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Phytochemical constituents of pressurized liquid extract from *Ziziphus jujuba* Mill. (Rhamnaceae) fruits and *in vitro* inhibitory activity on α-glucosidase, pancreatic α-amylase and lipase

Dora Trifonova[1], Ivanka Stoilova[1], Andrey Marchev[2], Petko Denev[3], Galena Angelova[1*], Anna Lante[4] and Albert Krastanov[1]

[1]*University of Food Technology, Department of Biotechnology, 4002 Plovdiv, Bulgaria*
[2]*Bulgarian Academy of Sciences, Department of Applied Microbiology, Laboratory of Applied Biotechnologies, 4000 Plovdiv, Bulgaria*
[3]*Bulgarian Academy of Sciences, Institute of Organic Chemistry with Centre of Phytochemistry, Laboratory of Biologically Active Substances, 4000 Plovdiv, Bulgaria*
[4]*Università di Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Viale dell`Universita 16, Agripolis, 35020 Legnaro, Padova, Italy*
[*]*Corresponding author:* galenaa@yahoo.com

Abstract

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In the current study the phytochemical composition of extract from *Ziziphus jujub*a Mill (Rhamnaceae) fruits obtained by pressurized liquid extraction (PLE) was investigated. The phytochemical analysis showed the presence of triterpenes, flavonoids and phenolic acids. The fruit extract had a total phenolic content of 21 ±2.01 mg gallic acid equivalents per g of extract (mg GAE/g extract), and total flavonoid content 2.48 ± 0.24 mg catechin equivalents per g dried extract (mg CE/g extract). This study evaluated the *in vitro* antioxidant activity of jujube extract and its inhibitory effects on α-glucosidase and pancreatic α-amylase and lipase. The obtained results proved inhibition of α-glucosidase with 70.94% and inhibition of α-amylase with 56.08%. Enzyme kinetic studies indicated that the inhibitors in extract have the most potent inhibition of lipase compared to other two enzymes with inhibitory constant K_i of 0.074 mg/cm^3. These results suggest the possible application of extract from *Z. jujub*a fruits obtained by PLE in the management of metabolic disorders as a whole. These are the first studies on this plant species cultivated in Bulgaria.

*Keywords: Ziziphus jujub*a*;* phytochemical profile; digestive enzymes; anti-diabetic; anti-obesity properties

Introduction

Plants are a source of bioactive compounds such as antioxidants and enzyme inhibitors. Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of crucial enzymes like α-amylase and α-glucosidase associated with diabetes mellitus type 2 and lipid peroxidation in tissues (Reddy et al., 2010). *In vivo* evidence supports the major contribution of hyperglycemia in producing oxidative stress and dysfunction in blood vessels of patients with diabetes (Ceriello, 2006). Oxidative stress is a major contribu-

tor to the β-cell damage and it was observed that diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis (Quilliot et al., 2005). That is why, one of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding absorption of glucose via inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase and α-amylase, which are the enzymes acting in the final step of the digestive process of carbohydrates (Bhandari et al., 2008; Khathi et al., 2014). Furthermore, lipase inhibitors have gained attention for their antiobesity activities to reduce the lipid absorption and to protect the pancreas that will enable the β-cells to produce normal levels of insulin (Nakai et al., 2005; Lin & Lin, 2008). Commercial inhibitors, such as acarbose, voglibose and miglitol used for controlling the blood sugar levels and the drugs Orlistat and Sibutramine which reduce intestinal fat absorption via inhibiting pancreatic lipase in patients cause numerous side effects (Mizuno et al., 2008). For this reason, a wide variety of natural sources have been explored for their ability to inhibit α-glucosidase (e.g. catechins and thea flavins) (Matsui et al., 2007), α-amylase (rosmarinic acid extracts) (McCue and Shetty, 2004) and pancreatic lipase (eucalyptus globulus extract) (Belfeki et al., 2016). The fruits of *Ziziphus jujube* Mill. are rich sources of biologically active molecules, such as polysaccharides, phenolic compounds, terpenes, saponins, etc. (Shoei et al., 1996; Lee et al., 2003; Hudina et al., 2008; San and Yildirim, 2010). These substances possess unique and multifactorial properties and could improve insulin sensitivity by multiple mechanisms (Castellano et al., 2013).

There are some researches concerning phytochemical composition of *Z. jujuba* (Rhamnaceae) fruits (Afrisham et al., 2015; Gao et al., 2013; Guo et al., 2015; Bai et al., 2016). The investigated in this search plant species is cultivated in Bulgaria but its phytochemical content is still poorly studied (Stoilova et al., 2017). Therefore, the aim of this study was to investigate the phytochemical constituents and in vitro inhibitory effect of extract from Bulgarian Z. jujuba (Rhamnaceae) fruits obtained by pressurized liquid extraction (PLE) against three digestive enzymes - α-glucosidase, α-amylase and pancreatic lipase. The increased biological activity of the extract would extend its benefits to human health.

Materials and Methods

Plant material

The plant *Z. jujub*a (*Rhamnaceae*) was cultivated in Bulgaria and was identified by Dr. Ina Aneva and voucher specimen (no. SOM 1355) was deposited at the herbarium of the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences. The fruits of jujuba (Rhamnaceae) were dried at 40°C, afterwards grinded, frozen and kept at – 20°C prior to extraction.

Chemicals and Reagents

α-Glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), α-amylase from porcine pancreas (EC 3.2.1.1), lipase (EC 3.2.1.1), p-nitrophenyl-α-D-glucopyranoside (pNPG), p-nitrophenyl palmitate (PNP), acarbose, 4-hydroxybenzoic acid hydrazide (PAHBAH), starch soluble (extra pure) were obtained from Sigma-Aldrich (Darmstadt, Germany). Phenolic standards: gallic, vanillic, ferulic, p-coumaric, p-hydroxy benzoic, syringic, trans-cinnamic, chlorogenic and rosemaryn acid, myricetin, quercetin, luteolin, kaempferol, apigenin, rutin, hyperosid, carnosic acid, betulin, betulinic acid, oleanolic and ursolic acid acid were purchased from SIGMA- ALDRICH (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) and other reagents were of analytical and HPLC grade.

Plant extract obtained by pressurized liquid extraction

The PLE was carried out in an automatic equipment (NM LAB/M Deputex 88, Limena, Padova, Italy) for 1.45 h, variable pressure 5-10 bar, with ethanol/water solutions (70:30 v/v acidified with 0.1 M HCl, pH 3), previously deoxygenated by flushing with nitrogen as reported by Rosseto et al. (2005). The collected extract was vacuum concentrated, dried at 40°C and stored at -20°C prior to be used.

Phytochemical analyses by HPLC

The obtained extracts were analyzed on HPLC system consisting of Waters 1525 Binary pump (WATERS, Milford, MA, USA), Waters 2487 Dual λ Absorbance Detector (WATERS, Milford, MA, USA), controlled by Breeze 3.30 software and equipped with reverse-phase Supelco Discovery HS C18 (25 cm x 4.6 mm, 5 µm) column at 26°C. The extracts were dissolved in appropriate concentrations with methanol and filtrated through 0.45 µm syringe filters. The constituents were identified according to their retention time (RT) and quantified according to calibration curve for each compound. For determination of triterpenes the method developed by Marchev et al. (2012) was applied. In brief, a mobile phase consisting of methanol and 0.1% formic acid (92:8) and 0.4 cm^3/min flow rate was used. The detection of the substances was monitored at 210 nm. For flavonoids analysis a gradient elution was applied. The mobile phases were 2.0% acetic acid (phase A) and methanol (phase B) with wavelength of detection 308 and 380 nm. Quercetin glycosides were detected at 380 nm by applying gradient elution with 2.0% acetic acid (phase A) and acetonitrile (phase B).

Rosmarinic acid was detected at 327 nm using mobile phase of methanol:H_3PO_4:H_2O = 50:0.3:49.7. The phenolic acids were quantified at 280 and 320 nm with mobile phase 2.0% acetic acid (phase A) and 0.5% acetic acid: acetonitrile (1:1). Detailed description and gradients of the mobile phase are described by Marchev et al. (2011).

Assay of total sugars

The total sugars were determined according to the method of Dubois et al. (1956).

Assay of total polyphenols content

The total polyphenols content (TPC) was determined according to Kerina et al. (1995). Briefly, to 0.1 cm^3 of extract (1:10 diluted) 0.5 cm^3 Folin-Ciocalteu reagent and 2.0 cm^3 10% $NaCO_3$ were added. After 10 min 1.0 cm^3 from the mix was added to 4.0 cm^3 of distilled water. The absorbance was read at 620 nm on spectrophotometer Spectrostar Nano (BMG, Ortenberg, Germany) against a blank sample containing water instead of extract. The phenolic amount was calculated through calibration curve with 0.01% solution of gallic acid and expressed as mg gallic acid equivalents per gram of extract (mg GAE.g^{-1} extract).

Assay of total flavonoids content

The total flavonoids content was determined according to Zhishen et al. (1999) with minor modifications. To 1 cm^3 appropriate diluted in DMSO extract 0.15 cm^3 $NaNO_2$ (5%) and 0.15 cm^3 $AlCl_3$ (10%) were added. Five minutes later 1.0 cm^3 NaOH (1M) was added and after 6 more min the volume was adjusted to 5 cm^3 with distilled H_2O. The reaction was conducted for 30 min at ambient temperature and afterwards the absorbance was measured at 510 nm against blank sample, which contained distilled water instead of extract. The amount of flavonoids was calculated through a calibration curve with (+) – cathecin and expressed as mg catechin equivalents per g extract (mg CE.g^{-1} extract).

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)

The radical scavenging ability was determined according to the method of Mensor et al. (2001). One cm^3 of 0.3 mM alcohol solution of DPPH was added to 2.5 cm^3 of the samples with different extract concentrations (EC). The samples were kept at room temperature in the dark and after 30 min the optic density was measured at 518 nm. The optic density of the samples and the control was measured in comparison with ethanol. Butylated hydroxytoluene (BHT) and Butylated hydroxyanizole (BHA), synthetic antioxidants, were used as a positive control.

Oxygen radical absorbance capacity (ORAC)

ORAC was measured according to the method of Ou et al. (2001) with some modifications (Ciz et al., 2010). The method measures the antioxidant scavenging activity against peroxyl radical generated by thermal decomposition of 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) at 37°C. Fluorescein was used as the fluorescent probe. The loss of fluorescence of fluorescein was an indication of the extent of its oxidation through reaction with the peroxyl radical. The protective effect of an antioxidant was measured by assessing the fluorescence area under the curve (AUC) plot relative to that of a blank in which no antioxidant was present. Solutions of AAPH, fluorescein and trolox were prepared in a sodium phosphate buffer (75 mM/dm^3, pH 7.4). Samples were diluted in the phosphate buffer as well. Reaction mixture (total volume 0.2 cm^3) contained fluorescein (0.17 cm^3, final concentration 5.36×10^{-8} M/dm^3), AAPH (0.02 cm^3, final concentration 51.51 mM/dm^3), and sample volume of 0.01 cm^3. The fluorescein solution and sample were incubated at 37°C for 20 min directly in a microplate reader, and AAPH (dissolved in buffer at 37°C) was added. The mixture was incubated for 30 s before the initial fluorescence was measured. After that, fluorescence readings were taken at the end of every cycle (1 min) after shaking. For the blank, 0.01 cm^3 of phosphate buffer was used instead of the extract. Each herb extract was analyzed in two runs with four replicates and results are presented as mean value ± standard deviation from the eight values. The antioxidant activity is expressed in micromole trolox equivalents (TE) per gram of dry weight. Trolox solutions (3.125, 6.25; 12.5; 25 and 50 μM/dm^3) were used for defining the standard curve.

Hydroxyl radical antioxidant capacity (HORAC)

The HORAC assay developed by Ou et al. (2002) measures the metal-chelating activity of antioxidants in the conditions of Fenton-like reactions employing a Co(II) complex and hence the protecting ability against formation of hydroxyl radical. Hydrogen peroxide solution of 0.55 M was prepared in distilled water, and 4.6 mM Co(II) was prepared as follows: 15.7 mg of $CoF_2\times4H_2O$ and 20 mg of picolinic acid were dissolved in 20 cm3 of distilled water. Fluorescein (0.17 cm^3, final concentration 60 nM) and 0.01 cm^3 of sample were incubated at 37°C for 10 min directly in a FLUOstar plate reader, then 0.01 cm^3 of H_2O_2 (final concentration 27.5 mM) and 0.01 cm^3 of Co(II) (final concentration 230 μM) solutions were added. Initial fluorescence was measured immediately, then readings were taken every minute after shaking. For the blank sample, sodium phosphate buffer (75 mM, pH=7.4) was used. Gallic acid solutions (100, 200, 600, 800 and 1000 μM in phosphate buffer) were used for standard

curve. The AUC values were calculated as they were for the ORAC assay. Each herb extract was analyzed in two runs with four replicates and results are presented as mean value ± standard deviation from the eight values. ORAC and HORAC analyses were carried out using a FLUOstar OPTIMA plate reader (BMG Labtech, Germany) with excitation wavelength of 485 nm and emission wavelength of 520 nm.

Inhibition of α-glucosidase activity

Yeast α-glucosidase has been frequently used to identify its inhibitors from medicinal plants. The method of Suresh et al. (2004) was employed with some modifications. The assay uses pNPG as substrate, which is hydrolyzed by α-glucosidase to release p-nitrophenol, a color agent that can be monitored at 405 nm. Briefly, 0.02 cm^3 of a sample solution was mixed with 0.07 cm^3 of the enzyme solution (1 unit/cm^3) in 0.1 M phosphate buffer (pH 6.8), since α-glucosidase is sensitive to different pH values, and incubated at 37ºC for 6 min under shaking. After incubation, 0.1 cm^3 pNPG (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mM) solution in the above buffer was added to initiate the colorimetric reaction at 37 ºC. The released p-nitrophenol was monitored at 405 nm every min for a total time of 30 min by a Spectrostar Nano (BMG, Ortenberg, Germany) 96 micro well plate reader against a blank sample without enzyme. The solvent DMSO (without extracts), enzyme and substrate were set up in parallel as a control and each experiment was performed in triplicates. One unit of enzyme activity was defined as the amount of enzyme that released one μM of p-nitrophenol per minute under the assay conditions described.

Inhibition of pancreatic α-amylase activity

The α-amylase activity was determined using the modified version of the method according to McDougall et al. (2005) and Grussu et al. (2011). Briefly, 0.05 cm^3 of the extracts, which were dissolved in DMSO was mixed with 0.2 cm^3 of the enzyme solution (0.5 U/cm^3) in 0.2 M sodium phosphate buffer (pH 6.9) and incubated at 37°C for 5 min. After incubation, 0.25 cm^3 of the soluble starch in concentration 0.1, 0.5, 1, 1.3 and 1.5 % was added to initiate the enzyme reaction. 100 mg PAHBAH substance was dissolve successively with 0.5 M HCl and 0.5 M NaOH to give the working PAHBAH reagent. Triplicate samples (250 μl) of assays were taken at fixed times and added to 1.75 cm^3 of PAHBAH reagent in a 2 cm^3 tube. After heating for 15 min at 100°C and cooling, the absorbance at 410 nm was measured by a spectrophotometer SPECTROstar Nano (BMG, Offenburg, Germany). Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the plant extracts. Mixtures without enzyme, containing only plant extract and acarbose served as blanks to eliminate the effect of reducing substances. One unit of enzyme activity was defined as the amount of enzyme that released one μM of glucose per minute under the assay conditions described.

Inhibition of pancreatic lipase activity

The pancreatic lipase activity was measured by using p-nitrophenyl palmitate (pNPP) in concentration from 0.1 to 1.5 mM as substrate buffered with Tris-HCl pH 9.0, as reported by Chanmee et al. (2013). An aliquot of 0.07 cm^3 of the pancreatic lipase solution (1 U/cm^3) in a Tris-HCl (pH 9.0) buffer solution was added into 0.02 cm^3 of the dissolved in DMSO sample and mixed with 0.1 cm^3 of the PNP solution dissolved in the above buffer in the well of a 96-well microplate to start the enzyme reaction. The plate was immediately placed in the 37°C preheating a spectrophotometer SPECTROstar Nano (BMG, Offenburg, Germany). The amount of p-nitrophenol released by the lipase was monitored at 405 nm every minute for a total time of 30 min against a blank sample without enzyme. DMSO (without extracts), enzyme and substrate were set up in parallel as a control and each experiment was performed in triplicates. One unit of enzyme activity was defined as the amount of enzyme that released one μM of p-nitrophenol per minute under the assay conditions described.

Kinetics of inhibition of α-amylase, α-glucosidase and lipase by ***Z. jujuba extract***

The kinetics of inhibition of α-glucosidase, lipase and α-amylase by *Z. jujuba* extract were measured by increasing substrate concentrations of pNPG (0.5- 5.0 mM), pNPP (0.1 – 1.5 mM) and starch (0.1-1.5%), respectively in the absence and presence of extract of *Z. jujub*a at concentrations of 0.66 and 1.33 mg/cm^3 for both α- glucosidase and α-amylase inhibitory assay; for lipase inhibitory assay at concentrations of 0.11 and 0.33 mg/cm^3. Acarbose at concentrations 0.66 and 1.33 mg/cm^3 was included as a possitive control (Stoilova et al., 2017). The initial rates of reactions were determined from calibration curves constructed using varying concentrations of p-nitrophenol and starch for the α-glucosidase, lipase and α-amylase inhibition assays, respectively. The initial velocity data obtained were used to construct Lineweaver-Burke's plot to determine the K_m (Michaelis constant) and V_{max} (maximum velocity) of the enzyme. The type of inhibition was determined by Lineweaver-Burk double reciprocal plot analysis of the data, which was calculated from the result according to Michaelis-Menten kinetics (Palmer, 2004). Each experiment was performed in triplicates. The inhibition of α-glucosidase, α-amylase and lipase by jujube extract was

calculated according to the following formula: % Inhibition = [(EAc− EAe)/EAc] × 100 where EAc and EAe are the enzyme activity of the control sample and enzyme activity in presence of jujube extract, respectively.

Statistical analysis

The experiments were performed in triplicate. The results were expressed as mean ± SD. Statistical analyses were performed using Microsoft Excel 2010.

Results and Discussion

Phytohemical composition of Z. jujuba extract

Phytochemical composition of Z. *jujuba* extract obtained by PLE includes total sugars 840 ±42.0 mg.g^{-1} extract, total phenolic compounds 21 ±2.01 mg.g^{-1}, total flavonoids 2.48 ± 0.24 mg.g^{-1}and proteins 1 ±0.08 mg.g^{-1} extract. Results of the HPLC analysis of *Z. jujuba* extract are presented in Table 1. In total three phenolic acids, three flavonoids and five triterpenes were identified. The presence of terpenoids and flavonoids in the *Z. jujuba* fruits has been reported by Lee et al. (2003) and Shoei et al. (1996). In our study, the total concentration of triterpenoids (831 µg.g^{-1} extract) is higher than this of phenolic acids (143 µg.g^{-1} extract) and flavonoids (101 µg.g^{-1} extract). Obviously the significant part of the phenolic and flavonoid compounds remains unidentified. In our previous study of *Z. jujuba* extract obtained by ultrasound extraction and the same extragent (70% ethanol), eleven phenolic acids with a total concentration of 2569 µg.g^{-1} extract, five flavonoids and quercetin glycosides - rutin and hyperoside (3519 µg.g^{-1} extract) have been identified (Stoilova et al., 2017).

Among the five triterpenes in the studied extract, betulin (360 ±9.08 µg.g^{-1} extract) is in the highest concentration and betulinic (116 ±2.93 µg.g^{-1} extract) and oleanolic acid (100 ±2.53 µg.g^{-1} extract) are in approximately the same concentrations. Chlorogenic acid (55.6 ±0.96 µg.g^{-1} extract) and caffeic acid (70 ±1.23 µg.g^{-1} extract) are found to be in higher concentration than rosmarinic acid (16.67 ±0.42 µg.g^{-1} extract). The identified flavonoids in the extract are myricetin (69.2±1.74 µg.g^{-1} extract), kaempferol (26.9±0.68 µg.g^{-1} extract) and luteolin (5.59±0.14 µg.g^{-1} extract). The presence of phenols including flavonoids and triterpenes, in the extract is determinant for its biological activity. Furthermore, the influence of the chemical profile as a crucial factor for the biological activity of an PLE should be elucidated.

Antioxidant activity of Z. jujuba extract

The antioxidant capacities of extract were analyzed by DPPH, ORAC and HORAC methods based on different mechanisms of action. The radical DPPH scavenging activity of *Z. jujuba* extract is compared with that of antioxidants widely used in food preservation, such as BHT and BHA. The of *Z. jujuba* extract proves to be a weak 2,2-DPPH$^{\bullet}$ reducer (IC_{50} = 2.611 µg/cm^3, R^2 = 0.9996), compared with BHT (IC_{50} = 0.5602 µg/cm^3, R^2 = 0.999) and BHA (IC_{50} = 0.1146 µg/cm^3, R^2 = 0.9953) at a DPPH concentration of 0.3 mM. The extent of radical inhibition in the DPPH assay is dependent on the used DPPH concentration in an inversely proportional manner. In comparison with the present study, the inhibitory concentration calculated as IC_{50} values for *Z. jujuba* bark extract possess maximum activity 4.211 ± 0.22 mg/cm^3 at a DPPH concentration of 0.05 mM (Mandakini & Sameera, 2015).

ORAC and HORAC methods

The oxygen radical absorbance capacity (ORAC), which is adopted to evaluate the peroxyl radical scavenging activity of the *Z. jujuba* pressurized liquid extract, is more efficient and accurate than traditional free radical based antioxidant assays, such as DPPH and ABTS. ORAC method shows the ability of antioxidants to scavenge peroxyl radicals via hydrogen atom transfer. These radicals are physiologically the most important ones and the hydrogen atom

Table 1. Bioactive compounds of *Z. jujuba* extract and fruits

Components	Triterpenes		Phenolic acids			Flavonoids		
	Concentration, µg.g^{-1} extract	Concentration, µg.g^{-1} biomass	Components	Concentration µg.g^{-1} extract	Concentration, µg.g^{-1} biomass	Components	Concentration, µg.g^{-1} extract	Concentration, µg.g^{-1} biomass
Carnosic acid	68 ±1.74	23 ±0.59	Chlorogenic acid	55 ±0.96	19 ±0.33	Myricetin	69 ±1.74	23.7±0.60
Betulin	360 ±9.08	123 ±3.11	Caffeic acid	70 ±1.23	24.28±0.42	Luteolin	5.59±0.14	1.92±0.05
Betulinic acid	116 ±2.93	39 ±1.00	Rosmarinic acid	16.67 ±0.42	5.71±0.14	Kaempferol	26.9±0.68	9.23±0.23
Oleanolic acid	100 ±2.53	34 ±0.87						
Ursolic acid	184 ±4.66	63 ±1.59						

transfer is the most physiologically relevant mechanism of antioxidant action. The HORAC method measures the metal-chelating activity of antioxidants under conditions of Fenton-like reactions and hence indicates the protecting ability against formation of hydroxyl radical. ORAC activity of *Z. jujuba* extract is 291.2±15.65 μmol TE.g^{-1} and HORAC – 141.4 ±8.1 μM GAE.g^{-1} which is an additional proof that compounds with high redox potential are found in *Z. jujuba* fruits extract. The analyzed extract includes a variety of metabolic compounds that could contribute to its antioxidant activity as quercetin and myricetin which are powerful inhibitors of iron-induced lipid peroxidation. Both kaempferol and quercetin turned out to be exceptionally good scavengers of linoleic acid peroxyl radicals (Erben-Russ et al., 1987). Cholbi et al. (1991) described the activity of apigenin, luteolin, as inhibitors of CCl4-induced rat liver NADPH-dependent microsomal lipid peroxidation. Oleanolic acid has a protective effect on β-cells at oxidative stress caused by the overproduction of mitochondrial reactive oxygen types (Castellano et al., 2013).

The presence of terpenes, phenolic acids and flavonoids in the investigated by us *Z. jujuba* extract is a prerequisite for a potential inhibitory activity of *Z. jujuba* against α-glucosidase and α-amylase.

Inhibition of α-glucosidase activity

The inhibition kinetics of α-glucosidase is studied using Lineweaver–Burk plots and the corresponding secondary plots (Figure 1A, B, C). The primary plots of the inhibition give straight lines with a point of intersection in the third quadrant. The linear regression equation is shown on Figure 1A. In the presence of different extract concentrations K_m and V_{max} values decrease and are different from that of the control sample (Table 2).

Therefore the inhibition is non-competitive-uncompetitive mixed. This could be also confirmed by the values of the dissociation constant: KI (1.259 mg/cm^3) and K_i (1.463 mg/cm^3), determined by the equations of the secondary graphics (Figure 1B, C). In case of non-competitive-uncompetitive inhibition KI < K_i (Palmer, 2004). The lower KI (ESI) value than the (EI) is evidence for the higher affinity of the inhibitors in the extract to bind with ES- complex than the free enzyme. The results are summarized in Table 2. The obtained kinetic parameters are compared with those of synthetic acarbose inhibitor, the details of which have been reported in a previous study (Stoilova et al., 2017).

The constant of inhibition (K_i) of acarbose (0.333 mg/cm^3) is with lower value than K_i of the extract (1.463 mg/cm^3) (Table 2). This means that the inhibition power of *Z. jujuba* extract is lower than that of acarbose. The studied extract exhibits non-competitive – uncompetitiv mixed inhibition, analogous to that performed by the synthetic inhibitor (SI). The presence of the extract in the reaction mixture causes strong inhibition of α–glucosidase (70.94%) comparable to that of acarbose (82.98%). Flavonoid structure, position and number of hydroxyl groups are determining factors for α-glucosidase inhibition. According to Tadera et al. (2006) α-glucosidase inhibitory activity of flavonoids increases with increasing the number of the hydroxyl groups in the B-ring, while the substitution of the hydroxyl group at posi-

Table 2. Inhibition and kinetic parameters of pNPG hydrolysis by α-glucosidase and kinetic parameters of starch hydrolysis by α-amylase in the absence and presence of inhibitors in *Z. jujuba* extract and acarbose

pNPG hydrolysis by α-glucosidase	Inhibition, %	Kinetic parameters			
		V_{max}, mM/(cm^3.min)	K_m, mg/cm^3	KI, mg/cm^3	K_i, mg/cm^3
Uninhibited reaction	–	66.23	1.481	–	–
EC 1.33 mg/cm^3	61.93	20.83	1.084	1.259	1.463
EC 3.33 mg/cm^3	70.94	14.31	1.035		
*acarbose 0.66 mg/cm^3	70.94	17.30	1.339	0.096	0.333
*acarbose 1.33 mg/cm^3	82.98	7.24	0.096		
Starch hydrolysis by α-amylase	Inhibition, %	V_{max}, μM/(cm^3min)	K_m, mg/cm^3	KI, mg/cm^3	K_i, mg/cm^3
Uninhibited reaction	–	0.852	0.964	–	–
EC 0.666 mg/cm^3	41.06	0.498	0.667	0.866	2.583
EC 1.333 mg/cm^3	56.08	0.339	0.582		
*acarbose 0.045 mg/cm^3	10.50	0.851	1.374	–	0.122
*acarbose 0.066 mg/cm^3	21.73	0.819	1.484		

*(Stoilova et al., 2017)

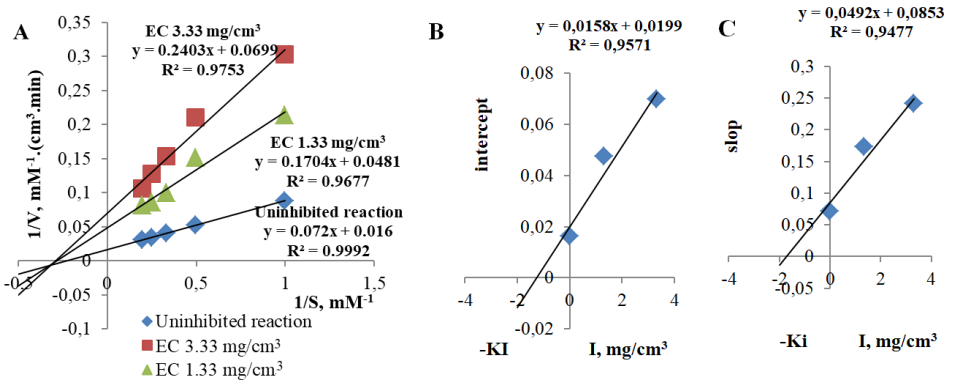

Fig. 1. Lineweaver–Burk plots of *Z. jujuba* extracts with α-glucosidase (A) and secondary plots (B and C) for determination of dissociation constants of ESI (KI) and EI (Ki). Reciprocal plots were obtained by variable extract concentrations. Each point represents the mean of three experiments

tion 3 decreases activity. Therefore, the presence of flavonoids such as myricetin, kaempferol, luteolin in the studied extract is premise for its inhibitory activity on α-glucosidase. Mizuno et al. (2008) report for inhibitory effect of these flavonoids in decreasing order (in %): myricetin (94) > luteolin (92) > quercetin (91) > kaempferol (82). Evidence has been accumulated showing that chlorogenic acid exhibits hypoglycemic and hypolipidemic effects (Bassoli et al., 2008; Rodriguez de Sotillo & Hadley, 2002) and acid inhibits rat intestinal α-glucosidase in a non-competitive manner (Matsui et al., 2006). There are few reports that triterpeniods have α-glucosidase inhibitory activity. Kumar et al. (2013) confirm that betulinic acid inhibits α-glucosidase activity with 52.2 ± 3.8% at concentration of 50 μg/cm³. Ali et al. (2002) proves that oleanolic acid inhibits α-glucosidase uncompetitive.

Inhibition of α-amylase activity

Lineweaver-Burk primary graphic for inhibition of α-amylase by extract and the corresponding regression equations are shown on Figure 2A. In the presence of different extract concentrations (EC), K_m and V_{max} values decrease and are different from that of the control sample (Table 2). In the primary graphic the obtained correlations are crossing in 3nd quadrant.

The dissociation constant are determined by the equations of the secondary graphics (Figure 2B, C) for ESI-complex, KI (0.866 mg/cm³) and inhibitory constant for the EI-complex, K_i (2.583 mg/cm³). The lower value of KI corresponds to stronger activity of the inhibitors in the extract to bind with ES than with enzyme. In the case of non-competitive-uncompetitive inhibition KI < K_i (Palmer, 2004).

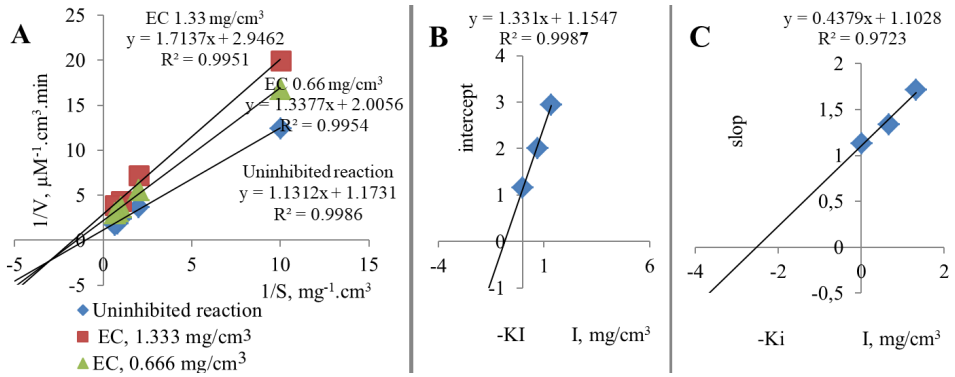

Fig. 2. Lineweaver–Burk plots of *Z. jujuba* extracts with α-amylase (A) and secondary plots (B and C) for determination of dissociation constants of ESI (KI) and EI (Ki). Reciprocal plots were obtained by variable extract concentrations. Each point represents the mean of three experiments

The kinetic parameters of starch hydrolysis by α-amylase in the presence of inhibitors in *Z. jujuba* extract and acarbose show differences in the type of inhibition. The inhibition by *Z. jujuba* extract is non-competitive – uncompetitive mixed and competitive by acarbose.

And with this enzyme, the inhibitory potency of jujube extract is lower than that of acarbose, but with the higher tested concentration (1.333 mg/cm^3) *Z. jujuba* extract induces a significant inhibition of α-amylase of 56.08%. The comparatively high α-amylase inhibitory activity is associated with the strong affinity of the inhibitors in the extract for binding with ES (KI 0.866 mg/cm^3) complex. The high α-amylase inhibitory activity suggests the presence of more active compounds that could inhibit the breakdown of complex carbohydrates to oligosaccharides.For some of the identified components in the extract by us, α-amylase inhibitory activity has been reported.

Pentacyclic triterpenoids, such as oleanolic acid (Hyun et al., 2014), betulinic acid, oleanolic acid, ursolic acid (Castellano et al., 2013) and ursolic acid (Chakrabarti and Rajagopalan, 2002) have been shown to inhibit α-amylase. Despite the fact that triterpenoids are widely distributed in plants the mechanism by which their activity occurs is still unknown (Sales de Paloma et al., 2012). It have been demonstrated that flavonoids such as luteolin (Chakrabarti et al., 2002), myricetin (Tadera et al., 2006) and kaempferol (McDougall et al., 2005; Lo Piparo et al. 2008) effectively inhibite α-amylase. Differences in percentage of inhibition by myricetin were observed. Its inhibitory activity ranges from 35.9% according to Ng, (2015) to 64% according to Tadera et al. (2006). It is known that phenolic acids such as chlorogenic (Funke and Melzing, 2006) and rosmarinic (McCue et al., 2004) posses α-amylase inhibition activity. Caffeic acid, isolated from *Elusine coracana* extracts has revealed inhibitory activity to α-amylase with IC_{50} value of 23.05 mg/cm^3 (Shobana et al., 2009).

The knowledge of type of inhibition reveals the precise mechanisms of action of enzyme inhibitors. The inhibitors in the studied extract exhibit the same type of inhibition to both enzymes. The dissociation constants KI and K_i at inhibition of α-glucosidase and α-amylase by the *Z. jujuba* extract show, that the inhibitors in the extract are not substrate analogues both for α-glucosidase and for α-amylase. Main constituents in the studied *Z. jujuba* extract are triterpenes, which distinguishes them from other extracts that contain mainly phenolic compounds. The variety of metabolites with different chemical structures in the extract is the possible explanation for the observed pattern of inhibition. Besides, obtained results prove high inhibition of α-glucosidase (70.94% at 3.33 mg/cm^3 extract) and lower inhibition of α-amylase (56.08% at 1.333 mg/cm^3 extract concentration). These differences in extract activity confirm their specificity towards the inhibition of the two enzymes. In the present research the potential of *Z. jujuba* extract as inhibitor of the enzyme α-glucosidase and α-amylase is described by K_i value not by $IC_{50.}$ The IC_{50} value depends on concentrations of the enzyme, the inhibitor and the substrate along with other experimental conditions. K_i value is thermodynamic constant that is independent of the substrate but depends on the enzyme and inhibitor (Cer et al., 2009). Determination of K_i value allows more easily comparing inhibitors determined by different surveys. Afrisham et al. (2015) studied the inhibitory activity of phenolic content in methanol extract of *Z. jujube* on α-amylase and established strong inhibitory effect with IC_{50} value of 827 μg/cm^3. Similar effect of inhibition on α-amylase (K_i 0.791 mg/cm^3) we received in our previous research with 70 % ethanolic *Z. jujuba* extracts (Stoilova et al., 2017). The results for α-amylase inhibition in the current study showed the effect of PLE both on phytochemical content of *Z. jujuba* extract and on resulting biological activities.

Inhibition of pancreatic lipase activity

The results of kinetics of inhibition of jujube extract against pancreatic lipase activity are reflected on Figure 3A, B,C and Table 3. Based on the Lineweaver-Burk primary graphic, equations for the control and for different concentrations of the extract have been obtained (Figure 3A). The obtained lines are crossing in the 2nd quadrant; K_m values are increasing, while V_{max} values are decreasing being different from control values of the (Table 3). It should be noted the minimum increase of K_m values with increasing the extract concentration. This fact reveals that the inhibitors have a weak impact towards the affinity of the enzyme to the substrate. KI value (0.466 mg/cm^3) and K_i value (0.074 mg/cm^3), determined by the equations of the secondary graphics (Figure 3B, C) show that the inhibitors in the extract have higher binding affinity to enzyme in comparison to the ES-complex. These results account for a mixed competitive-non-competitive inhibition (Palmer, 2004). In the inhibition of lipase, the low value of K_i indicates that jujube extract contains compounds which could serve as substrate analogues and therefore to compete for lipase active site. Such inhibitors could be fatty acids found in jujube fruit by San et al. (2010). Authors have determined that lipid content of fresh fruits ranged from 0.06% to 0.10% among the four jujube selections. The extremely potent anti-lipase activity is probably due to synergy between extract's compounds. Strong hypoglycaemic and hypolipidemic effects have been proven for chlorogenic acid (Bassoli et al., 2008; Rodriguez de Sotillo et al., 2002) and oleanolic acid (Castellano et al., 2013).

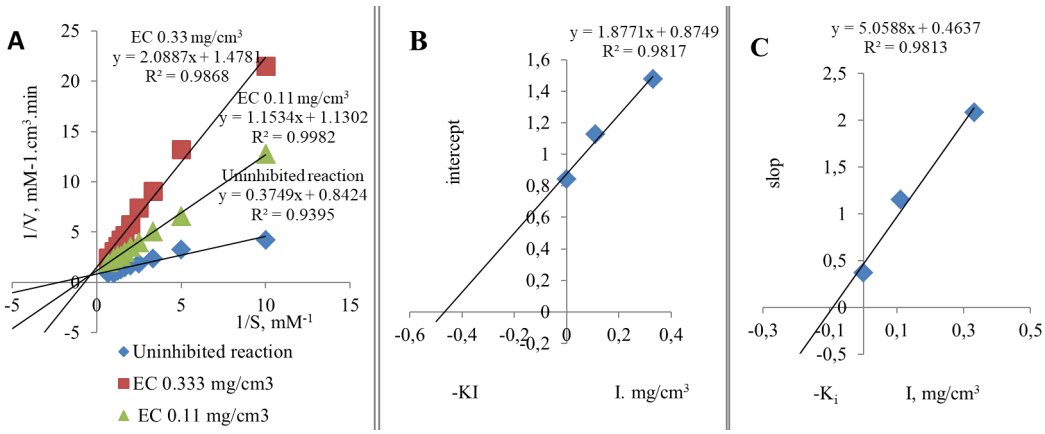

Fig. 3. Lineweaver–Burk plots of *Z. jujuba* extracts with lipase (A) and secondary plots (B and C) for determination of dissociation constants of ESI (KI) and EI (K_i). Reciprocal plots were obtained by variable extract concentrations. Each point represents the mean of three experiments

Table 3. Inhibition and kinetic parameters of p-NPP hydrolysis by lipase in the absence and presence of inhibitors in *Z. jujuba* extract

pNPP hydrolysis by lipase	Inhibition, %	Kinetic parameters			
		Vmax, mM/(cm³.min)	K_m, mg/cm³	KI, mg/cm³	K_i, mg/ cm³
Uninhibited reaction		1.187	0.134	-	-
EC 0.11 mg/cm³	54.97	0.877	0.304	0.466	0.074
EC 0.33 mg/cm³	63.22	0.676	0.425		

It is known that naturally-occurring polyphenols can inhibit lipase and several studies have focused on polyphenols from teas, herbal and fruit sources (McDougall et al., 2009). Lipase inhibitors have attracted significant attention because of their antiobesity activities, reduction of lipid absorption and consequently control of lipidemia.

In comparison with the present study, inhibition constant K_i value of tea saponin has been determined to be 0.25 mg/cm³ (Birari and Bhutani, 2007), and the inhibition constant value of EtoAc extracts of muscadine fruit was 46.22 mg/cm³ (You et al., 2012). Belfeki et al. (2016) reported that methanol extract of *E. globulus* leaves reveals inhibition constant (KI) of 0.271 mg/cm³ towards *Aspergillus niger* lipase and 0.188 mg/cm³ for olive lipase, whereas *M. viridis* leaves methanol extract exhibit KI of 0.303 mg/cm³ towards *Aspergillus niger* lipase and 0.421 mg/cm³ for olive lipase, respectively. The conclusion of Dechakhamphu & Wongchum (2015) is that phenolic, flavonoid and alkaloid compounds are key agents for pancreatic lipase inhibition *in vitro*.

The established high concentration of phenols found in *Z. jujuba* extract obtained by PLE is a prerequisite for its potent anti-lipase activity. Probably this extract, contains also other compounds which are responsible for the high anti-lipase activity. Compared to present study, ultrasonic extract of *Z. jujube* fruit with the same extragent does not show anti-lipase activity (Stoilova et al., 2017).

Glucose and lipid metabolic disorders are closely related with the occurrence and progression of diabetes, obesity and cardiovascular diseases (Bray, 2002). Obesity is one of the most common nutritional diseases worldwide leading to serious complications, such as Diabetes milletus type 2 (Knowler et al., 2002). Diets are high in fat and tend to promote obesity, and the pharmacologic inhibition of the digestion and absorption of dietary fat has been used as a strategy to treat obesity (Ballinger & Peikin, 2002). The inhibition of pancreatic lipase from jujube extract shows its potential as an effective means of regulating obesity. The management of type 2 diabetes requires an integrated approach, which includes the use of therapies to control glycemia and lipidemia in its late stages (Castellano et al., 2013). In connection with this the *Z. jujuba* extract obtained by PLE possess potential not only to reduce hyperglycemia but also to regulate obesity. Hyperglycemia induces generation of free radicals, which are responsible for oxidative stress (Castellano et al., 2013; Ceriello, 2006), which ultimately results in pancreatic B-cell failure (Quilliot et al., 2005). The high antioxidant

activities of the *Z. jujuba* extract is an opportunity for its use as a therapeutic agent, for treating oxidative stress based metabolic disorders. Besides this, the current therapeutic strategy for the control of postprandial hyperglycemia is the inhibition of acting glycoside hydrolases α-glucosidase and α-amylase, resulting in an aggressive delay of carbohydrate digestion to absorbable monosaccharides. In addition to its high antioxidant activities, the studied extract also shows anti-α-glucosidase and anti-α-amylase activity and powerful anti-lipase activity. Therefore *Z. jujuba* extract obtained by PLE has the potential to control the metabolic disorders as a whole and its therapeutic control is to be explored *in vivo.*

Conclusion

On the basis of the obtained data, it could be concluded that extract of *Ziziphus jujuba* Mill (Rhamnaceae) fruit obtained by PLE has a considerable antioxidant potential and exhibits *in vitro* inhibitory activity against α-glucosidase and α-amylase which are concerned in regulation and absorption of carbohydrates. Our results reveal the potent anti-lipase activity of the studied extract supports its use as a novel natural agent, possesing interesting health promoting effects.

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References

Afrisham, R., Aberomand, M., Ghaffari, M. A., Siahpoosh, A. & Jamalan, M. (2015). Inhibitory effect of *Heracleum persicum* and *Ziziphus jujuba* on activity of alpha-amylase. *Journal of Botany*, Volume 2015, ID 824683. http://dx.doi.org/10.1155/2015/824683

Ali, M. S., Jahangir, M., Hussan, S. S. & Choudhary, M. I. (2002). Inhibition of alpha-glucosidase by oleanolic acid and its synthetic derivatives. *Phytochemistry*, *60,* 295–299.

Bai, L., Zhang, H., Liu, Q., Zhao, Y., Cui, X., Guo, S. Zhang L, Ho, Ct. & Bai, N. (2016). Chemical characterization of the main bioactive constituents from fruits of *Ziziphus jujuba. Food & function*, *7(6),* 2870-2877. http://dx.doi.org/10.1039/C6FO00613B

Ballinger, A. & Peikin, S. I. R. (2002). Orlistat: its current status as an anti-obesity drug. *Eur. J. Pharm*., *440 (2-3),* 109-117.

Bassoli, B. K., Cassolla, P., Borba-Murad, G. R., Constantin, J., Salgueiro-Pagadigorria, C. L., Bazotte, R. B., Da Silva, R. S. & De Souza, H. M. (2008). Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. *Cell Biochemistry and Function*, *26 (3),* 320–328. https://doi.org/10.1002/cbf.1444

Belfeki, H., Mondher, M. & Mnasser, H. (2016). Antioxidant and anti-lipases activities in vitro of *Mentha viridis* and *Eucalyptus globulus* extracts. *Industrial Crops and Products*, *89,* 514–521.

Bhandari, M. R., Nilubon, J. A., Gao, H. & Kawabata, J. (2008). α-Glucosidase and amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata Haw). *Food Chemistry*, *106,* 247-252.

Birari, R. B. & Bhutani, K. K. (2007). Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug Discovery Today*, *12,* 879–889. https://doi.org/10.1016/j.drudis.2007.07.024

Bray, G. A. (2002). The underlying basis for obesity: relationship to cancer. *J. Nutr*., *132 (11),* 3451S-3455S. https://doi.org/10.1093/jn/132.11.3451S.

Castellano, J. M., Guinda, A., Delgado, T., Rada, M. & Cayuela, J. A. (2013). Biochemical basis of the antidiabetic activity of oleanolic acid and related pentacyclic triterpenes. *Diabetes*, *62,* 1791–1799. https://doi.org/10.2337/db12-1215

Cer, R. Z., Mudunuri, U., Stephens, R. & Lebeda, F. J. (2009). IC50-to-Ki: a web-based tool for converting IC_{50} to K_i values for inhibitors of enzyme activity and ligand binding. *Nucleic Acids Research*, *37* (Web Server issue), W441–W445. https://dx.doi.org/nar /gkp253

Ceriello, A. (2006). Oxidative stress and diabetes-associated complications, *Endocr. Pract*., *12 (1),* 60–62. https://doi.org/10.4158/EP.12.S1.60

Chakrabarti, R. & Rajagopalan, R. (2002). Diabetes and insulin resistance associated disorders: Disease and the therapy. *Current Science*, *83 (12),* 1533-1538.

Chanmee, W., Chaicharoenpong, Ch. & Petsom, A. (2013). Lipase inhibitor from fruits of *Salanum stramonifolium* Jacq. *Food and Nutrition Sciences*, *4,* 554-558. http://dx.doi.org/10.4236/fns.2013.45071

Cholbi, M. R., Paya, M. & Alcaraz, M. J. (1991). Inhibitory effects of phenolic compounds on CCl 4-induced microsomal lipid peroxidation. *Experientia*, *47,*195–199.

Ciz, M,, Cizova, H., Denev, P., Kratchanova, M., Slavov, A. & Lojek, A. (2010). Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control*, *21,* 518–523. https://doi.org/10.1016/j.foodcont.2009.07.017

Dechakhamphu, A. & Wongchum, N. (2015). Screening for anti-pancreatic lipase properties of 28 traditional Thai medicinal herbs. *Asian Pac. J. Trop. Biomed*, *5 (12),* 1042–1045. https://doi.org/10.1016/S1726-4901(10)70068-X

Dubois, M., Gilles K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28,* 350–356.

Erben-Russ, M., Bors, W. & Saran, M. (1987). Reactions of linoleic acid peroxyl radicals. *Int. J. Radiat. Biol., 52,* 393–412.

Funke, I. & Melzing, M. F. (2006). Traditionally used plants indiabetes therapy - phytotherapeutics as inhibitors of a-amylase activity. *Rev. Bras. Farmacogn., 16,* 1-5. http://dx.doi.org/10.1590/S0102-695X2006000100002

Gao, Q. H., Wu, C. S. & Wang, M. (2013). The jujube (*Ziziphus jujuba* Mill.) fruit: a review of current knowledge of fruit composition and health benefits. *Journal of Agricultural and Food Chemistry*, *61(14),* 3351-3363. https://doi.org/10.1021/jf4007032

Grussu, D., Stewart, D. & Mcdougall, J. G. (2011). Berry poly-

phenols inhibit α-amylase in vitro; Identifying active components in rowanberry and raspberry. *J. Agric.Food Chem*., *59,* 2324-2331. https://doi.org/10.1021/jf1045359

Guo, S., Duan, J. A., Qian, D., Tang, Y., Wu, D., Su, S., Wang, H. & Zhao, Y. (2015). Content variations of triterpenic acid, nucleoside, nucleobase, and sugar in jujube (*Ziziphus jujuba*) fruit during ripening. *Food Chemistry, 167,* 468-474. https://doi.org/10.1016/j.foodchem.2014.07.013

Hudina, M., Liu, M., Veberic, R., Stampar, F. & Colaric, M. (2008). Phenolic compounds in the fruit of different varieties of Chinese jujube (*Ziziphus jujuba* Mill.). *Journal of Horticultural Science and Biotechnology*, *83 (3),* 305–308. https://doi.org/10.1080/14620316.2008.11512382

Hyun, T. K., Eom, S. H. & Kim, J. S. (2014). Molecular docking studies for discovery of plant-derived α-glucosidase inhibitors. *Plant Omics Journal*, *7 (3),* 166-170.

Kerina, H. J., Trudgill, P. W. & Hopper, D. J. (1995). Evidence of two pathways for the metabolism of phenol by *Aspergillus fumigatus*. *Archives of Microbiology*, *163,* 176-181.

Khathi, A., Serumula, M., R.., Myburg, R. B., Van Heerden, F. R. & Musabayane, C. T. (2014). Effects of *Syzygium aromaticum*-derived triterpenes on postprandial blood glucose in streptozotocin - induced diabetic rats following carbohydrate challenge. *PLOS ONE,* 9. https://doi.org/10.1371/journal.pone.0081632

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A. & Nathan, D. M. (2002). Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med*., *346 (6),* 393-403. https://doi.org/10.1056/NEJMoa012512

Kumar, S., Kumar, V. & Prakash, O. (2013). Enzymes Inhibition and Antidiabetic Effect of Isolated Constituents from *Dillenia indica. BioMed Research International, 2013,* ID 382063. http://dx.doi.org/10.1155/2013/382063

Lee, S., Min, B. S., Lee, C-Gyu.; Kim, K-Sig. & Kho, Y. H. (2003). Cytotoxic triterpenoids from the fruits of *Ziziphus jujuba. Planta Medica*, *69,* 18-21.http://dx.doi.org/10.1055/s-2003-45155

Lin, C. L. & Lin, J. K. (2008). Epigallocatechin gallate (EGCG) attenuates high glucose-induced insulin signaling blockade in human hepG2 hepatoma cells. *Mol. Nutr. Food Res., 52,* 930–939. https://doi.org/10.1002/mnfr.200700437

Lo Piparo, E., Scheib, H., Frei, N., Williamson, G., Grigorov, M. & Chou, C. J. (2008). Flavonoids for controlling starch digestion: Structural requirements for inhibiting human α-amylase. *J. Med. Chem., 51,* 3555-3561. https://doi.org/10.1021/jm800115x

Mandakini, B. P. & Sameera, N. S. (2015). Pharmacological evaluation of crude and partially purified fractions of *Z. jujuba* Mill. Gard. Bark. *World J. Pharm. Sci., 3 (2),* 263-271.

Marchev, A., Georgiev, V., Ivanov, I., Badjakov, I. & Pavlov, A. (2011). Two-phase temporary immersion system for *Agrobacterium rhizogene*s genetic transformation of sage (*Salvia tomentosa* Mill.). *Biotechnology Letters, 33 (9),* 1873-1878. https://doi.org/10.1007/s10529-011-0625-5

Marchev, A., Ivanov, I., Georgiev, V. & Pavlov, A. (2012). Determination of di-and triterpenes in *Salvia tomentosa* Mill. cell suspension culture by high-performance liquid chromatography. *Scientific Works – University of Food Technologies*, *LIX,* 229-233.

Matsui, T., Ogunwande, I. A., Abesundara, K. J. M. & Matsumoto, K. (2006). Anti-hyperglycemic potential of natural products. *Mini-Reviews in Medicinal Chemistry*, *6 (3),* 349–356. https://doi.org/10.2174/138955706776073484

Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Tamaya, K., Miyata, Y., Tanaka, K. & Matsumoto, K. (2007). Alpha-glucosidase inhibitory profile of catechins and thea flavins. *Journal of Agricultural and Food Chemistry*, *55,* 99-105. https://doi.org/10.1021/jf0627672

Mc Cue, P. P. & Shetty, K. (2004). Inhibitoy effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. *Asia Pac. J. Clin. Nut., 13,* 101-106.

Mcdougall, G. J., Shpiro, F., Dobson, P., Smith, P., Blake, A. & Stewart, D. (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *J. Agric. Food Chem., 53,* 2760-2766. https://doi.org/10.1021/jf0489926

Mensor, L. L., Menezes, F. S., Leitao, G. G., Reis, A. S., Santos, T. S., Coube, C. S. & Leitao, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH. Free Radical Method. *Phytotherapy Research*, *15,* 127-130. https://doi.org/10.1002/ptr.687

Mizuno, C. S., Chittiboyina, A. G., Kurtz, T. W., Pershadsingh, H. A. & Avery, M. A. (2008). Type 2 diabetes and oral antihyperglycemic drugs. *Curr. Med. Chem*., *15,* 61-74.

Nakai, M., Fukui, Y., Asami, S., Toyoda-Ono, Y., Iwashita, T., Shibata, H., Mitsunaga, T., Hashimoto, F. & Kiso, Y. (2005). Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. *Journal of Agricultural Food Chemistry*, *53,* 4593-4598. https://doi.org/10.1021/jf047814

Ng, K. (2015). Evaluation of α-amylase and α-glucosidase inhibitory activity of flavonoids. *International Journal of Food and Nutritional Science*, *2 (6),* 1- 6. http://www.dx.doi.org/10.15436/2377-0619.15.042

Ou, B., Hampsch-Woodill, M., Flanagan, J., Deemer, E. K., Prior, R. L. & Huang, D. J. (2002). Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *J. Agr. Food Chem*., *50*, 2772–2777.

Ou, B., Hampsch-Woodill, M. & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescence probe. *J. Agr. Food Chem*., *49,* 4619–4626.

Palmer, T. (2004). Enzymes. Biochemistry, biotechnology, clinical chemistry. East-West Press Edition, New Delhi, 42-146.

Quilliot, D., Walter, E., Bonte, J. P., Fruchart, J. C., Duriez, P. & Ziegler, O. (2005). Diabetes mellitus worsens antioxidant status in patients with chronic pancreatitis. *Am. J. Clin. Nutr*., *81,* 1111–1117. https://doi.org/10.1093/ajcn/81.5.1117

Reddy. N. V. L. S., Anarthe. S..J. & Raghavendra, N. M. (2011). *In vitro* antioxidant and antidiabetic activity of *Asystasia gangetica* (Chinese violet) Linn. (Acanthaceae). *J. Res. Biomed. Sci., 1 (2),* 72-75.

Rodriguez De Sotillo, D. V. & Hadley, M. (2002). Chlorogenic acid modifies plasma and liver concentrations of: cholester-

ol, triacylglycerol, and minerals in (fa/fa) Zucker rats. *Journal of Nutritional Biochemistry*, *13 (12),* 717–726.

Rossetto, M., Lante, A., Vanzani, P., Spettoli, P., Scarpa, M. & Rigo, A. (2005). Red chicories as potent scavengers of highly reactive radicals: a study on their phenolic composition and peroxyl radical trapping capacity and efficiency. *J. Agric. Fd Chem*., *53,* 8169–8175. https://doi.org/10.1021/jf051116n

Sales De Paloma, M., Monteiro De Souza, P., Simeoni, L. A., Pérola De Oliveira, M. & Silveira, D. (2012). α-amylase inhibitors: A review of raw material and isolated compounds from plant source. *J. Pharm. Pharmaceut. Sci*., *15 (1),* 141 - 183.

San, B. & Yildirim, A. N. (2010). Phenolic, alpha-tocopherol, beta-carotene and fatty acid composition of four promising jujube (*Ziziphus jujuba* Miller) selections. *Journal of Food Composition and Analysis, 23,* 706–710. https://doi.org/10.1016/j.jfca.2010.02.008

Shobana, S., Sreerama, Y. N. & Malleshi, N. G. (2009). Composition and enzyme inhibitory properties of *finger millet (Eleusine coracana* L.*)* seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chem., 115,* 1268-1273. http://dx.doi.org/10.1016/j.foodchem.2009.01.042

Shoei, S. L., Buh, F. L. & Karin, C. L. (1996). Three triterpene esters from *Zizyphus jujuba. Phytochemistry, 43 (4),* 847-851.

Stoilova, I., Trifonova, D., Marchev, A., Stanchev, V., Angelova, G. & Krastanov, A. (2017). Phytochemical constituents and *in vitro* anti-diabetic properties of *Ziziphus jujuba* (Rhamnaceae) fruits. *International Journal of Pharmacognosy and Phytochemical Research, 9 (2),* 150-158. https://doi.org/10.25258/phyto.v9i2.8056

Suresh, B. K., Tiwari, A. K., Srinivas, P. V., Ali, A. Z., China, R. B. & Rao, J. M. (2004). Yeast and mammalian alpha-glucosidase inhibitory constituents from Himalayan rhubarb Rheum emodi Wall.ex Meisson., *Bioorg. Med. Chem. Lett*., *14,* 3841-3845. https://doi.org/10.1016/j.bmcl.2004.04.062

Tadera, K., Miinami, Y., Takamatsu, K. & Matsuoka, T. (2006). Inhibition of a-glucosidase and a-amylase by flavonoids. *J. Nutr. Sci. Vitaminol*., *52,* 149-153.

You, Q., Chen, F., Wang, X., Jiang, Y. & Lin, S. (2012). Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. *LWT-Food Sci. Technol., 46,* 164–168. https://doi.org/10.1016/j.lwt.2011.10.011

Zhishen, J., Mengcheng, T. & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64 (4)*, 555–559.

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