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ORIGINAL ARTICLE

ANTIOXIDANT AND ANTIDIABETIC POTENTIALS OF A DIMETHOXYLATED FLAVONE OBTAINED FROM *HELIANTHUS ANNUUS* L. LEAF EXTRACT

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ABSTRACT

Pectolinarigenin (5,7-dihydroxy-6,4'-dimethoxyflavone) is a methoxylated flavone found in most medicinal plants. This study interrogated the antioxidant and antidiabetic potentials of a pectolinarigenin obtained from *Helianthus annuus* L. leaves. The pectolinarigenin was obtained via a combination of solvent-solvent partitioning, column chromatography (CC), thin layer chromatography and high-performance liquid chromatography (HPLC) techniques. The antioxidant potential of the pectolinarigenin was investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide scavenging tests while the antidiabetic effect was interrogated in alloxan-induced diabetic rats. The pectolinarigenin significantly inhibited DPPH (54.9 %) and nitric oxide (46.1 %) compared with 96.9 and 53.2 % reductions respectively by vitamin C. Pectolinarigenin also reduced fasting blood sugar levels in a time-dependent manner and elicited optimum activity (66.3 %) within 6 h post-treatment at 10 mg/ kg compared with 68.9 % of glibenclamide (2 mg/kg). The pectolinarigenin elicited significant ($p < 0.05$) antioxidant and antidiabetic properties and might be useful in the development of novel antidiabetic, antioxidant and anti-inflammatory compounds.

Keywords: Antidiabetic, antioxidant**,** *Helianthus annuus,* pectolinarigenin, fractionation.

INTRODUCTION

Diabetes mellitus (DM) is a non-communicable disease with high prevalence in both developed and developing countries (Bhutkar and Bhise, 2013). The prevalence ranges from 6-11.4% depending on the region (International Diabetes Federation, 2019). DM is characterized by hyperglycemia emanating from reduced insulin secretion and/or sensitivity (American Diabetes Association, 2009). Hyperglycemia predisposes to conditions like oxidative stress, retinopathy, neuropathy, nephropathy and cardiovascular diseases. The pathogenesis of these diabetic complications is attributed to oxidative stress and antioxidants have been shown to ameliorate diabetic complications.

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The World Health Organization reported that about 80 % of world population depends on herbal products for their primary healthcare needs (Ekor, 2014). There is increasing patronage of herbal preparation in the management of DM due to problems associated with orthodox antihyperglycemic medicines. Herbal medicines are being used because they are perceived to have less side effects, cheaper, readily available and more efficacious (Ekor, 2014). Several bioactive principles with proven antidiabetic properties have been isolated from medicinal plants (Aba and Asuzu, 2018). Flavones are among the most frequently encountered subclass of flavonoids in medicinal plants (Berim and Gang, 2016). Some examples of flavones are apigenin, luteolin, tangeritin, chrysin, 6-hydroxyflavone, baicalein, scutellarein, wogonin, diosmin and flavoxate (Unnikrishnan *et al*., 2014). Many flavones have been isolated from plants (Miean and Mohamed, 2001; Zhao *et al*., 2010). Antioxidant, antihyperglycemic, antitumor, anti-inflammatory, hepatoprotective and antibacterial activities of some flavones have been documented (Chen *et al*., 2012; Farhadi *et al*., 2019).

Helianthus annuus L (sunflower) is grown mainly for its nutritional and medicinal values (Guo *et al*., 2017). It is also used in folklore medicine in the treatment of heart disease, respiratory tract infections, wound and diabetes mellitus (Guo *et al*., 2017). Some bioactive flavonoids, terpenes, caffeic acid and several essential oils have been isolated from *H. annuus* L. (Ceccarini *et al*., 2004; Amakura *et al*., 2013; Bashir *et al*., 2015). The antimicrobial, anti-inflammatory, anti-hypertensive, wound-healing, and antidiabetic properties as well as the antioxidant potential of heliangolide derivative isolated from *H. annuus* have been reported (Onoja and Anaga, 2014; Onoja *et al*., 2020). Despite its rich source of flavonoids, the antidiabetic and/or antioxidant properties of flavonoids from *H. annuus* have not been reported. In this paper, therefore, we report the isolation, characterization and antidiabetic/antioxidant potential of a flavone of *H. annuus* leaf.

MATERIALS AND METHODS

Plant collection and extract preparation

Helianthus annuus leaves were harvested in the wild within the environment of University of Nigeria, Nsukka, in June 2016 and identified by Mr A. O. Ozioko. A voucher sample (UNN/VPP/2012/2) was kept in the departmental herbarium for reference. The plant samples were air dried on laboratory bench. Cold maceration in 80 % methanol (Fischer Chemicals, UK) for 48 hours was adopted in preparation of hydromethanol extract of *H. annuus* leaves (HEHA) as reported by Onoja and Anaga (2017).

Solvent-solvent partitioning of HEHA

The HEHA was partitioned as described by Bibi *et al*. (2011). Briefly, the extract (50 g) was re-dissolved in 10 % methanol (MeOH) and successively partitioned in equal volumes each of n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and water using separating funnel. The crude extract yielded *n*-hexane (3.11 g), DCM (6.80 g) ethyl acetate (11.14 g), and water (17.23 g) after evaporation to dryness.

Thin layer chromatographic (TLC) analysis

Pre-coated silica gel 60 F_{254} plates, 20 × 10 cm (Merck KGaA GmbH, Germany) and solvent system composed of $CHCl_3$, EtOAc and MeOH (ideal ratio was determined for each experimental) at room temperature was used for TLC analysis. Appropriately developed plates were viewed under UV light at 254/365 nm or with anisaldehyde-sulphuric acid (Merck KGaA, Germany) detecting reagent.

Column chromatographic fractionation of EtOAc fraction

The column chromatographic fractionation of the EtOAc fraction was as previously described by Onoja *et al*. (2014). Briefly, 10.0 g of EtOAc fraction was dissolved in the first mobile phase (n-

hexane/EtOAc/MeOH, 7:2.8:0.2) and loaded on top of the silica gel 60 (0.06–0.20 mm particles) slurry packed column, 90 × 8 cm (GE Healthcare Europe GmbH, Germany), fraction/silica gel ratio, 1:70, at ambient temperature. The fraction was eluted with a mobile phase composed of n-hexane, EtOAc and MeOH starting with 70 % n-hexane, 28 % EtOAc and 2 % MeOH and gradually increasing the proportion of EtOAc up to 80 % keeping MeOH at 2 % at a flow rate of 1 ml/min. The collected eluates (10–15 mL) from the column chromatography (CC) fractionation of EtOAc fraction were analysed by TLC and subsequently pooled into five CC fractions (tubes 17–239) based on their TLC profiles as follows: M.1, 1220 mg (tubes 17–76), M.2, 1400 mg (tubes 79–128), M.3, 560 mg (tubes 130–148), M.4, 3050 mg (tubes 151–197) and M.5, 631 mg (tubes 200–239).

Following the results of the biological assays, M.4 (most active fraction) was further subject to CC separation on a 50 x 5 silica gel column, fraction/silica gel ratio, 1:50, at ambient temperature using isocratic mobile solvent EtOAc, MeOH, HAc (8.9:1.0:0.1) 1500 ml at flow rate of 1 ml/min. The eluates were analysed and pooled into five sub-fractions (M.4.1, M.4.2, M.4.3, M.4.4 and M.4.5) based on the TLC profile.

Semi-preparative HPLC isolation of compound

Semi-preparative HPLC system (Jasco, Germany) was used to purify sub-fraction M.4.4 (650 mg), obtained from the CC as described by Onoja *et al*. (2020). Reverse phase column Reprosil 100 C-18 (250 × 20 mm, 5 µm) with binary gradient of the mobile phase (water and methanol) at a flow rate of 10 mL min^{-1}, column temperature of 40 ˚C and sample injection loop of 1000 µL was for the separation. The mobile phase (water and methanol) gradient was optimized as follows: 60–30 % of water (10 min), 30–20 % water (5 min), 20–10 % water (5 min), 10–0 % water (5 min) and 0 % water (10 min) and additional 5 minutes to return the system to the initial mobile phase.

Structural characterization of the isolated compound

Structural characterization of the isolated compound was conducted as reported by Onoja *et al.* (2020). 1D- and 2D-NMR spectra were recorded on the Agilent DD_2 600 MHz spectrometer (Agilent Technologies, USA) at 25 °C in $CDCl_3$. The recorded spectra were referenced to the solvent signals of ^1H 7.260 ppm and ^{13}C 77.000 ppm ($CDCl_3$) and processed with MestRENOVA v. 11 (Mestrelab Research, Chemistry Software Solutions, USA) software. The chemical shift (d) of ^1H and ^{13}C NMR data were measured in ppm, while the multiplicity and the coupling constant (J) of ^1H were measured in Hz. All the spectra data were compared with the literature data.

Antioxidant activity

The solvent-fractions in the concentration range 25–400 ppm and sub-fractions (400 ppm) were used in the following experiment in triplicate and vitamin C was used as reference standard.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition test

The DPPH (Merck KGaA, Germany) inhibition potentials of solvent-fractions, CC-fractions and sub-fractions derived from the HEHA were investigated (Onoja *et al*., 2017). DPPH is a stable free radical that changes colour from violet to yellow colour within 30 minutes, when it reacts with hydrogen donors at room temperature.

Nitric oxide scavenging (NOS) activity

The nitric oxide scavenging potential of solvent-fractions, CC-fractions and sub-fraction derived from the HEHA were evaluated using Griess reaction method (Marcocci *et al*., 1994). The principle is based on the indirect estimation of amount nitric oxide released from sodium nitroprusside in an aqueous medium (physiological pH) with Griess reagent.

Experimental animals

Mature (70 days old) male albino Wistar rats (170 ± 5 g) were procured for the study. The rats were kept in aluminum cages in an adequately ventilated house at room temperature (25–27°C) and natural light/darkness cycle. Two weeks was allowed for the adaptation of the rat to the environment. The ethical clearance for the study was obtained from the Michael Okpara University of Agriculture, Umudike, Animal Ethical Committee (MOUAU/CVM/REC /201816).

Antidiabetic activity

Alloxan monohydrate-induced diabetic rats were used for the study (Sebai *et al*., 2015; Onoja *et al*., 2020). The antidiabetic activity of the solvent-fractions was evaluated at 300 mg/kg while the sub-fractions were evaluated at 50 mg/kg. The negative control groups received 5 % Tween-20 (5 mL/kg) and distilled water (5 mL/kg) for the solvent-fractions and sub-fractions, respectively. Glibenclamide (2 mg/kg) was used as the reference standard. All treatments were administered *par os* via gastric gavage. The fasting blood sugar (FBS) level was evaluated 1, 3, and 6 h post treatment via a snip on the tail vein, with Accu-Chek active glucometer (Roche, UK).

STATISTICAL ANALYSIS

The data were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). Dunnett's test (SPSS, version 20) was used to separate mean difference and significance was accepted at $p< 0.05$.

RESULTS AND DISCUSSION

Characterization of isolated compound

The isolation and characterization of a bioactive antidiabetic and antioxidant flavone (pectolinarigenin) was reported herein. Bioassay-guided isolation method was adopted in the isolation of the pectolinarigenin from EtOAc-soluble fraction of *Helianthus annuus* leaf. Pectolinarigenin was isolated from the most active sub-fraction of HEHA as cream amorphous substance, mol. formula $C_{17}H_{11}O_6$; calc. mol mass 314.2903 g/mol, UV^{MeOH} 200 nm, tR 7.359 min (Fig. 1), hRf 0.46 (EtOAc, MeOH, HAc 9.4:0;5:0.1) ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (150 MHz): see Table 1.

The EtOAc soluble of HEHA, subjected to repeated CC fractionation and semi-preparative HPLC purification, yielded a known amorphous substance, a dimethoxylated, dihydroxyl flavone called pectolinarigenin (Jeong *et al*., 2013; Lu *et al*., 2014; Liao *et al*., 2010) that was identified by ^{1}H and ^{13}C NMR spectra data. Two-dimensional NMR was used to unravel ^{1}H and ^{13}C chemical shift assignments of the flavonoid. The detailed COSY and HMBC correlations are illustrated as insert in Fig. 1.

The separation and purification processed improved the antidiabetic and antioxidant activities of the fractions. The selection of the most active fraction for further purification was based on the time of onset and the degree of reduction in the fasting blood sugar levels. This is because the best candidate antidiabetic drug is the one that can reduce blood glucose level within a short while to control postprandial hyperglycemia, which is problematic in diabetic patients (Patel *et al*., 2016).

The structure was further confirmed by spectral data of pectolinarigenin previously isolated from *Cirsium chranroenicum* (Lim *et al*., 2008). To the best of our knowledge, we are the first to report the isolation of this flavone from *Helianthus annuus*. Pectolinarigenin (5,7-dihydroxy-6,4'-dimethoxyflavone) is a methoxylated flavone. The methoxylation of flavones increase their metabolic stability and membrane transport in liver and intestine, thus improving the bio-availability. Pectolinarigenin has been isolated from the aerial part of *Cirsium chanroenicum, Linaria japonica, Chromolaena odorata, Cirsium setidens, Clerodendrum phlomidis,*

Arnica montana, Arnica angustifolia, Teucrium chamaedrys, Cirsium japonicum, Japanese *Chrysanthemum spp, Ruellia* spp (Liao *et al*., 2010; Jeong *et al*., 2013; Lu *et al*., 2014). The antidiabetic effects of pectolinarigenin isolated from *Cirsium japonicum* has been reported (Liao *et al*., 2010). Other pharmacological activities such as anti-inflammatory, cytotoxic, α-glucosidase inhibitor, α-amylase inhibitor, hepatoprotective and antioxidant activities of pectolinarigenin have been documented (Lim *et al*., 2008; Yoo *et al*., 2008; Noh *et al*., 2013; Lu *et al*., 2014; Wang *et al*., 2016; Zhang *et al*., 2016).

Antioxidant activities

The fractions produced concentration-dependent increases in the DPPH photometric assay (Table 2). The antioxidant activities of the fractions were lower ($p < 0.05$) when compared to vitamin C. The antioxidant activities of the fractions are arranged in the following descending order: EtOAc > n-hexane > DCM > MeOH. The 50 % inhibitory concentration (IC_{50}) of the fractions were > 400 ppm.

In the NOS model (Table 2), all the fractions (except hexane) produced concentration-dependent increase in NOS activity. The IC_{50} of DCM, n-hexane and methanol-fractions were > 400 ppm while the IC_{50} of EtOAc and vitamin C were ≤ 400 ppm.

The results of the antioxidant activities of CC fractions (M.1 – M.5) and sub-fractions (M.4.1 – M.4.5) are presented in Table 3. In DPPH assay, the IC_{50} of the fractions were less than 400 ppm concentration (except M.1). The antioxidant activities of the fractions were lower ($p < 0.05$) when compared to vitamin C. The NOS capacities of M.2–M.5 at 400 ppm concentration were comparable to vitamin C. The IC_{50s} were also > 400 ppm whereas the IC_{50} of vitamin C was < 400 ppm.

The results of the antioxidant activities of CC sub-fractions (M.4.1 – M.4.5) are also presented in Table 3. In DPPH photometric assay, all sub-fractions (M.4.1–M.4.5) exhibited antioxidant activities which were lower ($p < 0.05$) when compared to vitamin C. The IC_{50s} of M.4.1 and M.4.2 were > 400 ppm while the IC_{50s} of M.4.3, pectolinarigenin and M.4.5 were < 400 ppm. The NOS activities of M.4.1, M.4.3 and pectolinarigenin were not significant ($p > 0.05$) when compared to NOS of vitamin C while the NOS of M.4.2 and M.4.5 were lower ($p < 0.05$) when compared to NOS of vitamin C.

Flavonoids exhibit antioxidant activity via direct or indirect mechanisms. The direct mechanisms involve scavenging of free radicals, inhibition of oxidase, activation of antioxidant enzymes, chelation of metals etc., while the indirect mechanisms are inhibition of enzymatic processes associated with free radical liberation and activation of endogenous antioxidant enzymes (Unnikrishnan *et al*., 2014). The pectolinarigenin could elicit its antioxidant activity via any of the aforementioned mechanism. The scavenging of nitric oxide radical corroborated the inhibition of nitric oxide production *in vivo* as reported by Lim *et al*. (2008). The antioxidant potential of pectolinarigenin is advantageous in quenching of free radicals that abound in diabetic conditions (Lobo *et al*., 2010). The reported anti-inflammatory potential of pectolinarigenin will help in the control of inflammatory reaction that characterizes hyperglycemia and diabetes mellitus (Lim *et al*., 2008). Diabetes mellitus has been described as a chronic inflammatory condition and anti-inflammatory drugs are reported to be useful in its management (Tsalamandris *et al*., 2019).

Antidiabetic activity

The results of the antidiabetic activities of the fractions are presented in Table 4. The EtOAc-fraction and glibenclamide-treated groups showed significant ($p<0.05$) time-dependent increases in the percentage reduction of FBS when compared to 5 % Tween-20 (5 mL/kg)-treated group.

Table 1
Spectral data of pectolinarigenin

Carbon position	^{13}C, δ (ppm)	^{1}H, δ, ppm (mult., *J* (Hz))	^{13}C / ^{1}H HSQC
2	164.3		-C-
3	103.9	6.56, 1H, *d*, 2.7	-CH-
4	183.1	-	-C-
5	152.3	-	-C-
6	130.4	-	-C-
7	155.1	-	-C-
8	93.6	6.58, 1H, *d*, 2.7	-CH-
9	153.3	-	-C-
10	105.9	-	-C-
1’	-	-	-
3’	114.7	7.01, 1H, *dd*, 3.2, 8.9	-CH-
2’	128.2	7.83, 1H, *dd*, 2.9, 8.7	-CH-
4’	162.8	-	-C-
6’	128.2	7.83, 1H, *dd*, 8.7, 2.9	-CH-
5’	114.7	7.03, 1H, *dd*, 8.9, 3.2	-CH-
4’-OCH_3	55.7	3.89, 3H, *s*	-CH_3
6-OCH_3	61.0	4.03, 3H, *s*	-CH_3
7-OH	-	6.50, 1H, *s*	-OH

^{1}H and ^{13}C spectra were recorded at 600 and 150 MHz in $CDCl_3$ respectively

Table 2
Antioxidant activities of solvent fraction of HEHA

Assay models	Treatment/ppm	25	50	100	200	400
DPPH RSA	Hexane	21.03 ± 0.18	23.56 ± 0.42	31.37 ± 0.14	35.58 ±0.43	43.95±0.36
	DCM	23.56 ± 0.28	25.00 ± 0.07	26.44 ± 0.14	34.74 ± 0.35	41.11 ± 0.69
	EtOAc	15.50 ± 0.35	28.00 ± 0.42	44.19 ± 0.31	45.07 ± 0.46	52.40 ± 0.42
	Aqueous	19.71 ± 0.21	19.23 ± 0.14	22.36 ± 0.42	28.37 ± 0.28	35.58 ± 0.42
	Vitamin C	85.84 ± 0.18	89.26 ± 0.83	93.14 ± 0.53	96.32 ± 0.10	96.61 ± 0.57
NOS activity	Hexane	4.96 ± 1.91	4.62 ± 0.97	2.65 ± 0.48	1.88 ± 0.09	-0.26 ± 1.04
	DCM	19.40 ± 0.76	20.94 ± 0.62	23.85 ± 0.97	24.02 ± 1.50	24.53 ± 1.50
	EtOAc	19.23 ± 2.05	17.35 ± 1.85	17.95 ± 1.71	19.74 ± 1.07	24.19 ± 0.94
	Aqueous	14.62 ± 2.33	19.49 ± 0.51	17.61 ± 1.26	17.35 ± 0.17	28.46 ± 0.44
	Vitamin C	12.17 ± 1.82	27.97 ± 4.47	32.14 ± 3.15	23.40 ± 1.17	50.93 ± 4.08

RSA (radical scavenging activities), data expressed as percent mean±SEM, *p<0.05 is considered significant when compared with control. DCM = dichloromehane, EtOAc = ethyl acetate

Table 3
Antioxidant activities of CC fractions and sub-fractions of HEHA

Treatments, CCF (400 ppm)	Models		Treatments, CCsF (400 ppm)	Models	
	DPPH (%)	NOS (%)		DPPH (%)	NOS (%)
M.1	43.84±1.54	38.29±4.70	M.4.1	44.76±1.23	45.40±7.33
M.2	56.67±1.23	47.37±1.97	M.4.2	32.03±0.41	37.42±7.22
M.3	54.41±1.03	48.74±5.85	M.4.3	53.29 ± .51	45.73±3.72
M.4	57.29±0.21	47.32±5.31	Pectolinarigenin	54.93±0.92	46.06±2.30
M.5	52.46±0.31	43.98±6.24	M.4.5	50.72±0.21	33.70±3.17
Vitamin C	96.89±0.16	53.27±2.28	Vitamin C	96.89±0.16	53.27±2.28

Data expressed as mean ± SEM, *p<0.05 when compared with controls; DPPH = 2, 2-diphenyl-1-picrylhydrazyl, NOS = nitric oxide scavenging, CCF = column chromatography fractions, CCsF = column chromatography sub-fractions

Table 4
Effects of solvent fractions on FBS level in diabetic rats

Treatment/dose (mg/kg)	Mean reduction in FBS (%)		
	1 h	3 h	6 h
5% Tween-20, 5 ml/kg	-10.09 ± 5.83	6.21 ± 5.56	35.80 ± 10.99
Hexane soluble, 300	-12.09 ± 7.79	8.21 ± 1.78	38.89 ± 7.10
DCM soluble, 300	-13.89 ± 10.94	-8.20 ± 16.59	22.87 ± 11.46
EtOAc soluble, 300	37.50 ± 5.76	59.98 ± 8.43	53.34 ± 7.39
aqueous soluble, 300	11.51 ± 3.59	16.65 ± 6.06	36.19 ± 1.77
Glibenclamide, 2	6.80 ± 6.02	47.92 ± 14.47*	60.73 ± 10.78

Data expressed as mean ± SEM, *p<0.05 when compared with 5% tween-20 treated group, DCM = dichloromehane, EtOAc = ethyl acetate

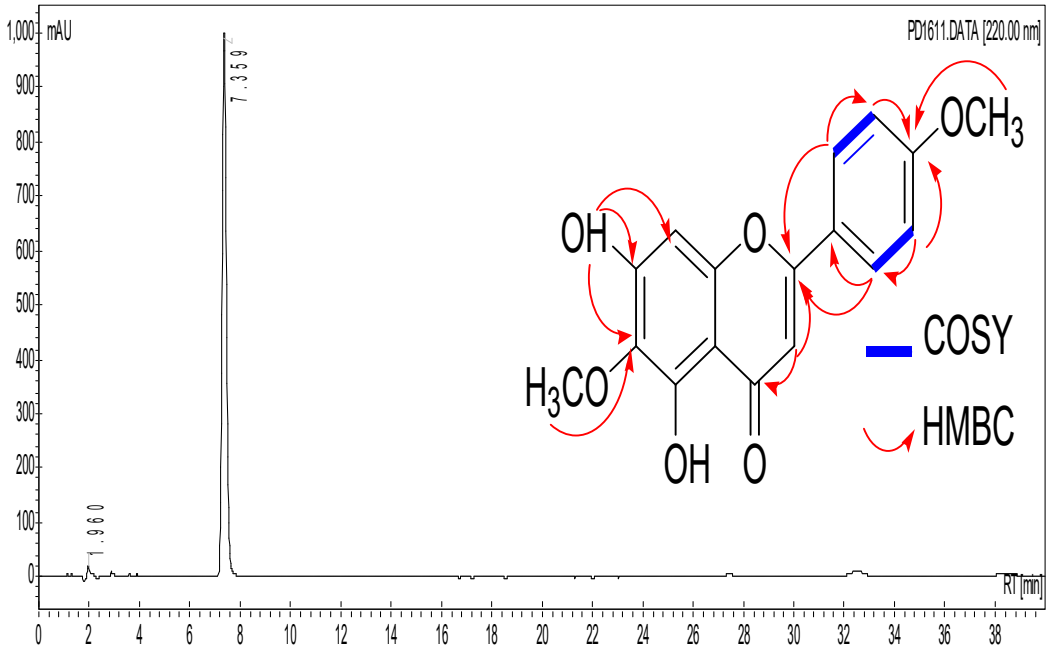

Fig. 1: HPLC chromatogram of pectolinarigenin (tR 7.359 min, structure and key HMBC and COSY correlations insert). COSY = proton-proton correlation spectroscopy, HMBC = carbon-proton heteronuclear multiple bond correlation,

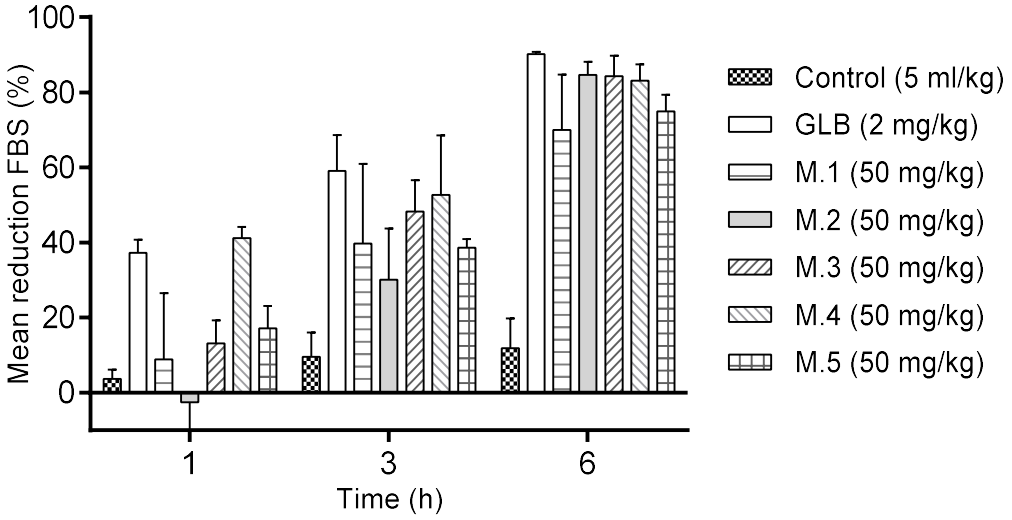

Fig. 2: Effects of CC fractions on FBS of alloxan-induced diabetic rats. *p < 0.05 when compared with negative control group. GLB = glibenclamide, M.1-M.5 = column chromatography (CC) fractions

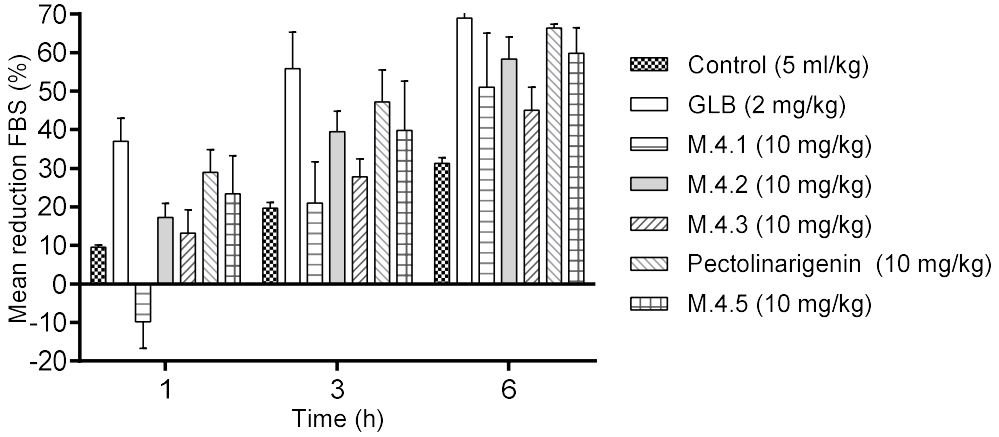

Fig. 3: Effects of CC sub-fractions on FBS of alloxan-induced diabetic rats. *p < 0.05 when compared with negative control group. GLB = glibenclamide, M.4.1-M.4.5 = column chromatography (CC) sub-fractions.

The effects of CC fractions (M.1 – M.5) on the FBS of alloxan-induced diabetic rats are presented in Fig. 2. At 1 h post treatment, M.4 and GLB caused significant ($p < 0.05$) increases in percentage reduction of FBS in the treated-groups when compared to distilled water-treated group. At 3 h post treatment, GLB, M.3, M.4 and M.5 produced significant

($p < 0.05$) increases in percentage reduction of FBS when compared to distilled water-treated group. At 6 h post treatment, GLB and all the fraction caused significant ($p < 0.05$) increase in percentage reduction of FBS when compared to distilled water-treated group.

The effects of CC sub-fractions on FBS of alloxan-induced diabetic rats are presented in Fig. 3. The GLB and sub-fractions (except M.4.1) caused time-dependent increases in the percentage reduction of FBS in treated rats. At 1 and 3 h post treatment, the percentage reductions in FBS of GLB and M.4.4-treated rats were elevated ($p < 0.05$) when compared with the negative control. At 6 h post treatment, the percentage reduction in FBS of GLB, M.4.2, pectolinarigenin and M.4.5-treated rats were elevated ($p < 0.05$) when compared with the negative control.

Our findings are in agreement with the reports of Liao and coworkers on the antidiabetic properties of pectolinarigenin isolated from *Cirsium japonicum* (Liao *et al*., 2010). According to the study, pectolinarigenin reduced blood glucose, cholesterol and triglyceride levels, but did not alter the levels of plasma insulin, GLUT-4 and leptin in the treated diabetic rats. The pectolinarigenin also increased the expression of adinopectin in plasma and adipose tissue, increased the activities of glycolytic enzymes and reduced the activities of gluconeogenic enzymes in diabetic rats (Liao *et al*., 2010). These findings by previous researchers suggest that the mechanism of the antidiabetic activities of pectolinarigenin is extrapancreatic; it improves insulin sensitivity, enhances glucose utilization by skeletal muscles and reduces hepatic glucose production (Liao *et al*., 2010).

CONCLUSION

We are the first to report the isolation of pectolinarigenin (5,7-dihydroxy-6,4'-dimethoxyflavone) from *Helianthus annuus*. It elicited significant antioxidant and antidiabetic activities and might be useful in the development of novel antidiabetic, antioxidant and anti-inflammatory compounds.

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