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Partial Purification of β–glucosidase enzyme from soybean (Glycine max) and determination of inhibitory effects two quercetin derivatives on enzyme activity

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Abstract. Glucosidases are enzymes that catalyze the hydrolysis of the glycosidic linkage of glycosides, leading to the formation of a sugar hemiacetal or hemiketal and the corresponding free aglycon. Activity of glucosidases is crucial for several biochemical processes. Thus, discovery of new glucosidase inhibitors is crucially important owing to potential therapeutic applications of this enzyme in the treatment of diabetes, human immunodeficiency virus infection, metastatic cancer, lysosomal storage disease etc. In the current study, inhibitory potential of 'quercetin' and its isomeric form 'morin hydrate' on the activity of β-glucosidase enzyme, present in the extract of soybean (*Glycine max* L.) seeds, were investigated. The compounds exhibited moderate inhibitory action in low milimolar concentrations. I_{50} values were calculated as 0.188 and 0.138 mM for quercetin and morin hydrate, respectively. The results have confirmed that these compounds can be used as leads for designations of novel glucosidase inhibitors which would be used in medicinal biotechnology and food science and technology.

Key words: beta–glucosidase, inhibition, quercetin, morin hydrate, soybean.

INTRODUCTION

Glucosidases (glycoside hydrolase) are enzymes that catalyse the hydrolysis of glycosidic bonds to form monosaccharides and oligosaccharides and are involved in rearrangement of glycoproteins, glycoconjugates and polysaccharides.

β-glucosidases fall into GH1, GH3, GH5, GH 9 and GH30 families of glycoside hydrolases. Among these, the family containing the largest number of characterized β-glucosidase is GH1 (Niemeyer, 1988; Henrissat, 1991; Henrissat & Davies, 1997). In terms of diversity, β-glucosidases exhibit their biological functions most extensively in plants. It was shown that about 40 GH1 β-glucosidases are expressed in a typical plant (Xu et al., 2004; Opassiri et al., 2006).

β-glucosidases have become the focus of many studies due to their key roles in many biological and biotechnological processes such as growth and development, defense and signaling mechanisms, biomass conversion, nutrient detoxification and nutritional quality. β-glucosidases are of great importance especially for the biomass conversion process. β-glucans including cellulose are globally the most abundant renewable biomass resources and β-glucosidase is the key element for conversion of β-glucans (Gilbert et al., 2008). Some other features of β-glucosidases make them useful for various industrial fields; such as improvement of nutritional quality, flavour and

stability in food sector (Opassiri et al., 2006; Nguyen et al., 2010; Chandra et al., 2013; Souza et al., 2014) bioavailability enhancement in pharmaceutical industry (Kim et al., 2013; Handa et al., 2014), as well as synthesis of certain oligosaccharides and glycosides as food supplements through their ability of catalysing reverse hydrolysis and transglycosylation reactions (Sa´nchez-Pe´rez et al., 2008; Pal et al., 2010; He et al., 2013).

Quercetin is a well known and remarkable flavonoid with regard to its biological protective activities and one of the most potent antioxidants among polyphenols, which is found in many herbal sources including apple, citrus, red grape, onion, tea, etc (Fig. 1) (Formica & Regelson, 1995; Rice–Evans et al., 1997; Prior, 2003). Quercetin is also known to posses antiviral, antibacterial, anticarcinogenic and antiinflammatory effects, and several isoforms of quercetin like aglycone and rutin glycosides in different doses are commercially available as supplements for supportive treatment of many diseases and disorders (Formica & Regelson, 1995; Di Carlo et al., 1999; Harborne & Williams, 2000).

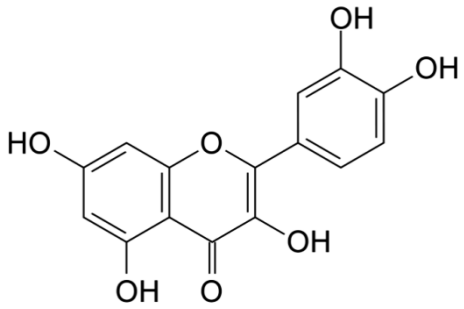

Figure 1. Structural formula of quercetin.

Morin hydrate is another attractive bioflavonoid, which is a polyphenolic compund in yellow crystalline structure, found in white mulberry, almond, sweet chestnut and some other fruits (Fig. 2). Morin hydrate serves a variety of pharmacological activities including free radical scavenging, anti–inflammatory effect, protection against DNA damage and low density lipoprotein oxidation, anticancer properties, making it beneficial for therapeutic applications of diabetes, cardiovascular and neurodegenerative diseases (Gopal, 2013).

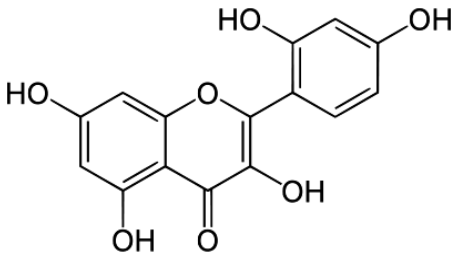

Figure 2. Structural formula of morin hydrate.

The objective of this study is to determine the potential effects of mentioned flavonoids, quercetin and morin hydrate, on soybean β-glucosidase enzyme as novel inhibitors. There has been a growing interest in manipulating the activity of glucosidase enzymes, in consequence of their important roles in many metabolic processes. Several kinds of chemically synthesized or naturally isolated glucosidase inhibitors are of great concern (Berecibar et al., 1999; Asano, 2003; Kim et al., 2006; Li et al., 2006; Pandey et al., 2006, Khalaf et al., 2015) as valuable biochemical tools and potential therapeutic agents, making substantial contributes to reveal the activity of the enzyme, understanding structures of potential inhibitors and discovery of compounds with a variety of promising applications.

MATERIALS AND METHODS

Materials

All chemicals used in soybean extraction, enzyme inhibition and purification steps, including p-Nitrophenyl β-D-glucopyranoside (p-NPG), dipotassium phosphate, quercetin, morin hydrate, sodium chloride, ammonium sulphate, ethanol, hydrogen chloride, sodium hydroxide were obtained from Sigma-Aldrich Co.

Plant material

Raw seeds of a soybean (*Glycine max* L.) genotype supplied by Ondokuz Mayıs University, Faculty of Agriculture were used for extraction.

Extraction and *in vitro* glucosidase inhibition assay

Soybean seeds were powdered in liquid nitrogen, following a physical fractionation. B-glucosidase activity was determined pursuing the procedure described by Ribeiro et al. (2006), with slight modifications. 100 mg of ground soybean sample was kept in a 0.05M phosphate buffer (pH 4.5), containing 0.1M NaCl, for 1 h at 4 °C. Following a centrifugation step, supernatant was filtered and directly used for further analyses.

For β-glucosidase activity, p-nitrophenyl-β-D-glucopiranoside (p-NPG) was used as substrate. 500 µl of p-NPG in 0.1M phosphate buffer (pH 5.0) was transferred to cuvette and pre-heated in a water bath for 5 min at 30 °C. Enzymatic reaction was initiated by adding 125 µl of supernatant containing β-glucosidase into the cuvette and final volume was completed to 1ml with distilled water; and then the absorbance value at the beginning of the reaction was read in a spectrophotometer at 420 nm. Samples in the cuvette were again put in the water bath for 10 min, at 30 °C. Final absorbance value was read in the spectrophotometer after this duration, to be compared with the initial value and was recorded as 100% control activity in the absence of an inhibitor. For inhibition assays, 1mM solutions of quercetin and morin hydrate were also included in the cuvette mixture. Different volumes of these compunds were added into the mixture in order to increase the inhibitor concentartion gradually; and equal volumes of water was diminished from the mixture. A graphic consisting of percent activity versus natural molecule concentration was drawn for each of the compounds. Cuvette contents used in the β-glucosidase activity tests are shown in Tables 1, 2.

Table 1. Details of cuvette components used to determine I_{50} values of quercetin flavonoid on soybean β-glucosidase

p-NPG/ K_2HPO_4 (pH 5) (µl)	Soybean extract (µl)	H_2O (µl)	Inhibitor (Quercetin) (µl)	Inhibitor concentration (mM)	Total volume (ml)
500	125	375	–	–	1
500	125	340	20	0.020	1
500	125	325	35	0.035	1
500	125	300	60	0.060	1
500	125	275	150	0.150	1
500	125	225	200	0.200	1

Table 2. Details of cuvette components used to determine I_{50} values of morin hydrate flavonoid on soybean β-glucosidase

p-NPG/ K_2HPO_4 (pH 5) (µl)	Soybean extract (µl)	H_2O (µl)	Inhibitor (Quercetin) (µl)	Inhibitor concentration (mM)	Total volume (ml)
500	125	375	–	–	1
500	125	355	20	0.020	1
500	125	340	35	0.035	1
500	125	305	70	0.070	1
500	125	245	130	0.130	1
500	125	225	150	0.150	1

Partial purification of the enzyme

Soybean extract was precipitated with ammonium sulfate, where the total concentration varided between 10–100%. Precipitates of every stage were collected by centrifugation at 11,000 rpm for 30 min and redissolved in 0.05M K_2HPO_4 buffer (pH 4.5). All the process was carried out on ice. The highest enzyme activity value for soybean β-glucosidase was observed at 30–40% concentration interval.

RESULTS AND DISCUSSION

Effects of flavonoid compounds on soybean β-glucosidase

The potential inhibitor compounds quercetin and morin hydrate inhibited β-glucosidase at millimolar levels. I_{50} values of flavonoids quercetin and morin hydrate were calculated as 0.188 and 0.138, respectively. Observed percent activity values of enzyme in response to different concentrations of both compounds are presented in Tables 3, 4, and Figs 3, 4.

Table 3. Effect of flavonoid quercetin on soybean β-glucosidase enzyme activity

Concentration (mM)	Activity %
0.000	100.0
0.020	93.8
0.035	82.2
0.060	73.4
0.150	56.0
0.200	48.3

Table 4. Effect of flavonoid morin hydraye on soybean β-glucosidase enzyme activity

Concentration (mM)	Activity %
0.000	100.0
0.020	92.7
0.035	85.8
0.070	64.0
0.130	54.9
0.150	42.2

Activity of glucosidases, including β-glucosidase, fulfils key roles in many biochemical processes. Their functions in the organism are also associated with several diseases and disorders; thus discovery of potential inhibitors of glucosidases as therapeutic agents against diabetes, obesity, viral infections, lysosomal storage diseases and cancer has been a challenging subject (Kordik & Allen, 1999; Platt & Butters, 2000; Lillelund et al., 2002; Papandréou et al., 2002; Gunasekaran et al., 2014).

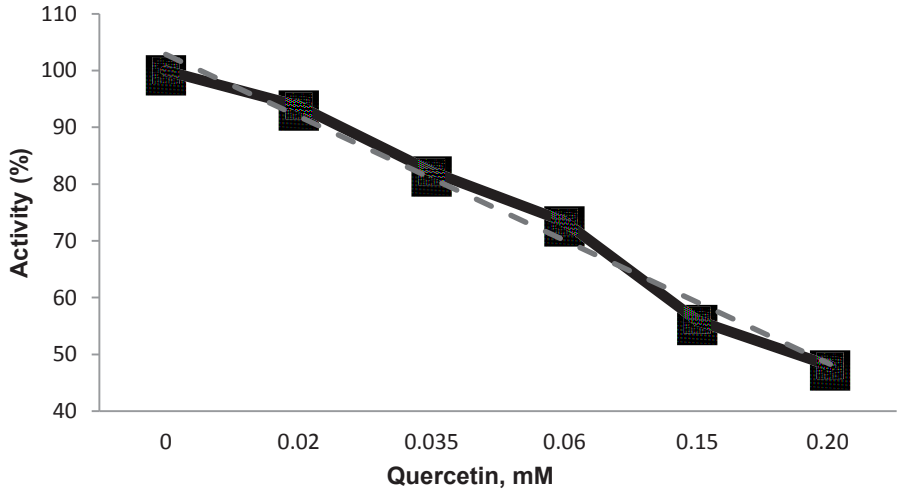

Figure 3. Effect of quercetin on soybean β-glucosidase enzyme activity.

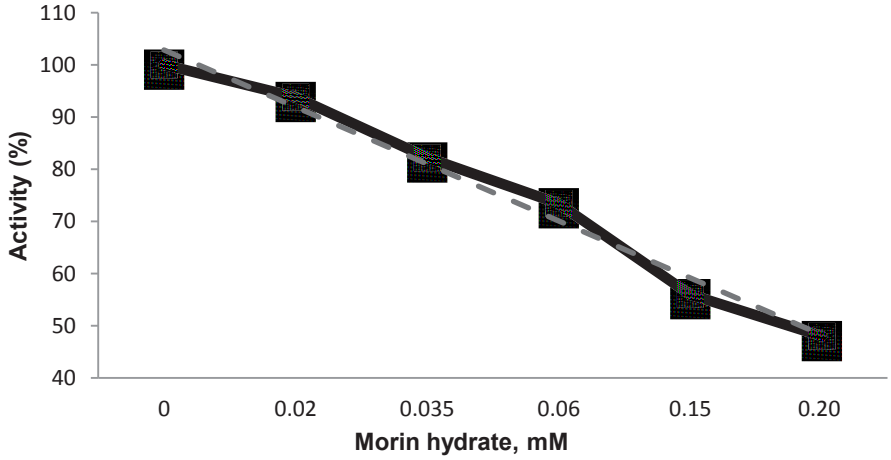

Figure 4. Effect of morin hydrate on soybean β-glucosidase enzyme activity.

Altering or blocking particular metabolic activities, glucosidase inhibitors served for understanding functions of glucosidases and enabled novel approaches to be utilized in several fields, other than medicine. For instance, glucosidase inhibitors can also function as agricultural chemicals such as antifungals insecticides (Asano, 2003).

CONCLUSIONS

Our study aimed to examine the inhibitory potential of two common compounds in nature, flavonoid quercetin and its isomeric form morin hydrate, on soybean β-glucosidase enzyme. The enzyme was partially purified, and both of the natural compounds were able to inhibit the β-glucosidase enzyme within the extract of soybean seeds at low concentrations.

Inhibitors of glucosidases are basically in glycosidic structure. In common to the two flavonoids investigated in this research, compunds which do not apparently bear structural similarities to carbohydrates constitute a new category of inhibitors and understanding of their functions and mechanisms are crucial for providing new approaches in terms of dicovery of new therapeutic agents. Inhibition of key enzymes by natural molecules has been recently the basis of pharmacology, biochemistry and chemistry research. Concerning the therapeutic potential of glucosidase inhibitors, current study provides precious information for further investigations.

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