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## Hypoglycemic activity of *Pyrus bioessieriana Buhse* leaf extract and arbutin: Inhibitory effects on alpha amylase and alpha glucosidase

### Abstract

**Background:** The mechanism of hypoglycemic and hypolipidemic activities of *Pyrus bioessieriana Buhse* leaf extract (PbBLE) and its phytochemical component arbutin, have not been well determined. The present study was performed to understand the hypoglycemic activity mechanisms of pbBLE and arbutin more clearly.

**Methods:** In vitro enzymatic carbohydrate digestion with PbBLE and arbutin was assessed using  $\alpha$ -amylase and  $\alpha$ -glucosidase powders. The enzyme solutions were premixed with PbBLE and arbutin at different concentrations (0.1, 1, 10 and 100 mg/ml). Substrate solutions and colorimetric reagents were added to the reaction. The release of glucose was determined by spectrophotometric method. Acarbose was used as the positive control.

**Results:** The extract (10, 100 mg/ml) completely inhibit  $\alpha$ - amylase and  $\alpha$ - glucosidase activities. The extract produced higher reduction of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity than arbutin. Inhibition at various concentrations (0.1, 1, 10, 100 mg/ml) were significantly different ( $p < 0.05$ ).

**Conclusion:** Our results exhibited that both the extract and arbutin were able to suppress the enzymes strongly.

**Keywords:** Hypoglycemic; *Pyrus bioessieriana Buhse* extract; Arbutin;  $\alpha$ -Amylase/  $\alpha$ -glucosidase inhibitors

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**D**iabetes mellitus (DM) is a lifelong disease and there is not yet a cure, but symptoms and problems can be prevented (1). DM is a chronic disease, resulting from insulin deficiency or decreased responsiveness of the organs to insulin or both (2) and has a significant impact on health, quality of life and life expectancy of patient, as well as on the healthcare system (3). Two types of DM are currently known, Type 1, insulin-dependent diabetes mellitus (IDDM), and type 2, non-insulin-dependent diabetes mellitus, (NIDDM) (3). The patients with type 1 DM are absolutely dependent upon insulin for life but the management of type 2 often needs combined regimens, including diet, medicine and exercise (4). The progressive nature of the disease necessitates continuous reassessment of glycemic control in people with diabetes and appropriate adjustment of therapeutic regimen. Due to the life-long treatment, prohibitive cost and unavailability of treatment in rural areas, the disease is a great burden to the patients (1). Herbal medicines have received significant attention because of their effectiveness, availability, fewer side effects and relatively low cost (5). Carbohydrates are the major constituents of the human diet (6). Pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase digest carbohydrates and facilitate absorption of monosaccharide such as glucose and fructose. One of the therapeutic approaches for reducing blood glucose in patients with diabetes is to prevent the absorption of carbohydrates after food uptake (7). In this regard,  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors play a critical role in reducing the postprandial increase of blood glucose level after a mixed carbohydrate diet (8-10).

In addition, the commercially available drugs for DM (such as acarbose) are limited by gastrointestinal disturbance effects (11). Therefore, there is an urgent need for searching therapies that may have less severe or no side effects.

In this study, we have selected *Pyrus bioessieriana Buhse* leaf extract (PbBLE) and its phytochemical components, arbutin, to evaluate their  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory properties. *Pyrus bioessieriana Buhse* (locally known as wild pear) is a species of pear that is native to Iran. The leaves are used for treatment of inflammation of the bladder, bacteriuria, high blood pressure, urinary stones and also used as diuretic (12). The dried leaves of this tree contain a considerable amount of arbutin that is abundant in a number of different plant species including Ericaceae and Rosaceae (*Pyrus bioessieriana Buhse*) (13). Arbutin is a glycosylated hydroquinone that inhibits melanin formation by blocking tyrosinase, it also has been shown to have antioxidant (14), antihyperglycaemic and antihyperlipidemic properties (15).

In this study, *in vitro* models were used to understand the mechanism of action of PbBLE and its active component on carbohydrate metabolism.

## Methods

**Materials:**  $\alpha$ -amylase from porcine pancreas,  $\alpha$ -glucosidase type I from bakers yeast, acarbose, arbutin, p-nitrophenyl  $\alpha$ -D-glucopyranoside and 3, 5 dinitrosalicylic acid, potato starch were purchased from Sigma-Aldrich (USA).

**Plant material and preparation:** The leaves of *Pyrus bioessieriana Buhse* were obtained from their natural habitat in the north of Iran; Babol. The plant material was identified and authenticated by the Mazandaran Department of Agricultural Sciences and Natural Resources. The authenticated dried leaves of *Pyrus bioessieriana Buhse* were extracted with aqueous methanol (63%) for 36 h room temperature. The extract was evaporated to dryness under reduced pressure with a rotator evaporator. The dried residue was dissolved in water and used for *in vitro* experiments (13, 15).

**Assay for  $\alpha$ -amylase inhibitory activity:** The  $\alpha$ -amylase inhibitory assay was performed by the methods of Tingting Wu (16) and Monica R. Louizzo (17) with a little modification. The  $\alpha$ -amylase solution was prepared by mixing 1 mg of enzyme in 1 ml phosphate buffer (0.002 M

sodium phosphate buffer, pH 6.9 with 0.006M sodium chloride). The colorimetric reagent was prepared by mixing sodium potassium tartrate solution (12.0 g sodium potassium tartrate tetrahydrate in 8.0 ml of 2M NaOH) and 90 mM of 3, 5 dinitrosalicylic acid solution (0.2 g of 3, 5 dinitrosalicylic acid in 10 ml of distilled water) 1:1 (v/v). The methanolic extract of *Pyrus bioessieriana Buhse* and arbutin at different concentrations (0.1, 1, 10 and 100 mg/ml) were premixed with  $\alpha$ -amylase solution and then were pre incubated in 25°C for 10 minutes. The reaction was initiated by adding 1% starch solution dissolved in sodium phosphate buffer. The mixtures were incubated at 25°C for another 10 minutes. After incubation, the reaction was stopped with 200 $\mu$ l of colorimetric reagent. The test tubes were maintained in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted by adding 4 ml distilled water and absorbance was measured at 540nm.

The absorbance of the sample blanks (buffer instead of enzyme solution) and the control (buffer in place of samples) were recorded as well. Acarbose was used as the positive control. The inhibitory activity of the extract and arbutin was calculated by the following equation:

Inhibition activity (%) =

$$\left( \frac{OD\ 540nm, control - OD\ 540nm, test}{OD\ 540nm, control} \right) \times 100$$

**Assay for  $\alpha$ -glucosidase inhibitory activity:** The  $\alpha$ -glucosidase inhibitory assay was performed according to the procedures reported by Suresh V. Nampoothiri (6) with modifications. The methanolic extracts at various concentrations (200 $\mu$ l) and  $\alpha$ -glucosidase (20 $\mu$ l, 10 U/ml) in 0.005M phosphate buffer (pH 6.8) were mixed and incubated for 5 minutes at 37°C. Para-nitrophenyl- $\alpha$ -D-glucopyranoside (1mM, 200 $\mu$ l) was added to initiate the reaction and the mixture was further incubated at 37°C for 20 minutes. The reaction was terminated by the addition of 500 $\mu$ l of 1M Na<sub>2</sub>CO<sub>3</sub> and diluted by adding 500 $\mu$ l of distilled water. The absorbance was recorded at 405nm. Acarbose was used as the positive control.

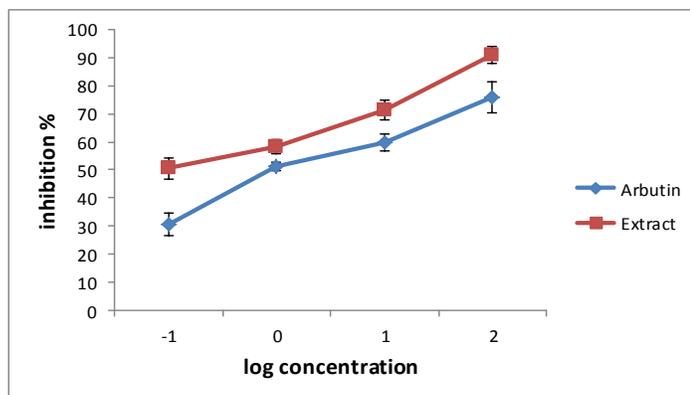
Inhibition activity (%) =

$$\left( \frac{OD\ 405nm, control - OD\ 405nm, test}{OD\ 405nm, control} \right) \times 100$$

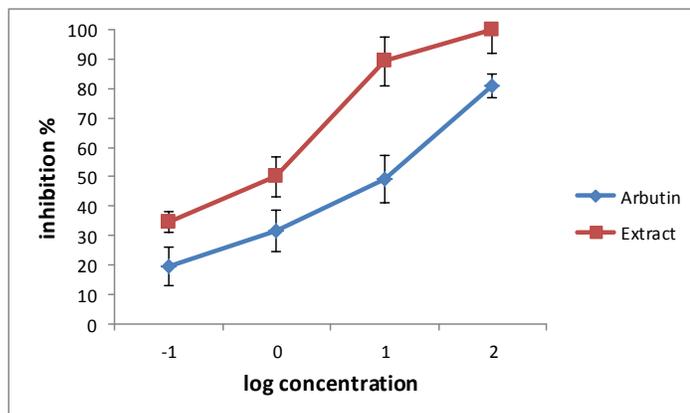
**Statistical analysis:** Each experiment was performed in triplicate. The results are presented as mean  $\pm$  standard deviation (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA) with subsequent post hoc comparisons by LSD (SPSS 16.0). The criterion for statistical significance is expressed as  $p < 0.05$ .

## Results

Both the extract and arbutin exhibited inhibitory activity in a dose-dependent manner (figures 1, 2). An almost complete inhibition of the  $\alpha$ -amylase and  $\alpha$ -glucosidase activities were obtained at 10 and 100mg/ml dosages of the extract, respectively. Arbutin, at the highest concentration was used (100mg/ml), inhibited only 75% of  $\alpha$ -glucosidase activity and 81% of  $\alpha$ -amylase activity.



**Figure 1.**  $\alpha$ -glucosidase inhibitory activities of the extract and arbutin. Inhibition by various concentrations are significantly different ( $p < 0.05$ )



**Figure 2.**  $\alpha$ -amylase inhibitory activities of the extract and arbutin. Inhibition by various concentrations are significantly different ( $p < 0.05$ )

## Discussion

Diabetes mellitus is an endocrine disease that is developing along with an increase in both obesity and ageing in the general population (2). The treatment aim of diabetic patients is to maintain near normal levels of glycemic control, in both the fasting and postprandial states. In particular,  $\alpha$ -amylase and  $\alpha$ -glucosidase participate in glucose digestion and are considered as key enzymes that can control postprandial hyperglycemia. In this present study, we have investigated the inhibitory effects of PbBLE and arbutin on  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. Several biological activities of PbBLE and arbutin have been described by some colleagues. According to the previous studies, PbBLE has an antioxidant activity in serum, liver, kidney and pancreas in normal rats (18). In addition, PbBLE can reduce the glucose diffusion and absorption through dialysis bag in laboratory models (19). In other studies, the antibacterial, antifungal, antilarvae, antioxidant, anticholinestrase (13) and antihyperglycemic activities of PbBLE and arbutin have been performed (15). Arbutin also has protective effects against tert-butyl hydroperoxid induced toxicity in hepatic cell lines (20). In this *in vitro* experiment, we focused to study the possible mechanism of PbBLE and arbutin as  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors.

The results of this *in vitro* experiment showed that PbBLE were able to inhibit both the enzymes significantly. Arbutin also showed strong inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase, though less potent than the extract, that it can be attributed to other compounds found in the extract such as flavonoids, phenols and glycosides (21). Some of these inhibitors have been used in clinical trials, for example, in a randomized design, *Salacia oblonga* extract was fed to a number of healthy subjects and results show a great reduction in plasma glucose and insulin. Also, breath hydrogen excretion was 60% greater ( $P < 0.001$ ) compared with control (22). At the same time, other studies have reported the high efficacy and safety of  $\alpha$ -glucosidase inhibitors added to metformin compared with metformin alone in patient with type 2 diabetes (23).

Daily consumption of *Reliv Glucaffect* was found to statistically significantly be lower blood fasting glucose from 145.3 mg/dL to 101.1 mg/dL and body weight from an average of 88.5 kg (BMI 26.8 kg/m<sup>2</sup>) to 81.3 kg (BMI 24.5 kg/m<sup>2</sup>) as compared to the control group (24). Our research is the first study of *Pyrus brossieriana* Buhse and its active

component, arbutin, in relation to their  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities. Based on the results presented here, we can claim that besides any other possible mechanism of action, PbBLE and arbutin have inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase. With these results, we can further support the traditional use of the plants based on their inhibitory activities on glucose absorption in the intestine.

The weakness of this study was related to the old fashioned equipment used in colorimetric assay. This study confirmed the potential anti-diabetic activity of the PbBLE and arbutin, focusing on the inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase. In our *in vitro* model, the results give scientific support to the use of *Pyrus bhosseriana* Buhse leaf extract and arbutin for the treatment of diabetes, however, further investigation is required to validate its use prior to clinical implementation as therapeutic agent.

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**Conflict of Interest:** There was no conflict of interest.

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