NATURAL DRUGS

BIOLOGICAL ACTIVITY AND POLYPHENOL CONTENT IN SELECTED HERBAL TEA BLENDS USED IN DIABETES

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Abstract: This study aimed to evaluate the antioxidant properties, α -glucosidase inhibitory activity, and the total content of polyphenols and flavonoids in the selected anti-diabetic herbal blends (**1-6**), used traditionally in diabetes. The strongest antioxidant activity (DPPH and CUPRAC) was observed in extracts from blends **1** and **6** ($IC_{50} < 70 \mu\text{g/mL}$, $IC_{0.5} < 110 \mu\text{g/mL}$, respectively). Extracts from these blends also contained most of the polyphenolic compounds ($> 40 \text{ mg GAE/g}$), had a high content of flavonoids (**6** – 10.96 mg QE/g , **1** – 7.51 mg QE/g) and the highest α -glucosidase inhibiting capacity (for **1** $> 90\%$, for **6** $> 90\%$ and 5.55% , while for acarbose 1.76% and 0.93% at the $100 \mu\text{g/mL}$ and $10 \mu\text{g/mL}$, respectively). The antioxidant activity of **1** and **6** correlated with the content of polyphenols and α -glucosidase inhibitory properties and indicates the existence of this mechanism in supporting the treatment of type 2 diabetes and diabetic complications by the above preparations.

Keywords: herbal blends, antidiabetes, antioxidant, α -glucosidase inhibition

The twentieth century brought a rapid rise in the incidence of diabetes, not only in highly developed countries but also in low- and middle-income countries. Diabetes prevention and treatment is an important public health problem. There are two types of diabetes: type 1 is characterized by deficient insulin production in the body and type 2, which results from the body's ineffective use of insulin (1). The consequences of the lack of insulin include disorders of carbohydrates, proteins and fats metabolism. Chronic hyperglycemia, as an effect of uncontrolled diabetes, leads to serious damage to the heart, blood vessels, eyes, kidneys, and nerves. The causes of diabetes are not fully known yet. It is assumed that they include genetic predisposition, immune system disorders and also viral infections, improper diet, obesity, the influence of unfavorable environmental factors, like the ones connected to the formation of excess of reactive forms of oxygen (the free radical theory) (2).

There are no simple ways to treat diabetes. Pharmacological treatment of hyperglycemia and concomitant diseases is introduced, as well as a change of lifestyle, proper diet and physical exercise (1). One of the ways to support diabetes treatment is to use herbal preparations. The literature

describes more than 400 plant species that have hypoglycemic properties (3). Herbal medicines could act as hypoglycemic agents through various mechanisms: by triggering an increase in insulin output or through altering sensitivity to insulin, facilitating metabolites in insulin-dependent processes or inhibiting intestinal absorption of glucose. Recently, α -glucosidase inhibitors have been of interest as they slow down the digestion and absorption of carbohydrates by competitive blocking of the activity of glucosidase secreted by intestinal epithelium, responsible for carbohydrate degradation. α -Glucosidase inhibitors play an important role in reducing postprandial hyperglycemia. Acarbose is the most commonly used α -glucosidase inhibitor in medicine (4).

In this light, the results obtained by Yin et al. are promising, as they indicate that 411 compounds extracted from plants, with various structures that belong to terpenes, alkaloids, quinones, flavonoids, phenols, phenylpropanoids, and steroids, have the α -glucosidase inhibiting properties (5). Some of the 44 flavonoids examined by Proenca et al. were characterized by a capacity for inhibiting this enzyme stronger than the capacity of acarbose (6). This activity was proven also for some phenolic acids (7).

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Therefore, further studies of natural sources of α -glucosidase inhibitors are promising. Traditional medicine, as support in diabetes treatment, recommends also various herbal blends, whose composition is defined on the basis of the knowledge on the activity of their individual components. The lack of research that would confirm their beneficial effect on diabetes made us take up this subject matter.

The aim of this study was to compare biological activity (inhibition of α -glucosidase and antioxidant properties) and the total content of polyphenols and the total content of flavonoids in *in vitro* studies in the selected anti-diabetic herbal blends (1-6), prepared by the authors, following various recipes (Table 1).

Materials and methods

Chemicals

Aluminum trichloride, methanol, ethanol, sodium acetate-3-hydrate, sodium carbonate, sodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate 2-hydrate were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland); Folin-Ciocalteu's phenol reagent, 2, 4, 6- tripyridyl-s-triazine, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, ascorbic acid, gallic acid, acarbose, PNPG (4-nitrophenyl α -D-glucopyranose) – Sigma-Aldrich, St. Louis, MO USA, and quercetin from Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Materials

All plants were purchased from Polish producers of medicinal herbs (Zakład Zielarski KAWON-HURT Nowak sp. j., Zakład Konfekcjonowania Ziół FLOS, and DARY NATURY – producent ekologicznej żywności i ziół). The selected anti-diabetic

herbal blends (1-6), prepared by the authors, following various recipes (Table 1).

Extracts preparation

To four grams analyzed herbal blends added 400 mL water at room temperature, the mixture was brought to boiling on a water bath, then heated for 5 min at 95°C on a water bath. The extract was concentrated under a vacuum at 40°C to volume 25 mL to yield a stock solution of 160 mg/mL that was used in further experiments.

Phytochemical analysis

Total phenolics content (TPC) determination

The total phenolics content was determined by the Folin-Ciocalteu method (14) with slight modification. The TPC was quantified from the standard calibration curve of gallic acid ($y = 9.8399x + 0.0289$ $r^2 = 0.9993$) in the concentration range (0.2-0.8 mg/mL) and expressed as gallic acid equivalents (mg GAE/g of the dry blend).

Determination of flavonoid content (TFC)

Total flavonoids content was determined according to AlCl_3 methods (15) after modification allowed using a microplate reader. The total flavonoid content was calculated using a calibration curve of quercetin (0.05-0.003 mg/mL) ($y = 93.229x + 0.0116$; $r^2 = 0.9996$) and the results were expressed as quercetin equivalent (mg QEs/g of the dry blend).

Bioactivity assay of the extracts

Antioxidant activity

Two methods were used for the measurement of antioxidant potential: DPPH radical scavenging

Table 1. Composition and origin of the tested herbal blends.

No	Composition of herbal blends	References
1	<i>Gei urbani rhizoma</i> 30, <i>Anserinae herba</i> 30, <i>Phaseoli pericarpium</i> 20, <i>Myrtilli folium</i> 10, <i>Rubi fruticosi folium</i> 10	8
2	<i>Urticae folium</i> 35, <i>Cichorii radix</i> 35, <i>Phaseoli pericarpium</i> 30	9
3	<i>Galegae herba</i> 25, <i>Phaseoli pericarpium</i> 25, <i>Myrtilli folium</i> 20, <i>Urticae folium</i> 20, <i>Salviae folium</i> 5, <i>Taraxaci radix</i> 5	10
4	<i>Foenugraeci semen</i> 38, <i>Urticae folium</i> 24, <i>Phaseoli pericarpium</i> 20, <i>Taraxaci radix</i> 10, <i>Mentae piperitae folium</i> 5, <i>Salviae folium</i> 3	11
5	<i>Myrtilli folium</i> 15, <i>Chamomillae anthodium</i> 10, <i>Salviae folium</i> 5, <i>Galegae herba</i> 5, <i>Phaseoli pericarpium</i> 5, <i>Agropyri rhizoma</i> 5, <i>Taraxaci radix</i> 5, <i>Hyperici herba</i> 5, <i>Maydis stigma</i> 5, <i>Lamii albi flos</i> 2	12
6	<i>Galegae herba</i> 10, <i>Juniperi fructus</i> 10, <i>Alchemillae herba</i> 10, <i>Tiliae inflorescentia</i> 10, <i>Fragariae herba</i> 10, <i>Uvae ursi folium</i> 10, <i>Myrtilli folium</i> 10, <i>Visci herba</i> 10, <i>Hyperici herba</i> 10, <i>Centaurii herba</i> 10	13

Table 2. Total content of polyphenols (TPC) and flavonoids (TFC), antioxidant and α -glucosidase inhibitory activity of herbal tea blends.

No	Compounds content (mg/g blend)		Biological activity			
			Antioxidant activity		Inhibition of α -glucosidase (%)	
	TPC	TFC	DPPH IC ₅₀ μ g/mL	CUPRAC IC _{0.5} μ g/mL	100 μ g/mL	10 μ g/mL
1	59.22 $\pm$ 5.38	7.51 $\pm$ 0.56	43	90	99.79	91.86
2	7.68 $\pm$ 0.13	1.08 $\pm$ 0.10	605	944	0.39	n. a.
3	17.80 $\pm$ 0.72	5.11 $\pm$ 0.46	225	372	0.10	n. a.
4	14.63 $\pm$ 0.70	5.47 $\pm$ 0.63	275	476	0.44	0.40
5	35.39 $\pm$ 1.50	12.16 $\pm$ 0.97	140	226	1.17	0.80
6	41.94 $\pm$ 0.94	10.96 $\pm$ 1.17	65	113	95.56	5.55
Vit. C	n. d.	n. d.	5.5	14.4	n. d.	n. d.
Ac	n. d.	n. d.	n. d.	n. d.	1.76	0.93

Abbreviations: n. d. - not determined; n. a. -not active; Ac - acarbose

and cupper reducing power (CUPRAC). The DPPH radical scavenging assay of the extracts was determined using a 96-well microplate, according to a previously reported method (16, 17) with slight modification. Vitamin C at different concentrations (120 – 15 μ g/mL) was used as standard (concentration in the sample tested was 12 – 1.5 μ g/mL). The DPPH radical scavenging activity (AA%) of the analyzed extracts/standard was calculated as follows:

$$AA\% = [(A_{DPPH} - A_{sample}) / A_{DPPH}] \times 100\%$$

where A_{DPPH} was the absorbance of the control [DPPH + methanol without sample], A_{sample} was the absorbance of the sample [DPPH + extract/standard].

The results were expressed as IC₅₀. IC₅₀ values were calculated from the plotted graph of scavenging activity of DPPH (%) against the concentrations of the extract/standard. IC₅₀ is defined as a total antioxidant required to inhibit the initial DPPH radical by 50%.

Determination of the ability to reduce the copper ion (II) was carried out by CUPRAC (18) according to the methodology described by Kikowska et al. (19). Positive control ascorbic acid was used in concentration 120 – 15 μ g/mL (in the tested sample was 30 – 3.75 μ g/mL). The results were expressed as the IC_{0.5} value which corresponds to the extract concentration indicating 0.5 absorbance.

α -Glucosidase inhibitory activity

The α -glucosidase inhibition capacity of the herbal blends tested was evaluated using the method described by Chipiti et al. (20) with its own modifi-

cation. For the analysis, concentrations of 100 μ g/mL and 10 μ g/mL of the tested blends and the acarbose as a standard substance were chosen.

The results are presented as % inhibition of α -glucosidase activity calculated from the formula: $[(A_{PK} - A_{PB}) / (A_{PK})] \times 100\%$; where: A_{PK} - absorbance of control A_{PB} - absorbance of the tested sample.

Statistical analysis

Statistical analysis was performed with Microsoft Excel 2016 software (Microsoft, Redmond, WA). Analyses were performed in duplicate, and the results are the average of six measurements (n = 6). Results were expressed as means $\pm$ SD. The median effect concentrations (IC₅₀ and IC_{0.5} values) were determined using a concentration–response curve.

RESULTS AND DISCUSSION

The results show that the content of polyphenols in the tested blends is varied and it is from 59.22 mg GAE/g to 7.68 mg GAE/g in blend **1, 6, 5, 3, 4 and 2**. The content of flavonoids was also different, the highest in blend **5 and 6** and the lowest in blend **2** (Table 2). The highest of polyphenols and flavonoids content in blends **1, 5 and 6** may be justified by the presence of such ingredients as: leaves of blueberry, fragaria and sage, flowers of matricaria, white nettle and tilia, herbs of St John's wort, common centaury, and common lady's mantle, in which the presence of numerous compounds like flavonoids, ellagitannins, catechins, phenolic acids and their derivatives were found (21-24).

When supporting diabetes treatment it is important to use, apart from the agents that decrease the glucose levels, natural substances with antioxidant effect, that support natural antioxidant system of the body, like phenols, flavonoids and vegetable raw materials that decrease the symptoms of oxidative stress, reduce formation of oxidative damages of lipids, proteins, and nucleic acids, among others. Given the fact that in diabetics, due to hyperglycemia, free radicals are formed and endogenous system for antioxidative protection is weakened more often than in the healthy, limiting the effects of oxidative stress may inhibit the development of vascular and neurologic complications of diabetes.

The antioxidant activity of the extracts was evaluated by radical scavenging assay (DPPH) and cupric ion reducing activity (CUPRAC). The tested extracts of blends **1** and **6** with the highest total content of polyphenols and flavonoids showed also the strongest antioxidative effect – in the case of blends **1** and **6** the value $IC_{50} < 70 \mu\text{g/mL}$ (in analysis with DPPH radical), and value $IC_{0.5}$ determined by CUPRAC method was $IC_{0.5} < 113 \mu\text{g/mL}$. Those blends were about ten times weaker when compared to vitamin C. The obtained values IC_{50} and $IC_{0.5}$ for the blends **1** and **6** were even a dozen of times higher when compared to other tested blends. The extract of blend **2** showed the weakest antioxidative activity, and its polyphenols content was also the lowest (Table 2).

Slowing down the absorption of carbohydrates through inhibition of their decomposition, may, besides the proper diet with the low glycemic index, significantly contribute to decreasing glycemia. In order to assess the efficacy of hypoglycemic effect of herbal blends **1** – **6**, we assessed their capabilities for inhibition of α -glucosidase, an enzyme responsible for decomposition of complex carbohydrates to simple sugars, including to glucose. Both, the blends and acarbose, which was simultaneously used as a drug, were tested in the concentrations of $100 \mu\text{g/mL}$ and $10 \mu\text{g/mL}$. The analysis of the obtained results showed that, similarly like in the case of antioxidative activity, the blends **1** and **6** had the greatest α -glucosidase inhibition capacity. In the case of blend **1**, it reached the value of 99.79% and 91.86%, for blend **6**, the value was 95.56% and 5.55%, in the concentrations of $100 \mu\text{g/mL}$ and $10 \mu\text{g/mL}$, respectively, and it was many times stronger than the tested one in the same concentrations of the reference acarbose (1.76% and 0.93%, respectively) (Table 2). The literature data indicate that the beneficial effect of plants in diabetes may result from α -glucosidase inhibition (7, 25). In the case of blends **2**, **3**, **4** and **5**

the α -glucosidase inhibiting activity resulted to be very weak (0.10-1.17%). It may be assumed that the effect of those blends is based on other mechanisms. Among the ingredients of the tested blends **1** – **6** α -glucosidase inhibiting properties were proved for *Cichorii radix* (26), *Vaccinium myrtilli folium* (7), *Foenugraeci semen* (27) and small ones in case of *Taraxaci radix* (28). The hypoglycemic effect of *Galegae herba* was connected to its anti-inflammatory and immunostimulating activity (29). For the herbs used traditionally in diabetes, namely *Urticae folium* (30), *Phaseoli pericarpium* (31), *Salviae folium* (32, 33), *Agropyri radix* (34), *Rubi fruticosi folium* (35), *Maydis stigma* (36), *Menthae piperitae folium* (37), *Hyperici herba* (38) and *Visci herba* (39), the influence on decreasing the sugar levels was found in tests on animals. Antidiabetic activity of *Alchemillae herba*, *Juniperi fructus* and *Uvae ursi folium* is reported only in traditional medicine. In the case of *Anserinae herba*, *Gei urbani rhizoma*, *Chamomillae anthodium*, *Tiliae inflorescentia*, *Centaurii herba*, *Fragariae folium*, *Lami albi flos* there are no reports on the blood-sugar-decreasing effect. Those raw materials are the components of the blend traditionally used in diabetes, and they may have a facilitating effect as antioxidants, anti-inflammatory agents, and their activity is conditioned by the presence of flavonoids, anthocyanins, proanthocyanidins, phenolic acids, ellagitannins, and other polyphenols. Their influence on hypoglycemic effect may also result from the synergism between active compounds present in a defined composition of herbal materials (40, 41).

The patients with type 2 diabetes are prescribed with synthetic α -glucosidase inhibitors which may cause undesirable effects from the gastrointestinal system: diarrhea, pain and bloating and other intestinal disorders. Sometimes it makes the patients stop taking those medicines. Plant medicines may alleviate those disorders because they contain compounds that have muscle-relaxing, anti-inflammatory and astringent properties. Yet another aspect of the interest in natural products is the potential opportunity to reduce doses of synthetic antidiabetic drugs (5). Oboh et al. proved that acarbose used together with gallic acid (1 : 1) resulted in α -glucosidase inhibition in $65.7 \pm 1.4\%$, almost as strongly as acarbose ($66.2 \pm 0.7\%$) and much stronger than pure gallic acid ($43.9 \pm 0.7\%$). The mixture of both compounds inhibited lipid peroxidation in a homogenate of rat pancreas tissues and showed *in vitro* antioxidative activity. The use of acarbose together with gallic acid (1 : 1) in antidiabetic therapy may result in a reduction of acarbose side effects (42).

CONCLUSION

The results of our research allowed us to assess and compare *in vitro* the potential of antioxidative effect and α -glucosidase inhibition of the selected herbal blends traditionally used in diabetes, whose activity in this field has not been studied yet. We have shown a strong α -glucosidase inhibiting activity of two blends that are, at the same time, characterized by a high content of polyphenols and strong antioxidative activity, which is a desirable effect in diabetes management. The results may provide important guidance when selecting an antidiabetic blend.

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Conflict of interest

The authors declare no conflicts of interest.

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