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In vitro α-amylase and α-glucosidase inhibitory activity of *Ononis angustissima* extracts

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ABSTRACT

The purpose of present study was to investigate the effects of extracts of *O. angustissima* a traditional medicinal plant, on α-amylase and α-glucosidase activities *in vitro*. The air-dried aerial parts of *O. angustissima* flowers, leaves and stems were extracted with distilled water (AE) and aqueous methanol (MeOH-H_2O); ethyl acetate (EtAOc) and n-butanol extracts (n-BuOH) were obtained from MeOH-H_2O crude extracts per liquid/liquid extraction. The results of the both enzyme inhibition activity was found in a dose-dependent manner, The strongest activity in case of α- glucosidase was shown by MeOH-H_2O and n-BuOH extracts of *O. angustissima* with IC_{50} value 0.94 and 0.99 mg/mL, respectively, whereas AE and EtOAc extract showed 1.10 and 1.17 mg/mL as IC_{50} value respectively compared with the standard acarbose having IC_{50} value 0.046 mg/mL. Whereas the higher activity in case of α-amylase inhibition, was found in MeOH-H_2O extract (IC_{50} = 2.01 mg/mL) followed by AE (2.52 mg/mL) and n-BuOH extract (2.88 mg/mL) respectively, compared with acarbose having IC_{50} value of 0.044 mg/mL. All extract from this plant possess moderate α-amylase inhibition with potent α-glucosidase inhibitory activity which may offer better therapeutic strategy to minimized postprandial hyperglycemia and its complications.

INTRODUCTION

Diabetes mellitus (DM), a chronic metabolic disorder characterized by high blood glucose levels, continues to be a major medical concern worldwide due to its high prevalence and potential deleterious effects. One goal of therapy for diabetic patients, especially type 2, is the maintenance of normal blood glucose levels after meal (DeFronzo, 1999). Postprandial hyperglycemia plays an important role in the development of type 2 diabetes and its complications. One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption glucose by inhibition of carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase (DeFronzo, 1999; Chiasson *et al*, 2002). Due to its high prevalence and potential deleterious effects, type 2 diabetes mellitus (T2DM) continues to be a major medical concern and major metabolic disorder and a multifactorial disease that is increasing worldwide (Zimmet, 2011). Recently, it has become one of the most common endocrine disorders and it has been reported that postprandial hyperglycemia is an important contributing factor for the development of diabetic complications (Monami *et al.*, 2013). Postprandial hyperglycemia is one of the earliest observable abnormalities of glucose homeostasis associated with type 2 diabetes mellitus (T2DM) (Baron, 1998). It plays an important role in the development of Type 2 diabetes mellitus and its associated chronic complications, such as micro- and macro-vascular disorders (neuropathy, cardiovascular, and cerebrovascular diseases) (Boutati and Raptis, 2004). It has been established that postprandial hyperglycemia strongly depends on the absorbed monosaccharides and the velocity of absorption in the small intestine and it is mediated by carbohydrates hydrolyzing enzymes such as pancreatic α-amylase and intestinal α-glucosidase: two members of exo-acting glycoside hydrolase enzymes (glucosidase).

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Postprandial glucose levels can be regulated through α-glucosidase inhibition. Inhibition of these enzymes delay and in some cases halt carbohydrate digestion thus prolonging overall carbohydrate digestion time causing a reduction in the rate of glucose absorption and consequently reducing postprandial plasma glucose rise (Henfeld *et al.,* 2004).

Nowadays, alpha-glucosidase inhibitors like acarbose, miglitol and voglibose are oral blood glucose lowering drugs communally used. They are also the only drug class that does not target a pathophysiological object in T2DM. They decrease postprandial hyperglycemia without inducing insulin secretion; these compounds do not induce hypoglycemia and have a good safety profile, although gastrointestinal adverse effects may limit long-term compliance to therapy (Neuser, 2005).

The research for new group of agents from naturel resources especially from traditional medicines became an attractive approach for the treatment of postprandial hyperglycemia.

These traditional medicines are relied upon for health care in many parts of the world (WHO, 1980). Several medicinal plants species have been used to control diabetes in the traditional medicinal systems of many cultures worldwide (Grover *et al.,* 2002; Bnouham *et al.,* 2006; Mentreddy, 2007; Sales *et al.,* 2012). A number of them are known to exert their antihyperglycemic activity via the inhibition of carbohydrate hydrolyzing enzymes. Therefore, natural inhibitors from plant sources can offer an attractive strategy for the effective control of postprandial hyperglycemia without or less unwanted secondary effects (Ali and Houghton, 2006; Tundis, 2010). The potential role of medicinal plants as inhibitors of α-amylase and α-glucosidase has been reviewed by several authors. A variety of plants has been reported to show an enzymatic inhibitory activity and so many are relevant to the treatment of type 2 diabetes (Benalla, 2010; Sudha, 2011; Sales, 2012).

Published research suggests that there is a direct relationship between of phenolic compound, flavonoids, and condensed tannin in the plant extract and the ability to inhibit α-glucosidase and α-amylase activities (Tadera *et al.,* 2006; Adisakwattana and Chanathong, 2011). Many phenolic compounds such as flavonoids and anthocyanin have positive effects on diabetes, by inhibiting the two keys enzymes hydrolyzing carbohydrates in the digestive tract (Lo Piparo *et al.,* 2008; Jo *et al.,* 2010; Rubilar *et al.,* 2011; Sales 2012; Wongsa *et al.,* 2012)

Ononis angustissima Lam. subsp. polyclada Murb is perennial herbs and shrubs belongs to the Fabaceae (Dobignard, 2013; Benabderahmane *et al.,* 2014). *Ononis* is a genus (tribe Trifolieae) comprising 75 species occurring in the Canaries, the Mediterranean region, North of Africa, North of Américа and from Europe to Central Asia (Mezrag *et al.,* 2013).

Biological type of *O. angustissima*, identified as champhyte and the phytogeography type as Algerian endemic (Bouheroum, 2009; The Algerian Journal of Arid Areas, 2012) of the north of the septentrional area of the Sahara and hammadas (Cheriti *et al.,* 2011; Benabderahmane *et al.,* 2014). It's reported to be a medicinal plant, It use as decoction for its hemostatic properties due to its specific secondary metabolites content with original chemicals and biological characteristics (Chahma and Djebar, 2008; Chehma and Youcef, 2009). It was also used in the treatment of diabetes in traditional Algerian medicine (Khacheba *et al.,* 2014)

Recently, *O. angustissima* was studied for its phytochemical composition, several flavonoids were identified (Bouheroum, 2009; Mezrag, 2013) two compounds are new in the genus: 2, 3, 4-Trihydroxyphenyl)-3-phenylprop-2-en-1-oneor 2', 3', 4'-Trihydroxychalcone and 6-Hydroxyaurone, thus they are isolated for the first time in this species (Benabderahmane *et al.,* 2014). It was also studied for its antioxidant activity (Djeridane *et al*., 2010; Ghribi *et al.,* 2015).

The aim of this study is to investigate the inhibitory potential of aerial part extracts of *O. angustissima* on mammalian carbohydrate digesting enzymes. It evaluated *in vitro* inhibition of rat intestinal α-glucosidase (EC 3.2.1.20) and porcine pancreatic α-amylase (EC 3.2.1.1) activities by its crude aqueous and hydromethanolic extracts with its butanolic and ethyl acetate fractions, besides phytochemical analysis.

MATERIALS AND METHODS

Chemicals and reagents

Porcine pancreatic α-amylase (EC 3.2.1.1) (PPA), 3,5-Dinitrosalicylic acid (DNSA color reagent), Soluble potato starch, p-nitrophenyl- α -D-glucopyranoside (p-NPG), catechin, Gallic acid were obtained from Sigma-Aldrich Chimie GmH, Germany. Acarbose from Glucobay (Bayer, Germany), Sigma-Aldrich Chimie GmH, Germany. All other chemical reagents used in this study were of analytical grade.

Plant material

Fresh aerial parts (flowers, stems and leaves) of *O. angustissima* were collected from Ain Safra (western Algeria) during spring. The plant was authenticated by a taxonomist at the department of Ecology, University of Tlemcen, Algeria. Voucher specimen was deposited at the Herbarium of the department. In laboratory the Plant materiel were washed under running tap water and shade dried at ambient temperature. Thereafter dry up sample entirely broken manually, cut into small pieces and conserved sheltered from humid conditions until use.

Preparation of extracts

Aqueous extract (AE)

The dried aerial part of *O. angustissima*: flowers, stems and leaves (30 g) were extracted under reflux in distilled water (450 mL) for 30 min at 50 °C. Aqueous extract (AE) was filtered and evaporated to dryness.

Hydromethanolic extract (MeOH-H_2O)

60 g of *O. angustissima* dried aerial part (flowers, stems and leaves) were extracted by maceration in 480 mL H_2O/CH_3OH

mixture 30/70 (room temperature for 48 h); thereafter, the mixture was filtered and evaporated to dryness.

Ethyl acetate (EtAOc) and butanol (n-BuOH) extracts

Ethyl acetate and butanolic extracts were recovered from hydro-methanolic solution previously described (H_2O/CH_3OH mixture 30/70). After extraction and concentration of MeOH-H_2O; the aqueous phase underwent liquid-liquid extraction successively with chloroform and hexane, followed by a 2-fold extraction with ethyl acetate, this organic phase was evaporated to dryness. Then, the recovered aqueous phase was followed by extraction with n-Butanol; organic phase was evaporated to dryness.

Phytochemical screening

Phytochemical examinations were performed for all the extracts using standard methods. Alkaloids (Dragendorff and Mayer reagent), reducing compounds (Fehling reagent), coumarin, quinones, flavonoids (cyanidine reaction), tannins (iron chloride), saponin and terpenoids (Liebermann Burchard reaction) (Trease and Evans, 1989; Harbone, 1998).

Determination of total phenolic contents (TPC)

The amount of phenols in each extracts of *O. angustissima* was determined, with Folin-Ciocalteu reagent using the method of Vermerris *et al.,* (2006) with slight modifications. Samples were compared to each other. Briefly, 2 mL of Na_2CO_3 (2% w/v) was added to 0.1 mL of sample (extracts or standard) and 100 µL of Folin-Ciocalteu reagent (0.2 N). The resulting mixture was incubated at room temperature for 30 min before absorbance measurement at 700 nm. Results were expressed as mg gallic acid equivalents (GAE) in g of extract (mg GAE/g). Gallic acid was used as standard.

Determination of total flavonoids contents (TFC)

Total flavonoids content of the extracts was determined according to colorimetric method described by Zhishen *et al.,* (1999) with few modifications. 500 µL of each sample or catechin as standard was mixed with 2 mL of distilled water and 150 µL of sodium nitrite ($NaNO_2$ 15%). After, addition of 150 µL of aluminum chloride (10%) and 2 mL of sodium hydroxide, the reaction mixture was incubated 15 min and then the absorbance was measured at 510 nm. The results are expressed in milligrams equivalent catechin per gram of extract (CEq mg/g) given in table 2 using the linear equation of the calibration curve of catechin.

***In vitro* porcine pancreatic α-amylase inhibition assay**

The α-amylase inhibitory activity was determined by assay adapted from method of Bernfeld (1955).The reaction mixture contain: 200 µl of the tested extracts of *O. angustissima* (0.18-10 mg/mL), 200 µL of 0.02 M sodium phosphate buffer (pH 6.9; 6.7 mM NaCl) containing 1.3 U/mL of porcine pancreatic α-amylase solution (PPA). The reaction medium was pre-incubated at 37 °C for 5 min, and then 200 µL of 0.4 % starch solution in the above buffer were added and incubated at 37 °C for 10 min. 600 µL of DNSA solution was added to the reaction and placed in a boiling water bath for 7 min, then cooled down in cold water. The reaction mixture was then diluted after adding 1 mL of distilled water and the absorbance was measured at 540 nm. To eliminate the absorbance produced by plant extract, appropriate extract controls with extract and except the enzyme were also included. Commercial inhibitor acarbose was used as a positive control at a concentration range of 0.040-2.670 mg/mL. As a blank buffer solution was used instead of substrate. The tube with enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α-amylase activity (%) = ((Abs C - Abs S)/Abs C)*100

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

***In vitro* intestinal rat α-glucosidase inhibition assay**

Preparation of the crude enzyme solution from small intestinal mucosa of rat

Inhibitory activities on α-glucosidase were measured using a crude extract obtained from small intestinal rat mucosa, as mammalian source of enzyme according to slightly modified method of Dahlqvist (1964). p-nitrophenyl-α-D-glucopyranoside (p-NPG) was used as substrate which is hydrolyzed to p-nitrophenol, a colored product that can be monitored at 405 nm (Bergmeyer and Bernt, 1974).

Preparation of the crude enzyme solution

After 20 hours of fasting, rats are scarified under light anesthesia. Their small intestine (the 20 cm of jejunal portion about 10 cm below the pylorus) was removed, rinsed with ice-cold saline and the mucosa was delicately scraped off with a slide glace. Mucosa from 5 rats was pooled and homogenized with 67 mM sodium phosphate buffer (pH 6.8) sonicated and centrifuged at 4 °C for 20 min at 5000 rpm. The supernatant was used for the assay of α-glucosidase. Its specific activity was determined (one unit of enzyme activity is defined as the amount of enzyme required to liberate 1 µmole of p-nitrophenol from p-NPG per minute at 37 °C at pH 6.8 per mg of protein. Protein content was determined using Lowry's method. The enzymatic solution (about 0.94 U/mg prot) was aliquoted in 1 mL cryotubes and stored at -20 °C until used.

α-glucosidase inhibitory assay

200 µL of a crude enzyme solution of rat intestinal α-glucosidase (adjusted to 0.2 U/mL as initial concentration in phosphate buffer 67 mM, pH 6.8) was mixed with 200 µL of the sample: plant extract (0.06-1.18 mg/mL) or acarbose, positive control, (0.001-0.118 mg/mL) solutions and 1 mL of phosphate buffer. The mixture was pre-incubated at 37 °C for 10 min after which 300 µL of p-NPG solution (10 mM) was added and the

reaction mixture further incubated at 37 °C for 40 min. The reaction was terminated by adding 3 mL of sodium carbonate Na_2CO3 (100 mM) into the mixture to stop the reaction. The absorbance of the liberated p-nitrophenol was measured at 405 nm. As a blank, buffer solution was used instead of substrate. The tube with enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity. The enzyme inhibition rate expressed as percentage of inhibition was calculated using the following formula:

Inhibition of α- glucosidase activity (%) = ((Abs C - Abs S)/Abs C)*100

Where Abs C is the absorbance of the control (100 % enzyme activity) and Abs S is the absorbance of the tested sample (plant extract or acarbose).

Statistical analysis

The experiments were performed in triplicates and the results were expressed as the mean values ± SD. The IC_{50} i.e. the concentration of extract/acarbose resulting in 50% inhibition of enzyme (α-amylase, α-glucosidase) was calculated by logarithmic regression analysis using Microcal Origin 5.0 (Microcal software, Inc. 1.0.0.1).

RESULTS AND DISCUSSION

Phytochemical screening

Table 1 provide the preliminary phytochemical screening of *O. angustissima* extracts, The results shows the wealth of MeOH-H_2O, n-BuOH and EtAOc extracts on alkaloids, tannins and flavonoids, these latter components are weakly present in EA extract. Terpenoids were abundantly present in all extracts, while a low detection of coumarins, amino acids and reducing sugar was observed in all extracts. Phytochemicals in plant extracts depends on the plant material (leaves, steams, seeds, roots) and its origin (moist or arid region). Also, the solvent polarity and the method of extraction may influence the composition of each extract on chemical constituents.

Table 1: Phytochemical screening of *O. angustissima* aerial part extracts.

	AE	MeOH-H_2O	n-BuOH	EtAOc
Flavonoids	+	+++	+++	+++
Alkaloids	+	+++	+++	+++
Tannins	+	+++	+++	+++
Saponins	++	+++	++	+++
Terpenoids	+++	+++	+++	+++
Quinones	++	+++	++	+++
Coumarins	++	+	+	-
Anthraquinones	-	-	-	-
Redusing compounds	++	++	+	+
amino acid	+	++	+++	+

Total polyphenol and flavonoid content

Table 2 includes the polyphenol and flavonoids content in *O. angustissima* extracts. The obtained results reveals a comparative rate of polyphénols in hydromethanolic MeOH-H_2O, n-BuOH and EtOAc extracts, 78.11, 74.55 and 72.21 mg GAE/g, respectively. In the aqueous extract EA, we determined 65.29 mg GAE/g.

Concerning flavonoids content (Table 2), hydromethanolic, and EtOAc extracts contain the highest level, about 32 mg CEq/g. However n-BuOH and AE extracts present lower concentration of flavonoids, ranging from 12 to 16 mg CEq/g of extract.

Table 2: Total polyphenols and flavonoids content of *O. angustissima* aerial part extracts.

Extracts	Polyphenols*	Flavonoids*
AE	65.29±0,03	12.60±0.01
MeOH-H_2O	78.11±0,01	34.14±0.03
n-BuOH	74.55±0,01	16.66±0.00
EtOAc	72.21±0.04	32.01±0.01

* mg gallic acid equivalent /g extract.

Further, the results of recent studies of Djeridane *et al.*, (2010) which have shown that the ethyl acetate extract obtained from roots of *O. angustissima* contain 12.03 mg GAE/g of dry matter and 02.35 mg rutin eq/g of dry matter. Khacheba *et al.*, (2014) determined the amount of the polyphenol and flavonoids of the aqueous extracts obtained from leaves of this plant (1.91 ± 0.52 mg GAE/g dw of polyphénols, and 1.16 ± 0.51 mg rutin eq/g dw of flavonoids). Plant extracts content on phytochemicals is dependent on used material, its origin, the harvest period and moisture. In addition, solvent with different polarities have significant effects on the phenolic content. According to some researchers, water and methanol are frequently used for extraction of polyphenolic compounds from different plants (Siddhuraju and Becker, 2003; Sultana *et al.*, 2009).

***In vitro* α-amylase inhibition assay**

The results in figure 1 showed the percentage inhibition of *O. angustissima* extracts and acarbose against α-amylase. All of them demonstrated a significant dose-depended reduction in α-amylase activity. The most important inhibition appeared in the MeOH-H_2O extract while the EtOAc extract present a weakest effect. Comparatively to acarbose, reference compound, using IC_{50} values (resulting in 50% inhibition of enzyme activity) it is estimated to be 0.044 mg/mL for acarbose (Table 3) and 33 to 74 fold higher for plant extracts tested.

Table 3: IC_{50} value for Inhibitory activity against porcine pancreatic α-amylase and intestinal rat α-glucosidase.

	IC_{50} (mg/mL)	
	α-amylase	α-glucosidase
Acarbose	0.044±0.01	0.046±0.01
EA	2.52±0.02	1.10±0.01
MEOH-H2O	1.46±0.01	0.99±0.01
n-BuOH	1.17±0.01	0.94±0.01
EtOAc	3.26±0.02	2.58±0.02

These results suggest that the bioactive exerting the inhibitory effect against α-amylase may be present in all plant extracts at different concentration, diluted among other not active compounds. Phytochemical analysis revealed that the extracts are rich in polyphenolic components. MeOH-H_2O extract showed the

higher amounts in comparison to other extracts, it contains 78.11 mg GAE/g extract of phenolic compounds and 34.14 mg CEq/g extract of flavonoids.

Previous studies concerning other plants from Fabaceae family were screened for α-amylase activity and showed inhibitory activity. They been reported with α-amylase inhibitory activity, namely: *Galega officinalis*, *Phaseolus vulgaris* and *Tamarindus indica* which shown respectively 35, 45-75, 90% inhibition of α-amylase at concentration of 200 mg/mL (Sales *et al.*, 2012). Extracts from *O. angustissima* are more potent inhibitors of α-amylase since they reached 77% at concentration of 3.3 mg/mL comparing to: *Galega officinalis* L. known for its antidiabetic property containing galegine (guanidine), as source of an oral antidiabetic drug, Metformine, acting by reduction of hepatic gluconeogenesis (Fabrican and Fransworth, 2001); while extract of *Trigonella foenum- graecum* have an IC_{50} value of 1.92 mg/mL (Nickavar and Yousefian, 2011) so close to IC_{50} of *O. angustissima* (1.46-3.26 mg/mL). It's well documented that polyphenols have α-amylase and α-glucosidase inhibitory activity that depends on phenolic profile (Kwon *et al.*, 2008; Kang *et al.*, 2014). Flavonoids are a major group of polyphenolic compounds which have been reported to possess inhibitory activity against α-amylase and α-glucosidase (Kim *et al.*, 2000; Tadera *et al.*, 2006; Williams G, 2013). In relation to their structure, number and position of their hydroxyl groups in the molecule which are determining factors for enzymes inhibition. The inhibitory activity increased considerably with an increase in the number of the hydroxyl group on the B ring (Tadera *et al.*, 2006). Baicalein, a flavone glycoside, inhibits α-amylase and α-glucosidase activity by 41% and 57% at 1 mM (IC_{50}=192.67 mg/mL), respectively. Also, Myricetin (flavonol) inhibits α-amylase and α-glucosidase activity by 36% and 48% at 1 mM (IC_{50}=327.77 mg/mL), respectively (Ng *et al.*, 2015). According to the author, the inhibitory activity of flavonoids was attributed to two interactions: the hydroxyl groups in the flavonoid molecular structure can form hydrogen bonds with the OH groups in active side chains of functional amino acids of the enzyme, and conjugated π-system is likely to be formed between the AC ring system and the indole Trp59 in the enzyme. Both the interactions can hinder the reaction between α-amylase and starch wich inhibits the starch digestion (Ng *et al.*, 2015).

Anthocyanins competitively inhibited porcine pancreatic α-amylase, Cyanidin-3-glucoside have the highest inhibition activity with the *Ki* value of 0.014 mM, followed by cyanidin-3-rutinoside, cyanidin-3,5-glucoside, and peonidin-3-glucoside with the *Ki* value of 0.019, 0.020, and 0.045 mM, respectively (Sui *et al.*, 2016). By the molecular docking study, the author showed that anthocyanins occupied the active site of porcine pancreatic α-amylase forming hydrogen bonds; GLU233 was found to be the common key side chain for imparting the inhibition activity.

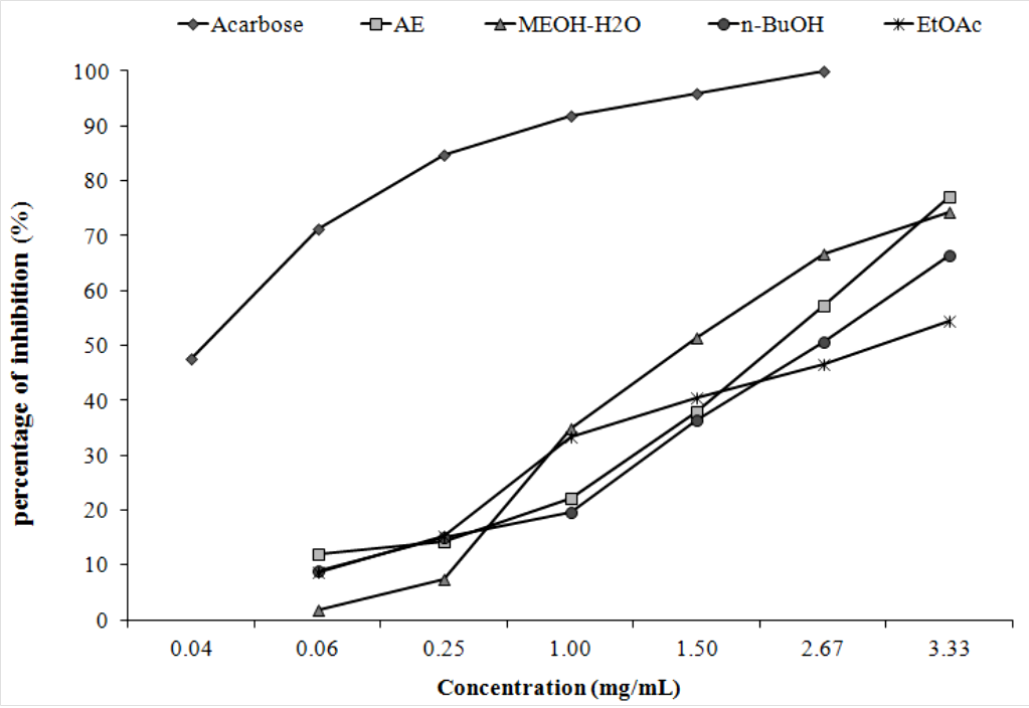

Fig. 1: *In vitro* inhibitory activity of *O. angustissima* aerial part extracts against porcine pancreatic α-amylase (percentage of inhibition (%) ± SD).

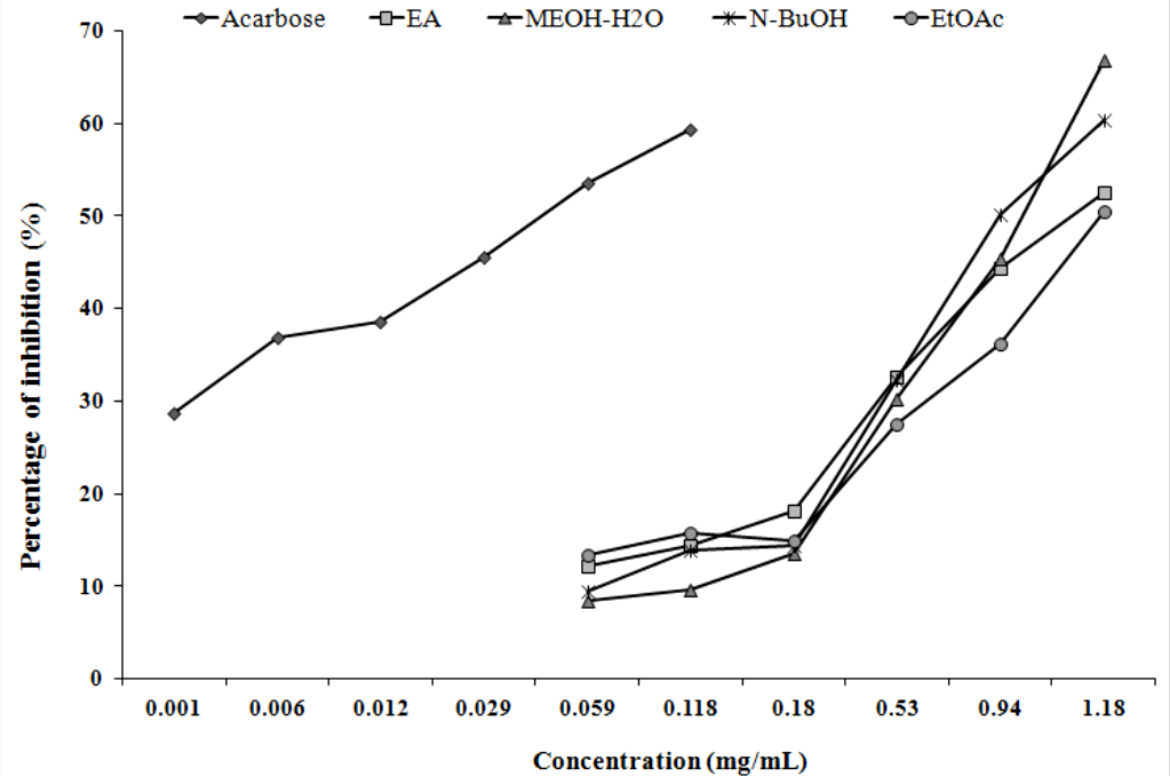

Fig. 2: *In vitro* inhibitory activity of *O. angustissima* aerial part extracts against intestinal rat α-glucosidase (percentage of inhibition (%) ± SD).

***In vitro* α-glucosidase inhibition assay**

The concentration-dependent α-glucosidase inhibitory activities and the IC_{50} values were estimated as indicated in figure 2 and table3, respectively. The four plant extracts tested showed a concentration-dependent inhibitory activity with a similar evolution profile using graded concentrations. For comparison, IC_{50} values of samples are relatively close but higher than IC_{50} of acarbose (0.046 mg/mL). This last is about 20 fold lower than IC_{50} of plant extracts. This observation suggest that bioactive compounds inhibiting α-glucosidase activity are present in all extracts and will be extracted by different solvents used in this study. When comparing the resulting α-amylase and α-glucosidase IC_{50} values, plant extracts showed a higher α-glucosidase inhibitory activity than α-amylase (Table 3).

When regard to the antidiabetic effect of acarbose, the use of this drug is reported to be associated with gastrointestinal side effects caused by the excessive inhibition of pancreatic α-amylase resulting to abnormal bacterial fermentation of undigested carbohydrates in the large intestine (Bischoff *et al.*, 1985). It is supported that any bioactive composed having lower inhibitory activity against α-amylase and stronger inhibitory activity against α-glucosidase may be an effective therapeutic agent for the control of postprandial hyperglycaemia with fewer side effects than acarbose (Kwon *et al.*, 2008).

A systematic review of literature revealed that terpenes, alkaloids, flavonoids and phenols showed potent inhibitory activity toward α-glucosidase (Yin *et al.*, 2014). Yeast α-glucosidase was potently inhibited by members of the anthocyanidin, isoflavone and flavonol subgroups with IC_{50} values below 15 mM, rat α-glucosidase was only slightly inhibited by compounds belonging to the anthocyanidin and isoflavone subgroups (Wenzel, 2013). Similarly, quercetin possess high inhibitory effect. The IC_{50} values against yeast α-glucosidase (*Ki*=8.5 mol/L), rat intestinal sucrose, and amylase were 8.86 g/mL, 216 mol/L and 71 mol/L, respectively (Yin *et al.*, 2014). Likewise, rutin a glycosylated flavonoid, showed an acarbose-like inhibitory effect on maltase activity and this was highly specific to maltase (Pereira *et al.*, 2011). The activity is relative to the chemical structure of these compounds. In the flavonoid structure, the C5, C6 and C7 OH groups of the A-ring are essential elements for inhibitory activity (Ng *et al.*, 2015). Also, the 2,3-double bond, 5-OH, the linkage of the B ring at the 3 position, and the hydroxyl substitution on the B ring enhanced the inhibition, while 3-OH reduced it (Tadera *et al.*, 2006). In this work, we used mammalian source of digestive enzymes α-amylase and α-glucosidases for in *vitro* inhibitory assay, which are structurally and mechanistically closely related to human enzymes (Bayer *et al.*, 1995). Porcine pancreatic α-amylase and yeast α-glucosidases are usually used for anti-diabetes neutraceutical and medicinal investigations as a model for screening potential inhibitors, because they are readily available in a relatively pure form (McDongall and Stewart, 2005).

Intestinal α-glucosidase is a key enzyme for carbohydrate digestion; it has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia. However, mammalian α-glucosidases crude extract from rat intestinal mucosa contains more than one enzyme activity, it is a mixture of sucrase, maltase,

isomaltase, glucoamylase (Jones *et al.,* 2011; Dhital *et al.,* 2013). pNPG, the chromogenic substrate is a common and non-specific substrate, which react indifferently with all these enzymes present in the mixture but inhibition reaction could be more specific and concern only a kind of these enzymes. This leads to an underestimated rate of inhibition activity. So it is more relevant and interesting when evaluating inhibitory activity of α-glucosidase to use specific substrate for each one, as maltose, saccharose, isomaltose for more accuracy and in an other hand, to be closer to physiological conditions, in the small intestine where the major carbohydrate are disaccharides.

CONCLUSION

Obesity and the onset of diabetes are two closely liked medical complications prevalent globally. Postprandial hyperglycemia is one of the earliest abnormalities of glucose homeostasis associated with type 2 diabetes. Postprandial glucose levels can be regulated through α-glucosidase inhibition. Medicinal plants constitute an important source of potential therapeutic agents for Type 2 Diabetes Mellitus. One vital therapeutic approach is the use of agents that can decrease postprandial hyperglycemia by inhibiting carbohydrate digesting enzymes resulting in a delay of carbohydrate digestion to absorbable monosaccharide. In this preliminary work, we attended to evaluate the α-amylase and α-glucosidase inhibitory activities of some extracts of *O. angustissima* to clarify its traditional use as antidiabetic treatment. Obtained results of the both enzyme inhibition activity, found in a dose-dependent manner constitute the first report for this plant. Further, *in vitro* and *in vivo* researches are required to confirm the present results, isolate and determine active substances and phenolic components contained in the extract of this plant that may be responsible for improvements in health conditions by regulating digestives enzymes inhibitory activities. *In vivo* studies are necessary to recognize a potential substance for clinical use in the therapy of diabetes and related disorders, so it is desirable to optimize secondary metabolite production and purification of compounds for the pharmaceutical applications. Furthermore, other studies *in vitro* and *in vivo* are needed to confirm these findings and characterize and determine bioactive components responsible of this effect. The present study confirms the traditional use of *O. angustissima* to treat diabetes mellitus.

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