Egypt. J. Chem. **Vol. 65,** No. 12 pp. 695 - 705 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

PHYTOCHEMICAL PROFILE, ANTI-LIPID PEROXIDATION AND ANTI-DIABETIC ACTIVITIES OF THYMUS ALGERIENSIS BOISS. & REUT

Zaoui Heyem[a], Boutaoui Nassima[b], Menad Ahmed[a], Ramazan Erenler[c], Zaiter Lahcene[b], Benayache Fadila[b], Ameddah Souad[a]*

[a]*Laboratoire de Biologie et Environnement, Faculté de Sciences de la Nature et de la vie, Université Constantine1, 25000 Constantine, Algérie*

[b]*Unité de recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques (VARENBIOMOL), Département de Chimie, Faculté des Sciences Exactes, Université Constantine1, 25000 Constantine, Algérie*

[c]*Department of Chemistry, Faculty of Arts and Sciences, Tokat Gaziosmanpaşa University, Tokat, Turkey*

Abstract

Thymus algeriens Bioss & Reut is an Algerian native plant traditionally used for culinary and medicinal purposes. Biological activities varied with their chemical composition, which depends on the floral and geographical origin. This study was designed to screen the phenolic content of the n-BuOH extract of *Thymus algeriensis* (BETA), anti-lipid peroxidation and anti-diabetic properties on different *in vitro* models. The results showed that BETA reduced TBARS formation in a concentration-dependent manner; protected β-carotene from auto-oxidation and prevented oxidation of ferrous in the linoleic acid/ferric thiocyanate assay. Furthermore, BETA showed effective inhibitions of the key enzymes linked to type 2 diabetes; *α*-amylase and *α*-glucosidase. BETA blocked also the formation of AGE as shown by the results of glucose-BSA (BSA-GLU), BSA-Methylglyoxal system (BSA-MGO) and glycated hemoglobin. The effectiveness of BETA in combating diabetics might be influenced by the synergistic action of its phenolic compounds; mentioned by the phenolic content and LC/MS analysis; particularly their richness on the rosmarinic acid and ferulic acid, cafeic acid as well quercetin. This suggests the potential of BETA to mitigate multiple complications stemming from oxidative stress and protein glycation in diabetes.

Keywords: Thymus algeriensis; LC-MS; Anti-glycation; Anti-lipid peroxidation; α-glucosidase; *α*-amylase

1. Introduction

Diabetes mellitus, a chronic metabolic disorder, is at present major worldwide health problem [1]. Multiple signaling pathways underlying hyperglycemic cellular damage in type 2 diabetes mellitus [2]. Elevated blood sugar levels have been implicated in the induction of oxidative stress via a number of mechanisms, viz., autoxidation of glucose [3] and advanced glycation products (AEG) formation. Several approaches of therapy were considered, the combat of oxidative stress is one of the therapeutic approaches in treating diabetic patients [4,5]. One promising approach for management of diabetes, particularly type 2 diabetes, is to decrease postprandial hyperglycemia by inhibiting carbohydrate-hydrolyzing enzymes in gastrointestinal tract [6]. The Inhibition of AGE formation has been an effective way for retarding the full range of diabetes complications. Since all the existing ant-diabetic agents are having considerable unwanted effects [4,7], there is an urgent demand for new and non-toxic agent. Traditional plant medicines are used throughout the world for a range of diabetic presentations [8]. Indeed, inhabitants of the world, including Algerians, believed that eating and consuming fresh medicinal plants may treat illnesses and ailments [9,10]; among those plants was Thymus algeriensis, which belongs to the Lamiaceae family [11]. In fact, this family is among the largest families of flowering plants with about 400 genera and over 7000 species distributed around the world [12]. *T. algeriensis* is the most widespread North African species. It is endemic in Libya, Tunisia, Algeria and Morocco [13]. *T. algeriensis* is largely used, fresh or dried, mainly as a culinary herb, essentially used in Algeria both as a popular herb and as a spicy herb [14,15]. The plant is largely used as a culinary herb and as a traditional medicine to treat digestive and respiratory infections (e.g., gastrointestinal dysentery) [16]. In addition, it was used to enhance the immune system and help fight colds [17]. These properties

*Corresponding author e-mail: amedsouad@yahoo.fr.; (Souad Ameddah).

Receive Date: 09 March 2022, Revise Date: 25 March 2022, Accept Date: 21 April 2022

DOI: 10.21608/EJCHEM.2022.126336.5600

depend greatly on their chemical compositions and are mainly attributed to their richness on phenolic acids [16]. However, it has been reported that a differences among mentioned phenolic compounds levels can be related to environmental conditions, such geographic origin [18], genetic and extraction methods [19], could influence the variability of these chemical composition. *T. algeriensis* has been previously studied with an exclusive attention to ethanolic extract and the methanol extracts [16,20] whereas, few studies carried out on the n-BOH extract. In continuation of our research on the genus *Thymus* and taking into account that there was no information available in the literature about the anti-diabetic studies on *T. algeriensis*, our investigation seeking the *in vitro* antidiabetic activity of the *n*-BuOH extract of *T. algeriensis* (BETA). The present study also is an attempt to identify and enrich the knowledge on the variation of the chemical composition of *T. algeriensis* (MSila region; Algeria) by using LC/MS, analyses.

2. Materials and methods

2.1. Chemicals

The chemical products and reagents used were: β-carotene, linoleic acid, galantamine,4-Nitrophenylα-D-glucopyranoside (≥99%), α-Glucosidase from Saccharomyces cerevisiae (Type I, ≥10 units/mg protein), acarbose (≥95%), amylase, *p*-nitrophenyl-a-D-glucopyranose (*p*-NPG), 3,5- dinitro-salicylic acid (DNS), serum albumin (BSA), methylglyoxal (MG), 40% aqueous solution), bovine hemoglobin (Hb). All the chemicals used including the solvents, were of analytical grade were purchased from Sigma Chemical Co. (Sigma-Aldrich (St. Louis, MO, USA).

2.2. Phytochemical studies

2.2.1. Plant material

Samples of full bloom plants were collected from wild population in MSila region (Coordinates: 35420 N 4330 E), Algeria, identified by Professor Mohamed Kaabeche (Biology Department, University of Setif 1, Algeria). A voucher specimen has been deposited in the Herbarium of the VARENBIOMOL research unit, University Frères Mentouri Constantine1. Aerial parts were manually separated, dried at controlled temperature (40 °C) in the dark until constant weight. Then plant material was powdered to a uniform granulometry and stored in the dark at –20 °C, in vacuum bags, until extractions.

2.2.2. Extraction

The air-dried aerial parts (leaves and flowers, 2.0 kg) of *T. algeriensis* were powdered and macerated at room temperature with EtOH–H_2O 70:30, (v/v) (15 L) for 24 h, four times with fresh solvent. After filtration, and evaporation. The remaining aqueous solution was extracted successively with CH_2Cl_2, EtOAc, and *n*-BuOH, giving CH_2Cl_2 (7.42 g), EtOAc (4.19 g) and *n*-BuOH (BETA) (33.15 g) extracts. In our investigation, we are interested to BETA.

2.2.3. Determination of total phenolic content (TPC)

The total phenolic (TPC) was determined with the Folin–Ciocalteu reagent following the method of Singleton et al. [21] with a slight modification using Gallic acid as standard. Results were expressed as micrograms of gallic acid equivalents per milligrams of extract (µg GAE/mg).

2.2.4. Determination of total flavonoid content (TFC)

The concentration of flavonoids was achieved using the method described Ordonez et al. al. [22] by using the aluminum chloride reagent results were expressed as µg quercetin equivalents (QE)/mg extract.

2.2.5. LC-MS analysis

The qualitative and quantitative analyses of polyphenolic compounds were performed by LC–MS. Thermo Scientific - Dionex Ultimate 3000 -TSQ Quantum with Thermo ODS Hypersil 250 × 4.6 mm, 5 µm column were used for quantitative analysis. The injection volume was 20 µL. The mobile phase included eluent A, water with 0.1 % formic acid and B, methanol. The flow rate was 0.7 mL/min at 40 °C. The gradient program was fixed as follow: 0-1 min, 100 % A, 5-20 min, 95 % A, 1-22 min, 5 % A, 25 min, 5 % A, 30 min 0 % A. Total process time was 30 min. Extracts were prepared to be 1 mg/1 mL and analyzed. The relationship between peak area and concentration was found to be linear from 0.5 to 10 mg/L (ppb) for each compound. Linearity was assessed using linear regression analysis of six points for each compound. Linear plot consists of three replicates per point. For all analyses, the correlation coefficients (R_2 values) were found to be ≥0.99. The limit of detection (LOD) and the limit of quantification (LOQ) values of compounds for LC-MS/MS method are given in Table. LOD and LOQ were determined by using measurements of reagent blanks spiked with low concentrations of analyte according to EURACHEM GUİDE. The blank solution was spiked to 0.1 ppm standard. Calculate LOD and LOQ as LOD = 3*S0 and LOQ= 10*S0, where S0 is the standard deviation. The standards used for LC-MS/MS analysis were chlorogenic acid, naringenin, p-hydroxybenzoic acid, kaempferol, gentisic acid, p-coumaric acid, quercetin, gallic acid, rosmarinic acid, ferulic acid and caffeic acid.

2.3. In vitro antioxidant effect

2.3.1. Lipid peroxidation (LPO) inhibition induced by Fe^{2+}/ascorbic acid system

The reaction mixture containing rat liver homogenate (0.1 mL, 25 % w/v) in Tris-HCl (30 mM), ferrous ammonium sulfate (0.16 mM), ascorbic acid (0.06 mM) and different concentrations of the extract was incubated for 1hr at 37 °C and the resulting

thiobarbituric reacting substance (TBARS) was measured at 532 nm [23].

2.3.2. β-carotene/linoleic acid bleaching assay

The antioxidant activity was evaluated using the β-carotene-linoleic acid model system [24]. BHA and trolox were used as standards. Antioxidant activities (inhibition percentage) of the samples were calculated using the following equation: Inhibtion % = (Absorbance of β-carotene at 120 min /Initial absorbance of β-carotene) × 100.

2.3.3. Peroxidation inhibition of linoilic acid ferric thiocyanate assay

The peroxy radical scavenging activity was determined by thiocyanate method [25]. BHA and trolox were used as standards. Antioxidant activities (inhibition percentage) of the samples were calculated using the following equation: Inhibtion % = (Absorbance at 72 hr-initial absorbance/Absorbance at 72 hr) × 100

2.4. Antidiabetic studies

2.4.1. α-amylase inhibitory activity

α-amylase inhibitory activity of extract was carried out according to the method of [26] with minor modification. The absorbance was read at 580 nm and the percentage inhibition was calculated using the equation: inhibition of enzyme activity was calculated as follows: Inhibitory effect (%) = (Absorbance of control – Absorbance of sample) / Absorbance of control × 100.

2.4.2. α-glucosidase inhibitory activity

The α-glucosidase inhibitory assay was performed by applying the literature procedure with minor modifications according to Matsuura et al. [27]. Absorbance of the resulting *p*-nitro phenol (pNP) was determined at 405 nm using spectrophotometer and was considered directly proportional to the activity of the enzyme acarbose was used as a positive control. Inhibitory effect (%) = (Absorbance of control – Absorbance of sample)/Absorbance of control × 100.

2.4.3. Anti-glycation (anti-AGE) Activity

2.4.3.1. In-vitro BSA-glucose assay

The anti-AGE of BETA, metformin and aminoguanidine were performed by using BSA-glucose assay [27]. After the incubation for 7 days at 37 °C in dark, the samples were measured at fluorescence intensity (excitation wave length of 370 nm and emission wave length of 440 nm) using Omega micro plate reader. The percent inhibition was calculated by using equation: AGE inhibition = [(F_0-F_t)/F_0 x 100], where F_t and F_0 represent the fluorescence intensity of the sample and the control mixtures, respectively.

2.4.3.2. In-vitro BSA-methylglyoxal (MGO) model

The BSA- MGO reaction mixture was incubated at 37 °C for 7 days, aminoguanidine an inhibitor of glycation of proteins was used as a positive control the fluorescence intensity of the samples was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm, using an Omega microplate reader [28]. The percentage of inhibition was calculated by using the above formula.

2.4.3.3. Anti-hemoglobin glycation

Glycation of hemoglobin was performed according to the methodology described by Gutierrez et al. [29] and Liu et al [30]. The concentrations of glycated hemoglobin were measured after the incubation period of 72 hr at a wavelength of 443 nm. The test the inhibition of glycated hemoglobin was calculated using above equation.

2.5. Statistical analysis

Statistical analysis was performed using Microsoft Office Excel 2016. All the results were expressed as mean value ± SD of three measurements. IC_{50}-value (µg/mL) is the effective concentration, which proves 50 % of activity, was calculated for each assay. Significant differences between means were determined by Student's t-test, differences between groups were carried out by analysis of variance (ANOVA) followed by Tukey test. p values <0.05 were considered as significant.

3. Results and discussion

3.1. Polyphenol and flavonoid contents

Table 1

Polyphenol and flavonoid contents of BETA extract

Extract	TPC (µg GAE/ mg extract)*	TFC (µg QE/ mg extract)**
BETA	299. 48 ± 1.38	168.17 ± 0.80

Results are expressed as means± SD deviation of three measures.

*Total phenolic is expressed as µg Gallic acid equivalents/mg of extract; **Total flavonoids are expressed as µg Quercetin equivalents/ mg of extract.

T. algeriensis has been reported for its richness in secondary metabolites particularly flavonoids and phenolic acids [18]. In our present results a high phenolic and flavonoid contents were found in the of *n*-BuOH plant extract of *T. algeriensis* with values of 299.48±1.38 µg GAE/mg extract and 168.17±0.80 µg QE /mg extract. That it seems that they belong to the interval of values reported in the literature between 18.73 µg GAE/mg and 318.07 ± 0.88 µg GAE /mg [31,32]. Our results are in accordance with the findings obtained from other studies carried out on *T. algeriensis*, for the same fraction. Mokhtari et al. [32] revealed a value of 318.07 0.88 µg GAE/mg and 198.17±0.12 µg QE/mg for TPC and TFC values respectively, more or less similar to our results. In addition, Righi et al. [33] reported that the crude hydromethanolic extract of *T. algeriensis* exhibited a TPC of approximately 300 µg GAE/mg, consisting of flavonoids (16 µg QE/mg). However, Hazzit and Baaliouamer. [14] found that *T. algeriensis* from Algeria showed a chemical polymorphism, even for samples from the same location. From the semi-arid area surrounding Bechar, in the south- west of Algeria,

different results (18.73 mg GAE/g extract) were obtained by the authors [31,34], TPC was detected at 81.5 mg GAE/g of *T. algeriensis*. While at the flowering stage of *T. algeriensis* in the Algerian Saharan Atlas zone (Laghouat region), TPC and TFC values were recorded respectively at 125±1 mg/g extract and 118±1 mg/g of ethanolic extract [20]. Numerous studies conducted have established that *Thymus* species are rich and promising sources of phenolic compounds and flavonoids. The amounts of phenolic and flavonoid compounds in crude extracts can also vary due to environmental factors, cultivation practices, growing locations and storage [35]. It should be mentioned that an increase of the phenolic metabolism in these plants may be related to the hard climate conditions (hot temperature, height solar exposure, dryness, short growing season) [36].

3.2. LC-MS analysis

It has been reported that *Thymus* species showed a great variability in the contents of the phenolic compounds [37]. Several phytochemical studies were performed on *Thymus algeriensis*, differences among phenolic compounds levels can be related to environmental conditions [20]. Many factors can be responsible for this variability in the chemical composition of thyme extracts. The most important are the climate, the soil, the harvest period and the method of preservation and extraction [19,38]. The chemical compositions of this species of thyme found in the literature relating to different countries (Libya, Algeria, Tunisia and Morocco) are characterized by different chemotypes and a great variability [39]. In our results, the LC-MS profile of the *n*-BuOH fraction of the *T. algeriensis* (BETA) is illustrated in Fig.1. Several compounds were detected; only eleven phenolic compounds were identified in the BETA by comparing their retention times with those of available commercial standards (Table 2 and Fig.1).

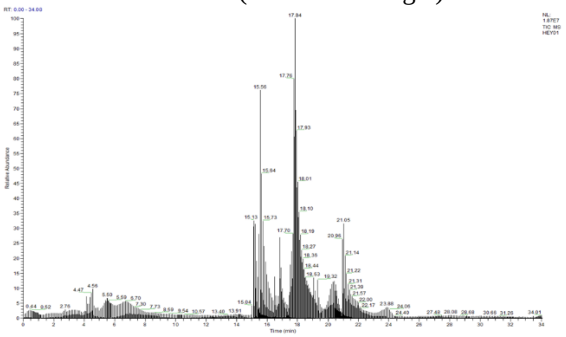

Figure 1 : LC-MS analysis of the phenolic constituents of BETA

Table 2 : LC-MS of phenolic compounds from BETA

N°	Name	MS/MS Ions studied				LOD	LOQ	RT	mg phenolic/Kg Extract
		Parent	Product	CE	Polarity				
1	Gallic acid	169.7	80.5 126.2	25 16	- -	0.058	0.091	10.1	**102,786**
2	Gentisic acid	153.7	109.5	21	-	0.026	0.039	13.87	**272,822**
3	Chlorogenicacid	353.4	86.5 192.1	43 21	- -	0.051	0.072	14.25	**916,221**
4	*p*-Hydroxybenzoicacid	137.9	66.6 94.6	38 17	- -	0.243	0.519	14.64	**755,211**
5	Cafeicacid	179.7	135.2 136.2	27 18	- -	0.042	0.058	15.26	**3404,063**
6	*p*-Cumaricacid	163.9	94.3 120.2	33 17	- -	0.069	0.109	16.97	**715,134**
7	Rosmarinicacid	359.2	134.3 162.2	44 20	- -	0.029	0.050	17.86	**54228,711**
8	Ferulicacid	195.4	89.4 177.4	30 7	+ +	0.063	0.118	17.88	**59826,860**
9	Naringenin	273	147.1 153	20 24	+ +	0.052	0.068	20.46	**141,739**
10	Quercetin	301	152.1 179.9	23 20	- -	0.141	0.181	20.51	**639,517**
11	4-OH benzaldehyde	121.9	93.5 121.1	25 20	+ +	0.032	0.059	15.32	**29,089**

The most abundant molecule was the ferulic acid (59826.860 mg/kg extract), followed by rosmarinic acid (54228.711 mg/kg extract), cafeic acid (3404.063 mg/kg extract), chlorogenic acid (916.221 mg/kg extract), *p*-hydroxybenzoicacid (755.211 mg/kg extract), *p*-cumaric acid (715.134 mg/kg extract), gentisic acid (272.822 mg/kg extract), gallic acid (102.786 mg/kg extract), 4-OH benzaldehyde (29.089 mg/kg extract) and the flavonoids, naringenin

(141.739 mg/Kg), quercetin (639.517 mg/kg extract). The results obtained by Boutaoui et al. [40] on the same fraction, reported the presence of three 3 common products and other different compounds in which epicatechin had the highest concentration. Rezzoug et al. [20], reported that the ethanolic extract of *T. algeriensis* from the Algerian Saharan Atlas (Laghouat), contains six different compounds, in which epicatechin was also found to be the major compound. The current investigation has shown that the main constituents of *T. algeriensis* are rosmarinic acid and ferulic acid followed by caffeic acid. Our results are in agreement with results obtained from other studies which reported the major constituent of *T. algeriensis* is rosmarinic acid. Mansour et al. [41] revealed that from the methanol extract of the *T. algeriensis* (Ain Dem- AIN Defla" region; Algeria), thirty-five secondary metabolites were characterized phenolic acids, mainly rosmarinic acid and its derivatives, dominated the T. *algeriensi s*extract. Righi et al. [33], also revealed the presence of rosmarinic acid in the methnolic extract from flowering *T. algeriensis* (Taglait, Bordj BouArreridj region; Algeria). Mokhtari et al. [32] revealed the presence of ten compounds including rosmarinic acid in the *n*-BuOH extract of *T. algeriensis* (Chelia mountain, Banta; Algeria). The different mentioned results show that the chemical composition of thyme extract can vary depending on the geographic location. In addition, the phenol content of the plant extract depends on various parameters such as genetic and ecological factors, the part of the plant used, the extraction method used and the same age of the plant [18,42]. In agreement with our findings, several studies note that despite the fact that *Thymus* plants are generally known for their richness in rosmarinic acid, the specific phenolic composition of thyme extracts is dependent on several factors [19,38].

3.3. LPO inhibitory activity of BETA

Table 3: Lipid peroxidation inhibition of BETA extract (IC50 value: µg/mL).

Extract/ Standard	$FeCl_2$/ Ascorbate	β-Carotene/ Linoleic acid	Linoleic acid ferric/ thyiocyanate
BETA	177.20±2.87	234.71±9.25	201.63±8.97
Trolox[a]	62.06±1.368	64.63±18.42	47.58±8.47
BHA[a]	68.94±12.22	45,77±7.66	38.73±8.7

IC_{50} values is defined as the concentration of 50% inhibition percentages and calculated by semi logarithmic regression analysis and expressed as Mean ±SD (n=3).
a: reference compounds, BHA (butylatedhydroxyanisole) and Trolox

3.3.1. LPO inhibition (Fe^{2+}/ascorbic acid system)

Lipid peroxidation is thought to be an important factor in the pathophysiology of a number of diseases [43]. Lipid peroxidation is probably the most extensively investigated free radical-induced process [44]. Table 3 showed that BETA extract reduced TBARS format ion in a concentration-dependent manner. BETA reduces (63.54±0.90 %) the MDA generated by Fe^{3+}/ascorbate at a concentration 400 µg/mL with an IC_{50} value: 177.20±2.87 µg/mL. This effect was comparable to the two standard references trolox and BHA which achieved 80.65±1.55 %; and 83.09±0.74 % respectively only at 200 µg/mL, with an IC_{50}: 62.06±1.368 µg/mL and 68.94±12.22 µg/mL values respectively.

3.3.2. β-Carotene/linoleic acid bleaching inhibition of BETA

The oxidation of linoleic acid produces hydroperoxides derived free radicals that attack the chromophore of *β*-carotene resulting in bleaching of the reaction emulsion [24]. Table 3 showed that there was a decrease in absorbance value due to the oxidation of *β*-carotene at 400 µg/mL, the *n*-BuOH exhibited high antioxidant activity (76.811.60 %; IC_{50}: 234.71±9.25 µg/mL) as compared to standards substances BHA and trolox (93.36±0.11 %; IC_{50} :45.77±7.66 µg/mL and 84.43±1.19 %; IC_{50}: 64.63±18.42 µg/mL) respectively. The presence of varying amount of antioxidant compounds in BETA can hinder the extent of *β*-carotene bleaching by neutralizing the linoleate free radicals and other free radicals formed in the system [45].

3.3.3. Peroxidation inhibition of linoilic acid ferric thiocyanate assay

In this system, the ferrous ion is oxidized by linoleate radicals such as hydroperoxides to form the ferric ion which is monitored as a thiocyanate complex at 500 nm. The antioxidant compounds in the plant extract prevent oxidation of ferrous ion inhibiting the linoleate radicals in system [25]. The oxidation of linoleic acid was effectively inhibited by BETA. The BETA showed high antioxidant activity with 75.27±0.81 % at 400 µg/mL with IC_{50}: 201.63±8.97 µg/mL; by inhibiting the formation of ferric ion. This inhibition was clearly compared to the two standard references trolox and BHA (83.55±0.46 %; IC_{50}: 47,58±8,47 µg/mL and 87.44±0,82 %; IC_{50}: 38.73±8.7 µg/mL) respectively. According to our finding, the beneficial effect of BETA may be attributed to the presence of high amounts of polyphenols. Our phytochemical studies on BETA revealed its richness in polyphenols such as ferulic acid, rosmarinic acid, cafeic acid, chlorogenic acid and quercetin mentioned in our work have been revealed antioxidant agents [46]. Our antioxidant findings are in good agreement with several studies in the literature, which show that *Thymus* species are candidate as potential antioxidant agents, their richness in rosmarinic acid and its derivatives have been previously reported for their antioxidant properties [47]. This potentiality could be attributed to its unsaturated double bound and the presence of dihydroxyphenol or catechol structure that

facilitates the delocalization of the resulting quinones [48]. The presence of catechol structure in the C11 and C12 position is also an important element in the antioxidant activity of romarinic acid. The presence of a carboxylic group in a phenolic acid dimer like rosmarinic acid enhances the antioxidant activity of this compound [47]. Increased oxidative stress is widely accepted as a participating in the development and progression of diabetes [3].

3.4. Antidiabetic activities of BETA

3.4.1. Anti-α-amylase and anti-α-glucosidase activity

Table 4:% α-amylase inhibitory effect and α-glucosidase inhibitory effect of BETA

α-Amylase		α-Glucosidase		
Concentration (µg/mL)	BETA	acarbose[a]	BETA	acarbose[a]
50	33.43±1.14	48.96±2.75	26.04±0.69	43.99±0.90
100	43.23±1.83	67.21±3.08	34.33±2.04	47.91±1.38
200	61.03±1.45	86.37±4.75	45.65±0.69	64.35±1.20
400	66.16±1.81	92.70±1.45	60.43±1.14	88.18±5.77
600	76.57±0.45	93.46±0.94	64.80±1.20	90.89±0.69
IC_{50}	132.53±5.24	41.64±4.94	237.79±15.20	85.24±6.69

IC_{50} values is defined as the concentration of 50% inhibition percentages, expressed as Mean±SD (n=3). a : reference compounds.

The inhibition of *α*-amylase and *α*-glucosidase activities is one of the primary approaches to manage hyperglycemic conditions of T2D patients [49]. According to current opinions, it is believed that inhibitory effects against the two key enzymes, namely *α*-amylase and *α*-glucosidase, may from the present results, BETA effectively inhibits the action of both *α*-amylase and *α*-glucosidase enzyme in a concentration dependent manner. The percentage inhibition of the two enzymes varied from 33.43±1.14 % to 76.57±0.45 % and from 26.04±0.69 % to 64.80±1.20 % respectively at a concentration ranging from 50 to 600 µg/mL compared to acarbose (from 43.99±0.90 % to 90.89±0.69 %) in the same range of concentration. The IC_{50} values of BETA for *α*-amylase and for the enzyme *α*-glucosidase inhibition were respectively (132.53±10.24 µg/mL and 237.79±15.20 µg/mL) comparable to that of acarbose (IC_{50}: 41.64±4.94 µg/mL and 85.24±1.69 µg/mL) respectively (Table 4). Acarbose is widely used in the treatment of patients with type 2 diabetes via inhibiting the upper gastrointestinal glucosidases that convert complex polysaccharides into monosaccharides [51]. Dietary polyphenols, in addition to their antioxidant, have been reported to exert anti-hyperglycemic by binding to glucose transporters [52] and competitively inhibiting digestive enzymes [53]. In the present study, the polyphenol-rich BETA extract showed high inhibitory effects against both *α*-glucosidase and *α*-amylase (Table 4), revealing that it could implement a potential anti-diabetes function by inhibiting the two enzymes. In addition, many herbs rich in phenolic compounds have been reported to exhibit strong interaction with proteins and can inhibit their enzymatic activities by forming complexes and changing conformation [26]. This inhibitory activity of the *T. algeriensis* extract could be due to the presence of several phytochemicals such as rosmarinic acid [54-56]. Many flavonoids, such as rutin, myricetin, kaempferol and quercetin, have been previously reported to inhibit *α*-glucosidase and *α*-amylase, these flavonoids exhibit both hypoglycemic and antioxidant effects in diabetic [57]. Collectively, our findings based on the refined and their roles could be directly associated with their specific structural characteristics, such as the position and number of hydroxyls and the number of double bonds on aromatic rings A and B as well as the heterocyclic ring C [58].

3.4.2. *Anti-glycation activity of BETA*

Table 5
Anti-aglycation of BETA extract (IC_{50} value : µg/mL).

Extract/Satandards	BSA-glucose	BSA-MGO	Hb-glygation
BETA	284.9±19.53	203.65±5.7	254.35±12.43
Aminoguanidine[a]	75.72±7.12	70.16±2.72	190.15±9.26
Metformine[a]	111.18±7.71	76.20±3.83	154.72±6.48

IC_{50} values is defined as the concentration of 50% inhibition percentages, expressed as Mean ± SD (n=3). a: reference compounds.

Formation of AGEs that leads to the glycation reaction of proteins is a hallmark of long-standing hyperglycemia [59]. It has been revealed that AGEs play key role in the development of diabetic complications [60]. Despite glycation inhibitors; metformin and aminoguanidine; which inhibit the crosslinking of AGE-BSA in glucose- BSA or free methylglyoxal scavenger respectively, they have been limited, due to their cytotoxicity, or because they are not effective enough to inhibit the glycation process in chronic hyperglycemia and other side effects [7].

Thus, there is an urgent demand for new and non-toxic agent. In the present study, the inhibitory activity of BETA on the AGE was evaluated using glucose-BSA and MGO-BSA glycation protein and Hb glycation model. Table5 showed that BETA proved dose dependent antiglycation activity when used glucose-BSA glycation model. At 600 µg/mL, BETA exhibited (75.56±2.04 %) inhibition of glucose-BSA glycation with an IC_{50}: 284.9±19.53 µg/mL. The two standard drugs, metformin and aminoguanidine, revealed (80.98±2.30 %; 85.178±3.56 %) inhibition of AGEs only at 300 µg/mL with IC_{50}: 111.18±7.71 µg/mL and 75.72±7.12 µg/mL respectively. 3-methylglyoxal (MGO) is considered to be one of the most reactive precursors of AGEs and is the reactive capable of causing dicarbonyl stress, influencing normal physiological functions [61]. In our study, BETA also had an inhibitory effect on AGEs' formation when tested on MGO-BSA glycation at 600 µg/ mL (76.8±0.48%; IC_{50}: 203.65±0,70 µg/mL). Inhibition of MGO-BSA glycation for metformin and aminoguanidine were found to be 84.71±2.41 %; IC_{50}: 76.20±3.83 % and 88.71±2.41 %; IC_{50}: 70.16±2.72 µg/mL respectively. Autoxidation and disruption of the electron transport chain protein glycation is a key molecular basis of diabetic complications resulting from chronic hyperglycemia [61]. It is believed that the formation of AGEs over a prolonged period *in vivo* especially affects long-lived proteins such as hemoglobin, hemoglobin A1c, a well-known non-enzymatic glycation product. Hemoglobin A1c is a clinical index for diabetes mellitus, where in the N-terminal amino group of valine is glycated [30]. BETA succeeded in restricting hemoglobin glycation upon exposure to 2 % glucose over a 72 hours period and was able to reach 77.82±2.36 % at 600 µg/mL with an IC_{50} value of 254.35±2.43 µg/mL, compared to metformin and aminoguanidine (90.30±1.39 %; IC_{50}: 154.72±6.48 µg/mL and 87.27±2.91 %; IC_{50}: 190.15±3.26 µg/mL) respectively (Table 5). It has been reported that the early stage of the Maillard reaction is accompanied by the production of a large amount of free radicals [27,62]. Therefore, we considered the anti-lipid peroxidation and free radical scavenging which has been demonstrated by BETA may have an effective effect on reducing AGEs at the early stage of glycation. Interestingly, the anti-AGE effect of BETA could be due to its richness in polyphenols identified by LC/MS analysis. The most abundant and potent agents reported were ferulic acid and rosmanic acid, followed by caffeic acid and chlorogenic acid. The possible synergistic interactions of these phenolic compounds have shown antioxidant properties, which may be beneficial for the anti-AGE effect [63]. Ferulic acid and chlorogenic acid have been reported to all significantly inhibit glucose-mediated protein modification [64], and all can significantly inhibit glucose-mediated protein modification [65]. Caffeic and chlorogenic have been reported to be the major inhibitors of AGE generation by methylglyoxal in model proteins [58] and could inhibit AGE production and the subsequent crosslinking of proteins activity [66,67]. Quercetin was shown to be effective at the late stage of protein glycation in the BSA-glucose model and significantly reduced the production of Amadori hemoglobin. HbAlc, at the start of the glycation phase [48] Aminoguanidine, an AGE inhibitor, has been found to be able to inhibit AGE production by scavenging MG and ethanedial [57]. The basic structure of phenolic acid has many hydroxyl groups; therefore, it may have excellent antiglycative and MG trapping functions when a benzene ring has one to twoOH groups. The compound 2,4,6-trihydroxybenzoic acid compound is believed to have good MG scavenging structure [64]. LC/MS analysis revealed that BETA possesses several antioxidants such as phenols and flavonoids, which have been suggested to inhibit the formation of AGEs, these properties, have been attributed to the structure of these compounds.

4. Conclusion

Based on these findings; it can be concluded that BETA exhibited a good inhibition of lipid peroxidation on different in vitro models (Fe^{2+}/ascorbic acid system; β-carotene-linoleic acid model system and linoleic acid ferric thiocyanate assay). The effectiveness of BETA in combating diabetics was clarified on several in vitro studies (α-amylase and α-glucosidase inhibition activity, anti-AGE activity (MGO-BSA; MGO-BSA; hemoglobin glycated). The antidiabetic activities of BETA could be due to its richness in polyphenols identified by LC/MS analysis, mainly the potent agents, ferulic acid, rosmanic acid cafeic acid. Synergistic interactions between all their compounds; can qualify BETA as an effective agent against diabetic complications linked to oxidative stress. In addition, these effects need to be confirmed using in vivo models for its effective use as therapeutic agents.

5. Conflict of interest:

The authors declare that they have no conflict of interest.

6. Disclosure statement and author contributions

Souad Ameddah designed and supervised the study; Zaoui Heyem performed all experiments, wrote the manuscript; Nassima Boutaoui involved in the phytochemical studies under the supervision of Lahcene Zaiter; Benayache Fadila; and Benayache Samir, Ahmed Menad performed the data analysis, and Erenler Ramazan was involved in LC MS analysis. All co-authors approve the current version of the manuscript.

7. Acknowledgments
We thank Algerian government for financial support

8. References

[1] Gahlan AA, Derayea SM, Omar MA, Saleh GA, Haredy AM. Square Wave Anodic Voltammetric Determination of Antidiabetic Drug Linagliptin in The Dosage Form and Biological Fluids by Microparticles Copper Pencil Graphite Electrode. Egypt.J.Chem. 2021:64:3121-3130. doi: 10.21608/EJCHEM.2021.48845.2999

[2] Wild SG, Roglic A, Green R, Sicree R, King H. Global prevalence of diabetes: estimated for the year 2000 and projection for 2030. Diabetes Care. 2004: 5:1047–1053

[3] Kang MH, Lee MS, Choi MK, Min KS, Shibamoto T. Hypoglycemic activity of gymnema sylvestre extracts on oxidative stress and antioxidant status in diabetic rats. J. Agric. Food Chem. 2012;60: 2517-2524. doi: 10.1021/jf205086b

[4] Dastagir G, Rizvi MA. Review - Glycyrrhiza glabra L. (Liquorice). Pak J Pharm Sci. 2016;29: 1727-1733. PMID: 27731836.

[5] Deore AB, Sapakal VD, Naikwade NS. Role of oxidative stress in pathogenesis of diabetes and its complications. Pharmacologyonline. 2011;2 : 603-621. https://pharmacologyonline.silae.it/files/archives/2011/vol3/015.deore.pdf

[6] Van de Laar FA. A-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc Health Risk Manag. 2008;4: 1189-1195. doi.org/10.2147/vhrm.s3119.

[7] Afify M, Samy N, Hafez NA, Alazzouni AS, Mahdy ES, El Mezayen HAEM, Kelany MM. Evaluation of zinc-oxide nanoparticles effect on treatment of diabetes in streptozotocin-induced diabetic rats. Egypt.J.Chem. 2019:62:1771-1783. doi: 10.21608/ejchem.2019.11350.1735

[8] Raz I, Eldor R. Rational therapy for diabetes: early recognition of adverse effects and avoidance of disruptive false alarms. Diabetes/Metab Res. 2012;28: 321-324. doi.org/10.1002/dmrr.2265.

[9] Ahn J, Alford AR, Niemeyer ED. Variation in Phenolic Profiles and Antioxidant Properties among Medicinal and Culinary Herbs of the Lamiaceae Family. J. Food Meas. Charact. 2020;14: 1720-1732. doi.org/10.1007/s11694-020-00420-5.

[10] Chelalba I, Benchikha N, Begaa S, Messaoudi M, Debbeche H, Rebiai A, Youssef FS. Phytochemical composition and biological activity of Neurada procumbens L. growing in southern Algeria. J. Food Process Preserv. 2020;44: e14774. doi.org/10.1111/jfpp.14774.

[11] Dob T, Dahmane D, Benabdelkader T, Chelghoum C. Studies on the essential oil composition and antimicrobial activity of Thymus algeriensis Boiss. et Reut. Int. J. Aromather. 2006;16: 95-100. doi.org/10.1016/j.ijat.2006.04.003

[12] Figueiredo AC, Barroso JG, Pedro LG. Volatiles from Thymbra and Thymus species of the western Mediterranean basin, Portugal and Macaronesia. Nat Prod Commun. 2010;5(ç): 1465-1476. https://journals.sagepub.com/doi/pdf/10.1177/1934578X1000500924

[13] Quezel P, Santa S.New flora of Algeria and southern desert regions. CNRS. 1963;2: 559-565. https://bibliotheques.mnhn.fr/medias/detailstatic.aspx?INSTANCE=exploitation&RSC_BASE=HORIZON&RSC_DOCID=493163

[14] Hazzit M, Baaliouamer A. Essential oil composition of Thymus algeriensis Boiss. ET Reut. and Thymus numidicus Poiret from Algeria. Rivista Italiana EPPOS. 2007;43: 11-18.

[15] Jayari A, El Abed N, Jouini A, Wahab OMSA, Maaroufi A, Ahmed SBH. Antibacterial activity of Thymus capitatus and Thymus algeriensis essential oils against four food-borne pathogens inoculated in minced beef meat. J. Food Saf. 2018;38: 1-10. doi.org/10.1111/jfs.12409.

[16] Ziani BE, Heleno SA, Bachari K, Dias MI, Alves MJ, Barros L, Ferreira IC. Phenolic compounds characterization by LC-DAD-ESI/MSn and bioactive properties of Thymus algeriensis Boiss. & Reut. and Ephedra alata Decne. Food Res Int. 2019;116: 312-319. doi.org/10.1016/j.foodres.2018.08.041.

[17] Nikolic M, Glamoclija J, Ferreira ICFR, Calhelha RC, Fernandes Â, Markovic T, Sokovic M. Chemical composition, antimicrobial, antioxidant and antitumor activity of Thymus serpyllum L., Thymus algeriensis Boiss. and Reut and Thymus vulgaris L. essential oils. Ind Crops Prod. 2014;52. doi.org/10.1016/j.indcrop.2013.10.006

[18] Amarti F, Satrani B, Ghanmi M, Farah A, Aafi A, Aarab L, Chaouch A. Chemical composition and antimicrobial activity of essential oils of Thymus algeriensis Boiss. & Reut. and Thymus ciliatus (Desf.) Benth. from Morocco. Biotechnol. Agron. Soc. Environ. 2010;14: 141-148.

[19] Echeverrigaray S, Agostini G, Atti-Serfini L, Paroul N, Pauletti GF, dos Santos AC. Correlation between the chemical and genetic relationships among commercial thyme cultivars. J. Agric. Food Chem. 2001;49: 4220-4223. doi.org/10.1021/jf010289j.

[20] Rezzoug M, Bakchiche B, Gherib A, Roberta A, Kilinçarslan F, Kilinçarslan Ö, Mammadov R, Bardaweel SK. Chemical Composition and Bioactivity of Essential Oils and Ethanolic Extracts of Ocimum Basilicum L. And Thymus Algeriensis Boiss. &Reut From the Algerian Saharan Atlas. BMC Complement. Altern Med. 2019;146: 19. doi.org/10.1186/s12906-019-2556-y.

[21] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Meth Enzymol. 1999;299: 152-178. doi.org/10.1016/S0076-6879(99)99017-1.

[22] Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of Sechiumedule (Jacq.)Swart extracts. Food Chem. 2006;97: 452-458. doi.org/10.1016/j.foodchem.2005.05.024.

[23] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95: 351-358. doi.org/10.1016/0003-2697(79)90738-3.

[24] Miraliakbari H, Shahidi F. Antioxidant activity of minor components of tree nut oils. Food Chem. 2008;111: 421-427. doi.org/10.1016/j.foodchem.2008.04.008.

[25] Osawa T, Namiki M. A novel type of antioxidant isolated from leaf wax of Eucalyptus Leaves. Agric Biol Chem. 1981;45: 735-739. doi.org/10.1080/00021369.1981.10864583.

[26] Liu S, Li D, Huang B, Chen Y, Lu X, Wang Y. Inhibition of pancreatic lipase, a-glucosidase, a-amylase, and hypolipidemic effects of the total flavonoids from Nelumbonucifera leaves. J Ethnopharmacol. 2013;149: 263-269. doi.org/10.1016/j.jep.2013.06.034.

[27] Matsuura N, Aradate T, Sasaki C, Kojima H, Ohara M, et al. Screening system for the Maillard reaction inhibitor from natural product extracts. J Health Sci. 2002;48: 520-526. doi.org/10.1248/jhs.48.520.

[28] Peng X, Chang KW, Ma J, Chen B, Ho CT, Lo C, Chen F, Wang M. Cinnamon bark proanthocyanidins as reactive carbonyl scavengers to prevent the formation of advanced glycation products. J Agric Food Chem. 2008;56: 1907-1911. DOI: 10.1021/jf073065

[29] Gutierrez RMP, Cotera LBF, Gonzalez AMN. Evaluation of the Antioxidant and Anti-glication Effects of the Hexane Extract from Piper auritum Leaves in Vitro and Beneficial Activity on Oxidative Stress and Advanced Glycation End-Product-Mediated Renal Injury in Streptozotocin-Treated Diabetic Rats. Molecules. 2012;17: 11897-11919. doi.org/10.3390/molecules171011897

[30] Liu H, Wang W, Khoo C, Taylor J, Gu L. Cranberry phytochemicals inhibit glycation of human hemoglobin and serum albumin by scavenging carbonyls. Food Funct. 2011;2: 475-482. doi.org/10.1039/C1FO10087D.

[31] Bakchiche B, Gherib A, Aazza S, Gago C, Miguel M. Antioxidant activities of eight Algerian plant extracts and two essential oils. Ind Crops Prod. 2013;46: 85-96. doi.org/10.1016/j.indcrop.2013.01.020.

[32] Mokhtari M, Chabani S, Mouffouk S, Aberkane MC, Dibi A, Benkhaled M, Haba H. Phytochemicals, Antihemolytic, Anti-inflammatory, Antioxidant, and Antibacterial Activities from Thymus Algeriensis. J. Herbs Spices Med. Plants. 2021;27: 1-14. doi.org/10.1080/10496475.2021.1891174.

[33] Righi N, Boumerfeg S, Fernandes PAR, Deghima A, Baali F, Coelho E, Cardoso SM, Coimbra MA, Baghiani A. Thymus algeriensis Bioss & Reut: Relationship of phenolic compounds composition with in vitro/in vivo antioxidant and antibacterial activity. Food Res. Int. 2020;136: 109500. doi.org/10.1016/j.foodres.2020.109500

[34] Abid M, Yaich H, Cheikhrouhou S, Khemakhem I, Bouaziz M, Attia H, Ayadi MA. Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. J. Food Sci. Technol. 2017;54: 2890-2901. doi.org/10.1007/s13197-017-2727-0

[35] Akula R, Ravishankar GA. Influence of Abiotic Stress Signals on Secondary Metabolites in Plants. Plant Signal Behav. 2011;6: 1720-1731. doi.org/10.4161/psb.6.11.17613.

[36] Guesmi F, Ben Farhat M, Mejri M, Landoulsi A. In vitro assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of Thymus hirtus sp. algeriensis. Lipids Health Dis. 2014;13: 1-12. doi.org/10.1186/1476-511x-13-114

[37] Li X, He T, Wang X, Shen M, Yan X, Fan S, She G. Traditional Uses, Chemical Constituents and Biological Activities of Plants from the Genus Thymus. Chem. Biodivers. 2019;16: 9. doi.org/10.1002/cbdv.201900254.

[38] Jaafari A, Mouse H, Rakib E, Ait M'barek L, Tilaoui M, Benbakhta C, Boulli A, Abbad A,

Zyad A. Chemical composition and antitumor activity of different wild varieties of Moroccan thyme. Rev. Bras. Farmacogn. 2007;17: 477-491. doi.org/10.1590/S0102-695X2007000400002

[39] Lamiri A, Lhaloui S, Benjilali B, Berrada M. Insecticidal effects of essential oils against Hessian fly, Mayetiola destructor (Say). Field Crops Res. 2001;71: 9-15. doi.org/10.1016/S03784290(01)00139-3.

[40] Boutaoui N, Zaiter L, Benayache F, Benayache S, Carradori S, Cesa S, Giusti AM, Campestre C, Menghini L, Innosa D, Locatelli M. Qualitative and Quantitative Phytochemical Analysis of Different Extracts from Thymus algeriensis Aerial Parts. Molecules. 2018;463: 1- 11. doi.org/10.3390/molecules23020463.

[41] Mansour S, Rezq S, Cheurfa M, Abdelfattah MAO, Rasha Rashied MH, El-Shazl AM, Yasri A, Wink M, Mahmoud MF. Thymus algeriensis and Thymus fontanesii: Chemical Composition, In Vivo Anti inflammatory, Pain Killing and Antipyretic Activities: A Comprehensive Comparison. Biomolecules. 2020;10: 599. doi.org/10.3390/biom10040599.

[42] Gharibi S, Ebrahim B, Tabatabaei S, Saeidi G. Comparison of Essential Oil Composition, Flavonoid Content and Antioxidant Activity in Eight Achillea Species. J. Essent. Oil-Bear. Plants. 2015;18: 1382-1394. doi.org/10.1080/0972060X.2014.981600

[43] Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause, or consequence? Lancet. 1994;344: 721-724. doi.org/10.1016/s0140-6736(94)92211-x

[44] Al-Okbi S, Al-Siedy ES. Potential anti-dyslipidemia and hepatoprotection of functional food components represented by tetracosanol and mixture of policosanol in Triton X-100 induced dyslipidemic rats. Egypt.J.Chem. 2022:65:11. doi: 10.21608/EJCHEM.2022.121634.5452

[45] Koleva II, Teris AB, Jozef PH, Linssen AG, Lyuba NE. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Analysis. 2002;13: 8-17. doi.org/10.1002/pca.611.

[46] Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem. 2001;75: 197-202. doi.org/10.1016/S0308-8146(01)00198-4

[47] Del Baño MJ, Lorente J, Castillo J, Benavente-García O, del Río JA, Ortuño A, Quirin KW, Gerard D. Phenolic Diterpenes, Flavones, and Rosmarinic Acid Distribution during the Development of Leaves, Flowers, Stems, and Roots of Rosmarinus officinalis. Antioxidant Activity. Agric. Food Chem. 2003;51: 4247-4253. doi.org/10.1021/jf0300745

[48] Chen H, Virk MS, Chen F. Phenolic acids inhibit the formation of advanced glycation end products in food simulation systems depending on their reducing powers and structures. Int J Food Sc Nutr. 2016;67: 400-411. doi.org/10.3109/09637486.2016.1166187.

[49] Dwek R, Butters T, Platt F, et al. Targeting glycosylation as a therapeutic approach. Nat Rev Drug Discov. 2002;1: 65–75. doi.org/10.1038/nrd708

[50] Etxeberria U, de la Garza AL, Campión J, Martínez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. Expert Opin Ther Targets. 2012;16: 269-297. doi.org/10.1517/14728222.2012.664134.

[51] Ibrahim FM, Fouad R, El-Hallouty S, Hendawy SF, Omer EA, Mohammed RS. Egyptian Myrtus communis L. Essential oil Potential role as in vitro Antioxidant, Cytotoxic and α-amylase Inhibitor. Egypt.J.Chem. 2021:64:3005-3017.

[52] Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: An update. Mini Rev Med Chem. 2010;10: 315-312. doi.org/10.2174/138955710791331007.

[53] Bischoff H. Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest. 1994;24(S3): 3-10. doi.org/10.1111/j.1365-2362.1994.tb02249.x

[54] Kwon O, Eck P, Chen S, et al. Inhibition of the intestinal glucose transporter GLUT2 by flavonoids. FASEB J. 2007;21: 366-377. doi :10.1096/fj.06-6620com.

[55] Umeno A, Horie M, Murotomi K, Nakajima Y, Yoshida Y. Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules. 2016;21: 708. doi.org/10.3390/molecules21060708.

[56] Mccue P, Shetty K. Health Benefits of Soy Isoflavonoids and Strategies for Enhancement: A Review. Crit Rev Food. 2010;44: 361-367. doi.org/10.1080/10408690490509591.

[57] Li Z, DU J, Sun H, Mang J, HE J, Wang J, Liu H, Xu Z. Effects of the combination of methylprednisolone with aminoguanidine on functional recovery in rats following spinal cord injury. Exp. Ther. Med. 2014;7: 1605-1610. doi.org/10.3892/etm..1613.

[58] Gugliucci A, Bantos D, Schulze J, Souzza M. Caffeic and chlorogenic acids in Ilex paraguariensis extracts are the main inhibitors

of AGE generation by methylglyoxal in model proteins. Fitoterapia. 2009;339: 344-80. doi.org/10.1016/j.fitote.2009.04.007

[59] El-Mesallamy A, Hussein S, Hussein AAM, Mahmoud SA, El-Azab KM. Reno-protective effect of methanolic extract of Stevia rebaudiana Bertoni and bioactive phenolic compounds in type-1-diabetes. Egypt.J.Chem. 2018:61: 609-615. doi: 10.21608/EJCHEM.2018.3550.1297

[60] Peppa M, Vlassara H. Advanced glycation end products and diabetic complications: a general overview. Hormones (Athens). 2005;4: 28-37. doi: 10.14310/horm.2002.11140

[61] Lapolla A, Flamini R, DallaVedova A, Senesi A, Reitano R, Fedele D, Basso E, Seraglia R, Traldi P. Glyoxal and methyl glyoxal levels in diabetic patients: quantitative determination by a new GC/MS method. Clin Chem Lab Med. 2003;41: 1166-1173. doi.org/10.1515/CCLM.2003.180.

[62] Yeh W, Hsia S, Lee W, Wu C. Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. J Food Drug Anal. 2017;25: 84-92. doi.org/10.1016/j.jfda.2016.10.017.

[63] Rabiei S, Rezaei M, Abasian Z, Khezri M, Nikoo M, Rafieian-kopaei M, Anjomshoaa M. The protective effect of Liza klunzingeri protein hydrolysate on carbon tetrachloride-induced oxidative stress and toxicity in male rats. Iran J Basic Med Sci. 2019;22: 1203-1210. doi.org/10.22038/ijbms.2019.33201.7927.

[64] Lo CY, Hsiao WT, Chen XY. Efficiency of trapping methylglyoxal by phenols and phenolic acids. J Food Sci. 2011;76: 90-96. doi.org/10.1111/j.1750-3841.2011.02067.x.

[65] Silvan J, Assar S, Srey C, Dolores Del Castillo M, Ames J. Control of the Maillard reaction by ferulic acid. Food Chem. 2011;128: 208-213. doi.org/10.1016/j.foodchem.2011.03.047.

[66] Chao CY, Mong MC, Chan KC, Yin MC. Anti-glycative and anti-inflammatory effects of caffeic acid and ellagic acid in kidney of diabetic mice. Mol Nutr Food Res. 2010;54: 388-395. doi.org/10.1002/mnfr.200900087.

[67] Wu CH, Huang HW, Lin JA, Huang SM, Yen GC. The proglycation effect of caffeic acid leads to the elevation of oxidative stress and inflammation in monocytes, macrophages and vascular endothelial cells. J Nutr Biochem. 2011;22: 585-594. doi.org/10.1016/j.jnutbio.2010.05.002.