Original Article

BEE POLLEN EXTRACTS AS POTENTIAL ANTIOXIDANTS AND INHIBITORS OF α-AMYLASE AND α-GLUCOSIDASE ENZYMES - *IN VITRO* ASSESSMENT

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Abstract

The study was conducted to determine the antioxidant and anti-diabetic properties of bee pollen. The phenol content of extracts was 1.43, 2.04, 2.10 and 1.79 mg gallic acid g-1, respectively. Total flavonoid content was 0.78, 1.39, 0.86 and 0.79 mg gallic acid g-1 respectively. Bee pollen extracts have the potential to scavenge free radicals and lower blood glucose due to the presence of phenols and flavonoids. The aqueous-ethanol extract had the lowest IC_{50} for HRS and TAC, 0.53mg/mL and 0.25mg/mL respectively, which depicts that this extract alleviates the destructive effect of hydroxyl radicals. Methanol extract had the lowest IC_{50} 0.21 mg/mL) for DPPH inhibition, hence it was able to scavenge the DPPH radicals. Ethanol extract had the lowest IC_{50} (0.04 mg/mL) for 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid inhibition, hence it can protect living cells from ABTS cations. The aqueous-ethanol extract displayed higher inhibition of α-amylase (4.51 mg/mL) while the aqueous extract exhibited a higher inhibition of α-glucosidase (0.60 mg/mL), which slows down the breakdown of disaccharides and reduces sugar buildup in the bloodstream. Bee pollen has potential antioxidative and anti-diabetic activity.

Keywords: alpha-glucosidase, amylase, antidiabetic, antioxidant, bee-pollen

INTRODUCTION

Diabetes mellitus (DM) is a persistent metabolic disease marked by hyperglycemia, due to changes in insulin production and in the metabolism of some nutrients (Shobana, Sreerama, & Malleshi, 2009) and causes heart diseases and dyslipidemia (Vaidya & Goyal, 2010). The regulation of excessive glucose rise after eating junk food is vital for managing diabetes and decreasing persisting vascular disorders (Lebovitz, 2001), and a remedial technique is to hinder the uptake of glucose by cells after intake (Vaidya & Goyal, 2010). Polysaccharides are broken down to glucose by enzymes, assimilated by the intestine and moved to the cells of the body, but excessive glucose rise could be reduced by hindering the activity of the enzymes that breakdown sugar thus reducing the uptake of glucose (Shobana, Sreerama, & Malleshi, 2009).

Alpha glucosidase inhibitors are commonly used in combination with substances that stimulate insulin secretion for DM treatment (Saito et al., 1998). Unfortunately, these synthetic agents have many side effects including organ disorders. To prevent or cure DM and adiposity, much work is being done on non- harmful natural inhibitors of the enzymes that break down complex sugars, plant derived medications and food that regulates normal body functions (Tundis, Loizzo, & Menichini, 2010; Hiroyuki, Tomohide, & Kazunori, 2001; Matsui et al., 2001).

Products of oxidation are continuously produced in living cells and destroyed by antioxidants in the body. DM is correlated with free-radical generation of oxidative damage and excessive weight (Mohanty et al., 2011). Antioxidants monitor different oxidative processes within body tissues and delay or abort oxidation by removing oxidation products, binding to free metals and donating electrons. Plants rich in antioxidants protect the beta cells by inhibiting the spontaneous reaction of compounds with molecular oxygen and halt the complications of diabetes (Liu, 1997).

Over one thousand plants are hypoglycaemic

substances with minimal undesirable effects (Tundis, Loizzo, & Menichini, 2010). *Apis mellifera* honeybees produce bee pollen by collecting large quantities of plant pollen dust and nectar and combining them with their enzyme-rich saliva (Mărgăoan et al., 2010). Bee pollen contains potential antioxidants such as essential amino acids, flavonoids, phenolic acids, vitamins, chlorophyll and carotenoids (Mărghitaş et al., 2009). Phenolic compounds produced in plants regulate polysaccharide breakdown by inhibiting the enzymes involved (McDougall et al., 2005).

Hyperglycemia causes varying vascular diseases and rapid development of DM hence prompt diagnosis and proper management will assist in timely mediation (Ratner, 2001). The enzymes involved in carbohydrate digestion must be controlled for the effective management of blood glucose levels in DM and borderline patients (Tundis, Loizzo, & Menichini, 2010). Alpha-amylases, such enzymes breakdown internal alpha 1, 4 glucosidic linkages in polysaccharides to produce glucose and maltose (Shobana, Sreerama, & Malleshi, 2009). The α-glucosidase enzyme, found in the brush border of the small intestine, breaks down α 1, 6 bonds in polysaccharides to produce glucose.

MATERIAL AND METHODS

Reagents

Silymarin, gallic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) were purchased from Sigma-Aldrich (Johannesburg, South Africa).

Bee pollen collection and extract preparation

The bee pollen sample weighing 16.58g was obtained from the Apiary unit, University of Ilorin, Nigeria, oven dried at 40°C to a constant weight of 14.18 g and ground into powder. Four portions (0.5 g each) of the sample were exhaustively extracted with 5 ml of 50% aqueous-ethanol, methanol, water and ethanol respectively, filtered using Whatman No. 1 filter paper and concentrated at 45°C to obtain dry crude extracts. The yield was 0.98 g of dry plant material which were reconstituted in respective solvents to prepare concentrations of 1, 0.8, 0.6, 0.4 and 0.2 mg/mL for the studies.

Quantification of phenols

In a procedure according to Haley et al. (2002), 30 µl aliquots of bee pollen extracts, 30 µl distilled water and 30 µl of the Folin-Ciocalteu reagent (0.5 N) were dispensed into a 96 well micro titer plate, incubated for 15 min at 25°C. Then, 150 µl of 20% sodium carbonate was added, incubated for 30 min at 25°C and absorbance was read at 725 nm using a microplate reader (BIO-RAD model 680, Japan). Phenolic content was estimated from data collected using the standard curve obtained from Gallic acid concentrations.

Flavonoids quantification

A method by Yanping, Yanhua, & Dongzhi (2004) was used. A volume of 77 µl of the extract concentrations, 308 µl of distilled water and 23 µl 5% $NaNO_3$ solution were dispensed into an Eppendorf tube, incubated for 6 min period, and then 23 µl 10% $AlCl_3$ was added, incubated for 6 min at 25°C, after which 308 µl 1M NaOH and 761 µl of distilled water was added. A BIO-RAD micro-plate reader (model 680, Japan) was used for measuring absorbance at 510 nm after incubation at 25°C for 15 min.

Metal chelating assay

This assay was estimated with a method by Dinis, Madeira, & Almeida (1994). A volume of 40 µl of the extracts and 200 µl of 2 mM $FeCl_2$ solution were dispensed into a 96-well micro titer plate. The reaction was initiated by the addition of 80 µl 5 mmol/l ferrozine, and the contents were left to stand at 25°C for 10 min after which absorbance was read using a BIO-RAD (model 680, Japan) microplate reader at 562 nm. For the percentage of inhibition of ferrozine–Fe^{2+} complex formation, the IC_{50} value was calculated from the linear regression equation: y = mx+ c; y is the percentage activity and equals 50, m is the slope, c is the intercept

and x is the IC_{50} value.
Percent inhibition = [A control – A extract/ A control] x 100 "(Equation 1)" Where A control is absorbance of the control, and A extract is absorbance of the extract/standard.

Nitric oxide scavenging assay method by Kumaran & Karunakaran (2007) was used for the assay. The interaction of nitric oxide with oxygen produces nitrite ions which can be estimated with Greiss reagent. Nitric oxide scavengers compete with oxygen resulting in reduced nitrite ion production. A volume of 1000 µl of 10 mM sodium nitroprusside and 250 µl phosphate buffered saline (pH 7.4), 250 µl of (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) of bee pollen extracts were incubated at 25°C for 120 min in a 1.5 ml Eppendorf tube. With the aid of a pipette, 50 µl was withdrawn and added to 100 µl of sulphanilic acid reagent (3.3% sulphanilicacidin 20% glacial acetic acid) in a 96-well micro titer plate, incubated for 5 min at 25°C, 100 µl of 0.1% w/v naphthylethylenediamine dihydrochloride added and incubated at 25°C for 30 min. Absorbance of the formed chromophore was read at 540 nm using a 96-well microplate reader (BIORAD, model 680, Japan). The percentage inhibition and IC_{50} values were calculated.

Hydroxyl radical determination
The deoxyribose method was used to assess the ability of the extracts to scavenge hydroxyl radicals. Extract aliquots of 100 µl, 120 µl 20 mM deoxyribose, 400 µl 0.1 M phosphate buffer, 40 µl 20 mM hydrogen peroxide and 40 µl 500 µM ferrous sulphate were dispensed into 2 ml Eppendorf tubes, and then 100 µl of distilled water was added and incubated for 30 min at 37°C. Next, 0.5ml 2.8% trichloroacetic acid and 400 µl 0.6% thiobarbituric acid solutions were added to stop the reaction. A volume of 300 µl was pipetted into a 96-well micro titer plate and absorbance was taken at 532 nm using a microplate reader (BIO-RAD, model 680, Japan) after incubation in boiling water for 20 min. The percentage inhibition and IC_{50} values were calculated.

Determination of total antioxidant capacity
The applied phospho-molybdenum method (Prieto, Pineda, & Aguilar, 1999) is based on the reduction of Mo (VI) - Mo (V) by the extract and formation of a green phosphate/Mo (V) complex at acid pH. A volume of 25 µl of the extract, 250 µl of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were dispensed into a 96-well microtiter plate and incubated at 95°C for 90 min. Absorbance was measured at 695 nm using a 96-well microplate reader (BIO-RAD, model 680, Japan) against a blank (25 µl of methanol) after cooling of the content of the microtiter plate to 25°C. The percentage inhibition and IC_{50} values were calculated.

DPPH radical scavenging activity
The applied procedure is described by Braca, Tommasi, & Bari (2001). A volume of 150 µl of the plant extract was added to 150 µl of 0.004% methanolic solution of DPPH in a 96-well micro titer plate, left to stand for 30 min and absorbance determined at 517 nm. The percentage inhibition and IC_{50} values were calculated.

ABTS radical determination
The ability of the extracts to scavenge ABTS cation chromophore generated from the oxidation of ABTS solution and potassium persulphate was determined according to the procedure by Re, Pellegrini, & Proteggente (1999). A volume of 50 ml each of 7 mmol/l aq. ABTS and 2.45 mM potassium persulphate were left in the dark for 16 h and adjusted with ethanol to 0.700 at 734 nm using a WPA BIOWAVE II (Bichrom, England) spectrophotometer. A volume of 20 µl aliquot was added to 200 µl ABTS solution in a 96-well micro titer plate and absorbance was read at 734 nm using a microplate reader (BIO-RAD, model 680, Japan) after fifteen minutes of incubation. The percentage inhibition and IC_{50} values were calculated.

Reducing power
The procedure followed was according to the

method by Pulido, Bravo, & Saura-Calixto, 2001. A volume of 350 µl of the extracts was added to 350 µl phosphate buffer (0.2 mol/l, pH6.6) and potassium ferricyanide (350 µl, 1%) in 1.5 ml Eppendorf tubes. After it was incubated at 50°C for 20 min, 350 µl of 10% trichloroacetic acid was added and centrifuged at 650 g at 25°C for 10 min. The supernatant of the solution (100 µl) was then pipetted into a 96-well micro titer plate and 100 µl of distilled water and 20 µl $FeCl_3$ (0.1%) added. Absorbance was measured at 700 nm. High absorbance values indicated increased reducing power.

Calculation of IC_{50} for α-amylase and α-glucosidase

The activities of α-glucosidase and pancreatic α-amylase were determined in the presence of the bee pollen extracts at various concentrations of 0.2-1 mg/mL for α-amylase and α-glucosidase in order to calculate the IC_{50} values. IC_{50} is defined as the concentration of phenolics required to inhibit 50% of the enzyme activity.

Kinetics of enzyme inhibition

The mode of inhibition of α-amylase and α-glucosidase by water extract of bee pollen was determined using Michaelis-Menton and Lineweaver-Burk equations. The water extract was used because it had the lowest IC_{50} value for α-amylase and highest IC_{50} value for α-glucosidase respectively. A phosphate buffer was used as a substrate for α-amylase and α-glucosidase. Enzyme activities were determined in the absence or presence of different concentrations of water extract of bee pollen. The concentrations of crude bee pollen extract used for the inhibitory kinetics was 0.3-5 mg/mL for α-amylase and 0.1-2 mg/mL for α-glucosidase.

Statistical Analysis

The software used was Graph Pad Prism version 7.01 (GraphPad Software, San Diego, MA, USA). Data was subjected to one way analysis of variance (ANOVA) and means were compared using the Scheffe multiple comparison test.

RESULTS

Phenolic and flavonoid contents

The result is shown in Tab. 1. Phenolic content of the aqueous-ethanol, methanol, aqueous and ethanol extracts were 1.43, 2.04, 2.1 and 1.79 mg gallic acid g^{-1} respectively, while the flavonoid content were 0.78, 1.39, 0.86 and 0.79 mg gallic acid g^{-1} respectively. The phenol content of the extracts was higher than the flavonoids.

Metal chelating assay

Fig. 1a shows the result of the assay. The antioxidant activity of the bee pollen methanol extract was significantly ($p<0.05$) higher than that of aqueous-ethanol, water, ethanol, silymarin and gallic acid at most concentrations except at 0.8 and 1 mg/mL, where the gallic acid extract had a higher percentage inhibition. Also at 1 mg/mL, ethanol had a higher percentage inhibition than other extracts. Gallic acid had the lowest IC_{50} (Tab. 1) for metal chelating (0.86 mg/mL) activity.

Nitric oxide scavenging activity assay

In the nitric oxide scavenging activity assay (Fig. 1b), the percentage inhibition by gallic acid was significantly ($p<0.05$) higher than that of aqueous-ethanol, water, ethanol and silymarin at most concentrations but at 0.2 mg/mL methanol had higher inhibition. Gallic acid had the lowest IC_{50} (Tab. 1) for nitric oxide scavenging (4.57 mg/mL) activity.

Hydroxyl radical scavenging capacity

The percentage of inhibition by aqueous-ethanol extract of bee pollen was significantly ($p<0.05$) higher than that of methanol, water, ethanol, silymarin and gallic acid at all concentrations except for 1 mg/mL. The aqueous extract had the highest inhibition of the extracts (Fig. 1c), and aqueous-ethanol also had the lowest IC_{50} value of 0.53 mg/mL (Tab. 2). Aqueous-ethanol had the most marked capacity to scavenge hydroxyl radicals (IC_{50} 0.53 mg/mL) and total antioxidant capacity (IC_{50} 0.25 mg/mL) respectively.

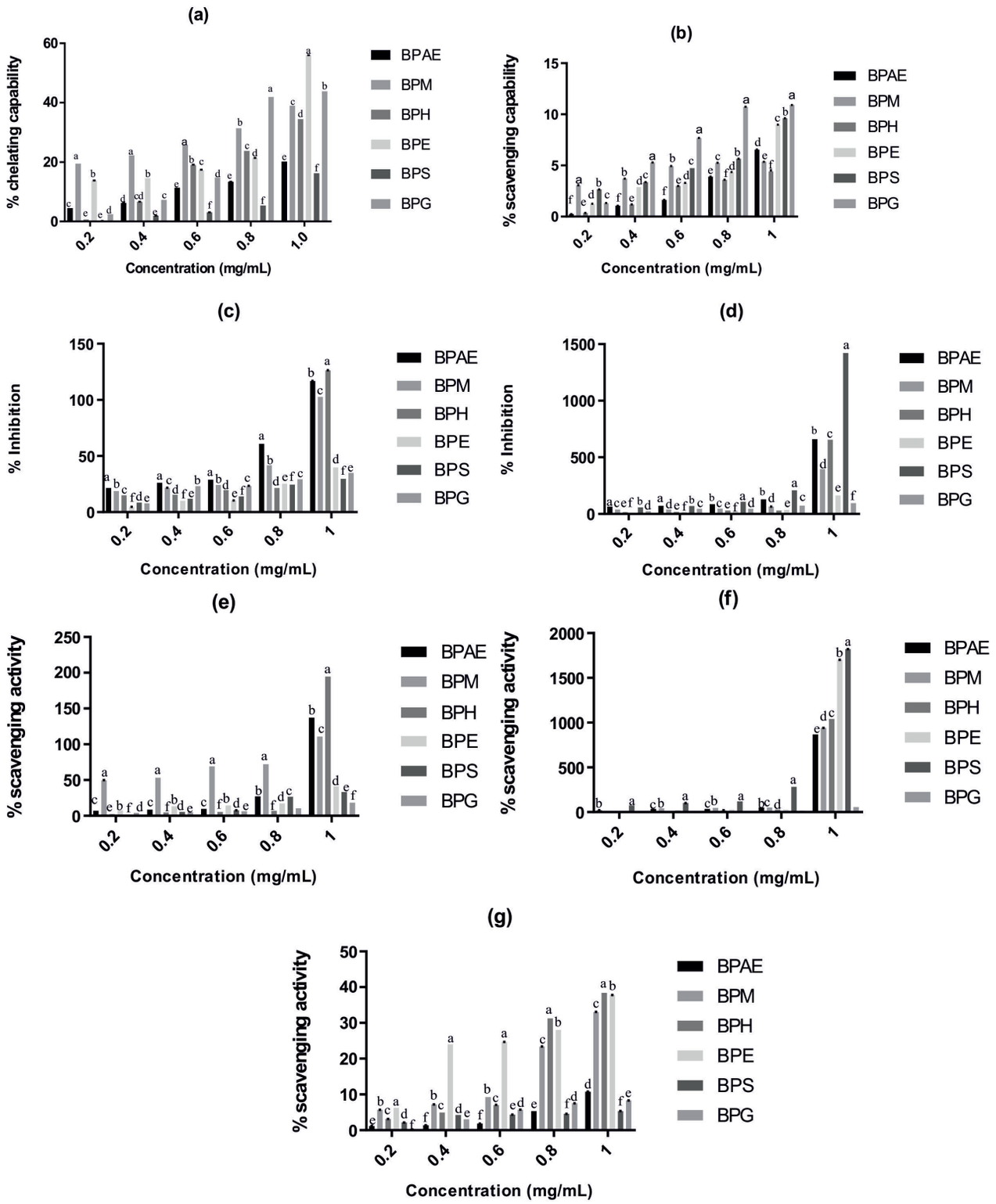

Fig. 1. Scavenging activities of bee pollen extracts on (a) metal ion chelation (b) nitric oxide (c) hydroxyl radical (d) total antioxidant capacity (e) 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (f) 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) (g), ferric reducing antioxidant capacity (FRAC).

Table 1

Total phenol and flavonoid content of bee pollen extracts

Phytochemicals	AEE	ME	AE	EE
Total phenol (mg gallic acid g^{-1})	1.43	2.04	2.10	1.79
Total flavonoid (mg gallic acid g^{-1})	0.78	1.39	0.86	0.79

AEE-aqueous-ethanol extract, ME-methanol extract, AE-aqueous extract, EE-ethanol extract

Table 2

IC_{50} values for free radical scavenging activity of bee pollen extracts

Samples	MC	NOS	HRS	TAC	DPPH	ABTS	FRAC
AE	2.78±0.01^{e}	7.12±0.01^{c}	0.53±0.01^{a}	0.25±0.01^{a}	0.61±0.01^{c}	0.07±0.01^{c}	4.85±0.01^{e}
Methanol	1.58±0.01^{d}	17.38±0.01^{e}	0.61±0.01^{b}	0.34±0.01^{c}	0.21±0.01^{a}	0.05±0.01^{bc}	1.6±0.01^{b}
Water	1.26±0.01^{c}	11.10±0.01^{d}	0.63±0.01^{b}	0.37±0.01^{d}	0.56±0.01^{b}	0.06±0.01^{bc}	1.27±0.01^{a}
Ethanol	1.14±0.01^{b}	6.65±0.01^{b}	1.35±0.09^{c}	0.51±0.01^{f}	1.51±0.01^{e}	0.04±0.01^{ab}	2.1±0.01^{c}
Silymarin	2.90±0.01^{f}	6.65±0.01^{b}	1.86±0.01^{e}	0.29±0.01^{b}	1.43±0.01^{d}	0.02±0.01^{a}	16.9±0.01^{f}
G. acid	0.86±0.01^{a}	4.57±0.01^{a}	1.54±0.01^{d}	0.44±0.01^{e}	3.18±0.01^{f}	0.98±0.01^{d}	5.54±0.01^{d}

MC-metal chelating, NOS- nitric oxide scavenging, HRS- hydroxyl radical scavenging, TAC-total antioxidant capacity, DPPH-1,1-diphenyl-2-picrylhydrazyl, ABTS-2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), FRAC- ferric reducing antioxidant capacity, AE- aqueous-ethanol, G. acid-Gallic acid.
^{abcdef} Means with different letter at the same concentration differ significantly ($p<0.05$)

Total antioxidant capacity

In Fig. 1d, the percentage inhibition through aqueous-ethanol extract of bee pollen was significantly ($p<0.05$) higher than that of methanol, water, ethanol and gallic acid at 0.2 and 0.4 mg/mL but silymarin had a greater inhibition at 0.6, 0.8 and 1 mg/mL. Aqueous-ethanol had also the lowest IC_{50} value of 0.25 mg/mL for a total antioxidant capacity which was significantly ($p<0.05$) lower than the IC_{50} for the other extracts and standards (silymarin and gallic acid) (Tab. 2).

DPPH free radical scavenging activity

The percentage inhibition through the methanol extract of bee pollen was significantly ($p<0.05$) higher than that of aqueous-ethanol, water, ethanol and silymarin at most concentrations except at 1 mg/mL where the aqueous extract had a significantly ($p<0.05$) higher inhibition than methanol (Fig. 1e). The great capacity of methanol extract to scavenge DPPH radical is shown by the IC_{50} value (0.21 µg/ml) activity which was significantly ($p<0.05$) lower than the IC_{50} for silymarin and gallic acid (1.43 and 3.18 mg/ml), respectively (Tab. 2).

ABTS assay

The percentage inhibition by silymarin was significantly ($p<0.05$) higher than that of aqueous-ethanol, methanol, water, ethanol and Gallic acid at all concentrations (Fig. 1f). Tab. 2 shows that Silymarin and ethanol had the lowest IC_{50} for ABTS (0.02 and 0.04 µg/mL respectively) but IC_{50} for ethanol, methanol and water were also similar. Gallic acid had the highest IC_{50} (0.98).

FRAC assay

The percentage inhibition by ethanol extract of bee pollen was significantly ($p<0.05$) higher than that of aqueous ethanol, methanol, water, silymarin and gallic acid were at 0.2, 0.4 and 0.6 mg/mL but water had a higher inhibition at 0.8 and 1 mg/mL (Fig. 1g). Tab. 2 shows that water had the lowest IC_{50} for FRAC (1.27 µg/mL) activity which was significantly ($p<0.05$) lower than the IC_{50} for the other extracts and standards (silymarin and gallic acid).

Table 3

IC_{50} values of bee pollen extracts on diabetes related enzymes

Samples	α Amylase	α Glucosidase
Aq Ethanol	4.51±0.01[a]	0.63±0.01[b]
Water	5.96±0.01[b]	0.60±0.01[a]
Acarbose	6.52±0.01[c]	11.30±0.01[c]

[abc] Means with different letter within rows differ significantly ($p<0.05$)

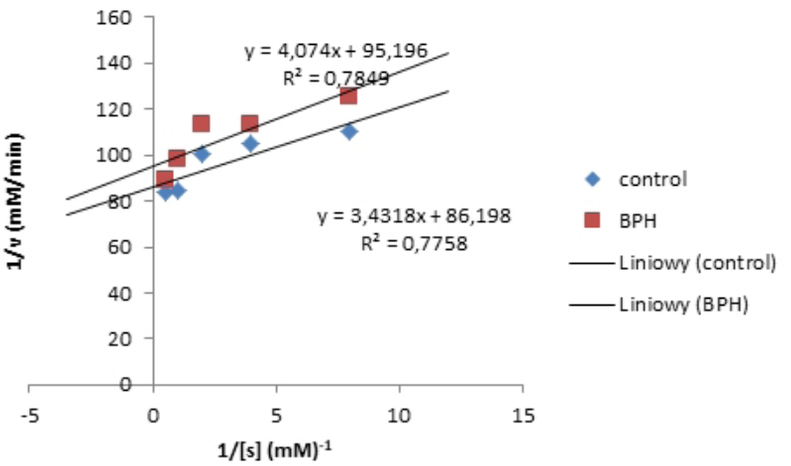

Fig. 2. Lineweaver-Burk plot of aqueous extract of bee pollen eliciting competitive inhibition on α-amylase activity

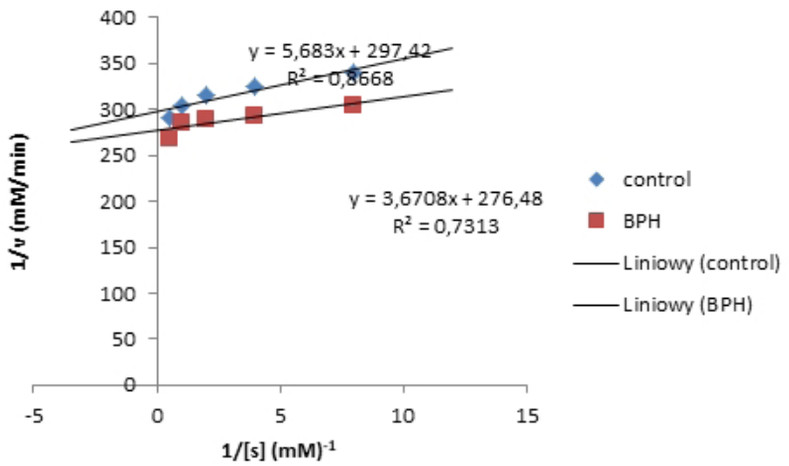

Fig. 3. Lineweaver-Burk plot of aqueous extract of bee pollen on α-glucosidase activity

Antidiabetic activity of Bee pollen

The aqueous-ethanol extract of bee pollen displayed the strongest inhibition of α-amylase (IC_{50} 4.51) and was more potent than the standard (acarbose) (IC_{50} 6.52). The water extract of bee pollen exhibited the strongest inhibition of α-glucosidase which is depicted by the lowest value of IC_{50} (0.60) which was also lower than the IC_{50} (11.30) of the standard (acarbose) (Tab. 3).

Enzyme kinetics

The Michaelis–Menton and Lineweaver–Burk equations used for the enzyme kinetic studies indicated, that in the presence of bee pollen the Michaelis–Menton constant (K_m) remained constant with a value of 0.01mg but the maximal velocity (V_{max}) increased to 0.03 and 0.04 µM/min, revealing a competitive type of inhibition for α-amylase (Fig. 2). For the α-glucosidase activity, the K_m (0.019 and 0.013 mg) and V_{max} (0.003 and 0.013 µM/min) differed, the lower the K_m value the higher the affinity of the enzyme for the inhibitor/substrate (Fig. 3).

DISCUSSION

The ability of the bee pollen extracts to scavenge free radicals and lower blood glucose could be attributed to the presence of phenols and flavonoids. Substances that hinder the activity of enzymes (α-glucosidase and α-amylase) that

break down polysaccharides obtained from plants are likely not harmful for the regulation of hyperglycaemia. It has been reported that foods with high polyphenol content have anti-diabetic potentials. Polyphenols for DM preserve Langerhans cell islets s against glucose overdose, are anti-inflammatory, scavenge free radicals, restrict α-amylases or α-glucosidase activity and reduce the formation of reactive derivatives of non-enzymatic glucose-protein condensation reactions (Xiao et al., 2015).

Hydrogen peroxide as an important reactive oxygen specie can penetrate biological membranes and is toxic if converted to a hydroxyl radical in the cell by reacting with Fe^{2+} and possibly Cu^{2+} ions (Gulcin, Elmastas, & Aboul-Enein, 2007). Hydroxyl radicals cause enormous damage in all living cells (Kazeem & Ashafa, 2015). The results obtained shows that bee pollen extracts can alleviate the destructive effect of hydroxyl radicals. Aqueous-ethanol extract of bee pollen had the most marked capacity to scavenge hydroxyl radicals (IC_{50} 0.53 mg/mL) and highest total antioxidant capacity (IC_{50} 0.25 mg/mL).

Plant antioxidants may prevent the production of free radicals, neutralize/scavenge free radicals produced in the body or reduce/chelate the transition metal composition (Amic et al., 2003). The results obtained shows that methanol had the lowest IC_{50} at 0.21 mg/mL. This implies that the methanol extract decolourized DPPH the most and had the best ability to scavenge the DPPH radicals (Wettasinghe & Shahidi, 2000).

Ethanol, methanol and water extract of bee pollen have the potential to scavenge ABTS radical cation $ABTS^{+}$ equally like silymarin, the standard. The ethanol extract scavenged ABTS cation chromophore produced from ABTS oxidation having an IC_{50} value of 0.04 mg/mL similar to the standard IC_{50} of 0.02 mg/mL. This depicts that the ethanol extract of bee pollen can protect living cells from ABTS cations.

Oxidative free radicals are involved in the pathologic process of diabetes mellitus. It is also believed that the metabolic disorders in diabetes mellitus may be due to enhanced cellular oxidative stress and reduced antioxidant potential (Modak et al., 2007). Bee pollen is recognized as a free radical scavenger and lipid peroxidation inhibitor (Almaraz-Abarca et al., 2004).

The standard curves of the % inhibition/scavenging effect and IC_{50} values of the extracts revealed a decrease in the concentration of these species which suggests that bee pollen has the ability to scavenge free radicals. This indicates that the extracts can halt the cascade of reactions involving free radicals (Sabiu, O'Neill, & Ashafa, 2016).

The aqueous extract had the most marked capacity to scavenge ferrous ions with an IC_{50} value of 1.27 mg/mL. The ability of antioxidants to chelate and deactivate transition metals such as Fe^{2+} prevents the initiation of lipid peroxidation and oxidative stress through metal-catalyzed reaction (Oboh, Puntel, & Rocha, 2006). The ability to chelate and deactivate metal ions is an important antioxidant mechanism which was displayed by the aqueous extract of bee pollen.

Diabetes mellitus can be managed through the strong hampering intestinal activity of α-glucosidases and mild obstruction of pancreatic α-amylase activity, which in turn controls postprandial plasma glucose levels and hence prevent potential chronic vascular complications (Krentz & Baile, 2005; Kwon, Apostolidis, & Shetty, 2008). Inhibitors of α-amylase and α-glucosidase derived from plants with excellent antioxidative potentials are attractive alternatives for DM patients (Kumavat, Shimpi, & Jagdale, 2012).

In this study, the aqueous extract of bee pollen demonstrated a good inhibitory activity of α-amylase and α-glucosidase which may be attributed to the high free radical scavenging activity of the aqueous extract. The inhibition of these enzymes slows down the breakdown of disaccharides to monosaccharides such as glucose, thereby reducing the amount of glucose absorbed into the blood circulation and ameliorating hyperglycemia (Kwon et al., 2007; Kazeem & Ashafa, 2015). The competitive inhibition of the enzymes by water extract of bee pollen

suggests that the inhibitory component of the extract binds reversibly to the active site of the enzyme and occupies it in a mutually exclusive manner with the substrate (Copeland, 2000).
The study indicates that bee pollen contains phenol and flavonoid compounds which are potential effective inhibitors of carbohydrate-hydrolyzing enzymes, indicating the therapeutic potential of bee pollen in the management of diabetes. Bee pollen extract may interfere with or delay the absorption of dietary carbohydrates in the small intestine, leading to suppression of postprandial blood glucose surges. Food-grade phenols and flavonoids from bee pollen extracts are potentially safer and can be used as hypoglycaemic agents.

Conflict of interest
The author has no conflict of interest.

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REFERENCES

Almaraz-Abarca, N., Campos, M.G., Avila-Reyes, A., Naranjo-Jimenez, N., Herrera-Corral, J., Gonzalez-Valdez, L.S. (2004). Variability of antioxidant activity among honey-bee collected pollen of different botanical origin. *Interciencia*, *29*(10), 574-578.

Amic, D., Davidovic-Amic, D., Bes, D. & Trinajstic, N. (2003). Structure-radical scavenging activity of cyaniding-3-galactoside and synergestic effect with acarbose. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *24*, 65-69.

Braca, A., Tommasi, N.D., & Bari, L.D. (2001). Antioxidant principles from Bauhinia terapotensis. *Journal of Natural Product*, *64*, 892–895. DOI: 10.1021/np0100845

Copeland, R.A. (2000). Enzymes: a practical introduction to structure, mechanism and data analysis. Wiley-VCH Inc, New York. Pp. 109-145.

Dinis, T.C.P., Madeira, V.M.C., & Almeida, L.M. (1994). Action of phenolic derivatives (acetaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archive of Biochemistry and Biophysics*, *315*, 161-169.

Gulcin, I., Elmastas, M., & Aboul-Enein, H.Y. (2007). Determination of antioxidant and free radical scavenging activity of basil (*Ocimum Basilicum* L. family *Lamiaceae*) assayed by different methodologies. *Phytotherapy Research*, *22*, 108-112. DOI: 10.1002/ptr.2069

Hiroyuki, F., Tomohide, Y., & Kazunori, O. (2001) Efficacy and safety of Touchi extract, an alpha-glucosidase inhibitor derived from fermented soya beans, in non-insulin dependent diabetes mellitus. *Journal of Nutritional Biochemistry*, *12*, 351-356. DOI: 10.1016/S0955-2863(01)00149-8

Kazeem, M.I., & Ashafa, A.O.T. (2015). In-vitro antioxidant and antidiabetic potentials of *Dianthus basuticus* Burtt Davy whole plant extracts. *Journal of Herbal Medicine*, *5*, 158-164. https://doi.org/10.1016/j.hermed.2015.06.003

Krentz, A.J., & Baile, C.J. (2005). Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs*, *65*, 385-411. DOI: 10.2165/00003495-200565030-00005

Kumaran, A., & Karunakaran, R.J. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT- Food science and technology*, *40*(2), 344-352. DOI: 10.1016/j.lwt.2005.09.011

Kumavat, U.C., Shimpi, S.N., & Jagdale, S.P. (2012). Hypoglycemic activity of *Cassia javanica* linn. in normal and streptozotocin-induced diabetic rats. *Journal of Advanced Pharmaceutical Technology and Re-*

search, *3*, 47-51. DOI: 10.4103/2231-4040.93562

Kwon, Y.I., Apostolidis, E., Kim, Y.C., & Shetty, K. (2007). Health Benefits of traditional corn, beans and pumpkin: invitro studies for hyperglycemia and hypertension management. *Journal of Medicinal Food*, *10*, 266-275. DOI: 10.1089/jmf.2006.234

Kwon, Y.I., Apostolidis, E., & Shetty, K. (2008). In vitro studies of eggplant (*Solanum melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. *Bioresources Technology*, *99*, 2981-2988. DOI: 10.1016/j.biortech.2007.06.035

Lebovitz, H.E. (2001). Effect of the postprandial state on non traditional risk factors. *American Journal of Cardiology*, *88*, 204-205.

Liu, H.Y., Toyn, J.H., Chiang, Y.C., Draper, M.P., Johnston, L.H., Denis, C.L. (1997). DBF2, a cell cycle regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. *EMBO Journal*, *16*(17), 5289-5298. DOI: 10.1093/emboj/16.17.5289

Mărgăoan, R., Mărghitas, L., Dezmirean, D., Mihai, C., Bobis, O. (2010). Bee Collected Pollen - General aspects and chemical composition. *Bulletin UASVM Animal Science and Biotechnologies*, *67*(1-2), 1-6.

Mărghitascedilla, L.A., Stanciu, O.G., Dezmirean, D.S., Bobişcedilla, O., Popescu, O., Bogdanov, S., Campos, M.G. (2009). In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. *Food Chemistry*, *115*(3), 878-883. DOI: 10.1016/j.foodchem.2009.01.014

Matsui, T., Ueda, T., Oki, T., Sugita, K., Terhara, N., Matsumoto, K. (2001). Alpha-glucosidase inhibitory action of natural acylated anthocyanins. I. Survey of natural pigments with potent inhibitory activity. *Journal of Agricultural Food Chemistry*, *49*, 1948-1951. https://doi.org/10.1021/jf001251u

McDougall, G.J., Shpiro, F., Dobson, P. Smith, P., Blake, A., Stewart, D. (2005). Different polyphenolic compounds of soft fruits inhibit amylase and α-glucosidase. *Journal of Agricultural Food Chemistry*, *53*, 2760–2766. DOI: 10.1021/jf0489926

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., Devasagayam, T.P. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of Clinical Chemistry and Nutrition*, *40*(3), 163-173. DOI: 10.3164/jcbn.40.163

Mohanty, P., Garg, R., Aljada, A., Ghanim, H., Dandona, P. (2011). Glucose Challenge Stimulates Reactive Oxygen Species (ROS) Generation by Leucocytes. *The Journal of Clinical Endocrinology and Metabolism*, *85*(8), 1945-7197. DOI: http://dx.doi.org/10.1210/jcem.85.8.6854

Oboh, G., Puntel, R.L., & Rocha, J.B.T. (2006). Polyphenols in red pepper [*Capsicum annum var. aviculare* (Tepin)] and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. *European Food Research and Technology*, *225*, 239-247. DOI: 10.1007/s00217-006-0410-1

Ortiz-Andrade, R.R., Garcia-Jimenez, S., Castillo-Espana, P., Ramirez-Avila, G., Villalobos Molina, R., Estrada-Soto, S. (2007). Alpha-glucosidase inhibitory activity of the methanolic extract from *Tournefotia hartwegiana*: an anti-hyperglycemic agent. *Journal of Ethnopharmacology*, *209*, 48-53. https://doi.org/10.1016/j.jep.2006.07.002

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal of Biochemistry*, *269*, 337-341. DOI: 10.1006/abio.1999.4019

Pulido, R., Bravo, L., & Saura-Calixto, F. (2001). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *48*, 3396-3402. https://doi.org/10.1021/jf9913458

Ratner, R.E. (2001). Controlling postprandial hyperglycemia. *The American Journal of Cardiology, 88*, 26H-31H. DOI: 10.1016/S0002-9149(01)01834-3

Re, R., Pellegrini, N., & Proteggente, A. (1999). Anti-

oxidant activity applying an improved ABTS radical cation decolourisation assay. *Free Radical Biological Medicine*, *26*, 1231-1237.

Sabiu, S., O'Neill, F.H. & Ashafa, A.O.T. (2016). Kinetics of α-amylase and α-glucosidase inhibitory potential of *Zea mays* Linnaeus (Poaceae), *Stigma maydis* aqueous extract: An *in vitro* assessment. *Journal of Ethnopharmacology*, *183*, 1-8. DOI: 10.1016/j.jep.2016.02.024

Saito, N., Sakai, H., Sekihara, H., & Yajima, Y. (1998). Effect of an α-glucosidase inhibitor (voglibose), in combination with sulphonyl urea, on glycemic control in type 2 diabetes patients. *Journal of International Medical Research*, *26*, 219-232. DOI: 10.1177/030006059802600501

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 Chemistry*, *115*(4), 1268-1273. DOI: 10.1016/j.foodchem.2009.01.042

Tundis, R., Loizzo, M.R., & Menichini, F. (2010). Natural Products as α-Amylase and α-Glucosidase Inhibitors and their Hypoglycaemic Potential in the Treatment of Diabetes: an Update. *Mini-Reviews in Medicinal Chemistry*, *10*(4), 315-331. DOI: http://dx.doi.org/10.2174/138955710791331007

Vaidya, H.B., & Goya, R.K. (2010). Exploring Newer Target Sodium Glucose Transporter 2 for the Treatment of Diabetes Mellitus. *Mini-Reviews in Medicinal Chemistry*, *10*(10), 905-913. DOI: 10.2174/138955710792007213

Wettasinghe, M., & Shahidi, F. (2000). Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borrage and evening primrose metals. *Food Chemistry*, *70*, 17-26. DOI: 10.1016/S0308-8146(99)00269-1

Xiao, J.B., & Hogger, P. (2015). Dietary Polyphenols and Type 2 Diabetes: Current Insights and Future Perspectives. *Current Medicinal Chemistry*, *22*(1), 23-38. DOI: 10.2174/0929867321666140706130807

Yanping, G. Z., Yanhua, L., & Dongzhi, W. (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. in vitro. *Journal of Food and Chemistry*, *16*, 5032-5039. DOI: 10.1021/jf049571r

Yu, L. Haley, S., Perret, J., Harris, M., Wilson, J., Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, *50*(6), 1619-1624. http://dx.doi.org/10.1021/jf010964p