A. SAVYCH (<http://orcid.org/0000-0001-6053-7625>),L. KRYSKIW (<https://orcid.org/0000-0001-5129-8167>),L. MOSULA (<https://orcid.org/0000-0003-3339-0562>)*Horbachevsky Ternopil National Medical University***DETERMINATION OF THE TOTAL CONTENT OF FLAVONOIDS IN HERBAL MIXTURES WITH ANTI-DIABETIC ACTIVITY****Key words:** herbal mixtures, flavonoids, UV-spectrophotometry, diabetes mellitus, phytotherapy, antioxidant activity, rutinA. О. САВИЧ (<http://orcid.org/0000-0001-6053-7625>), канд. фарм. наук, доцент,Л. С. КРИСЬКІВ (<https://orcid.org/0000-0001-5129-8167>), канд. фарм. наук,Л. М. МОСУЛА (<https://orcid.org/0000-0003-3339-0562>), канд. фарм. наук, доцент*Тернопільський національний медичний університет імені І. Я. Горбачевського МОЗ України***ВИЗНАЧЕННЯ ЗАГАЛЬНОГО ВМІСТУ ФЛАВОНОЇДІВ У РОСЛИННИХ ЗБОРАХ ІЗ ПРОТИДІАБЕТИЧНОЮ АКТИВНІСТЮ****Ключові слова:** рослинні збори, флавоноїди, УФ-спектрофотометрія, цукровий діабет, фітотерапія, антиоксидантна активність, рутин

Diabetes mellitus is characterized by a rapid increase in the rate of spread and severe complications e.g. angiopathy, which significantly impairs the prognosis and life quality of patients, so it belongs to the global health problems. [1, 2]. As of 2019, official International Diabetes Federation data report an increase in the number of patients to 642 million by 2040 [3]. An important problem of pharmacovigilance is that existing pharmacotherapy is able to reduce hyperglycemia, but it is not always possible to stabilize fluctuations of glycemia values during the day and keep it at optimal level [4, 5]. This leads to the formation of a cascade of pathological processes – excessive glycation and inactivation of the body's antioxidant defense system, triggering free radical oxidation of lipids and, consequently, the development of oxidative stress, which leads to the development and progression of diabetic complications [6, 7]. In turn, oxidative stress, which is the result of the accumulation of reactive oxygen species (ROS: hydroperoxyl, superoxide, hydrogen peroxide and hydroxyl radicals), nitrogen molecules (RNS: peroxynitrite of active nitrogen) and some derivatives of heavy metals (iron and copper) is the main event for the development of insulin resistance [8, 9]. This may reduce peripheral insulin sensitivity through major molecular mechanisms such as β -cell dysfunction, inflammatory responses, decreased regulation and / or localization of glucose transporter 4 (GLUT-4), mitochondrial dysfunction, and abnormal insulin signaling pathways [10, 11]. Hereat, one of the main priorities of the medical field is pharmacotherapy improvement and development of antidiabetic drugs with antioxidant properties for the treatment and prevention of this pathology [12].

Phytotherapy is among such promising areas, as compared with the use of traditional oral drugs, it has a number of significant advantages, including a safe pharmacological profile, mild therapeutic effect, low risk of side effects with long-term use, the possibility of combining with traditional therapies, due to the presence of complex of biologically active compounds it has a multifaceted effect. [13, 14]. Since herbal mixtures are likely to contain a wide range of biologically active compounds, it is possible to cover more links in the pathogenetic mechanism of diabetes and prevent its complications [15, 16], so the combination of medicinal plants is worth the attention of researchers [17, 18]. Plant biocompounds have a wide range of pharmacological action and a variety of mechanisms of influencing on the development of diabetes (pathogenesis of which

involves the development of insulin resistance, relative insulin deficiency, which leads to the decrease of secretory activity of β -cells of the pancreatic gland) and diabetic angiopathies (inactivation of antioxidant protection system, activation of lipid peroxidation and development of oxidative stress)

The pathogenesis of diabetes includes insulin resistance and, due to relative insulin deficiency, a decrease in the secretory function of pancreatic β -cells and a decrease in the protective activity of the antioxidant system, promotion of lipid peroxidation and oxidative stress, while plant biologically active compounds have different mechanisms of pharmacological correction of these metabolic abnormalities [19, 20].

In this regard, the important biologically active substances are hydroxylated polyphenolic compounds, namely flavonoids. The chemical structure of flavonoids is based on fifteen-carbon skeleton consisting of two benzene rings linked via a heterocyclic pyran ring [21]. The pharmacological activities of this biocompounds depend on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization [22]. In particular, functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [23, 24].

Antioxidant activity of flavonoids in the treatment and prevention of diabetes and its complications is important because they can include suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation [25, 26]; scavenging ROS; inhibition the enzymes involved in ROS generation – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, nicotinamide adenine dinucleotide phosphate (NADP) oxidase, and so forth [27, 28].

Therefore, the aim of study was to determine the total flavonoid content in some herbal mixtures with previously studied antidiabetic activity *in vivo* [29].

Materials and methods

Plant materials: For the study took plant raw materials collected in June-August 2020 in the Ternopil and Carpathian region, Ukraine (*Vaccinium myrtillus* leaf). The harvested raw materials were dried, homogenized and stored according to GACP guidelines [30]. Identification of plant raw materials was carried out at the Department of Pharmacognosy with Medical Botany of I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. Samples of harvested raw materials were transferred to the Departmental herbarium for further processing

Five different herbal mixtures with proven in preliminary *in vivo* antidiabetic activity tests [29] were chosen for investigation. The compositions of this mixtures were as shown in the table.

Chemicals and standards: Chemical reference substance (CRS) of rutin was of primary reference standard grade ($\geq 95\%$ purity HPLC) and was purchased from Sigma-Aldrich Chemical Company (Germany). Absolute ethanol, aluminium chloride and acetic acid was purchased from Sfera Sim LTD (Ukraine). Water used in the studies was produced by MilliQ Gradient water deionization system (USA).

Instrumentation and conditions: spectrophotometric measurements were performed on spectrophotometer UV-1800 Shimadzu (Japan) equipped with 1 cm matched quartz cells. Determination of the total flavonoid content was performed at a maximum absorption of 415 nm after complexation with aluminum chloride (AlCl_3). The measurements were performed 40 min after the addition of AlCl_3 [31].

Compositions of the herbal mixtures

Herbal mixtures	Herbal drug component	Portion in the mixture, %	Relative ratio
No 3	<i>Urtica dioica</i> leaf	26.32	5
	<i>Cichorium intybus</i> roots	26.32	5
	<i>Rosa majalis</i> fruits	21.05	4
	<i>Elymus repens</i> rhizome	15.79	3
	<i>Taraxacum officinale</i> roots	10.52	2
No 4	<i>Arctium lappa</i> roots	26.32	5
	<i>Elymus repens</i> rhizome	26.32	5
	<i>Zea mays</i> columns with stigmas	21.05	4
	<i>Helichrysum arenarium</i> flowers	15.79	3
	<i>Rosa majalis</i> fruits	10.52	2
No 7	<i>Inula helenium</i> rhizome with roots	10.0	1
	<i>Helichrysi arenarium</i> flowers	20.0	2
	<i>Zea mays</i> columns with stigmas	20.0	2
	<i>Origanum vulgari</i> herb	20.0	2
	<i>Rosa majalis</i> fruits	20.0	2
	<i>Taraxacum officinale</i> roots	10.0	1
No 13	<i>Cichorium intybus</i> roots	26.32	5
	<i>Elymus repens</i> rhizome	26.32	5
	<i>Helichrysum arenarium</i> flowers	21.05	4
	<i>Rosa majalis</i> fruits	15.79	3
	<i>Zea mays</i> columns with stigmas	10.52	2
No 19	<i>Urtica dioica</i> leaf	20.0	1
	<i>Taraxacum officinale</i> roots	20.0	1
	<i>Vaccinium myrtillus</i> leaf	20.0	1
	<i>Rosa majalis</i> fruits	20.0	1
	<i>Mentha piperita</i> herb	20.0	1

Exrtact: 1.00 g (accurate weight) of each powdered herbal raw materials was placed into a 150 mL flask. Then was added 30 mL of ethanol 50% (v/v) and was heated on a water bath under reflux for 30 minutes. The hot extract was filtered through cotton wool to a volumetric flask of 100 mL. The cotton wool and raw materials residue were returned to the flask and a new extractive cycle was performed by 15 min with 30 mL of ethanol 50% (v/v). This procedure was repeated two times. The filtrates were collected in the 100 mL volumetric flask, and the volume completed to the mark with ethanol 50% (v/v).

Stock solution: the solution resulting from the reflux was taken as stock solution.

Test solution: 1 mL of stock solution was placed into 25 mL volumetric flask and 2 mL of 3% AlCl_3 in 95% ethanol (w/v) solution was added. Then volume was completed to the mark with 95% ethanol.

Reference solution: 1 mL of stock solution was placed into 25 mL volumetric flask, then 1 drop of dilute acetic acid solution was added and volume was completed to the mark with 95% ethanol.

Standard solution: 0.05 g (accurate weight) of CRS of rutin was dissolved in 100 mL of 95% ethanol in a water bath. Then 1 mL of rutin solution was transferred in a 25 mL volumetric flask, 1 drop of dilute acetic acid solution was added to it and was completed to the mark with 95% ethanol.

The obtained results were processed according to the recommendations of the European Pharmacopoeia [32] using the methods of mathematical statistics of the MS Excel statistical package.

Results and discussion

The results of spectrophotometric determination of the total flavonoid content in terms of rutin are presented in figs. 1–2.

Fig. 1. Absorption spectra of AlCl_3 -flavonoid complexes for examined herbal mixtures (HM – herbal mixture)

The method of determination was based on the reaction of complexation of flavonoids with AlCl_3 . The maximum absorption of all samples was at $\lambda_{\text{max}} = 415 \pm 0.5$ nm.

Fig. 2. Total flavonoid content in the herbal mixtures determined spectrophotometrically

(Values are expressed as mean $\pm$ SD ($n = 4$, $P = 0.95$; HM – herbal mixture))

According to UV-spectrophotometric determination, it was found that the total flavonoid content was $1.47 \pm 0.04\%$ in the herbal mixture No. 3, $1.78 \pm 0.04\%$ in the herbal mixture No. 4, $1.29 \pm 0.02\%$ in the herbal mixture No. 7, $1.16 \pm 0.02\%$ in the herbal mixture No. 13 and $3.62 \pm 0.06\%$ in the herbal mixture No. 19 (fig. 2).

Thus, the sufficient content of flavonoids in the studied herbal mixtures (fig. 2) may indicate the ability of these mixtures to prevent the development of oxidative stress, which is the main pathogenic mechanism of diabetic angiopathies [12].

The obtained data testify to the expediency of using the studied herbal mixtures in order to optimize antidiabetic pharmacotherapy.

Conclusions

1. The results of UV-spectrophotometric analysis indicate the high content of flavonoids in the studied herbal mixtures that provides the powerful antioxidant activity, which is an important factor in the treatment of diabetes and in preventing the development of diabetic angiopathies. The best result on the total flavonoid content ($3.62 \pm 0.06\%$) was in the herbal mixture No. 19, which contains *Urtica dioica* L. leaf, *Taraxacum officinale* L. roots, *Vaccinium myrtillus* L. leaf, *Rosa majalis* L. fruits, *Mentha piperita* L. herb.

2. Conducted phytochemical studies may indicate a correlation between the content of flavonoids and pharmacological properties of the studied mixtures, because the functional hydroxyl groups of the latter possess strong antioxidant activity by neutralizing free radicals, chelation of heavy metal ions, increasing the activity of the body's antioxidant defense system and neutralization of lipid peroxidation products, which was confirmed in previous *in vivo* and *in vitro* studies.

Conflict of interests. There are no conflicts of interest regarding this study.

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DETERMINATION OF THE TOTAL CONTENT OF FLAVONOIDS IN HERBAL MIXTURES WITH ANTI-DIABETIC ACTIVITY

Key words: herbal mixtures, flavonoids, UV-spectrophotometry, diabetes mellitus, phytotherapy, antioxidant activity, rutin

A B S T R A C T

Diabetes mellitus is an important social and medical problem, as it causes the development of dangerous complications that lead to disability and mortality. This disease is characterized by a multi-vector pathogenesis that requires a comprehensive approach to treatment. Due to the use of mixtures of medicinal plants in the treatment of diabetes, it is possible to cover all aspects of the development of this disease and its complications, because the combination of different medicinal plants contain more biologically active substances that affect all parts of the pathogenetic mechanism of diabetes mellitus and its complications. Plant biocompounds have a wide range of pharmacological action and various mechanisms of influence on the development of diabetes and diabetic angiopathies (inactivation of the antioxidant defense system, activation of lipid peroxidation and

the development of oxidative stress). In addition, the niche of the pharmaceutical market of Ukraine with phytomixtures is almost empty.

The aim of the work was to study the total flavonoid content in some herbal mixtures, which have established hypoglycemic, hypolipidemic and antioxidant activity in previous studies *in vivo*.

The study of the total flavonoid content in herbal samples was carried out by UV-spectrophotometry with the recording of the absorption spectrum of complexation product of flavonoids with aluminum chloride ($\lambda_{\max}$ 415 nm) in terms of rutin.

According to the results of UV-spectrophotometric determination, it was found that the total flavonoid content in the herbal mixture No 3 was $1.47 \pm 0.04\%$, in the herbal mixture No 4 – $1.78 \pm 0.04\%$, in the herbal mixture No 7 – $1.29 \pm 0.02\%$, in the herbal mixture No 13 – $1.16 \pm 0.02\%$ and in the herbal mixture No 19 – $3.62 \pm 0.06\%$ in terms of rutin.

It was found that the best flavonoid content ($3.62 \pm 0.06\%$) was found in the plant mixture No 19, which contains nettle leaves, dandelion roots, blueberry leaves, rose hips and peppermint herb. Phytochemical studies may indicate a correlation between the content of flavonoids and pharmacological properties of the studied mixtures, because the functional hydroxyl groups of the latter cause strong antioxidant activity by neutralizing free radicals, chelation of heavy metal ions, increasing the activity of antioxidant protection products, which has been confirmed in previous *in vivo* and *in vitro* studies.

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ВИЗНАЧЕННЯ ЗАГАЛЬНОГО ВМІСТУ ФЛАВОНОЇДІВ У РОСЛИННИХ ЗБОРАХ ІЗ ПРОТИДІАБЕТИЧНОЮ АКТИВНІСТЮ

Ключові слова: рослинні збори, флавоноїди, УФ-спектрофотометрія, цукровий діабет, фітотерапія, антиоксидантна активність, рутин

АН О Т А Ц І Я

Цукровий діабет є важливою соціальною та медичною проблемою, адже спричиняє розвиток небезпечних ускладнень, що призводять до інвалідизації та смертності населення. Це захворювання характеризується багатовекторним патогенезом, що потребує комплексного підходу до лікування. Завдяки застосуванню зборів лікарських рослин у терапії цукрового діабету можна охопити усі ланки розвитку цього захворювання та його ускладнень, оскільки поєднання різних лікарських рослин містять більше біологічно активних речовин, які впливають на всі ланки патогенетичного механізму розвитку цукрового діабету та його ускладнень. Рослинні біосполуки мають широкий спектр фармакологічної дії та різноманітні механізми впливу на розвиток діабету та діабетичних ангіопатій (інактивація системи антиоксидантного захисту, активація перекисного окислення ліпідів та розвиток оксидативного стресу). Окрім цього, ніша фармацевтичного ринку України з фітозборів практично порожня.

Метою роботи було вивчити загальний вміст флавоноїдів у деяких рослинних зборах, що мають встановлену в попередніх дослідженнях *in vivo* гіпоглікемічну, гіполіпідемічну і антиоксидантну активність.

Дослідження загального вмісту флавоноїдів у рослинних зборах здійснювали методом УФ-спектрофотометрії з реєстрацією спектра поглинання продукту комплексоутворення флавоноїдів з алюміній хлоридом ($\lambda_{\max}$ 415 nm) у перерахунку на рутин.

За результатами УФ-спектрофотометричного визначення було встановлено, що загальний вміст флавоноїдів у рослинному зборі № 3 становить $1,47 \pm 0,04\%$, у рослинному зборі № 4 – $1,78 \pm 0,04\%$, у рослинному зборі № 7 – $1,29 \pm 0,02\%$, у рослинному зборі № 13 – $1,16 \pm 0,02\%$ та у рослинному зборі № 19 – $3,62 \pm 0,06\%$ у перерахунку на рутин.

Встановлено що за загальним вмістом флавоноїдів найкращий результат ($3,62 \pm 0,06\%$) виявився у рослинній суміші № 19, яка містить листя кропиви, корені кульбаби, листя чорниці, плоди шипшини та траву м'яти перцевої. Виконані фітохімічні дослідження можуть свідчити про кореляцію між вмістом флавоноїдів та фармакологічними властивостями досліджуваних сумішей, адже функціональні гідроксильні групи цих біологічно активних речовин зумовлюють потужну антиоксидантну активність шляхом знешкодження вільних радикалів, хелатування іонів важких металів, підвищення активності системи антиоксидантного захисту організму та нейтралізацію продуктів перекисного окиснення ліпідів, що було підтверджено у попередніх дослідженнях *in vivo* та *in vitro*.

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ОПРЕДЕЛЕНИЕ ОБЩЕГО СОДЕРЖАНИЯ ФЛАВОНОИДОВ В РАСТИТЕЛЬНЫХ СБОРАХ С ПРОТИВОДИАБЕТИЧЕСКОЙ АКТИВНОСТЬЮ

Ключевые слова: растительные сборы, флавоноиды, УФ-спектрофотометрия, сахарный диабет, фитотерапия, антиоксидантная активность, рутин

АННОТАЦИЯ

Сахарный диабет является важной социальной и медицинской проблемой, поскольку приводит к развитию опасных осложнений, приводящих к инвалидизации и смертности населения. Это заболевание характеризуется многовекторным патогенезом и требует комплексного подхода к лечению. Благодаря применению сборов лекарственных растений в терапии сахарного диабета можно охватить все звенья развития этого заболевания и его осложнений, поскольку сочетания различных лекарственных растений содержат больше биологически активных веществ, которые влияют на все звенья патогенетического механизма развития сахарного диабета и его осложнений. Растительные биосоединения имеют широкий спектр фармакологического действия и различные механизмы влияния на развитие диабета и диабетических ангиопатий (инактивация системы антиоксидантной защиты, активация перекисного окисления липидов и развитие оксидативного стресса). Кроме этого, ниша фармацевтического рынка Украины по фитосборам практически пуста.

Целью работы было изучение общего содержания флавоноидов в некоторых растительных сборах, которые имеют установленную гипогликемическую, гиполипидемическую и антиоксидантную активность в предыдущих исследованиях *in vivo*.

Исследование общего содержания флавоноидов в растительных сборах осуществляли методом УФ-спектрофотометрии с регистрацией спектра поглощения продукта комплексообразования флавоноидов с алюминия хлоридом ($\lambda_{\text{max}} 415 \text{ нм}$) в пересчете на рутин.

По результатам УФ-спектрофотометрического определения было установлено, что общее содержание флавоноидов в растительном сборе № 3 составляет $1,47 \pm 0,04\%$, в растительном сборе № 4 – $1,78 \pm 0,04\%$, в растительном сборе № 7 – $1,29 \pm 0,02\%$, в растительном сборе № 13 – $1,16 \pm 0,02\%$ и в растительном сборе № 19 – $3,62 \pm 0,06\%$ в пересчете на рутин.

Установлено, что по общему содержанию флавоноидов лучший результат ($3,62 \pm 0,06\%$) оказался у растительного сбора № 19, который содержит листья крапивы, корни одуванчика, листья черники, плоды шиповника и траву мяты перечной. Проведенные фитохимические исследования могут свидетельствовать о корреляции между содержанием флавоноидов и фармакологическими свойствами исследуемых смесей, ведь функциональные гидроксильные группы этих биологически активных веществ обуславливают мощную антиоксидантную активность путем обезвреживания свободных радикалов, хелатирования ионов тяжелых металлов, повышения активности антиоксидантной защиты организма и нейтрализацией продуктов перекисного окисления липидов, что было подтверждено в предыдущих исследованиях *in vivo* и *in vitro*.

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