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The Inhibitory Activity of α-Glucosidase in Methanol Extract of Some Antidiabetic Medicinal Plants in Sulaymaniyah Province

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ABSTRACT

Background and Objective: Control of hyperglycemia after meals is an important strategy in the management of type II diabetes, and the reduction of chronic complications associated with it. Therefore, inhibitors of carbohydrate-degrading enzymes such as α-glucosidase can be useful in the treatment of this disease. The aim of this study was to evaluate the α-glucosidase inhibitory activity in some traditional medicines in Sulaymaniyah province of Iraq.

Methods: Eight plant species were prepared with advisory from authentic spiceries in Sulaymaniyah. After air drying and preparation of their methanolic extracts, enzyme microplate assay was conducted on them in four different extract concentrations (1, 0.1, 0.01 and 0.001 mg/ml) along with negative and positive controls. Finally, enzyme kinetic analysis was performed on effective extracts.

Findings: Among the eleven studied plant species, *Rubus idaeus* L., *Rheum ribes* R. and *Salix alba* L. extracts at 1 mg/mL concentration showed highest inhibitory activity (98.13±1.05% (IC_{50}=0.046 mg/mL), 93.39±2.11% (IC_{50}=0.085 mg/mL) and 84.46±1.12% (IC_{50}=0.125 mg/mL), respectively) against α-glucosidase,. After kinetic analysis of α-glucosidase inhibition, *Rubus idaeus* L., *Rheum ribes* R. extracts showed mixed type of inhibition (competitive- uncompetitive) while extract of *Salix alba* showed uncompetitive type of inhibition.

Conclusion: The leave extract from both *Rubus idaeus*, *Salix alba* and root extract of *Rheum ribes* are remarkable α-glucosidase inhibitors, and may be used in the treatment of type II diabetics after clinical tests.

Keywords: *Diabetes, α-glucosidase inhibitor, Rubus idaeus L., Salix alba L., Rheum ribes R.*.

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Introduction

In the gastrointestinal tract, complex carbohydrates are digested by multiple breakdown reactions into monosaccharides which are absorbed in the small intestine. The digestion process begins with the secretion of amylases produced mainly by the pancreatic and salivary glands and catalyze the hydrolysis of starch into shorter polysaccharides. The final step in carbohydrates metabolism is mediated by α-glucosidase in the brush border of the enterocytes (1-3). α-Glucosidase inhibitors delay the digestion and absorption of complex carbohydrates in small intestine, and prevents sudden increase in after meal plasma glucose levels in people with type II diabetes (3, 4).

To date, more than 100 α-glycosidase inhibitors, including Castanospermine, Swanosonine, Trehazolin and Validamine have been isolated from plants and microorganisms (5). After a series of extensive clinical trials, Acarbose was introduced as a commercial drug in Germany in 1990 to treat diabetes (6). In 1996, Miglitol was patented by the US Food and Drug Administration under the brand name Glyset, and in 1999 it was recognized as the second inhibitor of the enzyme α-glucosidase with the least gastrointestinal side-effects (7). Due to the fact that the continuous use of these inhibitors causes many side-effects such as diarrhea, abdominal pain, flatulence and hepatotoxicity, it is necessary to develop new inhibitors with fewer side-effects. In recent decades, extensive efforts have been made to obtain α-glucosidase inhibitors from plant sources.

Brindis et al. evaluated the anti-hyperglycemic properties of aqueous extracts of the leaves and stems of *Coriandrum sativum* L. in normal rats and its anti α-glucosidase activity to confirm its use in folk medicine (8). Son et al. found that the hexane extract of the root of *Cudrania tricuspidata*, at a concentration of 300 mg/ml inhibits 77% of the activity of the α-glucosidase (9). Also, the results of a study by Pegah et al. showed that resveratrol and probiotics are effective in controlling diabetes by increasing GLP-1 levels and reducing insulin resistance (10). Oh et al. shows the inhibitory activity of alcoholic extract of tea plant and its pomace on α-glucosidase both in vitro and in vivo (11). Trinh et al. showed that by studying some native plants of Vietnam and their inhibitory activity on α-glucosidase, these plants can be effective in treating diabetes (12). Alam et al. showed in vitro antioxidant and α-glucosidase inhibitory activity of methanolic extract from *Clinacanthus natans* (13). Mohd Bukhari et al. showed that several samples of Malaysian plants act as anti-diabetic drugs by inhibiting α-glucosidase (14). In a study conducted by Zarei et al., the extracts of *Campanula involucrate, Hypericum scabrum* L., *Salvia suffruticosa* and *Silene ampulata* Bioss, showed more than 60% α-glucosidase inhibitory activity (15). Considering the virginity of medicinal plant resources in local cultures, the aim of this research was to search for potential α-glucosidase inhibitors in the extracts of a number of medicinal plants in Sulaymaniyah province of Iraq.

Methods

In this laboratory study, p-Nitrophenyl α-D-glucopyranoside (pNPG), Saccharomyces cerevisiae α-glucosidase (EC:3.2.1.20), acarbose, bovine serum albumin (BSA), and sodium carbonate were purchased from sigma Chemical Co. and other chemicals were purchased from Merck Co. Samples of eight plant species were prepared with advisory from authentic spiceries in Sulaymaniyah city. At the end of spring, aerial organs of the plants were collected from certain areas of range lands of Sulaymaniyah, under supervision of herbalists (Table 1). After systematic registration in Agricultural and Natural Resources Research Center of Sulaymaniyah, plants were completely dried in the shade and safe conditions at 25˚C. After complete drying, the plant materials were crushed into smaller pieces and turned into a soft powder by a household electric grinder.

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20 grams of the powder was put in a dark glass container to which 200 ml methanol was added. This mixture was stored at room temperature for 72 hours with stirring at certain intervals. The mixture was filtered by Whatman filter paper grade 42. Filtrate was concentrated by rotary evaporator at 68 °C and 50 rpm for 30 minutes. The concentrated extract was air drayed on 15 cm clock glass under the chemical hood. The extracts were collected by a clean blade and stored in 1.5 mL micro tubes in -20 °C until further use.

Table 1. Specifications of the studied plants

No.	English name	Scientific plant Name	Botanical family Name	Parts used	Location of plant		Place of plant
					N°	E°	
1	Raspberry	*Rubus idaeus L*	Rosaceae	Leaves	34°56'56''	45°44'11''	Sartak
2	Rhubarb	*Rheum ribes R*	Polygonaceae	Roots	35° 07'37''	45° 41'14''	Isma'il Store
3	White willow	*Salix alba L*	Salicaceae	Leaves	34°56'54''	45°43'39''	Sartak
4	Colocynth	*Citrullus colocynthis*	Cucurbitaceae	Fruit	34°56'14''	45°41'29''	Qaslan
5	Olives	*Olea europaea L*	Oleaceae	Leaves	34°56'18''	45°41'29''	Qaslan
6	Basil	*Ocimum basilicum L*	Lamiaceae	Leaves	35° 07'18''	45°40'37''	Zmnako
7	Okra	*Abelmoschus esculentus*	Malvaceae	Fruit	35°03'43''	45°39'48''	Bani khelan
8	Black mulberry	*Morus nigra*	Rosaceae	Leaves	35° 06'12''	45° 41'06''	Khelan

The assay was conducted on the activity of α-glucosidase by the modified Pistia-Brueggeman method with brief changes (16). All assays were directed in 96-well micro plates with a final volume of 200µl for each well, and using Tekan (Sunrise model) micro plate reader and performed as follows: 50µl of phosphate buffer (100 mM, pH=6.6), 50µl of plant extract at various concentrations (1, 0.1, 0.01, 0.001 mg/ml) and 50µl of enzyme solution of α-glucosidase (0.2 U/ml in phosphate buffer) was added to the test wells. Blank well contained all test well materials except the enzyme (50 µl of buffer was added instead). After 5 min incubation at 37°C, 50µl of p-Nitrophenyl α-D-glucopyranoside (p-NPG) substrate was added to both test and blank wells. After incubation for 30 minutes at 37 °C, 50µl of 0.1 M Sodium carbonate was added to stop the reaction. The absorbance was read at 405 nm. The measurements were performed in three replicates in the presence of negative and positive controls. In positive control, 1 mg/ml of Acarbose solution were used instead of the extract. At the end of the measurements, the empty plate absorption was subtracted from the absorption of the corresponding wells and also the absorption of the blank wells from the absorption of the fractional test wells. As a result, the final absorption is only the result of enzyme activity. For negative control, the final absorbance was calculated in the same way. The percentage of enzyme inhibition was calculated using the following equation:

$$\text{Inhibition percentage} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

The IC_{50} values for each extract were calculated using linear regression analysis of each percent inhibition plot. IC_{50} is the concentration of the inhibitor that causes 50% of the enzyme inhibition (17). Kinetic analysis was finally performed to determine the type inhibition of plant extract against α-glucosidase. Determination of inhibition of plant extracts against α-glucosidase activity was measured by four different concentrations of p-NPG substrates (0.311, 1.24, 2.48 and 3.11 mM) in the absence and presence of plant extracts at different concentrations. The type of inhibition was determined by Lineweaver-

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Burk plot analysis based on calculated data after Michaelis-Menten kinetics. For control and each concentration of inhibitor, V_{max} and K_m were determined.

Results

Since acarbose is one of the most common inhibitors of α-glucosidase, it was used as positive control in this study. Inhibitory activity of acarbose was measured at 7 different concentrations (Figure1), IC_{50}= 0.004 mg/mL.

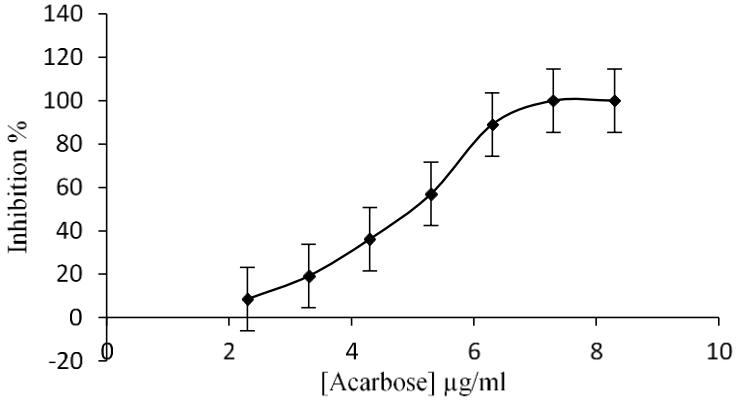

Figure 1. Variations in α-glucosidase percentage of inhibition vs. different concentrations of Acarbose

Table 2 shows the results of α-glucosidase inhibitory activity of methanolic extract of plant organs, in 4 concentrations. As shown, for all of the plant extracts, the highest inhibition percentage was related to the 1 mg/ml concentration.

Table 2. α-glucosidase percent inhibition for different aerial plant organs

No.	Botanical name	Family	Organ	Extract concentrations (mg/mL)							
				1		0.1		0.01		0.001	
				%I	σ	%I	σ	%I	σ	%I	σ
1	*Rubus idaeus*	Rosaceae	Leaves	98.13	1.05	47.18	1.2	17.51	0.31	16.82	0.41
2	*Rheum ribes* R	Polygonaceae	Roots	93.39	2.11	28.31	0.93	15.14	0.56	19.57	0.73
3	*Salixalba* L	Salicaceae	Leaves	84.46	1.12	25.62	0.56	21.77	0.85	17.40	1.16
4	*Morus nigr*a	Rosaceae	Leaves	68.95	2.15	26.41	1.53	19.02	0.14	12.99	0.48
5	*Abelmoschus esculentus*	Malvaceae	Fruit	23.66	0.70	15.12	0.85	12.23	0.81	15.36	0.19
6	*Citrullus colocynthis*	Cucurbitaceae	Fruit	19.15	1.44	19.74	0.33	17.04	0.38	15.05	0.74
7	*Olea europaea* L	Oleaceae	Leaves	18.29	0.97	13.85	0.71	15.52	0.57	0.29	0.034
8	*Ocimum basilicum* L	Lamiaceae	Leaves	10.05	0.59	4.57	0.36	2.67	0.09	1.2	0.063

To determine the type of inhibition, Lineweaver - Burk plots were drawn for methanolic plant extracts with highest inhibitory effect (Figures 2-4).

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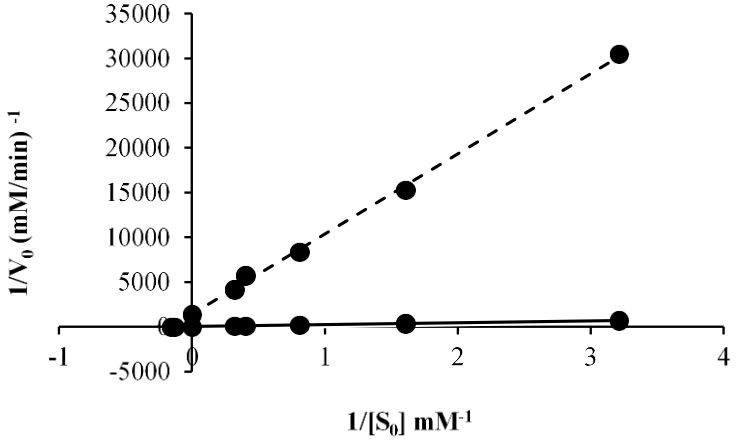

Figure 2. Lineweaver - Burk plot of α-glucosidase inhibition in the presence of *Rubus idaeus* L leaf extract (concentration of 1 mg/mL)

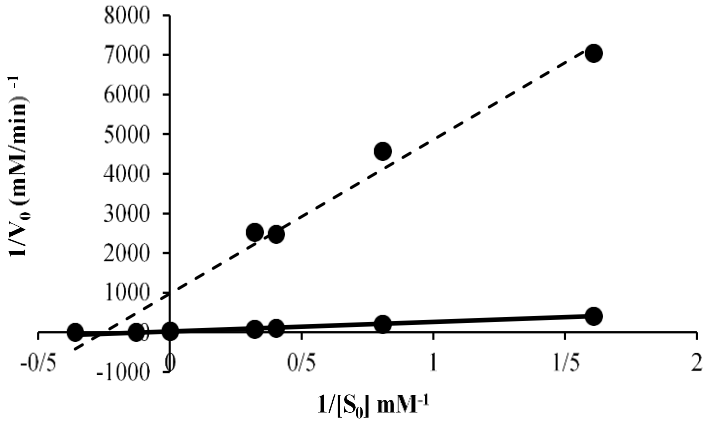

Figure 3. Lineweaver - Burk plot of α-glucosidase inhibition in the presence of *Rheum ribes* R root extract (concentration of 1 mg/mL)

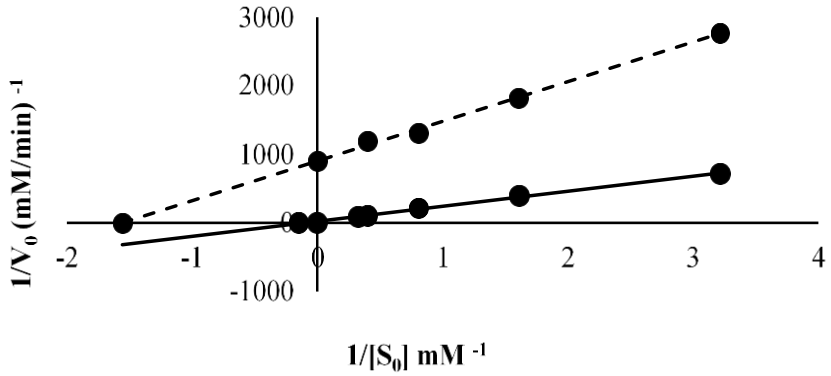

Figure 4. Lineweaver - Burk plot of α-glucosidase inhibition in the presence of *Salix alba* L leaf extract (concentration of 1 mg/mL)

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Based on the analysis results of the above plots, for *Rubus idaeus* plant leaf extract and *Rheum ribes* plant root extract, the type of inhibition was mixed (competitive - uncompetitive) and in the case of *Salix alba* leaf extract, it was uncompetitive inhibition type. Finally, the actual and apparent kinetic parameters of Michaelis enzyme constant (K_m) and maximum speed (V_{max}) were calculated in the absence and presence of methanol extracts of plant extracts (Tables 3 and 4).

Table 3. Changes in synthetic parameters of α-glucosidase at a concentration of 1 mg/ml of plant extracts

No	Botanical name	Family	Organ	Km (mM)	Km^{app} (mM)	Vmax (mM/min)	$Vmax^{app}$ (mM/min)
1	*Rubus idaeus* L	Rosaceae	Leaves	7.76±0.80	6.41±0.54	0.037±0.013	0.00071±0.00024
2	*Rheum ribes* R	Polygonaceae	Roots	7.87±0.76	2.78±0.19	0.035±0.006	0.00076±0.000086
3	*Salix alba* L	Salicacaee	Leaves	6.77±0.88	0.64±0.028	0.031±0.003	0.0011±0.00041

Table 4. Type of inhibition applied on α-glucosidase activity

No	Botanical name	Family	Organ	Type of inhibition
1	*Rubus idaeus* L	Rosaceae	leaves	Uncompetitive-noncompetitive
2	*Rheum ribes* R	Polygonaceae	Roots	Uncompetitive-noncompetitive
3	*Salix alba* L	Salicacaee	leaves	Uncompetitive

Discussion

In this study, the synthetic analysis showed that the inhibition type of the methanolic extract prepared from *Rubus idaeus* L. and *Rheum ribes* R. is of the mixed inhibition type (competitive - uncompetitive) and in the case of *Salix alba* L. extract, the classical uncompetitive type of enzyme inhibition. α-Glucosidase inhibitors are effective in improving the metabolic profile and potentially reducing the risk of long-term complications of hyperglycemia in patients with type 2 diabetes. They may be used as monotherapy or in combination with other antiglycemic drugs and insulin (18). Acarbose, voglibose, and miglitol are commercially available α-glucosidase inhibitors and are considered first-line therapy to reduce postprandial hyperglycemia. These drugs are combined with oral drugs such as metformin to control blood sugar and reduce the level of glucose bound to hemoglobin (HbA1c) (19).

The aim of this study was to find new potent inhibitors for α-glucosidase among plant extracts used as traditional remedies for diabetic patients in Sulaymaniyah province, Iraq. Eleven plant species from central regions of Sulaymaniyah province were collected after advisory from local spiceries. The methanolic extracts of these plants were tested for α-glucosidase inhibitory activity by means of microplate colorimetric method. Based on their potential inhibitory activity on α-glucosidase, they have been divided into two groups: strong and weak inhibitory plant extracts. Among them, *Rubus idaeus* L, *Salix alba* L and *Rheum ribes* R showed considerable potential to inhibit α-glucosidase.

Many studies have been done at this regard. From the Vietnamese plant *E. antiquorum*, seven α-glucosidase inhibitors were isolated and elucidated, among them ent-3α-acetoxy-16β,17-dihydroxyatisane exhibited the highest inhibitory activity against yeast α-glucosidase. The kinetic mechanism indicated that it retarded α-glucosidase in a noncompetitive manner (20). Loizzo et al. reported inhibitory activity towards digestive enzymes associated with diabetes of extracts of 9 plant species gathered in Lebanon (21). In 2012, six Philippine plants had been screened for its capability α-glucosidase inhibitory

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activity (22). Shim et al. observed the inhibitory effect of aqueous extract from the gall of *Rhus chinensis* on α-glucosidase activity (23). In 2010, Shai et al. selected six medicinal plant types for α-glucosidase inhibitory activities, and the kind of inhibition was shown as noncompetitive (24).

The results of this study show that the methanolic extract of *Rubus idaeus* L, *Salix alba* L and *Rheum ribes* R have a significant inhibitory effect on the enzyme α-glucosidase and kinetic study results showed that plants have great inhibitory activity towards α-glucosidase enzyme like other inhibitory drugs which are used for this regard.

Conflict of interest: The authors declare that there is no conflict of interest.

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