RESEARCH ARTICLE

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Alpha-glucosidase inhibitory effect and antioxidant properties of different extracts from *Lycium barbarum* L.

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ABSTRACT

Fruits of the plant *Lycium barbarum* L. (Goji berry) fruit have been widely used around the world for their medicinal purposes and as a functional food for more than 4500 years. Studies indicate the effects of the fruits of Goji berry on aging, neuroprotection, fatigue, energy expenditure, glucose control in diabetics, antioxidant properties, immunomodulation, anti-tumor activity, and cytoprotection. Some authors report that antioxidant molecules found in goji fruit might offer many health protective benefits by alleviating oxidative stress when used as an additive or in a mixture with other phytochemicals in fruit or herbal products. It was found that Goji berry fruit growing in Bulgaria has a total polysaccharide content of about 4%. The content of total polyphenol compounds in the different extracts was found to be between 5.5mg and 8mg GAE/g of dry product. Polyphenols from dry Goji berry fruit were extracted and studied by HPLC analysis for the type of polyphenols present. Antioxidant activity of extracts rich in polyphenols was determined by DPPH, FRAP and CUPRAC methods. The data was compared with a medicinal product containing an extract of Goji berry, where synergistic effects were also established. Inhibitory effects of the Goji berry extracts and their corresponding polyphenols were investigated in the presence of α-glucosidase for antidiabetic activity. The 50% inhibitory concentration found for organic extract was IC_{50}=91.7 μg GAE/g of fruits. The study demonstrated that some of the polyphenols in the investigated extracts exhibit competitive properties towards the enzyme.

Key words: Goji berry, α-Glucosidase, antioxidants, enzyme inhibition

Introduction

Diabetes is a chronic metabolic disorder caused by absolute or relative resistance to insulin. Diabetes type II (non-insulin dependent) manifests in 90% of all cases and the disease is expected to affect over 300 million people over the next 20 years.

In recent decades, medicinal plants have become increasingly popular in the treatment of diabetes. This is due to their inhibitory effects on key carbohydrate enzymes, such as pancreatic α-amylase and intestinal α -glucosidase. Inhibition of these enzymes significantly reduces the absorption of carbohydrates, thereby reducing the blood glucose concentration (Baron, 1998; Chethan et al., 2008; Kunyanga et al., 2012).

The functions of glucosidases in the body are numerous, requiring for the investigation of potential therapeutic inhibitors to be used in various diseases. Currently, three drugs are used therapeutically, such as anti-glucosidases: acarbose (Precose), miglitol (Glyset) and N-butyl-1-deoxynojirimycin (Zavesca). Acarbose and miglitol are used in the treatment of non-insulin-dependent type II diabetes as they reduce postprandial hyperglycemia by interfering with carbohydrate digestion (de Melo et al., 2006). As a result of the administration of these preparations, undesirable side effects occur.

Therefore, the search for other, both efficacious and safe α-glucosidase inhibitors of natural origin are sought to develop as functional agents for the treatment of diabetes. The main advantages of natural substances are their effectiveness, low frequency of side effects and cost. Extracts from different medicinal plants rich in polyphenols are very promising antidiabetic agents (Mogale et al., 2011; Anhê et al., 2013; Zhang et al., 2014).

The genus *Lycium* (Solanaceae) consists of approximately 70 species which are disjunctly distributed in temperate to subtropical regions in South America, North America, southern Africa, Eurasia, and Australia (Yaoa et al., 2018).

Goji berry fruits have been widely used in the form of concentrated extracts in different beverages and as an ingredient in yogurts for their benefits in relation to antiaging,

kidney and liver functions. Modern pharmacological research indicates beneficial effects of Goji fruits on aging, neuroprotection, general well-being, fatigue/endurance, metabolism/energy expenditure, and glucose control in diabetics, antioxidant properties, immunomodulation, antitumor activity and cytoprotection (Zhou et al., 2017). Goji fruits are rich in polysaccharides, carotenoids and related compounds, phenolic acids and flavonoids. Seven types of polyphenols, including phenylpropanoids, coumarins, lignans, flavonoids, isoflavonoids, chlorogenic acid derivatives and other constituents were isolated (Chen et al., 2018). Improved knowledge of the chemical composition of extracts from Goji berry fruits would contribute to their use on a world scale.

Several studies have reported that antioxidant molecules obtained from some *Lycium* species are responsible for many health protective benefits (Protti et al., 2017).

Therefore, it is hypothesized that Bulgarian *L. barbarum* can also exert good biological properties.

In this study, we aimed to evaluate the inhibitory effect on alpha-glucosidase activity and the antioxidant activity of extracts of fruits of *L. barbarum*, from Bulgaria. The data obtained were compared with a medicinal product containing an extract of Goji berry, where synergistic action is established.

Materials and Methods

Chemicals and reagents

Alpha-glucosidase (EC 3.2.1.20) – 19.3 U/mg from *Saccharomyces cerevisiae*, DPPH (2,2-Diphenyl-1-picrylhydrazyl), Rutine hydrate, Quercetin, Kaempferol, Myricetin, Catechin, Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), neocuproine, ammonium acetate, Copper (II) chloride dehydrate, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Iron(III) chloride hexahydrate, 3,5-Di-tert-4-butylhydroxytoluene (BHT) (products of Sigma-Aldrich Chemie), Folin–Ciocalteu's phenol reagent, formic acid, gallic acid, potassium chloride, sodium acetate were obtained from Merck (Darmstadt, Germany).

Preparation of Lycium barbarum L. extracts

Water extraction

The sample (10 g fruits) was mixed with water – at a solid/liquid ratio of 1:8. The phenolic compounds from Goji berry were extracted on a magnetic stirrer at a temperature of 25°C for 60 min and filtered through nylon cloth (double extraction).

Organic extraction

The extraction solvent was a mixture of organic solvent (acetone): water: acid (70/30/1 v/v/v). The selected acid was formic acid. The conditions for the extraction were as follows: solid/liquid ratio 1:8, on a magnetic stirrer at a temperature of 25°C for 60 min and filtered through nylon cloth (double extraction).

The collected water and organic extracts were concentrated to dry substance using a rotary evaporator at a temperature not exceeding 50°C.

Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to determine total phenolic content as described by Singleton & Rossi, 1965. The absorbance readings were taken at 760 nm using UV-VIS spectrophotometer DU 800 (Beckman Coulter ®, Brea, CA, USA) after incubation for 5 min at 50°C. Gallic acid was used as a reference standard. The results were expressed as milligram gallic acid equivalent per 100 g sample (GAE/100 g sample).

HPLC analysis of flavonols and flavanols

Flavonols composition and flavanols content of the extracts were assessed with an HPLC analysis using the chromatographic system VWR La Prep Σ (Knawer, Germany) which consists of LP 1100 HPLC pump, a LP 3104 UV absorbance detector, a column Chromolith® Performance RP-18e (100 x 4.6 mm x 2 µm), Merck, Germany. The managerial chromatography system and data processing used EZChrome Elite, the software of Agilent. The column temperature was maintained at 25°C. Mobile phase A was methanol, mobile phase B – acetonitrile and mobile phase C – water. The isocratic programme was as follows: 0-15 min, 46% A to 12% B to 42% C. The injection volume was 20 µl, the mobile phase flow was 0.78 ml/min and the detection wavelength was 360 nm for flavonols and 280 nm for flavanols. The samples were determined by the retention time of rutin, myricetin, quercetin, kaempferol and catechin standards.

α-Glucosidase assay

The α-glucosidase's inhibitory activity was determined according to the procedure of Dewi et al. (2007). Measurements were performed on 800 DU spectrophotometer (Beckman Coulter®, Brea, CA, the U.S.). One unit of enzymatic activity (U) is defined as the amount of enzyme which releases one µmol of p-nitrophenol per minute under the assay conditions.

Antioxidant Activity Assay

The antioxidant activities of the samples were determined using FRAP, CUPRAC and DPPH methods.

The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow (Fe^{3+}-$(TPTZ)_2$) complex to the blue (Fe^{2+}-TPTZ) complex by electron donating substance under acidic condition (Benzie & Strain, 1996). The 2850 µl of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 150 µl of the extracts were

allowed to react. Maximum absorbance values at 593 nm were recorded for 10 min at 37°C.

The CUPRAC assay was performed as described by Apak et al. (2004). The method involves mixing the solutions of 10 mM $CuCl_2$, 7.5 mM neocuproine, 1 M ammonium acetate at pH 7, and 50 µl of different extracts and measuring the absorbance at 450 nm after 60 min.

Radical scavenging activity (RSA) of extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed spectrophotometrically at 517 nm. The assay was done according to a method reported by Brand-Wiliams et al. (1995). A DPPH solution (80 µM) was freshly prepared in methanol. A volume of 2 ml of this solution was allowed to react with 150 µl of sample extracts in various concentrations and the absorbance was measured after 15 minutes in dark.

The final absorbance was compared with the standard curve in the range of 25 to 500 µM Trolox, dissolved in methanol. The data were expressed as µM Trolox equivalent/g sample (µM TE/g sample).

Results and Discussion

It can be seen from Table 1 that the water extraction has a higher content of polyphenolic substances than with the acetone. The amount of total polyphenols in the organic extraction is of the same order as in the water extraction. As a result, we cannot distinguish the two derived extracts from the fruits of Goji berry. When using the extractants, catechins and rutin are successfully isolated, but myricetin, quercetin and kaempferol are not extracted. About 50% more rutine and catechin are isolated when using acetone as an extractant.

According to Termentzi et al. (2006) and other authors, the better solvent for extraction of polyphenols from the fruits of Goji berry is the organic solvent. According to Benchennouf et al. (2016) of *L. barbarum* fruits cultivated in Greece, fractions rich in polyphenols were obtained by extraction with water, ethyl acetate, dichloromethane and butanol. The highest polyphenol content is the ethyl acetate fraction – 109.72 mg GAE/g of dry fruit, while the water content is 14.13 mg GAE/g dry fruit. Differences in the polyphenol content of Goji berry extracts cultivated in Bulgaria depends on the cultivation area and agroecological conditions.

HPLC analysis of goji berry water and organic extracts was conducted. Based on these qualitative HPLC analyses, catechin was among the major flavanol compounds – 27.1 mg/g sample for acetone extract, followed by water extract (Table 1). The flavonol content in Goji berry was represented by glycoside rutin – 3.2 mg/100 g sample for acetone extract. The other flavonols aglycones myricetin, quercetin and kaempferol were present in traces in the two extracts (an amount of less than 0.1 mg/100 g sample). This data coincided with the data of other authors (Protti et al., 2017, Wojdyło et al., 2018).

Influence of Goji berry extracts on α-glucosidase activity from Saccharomyces cerevisie

Table 1. *Total phenolic, flavanols and flavonols content in different extracts of Goji berry.*

	Water extraction	**Acetone extraction**
TPC (mg GAE/100 g sample)	8.04±0.5	5.46±0.3
Catechin (mg/g sample)	14.7±0.5	27.1±0.2
Rutin (mg/100 g sample)	1.4±0.09	3.2±1.9
Myricetin (mg/100 g sample)	*	*
Quercetin (mg/100 g sample)	*	*
Kaempferol (mg/100 g sample)	*	*

Legend: The contents of the corresponded compounds found in an amount of less than 0.1 mg/100 g sample

Studies conducted on the inhibitory effect of Goji berry extracts are attempts to detect naturally occurring glucosidase inhibitors with increased potency and fewer side effects to available drugs, such as acarbose and miglitol.

In vitro, the inhibition effect on α-glucosidase has shown that the resulting aqueous and acetone extracts rich in polyphenols from Goji berry fruits have an inhibitory effect on the enzyme.

The inhibitory effect of Goji berry extracts was conducted in the range of 0.445 to 0.089 mg GAE/g of fruit. The effectiveness of α-glucosidase inhibition ranged from 91.7 µg GAE/g of fruit to 259.6 µg GAE/g of fruit. The extract obtained with water showed a dose-dependent effect on the α-glucosidase inhibition. The minimum inhibitory concentration of the aqueous extract is IC_{50}=259.6 µg GAE/g of fruit (Figure 1).

In determining the α-glucosidase inhibitory potential, significant differences were observed between the extracts

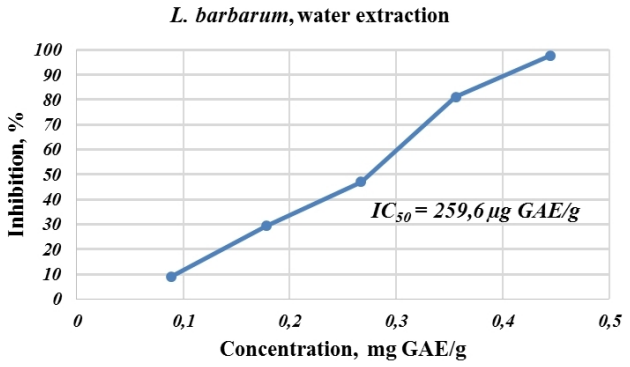

Figure 1. *Inhibitory effect of aqueous extract from Goji berry on α-glucosidase activity of Saccharomyces cerevisiae*

obtained. Acetone extract exhibits inhibitory properties at concentrations significantly lower than those in aqueous

extraction. The 50% inhibitory concentration found for this extract is IC_{50}=91.7 µg GAE/g of fruit as shown in Figure 2.

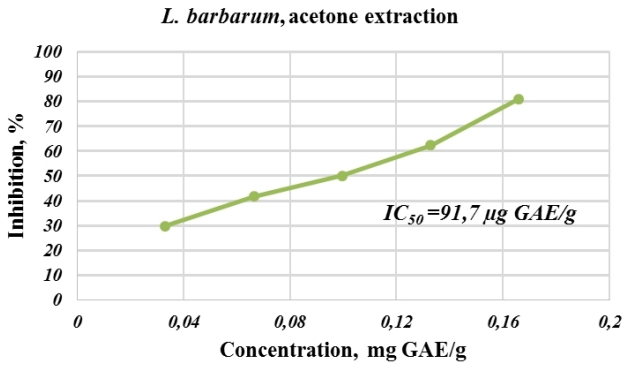

Figure 2. *Inhibitory effect of acetone extract from Goji berry on α-glucosidase activity of Saccharomyces cerevisiae*

McDougall et al. (2005) reported pomegranate, strawberry, raspberry, lingonberry, black currant, and blueberry extracts as effective inhibitors of carbohydrate enzymes, such as α-amylase and/or α-glucosidase. Inhibition of these enzymes is specifically useful in the treatment of non-insulin-dependent diabetes, as it slows down glucose release into the bloodstream. Guowen et al. (2010) treated mice with *L. barbarum* extracts and observed significantly reduced blood glucose levels as compared with a diabetic control group.

As a control in determining an inhibitory effect on α-glucosidase activity, acarbose is used (Chiasson, 2002). Acarbose has been found to have an IC_{50}=1.9 mg/ml. Comparison of α-glucosidase activity in acarbose with the activity of extracts obtained with different extractors indicates that Goji berry extracts, which are polyphenolic rich, are much better inhibitors.

To prevent or slow down the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Different plants contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics and carotenoids, and may help protect cellular systems from oxidative damage and also may lower the risk of chronic diseases. The hypothesis that dietary antioxidants lower the risk of chronic disease was determined from epidemiological studies (Allen & Tresini, 2000; Liu, 2004).

Various methods were used to evaluate the antioxidant properties of the extracts obtained. Diverse in vitro methods (DPPH, FRAP and CUPRAC) based on different mechanisms were used. There is still no standard method for determining the effects of different radicals. In addition, the analyzed samples are composed of a large number of individual compounds, making it difficult to evaluate them, as well as the methods used to measure the total antioxidant activity.

In Figure 3, the dependence of the antioxidant activity of the extracts on the total amount of polyphenols is presented. It has been found that the aqueous extract of Goji berry is more abundant in total polyphenols than the acetone extract. As a result, it is found that the antioxidant activity of the extracts correlates with the total amount of polyphenols.

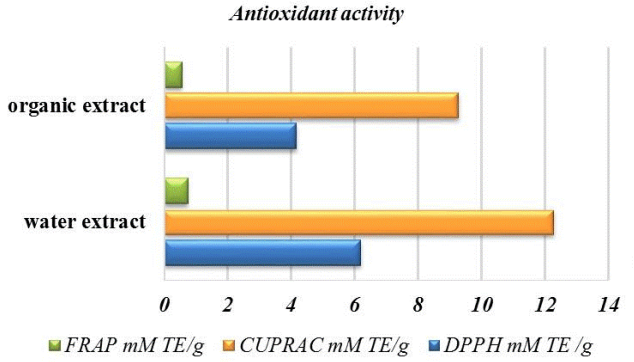

Figure 3. *Antioxidant activity of Goji berry extracts*

The data obtained for the antioxidant activity of both the extracts determined by the FRAP method coincides with the data of other authors (Henning et al., 2014), who also found that the reduction of Fe (III) to Fe (II) is in the range of about 0.9 mM TE/g.

Antioxidant activity determined by DPPH radicals was in the range of 4.2 mM TE/g to 6.2 mM TE/g. The antioxidant activity against the DPPH of the dietary supplement is determined. In addition, the different species and varieties of fruit have different phytochemical profiles. Determination of the antioxidant activity of the supplement is presented as an IC_{50}=0.81 µM TE/capsule. One component of this supplement is the Goji berry extract. Based on the results obtained, the combination of Goji berry and the other ingredients shows a synergistic effect in antioxidant activity. The synergistic effect of the combination with Goji berry is responsible for the powerful antioxidant effect in the dietary supplement.

Conclusions

In the present study, the bioactive polyphenols, antioxidant and the enzyme-inhibitory properties of Goji berry were evaluated. The results indicate that cultivated fruits of Goji berry from Bulgaria can be used as a valuable source of bioactive compounds in further functional foods or health-promoting formulations. The study demonstrated that some of the polyphenols in the investigated extracts exhibit competitive properties towards the enzyme. Enzyme inhibitory properties of Goji berry demonstrate that the extracts are potential natural glucohydrolase inhibitors.

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