M.M. Gryshko National Botanical Garden of the NAS of Ukraine accumulated polyphenol compounds with high antioxidant activity. Obtained data demonstrated that these plant species can be a potential source of natural antioxidants that can be used in the pharmacological investigations. It is necessary to a branch of biological science to investigate biochemical properties of not cultivated plants only but wild plants also to identify new sources of biologically active compounds.

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ВМІСТ ФЕНОЛЬНИХ СПОЛУК У РОСЛИННІЙ СИРОВИНІ *CICHORIUM INTYBUS* L., *LAMIUM PURPUREUM* L. ТА *VISCUM ALBUM* L.

Мета — оцінити антиоксидантний потенціал етанольних екстрактів відібраних в умовах Національного ботанічного саду імені М.М. Гришка НАН України дикорослих рослин шляхом визначення фенольних сполук.

Матеріал та методи. Використовували суху сировину *Cychorium intybus* L., *Lamium purpureum* L. та *Viscum album* L. Рослини *Cychorium intybus* і *Lamium purpureum* зібрано в Національному ботанічному саду імені М.М. Гришка НАН України, рослини *Viscum album* — з крони дерев *Tilia cordata* Mill. Протягом 2 год 0,2 г сухої рослинної сировини екстрагували з 20 мл 80 % етанолу. Загальний вміст поліфенолів в екстрактах вимірювали методом, описаним Singleton і Rossi (1965) з використанням реактиву Фоліна—Чокалтеу. Результати виражені у мг галової кислоти (еквівалент) на грам сухої речовини (мг ГКЕ/г). Визначення загального вмісту флавоноїдів проведено за модифікованим методом, описаним Shafii et al. (2017). Результати виражено у мг кверцетин-еквівалента на 1 г сухої речовини (КЕ/г). Визначення загального вмісту фенольних кислот в екстрактах проведено методом, описаним у Farmakopea Polska (1999). Результати виражено у мг кофейної кислоти (еквівалент) на 1 г сухої речовини (мг ККЕ/г). Антирадикальну активність зразків вимірювали з 2,2-дифеніл-1-пікрилгідразилом (ДФПГ) за методом Sanchez-Moreno et al. (1998). Антиоксидантну активність визначали фосфомолібденовим методом, описаним P. Prieto et al. (1999) з незначною модифікацією. Результати виражені у мг тролокс-еквівалента на 1 г сухої речовини (мг ТЕ/г). Експериментальні дані опрацьовано в Excel 2010. Кореляційний аналіз проведено з використанням критерію Пірсона.

Результати. Загальний вміст поліфенолів для рослин *C. intybus*, *L. purpureum* та *V. album* становив 33,91, 34,61 та 31,28 мг ГКЕ/г відповідно, флавоноїдів — 26,29, 28,89 та 25,10 мг КЕ/г, фенольних кислот — 4,56, 4,87 та 4,07 мг ККЕ/г відповідно, антиоксидантна активність екстрактів, визначена методом ДФПГ, — 8,35, 7,66 та 8,55 мг ТЕ/г, антиоксидантна активність, визначена фосфомолібденовим методом, — 93,01, 142,62 та 9,31 мг ТЕ/г. Установлено стійку прямо пропорційну кореляцію між накопиченням поліфенольних сполук і антиоксидантною активністю екстрактів.

Висновки. Дикорослі рослини *C. intybus*, *L. purpureum* та *V. album* у Національному ботанічному саду імені М.М. Гришка НАН України накопичували поліфенольні сполуки з високою антиоксидантною активністю. Отримані дані свідчать, що ці види можуть бути потенційним джерелом природних антиоксидантів, що можна використовувати у фармакологічних дослідженнях. Визначення біохімічних особливостей не лише культурних, а і дикорослих рослин є важливим напрямом сучасної біологічної науки для виявлення нових джерел біологічно активних речовин.

Ключові слова: *Cichorium intybus, Lamium purpureum, Viscum album*, поліфеноли, флавоноїди, фенольні кислоти, антиоксидантна активність.

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СОДЕРЖАНИЕ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ В РАСТИТЕЛЬНОМ СЫРЬЕ *CICHORIUM INTYBUS* L., *LAMIUM PURPUREUM* L. И *VISCUM ALBUM* L.

Цель — оценить антиоксидантный потенциал этанольных экстрактов отобранных в условиях Национального ботанического сада имени Н.Н. Гришко НАН Украины дикорастущих растений путем определения фенольных соединений.

Материалы и методы. Использовали сухое сырье *Cychorium intybus* L., *Lamium purpureum* L. и *Viscum album* L. Растения *Cychorium intybus* и *Lamium purpureum* собраны в Национальном ботаническом саду имени Н.Н. Гришко НАН України, растения *Viscum album* — с кроны деревьев *Tilia cordata* Mill. На протяжении 2 ч 0,2 г сухого растительного сырья экстрагировали в 20 мл 80 % этанола. Общее содержание полифенолов в экстрактах измеряли методом, описанным Singleton и Rossi (1965) с использованием реактива Фолина—Чокалтеу. Результаты выражены в мг галовой кислоты (эквивалент) на 1 г сухого вещества (мг ГКЭ/г). Определение общего содержания флавоноидов проводили модифицированным методом, описанным Shafii et al. (2017). Результаты выражены в мг кверцетин-эквивалента на 1 г сухого вещества (КЭ/г). Определение общего содержания фенольных кислот экстрактов проводили методом, описанным в Farmakopea Polska (1999). Результаты выражены в мг кофейной кислоты (эквивалент) на 1 г сухого вещества (мг ККЭ/г).

Антирадикальную активность образцов измеряли с 2,2-дифенил-1-пикрилгидразилом (ДФПГ) по методу Sanchez-Moreno et al. (1998). Антиоксидантную активность определяли фосфомолибденовым методом, описанным Prieto et al. (1999) с незначительной модификацией. Результаты выражены в мг тролоксэквивалента на 1 г сухого вещества (мг ТЭ/г). Экспериментальные данные обработаны в Excel 2010. Корреляционный анализ проводили с использованием критерия Пирсона.

Результаты. Общее содержание полифенолов для растений *C. intybus*, *L. purpureum* и *V. album* составило 33,91, 34,61 и 31,28 мг ГКЭ/г соответственно, флавоноидов — 26,29, 28,89 и 25,10 мг КЭ/г, фенольных кислот — 4,56, 4,87 и 4,07 мг ККЭ/г, антиоксидантная активность экстрактов, определенная методом ДФПГ, — 8,35, 7,66 и 8,55 мг ТЭ/г, антиоксидантная активность, определенная фосфомолибденовым методом, — 93,01, 142,62 и 9,31 мг ТЭ/г. Установлена стойкая прямо пропорциональная корреляция между накоплением полифенольных соединений и антиоксидантной активностью экстрактов.

Выводы. Дикорастущие растения *C. intybus*, *L. purpureum* и *V. album* в Национальном ботаническом саду имени Н.Н. Гришко НАН Украины накапливали полифенольные соединения с высокой антиоксидантной активностью. Полученные данные свидетельствуют, что данные виды растений могут быть потенциальным источником природных антиоксидантов, что можно использовать в фармакологических исследованиях. Определение биохимических особенностей не только культурных, но и дикорастущих растений является важным направлением современной биологической науки для для выявления новых источников биологически активных веществ.

Ключевые слова: *Cichorium intybus, Lamium purpureum, Viscum album,* полифенолы, флавоноиды, фенольные кислоты, антиоксидантная активность.