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The Inhibitory Effect of Some Iranian Plants Extracts on the Alpha Glucosidase

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Abstract

Objective
Diabetes mellitus is manifested by hyperglycaemia. Different treatments such as diets and drugs are recommended for diabetes control. For various reasons in recent years traditional plant (herbal) therapies as prescribed by indigenous systems of medicine with different mechanisms have commonly been used. The digestive enzymes such as alpha glucosidase are among these herbal remedies.
Materials and Methods
One hundred species of plants were collected or purchased from the Medicinal Herbal Markets and botanically identified. Methanolic and aqueous extracts were prepared by the maceration method. The enzymatic activities of alpha glucosidase were determined colorimetrically by monitoring the release of p-nitrophenol from the appropriate p-nitrophenol glycoside substrate, after 30 mins incubation at 37 °C in the phosphate buffer (pH= 6.8).
Results
Among 200 prepared extracts, *Verbascum kermanensis*, *Rosa damascene, Rosmarinus officinalis*, *Levisticum officinale*, *Zataria multiflora Sanguisorba minor*, *Alhagi camelorum*, *Pistacia vera*, *Vaccinium arcto-staphylus*, *Zhumeria majdae, Alpinia officinarum*, *Salvadora persica,* and *Thymus serpyllum* showed more than 50% inhibitory effect on the alpha glucosidase.
Conclusion
These active plants have no records in the literature for their anti diabetic effect and might be the new agents for diabetes control. This needs further *in vitro* and *in vivo* studies, some of which are under investigation.

Keywords: Alpha glucosidase, Diabetes, Herbals, Hyperglycemia, Inhibitors, Plant

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Introduction

Glycoside trimming enzymes are crucially important in metabolic pathways, such as glycoprotein and glycolipids processing and carbohydrate digestion in the intestinal tract. Amongst the vast variety of enzymes, glucosidases are postulated to be a powerful therapeutic target since they catalyze the cleavage of glycosidic bonds releasing glucose from the non-reducing end of an oligo- or polysaccharide chains (1). Glucosidase inhibitors are currently of interest owing to their promising therapeutic potential in the treatment of metabolic disorders such as diabetes (1).

Diabetes mellitus (DM) is a chronic disorder of metabolism caused by an absolute or relative lack of or resistance to insulin. It is characterized by exaggerated glycaemic response in postprandial and/or fasting state (2).

At the present time it is estimated that 150 million people worldwide have diabetes and this number will increase to 220 million by 2010 and 300 million by 2025. Globally, the percentage of type 2 diabetes is greater than 90% (3). Control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications (4).

One of the therapeutic approaches for reducing postprandial hyperglycemia is to prevent absorption of carbohydrates after food intake since only monosaccharides can be absorbed from the intestinal lumen and transported into the blood circulation. Complex polysaccharides must be digested by the enteric digestive enzymes including alpha glucosidase. Therefore, alpha glucosidase inhibitors such as acarbose, voglibose, and miglitol are widely used either alone, or in combination with other antidiabetic medications or insulin in patient with type 2 diabetes (5).

Screening of alpha glucosidase inhibitors from plants and synthetic sources are increasing. Inhibitors of these enzymes have recently been found from natural sources. In Mexico, 821 plant species have been registered from traditional medicine, and 51 of them have been employed as anti-diabetic agent (4).

In addition glucosidase inhibitors have therapeutic potential in the treatment of disorders such as human immunodeficiency virus (HIV) infection, metastatic cancer, and lysosomal storage diseases (1).

Therefore, search for alpha glucosidase inhibitor, especially in plants give rise to a reliable, cheap and safe medicine in the management and control of diabetes and other diseases.

Materials and Methods

Plants

Different parts of plants, such as flowers, fruits, leaves, aerial parts, roots or seeds with known or unknown antidiabetic effect were collected from various provinces throughout Iran or purchased from the medicinal herbal markets in Kerman city and all of them were botanically identified. A voucher specimen was deposited at the herbarium of the Herbal Medicines Research Center Faculty of Pharmacy, Kerman University of Medical Sciences, Iran (Table 1).

Methanolic and aqueous extracts were prepared from 20 g of air-dried tissue of each plant, pulverized by maceration in 200 ml methanol or distilled water at room temperature for 24 hrs. After filtration, extracted methanolic substrates were air dried and the aqueous extracts dried at 40 ^{o}C in incubator. The dried materials either powdered or waxy shaped compound were kept in the dark vials at -20 ^{o}C.

Enzyme assay

P-Nitrophenyl-α-D-Glucopyranoside (PNPG) and Bakers yeast alpha glucosidase were purchased (Sigma, USA). Yeast alpha glucosidase has frequently been used to investigate the inhibitory activity of traditional medicinal plants (6).

The enzymatic activities of alpha glucosidase were determined colorimetrically by monitoring the release of p-nitrophenol from the appropriate p-nitrophenol glycoside substrate (3). The assay mixture for these experiments contained 5 μmol PNPG, enzyme

solution (0.1 U), in 900 µL of sodium phosphate buffer (50 mM), pH 6.8 in the final volume of 1 ml. Each extract 100 µg was dissolved in 20 µL of distilled water and added to the test mixture before adding the substrate. Blank sample contained whole test mixture and the extract without enzyme solution. Distilled water added to the control sample (20 µL), and in the positive control 20 µL acarbose (100 µg) was enhanced.

The mixture incubated at 37 ^{o}C for 30 mins, the reaction terminated by adding 3 volumes of NH_4OH solution (0.05 M). The absorbance at 405 nm was determined by NOVA spectrophotometer (LKB, Sweden).

The inhibitory activity calculated using following formula (7):

Inhibitory activity (%) = $(OD_{control} - OD_{test}) /OD_{control} \times 100$

Each test performed 3 times and the mean value was used for the inhibitory activity of plants extracts.

Results

Positive control

Acarbose, a known drug for alpha glucosidase inhibition, at the concentration used in this study showed 51% inhibitory effect on the alpha glucosidase. The results are summarized in Table1.

Table 1: Characteristics of inhibitory effects of plants extracts on alpha glycosidase (Positive control: Acarbose (51±3)).

Plants name	Family	Used parts	Methanolic (%)	Aqueous (%)
Acantholepis orientalis	Asteraceae	Aerial parts	1±0.1	1±0.2
Achillea eriophora	Asteraceae	Aerial parts	4±1	4±1
Achillea wilhelmsii	Asteraceae	Aerial parts	4±0.2	3±0.3
Acroptilon repens	Asteraceae	Aerial parts	1±0.3	1±0.1
Alhagi camelorum	Fabaceae	Aerial parts	92±3	2±0.3
Alpinia officinarum	Zingiberaceae	Rhizomes	48±3	16±4
Arctium lappa	Asteraceae	Roots	2±0.1	80±3
Artemisia santolina	Asteraceae	Aerial parts	3±0.3	3±0.1
Berberis integrimma	Berberidaceae	Aerial parts	1±0.2	1±0.2
Berberis integrimma	Berberidaceae	Roots	2±0.4	3±0.4
Biebersteinia multifida	Gerariaceae	Aerial parts & fruits	1±0.1	3±0.2
Brassica nigra	Brassicaeae	Seeds	2±0.1	0±0
Bryonia aspera	Cucurbitaceae	Aerial parts	1±0.1	0±0
Bunium persicum	Apiaceae	Seeds	7±2	0±0
Camellia sinensis	Theaceae	Leaves	95±3	85±1
Cannabis sativa	Cannabaceae	Seeds	2±0.6	0±0
Cardaria draba	Brassicaeae	Aerial parts &flowers	2±3	3±0
Carthamus oxyacantha	Asteraceae	Aerial parts	0±0	3±0.2
Chaerophyllum khorassanicum	Apiaceae	Aerial parts	1±0.2	8±2
Cichorium intybus	Asteraceae	Roots	5±0.4	4±0.3
Cinnamomum zeylanicum	Lauraceae	Derm	100±3	98±2
Citrus aurantium	Rutaceae	Flowers	0±0	58±7
Citrus sinensis	Rutaceae	Fruits hull	0±0	5±0
Convolvulus pilosellaefolius	Concolvulaceae	Aerial parts	3±0.2	2±0.3
Cordia mixa	Boraginaceae	Fruits	3±0.3	1±0.3
Crocus sativa	Iridaceae	Leaves	1±0.5	0±0
Cuminum cyminum	Apiaceae	Seads	0±0	1±0.2
Ducrosia assadii	Apiaceae	Aerial parts	1±0.2	1±0

Table 1 contd.

Plants	Family	Used parts	Methanolic (%)	Aqueous (%)
Echium amoenum	Boraginaceae	Flowers	0±0	4±0.4
Equisetum arvense	Equisetaceae	Whole the plant	4±1	2±0.2
Eremostachys laciniata	Lamiaceae	Whole the plant	2±0.2	7±0.5
Eremurus persicus	Liliaceae	Aerial parts	2±0.3	3±1
Eremurus persicus	Liliaceae	Flowers	1±0.2	0±2
Eremurus persicus	Liliaceae	Fruits	1±0.3	2±0.9
Euphorbia hebecarpa	Euphorbiaceae	Aerial parts &flowers	9±1	4±1
Ferula Assa-foetida	Apiaceae	Aerial parts &flowers	2±1	0±0
Ferula oopoda	Apiaceae	Aerial parts	1±0.1	0±0
Ferulago angulata	Apiaceae	Aerial parts	11±1	4±2
Ficus carica	Moraceae	Leaves	3±0.3	4±0.5
Foeniculum vulgare	Apiaceae	Fruits	0±0	1±0.3
Francoeuria undulata	Asteraceae	Aerial parts	3±0.3	6±0.4
Fumaria parviflora	Fumariaceae	Aerial parts	3±0.2	3±0.3
Glycyrrhiza glabra	Fabaceae	Aerial parts	41±3	5±2
Gundelia tournefortii	Asteraceae	Aerial parts	1±0	0±0
Heracleum persicum	Apiaceae	Fruits	4±0.4	1±0.2
Hibiscus gossypifolius	Malvaceae	Flowers	0±0	1±0
Hyoscyamus senecionis	Solanaceae	Aerial parts &flowers	3±0.2	4±0.4
Hypecoum pendulum	Fumariaceae	Aerial parts	10±0.5	1±0
Juglans regia	Juglandaceae	Fruits hull	0±0	2±0.2
Juglans regia	Juglandaceae	Leaves	0±0	14±4
Laurus nobilis	Lauraceae	Leaves	10±0.9	4±0.4
Lawsonia inermis	Lythraceae	Leaves	64±4	6±2
Levisticum officinale	Apiaceae	Roots	98±2	89±3
LInum usitatissimum	Liliaceae	Seeds	0±0	0±0
Malva sylvestris	Malvaceae	Flowers	4±0.4	6±2
Marrubium anisodon	Lamiaceae	Aerial parts	3±0.2	6±0.5
Mentha longifolia	Lamiaceae	Aerial parts	3±1	30±4
Mentha piperita	Lamiaceae	Leaves	0±0	90±2
Myrtus communis	Myrtaceae	Leaves	97±3	99±3
Nepeta crispa	Lamiaceae	Aerial parts	0±0	0±0
Nepeta saccharata	Lamiaceae	Whole the plant	0±0	0±0
Nigella sativa	Ranunculaceae	Seeds	0±0	2±0.3
Onobrychis viciifolia	Fabaceae	Aerial parts	0±0	1±0.1
Otostegia persica	Lamiaceae	Aerial parts	0±0	2±0.1
Outreya carduiformis	Asteraceae	Aerial parts	0±0	0±0
Peganum harmala	Zygophyllaceae	Aerial parts	0±0	0±0
Peucedanum Aucheri	Apiaceae	Roots	0±0	2±0.2
Pimpinella anisum	Apiaceae	Seeds	0±0	2±0.1
Piper nigrum	Pipereaceae	Fruit	3±0.3	5±0.3
Pistacia vera	Anacardiaceae	Fruits hull	90±4	54±7

Table 1 contd.

Plants	Family	Used parts	Methanolic (%)	Aqueous (%)
Punica granatum	Lythraceae	Fruits hull	93±1	83±4
Quercus infectoria	Fagaceae	Galls	98±2	96±4
Rosa damascena	Rosaceae	Floret	98±1	81±3
Rosmarinus officinalis	Lamiaceae	Aerial parts	65±2	81±5
Rubia tinctorium	Rubiaceae	Roots	6±2	1±0.1
Salvadora persica	Salvadoraceae	Wood	53±5	0±0
Salvia rhytidea	Lamiaceae	Whole the plant	0±0	0±0
Scrophularia frigida	Scorophulariaceae	Aerial parts	6±2	5±0.3
Sanguisorba minor.	Rosaceae	Aerial parts	92±3	64±8
Scrophularia striata	Scorophulariaceae	Aerial parts	1±0	0±0
Solanum dulcamara	Solanaceae	Fruits	0±0	0±0
Sonchus asper	Asteraceae	Aerial parts	3±0.2	2±0.2
Sophora alopecuroides	Fabaceae	Aerial parts	3±0.4	0±0
Stachys inflata	Lamiaceae	Aerial parts	2±0.3	7±0.3
Stachys lavandulifolia	Lamiaceae	Aerial parts	6±0.4	3±0.4
Terminalia chebulla	Combretaceae	Fruits	90±3	94±2
Teucrium polium	Lamiaceae	Aerial parts	7±0.3	1±0
Teucrium scordium	Lamiaceae	Aerial parts	4±0.9	1±0
Thymus serpyllum	Lamiaceae	Aerial parts	0±0	67±3
Trigonella foenum graecum	Fabaceae	Seeds	3±0.3	0±0.5
Urtica dioica	Urticacea	Aerial parts	2±0.2	4±0.3
Urtica urens	Urticacea	Aerial parts	2±0.3	11±2
Vaccinium arcto-staphylus	Ericaceae	Fruits	98±3	49±4
Valeriana hispida	Valerinaceae	Rhizomes	1±0.9	1±0.4
Verbascum kermanensis	Scrophulariaceae	Leaves	52±3	72±9
Verbascum songaricum	Scrophulariaceae	Aerial parts	0±0	1±0.9
Zataria multiflora	Lamiaceae	Aerial parts	0±0	89±3
Zhumeria majdae	Lamiaceae	Leaves	63±4	29±4
Zingiber officinale	Zingiberaceae	Rhizomes	10±3	5±1
Ziziphus spina-christi	Rhamnaceae	Leaves	72±4	50±3

Extracts with 75-100% inhibitory effect on the alpha glucosidase (high potency)

We found that both methanolic and aqueous extract of *Levisticum officinale, Myrtus communis, Rosa damascene, Terminalia chebulla, Punica granatum, Quercus infectoria, Camellia sinensis, Cinnamomum zeylanicum*, had more than 75% inhibitory effect on alpha glucosidase. In addition, the aqueous extract of *Arctium lappa, Mentha piperita, Rosmarinus officinalis, Zataria multiflora* and methanolic extract of *Alhagi camelorum, Pistacia vera, Sanguisorba minor Vaccinium arcto-staphylus* had shown more than 75% inhibitory activity on alpha glucosidase.

Extracts with 50-75% inhibitory effect on the alpha glucosidase (moderate potency)

In our study the aqueous and methanolic extracts of *Verbascum kermanensis* and *Ziziphus spina-christi* and the aqueous extract of *Sanguisorba minor, Thymus serpyllum, Citrus aurantium* and methanolic extract of *Salvadora persica, Rosmarinus offinalis, Zhumeria majdae, Lawsonia inermis* showed 50%-75% inhibitory effect on the alpha glucosidase.

Extracts with 25-50% inhibitory effect on the alpha glucosidase (low potency)

The aqueous extract of *Zhumeria majdae, Verbascum kermanensis* and methanolic extracts of *Glycyrrhiza glabra* and *Alpinia officinarum,* had 25%-50% inhibitory activity on the alpha glucosidase.

Extracts with less than 25% or no inhibitory effect on the alpha glucosidase

Aqueous extract of *Juglans regia, Urtica urens* and methanolic extracts of *Laurus nobilis, Hypecoum pendulum* demonstrated 14%, 11%,10% and 10% inhibitory activity respectively and the rest of plants extracts showed <10% or no inhibitory activity in this study. No extract was found to enhance the enzyme activity.

Discussion

Postprandial hyperglycemia could induce the non-enzymatic glycosylation of various proteins; resulting in the development of chronic complications. Therefore, control of postprandial plasma glucose levels is critical in the early treatment of DM and in reducing chronic vascular complications (4). Inhibition of enzymes involved in the metabolism of carbohydrates including alpha-glucosidase is one of the therapeutic approaches for reducing postprandial hyperglycemia (5) .

Screening of plants for enzyme inhibitors such as angiotensin converting enzyme (ACE) and alpha glucosidase despite their natural constituents, have long been performed (8, 9). Although these plants are rich in tannin contents which may have an effect on the enzyme activity, no attempt have been made to relate these activities to their tannin content. Still many compounds have shown to be responsible for their functional activities. In this research there was no intention to correlate the activity of the crude extract to any active compound. Instead there were found many plants with high content of tannin which showed no or less inhibitory activity of glucosidase, showing no direct correlation with their tannin content as found by Cope *et al* (10). Beside that the inhibition was almost specific to this enzyme and when β-glucosidase, β-galactosidase and alpha mannosidase were examined, no inhibitory effect was found against these enzymes (results not shown).

In most cases the *in vitro* results of active plants showed a very good correlation with that of *in vivo* studies (paper submitted). Linewiver-burk plot analysis also showed a non competitive inhibition mode (results not shown) that is another phenomena for unique inhibitory action of the extract regardless of tannin content.

In this study we found 38 plants extracts with more than 50% inhibitory effect on the alpha glucosidase which might be effective in the diabetes control, Also this study manifested the dose dependent inhibitory effect of *Levisticum Officinale* on the postprandial glucose level.

Myrtus communis L. leaves as well as the volatile oil obtained from the leaves have been used to lower the blood glucose level in type-2 diabetic patients in Turkish folk medicine (11) and methanolic extract of *Terminalia chebula* are being used extensively in Indian herbal medicine. Oral administration of the extracts reduced the blood sugar level significantly in normal and in alloxan diabetic rats (12).

A prospective epidemiological study which was done in Japan found that men and women who drink 6 or more cups of green tea *(Camellia sinensis*) per day had one-third lower incidence of type 2 diabetes mellitus over 5 years (13). In another study, administration of green tea suppressed the elevation of serum glucose level significantly in non-diabetic rats (14).

In our study, aqueous and methanolic extract of *Myrtus communis, Terminalia chebula* and *Camellia sinensis* had a strong inhibitory effect on the alpha *glucosidase* (more than 90%). This may indicate that at least one reason for the reduction of blood glucose level by these plants. It could be consequence of a decrease in glucose absorption by the inhibiting this enzyme.

The extract of *Pistacia atlantica* have shown significant alpha amylase inhibitory activity (15). In our study the methanolic extract from fruits hull of *Pistacia vera* has

strong inhibitory effect (97%) on the alpha glucosidase, whereas its aqueous extract had moderate potency (61%). The difference between two methods of extraction could be due to different constituents of the fractions as seen for antioxidant properties of *Myrtus communis* L. extracts (16).

Glycyrrhiza glabra reduced the hyperphagia and polydipsia but did not alter the hyperglycaemia or hypoinsulinaemia (17). In contrast, Kimura reported that the water extract of *Glycyrrhiza glabra* markedly lowered blood glucose levels in diabetic animals (18).

We showed that methanolic extract of *Glycyrrhiza glabra* had low inhibitory activity (40%) for alpha glucosidase whereas its aqueous extract showed no inhibitory effect. *Zizyphus spina-christi* is one of the plants commonly used in Egyptian folk medicine for the treatment of different diseases (19). It has been shown that treatment of normal rats produced insignificant changes in some enzymes involved in carbohydrate metabolism. However, in diabetic rats, treatments with this herb significantly reduced the serum glucose level, liver phosphorylase and glucose-6-phosphatase activities, and significantly increased the serum pyruvate level and liver glycogen content (19).

In this study the aqueous and methanolic extract of *Zizyphus spina-christi* showed 53% and 76% inhibitory effect on the alpha glucosidase, respectively. So this plant might have other mechanism for reduction of blood glucose.

Aqueous extracts of *Cinnamomum zeylanicum* can reduce blood glucose concentration in diabetic rats and it seems that the cinnamon effect is probably linked to the potentiating action of cinnamon on insulin (20). Whereas part of this phenomenon could be related to a very strong inhibitory effect of this plant on the alpha glucosidase, showed in this study.

In Ayurvedic medicine almost all parts of *Punica granatum* (PG) have been used for the treatment of numerous disorders. Only PG flowering part (PGF), however have been recommended in Unani literature as a remedy for diabetes. PGF extract demonstrated a potent inhibitory effect on the alpha-glucosidase activity. The inhibition was dependent on the concentration of enzyme and substrate, as well as on the pretreatment length of the enzyme (3) which are similar to our findings that showed by fruits derm of *Punica granatum* and this part of palnts have strong inhibitory effect on the alpha glucosidase.

Arctium lappa (21) and *Mentha piperita* (22) demonstrated anti diabetic effect. We showed that the aqueous extract of these have strong inhibitory effect on the alpha glucosidase and seems reasonable that at least one mechanism for their antidiabetic effect is an inhibitory action.

Trigonella foenum greacum, have been reported to be beneficial for treating type 2 diabetes. The stimulating or regenerating effect on beta cells or extra pancreatic effects were proposed for the hypoglycemic action of this herb (23). In contrast, *Trigonella foenum greacum* had no inhibitory effect on the alpha glucosidase in our study. This phenomenon was true for *Urtica dioica* (24), *Ficus carica* (25), *Peganum harmala* (26), *Laurus nobilis*(27), *Teucrium polium*(28), *Zingiber officinale* (29) and *Cuminum cyminum* (30) which previously had shown anti diabetic effect. So other alternative mechanism's for the hypoglycemic effect of these plants must be involved.

A component isolated from the methanolic extract of the galls of *Quercus infectoria*, significantly inhibited the alpha-glycosidases. The inhibitory activity on alpha-amylase was approximately 10 times lower than that of acarbose. So this plant might reduce the side effects, by reducing the inhibition of alpha-amylase. Our results indicated that the aqueous extract of *Quercus infectoria* can inhibit the alpha glucosidase as well as its methanolic extract (31).

Other plants such as *Verbascum kermanensis*, *Rosa damascene, Citrus aurantium*, *Rosmarinus officinalis*, *Levisticum officinale*, *Zataria multiflora, Sanguisorba minor*, *Alhagi camelorum*, *Pistacia vera*, *Vaccinium arcto-staphylus*, *Zhumeria majdae, Alpinia officinarum*, *Salvadora persica,*

Lawsonia inermis and *Thymus serpyllum* which presented more than 50% inhibitory effect on the alpha glucosidase in this study.

Conclusion

There was no record in the literature for their anti-diabetic effect but they might be a good candidate for the control of diabetes. It is necessary to investigate the effects of active plants on blood glucose and the probable role of them on the insulin secretion; some of which are under investigation.

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