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).
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, FeCl3 6H2O, 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 r² = 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 AlCl3 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; r² = 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.

<table>
<thead>
<tr>
<th>No</th>
<th>Composition of herbal blends</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gei urbani rhizoma 30, Anserinae herba 30, Phaseoli pericarpium 20, Myrtilli folium 10, Rubi fruticosi folium 10</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Urticae folium 35, Cichorii radix 35, Phaseoli pericarpium 30</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Galegae herba 25, Phaseoli pericarpium 25, Myrtilli folium 20, Urticae folium 20, Salviae folium 5, Taraxaci radix 5</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Foenugraeci semen 38, Urticae folium 24, Phaseoli pericarpium 20, Taraxaci radix 10, Mentae piperitae folium 5, Salviae folium 3</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>Myrtilli folium 15, Chamomillae anthodium 10, Salviae folium 5, Galeage herba 5, Phaseoli pericarpium 5, Agropyri rhizoma 5, Taraxaci radix 5, Hyperici herba 5, Maydis stigma 5, Lamii albi flos 2</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>Galegae herba 10, Juniperi fructus 10, Alchemillae herba 10, Tiliae inflorescentia 10, Fragariae herba 10, Uvae usi folium 10, Myrtilli folium 10, Visca herba 10, Hyperici herba 10, Centaurii herba 10</td>
<td>13</td>
</tr>
</tbody>
</table>
Biological activity and polyphenol content in selected herbal tea...

and copper 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 ìg/mL) was used as standard (concentration in the sample tested was 12 – 1.5 ìg/mL). The DPPH radical scavenging activity (AA%) of the analyzed extracts/standard was calculated as follows:

\[
AA\% = \left[ \frac{A_{DPPH} - A_{sample}}{A_{DPPH}} \right] \times 100\%
\]

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 IC\(_{50}\). IC\(_{50}\) values were calculated from the plotted graph of scavenging activity of DPPH (%) against the concentrations of the extract/standard. IC\(_{50}\) 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 ìg/mL (in the tested sample was 30 – 3.75 ìg/mL). The results were expressed as the IC\(_{0.5}\) value which corresponds to the extract concentration indicating 0.5 absorbance.

\(\alpha\)-Glucosidase inhibitory activity

The \(\alpha\)-glucosidase inhibition capacity of the herbal blends tested was evaluated using the method described by Chipiti et al. (20) with its own modification. For the analysis, concentrations of 100 ìg/mL and 10 ìg/mL of the tested blends and the acarbose as a standard substance were chosen.

The results are presented as % inhibition of \(\alpha\)-glucosidase activity calculated from the formula: \([\frac{(A_{Rg} - A_{P})}{A_{Rg}}] \times 100\%\); where: \(A_{Rg}\) - absorbance of control \(A_{P}\) - 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 ± SD. The median effect concentrations (IC\(_{50}\) 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 matri- caria, 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).

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds content</th>
<th>Biological activity</th>
<th>Antioxidant activity</th>
<th>Inhibition of (\alpha)-glucosidase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mg/g blend)</td>
<td></td>
<td>DPPH IC(_{50}) ìg/mL</td>
<td>CUPRAC IC(_{50}) ìg/mL</td>
</tr>
<tr>
<td>1</td>
<td>59.22 ± 5.38</td>
<td>7.51 ± 0.56</td>
<td>43</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>7.68 ± 0.13</td>
<td>1.08 ± 0.10</td>
<td>605</td>
<td>944</td>
</tr>
<tr>
<td>3</td>
<td>17.80 ± 0.72</td>
<td>5.11 ± 0.46</td>
<td>225</td>
<td>372</td>
</tr>
<tr>
<td>4</td>
<td>14.63 ± 0.70</td>
<td>5.47 ± 0.63</td>
<td>275</td>
<td>476</td>
</tr>
<tr>
<td>5</td>
<td>35.39 ± 1.50</td>
<td>12.16 ± 0.97</td>
<td>140</td>
<td>226</td>
</tr>
<tr>
<td>6</td>
<td>41.94 ± 0.94</td>
<td>10.96 ± 1.17</td>
<td>65</td>
<td>113</td>
</tr>
<tr>
<td>Vit. C</td>
<td>n. d.</td>
<td>n. d.</td>
<td>5.5</td>
<td>14.4</td>
</tr>
<tr>
<td>Ac</td>
<td>n. d.</td>
<td>n. d.</td>
<td>n. d.</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Abbreviations: n. d. - not determined; n. a. - not active; Ac - acarbose
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<sub>50</sub> < 70 µg/mL (in analysis with DPPH radical), and value IC<sub>50</sub> determined by CUPRAC method was IC<sub>50</sub> < 113 µg/mL. Those blends were about ten times weaker when compared to vitamin C. The obtained values IC<sub>50</sub> and IC<sub>0.5</sub> 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 µg/mL and 10 µg/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 µg/mL and 10 µg/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 diabetics, namely Urticae folium (30), Phaseoli pericarpium (31), Salviae folium (32, 33), Agropyri radix (34), Rhus fructicosi folium (35), Maydis stigma (36), Menthae piperitiae 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 usi folium is reported only in traditional medicine. In the case of Anserinae herba, Gei urbani rhizoma, Chamomillae anthodium, Tiliae inflorescentia, Centaurni 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, ellagitanins, 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 ± 1.4%, almost as strongly as acarbose (66.2 ± 0.7%) and much stronger than pure gallic acid (43.9 ± 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.

Acknowledgment

This work was supported by the Department of Pharmacognosy Poznan University of Medical Sciences (502-01-3309419-02578).

Conflict of interest

The authors declare no conflicts of interest.

REFERENCES


Received: 29.05.2019