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ANTIOXIDANT ACTIVITY *IN VITRO* OF ANTIDIABETIC HERBAL MIXTURES

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Abstract

Medicinal plants and their combinations due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of development of diabetes mellitus and its complications. One of such combinations is two antidiabetic herbal mixtures with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in previous pharmacological study *in vivo.*

Thus, the aim of this research was to study an antioxidant activity *in vitro* by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power of the sample 1 (*Urtica dioica* leaf, *Cichorium intybus* roots, *Rosa majalis* fruits, *Elymus repens* rhizome, *Taraxacum officinale* roots), and the sample 2 (*Arctium lappa* roots, *Elymus repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits).

Antioxidant activity was investigated by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power of methanol extracts of antidiabetic herbal mixtures. The study showed that the IC50 of DPPH radicals inhibition in the sample 1 and sample 2 was 356.47±2.78 µg/mL and 344.29±3.11 µg/mL, respectively; the IC50 of chelating capacity in the sample 1 and sample 2 was 326.50±2.64 µg/mL and 288.38±2.64 µg/mL, respectively; the value of ferric reducing power of the sample 1 was 0.327 at 100 µg/mL and 0.689 at 1000 µg/mL; of the sample 2 – 0.363 at 100 µg/mL and 0.723 at 1000 µg/mL.

Established pharmacological properties make these herbal mixtures perspective remedies for the prevention and treatment of diabetes type 2 and its complications in order to reduce oxidative stress by capturing free radicals and binding heavy metal ions.

Keywords: *diabetes mellitus, antidiabetic herbal mixtures, antioxidant activity, DPPH radical scavenging assay, ferric reducing power assay, ferrous ion chelating assay*

Introduction

Diabetes mellitus is one of the priority problems of the WHO, which requires immediate solution, as the epidemiological situation is alarming – the number of patients is growing rapidly each year, leading to increased disability and mortality due to the development of macro- and microangiopathies [1]. According to the official data of the International Diabetes Federation (2019), the incidence of diabetes in the world is projected to increase 1.5 times by 2030, amounting to more than 500 thousand patients [2].

An important problem of pharmacovigilance is that existing pharmacotherapy can effectively reduce hyperglycemia, but it is not always able to stabilize fluctuations in glycemic values during the day and maintain it at an optimal level. This leads to the formation of a cascade of pathological processes – excessive glycation and inactivation of the body's antioxidant defense system, triggering the processes of free radical oxidation of lipids and, as a consequently, the development of oxidative stress, which leads to the development and progression of diabetic angiopathies [3, 4].

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. This may reduce peripheral insulin sensitivity through major molecular mechanisms such as β-cell dysfunction, inflammatory responses, decreased regulation and / or localization of GLUT-4, mitochondrial dysfunction, and abnormal insulin signaling pathways [1, 3]. Therefore, the optimization of existing antidiabetic pharmacotherapy, search and study of new drugs for the prevention and treatment of this disease and its complications are important issues in modern pharmacy and medicine.

One of these areas is using herbal remedies, either as monotherapy for the prevention of diabetes mellitus type 2 or in the mild stages of this disease and in the combination with traditional therapy in more severe forms [5, 6]. Phytotherapy is a justified method for the prevention and treatment because it has some advantages, such as relatively low toxicity, mild pharmacological effects and possibility to be used for long periods without significant side-effects, and it often well combines with synthetic drugs [7, 8, 9].

Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will influence on the all links of the pathogenetic mechanism of development of diabetes mellitus and its complications [10, 11, 12, 13, 14, 15].

Antioxidant activity is very important in the treatment and prevention of diabetes and its complications because they can include suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; scavenging ROS; inhibition the enzymes involved in ROS generation – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, nicotinamide adenine dinucleotide phosphate (NADH) oxidase, and so forth [16, 17].

Accordingly, the aim of this research was to study an antioxidant activity *in vitro* by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power of two antidiabetic herbal mixtures with established hypoglycemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* [8, 9, 18, 19] and the defined phytochemical composition that determines such pharmacodynamics [10, 11, 12, 13, 14, 15, 20].

Methods

Plant materials: The herbal raw materials, harvested from June to August 2020 in the Ternopil region, were used. After harvesting, the raw materials were dried, graind and stored according to the general GACP requirements [21]. The plants were identified by Department of Pharmacognosy with Medical Botany, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. The voucher specimens of herbal raw materials have been deposited in the departmental herbarium for future records.

Two different herbal mixtures were used for the study. The composition of the mixtures is given in Table 1.

Drugs and chemicals: All reagents including methanol, ascorbic acid, ethylenediaminetetraacetic acid disodium salt (EDTA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (ferrozine) were of analytical grade (≥ 95 % purity) and was purchased from Sigma-Aldrich Chemical Company (Germany). Water used in the studies was produced by MilliQ Gradient water deionizaton system (USA).

Instrumentation and conditions: spectrophotometric measurements were performed on spectrophotometer Shimadzu UV-1800 (Japan) equipped with 1 cm quartz cells.

Extraction procedure: The samples of herbal raw material were grinded into a powder by laboratory mill, then about 300-600 mg (accurately weighed) of each were extracted by 5-10 mL of 70 % methanol in the ultrasonic bath at 80 °C for 5 hours in glass tight vials with a teflon cover. The resulting extracts were centrifuged at 3000 rpm and filtered through disposable membrane filters with pores of 0.22 µm [22, 23].

DPPH radical scavenging assay: The DPPH radical scavenging capacity was determined following the method of Wu et al. [24]. 1 µL of samples of the studied extracts with a range of concentrations 100-1000 µg/mL were mixed with 2 mL of 0.04 mg/mL DPPH in ethanol. The resulting mixtures were shaken vigorously and allowed to stand at 25 °C for 30 min. Then, they were centrifuged at 1500 rpm for 10 min, after which, the absorbance of the supernatants were measured at 517 nm. Experiments were performed in triplicate. Ascorbic acid was used as a control. The DPPH radical scavenging capacity was calculated using the following formula:

$$\%\ \text{DPPH scavenging activity} = \frac{1-(A1-A2)}{A0} \times 100,$$

where A_0 – an absorbance of the control (without extracts);

A_1 – an absorbance of the sample with extracts;

A_2 – an absorbance without DPPH.

An antioxidant activity of the herbal extracts was expressed as IC50, which was defined as the concentration in µg of dry material per mL (µg/mL) that inhibits the formation of DPPH radicals by 50 %. Each value was determined from regression equation.

Ferric reducing power assay: Reducing power was determined according to the method used by Wu et al. [24]. 1 µL of samples of the studied extracts with a range of concentrations 100-1000 µg/mL in phosphate buffer 0.2 mol/L (pH 6.6) were added to 2 mL of 1 % potassium ferricyanide and incubated at 50 °C for 20 min. For the termination of reaction, it was added 2 mL of 10 % trichloroacetic acid. Then the obtaining solutions were centrifuged at 3000 rpm for 10 min and 2.5 mL of supernatants were mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride. The mixtures were allowed to rest for 10 min and absorbance was measured at 700 nm. Experiments were performed in triplicate. Ascorbic acid was used as a control.

Ferrous ion chelating assay: The ferrous ion chelating capacity was determined based on the method Wu et al. [24]. 3 µL of samples of the studied extracts with a range of concentrations 100-1000 µg/mL were added to 0.05 mL of 2 mmol/L ferrous chloride solution, and 0.2 mL of 5 mmol/L ferrozine solution. Then mixtures were shaken vigorously and incubated at 25 °C for 10 min. The absorbance was measured at 562 nm. Experiments were performed in triplicate. EDTA was used as a positive control. The ferrous ion chelating capacity was calculated using the following formula:

$$\%\ \text{chelating capacity} = \frac{A0-(A1-A2)}{A0} \times 100,$$

where A_0 – an absorbance of the control (without extracts);

A_1 – an absorbance of the sample with extracts;

A_2 – an absorbance without EDTA.

An antioxidant activity of the herbal extracts was expressed as IC50, which was defined as the concentration in µg of dry material per mL (µg/mL) that catalyzes the chelation of metal ions by 50 %. Each value was determined from regression equation.

Statistical analysis: Statistical significance was calculated by one-way analysis of variance (ANOVA) using Excel software package. The data are expressed as the mean ± SD (n=3). Differences between values were considered significant, when $p < 0.05$.

Results

The results of the study of antioxidant activity *in vitro* of two antidiabetic herbal mixtures are present in Figures 1-3. The study was performed in a range of concentration 100-1000 µg/mL and was evaluated by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power.

It was found the relationship between the increasing of DPPH radical scavenging activities and concentration of methanol extracts of antidiabetic herbal mixtures. During the study of inhibition of DPPH radicals it was established that the IC50 of sample 1 was 356.47±2.78 µg/mL and the IC50 of the sample 2 was 344.29±3.11 µg/mL (Figure 1). The IC50 value of control – ascorbic acid was 119.24±2.35 µg/mL. The decrease in absorbance of the DPPH radicals in the studied herbal mixtures was caused by the antioxidant scavenging of the radicals and the cleavage of the hydrogen atom due to the high content of phenolic compounds.

As shown in Figure 2, the value of ferric reducing power of the sample 1 was 0.327 at 100 µg/mL and 0.689 at 1000 µg/mL; of the sample 2 – 0.363 at 100 µg/mL and 0.723 at 1000 µg/mL. Ascorbic acid exhibited only slightly higher activity, with a value of ferric reducing power of 0.412 and 0.791 at 100 µg/mL and 1000 µg/mL, respectively (Figure 2).

During the study of chelating capacity it was established that the IC50 of the sample 1 was 326.50±2.64 µg/mL and IC50 of the sample 2 was 288.38±2.64 µg/mL (Figure 3). The IC50 value of positive control – EDTA was 110.55±1.93 µg/mL.

Discussion

An antioxidant study *in vitro* by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power showed that the herbal mixtures have the ability to reduce the manifestations of oxidative stress as a triggering of insulin resistance and diabetic complications by capturing free radicals and by binding heavy metal ions with free radical activity.

Their antioxidant activity, which has been confirmed by this research, makes the studied herbal mixtures effective in the treatment of type 2 diabetes, as they can suppress the formation of ROS either by inhibiting enzymes or by chelating trace elements involved in the generation of free radicals; can scavenging ROS; can inhibit enzymes involved in the generation of ROS – microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, NADH oxidase and others [16, 17, 25, 26].

Therefore, the establishment of powerful antioxidant activity *in vitro* by DPPH radical scavenging assay, ferric reducing power assay and ferrous ion chelating assay in the herbal antidiabetic mixtures launched them as perspective herbal medicines for prevention and treatment of diabetes mellitus type 2 and its complications.

Conclusions

The results of the study of antioxidant activity *in vitro* of two antidiabetic herbal mixtures indicate their powerful properties by DPPH radical scavenging activity, ferrous ion chelating capacity and ferric reducing power. The study showed that the IC50 of DPPH radicals inhibition in the sample 1 and in the sample 2 was 356.47±2.78 µg/mL and 344.29±3.11 µg/mL, respectively; the IC50 of chelating capacity in the sample 1 and in the sample 2 was 326.50±2.64 µg/mL and 288.38±2.64 µg/mL, respectively; the value of ferric reducing power of the sample 1 was 0.327 at 100 µg/mL and 0.689 at 1000 µg/mL; of the sample 2 – 0.363 at 100 µg/mL and 0.723 at 1000 µg/mL.

Established pharmacological properties make these herbal mixtures perspective remedies for the prevention and treatment of diabetes type 2 and its complications.

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Table 1. Composition of the herbal mixtures

Herbal mixtures	Herbal drug component	Portion in the mixture, %	Relative ratio
Sample 1	*Urtica dioica* leaf	26.32	5
	Cichorium intybus roots	26.32	5
	Rosa majalis fruits	21.05	4
	Elymus repens rhizome	15.79	3
	Taraxacum officinale roots	10.52	2
Sample 2	*Arctium lappa* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Zea mays columns with stigmas	21.05	4
	Helichrysum arenarium flowers	15.79	3
	Rosa majalis fruits	10.52	2

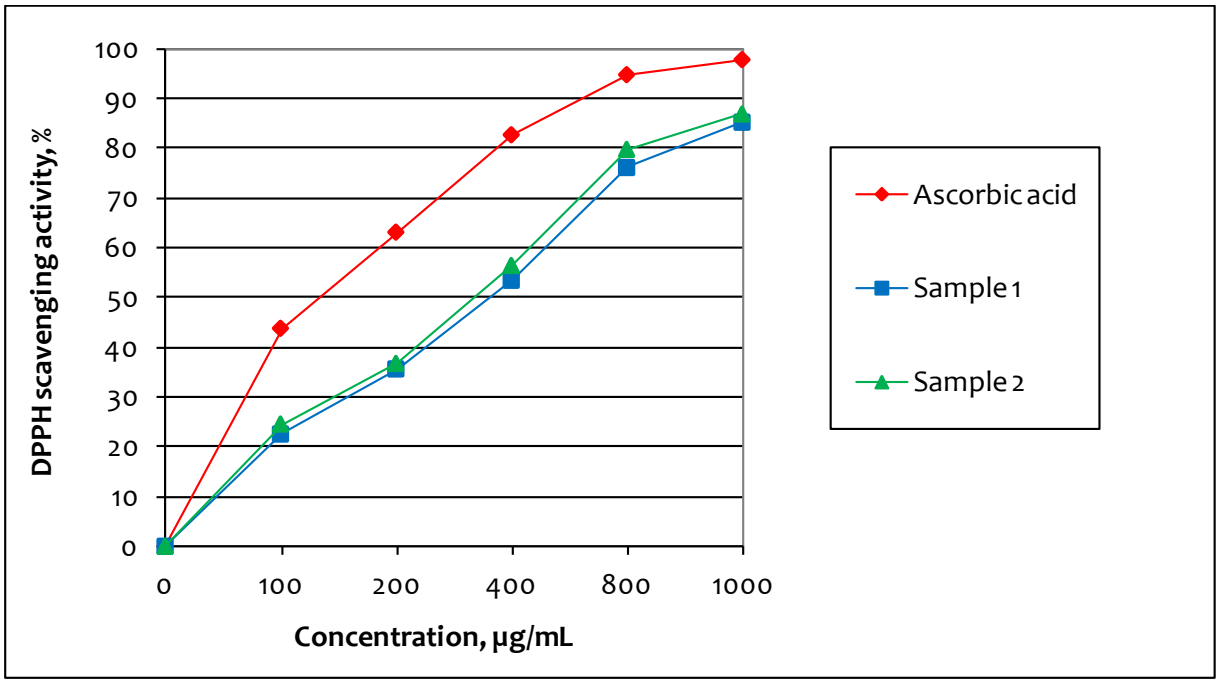

Figure 1. DPPH radical scavenging activities of methanol extracts of antidiabetic herbal mixtures and ascorbic acid. Values are expressed as mean ± SD (n=3).

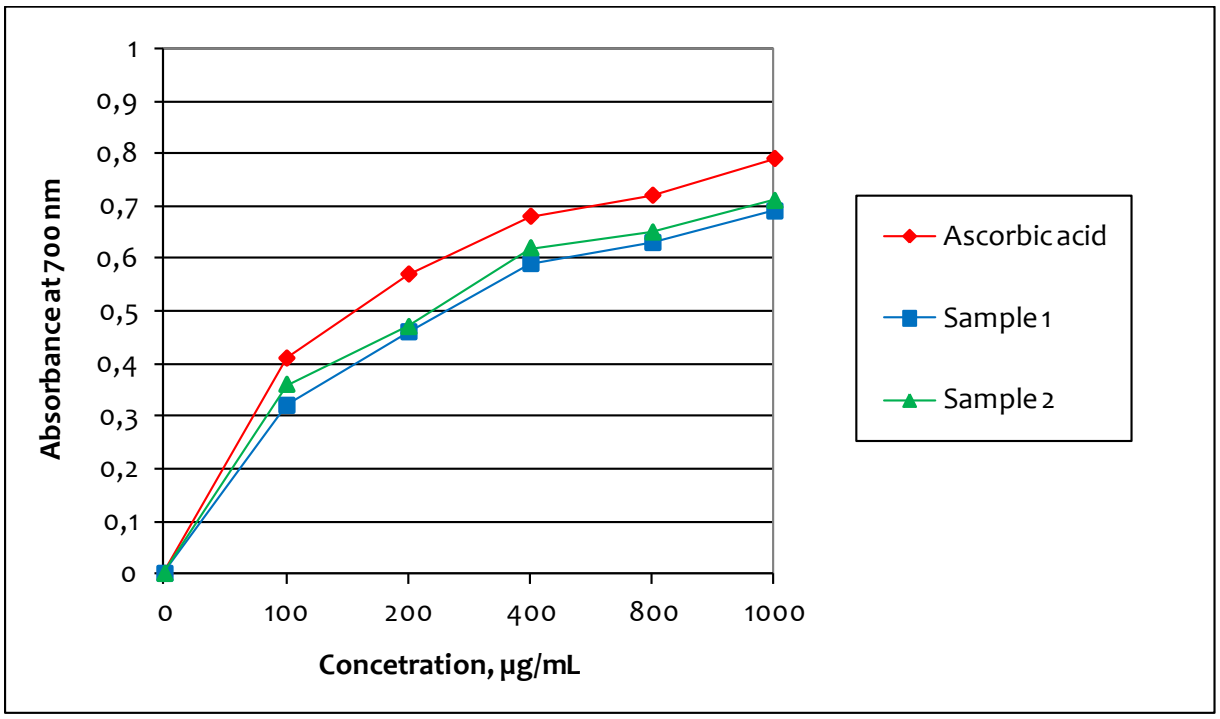

Figure 2. Ferric reducing power of methanol extracts of antidiabetic herbal mixtures and ascorbic acid. Values are expressed as mean ± SD (n=3).

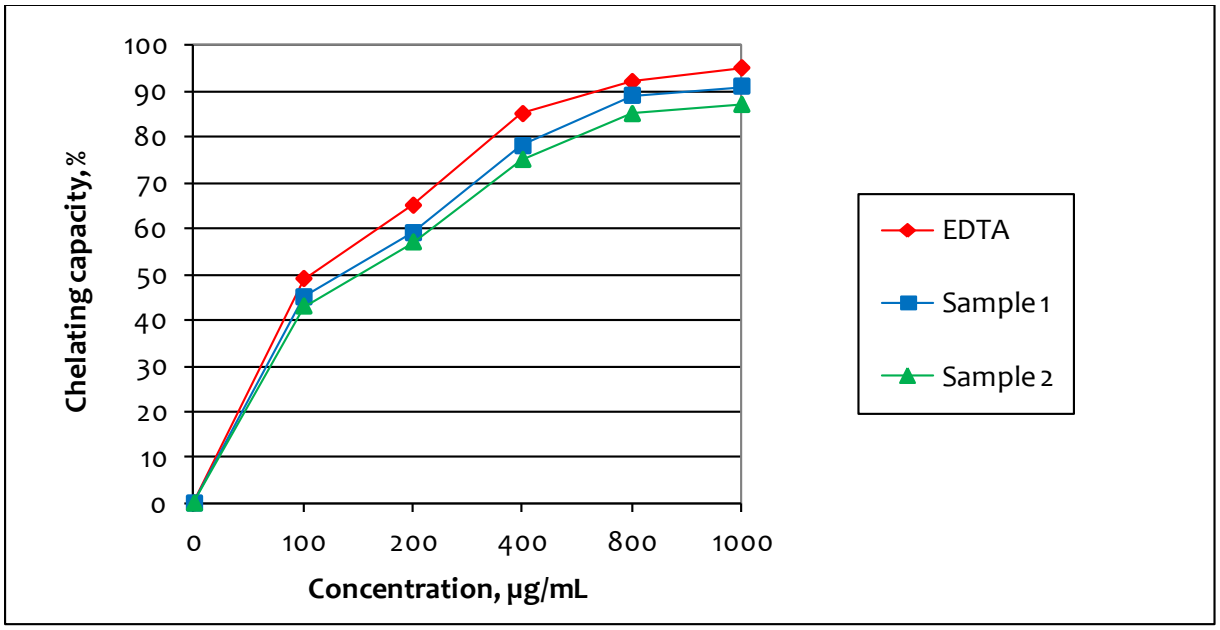

Figure 3. Ferrous ion chelating activities of methanol extracts of antidiabetic herbal mixtures and EDTA. Values are expressed as mean ± SD (n=3).