Research Article

GC/MS and HPLC Analysis of Alpha-glucosidase Inhibitor's Sub-fractions from Egyptian Propolis

Faten K. Abd El-Hady[1*], Ahmed M.A. Souleman,[2] Seham El Hawary[3], Nesma M. Salah[1], Zeinab A. El-Shahid[1]
[1]Chemistry of Natural Products Department, National Research Center, Egypt.
[2]Department of Phytochemistry and Plant Systematic, National Research Center, Egypt.
[3]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.
***Corresponding author's E-mail:** fatenkamal@hotmail.com

Accepted on: 29-03-2016; **Finalized on:** 30-04-2016.

ABSTRACT

There has been an enormous interest in the development of alternative natural medicines for type II diabetes, have the ability to delay or prevent glucose absorption. The aim of this study was to investigate the potential α-glucosidase inhibitory activity of propolis extract and its sub-fractions *in-vitro*. The ether sub-fraction 6(A) had moderate activity (32.7 %), in comparison with that of acarbose (49%). The ethyl acetate sub-fractions 1 and 2(B) showed low and high moderate α-glucosidase inhibitory activity (20 and 37% respectively). GC/MS analyses of sub-fraction (A) showed high percentage of phenolic acids (42.6 %); especially 3,4-dimethoxy-*trans*-cinnamic acid (30.7%) and moderate presence of p-methoxy-*trans*-cinnamic and 3,4-dimethoxy-*cis*-cinnamic acids. It contained five caffeate esters; caffeic acid methyl ester, 3-methyl-3-butenyl-*cis*-caffeate, 3-methyl-3-butenyl-*trans*-caffeate, 3-methyl-2-butenyl-*trans*-caffeate and benzyl-*trans*-caffeate. Only two flavonoids were identified; quercetin and myricetin. High percentage of aliphatic acids were found in sub-fraction B (39.8%); the highest percentage is hexadecanoic acid (26.9%) followed by octadecenoic acid and octadecanoic acid (4.8 and 3.39%). It mainly contained phenolic acids esters (10.6%), from which, six (*cis* and *trans*) ferulate esters; 3-methyl-3-butenyl-*cis*-ferulate, 2-methyl-2-butenyl-*cis*-ferulate, 3-methyl-2-butenyl-*cis*-ferulate, 3-methyl-3-butenyl-*trans*-ferulate, 2-methyl-2-butenyl-*trans*-ferulate, 3-methyl-2-butenyl-*trans*-ferulate and three coumarate esters; 3-methyl-3-butenyl *trans*-coumarate, 2-methyl-2-butenyl-*trans*-coumarate, 3-methyl-2-butenyl-*trans*-coumarate. Only pinocembrin flavanone was identified (6.8%). HPLC analysis revealed the presence of 8-methoxykaempferol (6.74µg), quercetin-7,3'-dimethylether, chrysin-7-methylether, biochanin A and dimethylallyl caffeate (9.0 µg) in sub-fraction (A). Quercetin-3,7-dimethylether (15.62µg) was significantly present in moderate concentrations in sub-fractions B, while quercetin-7,3'-dimethylether and the chrysin-7-methylether were present in low concentrations. It could be concluded that, Egyptian propolis bioassay guided fractionation on α-Glucosidase enzyme revealed that; some sub-fractions are highly active inhibitors and some are inactive.

Keywords: Propolis, α-Glucosidase inhibitors, Chemical Composition; GC/MS and HPLC analysis.

INTRODUCTION

Recently, Diabetes mellitus has been becoming an intimidating worldwide problem threatening all ages. In developing countries the number of diabetic patients is increasing rapidly. It has become a common disease due to aged population in the world, bad food habit and environmental pollution. The incidence of type II diabetes is increasing worldwide with hyperglycemia.[1] Hyperglycemia, a typical symptom in Type II diabetes and is characterized by a rapid increase in blood glucose levels due to starch hydrolysis by pancreatic α-amylase and glucose absorption by α-glucosidases in the small intestine.[2] One of the available glucose-lowering treatments is α-glucosidase inhibitors.

Alpha-glucosidases are a series of enzymes, which catalyze the final step in the digestive process of carbohydrates to release absorbable monosaccharides resulting in increased blood glucose levels[3], thus α-glucosidase inhibitors have become candidates to restrain the digestion and absorption of carbohydrates and hinder postprandial hyperglycemic deviation. Therefore, they have a potential to decrease progress of type II diabetes. However, some synthetic α-glucosidase inhibitors, such as acarbose, exhibit certain side effects[4], including liver disorders, renal tumors, and diarrhea all of which are associated with incomplete carbohydrate absorption.[5] As a result, many researchers have focused on natural extracts inhibiting α-glucosidase activity, especially those rich in polyphenols compounds.

Propolis is a very complicated mixture of chemical compounds obtained by bees from bark resinous exudates and leaf buds which mixed with wax and salivary secretions.[6] Propolis samples from different flora could be completely different in their chemistry and biological activity.[7] The main bioactive chemical compounds in propolis are reported to be phenolic acids, terpenes, cinnamic acid derivatives and flavonoids.[8-12] The solvents used to extract propolis play a key role in its different bioactivities, due to the diverse types of chemical components.[13]

Propolis was reported to possess a broad spectrum of biological activities such as antibacterial[8], antifungal[14], antiviral[8,10], antiinflammatory[15], antioxidant[9] and anticancer activities.[11,16]

The aim of this study was to evaluate the α-glucosidase inhibitory activity of Egyptian propolis fractions and sub-fractions with comparative correlation to chemical

composition of the highly active sub-fractions through GC/MS and HPLC analysis.

MATERIALS AND METHODS

Propolis extraction

Propolis (20 g) was cut into small pieces and extracted with distilled water (300 ml x 3) each for 2 hours at 85 °C to give propolis water extract, the residue was extracted with 70% ethanol (300 ml x 3) under reflux conditions each for 2 h which gave (PEE 70%), this extract (5.5 g) was dried under reduced pressure, the dried residue was suspended in water and then partitioned successively in turn with ether and ethyl acetate.

Bioassay guided fractionation and characterization of the fractions

Ether fraction (2.7g) was subjected to Sephadex LH-20 column chromatography (10 x 1 cm) and stepwise gradient elution was carried out using a solvent system of decreasing polarity starting with 100% distilled water then water–methanol. Fractions of 10 ml were collected and investigated by TLC (silica gel DF_{245} Merck) using different spraying reagents, similar fractions were combined and concentrated to dryness under reduced pressure to obtain one main fraction (1.7g) it was fractionated again into many sub-fractions on column packed with silica gel (0.06-0.2mesh, Merck), stepwise elution with petroleum ether, (pet.ether–ethylacetate) was carried out.

The ethyl acetate fraction (1.1 g) also was further subjected to column chromatography packed with silica gel (0.06-0.2mesh, Merck), elution was carried out with pet.ether, pet.ether–ethylacetate (9:0.5, 9:1, and 8:2), resulted in total four main sub-fractions.

α-Glucosidase inhibition assay

α-glucosidase Inhibitors which act as competitive inhibitors of intestinal α-glucosidase can delay the digestion and subsequent absorption of elevated blood glucose levels.

The α-glucosidase inhibitory activity was assessed by the standard method[17], with slight modifications. Briefly, a volume of 60 µl of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in 96 well plates at 37 °C for 20 min. After pre-incubation, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 °C for another 20 min.

Then the reaction was stopped by adding 160 µl of 0.2 M $NaCO_3$ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution in place of the extract.

For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Commercially available Acarbose was used as a standard and compared with all extracts.

HPLC analysis

After extraction of the materials, the sub-fractions were dissolved in MeOH. Both the mobile phase and the dissolved materials were filtered by a Millex-HX Nylon syringe filter (0.45 um, 25 mm; Millipore, Bedford, MA). The materials are subjected to chromatographic analysis with High-Performance liquid Chromatography (HPLC), Reverse phase with the following specifications; Shimadzu SCL-10Avp System controller. Dual pump shimadzu liquid chromatography (LC-10Avp), shimadzu degasser (DGU-14A), shimadzu UV-Vis detector (SPD-10Avp) and column: phenomenex RP-18 (UK; 250 x 4.00 mm, 5 micron). Elution was with water/formic acid (19:1 v/v; solvent A) and acetonitrile (solvent B), and the flow rate was 1 ml/min. Gradient elution started with 20% B, reaches 25% B at 25 min and 30% B at 35 min, and then the system became isocratic until 50 min, reaches 50% B at 60 min and 70% B at 67 min, at ambient temperature. The mobile phase solvents are HPLC grade and di-ionized H_2O. The compounds were detected with a UV detector and the chromatograms were recorded at 340 and 290 nm for flavones and flavanones, respectively.[18]

GC/MS analysis of highly α-glucosidase inhibitors propolis Fractions

Sample preparation for GC/MS analysis

1.5 mg of the dried matter was prepared for chromatography by derivatization for 30 min at 80 °C with 20 µl pyridine + 30 µl N,O, bis-(trimethylsilyl) trifluoroacetamide (BSTFA) and analyzed by GC/MS.[19]

GC/MS analyses

A Finnigan MAT SSQ 7000 mass spectrometer was coupled with a Varian 3400 gas chromatograph. DB-1 column, 30 m x 0.32 mm (internal diameter) , was employed with helium as carrier gas (He pressure, 20 Mpa/cm^2), injector temperature, 310°C; GC temperature program, 85 - 310°C at 3 °C/ min (10 min. initial hold). The mass spectra were recorded in electron ionization (EI) mode at 70 eV. The scan repetition rate was 0.5 s over a mass range of 39-650 atomic mass units (amu).

Identification of compounds

The identification was accomplished using computer search user-generated reference libraries, incorporating mass spectra.

Peaks were examined by single-ion chromatographic reconstruction to confirm their homogeneity.

In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed on the bases of its mass spectral fragmentation.

Reference compounds were co-chromatographed when possible to confirm GC retention times.

RESULTS

α-Glucosidase inhibitory activity

The main purpose of this study was to investigate the Potential α-glucosidase inhibitory effect of propolis extract and its sub-fractions. PEE 70% extract, ether, ethyl acetate fractions and their sub-fractions were investigated for their α-glucosidase inhibitory activity.

The PEE 70% extract, ether fraction and its six sub-fractions showed no inhibitory activity, except the sub-fraction (6) had moderate activity (32.7 %, Figure 1A), in comparison with that of acarbose (49%).

The ethyl acetate fraction and its sub-fractions 3 and 4 had no inhibitory activity, while the sub-fractions 1 and 2 showed low and high moderate α-glucosidase inhibitory activity (20 and 37% respectively, Figure 1B).

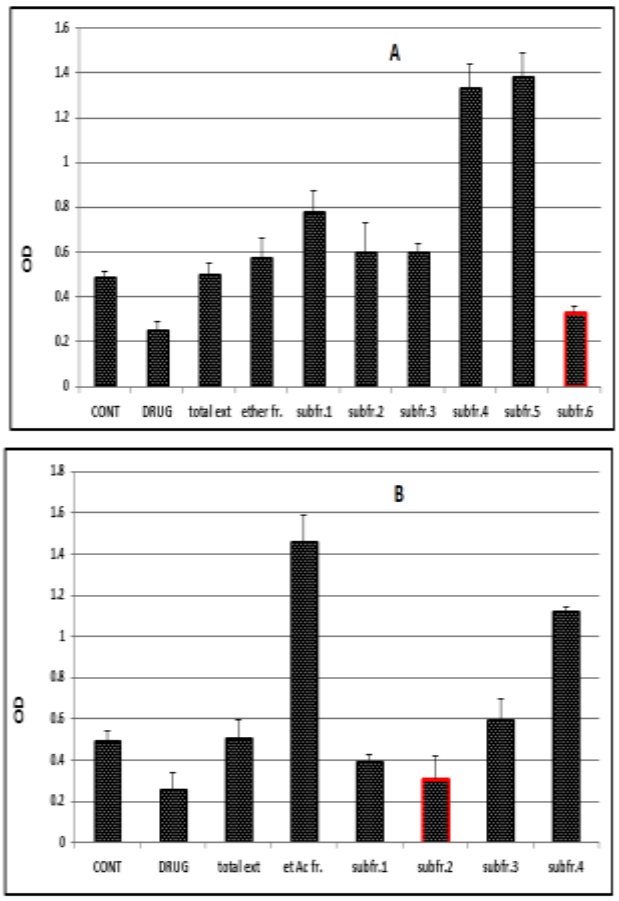

Figure 1: Bioassay guided fractionation of 70% PEE to show α-glucosidase inhibitory activity; Figure 1A= ether, Figure 1B= ethyl acetate fractions and their sub-fractions. Values are expressed as mean + SD, n=3 (400 µg/ml)

GC/MS analyses

The sub-fractions with high α-glucosidase inhibitory activity; A(ether sub-fraction 6) and B (ethyl acetate sub-fraction 2), were subjected to GC/MS analysis.

The presence of high percentage of Phenolic acids (42.6 %, in A); especially 3,4-dimethoxy-*trans*-cinnamic acid (30.7%). p-Methoxy-*trans*-cinnamic acid and 3,4-dimethoxy-*cis*-cinnamic acid showed moderate presence (1.61 and 5.34 % respectively), (Table 1, Figure 2). This fraction contained only five caffeic acid esters; caffeic acid methyl ester, 3-methyl-3-butenyl-*cis*-caffeate, 3-methyl-3-butenyl-*trans*-caffeate, 3-methyl-2-butenyl-*trans*-caffeate and benzyl-*trans*-caffeate[20] (0.44, 0.05, 0.49, 0.42 and 0.48% respectively) (Table 1, Figure 2). Only two flavonoids were identified by GC/MS in this fraction; quercetin and myricetin (0.08 and 0.36% respectively).

High percentage of aliphatic acids were found in fraction B (39.8%); the highest percentage is hexadecanoic acid (26.9%) followed by octadecenoic acid and octadecanoic acid (4.8 and 3.39%), (Table 1, Figure 3).

Fraction B mainly contained phenolic acids esters (10.6%), from which, six (*cis* and *trans*) ferulate esters; 3-methyl-3-butenyl-*cis*-ferulate, 2-methyl-2-butenyl-*cis*-ferulate, 3-methyl-2-butenyl-*cis*-ferulate (0.58, 0.44, 0.9 % respectively), 3-methyl-3-butenyl-*trans*-ferulate, 2-methyl-2-butenyl-*trans*-ferulate, 3-methyl-2-butenyl-*trans*-ferulate (1.33, 0.8, 1.12% respectively) and three -*trans*-coumarate esters; 3-methyl-3-butenyl-*trans*-coumarate, 2-methyl-2-butenyl-*trans*-coumarate, 3-methyl-2-butenyl-*trans*-coumarate[21] (2.58, 0.44, 1.23 % respectively) (Table 1, Figure 3). Only pinocembrin flavanone was identified by GC/MS analyses in this fraction with high percentage (6.8%, Table 1, Figure 3).

HPLC analysis of propolis

The highly active α-glucosidase inhibitors sub-fractions A and B were analyzed by HPLC. Fifteen flavonoid compounds and one caffeic acid ester were quantitatively identified in propolis sub-fractions A and B.

The flavonols 8-methoxykaempferol (6.74µg/mg sub-fraction), quercetin-7,3'-dimethylether (2.45µg), the flavone chrysin-7-methylether (2.53µg), the flavanone biochanin A (2.0 µg) and dimethylallylcaffeate (9.0 µg) were present in low concentrations in sub-fraction A. The flavanol quercetin-3,7-dimethylether (15.62, µg/mg sub-fraction) was significantly present in moderate concentrations in sub-fractions B, while quercetin-7,3'-dimethylether (1.40 µg) and the flavone Chrysin-7-methylether (2.43µg) were present in low concentrations (Table 2, Figure 4).

Table 1: Chemical composition assessed by GC/MS analysis of propolis sub-fractions (A) and (B)

No.	Compounds	RT	*Sub-fraction A	*Sub-fraction B
	Aliphatic acids			
1	2-hydroxyl-Propanoic acid	8.30	1.77	0.95
2	Hydroxyacetic acid	8.91	0.37	0.15
3	Ethanedioic acid	12.1	-----	0.1
4	Butanedioic acid	21.46	-----	1.57
5	1,2,3-Propanetriol triacetate (Triacetin)	22.96	-----	0.11
6	Decanoic acid,	27.72	-----	0.33
7	Undecanoic acid	31.74	-----	0.08
8	Dodecanoic acid	35.57	-----	0.8
9	Tetradecanoic acid	42.57	-----	0.93
10	n-Pentadecanoic acid	45.79	-----	0.19
11	Cis-9-Hexadecenoic acid	48.26	-----	0.28
12	Hexadecanoic acid	49.32	-----	**26.88**
13	cis-10-Heptadecenoic acid	51.15	-----	0.04
14	Octadecenoic acid	54.34	-----	**4.8**
15	Octadecanoic acid	54.94	0.07	3.39
	Total		***2.21***	***39.8***
	Aliphatic esters			
16	Hexadecanoic acid methyl ester	44.96	-----	0.15
17	Octadecanoic acid, methyl ester	51.25	-----	0.05
18	Hexanoic acid, 2-ethyl,diester with tetraethylene glycol	60.94	0.22	-----
19	Hexadecanoic acid, 2-hydroxy-1-hydroxy methyl- ethyl ester	63.46	0.07	-----
20	Hexadecanoic acid, 2,3-dihydroxy propyl ester	64.31	-----	1.63
21	Octadecanoic acid, 2,3-dihydroxy-propyl ester	68.89	**1.16**	-----
	Total		***1.45***	***1.83***
	Phenolic compounds			
22	4(t-Butyl)2(prop-2'enyl)phenol	17.97	-----	0.08
23	4-hydroxy-benzaldehyde	23.84	-----	0.05
24	Diphenyl ether	24.93	-----	0.08
25	1-Phenyl-3-hydroxy-1-propene	26.22	-----	0.22
26	8,8aDimethyl2(1methylethylidene)1,2,3,7,8,8ahexahydronaphthalene	26.8	-----	0.04
27	6(4-tert-Butylphenyl) 1,3,5-hexatriynyl	31.42	-----	**7.51**
28	1,3-Bis[2hydroxyphenyl]-2-propen-1-one	60.16	-----	0.06
	Total		***-----***	***8.04***
	Phenolic acids			
29	Benzoic acid	17.87	0.5	0.12
30	3-Phenyl-3-hydroxypropanoic acid	33.54		0.03
31	p-Hydroxybenzoic acid	34.68	**1.14**	-----
32	P-Methoxy-cis-cinnamic acid	37.16	0.03	-----
33	p-Methoxy trans-Cinnamic acid	41.92	**1.61**	0.06
34	3,4-Dimethoxy-cis-cinnamic acid	43.66	**5.34**	-----

35	Isoferulic acid	45.17	-----	0.03
36	p-Coumaric acid	45.59	-----	0.2
37	3,4-Dimethoxy-trans-cinnamic acid	49.37	**30.7**	-----
38	Ferulic acid	50.62	0.05	0.15
39	Caffeic acid	52.03	0.12	-----
40	1,2-Benzenedicarboxylic acid	62.79	3.06	-----
41	Rosmarinic acid,	68.39	-----	0.04
	Total		***42.55***	***1.43***
	Phenolic acids esters			
42	1,2-Benzenedicarboxylicacid, bis(2methylpropyl)ester	43.0	-----	0.06
43	Phthalic acid, butyl dodecyl ester	46.09	-----	0.27
44	Caffeic acid methyl ester	47.85	**0.44**	
45	3-Methyl-3-butenyl-*cis*-ferulate	51.85	-----	0.58
46	3-Methyl-3-butenyl-*trans*-coumarate	52.39	-----	**2.58**
47	2-Methyl-2-butenyl-*cis*-ferulate	52.7	-----	0.44
48	3-Methyl-2-butenyl-*cis*-ferulate	52.91	-----	**0.99**
49	3-Methyl-3-butenyl-*cis*-caffeate	53.56	0.05	
50	2-Methyl-2-butenyl-*trans*-coumarate	53.58	-----	0.44
51	3-Methyl-2-butenyl-*trans*-coumarate	53.99	-----	**1.23**
52	3-Methyl-3-butenyl-*trans*-ferulate	56.45	-----	**1.33**
53	2-Methyl-2-butenyl-*trans*-ferulate	57.62	-----	0.8
54	3-Methyl-2-butenyl-*trans*-ferulate	57.92	-----	**1.12**
58	3-methyl-3-butenyl-*trans*-caffeate	58.12	**0.49**	-----
59	3-methyl-2-butenyl-*trans*-caffeate	59.56	**0.42**	-----
60	Benzyl-*trans*-caffeate	67.05	**0.48**	-----
	Total		***1.88***	***10.16***
	Diterpenes			
61	Dehydroabietic acid	58.69	**-----**	0.11
	Flavonoids			
62	Pinocembrin	63.05	**-----**	**6.8**
63	Myricetin	70.33	**0.36**	-----
64	Quercetin	73.25	0.08	-----
	Total		***0.44***	***6.8***
	Others			
65	Glycerol	20.02	0.32	0.74
66	1,1,1-Tris(hydroxymethyl)propane	30.88	-----	0.08
67	Oleanitrile	49.90	-----	0.05
	Total		***0.32***	***0.87***

RT=retention time.*, TIC =The ion current generated depends on the characteristics of the compound concerned and it is not a true quantitation. t, tentatively identified from mass spectra

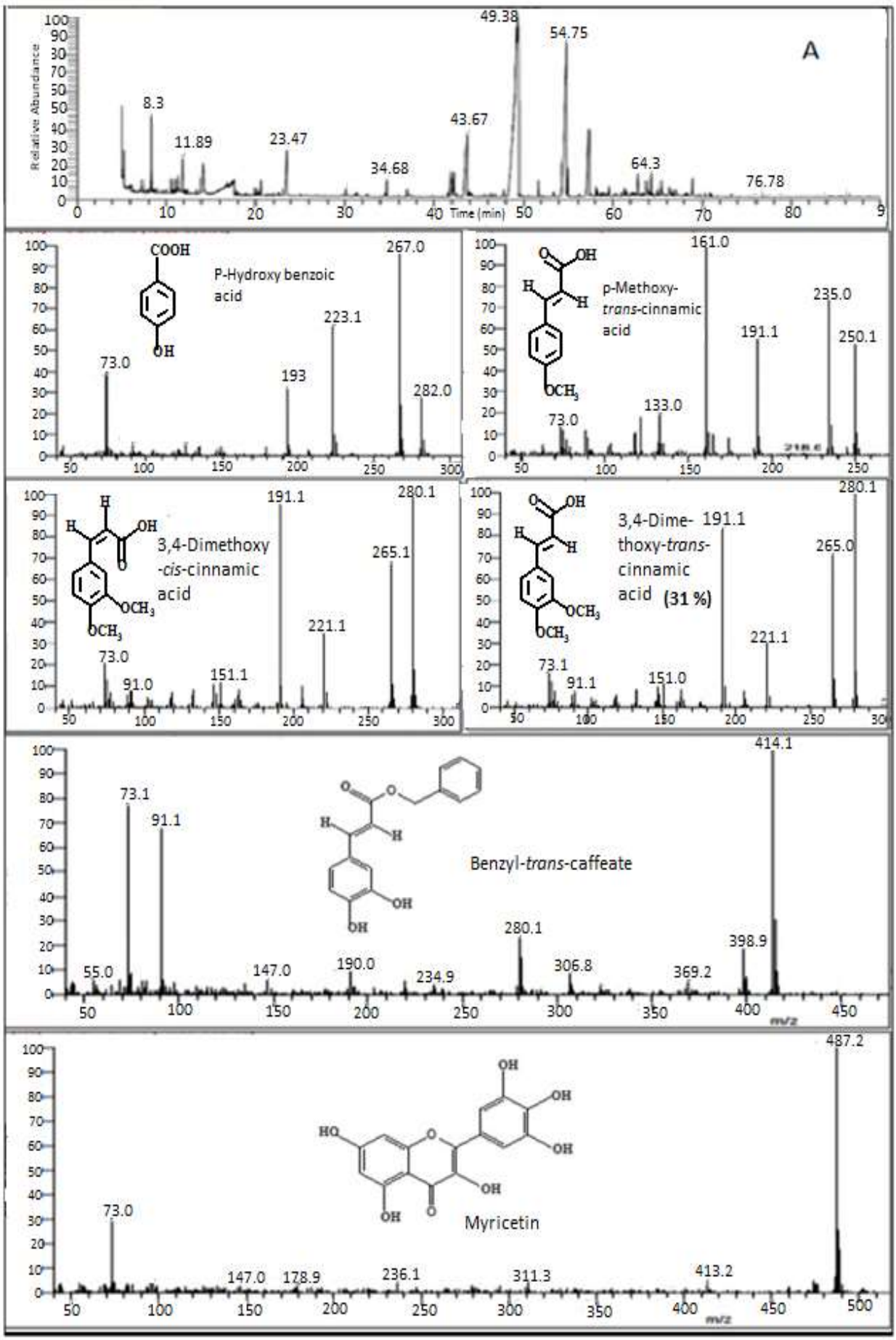

Figure 2: GC/MS Chromatogram of propolis sub-fraction (A) and mass spectra of prominent peaks

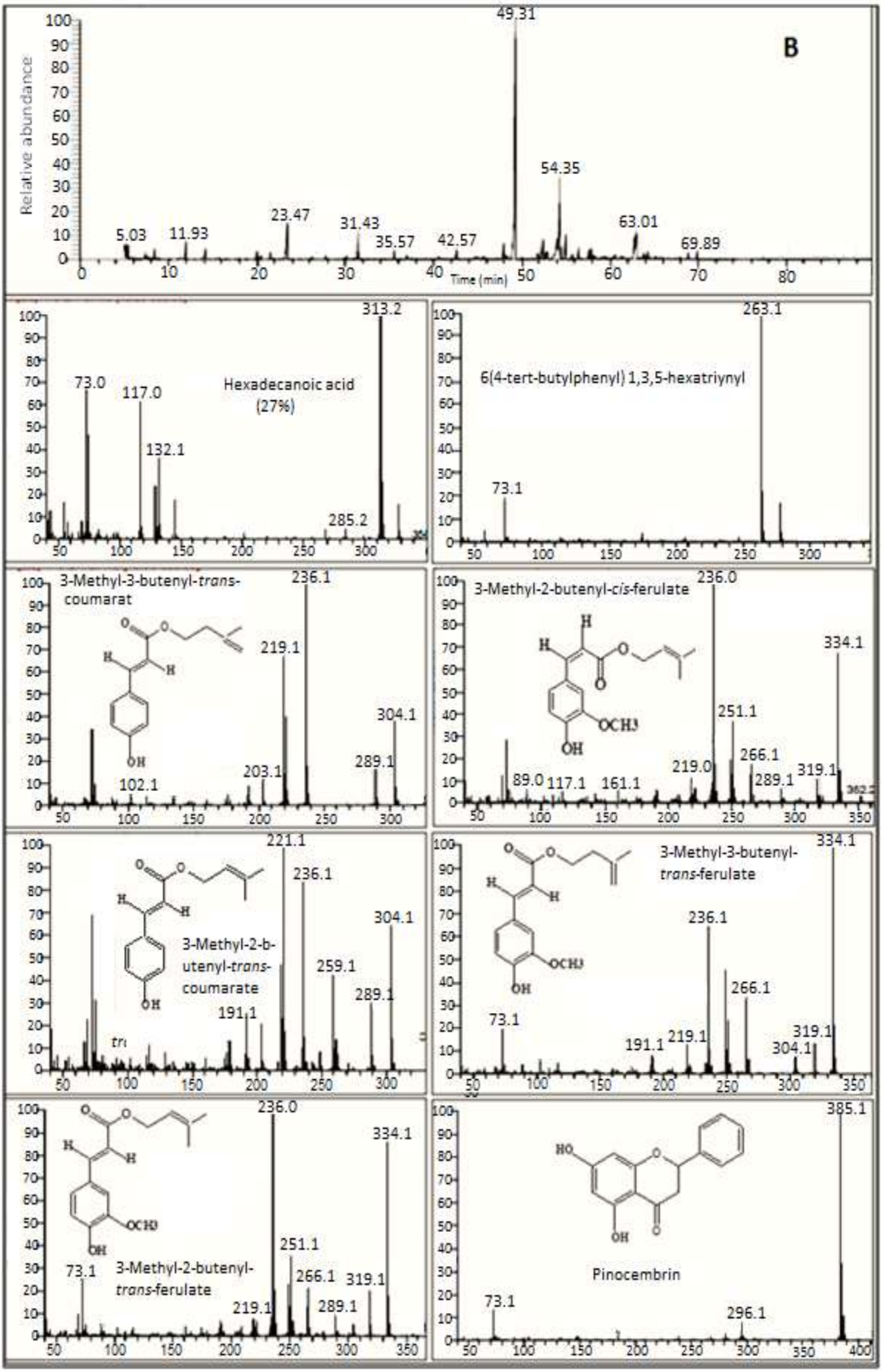

Figure 3: GC/MS Chromatogram of propolis sub-fraction (B) and mass spectra of prominent peaks

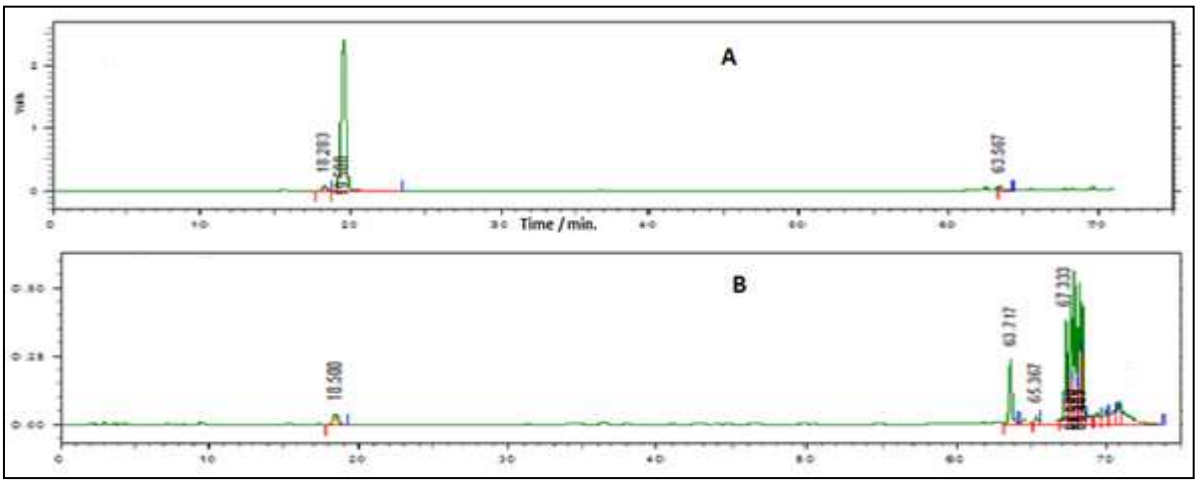

Figure 4: HPLC chromatograms of propolis sub-fractions (A) and (B)

Table 2: Flavonoids assessed by HPLC of propolis sub-fractions (A) and (B) (µg /mg sub-fraction)

No.	Name	Chemical name	RT	Sub-fraction A	Sub-fraction B
		Flavones			
1	Apigenin	5,7,4'-trihydroxyflavone	37.95	-----	0.04
2	Luteolin-3'-methylether	5,7,4'-trihydroxy-3'-methoxyflavone	42.06	0.02	0.24
3	Chrysin-7-methylether	5- hydroxy-7-methoxy flavone	61.91	**2.53**	2.43
4	Acacetin	5,7- dihydroxy-4'-methoxy flavone	65.4	-----	0.14
	Total			**2.55**	**2.85**
		Flavonols			
5	Myricetin	3,5,7,3',4',5'- hexahydroxyflavone	12.88	0.02	-----
6	Quercetin-3-methylether	5,7,3',4'-tetrahydroxy-3-methoxyflavone	29.33	-----	0.03
7	Quercetin-3,7-dimethylether	5,3',4'-trihydroxy-3,7-dimethoxyflavone	34.6	-----	15.62
8	8-Methoxykaempferol	3,5,7,4'- tetrahydroxy-8- methoxyflavone	37.58	6.74	-----
9	Kaempferol-3-methylether	5,7,4'- trihydroxy-3-methoxyflavone	44.46	-----	0.06
10	Quercetin-7-methylether	3,5,3',4'-tetrahydroxy-7-methoxyflavone	56.88	0.28	-----
11	Quercetin-7,3'-dimethylether	3,5,4'-trihydroxy-7,3'-dimethoxyflavone	66.13	2.45	1.40
	Total			**9.50**	**17.11**
		Flavanones			
12	Hesperetin	5,7,3'- trihydroxy-4'-methoxyflavanone	39.1	0.42	0.04
13	Pinocembrin	5,7-dihydroxyflavanone	62.73	-----	0.46
14	Biochanin A	5,7-dihydroxy-4'-methoxyflavanone	65.15	2.0	0.54
	Total			**2.42**	**1.04**
		Isoflavones			
15	Genistein	5,7,4'-trihydroxyisoflavone	35.8	-----	0.51
		Caffeic acid esters			
16	Dimethylallylcaffeate	3-methylbut-2-enyl caffeate	62.65	9.0	-----

DISCUSSION

Diabetes is characterized by high blood sugar levels which can cause serious complications such as organ failures and/or destruction of the kidneys, eyes, and various cardiovascular diseases. Therefore, the treatment methods mainly focus on reducing fluctuations in blood sugar levels and their related complications. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes, such as α-glucosidase.[22] α-Glucosidase hydrolyzes the disaccharides to monosaccharides to be available for the intestinal absorption. The inhibition of α-

glucosidase activity in the digestive tract is considered an effective way to control diabetes through lowering glucose absorption. Therefore, inhibition of α-glucosidase activity by safe effective natural products has long been considered.

Hyperglycemia is also believed to increase the production of free radicals and reactive oxygen species, leading to oxidative tissue damage and diabetic complications such as nephropathy, neuropathy, retinopathy, and memory impairment[23], which can be controlled by the highly antioxidants polyphenols compounds. Some studies demonstrated that the intake of flavonoids, including quercetin and myricetin is inversely associated with the risk of incident type II diabetes.[24] Therefore it has now been adopted that an effective antidiabetic compound should have both hypoglycemic, antioxidant properties and minimal side effects.[25]

This study investigated the effect of Egyptian propolis's different fractions against α-glucosidase activity. Studying the chemical composition of the highly active α-glucosidase inhibitors sub-fractions was carried out by using different chromatographic techniques as HPLC and GC/MS. The analysis revealed that they are more or less completely different in their bioactive compounds; i.e. in GC/MS analysis, aliphatic acids (A=2%, B= 40%), Phenolic compounds (A=0%, B=8%), Phenolic acids (A=42.5%, B=1.4%), Phenolic acids esters (A=1.9%; caffeate esters only, B=10% ferulate and coumarate esters) and Flavonoids (A= 0.4%, B=7%). In HPLC analysis of flavonoids (A= 14.5%, B= 21.5%).

In this context, many antioxidants polyphenol compounds found in this study-in Egyptian Propolis sub-fractions-were previously studied and significantly increase the α-glucosidase inhibitory activity *in-vitro*. Quercetin has been reported to possess high inhibitory effect against α-glucosidase with IC_{50} (8.86 µg/Ml).[26] Myricetin and rosmarinic acid strongly inhibited α-glucosidase (IC_{50}= 0.1mM and 33.0 ± 4.6 µmol/L, respectively)[27], p-coumaric acid and ferulic acid had α-glucosidase inhibitory activity (IC_{50}>30 mmol/L and IC_{50}= 4.9 ± 0.3 mmol/L).[28] Caffeic acid showed pronounced inhibition of α-glucosidase.[29] Ferulic acid derivatives showed strong inhibitory effects on α-glucosidase enzyme.[28] 4-Hydroxy-3-methoxy benzoic acid and 4-hydroxy-3,5-dimethoxy benzoic acid showed 27 and 35% inhibition of α-glucosidase activity, respectively.[30] 4-Methoxy-*trans*-cinnamic acid and its ethyl ester showed the highest potent inhibitory activity among other *trans*-cinnamic acid derivatives.[31]

The molecular structures of polyphenols as α-glucosidase inhibitors influence the inhibition in the following ways: the presence of -OH group at C-5 & 7 positions at A ring and C-3'OH of B ring significantly increase the α-glucosidase inhibitory activity.[32,33]

Tadera indicated that presence of two hydroxyl groups in B ring of poly phenolic compounds is necessary for strong inhibition of α-glucosidase, that is the cause; quercetin gave higher activity than apigenin. C-3 and C-5 hydroxylation on the A and C rings of flavones significantly increase the α-glucosidase inhibitory activity, while hydroxylation of C-4' for the B ring, is crucial for this activity.[34]

Phenolic acids with more than one hydroxyls showed strong inhibition, especially with ortho or meta-dihydroxyls.[35]

CONCLUSION

This is the first time to study Egyptian propolis bioassay guided fractionation on α-glucosidase inhibitory activity with comparison to its chemical composition by GC/MS and HPLC analysis.

Some sub-fractions are moderately active. The crude extract, original fractions and most of sub-fractions are inactive.

Acknowledgement: The authors are grateful for the financial support by the National Research Center of Egypt (Contract 2/3/5).

REFERENCES

1. Mohamed EAH, Siddiqui MJA, Ang LF, Sadikun V, Chan SH, Tan SC, Asmawi MZ, Yam MF. Potent α-glucosidase and α-amylase inhibitory activities of standardized 50% ethanolic extracts and sinensetin from Orthosiphon stamineus Benth as anti-diabetic mechanism. BMC Complementary and Alternative Medicine, 12, 2012, 176.
2. Deshpande MC, Venkateswarlu V, Babu RK, Trivedi RK. Design and evaluation of oral bioadhesive controlled release formulations of miglitol, intended for prolonged inhibition of intestinal alpha-glucosidases and enhancement of plasma glycogen like peptide-1 levels. Int. J. Pharm., 380, 2009, 16-24.
3. Shai LJ, Masoko P, Mokgotho MP. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. South African Journal of Botany, 76, 3, 2010, 465–470.
4. Fujisawa T, Ikegami H, Inoue K, Kawabata Y, Ogihara T. Effect of two alpha-glucosidase inhibitors, voglibose and acarbose on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism, 54, 2005, 387–390.
5. Ghadyale S, Takalikar V, Haldavnekar, Arvindekar A. Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by Cymbopogon martini (Roxb.). Evidence-Based Complementary and Alternative Medicine, 2012, Article ID 372909.
6. Burdock G A. Review of the biological properties and toxicity of bee propolis (propolis). Food and Chemical Toxicology, 36, 1998, 347–363.
7. Bankova V. Recent trends and important developments in propolis research. eCAM, 2, 2005, 29–32.
8. Abd El Hady FK, Hegazi AG. Egyptian propolis: 2. chemical composition, antiviral and antimicrobial activities of east nile delta propolis. Z. Naturforsch, 57c, 2002, 386–394.

9. Hegazi AG, Abd El-Hady FK. Egyptian propolis: 3. antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. Z. Naturforsch, 57c, 2002, 395-402.
10. Abd El Hady FK, Hegazi AG, Wollenweber E. Effect of Egyptian propolis on the susceptibility of LDL to oxidative modification and antiviral activity with special emphasis on chemical composition. Z. Naturforsch 62c, 2007, 645-655.
11. Abd El-Hady FK, Souleman AMA, El-Shahid ZA. Antiacetylcholinesterase and Cytotoxic Activities of Egyptian Propolis with Correlation to its GC/MS and HPLC Analysis. Int. J. Pharm. Sci. Rev. Res., 34(2), 2015, 32-42.
12. Abd El-Hady FK, Souleman AMA,, El Hawary S, Salah NM, El-Shahid ZA. Egyptian Propolis Bioassay Guided Fractionation and GC/MS, HPLC Analysis of Highly Anti-acetylcholinesterase Sub-fractions. Int. J. Pharm. Sci. Rev. Res., 35(1), 2015, 53-62.
13. Watanabe J, Kawabata J, Kurihara H. Isolation and identification of α-glucosidase inhibitors from Tochu-cha, Biosci. Biotechnol. Biochem., 61, 1997, 177–178.
14. Silici S, Koç NA, Ayangil D, Ankay S. Antifungal activities of propolis collected by different races of honeybees against yeasts isolated from patients with superficial mycoses. J. Pharmacol. Sci., 99, 2005, 39–44.
15. Tan-No K, Nakajima T, Shoji T, Nakagawasai O, Niijima F, Ishikawa M, Endo Y, Sato T, Satoh S, Tadano T. Anti-inflammatory Effect of Propolis through Inhibition of Nitric Oxide Production on Carrageenin-Induced Mouse Paw Edema. Biol. Pharm. Bull, 29(1), 2006, 96—99.
16. Oršolić NA. Review of propolis antitumour action *in-vivo* and *in-vitro*. Journal of Api Product and Api Medical Science, 2(1), 2010, 1-20.
17. Dong HQ, Li M, Zhu F, Liu FL, Huang JB. Inhibitory potential of trilobatin from Lithocarpus polystachyusRehd against α-glucosidase and α-amylase linked to type II diabetes, Food Chemistry, 130, 2012, 261-266.
18. Gil MI, Ferreres F, Ortiz A, Subra E, Toma` s-Barbera`n FA. Plant phenolic metabolites and floral origin of Rosemary honey, J. Agric. Food Chem. 43, 1995, 2833-2838.
19. Christov R, Bankova V, Hegazi AG, Abd El-Hady FK, Popov S. Chemical composition of Egyptian propolis. Z. Naturforsch, 53c, 1998, 197-200.
20. Greenaway W, Wollenweber E, Scaysbrook T, Whatley FR. Esters of Caffeic Acid with Aliphatic Alcohols in Bud Exudate of Populus nigra. Z. Naturforsch, 43c, 1988, 795-798.
21. May J, Scaysbrook T, Whatley F R. Identification by Gas Chromatography-Mass Spectrometry of 150 Compounds in Propolis. Z. Naturforsch, 46c, 1991, 111-121.
22. Thilagam E, Parimaladevi B, Kumarappan C, Mandal SC. α-Glucosidase and α-amylase inhibitory activity of senna surattensis. J Acu Meri Stud, 6(1), 2013, 24-30.
23. Moradi A, Asghari B, Saeidnia S, Ajani Y, Mirjani M, Malmir M, Bazaz RD, Hadjiakhoondi A, Salehi P, Hamburger M, Yassa N. *In vitro* α-glucosidase inhibitory activity of phenolic constituents from aerial parts of *Polygonum hyrcanicum* Fahimeh. Journal of Pharmaceutical Sciences, 20, 2012, 37.
24. Rigelsky JM, Sweet BV. Hawthorn, pharmacology and therapeutic uses. Am. J. Health-Syst. Pharm., 59, 2002, 417–422.
25. Bello A, Aliero AA, Saidu Y, Muhammad S. Phytochemical Screening, Polyphenolic Content And Alpha-Glucosidase Inhibitory Potential of Leptadenia hastata (Pers.) Decne. Nigerian Journal of Basic and Applied Science, 19(2), 2011, 181-186.
26. Schnitzler P, Neuner A, Nolkemper S, Zundel C, Nowack H, Sensch KH, Reichling J. Antiviral activity and mode of action of propolis extracts and selected compounds. Phytother. Res., 24, 2010, 20–28.
27. Masayoshi I, Ayako y, yumiko I, chikako K, Ayako M. Effect of Flavonoids on a-Glucosidase and f3-Fructosidase from Yeast, Agric. Bioi. Chem., 48(6), 1984, 1559-1563.
28. Jeong EY, Cho KS, Lee HS. α-Amylase and α-glucosidase inhibitors isolated from Triticum aestivum L. sprouts. J. Korean Soc. Appl. Biol. Chem., 55, 2012, 47–51.
29. Oboh G, Agunloye OM, Adefegha SA, Akinyemi AJ, Ademiluyi AO. Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (*in vitro*): a comparative study. J Basic Clin Physiol Pharmacol. 26(2), 2015, 165-70.
30. Lelono RAA, Tachibana S. Preliminary Studies of Indonesian Eugenia polyantha Leaf Extracts as Inhibitors of Key Enzymes for Type II Diabetes. J. Med. Sci., 13, 2013, 103-110.
31. Adisakwattana S, Sookkongwaree K, Roengsumran S, Petsom A, Ngamrojnavanich N, Chavasiri W, Deesamer S, Yibchok-anun S. Structure–activity relationships of trans-cinnamic acid derivatives on alphaglucosidase Inhibition. Bioorganic & Med. Chem. Let., 14, 2004, 2893-2896.
32. Gao H, Nishioka T, Kawabata J, Kasai T. Structure–Activity Relationships for α-Glucosidase Inhibition of Baicalein, 5,6,7-Trihydroxyflavone: The Effect of A-Ring Substitution. Biosci. Biotechnol. Biochem., 68, 2004, 369–375.
33. Li H, Song F, Xing J, Tsao R, Liu Z, Liu S. Screening and structural characterization of α-glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MSn and SORI-CID FTICR MS. Journal of the American Society for Mass Spectrometry, 20, 2009, 1496-1503.
34. Tadera K, Minami Y, Takamatsu K, Matsuoka T. Inhibition of α-glucosidase and α-amylase by flavonoids. Journal of Nutritional Science and Vitaminology, 52, 2006, 149-153.
35. Xie Y1, Chen X. Structures required of polyphenols for inhibiting advanced glycation end products formation. Curr Drug Metab., 14(4), 2013, 414-431.

Source of Support: Nil, **Conflict of Interest:** None.