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Chaenomeles Sinensis: A Potent α-and β-Glucosidase Inhibitor

[1]Shruti Sancheti, [1]Sandesh Sancheti and [1,2]Sung-Yum Seo
[1]Department of Biology, Kongju National University, Kongju 314-701, Korea
[2]Korean Collection of Herbal Extract, Inc., Kongju 314-701, Korea

Abstract: Problem statement: Glycosidase inhibitors are vital sources for the treatment of diabetes type II with a special importance in pharmacology, food industry and biotechnology, since for diabetes control, different diets and drugs, especially herbal medicines are recommended in this era. **Approach:** While screening for the potent natural glycosidase inhibitors, we found the fruits of *Chaenomeles sinensis* (*C. sinensis*), as the most effective glycosidase inhibitor. The crude 80% methanolic extract of the fruits and its n-hexane, methylene chloride, ethyl acetate, n-butanol and aqueous fractions were further investigated for α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase enzyme inhibition activities. **Results:** All the *C. sinensis* extracts showed remarkable α-glucosidase and β-glucosidase inhibitory activities (at a concentration of 5 μg 210 μL reaction^{-1}) ranging from 82-99 and 5-85%, respectively. Among all the inhibition studies, n-butanol fraction demonstrated the highest (99%) α-glucosidase inhibitory activity, whereas minor α-galactosidase (18-35%) and β-galactosidase (10-34%) inhibitions were examined in all the fractions of *C. sinensis*. **Conclusion:** *C. sinensis* fruits may prove as potent natural anti-diabetic source with noteworthy α-glucosidase and β-glucosidase inhibitions, because the inhibition of these enzymes provide a strong biochemical basis for the management of type II diabetes by controlling glucose absorption. These results provide intense rationale for further animal and clinical studies.
Key words: *Chaenomeles sinensis*, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase

INTRODUCTION

Diabetes Mellitus (DM) is a major chronic life-threatening disorder, in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin; resulting in an increased blood glucose level. DM is a serious metabolic disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. As per WHO, at least 171 million people worldwide have diabetes, which is likely to be more than double by 2030 and around 3.2 million deaths every year are attributable to complications of diabetes; six deaths every minute[1].

From the basic two types of DM, one being insulin dependent (type I) and another non-insulin dependent (type II), later is the most prevalent form and is developing with as increase in obesity and aging in the general population[2]. In recent years, there has been renewed interest in the DM treatment using herbal drugs as they are generally non-toxic and World Health Organization has also recommended the evaluation of the effectiveness of plants in condition where we lack safe modern drugs. Plant derivatives with hypoglycaemic properties have been used in folk medicine from very ancient time[3]. Despite the introduction of hypoglycaemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem to people[4]. Medicinal plants used to treat hyperglycemic conditions are of considerable interest to ethno botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant. So, traditional herbal therapies by indigenous systems of medicine for treating this ailment are being intensively explored.

In continuing this investigation, we assessed the various enzyme (such as, α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase) inhibition activities on Korean herbal plants, as these enzymes are involved in a variety of biochemical processes related to metabolic disorders and diseases, such as diabetes, viral or bacterial infections, lysosomal storage disorders and cancer. Therefore, an exhausted attempt has been given on the design of efficient glycosidase inhibitors for many promising applications[5-8]. In our screening, we found *C. sinensis* as the most promising candidate for further scrutiny.

Corresponding Author: Sung-Yum Seo, Department of Biology, Kongju National University, Kongju, Korea
Tel: 82-41-850-8503 Fax: 82-41-854-8503

The plant *Chaenomeles sinensis* (Thouin) Koehne (also called as *Pseudocydonia sinensis* (Thouin) CK Schneid, common name: Chinese-quince, ma gua) belonging to Rosaceae family is mainly distributed in Korea, China and Japan. Its fruits have been shown to have higher amounts of phenolics. This high phenolics content can be expected to act as a factor for improvement in health[9]. Its fruit is used as traditional Chinese medicine to treat throat diseases. It also reported antiinfluenza, antioxidant, antiulcerative, antiviral, antihemolytic, antipruritic and antibacterial (against *Streptococcus pyogenes* (which is a causative agent of tonsillitis and pharyngitis) activities[9-11].

To our knowledge, there are no prior reports discussed on the anti-diabetic activity of *C. sinensis.* Therefore, the objective of this study was to investigate the anti-diabetic potential of the crude extract of *C. sinensis* and its various fractions by confirming the *in vitro* enzyme inhibition studies on glycosidase.

MATERIALS AND METHODS

Plant material: The dried and matured fruits of *C. sinensis* were obtained from "Korean Collection of Herbal Extracts" a Biotech company in Korea. A collection of voucher specimen is available with the company (Korea Collection of Herbal Extracts, 2000).

Extraction: The dried and ripe fruits were chopped into small pieces and pulverized into a fine powder. The powdered *C. sinensis* fruits (2 Kg, dry weight) were kept for extensive decoction in 80% methanol for 2 months at room temperature. The extract was then concentrated using rotary vacuum evaporator at 20-30°C to obtain the dried crude extract (210 g).

Fractionation: The crude methanolic extract (210 g) was suspended in distilled water (1L) and partitioned with n-hexane, methylene chloride, ethyl acetate and n-butanol to yield the n-hexane (17 g), methylene chloride (12 g), ethyl acetate (26 g), n-butanol (77 g) and aqueous (64 g) fractions, respectively. The enzyme inhibition activity assays were performed using 500 μg mL^{-1} concentrations for the crude extract and various fractions.

Reagents: α-Glucosidase (from *Saccharomyces cerevisiae* type I), β-glucosidase (from almonds), α-galactosidase (from green coffee beans), β-galactosidase (from *Escherichia coli*), 4-nitrophenyl α-D-glucopyranoside, 4-nitrophenyl β-D-glucopyranoside, 4-nitrophenyl α-D-galactopyranoside, 2-nitrophenyl β-D-galactopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other commercially available reagents and solvents were used as received.

Enzyme assays: The enzyme inhibition activities for α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase were evaluated according to the method previously reported by Shibano *et al*.[12] with minor modifications.

α-Glucosidase assay: The reaction mixture consisted 50 μL of 0.1 M phosphate buffe r (pH 7.0), 25 μL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μL of test sample (concentration: 500 μg mL^{-1}) and 25 μL of α-glucosidase solution (a stock solution of 1 mg mL^{-1} in 0.01 M phosphate buffer, pH 7.0 was diluted 0.04 Units mL^{-1} with the same buffer, pH 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100 μL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using microplate reader. All experiments were carried out in triplicates.

β-Glucosidase assay: The reaction mixture consisted 50 μL of 0.1 M phosphate buffer (pH 7.0), 25 μL of 0.5 mM 4-nitrophenyl β-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μL of test sample (concentration: 500 μg mL^{-1}) and 25 μL of β-glucosidase solution (a stock solution of 1 mg mL^{-1} in 0.01 M phosphate buffer, pH 7.0 was diluted 0.04 Units mL^{-1} with the same buffer, pH 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100 μL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using microplate reader. All experiments were carried out in triplicates.

α-galactosidase assay: The reaction mixture consisted 50 μL of 0.1 M phosphate buffer (pH 7.0), 25 μL of 0.5 mM 4-nitrophenyl α-D-galactopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μL of test sample (concentration: 500 μg mL^{-1}) and 25 μL of α-galactosidase solution (a stock solution of 1 mg mL^{-1} in 0.01 M phosphate buffer, pH 7.0 was diluted 0.04 Units mL^{-1} with the same buffer, pH 7.0 just before assay). This reaction mixture was then incubated at 37°C

for 30 min. Then, the reaction was terminated by the addition of 100 μL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 410 nm using microplate reader. All experiments were carried out in triplicates.

β-Galactosidase assay: The reaction mixture consisted 50 μL of 0.1 M phosphate buffer (pH 7.0), 25 μL of 0.5 mM 2-nitrophenyl β-D-galactopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μL of test sample (concentration: 500 μg mL^{-1}) and 25 μL of β-galactosidase solution (a stock solution of 1 mg mL^{-1} in 0.01 M phosphate buffer, pH 7.0 was diluted 0.04 Units mL^{-1} with the same buffer, pH: 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100 μL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of o-nitrophenol released in the reaction mixture at 410 nm using microplate reader. All experiments were carried out in triplicates.

Statistical analyses: All assays were performed at least three times with triplicate samples. The inhibition rates for all the assays were calculated as a percentage of control (buffer containing MeOH) without inhibitor. All results are expressed as mean±SD.

RESULTS

The crude extract of *C. sinensis* and its various fractions were evaluated at 5 μg 210 μL^{-1} well concentrations for α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase enzyme inhibition studies.

All the *C. sinensis* extracts showed remarkable α-glucosidase inhibitory activities (at a concentration of 5 μg 210 μL $reaction^{-1}$) ranging from 82-99% and are detailed here (Fig. 1): n-butanol (99%) > water (97%) > ethyl acetate (92%) > n-hexane (91%) > crude extract (89%) > methylene chloride (82%).

Furthermore β-glucosidase inhibition results are: water (85%) > crude extract (58%) > ethyl acetate (48%) > methylene chloride (40%) > n-butanol (37%) > n-hexane (5%). This shows the outstanding activity of β-glucosidase inhibition in water extract only (Fig. 2).

The less significant α-galactosidase (18-35%) and β-galactosidase (10-34%) inhibitions were examined in all the *C. sinensis* fractions (Fig. 3 and 4).

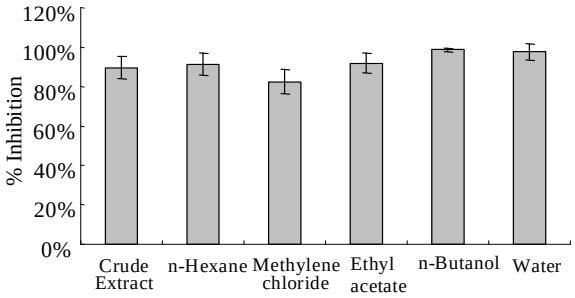

Fig. 1: α-Glucosidase inhibition by crude extract of *C. Sinensis* and its fractions at 5 μg 210 μL^{-1}

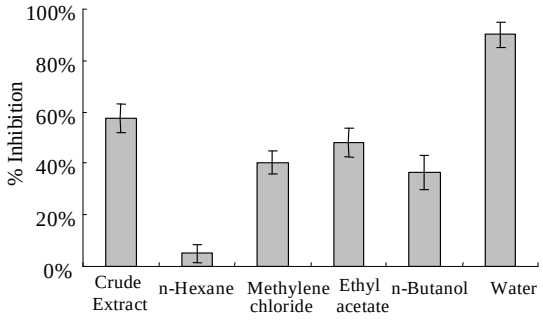

Fig. 2: β- Glucosidase inhibition by crude extract of *C. Sinensis* and its fractions at 5 μg 210 μL^{-1}

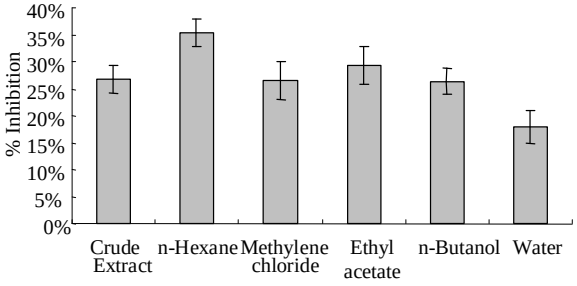

Fig. 3: α-Glucosidase inhibition by crude extract of *C. Sinensis* and its fractions at 5 μg 210 μL^{-1}

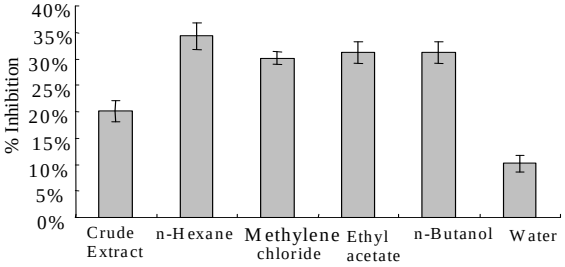

Fig. 4: β- Glucosidase inhibition by crude extract of *C. Sinensis* and its fractions at 5 μg 210 μL^{-1}

DISCUSSION

Glycosidase inhibitors are the important tools for studying the mechanisms of action of glycosidases and are also prospective therapeutic agents for some degenerative diseases, including diabetes, viral attachment and cancer[2,7,8]. As synthetic glycosidase inhibitors have many side/adverse effects, herbal medicines are being the center point for the researchers in the current era.

Therefore, in this study, for the first time, the *C. sinensis* crude extract (in 80% methanol) and its fractions were investigated for glycosidase inhibition activities. The present enzyme inhibition studies demonstrated that, the *C. sinensis* fruit extract is a potent novel α-glucosidase inhibitor with moderate β-glucosidase inhibition and lower α- and β-galactosidase inhibitions. The α-glucosidase inhibition may be related to the high phenolic content and antioxidant property of the fruits[9,10].

Therefore based on this *in vitro* study, we recommend that, the C. *sinensis* fruits may have beneficial effects in managing type II diabetes mellitus.

CONCLUSION

The results of the *in vitro* enzyme inhibition studies emphasize the potent effect of the *C. sinensis* fruit extract on diabetes treatment. Based on this, further pre-clinical and clinical studies can be pursued.

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