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Original Article

Searching for Alpha-Glucosidase Inhibitory Activity in Hexane Extracts by some Plants from Kurdistan Province

Mohammad Ali Zarei* , Mina Poursharifi

Department of Biological sciences and Biotechnology, Faculty of science, University of Kurdistan, Sanandaj, Iran

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ABSTRACT

Objective: Alpha-glucosidase, as a carbohydratase catalyses the liberation of alpha-glucose from the nonreducing end of some carbohydrates from foods in digestive tract. Consequently, retard absorption of glucose by the inhibition of carbohydrate hydrolyzing enzymes is one of the therapeutic approaches to decrease the postprandial hyperglycemia. Recently, a great deal of attention has been paid to natural products like plants, as a reliable source for bioactive compounds. The aim of this study was to find new potent inhibitors for Alpha-glucosidase among plant extracts**. Methods:** Hexan extracts of 60 plants species were screened for their Alpha-glucosidase inhibitory activity at 0.001, 0.01, 0.1 and 1 mg/mL concentration. Acarbose, dissolved in buffer, was used as a positive control. Every experiment was done in triplicate. Descurania Sophia (L.) Webb & Berth, Fumaria vailantii Loisel, Ferula Haussknechti Wolf ex Rech,Haplophyllum acutifolium (DC.)G.Don, Isatis cappadociaca Desv., Eremostachys laevigata Bunge, Silene aucheriana Boiss exhibited more than 60% alpha-glucosidase inhibitory activity.. **Results:** Among them, *Descurania Sophia*, *Fumaria vailantii* and *Ferula haussknechti* exhibited significant alpha glucosidase inhibitory activity with IC50 values of 0.9 µg/ml, 24.55 µg/ml and 2.71 µg/ml, respectively. Because of its high inhibitory activity and low IC50, *Descurania Sophia* extract would be interesting for further studies.

1.INTRODUCTION

Diabetes mellitus is one of the mainly common diseases in our planet. In the current years, the morbidity and death rates of diabetes increase quickly (Mary et al, 2001). So diabetes has become the third largest cause of death, which is just next to malignancy and cardiovascular disorders. There are two major kinds of diabetes: insulin-dependent diabetes mellitus or Type I diabetes (Bougneres et al, 2008), and non-insulin-dependent diabetes mellitus or, Type II diabetes (Sim et al, 2010). Type I diabetes result from autoimmune devastation of insulin-producing beta cells of the pancreas. The consequent lack of insulin leads to augmented blood and urine glucose. The typical symptoms are polyuria, polydipsia, polyphagia, and weight loss. Type II diabetes is a metabolic disorder that is characterized by elevated blood glucose, insulin resistance and its concomitant deficiency (Cooke et al, 2008). But the common character of two kinds of diabetes mellitus is the postprandial hyperglycemia.

***Corresponding Author:** Mohammad Zarei, Department of Biological sciences and Biotechnology, Faculty of science, University of Kurdistan, Sanandaj, Iran (mazarei@uok.ac.ir)

Hence the control of postprandial hyperglycemia is a necessity in diabetes mellitus (Puls et al,1973; Puls et al, 1977), since extremely elevated blood glucose may cause the initiation and progression of diabetic complications (Stout et al, 1990). Following food eating, carbohydrates are transformed into glucose by several enzymes. Between these, α-glucosidase is created by intestinal cells and breaks glycosidic bonds in oligosaccharides during the final step of hydrolysis (Li et al, 2009). Glucose is the main component of blood sugar. Hence, α-glucosidase maybe a perfect aim for the handling of at least, Type II diabetes mellitus (Seo et al, 2007). The α-glucosidase inhibitors are useful for delaying glucose absorption and preventing postprandial blood glucose level increase, consequently they take part in the therapy of Type II diabetes mellitus (Heiner et al, 2002). Obtainable hypoglycemic agents such as acarbose, metformin, miglitol and voglibose successfully manage glucose level but also are cause of major gastrointestinal side effects. Investigators in search for some inhibitors without side effects has been faced towards natural sources, specifically, plants (Chen et al, 2005; Gurudeeban et al, 2012). The aim of the authors was to examine possible α-glucosidase inhibitors in the extracts of a number of plants native to Kurdistan province of Iran. To reveal the character of these plants as potential sources for the development of novel drugs for handling of diabetes and associated diseases.

2.MATERIALS AND METHODS

2.1.Preparation of plant extract

Plants were collected from the central areas of Kurdistan Province, IRAN, from June to July 2012. Voucher specimens have been deposited in the Herbarium of the Institute of Agricultural Research of Sanandaj, and were authenticated by the botanist, Mr. Hossin Maroofi. Specimens were selected according to their availability and accessibility. They were dried out under shadow and grinded by electric grinder to a fine powder. About 10 g of each ground powder was soaked in 100 ml hexane for 24 h with sporadic shaking. The soaked substance was filtered throughout Whatman grade N0. 42 filter paper. The solvent was evaporated with rotary evaporator. The residues were air dried under chemical hood and finally collected in small microfuge tubes and stored at -20°C. Plant hexanic remains was weighed and dissolved in DMSO. This stock solution of 10 mg/ml was used diluted to 1, 0.1, 0.01, and 0.001 mg/ml final concentrations for determination of enzymatic activities.

2.2. Inhibition assay for alpha-glucosidase activity

Alpha-glucosidase inhibition was analyzed by means of kinetic end-point assay described by Pistia-Brueggeman by means of a little modification (Pistia-Brueggeman et al, 2003; Ankita et al 2011). All the enzyme, inhibitor and substrate solutions were made using the same buffer namely 50 µl of phosphate buffer (50 mM; pH 6.8). In a 96-well plate with 150 µl final reaction mixture volume, containing 50 µl of phosphate buffer, 10 µl of 1 U/ml alpha-glucosidase and 20 µl of plant extract of varying concentrations (1, 0.1, 0.001, 0.0001mg/ml) was pre-incubated for 5 min at 37°C, then 20 µl of 1 mM PNPG substrate was added to the mixture. Following additional incubation at 37°C for 30 min, the reaction was stopped by addition of 50 µl of 0.1M sodium carbonate. Acarbose was used as a positive control and double distilled water as negative control. The enzymatic hydrolysis of substrate was monitored by the quantity of p-nitrophenol released in the reaction mixture at 405 nm using microplate reader. Each experiment was performed in triplicates, along with proper blanks. The inhibition percentage of alpha-glucosidase was assessed by the next formula: % inhibition = {Abs (control) –Abs (Test)}/ Abs (control). IC50 value is defined as the concentration of extract inhibiting 50% of alpha-glucosidase action under the stated assay setting. In the case of significant inhibition, IC50 values were calculated. All values are represented as Mean ± Standard Deviation.

2.3.Kinetics of enzyme inhibition

With the aim of examining the mode of inhibition of plant extracts against alpha-glucosidase activity, its activity was measured with increasing concentrations of PNP-glycoside (0.25, 0.5, 1, 2 mM) in the absence or presence of plant extract at different concentrations (1, 0.1, 0.001, 0.0001mg/ml). Most favorable doses of plant extract were determined founded on the outcome from inhibitory activity assay as described earlier. Inhibition type for plant extract was determined by Lineweaver–Burk and secondary plot analysis of the data resulted from enzyme assays containing various concentration of PNP-glycoside and plant extract.

3. RESULTS

Sixty plant species from central regions of Kurdistan province were collected randomly. The hexane extracts of these plants were tested for alpha-glucosidase inhibitory activity by means of colorimetric method in 96-welled microplate. Based on their potential inhibitory activity on alpha-glucosidase, they have been divided into three groups: Strong, moderate and weak inhibitory plant extracts. Which, their inhibition percent were more than 60%, between 15 and 60%, and less than 15%, respectively. Seven plants exhibited extra alpha-glucosidase inhibitory activity, i.e. *Descurania Sophia* (L.) Webb & Berth, *Fumaria vailantii* Loisel, *Ferula Haussknechti* Wolf ex Rech, *Haplophyllum acutifolium* (DC.) G.Don, *Isatis cappadociaca* Desv., *Eremostachys laevigata* Bunge, *Silene aucheriana* Boiss. (Figure. 1). Among them *Descurania Sophia* (L.) Webb & Berth, *Fumaria vailantii* Loisel and *Ferula Haussknechti* Wolf ex Rech., exhibited significant alpha-glucosidase inhibitory activity with IC50 0.9 µg/ml, 24.55 µg/ml, 2.71 µg/ml, respectively. Acarbose, as a standard Alpha-glucosidase

inhibitor in this study, showed the IC50 of 1.49M. At the concentration of the test samples in the study, acarbose entirely inhibited the activity of alpha-glucosidase. Since IC50 for *Descurania Sophia* (L.) Webb & Berth,was very much lower than all other plant extracts, so its kinetics behavior was analyzed and according to Line waver-Burck kinetic analysis, its inhibition mode on alpha-glucosidase is uncompetitive at 1µg/ml extract concentration (Figure 2), (K_i=0.089 µg/ml), Uncompetitive - noncompetitive at 0.1µg/ml extract concentration (Figure 3), (K_i=0.03 µg/ml), Not defined at 0.01µg/ml extract concentration (Figure 4), and Uncompetitive at 0.001µg/ml extract concentration (Figure 5), (K_i=0.00012 µg/ml).

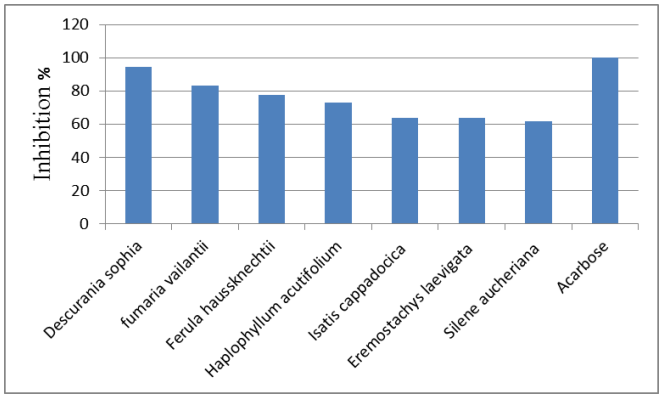

Figure 1. Inhibitory effect of seven more potent plant species hexane extracts on alph-glucosidase activity. Acarbose has been shown here as positive control

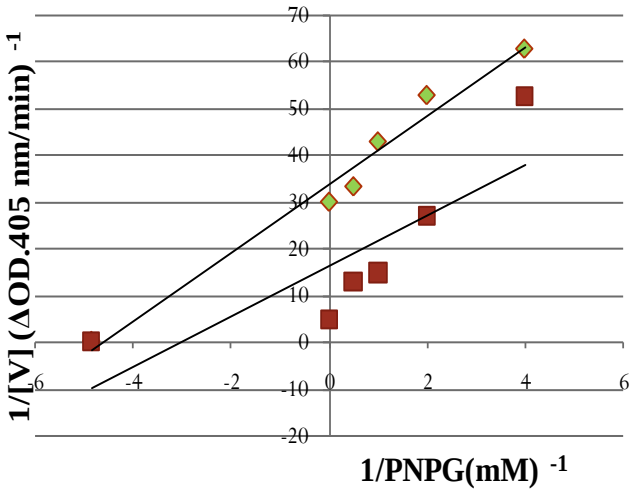

Figure 2. Double reciprocal plots for kinetic analysis of alpha-glucosidase inhibition by *Descurania Sophia (L.) Webb & Berth* hexane 1 µg/ml extracts with PNP-glycoside as substrate.

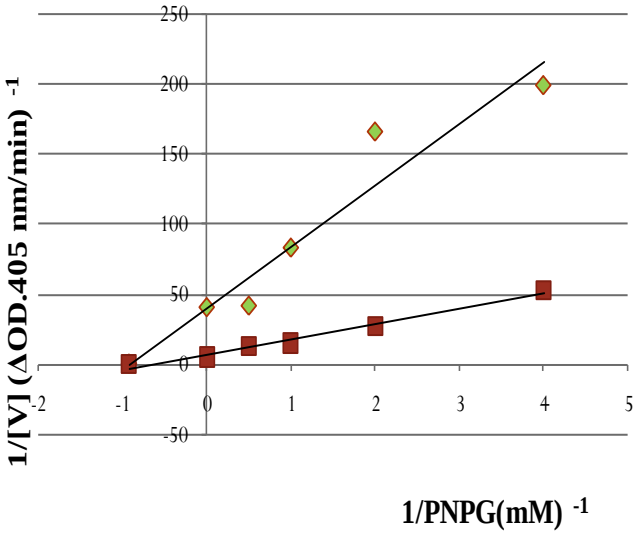

Figure 3. Double reciprocal plots for kinetic analysis of alpha-glucosidase inhibition by *Descurania Sophia (L.) Webb & Berth* hexane 0.1 µg/ml extracts with PNP-glycoside as substrate.

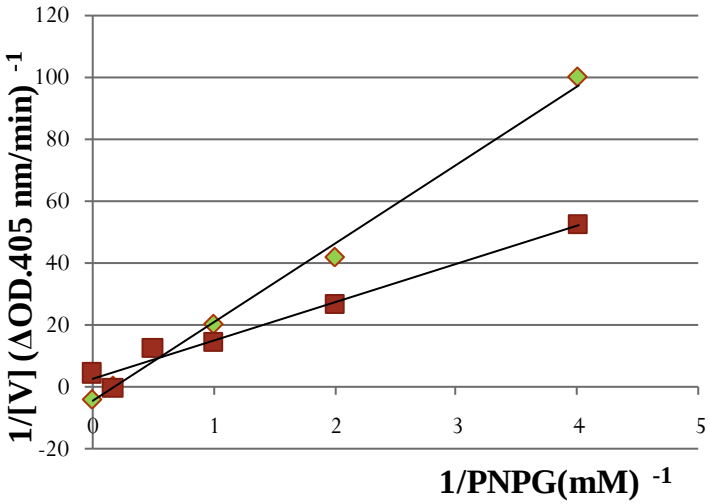

Figure 4. Double reciprocal plots for kinetic analysis of alpha-glucosidase inhibition by *Descurania Sophia (L.) Webb & Berth* hexane 0.01 µg/ml extracts with PNP-glycoside as substrate.

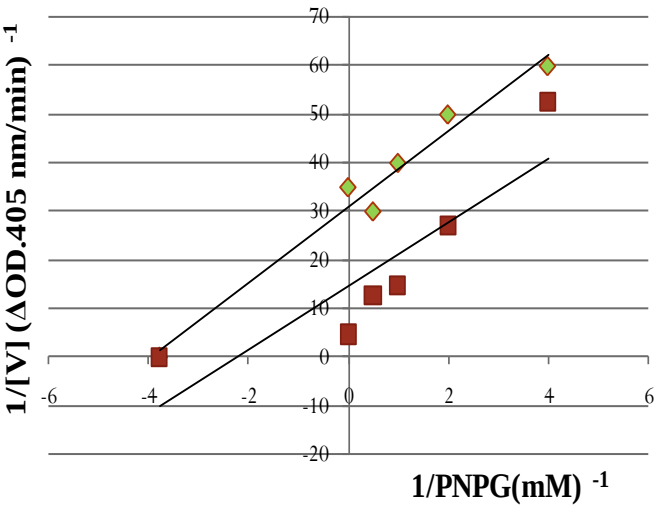

Figure 5. Double reciprocal plots for kinetic analysis of alpha-glucosidase inhibition by *Descurania Sophia (L.) Webb & Berth* hexane 0.001 µg/ml extracts with PNP-glycoside as substrate.

4. DISCUSSION

The present study was geared towards investigating the potential inhibitory effects of hexane extracts from 60 randomly selected plants of Kurdistan province of IRAN to inhibit key carbohydrate hydrolyzing enzyme *α*-glucosidase. Additionally, inhibition kinetics of potent plant extracts were also evaluated. Plant hexane extracts have been studied for *α*-glucosidase inhibitory activity formerly. In 1996 Matsui, surveyed alpha-glucosidase inhibitory of some food components to identify a prophylactic effect for diabetes in foods (Matsui et al,1996). In 2003 Shim examined the inhibitory effect of aqueous extract from the gall of *Rhus chinensis* (AEGRC) on alpha-glucosidase activity, their results suggested that AEGRC might exert anti-diabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the postprandial increase of blood glucose (Shim et al, 2003). Loizzo, reported the inhibitory activity against digestive enzymes related to diabetes of extracts of nine plant species collected in Lebanon, where they have a traditional use against diabetes, Their results supported the traditional use of some the species examined (Loizzo et al,2008). In a study by Eidi, an ethanol extract of the Coriander (Coriandrum sativum L.) seeds was investigated for effects on insulin release from the pancreatic beta cells in streptozotocin-induced diabetic rats. The extract decreased serum glucose in streptozotocin-induced diabetic rats and increased insulin release from the beta cells of the pancreas (Eidi et al, 2009). In 2010 Shai screened six medicinal plant species, with alleged antidiabetic properties for α-glucosidase inhibitory activities. Among them C. *abbreviate* possessed compounds that inhibit the glycosidic bond cleavage by α-glucosidase. The type of inhibition by C. *abbreviate* was confirmed as noncompetitive (Shai et al, 2010). In 2011 Ieyama and coworkers showed an effective α-glucosidase inhibitory activity for plant *Eleutherine Americana*, (Ieyama et al, 2011). In 2012, six selected Philippine plants were screened for their potential a-glucosidase inhibitory activity as the first report on thea-glucosidase inhibitory effect of the six Philippine plants, in support of their ethnomedicinal use for diabetes. The report partly defined the mechanism on why those medicinal plants possess antidiabetic properties (Lawag et al, 2012). Kumar et al investigated the enzyme inhibitory and antidiabetic activity for the constituents isolated from Dillenia indica. They concluded that indica might be a potential source of antidiabetic agents (Kumar et al, 2013). In 2014 Brindis F. evaluated antihyperglycaemic properties of the aqueous extract from the leaves and stems of Coriandrum sativum L. in normoglycaemic rats, and on α-glucosidase activity from Saccharomyces cerevisiae, in order to validate its use in folk medicine. Their results confirmed the antidiabetic properties of the extract of C. sativum L., probably by the inhibition of α-glucosidase in the gastrointestinal tract (Brindis et al, 2014). From natural and geographical point of view, Kurdistan Province has a great diversity of native plant species of which little is known, especially in relation to their bioactivity. So plant species from this area were randomly selected and their hexaneic extracts assayed in order to find plants with the highest α-glucosidase inhibitory effect. The hexaneic extracts of those plants were tested in four different concentrations for tyrosinase inhibitory activity because some inhibitors may be active in a defined concentration but not in others. The most potent extract was prepared from *Descurania Sophia* (L.) Webb & Berth., a plant without any earlier report of α-glucosidase inhibitory effect. *Descurainia sophia* is a member of the mustard family. Common names include flixweed, herb-Sophia and tansy mustard, and in Iran it is called “Khakeshir”. It reproduces by seeds. Its stem is erect, branched, and 4 to 30 inches high (Vural et al, 2009; Li et al, 2010). Analyses of the volatile constituents from the plants of Descurainia sophiacollected from different growing locations indicatecis-β-ocimene, menthol, neoisomenthyl acetate to be the predominant components of Descurainia sophia (Lee et al, 2013). In 2014 Amiri etal studied a total of 37 plants belonging to 32 genera and 26 families and documented them for their therapeutic use against jaundice. Descurainia sophia was among them and its Ethnomedicinal uses was reported as Jaundice, Blood and Liver Cleanser, Febrifuge, Laxative, Treat of Furuncles, Anti-thirst (Amiri et al, 2014). In conclusion the extracts from *Descurania Sophia* (L.) Webb & Berth, *Fumaria vailantii* Loisel, *Ferula Haussknechti* Wolf ex Rech, *Haplophyllum acutifolium* (DC.) G.Don, *Isatis cappadociaca* Desv., *Eremostachys laevigata* Bunge, *Silene aucheriana* Boiss., presented considerable inhibition of alpha-glucosidase and among them *Descurania Sophia*

(L.) Webb & Berth showed strongest inhibition (higher than 90%) at studied concentrations and its IC50 was considerably lower than others. So this plant species is valuable for further analysis to find effective secondary metabolites in its extracts which may be responsible for above mentioned strong inhibitory activity.

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