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STUDY OF POLYSACCHARIDE FRACTIONS CONTENT IN PLANT ANTIDIABETIC MIXTURES

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Abstract

Plant mixtures due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of diabetes mellitus development and its complications. Polysaccharides deserve the particular attention through their hypoglycemic, hypolipidemic, anticholesterolemic, antioxidant, anti-inflammatory and detoxifying activities. Therefore, the aim of present study was to investigate total content of polysaccharide fractions in PAMs: sample 1) *Urtica dioica* leaf, *Cichorium intybus* roots, *Rosa majalis* fruits, *Elymus repens* rhizome, *Taraxacum officinale* roots; sample 2) *Arctium lappa* roots, *Elymus repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits; sample 3) *Inula helenium* rhizome with roots, *Helichrysi arenarium* flowers, *Zea mays* columns with stigmas, *Origanum vulgare* herb, *Rosa majalis* fruits, *Taraxacum officinale* roots; sample 4) *Cichorium intybus* roots, *Elymus repens* rhizome, *Helichrysum arenarium* flowers, *Rosa majalis* fruits, *Zea mays* columns with stigmas; sample 5) *Urtica dioica* leaf, *Taraxacum officinale* roots, *Vaccinium myrtillus* leaf, *Rosa majalis* fruits, *Mentha piperita* herb, which were used in Ukrainian folk medicine for the prevention and treatment of DM type 2.

The results of the study showed that the highest content of PLF and WSPF was in PAM 1 (16.81±0.02 % and 18.32±0.03 %, respectively), PSF±0.02 was in PAM 4 (10.07 %), HF(A) in PAM 5 (13.11±0.04 %) and HF(B) in PAM 2 (10.07±0.03 %).

Present study revealed a numerous of polysaccharide fractions that are important for the treatment and prevention of DM type 2 and its complications.

Keywords: *plant antidiabetic mixture, diabetes mellitus, polysaccharides, pectic substances, hemicellulose, gravimetry*

Introduction

Diabetes mellitus (DM) is one of WHO's priority issues, which requires immediate resolution as the epidemiological situation is gaining alarming proportions – the number of diabetic patients is increasing every year, and with it the number of deaths and disabilities due to the development of diabetic angiopathies [1, 2, 3]. According to the official report from the International Diabetes Federation (2019), it is projected the increase of incidence of DM type 2 by 1.5 times by 2040 in the world, which will amount to more than 640 thousand patients [4]. Therefore, the implementation of pharmacotherapy optimization, the search and study of new drugs for the prevention and treatment of DM type 2 and its complications is a top issue of pharmacy and medicine.

One of these areas is using the phytodrugs, as monotherapy in the mild stages of morbidity and for its prevention, as well as in combination with traditional therapy in more severe forms of DM [5, 6, 7, 8, 9]. Phytotherapy is the justified method for prevention and treatment, as it has some advantages, such as relatively low-toxicity, mild pharmacological effects and possibility to be used for long periods of time without significant side-effects, and it often well combined with synthetic drugs [10, 11, 12, 13, 14, 15]. Particular attention deserves the combinations of different medicinal herbs, because such plant mixtures are expected to have more biologically active substances [16, 17, 18, 19, 20, 21, 22] with wide range of pharmacological action and a variety of mechanisms for influencing the DM development and diabetic angiopathies [23, 24, 25].

Therefore, the aim of our study was to investigate total content of polysaccharide fractions in plant antidiabetic mixtures (PAMs): a) *Urtica dioica* leaf, *Cichorium intybus* roots, *Rosa majalis* fruits, *Elymus repens* rhizome, *Taraxacum officinale* roots; b) *Arctium lappa* roots, *Elymus repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits; c) *Inula helenium* rhizome with roots, *Helichrysi arenarium* flowers, *Zea mays* columns with stigmas, *Origanum vulgare* herb, *Rosa majalis* fruits, *Taraxacum officinale* roots; d) *Cichorium intybus* roots, *Elymus repens* rhizome, *Helichrysum arenarium* flowers, *Rosa majalis* fruits, *Zea mays* columns with stigmas; e) *Urtica dioica* leaf, *Taraxacum officinale* roots, *Vaccinium myrtillus* leaf, *Rosa majalis* fruits, *Mentha piperita* herb, which were used in Ukrainian folk medicine for the prevention and treatment of DM type 2 [26, 27, 28, 29].

Methods

Plant materials: the herbal raw materials, harvested from June to August 2019 in the Ternopil region and Carpathians (*Vaccinium myrtillus* leaf) (Ukraine), were used. After harvesting, the raw materials were dried, graind and stored according to the general GACP requirements [30]. The plants were identified by Prof. S. M. Marchyshyn [31, 32, 33, 34, 35, 36, 37]. The voucher specimens of herbal raw materials have been deposited in Herbarium of Pharmacognosy with Medical Botany Department, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.

Five different PAMs with reliable hypoglycemic activity established during the screening tests [38] were used for the study. The composition of PAMs is given in Table 1.

Chemicals and reagents: all reagents were purchased from the Ltd. Sfera Sim (Lviv, Ukraine) [39, 40].

Determination of total content of polysaccharide lipophilic fraction (PLF) by gravimetry [41, 42]: the samples of herbal raw material 20 g (accurately weight) were placed into a 250 mL of flask. It was added 200 mL of water and then flask was connected to reflux and boiled with stirring during 30 min. The extraction was repeated twice, using the first time – 200 mL, the second – 100 mL of water. The aqueous extracts were combined, centrifuged and the liquid over sediment was decanted into a 500 mL of volumetric flask through 5 layers of gauze, embedded in a glass funnel with a diameter of 55 mm and pre-washed by water. The filters were washed and adjusted by water to volume to the mark (*solution A*).

25 mL of *solution A* was placed in a centrifuge tube, added 75 mL of ethanol 95 %, mixed and heated in a water bath to 30 °C during 5 min. After 60 min, the content of the tubes was centrifuged at 5000 rpm during 30 min. The liquid over sediment was filtered under vacuum at a pressure of 13-16 kPa

through a glass filter with a diameter of 40 mm, dried to constant mass at a temperature of 100-105 °C. The precipitate was quantitatively transferred to the filter, sequentially washing by 15 mL of 95 % ethanol/water (3:1, *v*/*v*), 10 mL of acetone and 10 mL of ethyl acetate. The filter with precipitate was dried in the air and then at a temperature of 100-105 ° C to constant weight. The total content of PLF (*X*, %) in terms of dry raw materials was calculated by the formula:

$$X = \frac{(m_2 - m_1)\times 500\times 100\times 100}{\mathrm{m}\times 25\times(100-\mathrm{W})},$$

where m_1 – mass of the filter, g;
m_2 – mass of filter with sediment, g;
m – mass of raw materials, g;
W – loss in weight on drying, %.

Determination of total content of water-soluble polysaccharides fraction (WSPF) by gravimetry [43]: study was performed from the waste products remaining after obtaining the lipophilic fractions. To remove the alcohol-soluble compounds, the waste products were extracted by ethanol 82 % in proportion (1:10) and infused at room temperature for 2 hours. The obtained extract was filtered, the waste products were again filled by the same volume of ethanol 82 % for 2 hours. After removal of the alcohol-soluble compounds, it was isolated WSPF.

The dry waste products were extracted by hot water in proportion (1:10) when heated to a temperature of 95 °C during 60 min with constant stirring. The re-extraction was performed in proportion of waste products/extractant (1:10). The obtained extract was separated from the raw materials, combined and evaporated to 1/5 of the first volume. WSPF was precipitated by a triple volume of ethanol 96 % at room temperature. The precipitates were separated, washed by ethanol 96 % and acetone, dried and weighed. It was received WSPF.

Determination of total content of pectic substances fraction (PSF) by gravimetry: the waste products remaining after removal WSPF were used to obtain the PSF. Extractions of waste products were performed twice with a mixture of 0.5% solutions of acid oxalate and ammonium oxalate (1:1, *v*/*v*) in proportion of waste products/extractant (1:20) at a temperature of 80-85 °C for 2 hours. The combined extracts were concentrated and precipitated by a five-fold volume of ethanol 96 %. The obtained precipitate was filtered, washed by ethanol 96 %, dried and weighed. It was received PSF.

Determination of total content of hemicellulose fraction (HF) by gravimetry: the waste products remaining after separation the WSPF and PSF were used to obtain the HF(A) and HF(B). The extraction was performed twice with solution of 7 % NaOH in proportion of waste products/extractant (1:5) at room temperature for 12 hours. The alkaline extract was filtered. It was added ice acetic acid to the formation of acidic environment. The obtained precipitate was filtered, washed, dried and weighed. It was received the HF(A).

Statistical analysis: Statistical evaluation was carried out with StatView. Data were expressed as mean ± SEM. Statistical differences were evaluated by One-way ANOVA [44, 45].

Results and Discussion

The results of gravimetric determination of total polysaccharide fractions content in five samples of PAMs are presented in Fig. 1.

The results of the study showed that PAM 1 contains 16.81±0.02 % of PLF, 18.32±0.03 % of WSPF, 6.18±0.02 of PSF, 12.07±0.04 % of HF(A) and 9.18±0.03 % of HF(B); PAM 2 contains 9.32±0.02 % of PLF, 17.21±0.03 % of WSPF, 9.12±0.02 of PSF, 11.64±0.04 % of HF(A) and 10.07±0.03 % of HF(B); PAM 3 contains 10.87±0.02 % of PLF, 17.34±0.03 % of WSPF, 5.98±0.02 of PSF, 10.59±0.04 % of HF(A) and 8.49±0.03 % of HF(B); PAM 4 contains 11.45±0.02 % of PLF, 17.92±0.03 % of WSPF, 10.07±0.02 of PSF, 10.48±0.04 % of HF(A) and 8.92±0.03 % of HF(B); PAM 5 contains 15.27±0.02 % of PLF, 18.19±0.03 % of WSPF, 8.25±0.02 of PSF, 13.11±0.04 % of HF(A) and 9.56±0.03 % of HF(B) (Fig. 1).

Polysaccharides obtained from plants are very important active substances for the prevention and treatment of DM type 2 and diabetic angiopathies, as they have hypoglycemic, hypolipidemic, anticholesterolemic, antioxidant, anti-inflammatory and detoxifying activities [46, 47, 48]. These compounds stimulate the growth of beneficial bacteria in the colon, including *Bifidobacteria* and *Lactobacilli*, thereby modulating the composition of microflora [49, 50, 51, 52]. It creates an environment

that protects against pathogens, toxins and free radicals resulting from lipid peroxidation [53, 54]. Powerful antioxidant properties of polysaccharides in number of studies *in vivo* and *in vitro,* whose mechanism of action is not understood exactly, have been observed [55, 56]. Plant carbohydrates can regulate the lipid metabolism by lowering of triglycerides and cholesterol, a disorder of which occurs in DM type 2 and leads to the development of cardiovascular diseases and microcirculatory complications – diabetic nephropathy, neuropathy and retinopathy, the formation of diabetic foot [44, 57]. The hypoglycemic activity of carbohydrates is realized by increasing of insulin secretion, inhibition of glucagon secretion, stimulation of β-cells proliferation and neogenesis [46, 48]. All these polysaccharide properties make them an important group of substances for prevention and treatment of DM type 2 and its extremely dangerous complications.

Conclusions

It was conducted the study of total polysaccharide fractions content in five samples of PAMs: sample 1) *Urtica dioica* leaf, *Cichorium intybus* roots, *Rosa majalis* fruits, *Elymus repens* rhizome, *Taraxacum officinale* roots; sample 2) *Arctium lappa* roots, *Elymus repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits; sample 3) *Inula helenium* rhizome with roots, *Helichrysi arenarium* flowers, *Zea mays* columns with stigmas, *Origanum vulgare* herb, *Rosa majalis* fruits, *Taraxacum officinale* roots; sample 4) *Cichorium intybus* roots, *Elymus repens* rhizome, *Helichrysum arenarium* flowers, *Rosa majalis* fruits, *Zea mays* columns with stigmas; sample 5) *Urtica dioica* leaf, *Taraxacum officinale* roots, *Vaccinium myrtillus* leaf, *Rosa majalis* fruits, *Mentha piperita* herb, which were used in Ukrainian folk medicine for the prevention and treatment of DM type 2.

The obtained phytochemical studies may indicate a correlation between the component composition and content of polysaccharide fractions in the samples of PAMs and their effectiveness in the treatment and prevention of DM type 2 and its complications.

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Table 1. Composition of plant antidiabetic mixtures

PAMs	Plant 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
Sample 3	*Inula helenium* rhizome with roots	10.0	1
	Helichrysi arenarium flowers	20.0	2
	Zea mays columns with stigmas	20.0	2
	Origanum vulgari herb	20.0	2
	Rosa majalis fruits	20.0	2
	Taraxacum officinale roots	10.0	1
Sample 4	*Cichorium intybus* roots	26.32	5
	Elymus repens rhizome	26.32	5
	Helichrysum arenarium flowers	21.05	4
	Rosa majalis fruits	15.79	3
	Zea mays columns with stigmas	10.52	2
Sample 5	*Urtica dioica* leaf	20.0	1
	Taraxacum officinale roots	20.0	1
	Vaccinium myrtillus leaf	20.0	1
	Rosa majalis fruits	20.0	1
	Mentha piperita herb	20.0	1

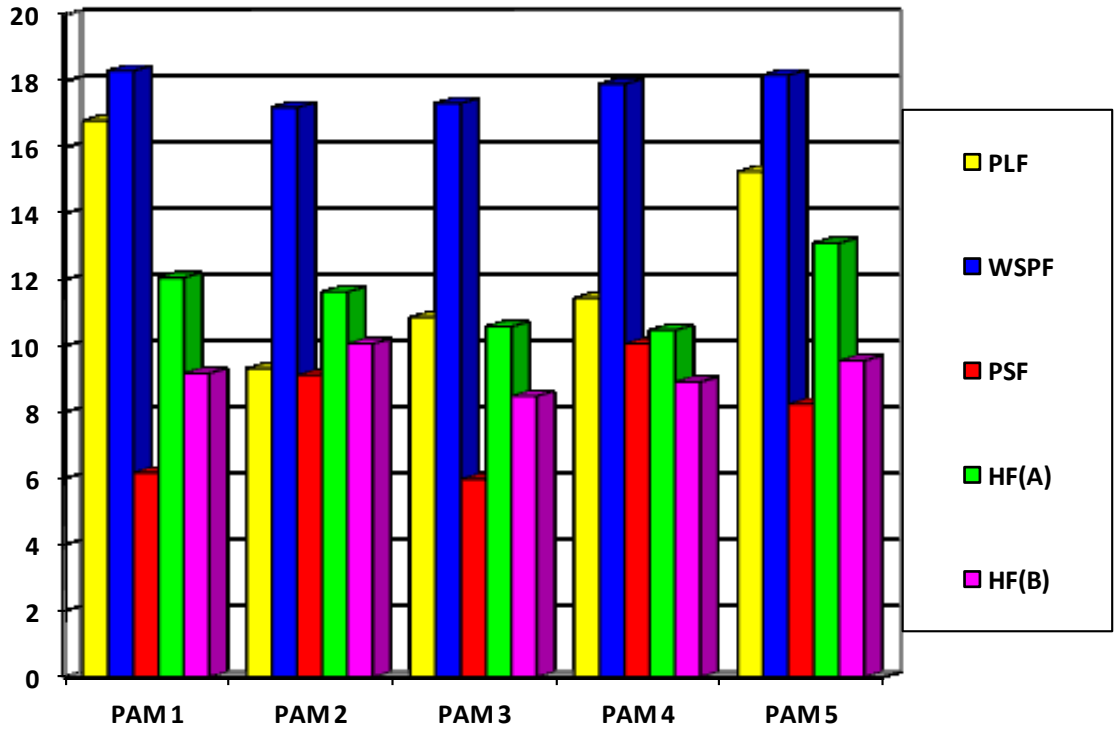

Figure 1. Total content of polysaccharide fractions in plant antidiabetic mixtures by gravimetry. Values are expressed as mean ± SD (n=5).