Pertanika J. Trop. Agric. Sc. 42 (1): 139 - 153 (2019)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

α-glucosidase Inhibitory and Antioxidant Activities of *Entada spiralis* Ridl. (Sintok) Stem Bark Extracts

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ABSTRACT

Entada spiralis Ridl. (Leguminosae), locally known as Sintok or Beluru, is a tropical woody climber that grows widely in Malaysia. It is a valuable and well-known plant in herbal medicine due to its various traditional and medicinal applications. Crude extracts were obtained from the stem bark by using petroleum ether, chloroform, and methanol as extracting solvents and were then bioassayed for their biological potential. The antioxidant and α-glucosidase inhibitory activities of the extracts were assessed by using DPPH, ABTS, β-carotene, and α-glucosidase inhibitory methods. Qualitative analysis showed the presence of most of the phytochemicals in methanol extract; however, chloroform and petroleum ether extracts contained terpenoid and tannins as their major phytoconstituents, respectively. The methanol extract contained the highest amount of total phenolics (42.5 ± 15.85 µg GAE/mg) and flavonoids (28.94 ± 2.93 µg QE/mg), and showed the most potent α-glucosidase inhibitory activity with an IC_{50} value of 20.67 µg/mL. The same methanol extract exhibited the highest β-carotene bleaching inhibition (27% at 1 mg/mL), while methanol and chloroform extracts exhibited good radical scavenging activities (IC_{50} 37.29 ± 0.05 and 90.84 ± 3.12 µg/mL, respectively) against ABTS and DPPH radicals. Bioassay-guided silica gel column chromatography purification of the most active methanol extract afforded 3, 4′,5,7-tetrahydroxyflavone (6 mg). The compound displayed promising inhibitory activities against free radicals as well as α-glucosidase enzyme. These results suggest the potential use of *E. spiralis* Ridl. stem bark as a therapeutic agent against hyperglycaemia.

Keywords: Antioxidant, α-glucosidase, β-carotene, crude extracts, DPPH, *Entada spiralis* Ridl., Leguminosae, 3,4′,5,7-tetrahydroxyflavone

ARTICLE INFO

Article history:
Received: 06 October 2017
Accepted: 26 September 2018
Published: 26 February 2019

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ISSN: 1511-3701
e-ISSN: 2231-8542

INTRODUCTION

Plants continue to play a vital role in the healthcare system. More than two thirds of the world population depend on medicinal plants for their primary healthcare (World Health Organization [WHO], 2009) and this has served as a catalyst for continuous research on traditional plants which has resulted in the discovery of several plant-derived drugs and active compounds that are used directly in medicine. It is assumed that almost 75 percent of the world's existing plant species have medicinal value and nearly all of these plants possess potent antioxidant potential (Krishnaiah et al., 2011). This potential is the result of the presence of both low and high molecular weight secondary metabolites which are derived or synthesised from primary metabolites such as sugars and amino acids through glycosylation, hydroxylation, and methylation (Kasote et al., 2015).

α-glucosidase is a membrane-bound enzyme situated at the gut wall of the small intestine. It catalyses the hydrolysis of terminal α-(1-4)-linked glucose, releasing a single α-glucose molecule (Chiba, 1997). One of the most acceptable ways of treating diabetes is by reducing postprandial hyperglycaemia (Sudha et al., 2011; Xie et al., 2003). The only way to achieve this is by retarding the actions of digestive enzymes to delay the digestion and absorption of glucose through the brush border (Ahmed et al., 2017; Hilmi et al., 2014; Kazeem et al., 2013). Commercial enzyme inhibitors such as voglibose, acarbose, and miglitol, have been reported to be accompanied with serious gastrointestinal side effects like diarrhoea, flatulence and bloating (Deacon, 2011; Martin & Montgomery, 1996), and this has further increased the search for digestive enzyme inhibitors from natural sources. Several plant extracts have been reported to be powerful starch-hydrolysing enzyme inhibitors. These plant-derived inhibitors are more acceptable due to their low cost and less side effects (Benalla et al., 2010; Bhat et al., 2011).

Some chemical compounds and reactions generate free radicals or oxygen species (pro-oxidants), while some compounds and reactions, on the other hand, scavenge and oppose their toxic actions (antioxidants). In a normal cell, there is an optimal balance between pro-oxidants and antioxidants. However, when there is an increase in the generation of free radical or oxygen species against the level of antioxidant in the body, the balance shifts towards pro-oxidants and this results in oxidative stress. Free radicals have been considered to be the major causative agents of cell damage, causing diseases such as diabetes mellitus, cancer, cardiovascular and liver problems (Boligon et al., 2014; Hasan et al., 2017; Yankuzo et al., 2011). Antioxidants are therefore needed to stabilise and neutralise free radicals to prevent them from attacking cells and tissues. There has been a global interest in plant-derived antioxidants because of their high efficacy and relatively less side effects (Dehghan, et al., 2016; Sarian et al., 2017).

Entada (synonym: *Entadopsis* Britton) belongs to the pea family of Leguminosae containing approximately 28 species,

with about six species found in Asia, 21 species in Africa, and two in America. Various enzyme-inhibiting and antioxidant activities of different species of *Entada* have been reported in the literature. Several compounds isolated from seed kernel of *Entada scandens*, whole plantlets of *Entada africana* and *Entada abyssinica* contain high antioxidant activity (Guissou et al., 2010: Teke et al., 2011). Ethanol extract of *Entada rheedii* seed coat, cotyledon, and pericarp demonstrated significant α-glucosidase inhibition with IC_{50} values of 98.73 ± 0.46, 28.08 ± 11.28, and 74.01 ± 2.02 mg/ mL, respectively (Ruangrungsi et al., 2008). Acidified methanol and 70% acetone extracts of *E. scandens* seed also displayed good α-amylase inhibitory potential of 89% and 34.82%, respectively (Gautam et al., 2012).

In Malaysia, the most common species of *Entada* is *Entada spiralis* Ridl (synonym: *Entada scheffleri*), locally known as Beluru or Sintok. It is a woody climber that can grow up to a height of 25 m. Sintok possesses a wide range of ethnomedicinal uses. The root decoction is used to treat venereal diseases and haemorrhoids while the stem bark is used for hair treatment, for cleaning insect bites and also used as body wash (Harun et al., 2015). A previous study on this plant showed the potency of the methanol fraction against human dermatophyte (Harun et al., 2011). However, despite the fact that many species from these genera have been reported by previous researchers as having a positive effect on body glucose level and enhancing antioxidant capacity, so far, no research has been conducted on *E. spiralis*, particularly on its potential as a digestive enzyme inhibitor and as an antioxidant agent. Hence, this study aimed to determine the α-glucosidase and antioxidant activities of different extracts of *E. spiralis* Ridl. stem bark.

MATERIALS AND METHODS

Chemicals and Reagents

Quercetin (QC), sodium carbonate (Na_2CO_3), aluminium chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *p*-nitrophenyl glucopyranoside (*p*-NPG) and α-glucosidase enzyme from *Saccharomyces cerevisiae*, Tween 40, β-carotene, and linoleic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent (FC), gallic acid, and ascorbic acid were obtained from Merck (Darmstadt, Germany). All chemicals and solvents used were of analytical grade.

General

FTIR spectrometer (Perkin Elmer Inc., Massachusetts, USA) equipped with horizontal attenuated total reflectance device was used to detect the functional groups. Melting point was measured using Smp 10, BIBBY STERILIN, Ltd, Stone, ST50SA, United Kingdom. UV-VIS spectrophotometer 1800 series, Shimadzu, Japan was used to detect the presence or

absence of chromophores. ^{1}H-, ^{13}C- NMR spectra were measured using FT-NMR cryoprobe Bruker advance 111 spectrometer (500 and 150 MHz, respectively), Bruker Scientific Technology Co., Ltd. Yokohama, Japan. Absorbance was measured using microplate Reader TECAN PRO 200, Tecan Trading AG, Switzerland. Chromatotron model 7924T (T-squared Technologhy, Inc), USA was used to purify the compound.

Plant Preparation and Extraction

Entada spiralis Ridl. stem barks were obtained from Tasik Chini Forest, Pekan District, Pahang, Malaysia (voucher specimen KMS-5228) were cut into smaller pieces, air-dried at room temperature, and pulverized into powdered form to give a final mass of 4.5 kg. The powder was macerated successively using petroleum ether, chloroform, and methanol to get petroleum ether extract (Ep), chloroform extract (Ec), and methanol extract (Em), respectively. Maceration with each solvent was repeated until exhaustion before proceeding to the next solvent and the resultant filtrates from each solvent were concentrated *in vacuo* using a rotary evaporator (IKA RV 10B S99, 40°C, 115 rpm) (Ahmed et al., 2012). The crude extracts were packed in a glass bottle and kept in the fridge until further analysis.

Fractionation and Purification of Methanol Extract: Isolation of Active Principle

Methanol extract (most active, 10 g) was subjected to silica gel (70–230 mesh) column chromatography (30 × 80 cm) and eluted with gradient solvent system of $CHCl_3$:MeOH 90%:10%–10%:90% to generate pooled fractions F1–F4. Dried F1 (1.7 g) was dissolved in ethanol to remove ethanol-insoluble portion. The ethanol-soluble portion (900 mg) was subjected to repeated centrifugal chromatography using silica gel PF254 with gypsum with the solvent system 90% DCM:10% $CHCl_3$ to afford C1 (6 mg) as the active principle.

Estimation of Phytoconstituents

Determination of Total Phenolic Content (TPC). The TPC in different *E. spiralis* extracts was determined using Folin–Ciocalteu method adapted from Ahmed et al. (2015) and Umar et al. (2010) with some modifications. Gallic acid was used as standard. 50 µL of 10% FC w/v (FC: H_2O) was introduced into a 96-well microplate followed by 10 µL of standard or sample (7.18–1000 µg/mL). Blanks contained only the solvent. 50 µL of 40% Na_2CO_3 was then added to each well and the plate was incubated for 2 h at room temperature. Absorbance was measured at 725 nm using a microplate reader. The assay was conducted in triplicate and total phenolic content was determined from the linear regression curve of absorbance against concentration. Results obtained were expressed as microgram of gallic acid equivalence per milligram dry weight of the extract (µg GAE/mg dw of extract).

Determination of Total Flavonoid Content (TFC). The methods reported by Abdel-Hameed (2009) and Ahmed et al. (2015)

were followed with slight modifications. Quercetin (QC) was used as standard. 100 µL of 2% $AlCl_3$ in methanol was added to 100 µL of extract (1 mg/mL) or standard (7.81–250 µg/mL). Blanks contained extracts with solvent without $AlCl_3$. Absorbance was measured at 415 nm after 15 min. The test was conducted in triplicate and a quercetin calibration curve was used to determine the concentration of each extract using the equation Y = mx + c. Results were expressed as microgram of QU equivalence per milligram dry weight of the sample.

Antioxidant Activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. This assay was conducted using DPPH by modifying methods from Ahmed et al. (2015) and Sulaimon et al. (2011). 150 µL of freshly prepared 0.4 M DPPH solution was carefully pipetted into a round-bottomed 96-well microplate. 100 µL of sample or standard with varying concentrations (7.81–500 µg/mL) were added. Blanks contained only the extraction solvent and DPPH. Ascorbic acid dissolved in distilled water and quercetin dissolved in MeOH were used as standards. The plate was left in the dark for 25 min to activate, after which it was placed in a microplate reader. Absorbance was read at 517 nm. The test was conducted in triplicate and the percentage inhibition of each sample/ standard was calculated using the following equation:

$$(\%\ DPPH\ inhibition) = \left[\left[\frac{Ac - As}{Ac}\right]\right] \times 100$$

where *Ac* represents the absorbance of DPPH radical in MeOH and *As* represents the absorbance of DPPH radical in the sample or standard. IC_{50} obtained from graphical plot from percentage inhibition against concentration was used to define the radial scavenging activity of each extract.

2, 2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), ABTS Assay. For ABTS assay, the Zheleva-Dimitrova et al. (2010) method was adopted with some modifications. Stock solutions of ABTS (7 mM) and potassium persulfate (2.45 mM) were prepared using distilled water. The working solution was prepared by adding 1 mL of ABTS solution to an equal volume of potassium persulfate solution. The reaction mixture was left overnight for 16 h to generate the intense blue-coloured ABTS radical $ABTS^{\bullet+}$. $ABTS^{\bullet+}$ (1 mL) was added to 50 mL of MeOH and distributed (100 µL each) into a 96-well microplate containing 100 µL of serially diluted sample or standard (7.81-125 µg/mL). Absorbance was measured at 734 nm against blank (containing sample and MeOH only). The test was conducted in triplicate. The extracts were compared with Trolox and ascorbic acid as standards and percentage inhibition was calculated as follows:

$$(\%\ ABTS\ inhibition) = \left[\left[\frac{Ac - As}{Ac}\right]\right] \times 100$$

where *Ac* is the absorbance of ABTS in MeOH and *As* is the absorbance of ABTS in the sample or standard. The radical scavenging activity was determined from the

IC_{50} obtained from the percentage inhibition curve against different concentrations of the sample or standard.

β -Carotene Bleaching Assay. This assay was conducted according to Duan et al. (2006) and Yim et al. (2010) with slight modifications. Briefly, emulsion of β-carotene (BC) and linoleic acid (LA) was obtained by dissolving 200 µg BC in 1 mL chloroform. This was rapidly transferred into a 250 mL round bottomed flask containing 20 µL of LA and 200 µL of Tween 40. Chloroform was removed at 45°C using a rotary evaporator, after which 50 mL of hot distilled deionized water (50°C) mixed with 5 mL of phosphate buffer (pH 6.8) was added. The mixture was stirred vigorously to form BC–LA emulsion. A control emulsion was prepared without BC. Next, 200 µL of the emulsion was added into a round-bottomed 96-well microplate containing 50 µL of extract or standard (1 mg/mL). Initial absorbance at time 0 was measured immediately at 450 nm. Subsequent absorbance at time t was recorded at every 10 min for 1 h. Control was measured using solvent and control emulsion. Quercetin was used as standard. The percentage of BC bleaching inhibition was calculated using the following equation:

$$(\%\ bleaching\ inhibition) = \left[\left[\frac{Rc - Rs}{Rc}\right]\right] \times 100$$

where *Rc* and *Rs* are the bleaching rates for the control and sample, respectively.

α-Glucosidase Inhibitory Assay. Effect of extracts or compounds on the inhibition of α-glucosidase was determined according to the method described by Jeong et al. (2013) with minor modifications. Briefly, α-glucosidase enzyme obtained from *Saccharomyces cerevisiae* (1 U/mL) was dissolved in freshly prepared 50 mM K_3PO_4 buffer (pH 6.9) as a stock solution. The substrate, 5 mM *p*-nitrophenyl glucopyranoside (*p*-NPG), was prepared in 0.1 M phosphate buffer (pH 6). 100 µL of the enzyme was then transferred into a 96-well microplate containing 50 µL of sample or standard and incubated for 10 min at room temperature. Next, 50 µL of *p*-NPG was added and the plate was incubated for another 5 min. Absorbance was immediately measured at 405 nm using a microplate reader. Quercetin was used as standard. The percentage inhibition of α-glucosidase was calculated using the following equation:

$$(\%) = \left[\left[1 - \frac{S - b}{C}\right]\right] \times 100$$

where *S* is the absorbance of the sample or standard, *b* is the absorbance of the blank containing 100 µL of MeOH + 50 µL of sample + 50 µL of substrate, and *C* is the control containing 100 µL of buffer and 100 µL of enzyme. The percentage of the extract required to inhibit 50% of the α-glucosidase activity (IC_{50}) was determined from the regression curve. The experiment was conducted in triplicate.

Statistical Analysis

Data were analysed using one-way analysis of variance (ANOVA). Results were expressed as mean ± standard error of mean (SEM) of triplicate measurements. Significant differences between parameters were determined using Tukey's HSD post hoc tests (significant at $p<0.05$).

RESULTS AND DISCUSSION

Structural Characterization of Active Principle

Active principle (C1) was obtained as a yellow amorphous powder (m.p.: 275–277°C). It displayed UV absorption (λ_{max}) at 225 nm, indicating the presence of aromatic rings. IR spectrum showed absorptions at 3291 cm^{-1} for O–H stretch, 2924 cm^{-1} for C–H stretch, 1257 cm^{-1} for C–O stretching vibration. The vibration at 1613 cm^{-1} indicated olefinic C=C stretch. ^{13}C-NMR spectrum showed a total of thirteen carbon signals, suggesting a flavonoid skeleton comprising six aromatic CH and nine quaternary carbons with C=O appearing at δ_C 176.64 ppm. Complete ^{13}C-NMR and ^{1}H-NMR (500 MHz, MD_3OD) spectra data are given in Table 1. ^{1}H-NMR spectrum of C1 displayed signals comprising of two meta-coupled doublets at 6.21 and 6.42 ppm each with J = 2.1 and 2.2 Hz assignable to H-6 and H-8 of ring A of flavone skeleton, respectively. Signals for the B-ring protons appeared at 6.94 (dd, J= 2.0, 7.0, 2H, H-3′& H-5′) and 8.12 (dd, J= 2.0, 7.0, 2H, H-2′ & H-6′). ^{13}C-NMR and ^{1}H-NMR of C1 are shown in Figure 1 Spectra data obtained were compared with the literature (Ahmed et al., 2014) and consequently the isolated flavonoid (C1) was identified as 3,4′,5,7-tetrahydroxyflavone (Figure 1), a known flavonol (kaempferol), which has already been reported for its antioxidant and antidiabetic activities (Sarian et al., 2017).

Table 1
Spectra data of C1 compared with literature

Position	δ_H (ppm), m J (Hz)	δ_C (ppm)	δ_H (ppm), m J (Hz)	Kaempferol δ_C (ppm) (Ahmed et al., 2014)
2	-	146.70	-	147.12
3	-	135.72	-	136.75
4	-	176.64	-	176.71
5	-	161.09	-	162.44
6	6.21 (1H, d), J=2.1	97.94	6.27 (d), J = 1.8	99.26
7	-	164.31	-	165.05
8	6.42 (1H, d), J= 2.2	93.12	6.54 (d), J = 1.8	94.61
9	-	156.88	-	157.89
10	-	103.13	-	104.27
1'	-	122.35	-	123.44
2' & 6'	8.12 (2H, dd) J = 2.0, 7.0	129.29	8.16 (dd) J = 1.8, 8.7	130.58
3' & 5'	6.94 (2H, dd) J = 2.0, 7.0	114.93	6.54 (dd) J = 2.4, 9.0	116.26
4'	-	159.15	-	160.26

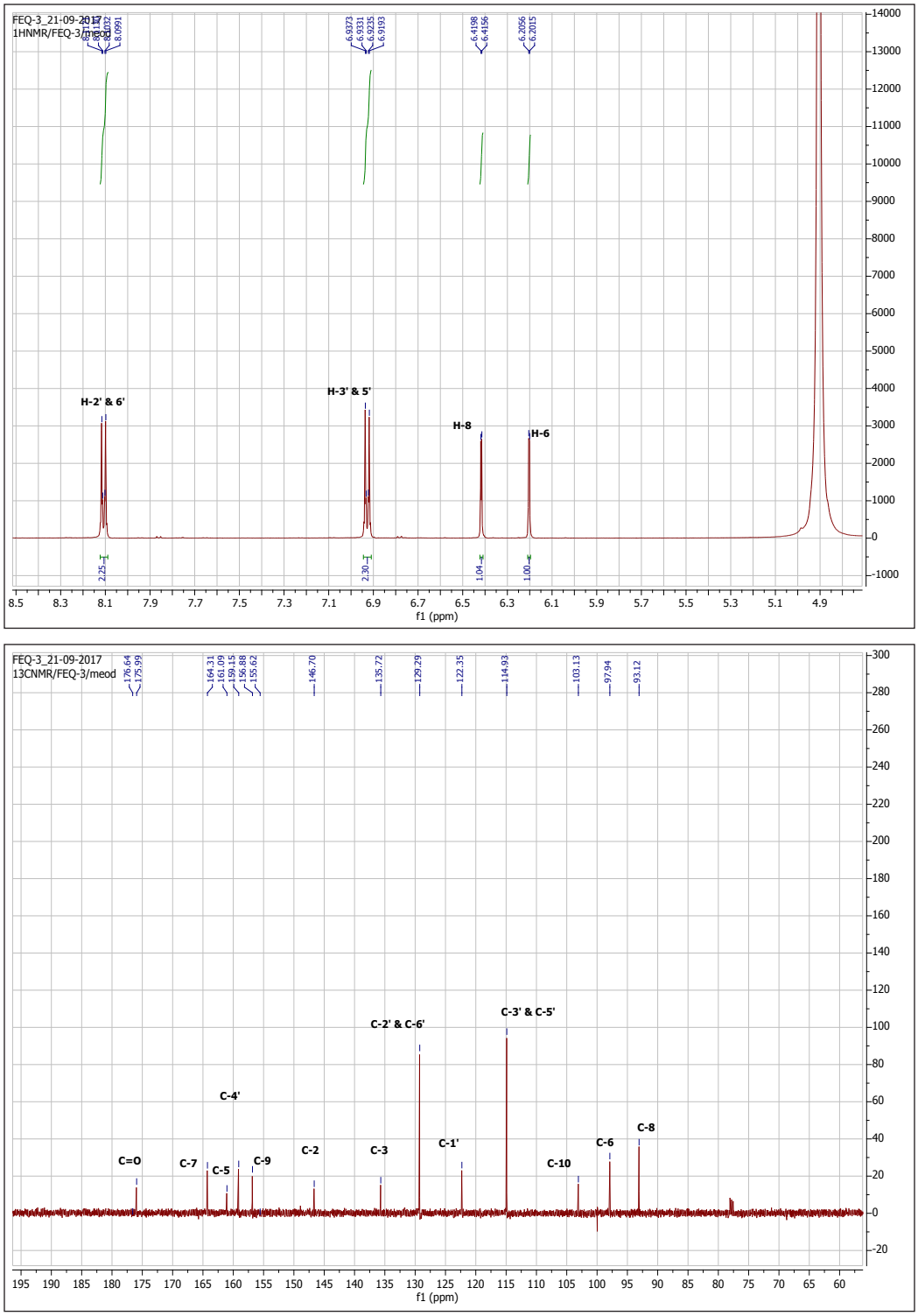

Figure 1. [1]H-NMR and [13]C-NMR spectra of C1

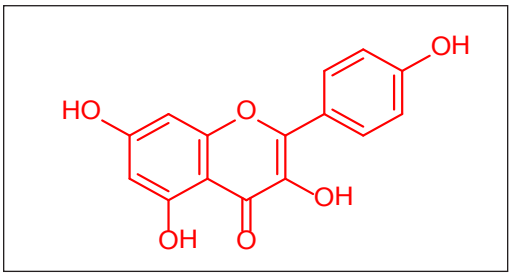

Figure 2. Structure of C1 isolated from active methanol extract of *E. spiralis*

Estimation of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Flavonoids are major phenolic compounds and are naturally occurring antioxidants. The biological activities, especially the free radical scavenging property of phenols, can be attributed to their reducing properties as a hydrogen or electron donor (Rice-Evans et al., 1997). Results obtained from TPC and TFC of different samples are shown in Table 2. TPC values were observed to vary significantly among the samples ($p < 0.05$). Methanol extract (Em) showed the highest value (42.5 ± 8.59 µg GAE/mg dw) as obtained from the calibration curve of gallic acid (R^2= 0.9941). Similar methanol extract from *E. pursaetha* seeds have been reported to possess 5.5 mg per catechol equivalent /g of the sample (Pakutharivu & Suriyavadhana, 2010). TPC of all *E. spiralis* extracts were observed in decreasing order from Em > Es > Ep with the values of 42.5 ± 8.58, 28.3 ± 1.38 and 2.6 ± 0.95 µg GAE/mg dw, respectively. Similarly, TFC followed the same trend. Overall, TFC values were observed to be lower than the TPC values. This is expected because flavonoids are a part of phenolic compounds.

Table 2
Total phenolic and flavonoid contents of various extracts of E. spiralis

Extract	TPC (µg GAE/mg dw)	TFC (µg QE/mg dw)
Em	42.56 ± 8.59	28.94 ± 2.93
Ec	28.30 ± 1.38	12.73 ± 1.93
Ep	2.62 ± 0.95	0.84 ± 0.24
R^2	0.9941	0.9747

Values are expressed as mean ± SEM (n=3) of triplicate measurements. Results were analysed using one-way ANOVA. Significant difference was determined using Tukey's HSD (at $p < 0.05$). TPC (total phenolic content), TFC (total flavonoid content), GAE (gallic acid equivalence), QE (quercetin equivalence), Em (*E. spiralis* methanol extract), Ec (*E. spiralis* chloroform extract), Ep (*E. spiralis* petroleum ether extract), dw (dry weight), R^2 (R-squared value obtained in each regression line with different concentrations of standards)

Antioxidant Activity

Radical Scavenging Activities of DPPH and ABTS. The maximum absorption of DPPH radical is 517 nm. This absorption diminishes when free radical is reduced to hydrazine derivatives by the action of antioxidants through the electron transfer or H-atom transfer process (Kosar et al., 2011). Also, the extent of decolourisation of $ABTS^{\bullet+}$ at 734 nm is used in measuring the antioxidant potential of extracts or individual compounds under investigation. Analysis of antioxidant activities of various extracts at different concentrations (7.81–125 µg/mL) on DPPH and ABTS radicals with

their corresponding IC_{50} values are shown in Table 3 with ascorbic acid (AC), Trolox (Tx) (ABTS only), and quercetin (QC) as reference standards. All the tested samples and standards showed radical scavenging activity in a concentration-dependent manner. For the DPPH method, inhibitory activities were observed in decreasing order from AC > QC > C1> Em > Ec > Ep.

Table 3
Radical scavenging activities of different extracts and compounds from E. spiralis *stem bark*

Sample	DPPH radical-scavenging IC_{50} (µg/mL)	ABTS radical-scavenging IC_{50} (µg/mL)
Em	42.67 ± 4.10^{C}	37.29 ± 0.05^{C}
Ec	472.83 ± 11.20^{D}	90.84 ± 3.12^{D}
Ep	1050.57 ± 23.21^{E}	232.08 ± 26.53^{E}
C1	31.69 ± 1.57^{A}	22.56 ± 3.15^{B}
QC	29.82 ± 3.73^{A}	-
Tx	-	15.23 ± 2.15^{A}
AC	24.67 ± 0.45^{B}	16.74 ± 1.76^{A}

Values are expressed as mean ± SEM of triplicate measurement. Results were analysed using one-way ANOVA. Significant difference was determined using Tukey's HSD (at $p < 0.05$). Em (*E. spiralis* methanol extract), Ec (*E. spiralis* chloroform extract), Ep (*E. spiralis* petroleum ether extract), Tx (Trolox), QC (quercetin), C1 (3,4′,5,7-tetrahydroxyflavone), AC (ascorbic acid), IC_{50} (concentration of a sample required to scavenge 50% of the free radicals). Values with the same letters are not significantly different.

The radical scavenging activity of C1 was statistically similar with QC (significance at $< p < 0.05$). All the extracts and the C1 showed better radical scavenging activity towards ABTS radicals compared to that of DPPH. The scavenging activity of Em for all concentrations (except at the lowest concentration) was significantly higher than Trolox ($p < 0.05$). There was no significant difference in the scavenging activity of Tx and AC (at $p < 0.05$). AC showed the highest and lowest radical scavenging activity against ABTS radical and hence, displayed the lowest IC_{50} (16.74 ± 1.76 µg/mL) while Ep showed the lowest activity and therefore, showed the highest IC_{50} value (232.08 ± 26.53 µg/mL). The lower activity exerted by Ec compared to Em could be attributed to the lesser amount of TPC and TFC in the extract since these contents are the major constituents responsible for antioxidant activity (Rice-Evans et al*.,* 1997). Similar lower activity of chloroform extract was reported from the root, leaves and stem bark of *E. africana*. All the chloroform extracts displayed higher EC_{50} values against DPPH radicals than that of methanol extracts (Tibiri et al*.,* 2010). The relatively low activities observed in scavenging potentials of Ep may be attributed to low content of phenolics in the extract (Oyedemi et al., 2013).

β-Carotene Bleaching Assay. This assay is based on the discolouration of β-carotene (BC) by a lipid or peroxy radical as a result of breakage of the π-conjugation by linoleic acid (LA) to C=C of BC, forming hydroperoxide free radical by auto-oxidation during incubation (at 50°C). However, the presence of an antioxidant retards this

reaction Duan et al., (2006). The extent of BC bleaching inhibitory activity of different extracts of *E. spiralis* and standard is shown in Figure 3. There was no significant difference in the inhibitory activity of Em and Ec, while Ep was observed to have the lowest activity. Meanwhile BC bleaching inhibition of *Polysiphonia urceolata* was reported to show inhibitory activity stronger than that of gallic acid used as positive control after 420 min (Duan et al*.,* 2006). Using the same reaction time, similar strong activity was observed in different edible mushrooms species (Barros et al*.,* 2007). This means that the relatively low activity displayed by all the extracts and control could be due to shortage in reaction time, suggesting that the time needs to be extended further to enhance maximum activity.

Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SEM of three determinations.

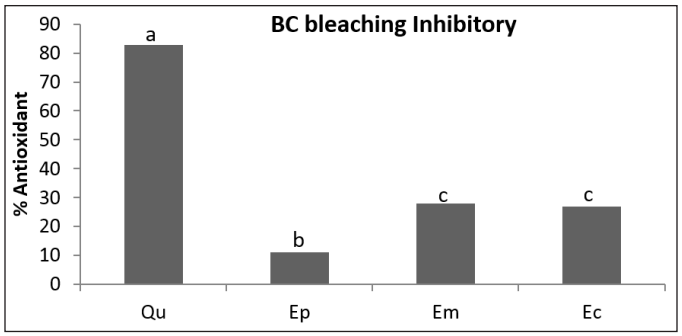

Figure 3. β-Carotene bleaching inhibitory activity of quercetin (Qu) and *E. spiralis* extracts

α-Glucosidase Inhibitory Assay. α-Glucosidase is a carbohydrate-hydrolysing enzyme responsible for postprandial hyperglycaemia (Kwon et al*.*, 2007). Its main function is to catalyse the hydrolysis of disaccharides to monosaccharides, which leads to a drastic increase in the level of glucose in the body (Matsui et al*.*, 2007).

Table 4
α-Glucosidase inhibitory activity of different extracts and compounds from E. spiralis

Conc (µg/mL)	% inhibition				
	QC	Em	Ec	Ep	C1
500	99.12 ± 4.12	90.10 ± 3.41	80.26 ± 7..23	75.46 ± 8.14	91.32 ± 4.56
250	90.74 ± 3.98	83.61 ± 2.89	65.86 ± 3.09	60.24 ± 5.36	87.59 ± 4.23
125	79.95 ± 4.19	73.58 ± 5.02	58.42 ± 4.71	52.29 ± 3.76	80.18 ± 5.95
62.5	70.68 ± 0.75	65.12 ± 6.21	50.89 ±5.11	50.02 ± 4.98	74.24 ± 3.51
31.25	60.91 ± 3.67	48.37 ± 4.09	48.81 ± 3.84	47.30 ± 4.23	58.46 ± 2.34
15.625	55.23 ± 2.61	40.88 ± 3.38	45.11 ± 9.08	44.56 ± 6.33	55.32 ± 3.98
7.8125	40.76 ± 0.21	35.34 ± 2.19	38.82 ± 6.75	28.64 ± 6.32	40.39 ± 4.10
IC_{50} (µg/mL)	18.15 ± 0.15	20.63 ± 0.44	74.96 ± 24.77	172.93 ± 1.77	19.98 ± 1.23

Values are expressed as mean ± SEM of triplicate measurement. Results were analysed using one-way ANOVA. Significant difference was determined using Tukey's HSD (at $p < 0.05$). QC (quercetin), C1 (3,4′,5,7-tetrahydroxyflavone), Em (*E. spiralis* methanol extract), Ec (*E. spiralis* chloroform extract), Ep (*E. spiralis* petroleum ether extract), IC_{50} (concentration of a sample required to inhibit 50% of the enzyme)

Inhibition of this enzyme is known to be one of the effective strategies in reducing and preventing postprandial hyperglycaemia in diabetes patients. α-Glucosidase inhibitory activities of the extracts as well as the isolated constituent from the active fraction are shown in Table 4. Compound 1 exhibited a remarkable inhibitory activity with low IC_{50} value of 19.98 ± 1.23 µg/mL. The inhibitory activity of QC was significantly higher ($p < 0.05$) in all concentrations, although there was no significant difference between its IC_{50} and those of C1 and Em. Ep showed the lowest inhibition value while Em and Ec were considered active, having their IC_{50} less than 100 µg/mL. Meanwhile, Em, which had the least IC_{50} (20.63 ± 0.44 µg/mL) among these extracts, was considered as the most potent α-glucosidase inhibitor. Strong inhibitory activities of methanol extracts from various plants have previously been reported (Gholamhosenian & Fallah, 2009; Ortiz et al., 2007). The low digestive enzyme-inhibitory activity of Ep may be due to the presence of biologically active phytochemicals in an insignificant amount.

CONCLUSION

This present study has showed that *E. spiralis* stem bark possesses a strong α-glucosidase inhibitory and antioxidant activities. It acts by scavenging free radicals and inhibiting α-glucosidase enzyme in a dose-dependent manner. Moreover, methanol extract had the highest phenolic and flavonoids contents among all the extracts and subsequently exerted the highest antioxidant and α-glucosidase inhibitory activities. Fractionation and purification of this extract led to isolation of a bioactive compound, which was characterized as 3,4′,5,7-tetrahydroxyflavone and also known as kaempferol. The presence of 3,4′,5,7-tetrahydroxyflavone in the stem bark of *E. spiralis* as an antioxidant and α-glucosidase inhibitor is being reported for the first time. This plant may therefore have potential therapeutic applications for diabetes mellitus type 2 management.

ACKNOWLEDGMENT

The corresponding author would like to acknowledge the support provided by the Ministry of Science, Innovation and Technology, Malaysia (E-science SF13-005-0055), the Ministry of Higher Education through Fundamental Research Grant Scheme (FRGS 13-089-0330), and also the technical support provided by the Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, IIUM, 25200 Kuantan, Pahang, Malaysia.

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