ISSN 1029 - 4244 (Print)
eISSN 2415-3303 (Online)

Львівський медичний часопис 2022. Т. 28. № 1-2
Acta Medica Leopoliensia 2022;28(1-2)

© 2022 by the author(s).
This work is licensed under **Creative Commons Attribution 4.0 International License** *https://creativecommons.org/licenses/by/4.0/*

DOI: https://doi.org/10.25040/aml2022.1-2.089
УДК: 615.274:582.991:582.943].07

МЕТОДИЧНИЙ ПІДХІД ДО ВИЗНАЧЕННЯ АНТИОКСИДАНТНОЇ АКТИВНОСТІ НАСТОЙОК ЕХІНАЦЕЇ ПУРПУРОВОЇ ТА МОНАРДИ ТРУБЧАСТОЇ ЯК КРИТЕРІЮ ЯКОСТІ ПІД ЧАС РОЗРОБКИ ЇХ СКЛАДУ Й ЛАБОРАТОРНОЇ ТЕХНОЛОГІЇ

Єзерська О.І.[1] ORCID: 0000-0001-8543-2723
Гудзь Н.І.[1] ORCID: 0000-0002-2240-0852
Кобилінська Л.І.[2] ORCID: 0000-0002-8965-8436
Філіпська А.М.[1] ORCID: 0000-0002-5759-1521
Туркіна В.А.[3] ORCID: 0000-0002-0660-8485
Бріндза Я.[4] ORCID: 0000-0001-8388-8233
Вєчорек П.П.[5] ORCID: 0000-0002-0016-0114
Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна
[1] Кафедра технології ліків та біофармації
[2] Кафедра біологічної хімії
[3] Науково-дослідний інститут епідеміології та гігієни
[4] Словацький сільськогосподарський університет в Нітрі, Нітра, Словацька Республіка
Інститут наук про рослини й навколишнє середовище,
[5] Опольський університет, Ополе, Польща
Кафедра аналітичної хімії

Ключові слова: *ехінацея пурпурова, монарда трубчаста, екстрагування,загальна антиоксидантна активність, рутин*
Для цитування: *Єзерська О.І., Гудзь Н.І., Кобилінська Л.І., Філіпська А.М., Туркіна В.А., Бріндза Я., Вєчорек П.П. Методичний підхід до визначення антиоксидантної активності настойок ехінацеї пурпурової та монарди трубчастої як критерію якості під час розробки їх складу й лабораторної технології. Львівський медичний часопис. 2022. Т. 28. № 1-2. С. 89-98. DOI: https://doi.org/10.25040/aml2022.1-2.089*
Для кореспонденції: *Єзерська Оксана Іванівна, вул. Пекарська, 69, м. Львів, 79000*
e-mail: o.yezerska@gmail.com
Стаття надійшла: 21.02.2022 ***Прийнята до друку:*** 27.05.2022

METHODICAL APPROACH TO THE DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF THE ECHINACEA PURPUREA AND MONARDA FISTULOSA TINCTURES AS A QUALITY CRITERION OF THE DEVELOPMENT OF THEIR COMPOSITION AND LABORATORY TECHNOLOGY

Oksana Yezerska[1] *ORCID: 0000-0001-8543-2723*
Nataliia Hudz[1] *ORCID: 0000-0002-2240-0852*
Lesya Kobylinska[2] *ORCID: 0000-0002-8965-8436*
Anna Filipska[1] *ORCID: 0000-0002-5759-1521*
Vira Turkina[3] *ORCID: 0000-0002-0660-8485*
Jan Brindza[4] *ORCID: 0000-0001-8388-8233*
Piotr Pawel Wieczorek[5] *ORCID: 0000-0002-0016-0114*
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
[1] Department of Drug Technology and Biopharmacy
[2] Department of Biological chemistry
[3] Research Institute of Epidemiology and Hygiene
[4] Slovak University of Agriculture in Nitra, Nitra, Slovak Republic
Institute of Plant and Environmental Sciences
[5] University of Opole, Opole, Poland
Department of Analytical Chemistry

Keywords: *Echinacea purpurea, Monarda fistulosa, extraction, antioxidant activity, rutin*
For citation: *Yezerska O, Hudz N, Kobylinska L, Filipska A, Turkina V, Brindza J, Wieczorek PP. Methodical approach*

to the determination of the antioxidant activity of the Echinacea purpurea and Monarda fistulosa tinctures as a quality criterion of the development of their composition and laboratory technology. Acta Medica Leopoliensia. 2022;28(1-2):89-98. DOI: https://doi.org/10.25040/aml2022.1-2.089
For correspondence: *Yezerska Oksana, Pekarska, 69, Lviv, Ukraine, 79000, e-mail: o.yezerska@gmail.com*
Received: February 21, 2022 ***Accepted:*** May 27, 2022

Реферат

Мета. *Метою дослідження було опрацювання методичного підходу до визначення антиоксидантної активності настойок із трави монарди трубчастої та квітів і коренів ехінацеї пурпурової, а також розроблення їх технології в лабораторних умовах.*

Матеріали і методи. *Використано методи аналізу, синтезу, систематизації та порівняння інформації наукових даних; визначення розміру частинок лікарської рослинної сировини; методи мацерації та ремацерації для виготовлення досліджуваних настойок; тест DPPH для оцінки загальної антиоксидантної активності розроблених настойок.*

Результати й обговорення. *Опрацьовано методичний підхід до визначення антиоксидантної активності настойок із трави монарди трубчастої та квітів і коренів ехінацеї пурпурової, суть якого полягала в підборі відповідного розведення настойок. Настойки виготовляли за допомогою мацерації та/або ремацерації в лабораторних умовах. Співвідношення подрібненої трави монарди трубчастої до настойки були близькими до співвідношень, які використовують у фармацевтичній промисловості, а саме 1 до 5 і 1 до 10. Коефіцієнти спиртопоглинання 70% етанолу для коренів ехінацеї пурпурової (розмір 2-5 мм), квітів ехінацеї пурпурової (розмір 1-3 мм), трави монарди трубчастої (розмір 0,5-3 мм) дорівнювали 1,2, 2,25 та 5,0 мл/г відповідно. Дослідження показали, що настойки ехінацеї пурпурової вміщують сполуки з антиоксидантними властивостями. Загальна антиоксидантна активність цих настойок становила у межах від 254,8 до 815,8 мг рутин-екв. в 1 л настойки або 1,12-4,43 мг рутин-екв. в 1 г сировини залежно від частини рослини, розміру часток і типу екстракції. Антиоксидантна активність настойок трави монарди трубчастої дорівнювала 2203,6 мг рутин-екв. в 1 л настойки для співвідношення 1 до 9,5 і 20,3 мг рутин-еквівалентів в 1 г сировини та 2119,4 мг рутин- екв. для настойки у співвідношенні 1 до 4,5 і 1 : 9,7 мг рутин-екв. в 1 г сировини.*

Висновки. *Опрацьовано підхід для визначення антиоксидантної активності розроблених настойок, а саме експериментально встановлено розведення настойок для аналітичної методики визначення антиоксидантної активності. Результати досліджень показали, що настойки ехінацеї пурпурової володіють антиоксидантною активністю. Настойки трави монарди трубчастої також багаті сполуками з антиоксидантними властивостями. Опрацьовано лабораторну технологію шести настойок. Подальші дослідження буде спрямовано на вивчення вищезгаданих настойок на мікроорганізмах і лабораторних тваринах.*

Abstract

Aim. *The aim of the study was to develop the methodical approach to determine the antioxidant activity of the tinctures of Monarda fistulosa herb and flowers and roots of Echinacea purpurea, as well as to develop their technology in laboratory conditions.*

Materials and Methods. *The following methods were used: analysis, synthesis, systematization, and comparison for processing of published scientific data on antioxidant activity; method for measuring the particle size of raw herbal materials; maceration and remaceration methods for obtaining the tested tinctures; DPPH test for the valuation of the antioxidant activity of the developed tinctures.*

Results and Discussion.
The methodical approach to determining the antioxidant activity of the tinctures of Monarda fistulosa herb and flowers and roots of Echinacea purpurea was elaborated, the essence of which consisted in the selection of the appropriate dilution of the tinctures. Tinctures were prepared with the help of maceration or/and remaceration in laboratory conditions. The ratios of herbal raw materials (HRM) to the final tincture were close to ratios that are widely employed in the pharmaceutical industry, namely 1 to 5 and 1 to 10. The coefficients of alcohol absorption for the roots of Echinacea purpurea (size 2-5 mm), flowers of Echinacea purpurea (size 1-3 mm), herb of Monarda fistulosa (size 0.5-3 mm) were measured. They were measured as 1.2, 2.25, and 5.0 ml/g, respectively, for 70% ethanol. The studies revealed that Echinacea purpurea tinctures are a valuable source of antioxidant compounds. The antioxidant activity of these tinctures was 254.8-815.8 mg rutin-equivalents in 1 L of the tinctures or 1.12-4.43 mg rutin-equivalents in 1 g of the HRM depending on the part of the plant, particle size and extraction type. The antioxidant activity of the tinctures of the Monarda fistulosa herb was equal to 2203.6 mg eq-rutin/L and 20.3 mg eq-rutin/g for the tincture and HRM at a ratio of 1 to 9.5 and 2119.4 mg eq-rutin/L and 9.7 mg eq-rutin/g for the tincture at a ratio of 1 to 4.5.

Conclusions. *The approach to the determination of the antioxidant activity of the tested tinctures was elaborated, namely the dilutions of the tinctures were established for the analytical procedure of the determination of the antioxidant activity. Our studies demonstrated that tinctures of Echinacea purpurea contained compounds with antioxidant activity. The tinctures of Monarda fistulosa herb are very rich in compounds with antioxidant properties. The laboratory technology of six tinctures was elaborated. Further studies will be directed at laboratory studies on microorganisms and animals*

Introduction

The adaptation of organisms to changes in the environment is regulated by the nervous, endocrine and cardiovascular systems. The mechanism of adaptogens' actions is to enhance the nonspecific resistance of the organism to the negative influence of biological, physical and chemical factors. Free radical oxidation at almost all stages of its course forms many products because of the interaction of free radicals both with each other and with biological macromolecules [10, 16]. Therefore, free radical oxidation, along with reactive oxygen species, produces other active free radicals (peroxides, epoxides, aldehydes, ketones, alcohols, dialdehydes, etc.), which can interact covalently with certain functional groups of proteins, leading to their destruction. Free radical oxidation can induce the modification of proteins, including enzymes, changes in their activity, destruction of antioxidants (vitamins, ubiquinone, steroid hormones, etc.), changes in phospholipid composition, the appearance in the hydrophobic part of oxidation products that initiate ionic transport processes, changes in conformation composition, and hence the structural and functional properties of the membranes, and in the DNA structure of damaged cells [16].

The antioxidant system is a powerful mechanism that prevents the development of free radicals and peroxide reactions in the organism [10]. It should be noted that until recently, even in scientific circles there is an opinion that antioxidants are found only in vegetables and fruits. However, recent studies demonstrate that almost all plants are rich in antioxidants [4]. This ubiquity of antioxidants in the plant world is due to the fact that these molecules perform protective functions, helping the plant themselves to fight pests and ultraviolet radiation [4]. Antioxidants eliminate some free radicals from body cells, prevent or reduce damage caused by oxidation [10]. This means that herbal preparations rich in antioxidants can decrease the risk of many diseases, including heart disease and some cancers. The activity of herbal preparations is related to their chemical composition and biologically active substances which include such specific substances as glycosides, flavonoids, polysaccharides, glycopeptides. Vitamin C (ascorbic acid), vitamin E (tocopherol), vitamin K in reduced hydroquinone form, provitamin A (beta-carotene) and its carotenoid precursors, ubiquinone (coenzyme Q10) in reduced form have a non-specific antioxidant effect [1, 12]. These and similar substances usually increase each other's actions, for example, vitamins C (water-soluble) and E (fat-soluble) complement each other, protecting cell membranes from lipid peroxidation [1].

A plethora of the pharmacological activities of medicinal plants in combination with high bioavailability and low toxicity can broaden the list of the diseases for which the administration of herbal medicines is indicated. The administration of herbal preparations is the most adequate preventive and complementary method of the prevention and treatment of many diseases under unfavorite ecological and epidemic conditions, especially under conditions of spreading the coronavirus disease COVID 19. Thus, studying herbal preparations with antioxidant, antimicrobial, adaptogenic and immunomodulatory activities is considered a topical issue of modern medicine and pharmacy.

Monarda fistulosa is a promising essential oil-bearing plant of the *Lamiaceae* family, which contains biologically active substances of different classes. Among these compounds are flavonoids (luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, luteolin, apigenin), phenolic acids (chlorogenic acid, caffeic acid, rosmarinic acid), carotenoids, vitamins B_1, B_2, ascorbic acid, etc. [13]. Thus, the herb of *Monarda fistulosa* could be regarded as a raw material for the preparation of herbal medicinal products with antioxidant properties.

Echinacea is a genus of the *Compositae* family [14]. The most common valuable species are *Echinacea purpurea* (L.) Moench., *Echinacea pallida* Nutt. and *Echinacea angustifolia* DC [5]. *Echinacea purpurea* grows in the eastern part of North America. However, some species of the genus *Echinacea* are

cultivated in many countries and is mainly used to treat infectious diseases of the upper and lower respiratory systems, as well as to treat toothache, intestinal pain, skin disorders, seizures, chronic arthritis and cancer, etc. All the parts of this plant are used in traditional medicine. *Echinacea purpurea* contains derivatives of caffeic acid (esters of caffeic acid with sugars), conjugates of caffeic acid with quinic and tartaric acids, as well as glycosides of apigenin, luteolin, kaempferol, quercetin, isorhamnetin, etc. Among such conjugates is chicory acid as the main phenolic compound of *Echinacea* [6]. *Echinacea angustifolia* and *Echinacea pallida* showed more anti-inflammatory potential as the mice demonstrated significantly higher production of such anti-inflammatory cytokines as IL-4 and IL-10 after the administration of the *Echinacea* tinctures [18]. *Echinacea purpurea* roots contain a potent water-soluble antiviral compound against the two membrane-containing viruses (herpes simplex type 1 and influenza virus) [6]. The tinctures of the aerial parts of *Echinacea purpurea* on the base of 70% ethanol demonstrated the antioxidant and antimicrobial activity towards *Candida albicans* and *Saccharomyces cerevisiae* [14]. Thus, tinctures of *Echinacea purpurea* could be used for the treatment of infectious diseases of the oral cavity. The primary aim of this publication was to elaborate an approach in order to evaluate the antioxidant activity of the tinctures prepared from the herb of *Monarda fistulosa* and flowers and roots *Echinacea purpurea*, grown in the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute of the National Academy of Agrarian Sciences. The secondary aim was to elaborate the analytical procedure of measuring the antioxidant activity of the prepared tinctures. Finally, the aim of this paper was to elaborate the laboratory technology of the tinctures of the herb of *Monarda fistulosa* and flowers and roots *Echinacea purpurea*.

Materials and Methods

The following methods were used: analysis, synthesis, systematization, and comparison for processing of published scientific data on antioxidant activity; method for determining the particle size of raw herbal materials; maceration and remaceration methods for obtaining the tested tinctures; DPPH test for the valuation of the antioxidant activity of the developed tinctures [9].

Plant materials

The flowers of *Echinacea purpurea* were collected in the summer of 2020 and roots were collected in May of 2021 (Sector of Mobilization and Conservation of Plant Resources of the Rice Institute). The herb of *Monarda fistulosa* was collected in the summer of 2020 (Sector of Mobilization and Conservation of Plant Resources of the Rice Institute). The specimens are stored in the herbarium of the Sector of Mobilization and Conservation of Plant Resources of the Rice Institute. The voucher specimens are identified as EPr-1, EPf-1, MFh-1, respectively. In general, the ratio of the herbal substance to a final product was used as approximately 1 to 5 and 1 to 10 at an appropriate particle size (70% ethanol). The residue can be removed by filtration through paper filters. The characteristics of the herbal raw materials (HRM) and their tinctures are provided in Table 1.

Determination of antioxidant activity (DPPH radical-scavenging activity)

The antioxidant activity was evaluated by the DPPH test. The DPPH test is based on the reaction with electron donors or hydrogen radicals (H•) [2, 8, 9]. The antioxidant activity of the tested tinctures was measured according to own elaborated analytical procedure of the DPPH test. Briefly, 0.003% solution of DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich, Germany) was prepared, using 96% ethanol. This solution of DPPH was used on the day of its preparation after the previous measurement of its absorbance at a wavelength of 515 nm. The absorbance of the DPPH solution should range from 0.760 to 0.840, namely 0.800±5%. 0.05 ml of the tested tinctures or their different dilutions was added to 1.95 ml of the DPPH solution in 2 ml tubes. The blank consisted of the same volume of the tincture or its dilution and 1.95 ml of 96%

Table 1

Characteristics of the HTM and their tinctures

N	Name of HRM	Time of the collection	Features of the tinctures (a ratio of HRM to tincture), particle size, identification	Type of the extraction
1	*Monarda fistulosa* herb	The herb was collected in the summer of 2020	3.80 g to 36.5 ml, particle size 0.5-3 mm (1/21)	Remaceration
2			3.83 g to 17.5 ml, particle size 0.5-3 mm (2/21)	Remaceration
3	*Echinacea purpurea* roots	The roots were collected in April of 2021	3.50 g to 19 ml, particle size 2-5 mm (1/21)	Remaceration
4			3.49 g to 14.4 ml, particle size 2-5 mm (2/21)	Maceration
5	*Echinacea purpurea* flowers	The flowers were collected in the summer of 2020	5.06 g to 28.0 ml, particle size 1-3 mm (3/21)	Maceration
6			5.0 g to 26.0 ml, particle size 1-3 mm (4/21)	Remaceration

ethanol while 96% ethanol was as the blank for 0.003% solution of DPPH. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 40 min. The absorbance of the resulting mixtures was read at a wavelength of 515 nm in 40 min, using the spectrophotometers: Genesys 20 (USA). The DPPH radical-scavenging activity was computed according to the following equation:

$$\text{DPPH radical-scavenging activity (\%)} = (A_{control} - A_{sample} \times 100\%)/A_{control}$$

where $A_{control}$ is the absorbance of the solution of DPPH against 96% ethanol, A_{sample} is the absorbance of the reaction mixtures of the tinctures or their dilution with DPPH at a wavelength of 515 nm against the same volume of the tincture or its dilution and 1.95 ml of 50% ethanol. The reaction mixture for measuring $A_{control}$ consisted of 1.95 ml of 0.003% solution of DPPH and 0.05 ml of 96% ethanol [9].

Results and Discussion

The tinctures were prepared using maceration or/and remaceration in laboratory conditions. The ratios of HRM to the final tincture were close to ratios that are widely used in the pharmaceutical industry, namely 1 to 5 and 1 to 10 [3, 15]. The features of the preparation of the tested tinctures are provided in Table 2.

Ethanol absorption coefficients are important technological parameters in the manufacture of herbal preparations. The coefficients of alcohol absorption for the roots of *Echinacea purpurea* (size from 2 to 5 mm), flowers of *Echinacea purpurea* (size from 1 to 3 mm), herb of *Monarda fistulosa* (size from 0.5 to 3 mm) were determined. They were equal to 1.2, 2.25, and 5.0 ml/g, respectively, for 70% ethanol (Table 3). These studies conform with our previous studies performed with the herb of *Monarda fistulosa* of 2019 year of the collection. The ethanol absorption coefficient was equal to 4.8 ml/g if the particle size was 0.5 to 5 mm [17]. Simultaneously, the laboratory technology of six tinctures as a variety of liquid extracts by the method of simple maceration and remaceration was elaborated: the tincture of roots of *Echinacea purpurea*, tincture of *Echinacea purpurea*

Table 2

Features of the preparation of the tested tinctures

Name of HRM, identification of the tincture	Extraction:added volume/obtained volume (ml), time of the extraction				Total volume, ml	Final ratio of HRM to the tincture (g to ml)
	1	2	3	4		
Monarda fistulosa herb (1/21)	38.0/20.0, 24 hours	7.5/5.0, 4 hours	7.0/5.5, 4 hours	8.0/6.5, 4 hours	36.5	1 to 9.5
Monarda fistulosa herb (2/21)	21.0/1.0, 24 hours	9.0/9.0, 4 hours	5.0/5.0, 4 hours	6.0/4.5, 4 hours	17.5	1 to 4.5
Echinacea purpurea roots (1/21)	11.0/7.0, 24 hours	7.0/7.0, 5 hours	5.0/5.0, 5 hours	–	19.0	1 to 5.5
Echinacea purpurea roots (2/21)	20.0/14.4 7.5 days	–	–	–	14.4	1 to 4.1
Echinacea purpurea flowers (3/21)	40.0/28.0, 7 days	–	–	–	28.0	1 to 5.6
Echinacea purpurea flowers (4/21)	31.0/19.5 24 hours	5.0/5.5 4 hours	1.0/1.5 4 hours	–	26.0	1 to 5.2

Table 3

Calculations of the alcohol absorption coefficients of the herbal raw materials for 70% ethanol

Name	Mass of the herbal raw materials, g	Particle size, mm	Volume of the taken 70% eehanol for filling, ml	Volume of the tinctures after the ethanol absorption, ml	Calculations	Mean values of the alcohol absorption coefficients
Echinacea purpurea flowers	5.06	1-3	40	29	(40-29)/5.06=2.2	2.25
	5.00		31	19.5	(40-19.5)/5.0=2.3	
Echinacea purpurea roots	3.49	2-5	20	16	(20-16)/3.49=1.2	1.20
	3.50		11	7	(11-7)/3.50=1.2	
	3.57		20	7	(20-7)/3.57=3.64	
Monarda fistulosa herb	3.80	0.5-3	38	20	(38-20)/3.80=4.74	4.98
	3.83		21	1	(21-1)/3.83=5.22	

flowers, the tincture of the herb of *Monarda fistulosa*. Sometimes the total volume of the tincture was not equal to the sum of the separated volumes obtained in the process of remaceration which can be explained by deviations of measurements and losses of the solvent during the measurements in laboratory conditions.

The antioxidant activity of the tested tinctures was evaluated by the DPPH test [8,9]. Antioxidant activity could be considered as a criterion of quality of herbal preparations [7-9,13]. The analytical technique of the antioxidant activity measurement of the tincture of the herb of *Monarda fistulosa*, the tincture of the roots of *Echinacea purpurea* and tincture of the *Echinacea purpurea* flowers was elaborated by the DPPH test, namely a dilution of the tincture was selected and rutin was chosen for constructing the calibration curve. The results were expressed as mg eq-rutin/L of an tincture and mg eq-rutin/g of the HRM. The calibration curve was constructed in the concentration range of 95 to 305 mg/L of rutin. The equation was y=0.228x+7.0992, R^2=0.9945. Rutin was chosen as a commercially available marker [9].

The studies showed that *Echinacea purpurea* tinctures are a valuable source of antioxidant compounds. The antioxidant activity of these tinctures was 254.8-815.8 mg rutin-equivalents in 1 L of the tinctures or 1.12-4.43 mg rutin-equivalents in 1 g of the HRM depending on the part of the plant, particle size and extraction type. Moreover, it was revealed that the type of extraction (maceration and maceration) did not have an influence on the extraction of phytoconstituents with antioxidant activity from flowers at the particle size in the range of 1 to 3 mm (1.41 and 1.38 mg of rutin-equivalents in 1 g of the flowers, respectively). However, the mode of extraction significantly influenced the extraction of compounds with antioxidant activity from the roots at the particle size in the range of 2 to 5 mm. The remaceration and maceration for the roots gave the following antioxidant activity: 4.43 and 1.12 mg of rutin-equivalents in 1 g of the roots. If we compared the roots and flowers, the remaceration resulted in the following antioxidant activity: 4.43 and 1.41 mg of rutin-equivalents in 1 g of the roots and flowers, respectively. Such a difference can be interpreted by the specific histological structure of roots and flowers. The roots of *Echinacea purpurea* are softer compared to flowers that can assist the extraction of biologically active substances from roots.

As can be seen from Fig. 1 and 2, and Table 4, the DPPH scavenging ability increased proportionally with reducing in the dilution of tincture 1/21, namely the higher dilution of the tincture, the lesser was its scavenging ability. Moreover, the coefficient of correlation was higher in the case of four points (undiluted tincture, dilutions of 1 to 10, 1 to 5 and 1 to 2.5). Our studies are in line with the studies performed by Lee et al. These authors determined that the Echinacea purpurea tincture had close values (85.1 and 91.4%) of scavenging abilities at its concentrations of 0.5 and 1.0 mg/ml, respectively. Ascorbic acid showed similar results. The half-effective dose (ED_{50}) of ascorbic acid was approximately at 0.01 mg/ml. The DPPH scavenging ability of ascorbic acid at a

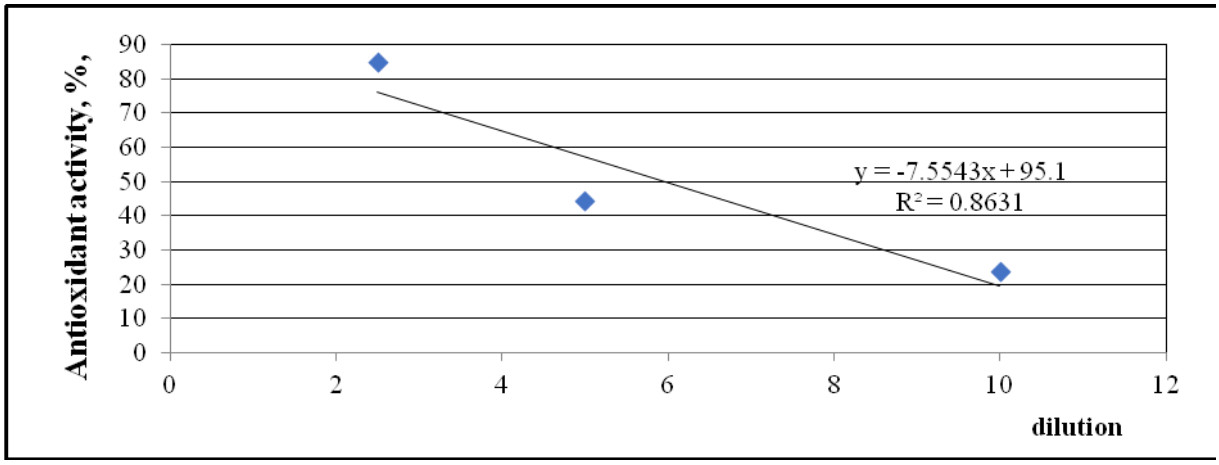


Fig. 1

Elaboration of the DPPH free radical scavenging activity assay: choosing a volume of tincture 1 of the roots of Echinacea purpurea at three dilutions

concentration of 0.016 mg/ml was 87.6%, a further increase in the concentration from 0.08 mg/ml to 1 mg/ml did yield the same value of the antioxidant activity which were close to 100% [11]. In our studies, the undiluted and dilute tincture in 2.5 times demonstrated very close values as well.

For computing of rutin equivalents, we employed the value of antioxidant activity which was the closest to the value of ED_{50}, namely 44.3% for tincture 1/21 and 38.1% for tincture 2/21, namely dilutions 1 to 5 and 1 to 2, respectively. For tinctures 3/21 and 4/21, we performed studies with one dilution (1 to 2).

Our studies conform with data of Stanisavljevic et al. who measured the antioxidant activity depending on the concentration of the dried tincture [14]. It was revealed that an increase in the concentration of this tincture of more than 0.2 mg/ml (0.5 and 1.0 mg/ml) did not increase the antioxidant activity [14].

Therefore, our technological and analytical studies showed that the morphological part of *Echinacea purpurea* (roots or flowers) and extraction type (maceration or remaceration) have a great influence on the extraction of biologically active substances with antioxidant activities that is very important for the development of tinctures used in the pharmaceutical, food and cosmetic industries. The next studies will be related to measuring the total flavonoid content in the tinctures.

The antioxidant activity of the tinctures of the *Monarda fistulosa* herb was equal to

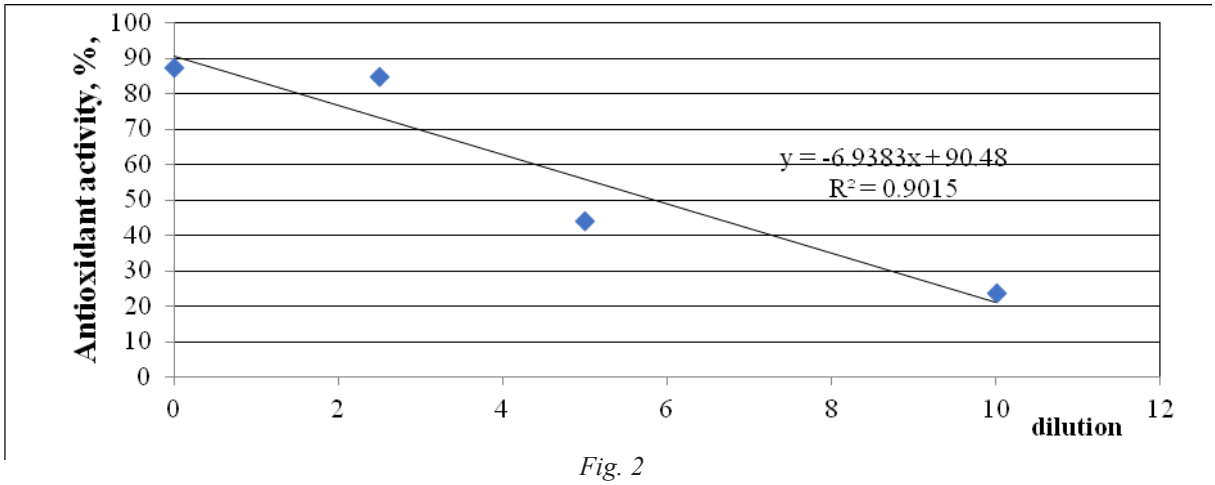


Fig. 2

Elaboration of the DPPH free radical scavenging activity assay: choosing a volume of tincture 1 of the roots of Echinacea purpurea at four dilutions

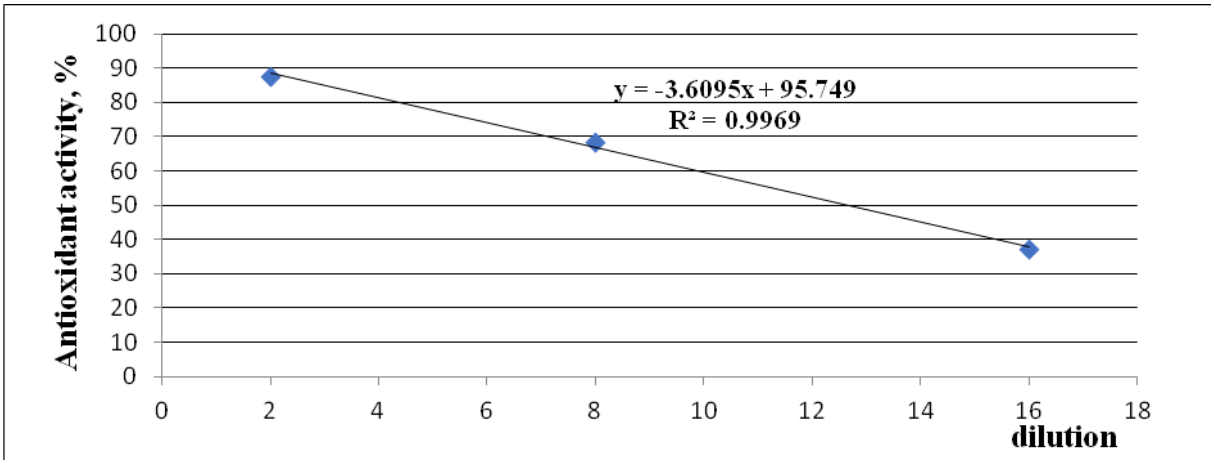


Fig. 3
Elaboration of the DPPH free radical scavenging activity assay: choosing a volume of tincture 2 of Monarda fistulosa

2203.6 mg eq-rutin/L and 20.3 mg eq-rutin/g for tincture 1 and 2119.4 mg eq-rutin/L and 9.7 mg eq-rutin/g for tincture 2 and HRM, respectively. Therefore, the ratio of the raw material to solvent has no significant influence on the extent of extraction [7]. These results revealed that a ratio of the herbal substance to a final product 1 to 4.5 did not give an adequate depletion of the HRM. Thus, at a ratio of 1 to 4.5, the same degree of extraction occurred like at a ratio of 1 to 9.5. For computing of rutin equivalents, we employed the value of antioxidant activity which was the closest to the value of ED_{50}, namely 37.3% for tincture 2/21. For tinctures 1/21, we performed the study with one dilution (1 to 16).

It was revealed that the ability to capture free radicals of DPPH from the tested six tinctures decreased with increasing dilutions of the tinctures or in other words with decreasing concentrations of antioxidants. The reduction in the DPPH was directly proportional to the concentration of antioxidants present in the reaction mixture.

It is impossible to compare our results of DPPH inhibition with the data of the other researchers of *Monarda fistulosa* as we did not find the data of the studies of the tinctures of this species. However, we revealed other results of the studies related to *Monarda fistulosa*. The DPPH radical scavenging assay of the dry extract obtained from the hydrodistilled residue by-product of *Monarda fistulosa* L. herb had a strong antiradical activity with an ED_{50} value of 0.285 mg/ml. Shanaida et al. used a similar analytical procedure of the DPPH test: 0.1 ml of each test sample in the different concentrations (0.1-1.0 mg/ml) was mixed with 1.9 ml of DPPH solution in methanol (25 μg/ml), and this mixture was incubated for 30 min in darkness at room temperature. The absorbances were measured at a wavelength of 517 nm [13].

The results of the antioxidant activity of six tinctures are provided in Table 4.

As can be seen from table 4, the tinctures of the *Monarda fistulosa* herb are very rich in compounds with antioxidant activity. For further studies we have chosen a ratio of 1 to 10 for the *Monarda fistulosa* herb and a ratio of 1 to 5 for the roots and flowers of *Echinacea purpurea*.

Conclusions

The approach to measuring the antioxidant activity of tested tinctures was elaborated. The essence of this approach is to employ the appropriate dilution of a tincture that gives the value of antioxidant activity which is the closest to the value of ED_{50}. It was established in the experimental studies that the tinctures of *Monarda fistulosa* herb should be diluted in 16 times and the tinctures of *Echinacea purpurea* should be diluted in 2-5 times depending on the plant part and mode of the extraction. These studies also offer that the tinctures of *Echinacea purpurea* and *Monarda fistulosa* herb with using 70% ethanol are herbal preparations with

Table 4

The antioxidant activity of the tested tinctures

Tincture	Antioxidant activity			
	undiluted tincture, %	diluted tincture, %	mg eq-rutin/L	mg eq-rutin/g
Echinacea purpurea roots (1/21)	87.40%	84.9% (1 to 2.5) 44.3% (1 to 5) 23.9% (1 to 10)	815.8	4.43
Echinacea purpurea roots (2/21)	–	18.5% (1 to 5) 38.1% (1 to 2)	271.9	1.12
Echinacea purpurea flowers, maceration (3/21)	–	36.2% (1 to 2)	254.8	1.41
Echinacea purpurea flowers, remaceration (4/21)	–	37.4% (1 to 2)	266.1	1.38
Monarda fistilosa, herb, remaceration (1/21)	–	38.5% (1 to 16)	2203.6	20.3
Monarda fistilosa, herb, remaceration (2/21)	–	37.3% (1 to 16) 68.5% (1 to 8) 87.6% (1 to 2)	2119.4	9.68

antioxidant activity. The laboratory technology of six tinctures was elaborated. Further studies will be directed at laboratory studies on microorganisms and animals. The proposed approach can be used in scientific studies related to the determination of the antioxidant activity of other herbal preparations.

Acknowledgment

Co-author Nataliia Hudz also thanks SAIA for the scholarship for the research related to the development of herbal products on the base of adaptogens and performed at the Slovak University of Agriculture in Nitra (Slovakia), 2021.The coathours are thankful to Liudmyla Svydenko for providing the herbs from Resources of the Rice Institute of the NAAS, Plodove, Kherson region, 74992 Kherson, Ukraine.

References

1. Aarland RC, Banuelos-Hernandez AE, Fragoso-Serrano M, Sierra-Palacios ED, Diaz de Leon-Sanchez F, Perez-Flores LJ, Rivera-Cabrera F, Mendoza-Espinoza JA. Studies on phytochemical, antioxidant, anti-inflammatory, hypoglycaemic and antiproliferative activities of Echinacea purpurea and Echinacea angustifolia tinctures. Pharm Biol. 2017; 55(1): 649-656. https://doi.org/10.1080/13880209.2016.1265989.
2. Banica F, Bungau S, Tit DM, Behl T, Otrisal P, Nechifor AC, Gitea D, Pavel F-M, Nemeth S. Determination of the total polyphenols content and antioxidant activity of Echinacea purpurea tinctures using newly manufactured glassy carbon electrodes modified with carbon nanotubes. Processes. 2020; 8(7): 833. https://doi.org/10.3390/pr8070833.
3. Cechinel-Filho V. Plant bioactives and drug discovery: principles, practice, and perspectives. John Wiley & Sons, Inc.: Hoboken, NJ, USA. 2012; 586 p. https://doi.org/10.1002/9781118260005
4. Dobrange E, Peshev D, Loedolff B, Van den Ende W. Fructans as immunomodulatory and antiviral agents: the case of Echinacea. Biomolecules. 2019; 9(10): 615. https://doi.org/10.3390/biom9100615.
5. Hassan RA, Safaa MA, Azza HM, Sahar EH. Antioxidant and Antimicrobial Activities of Echinacea purpurea L. Extracts. J Agricul Chem Biotechnol. 2020; 11(10): 279-283. https://doi.org/10.21608/jacb.2020.123553.
6. Hudson J, Vimalanathan S, Kang L, Amiguet VT, Livesey J, Arnason JT. Characterization of Antiviral Activities in Echinacea. Pharm Biol. 2005; 43(9): 790-796. https://doi.org/10.1080/13880200500408491.
7. Hudz N, Horcinova-Sedlackova V, Simkova J, Svydenko L, Brindza J, Wieczorek PP. Evaluation of the Monarda fistulosa Tinctures. The Joint International Pharmacy Symposium "Contemporary Pharmacy: Issues Challenges and Expectations 2021" and "11th Conference: Pharmacy Science and Practice": abstract book of the international conference, Kaunas, 22

October 2021. 2021: 28.
8. Hudz N, Ivanova R, Brindza J, Grygorieva O, Schubertova Z, Ivanisova E. Approaches to the determination of antioxidant activity of tinctures from bee bread and safflower leaves and flowers. Potr S J F S. 2017; 11(1): 480-488.
9. Hudz N, Sedlackova VH, Yezerska O, Zhurba M, Brindza J. Methodological approach to the elaboration of the analytical procedure of the antioxidant activity determination of the Schisandra chinensis (Turcz) Baill. tinctures. Agrobiodivers Improv Nutr Health Life Qual. 2021; 5(2): 250-257. https://doi.org/10.15414/ainhlq.2021.0024
10. Kobylinska LI, Tymochko MF. The role of prooxidant-antioxidant balance in the adaptational reactions of organism. Exp and Clin Physiol and Biochem. 2000; 4: 52-58. Ukrainian (Кобилінська ЛІ, Тимочко МФ. Роль прооксидантно-антиоксидантного балансу в адаптаційних процесах організму. Експ. та клін. фізіологія і біохімія. 2000; 4: 52-58).
11. Lee TT, Chen CL, Shieh ZH, Lin JC, Yu B. Study on antioxidant activity of Echinacea purpurea L. tinctures and its impact on cell viability. Afr J Biotechnol. 2009; 8(19): 5097-5105.
12. Oniszczuk T, Oniszczuk A, Gondek E, Guz L, Puk K, Kocira A, Kusz A, Kasprzak K, Wojtowicz A. Active polyphenolic compounds, nutrient contents and antioxidant capacity of extruded fish feed containing purple coneflower (Echinacea purpurea (L.) Moench.). Saudi J Biol Sci. 2019; 26(1): 24-30. https://doi.org/10.1016/j.sjbs.2016.11.013.
13. Shanaida M, Hudz N, Jasicka-Misiak I, Wieczorek P.P. Polyphenols and pharmacological screening of a Monarda fistulosa L. dry tincture based on a hydrodistilled residue by-product. Front Pharmacol. 2021; 12: 563436. https://doi.org/10.3389/fphar.2021.563436.
14. Stanisavljevic I, Stojicevic S, Velickovic D, Veljkovic V, Lazic M. Antioxidant and Antimicrobial Activities of Echinacea (Echinacea purpurea L.) Tinctures Obtained by Classical and Ultrasound extraction. Chin J Chem Eng. 2009; 17(3): 478-483. https://doi.org/10.1016/S1004-9541(08)60234-7.
15. State Pharmacopoeia of Ukraine 2nd ed. / Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. Kharkiv: Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. 2015; Vol. 1: 1028 p. Ukrainian (Державна Фармакопея України: в 3 т. / Державне підприємство "Український науково-експертний фармакопейний центр якості лікарських засобів". 2-е вид. Харків: ДП "Український науково-експертний фармакопейний центр якості лікарських засобів". 2015. Т. 1. 1128 с.)
16. Tymochko MPh, Kobylinska LI. Free radical reactions and their metabolic role. Medical Chemistry. 1999; 1(1): 19-25. Ukrainian (Тимочко МФ, Кобилінська ЛІ. Вільнорадикальні реакції та їх метаболічна роль. Мед. хімія. 1999;1(1): 19-25).
17. Yezerska O, Darmograj R, Hudz N, Levon V. Prospects of the application of some species of the Lamiaceae family and some features of the development of their tinctures: prospects of the application of some species of the Lamiaceae family. Agrobiodivers Improv Nutr Health Life Qual. 2021; 5(1): 72-79. https://doi.org/10.15414/ainhlq.2021.0008.
18. Zhai Z, Liu Y, Wu L, Senchina DS, Wurtele ES, Murphy PA, Kohut ML, Cunnick JE. Enhancement of innate and adaptive immune functions by multiple Echinacea species. J Med Food. 2007; 10(3): 423-434. https://doi.org/10.1089/jmf.2006.257