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Article

Bioactivity-guided isolation of the antidiabetic principle in *Pterocarpus Santalinoides* leaf extract

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Pterocarpus santalinoides is used in Nigerian ethnomedicine to treat diabetes mellitus. This study aimed to establish the antidiabetic property of the plant, and isolate and characterize its active principle. Dried and pulverized leaves (500 g) of *P. santalinoides* were extracted with 1.8 L of 80 % hydromethanol by cold maceration. The dried extract (10 g) was partitioned into *n*-hexane, ethyl acetate (EtOAc), *n*-butanol, and water. Antidiabetic activitiy-guided isolation by column chromatographic separation of the EtOAc soluble and purification of the sub-fractions by repeated preparative thin layer chromatography (pTLC) yielded a C-glycosyl flavonoid, identified as isovitexin. The chemical structure was elucidated based on high-resolution mass spectroscopy, 1D, and 2D nuclear magnetic resonance spectroscopic analyses. Alloxan-induced diabetic rat model was adopted for antidiabetic screening. The extract of *P. santalinoides* (100-200 mg/kg), fraction F4 (50 mg/kg), sub-fraction F4.3 (10 mg/kg), and the semi-purified compound F4.3.2 (5 mg/kg) significantly ($p < 0.05$) reduced the fasting blood glucose of alloxan-induced diabetic rats, causing 48.4, 69.4, 57.7 and 64.5 % antidiabetic activity respectively, compared with > 68 % recorded in glibenclamide (2 mg/kg) control. These results reveal that isovitexin is the antidiabetic principle in *P. santalinoides*.

Keywords: *Pterocarpus santalinoides*. Diabetes. Chromatography. Fraction. Isolation. Isovitexin.

INTRODUCTION

The prevalence of diabetes has been on the increase despite existing drugs and other management strategies (Wild *et al*., 2004; Kaveeshwar, Cornwall, 2014). It is projected that in 2030, the number of individuals with diabetes will rise to 366 million (Omeh *et al*., 2014). Worrisome also is the economic implication of the disease, which as of 2014 was estimated to be 612 billion USD (IDF, 2014).

Currently existing antidiabetic drugs are associated with side effects such as hypoglycemia, weight gain and gastrointestinal disturbances (Rang *et al*., 2007; Harvey, Champe, 2009) while surgical interventions (pancreatic transplant, Islet cell transplantation and bariatric surgery) are very expensive, besides significant failure rate and post-surgical complications (Lakey, Burridge, Shapiro, 2003; Keider, 2011). These factors, no doubt contribute to the rising interest in the search for natural products that possess antidiabetic activity (Gokce, Haznedaroglu, 2008).

Pterocarpus santalinoides L'Herit ex DC belongs to the *Fabaceae* family (Madubuike, Ezeja, Ezeigbo, 2012) and grows mainly along riverine forests in West Africa and tropical South America (Ogan, 1990). In Nigerian traditional medicine, *P. santalinoides* leaf is used to treat stomach aches, diarrhea, diabetes and enhance wound healing (Aba, Udechukwu, 2018). Researchers have reported antidiarrheal (Nworu *et al*., 2009; Madubuike, Ezeja, Ezeigbo, 2012), hepatoprotective (Offor *et al*., 2015),

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antimicrobial (Odeh, Tor-Ayiin, 2014) and antidiabetic/hypoglycemic (Okwuosa *et al*., 2011; Aja *et al*., 2015; Aba, Udechukwu, 2018; Madubuike, Anaga, Asuzu, 2020a; Madubuike, Anaga, Asuzu, 2020b) activities of the plant. The present study aimed to report the antidiabetic activity *of P. santalinoides* in rats.

MATERIAL AND METHODS

Reagents and chemicals

Analytical grades of methanol, *n*-butanol, EtOAc, *n*-hexane, formic acid, p-anisaldehyde, sulfuric acid, acetonitrile, sulfanilic acid, and silica gel were procured from Merck KGaA GmbH (Germany), d-methanol, 99.8 % D (Sigma-Aldrich, Darmstadt), glibenclamide (GNC, Nigeria) and Millipore water for UHPLC/+ESI-QqTOFMS/MS measurement was LC-MS grade (HiPerSolv CHROMANORM, VWR International, Belgium).

Plant collection and preparation of extract

Fresh leaves of *P. santalinoides* were collected from its natural habitat in Nsukka, Nigeria in October 2014. The plant specimen was authenticated by Mr. A. O. Ozioko, a taxonomist with the Bioresources Development and Conservation Programme (BDCP), Nsukka. A voucher specimen (catalogued: MOUAU/VPP/2014/017) was deposited in the institution's herbarium. The leaves were air-dried under ambient temperature (25 – 27 ºC) and reduced to a coarse powder. Five hundred grams of the pulverized powder was macerated in 1.8 L of 80% methanol, with intermittent shaking for 72 h (Madubuike, Anaga, Asuzu, 2020a). The residue was removed via filtration and the filtrate concentrated to dryness as methanol extract of *P. santalinoides* (MEP).

Solvent-solvent separation of MEP

The extract (10 g) was dissolved in 100 ml of 80 % aqueous methanol and successively partitioned in *n*-hexane, ethyl acetate (EtOAc), *n*-butanol (*n*-But) and water using a separating funnel. The crude extract yielded *n*-hexane (1.5 g), EtOAc (5.8 g) and *n*-butanol (2.4 g) after evaporation to dryness.

Column chromatographic separation of EtOAc soluble fraction

The EtOAc soluble portion (5 g) formed on evaporation to dryness was dissolved with initial mobile phase solvent (n-hexane-chloroform, 9:1) and introduced appropriately to the silica gel 60 (F254; powder: 0.063 – 0.200; mesh: 70 - 230) slurry packed column, 45 x 5 cm (extract: silica gel ratio, 1: 90) at room temperature. The fraction was eluted with a gradient of mobile phase consisting of n-hexane, chloroform, ethyl acetate and methanol, at a flow rate of 1 ml/min.

Thin layer chromatographic analysis of fractions

Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F_{254} plates, 20 x 10 cm with various solvent systems comprising chloroform, ethyl acetate and methanol in an appropriate combination ratio optimized for each experimental run at room temperature. The plates, developed over 8 cm front, were visualized under UV light at 254/365 nm and sprayed with anisaldehyde-sulphuric acid detecting reagent. The collected eluates (15 ml) from the column chromatographic separation of the EtOAc soluble portion was analyzed by TLC at predetermined intervals and subsequently pooled into ten fractions based on their TLC profiles.

Preparative TLC of the active fraction and sub-fraction

Separation of the active fraction and sub-fraction was achieved following the method of Stahl (1969), with some modifications. Silica gel 60 (35 g) was mixed with 87 ml of water in a ceramic mortar to form a slurry which was poured into a spreader adjusted to 0.5 mm and spread onto 5 chromatographic glass plates (20 x 20 cm). The prepared plates were oven-activated (110 ºC) for 1 hour, after which the active fraction/sub-fraction was dissolved in the appropriate solvent and streaked on the coated surface of the plates. The plates were carefully

placed in a multi-channeled chromatographic tank containing a predetermined solvent system (chloroform: ethyl acetate: methanol in 3: 2: 1 ratio) filled to the 0.5 cm mark. The eluting solvent was allowed to run for a distance of 15 cm after which they were removed and air-dried. The separated zones or bands were scraped into different centrifuge tubes, dissolved in methanol and centrifuged (2500 r. p. m) for 10 min, decanted and the supernatant evaporated to dryness. The obtained compounds (fractions/sub-fractions) were labeled and stored in a refrigerator at 4 °C until the time of use.

Structural characterization of the isolated compound

The high-resolution electrospray ionization mass spectrometry (HRESIMS) data of the isolated compound were analyzed with UHPLC/ESI-QTOF MS/MS. Chromatographic separations on a Dionex Ultimate 3000 RS Liquid Chromatography System with a Dionex Acclaim RSLC 120, C18 column (2.1 x 100 mm, 2.2 μm) using a binary gradient (A: water with 0.1% formic acid; B: acetonitrile with 0.1% formic acid) at 0.8 mL/min: 0 to 9.5 min: linear from 5% B to 100%B; 9.5 to 12.5 min: isocratic 100% B; 12.5 to 12.6 min: linear from 100% B to 5% B; 12.6 to 15 min: isocratic 5% B. The injection volume was 5 μL. The eluted compound was detected using a Dionex Ultimate DAD-3000 RS over a wavelength range of 200-400 nm and a Bruker Daltonics micrOTOF-QII quadrupole/time-of-flight mass spectrometer equipped with an Apollo electrospray ionization source in positive mode at 5 Hz over a mass range of *m/z* 50-1000. Both 1D (^{1}H and ^{13}C) and 2D ($^{1}H/^{1}H$ COSY, $^{1}H/^{13}C$ HSQC and $^{1}H/^{13}C$ HMBC) NMR spectra were measured on an Agilent DD2 600 MHz spectrometers. Overnight dried samples (using a desiccant-filled Desaga drying apparatus, Desaga, Germany) were typically prepared in deuterated methanol (CD_3OD). The recorded spectra were respectively referenced to the CD_3OD signals of ^{1}H; 3.310 ppm and ^{13}C; 49.000 ppm (CD_3OD) and processed with MestRENOVA v. 11 (Mestrelab Research, Chemistry Software Solutions) software. ^{1}H NMR data were reported indicating the chemical shift (δ) in ppm, the integral size (in cases where signals represent more than one proton, e.g., 2H or 3H), the multiplicity (*s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; *br*, broad; *dd*, doublet of doublets, etc.) and the coupling constant(s) (*J*) in Hz while the ^{13}C NMR data were reported indicating only the chemical shifts (σ) in ppm. All the spectra data were compared with the library data.

Experimental animals

Mature male albino Wistar rats (112.5±7.5 g) procured from the Laboratory Animal Unit of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, were used for the experiments. The rats were housed in stainless steel cages in a well-ventilated room at ambient temperature (25-27 ºC) and 12 h light-darkness cycle. They were given standard pelleted feed (Vital feed®, Nigeria) *ad libitum* and acclimatized for 2 weeks before use. The experimental protocol was approved by the institution's ethics committee for use of laboratory animals (Approval No. MOUAU/CVM/REC/19009), and the rats were handled in strict compliance with NIH Guidelines for Care and Use of Laboratory Animals (Pub. No. 85-23, Revised, 1985).

Induction of diabetes

Diabetes was achieved in male albino Wistar rats by a single injection of alloxan (160 mg/kg, intraperitoneally) after 18 h fast (Anaga, Asuzu, 2010). The fasting blood glucose (FBG) of the rats was determined with blood from the tail vein, using a glucometer test kit (Accu-Check Active®, Roche, Indiana, USA). Rats having FBG ≥ 126 mg/dl 72 h post-induction were considered diabetic and used for the study (Omeh *et al.*, 2014).

Antidiabetic study

Thirty diabetic rats were randomly assigned to 5 groups ($n = 6$). Rats in group 1 received 5 ml/kg of distilled water and served as the negative control. Group 2 rats (positive control) were given glibenclamide (2 mg/kg), while groups 3, 4, and 5 were treated with 50, 100

and 200 mg/kg of MEP, all by gavage. The FBG of the rats was determined at 0, 1, 3 and 6 h post-treatment (Ezeja *et al.*, 2015). Fractions (F1-F10) recovered from the column chromatography (CC) were tested at the dose of 50 mg/kg for antidiabetic activity in alloxan-induced diabetic rats following the procedures earlier described. Sub-fractions (F4.1-F4.5 and F4.3.1-F4.3.3) yielded by preparative thin layer chromatographic separation of the active fraction (F4), were also screened for antidiabetic activity as described previously.

Statistical analysis

Data obtained from the study were presented as mean ± S.E.M and analyzed using one-way analysis of variance (ANOVA) (SPSS software; v. 22). The variant means were separated by the least significant difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Extraction and isolation of active compound

Extraction of the pulverized leaves of *P. santalinoides* yielded 11.6 g of crude extract (designated as MEP). Solvent-solvent partitioning of MEP (10 g) gave the following soluble portions: *n*-hexane (1.5 g), EtOAc (5.8 g) and *n*-butanol (2.4 g). Separation of the *EtOAc* soluble (5 g) via CC yielded 10 fractions (F1-F10). Further separation of Fraction F4 (1.54 g) on silica gel afforded four sub-fractions (F4.1, F4.2, F4.3 and F4.4) and sub-fraction F4.3 yielded F4.3.1, F.4.3.3 and semi-purified F4.3.2 after it was re-chromatographed over silica gel (pTLC).

Characterization and structural elucidation of active compound

The isolated compound (F4.3.2) showed UV maxima of 215 nm (Figure 1A). Its high-resolution mass spectrophotometry (HRMS) resulted in a protonated molecular ion at *m/z* 433.3858 $[M+H]^+$, (Figure 1B) from where the elemental formula of $C_{21}H_{21}O_{10}$ was derived (calculated mass for $C_{21}H_{21}O_{10}$: 433.3862, $[M+H])^+$. The MS/MS fragmentation pattern (Figure 1C) showed multiple fragments of the compound. A fragment at m/z 416.0608 $[M+H-17]^+$ indicated the loss of a substituent of molecular formula OH. Similarly, a fragment at m/z 313.0382 $[M+H-120]^+$ was a result of the loss of a substituent of molecular formula $C_7H_4O_2$, whereas, a loss of molecular formula $C_{15}H_9O$ gave a fragment signal at m/z 164.9898 $[M+H-269]^+$.

The ^{1}H-NMR of F4.3.2 (in methanol-*d4*) showed the following signals: four aromatic protons of the AA′BB′ coupling system in B ring at δH 7.78 (2H, *d, J = 8.8* Hz) and 6.86 (2H, *d, J = 8.8* Hz); two singlet protons at δH 6.44 (1H, s) and 6.27 (1H, s) assigned to a flavone aglycone; one anomeric proton at δH 4.69 (1H, d, J = 9.9 Hz), assigned to a C-sugar unit (Table I, Supplementary 1A).

The ^{13}C-NMR spectra revealed the presence of 21 signals (Table I, Supplementary 1B). The ^{1}H-^{13}C single bond connectivity in the molecule was obtained from a 2D heteronuclear single quantum correlation (HSQC) spectroscopy. The spectra showed the presence of 1 methylene carbon (1 -CH_2), 11 methine carbons (11 -CH-) and 9 quaternary carbons (9 –C-) from the C-H cross peak correlations (Supplementary 1C). The heteronuclear multiple bond correlation (HMBC) spectroscopy showed that the position of the sugar moiety in the compound is at C-6. The Hydrogen-Hydrogen correlation spectroscopy (^{1}H-^{1}H COSY Spectroscopy) revealed the relationship between hydrogen atoms in the active principle.

All the spectral data were compared with literature and agreed with data for previously isolated isovitexin (5,7-dihydroxy-2-(4-hydroxyphenyl)-6-[(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]chromen-4-one) previously isolated from *Vitex grandifolia* (Bello *et al.*, 2019). The position of the sugar moiety at C-6 was resolved by the HMBC between H-1 (δH, 4.89, *J= 9.9* Hz) of the glucose and C-6 (δC 110.6) and C-5 (δC 162.0). The absence of any HMBC correlation with either C-8 (δC 97.4) or C-9 (δC 159.4) further confirmed the position at C-6 (Ramarathnam *et al*, 1989; Kim *et al.*, 2005; Yu *et al*., 2014).

Isovitexin, also known as apigenin-6-C-glucoside is an isomer of vitexin (apigenin-8-C-glucoside) and both compounds have been purified from some medicinal plants and exhibit similar biological properties (Yao *et al*., 2011).

However, existing literature shows no previous isolation of isovitexin from *P. santalinoides.* From the structure of flavonoids and known evidence of their bioactivities, the structure of phenolic hydrogen in isovitexin molecule, especially O – tri – or O – di – hydroxyl structure may be the active group (Guo *et al.*, 1999).

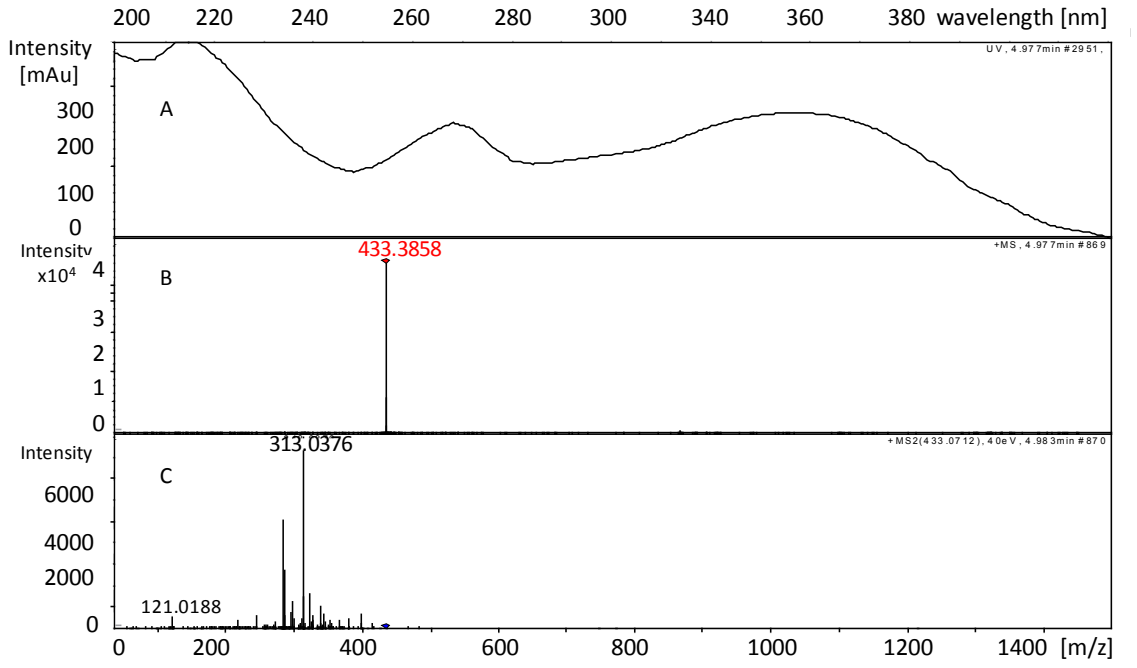

FIGURE 1 - UV-UHPLC (A) /EIC MS (B) /Fragmentation pattern (C) of F4.3.2 (M+H)+ 433.3858 Da, retention time, tR 4.977 min.

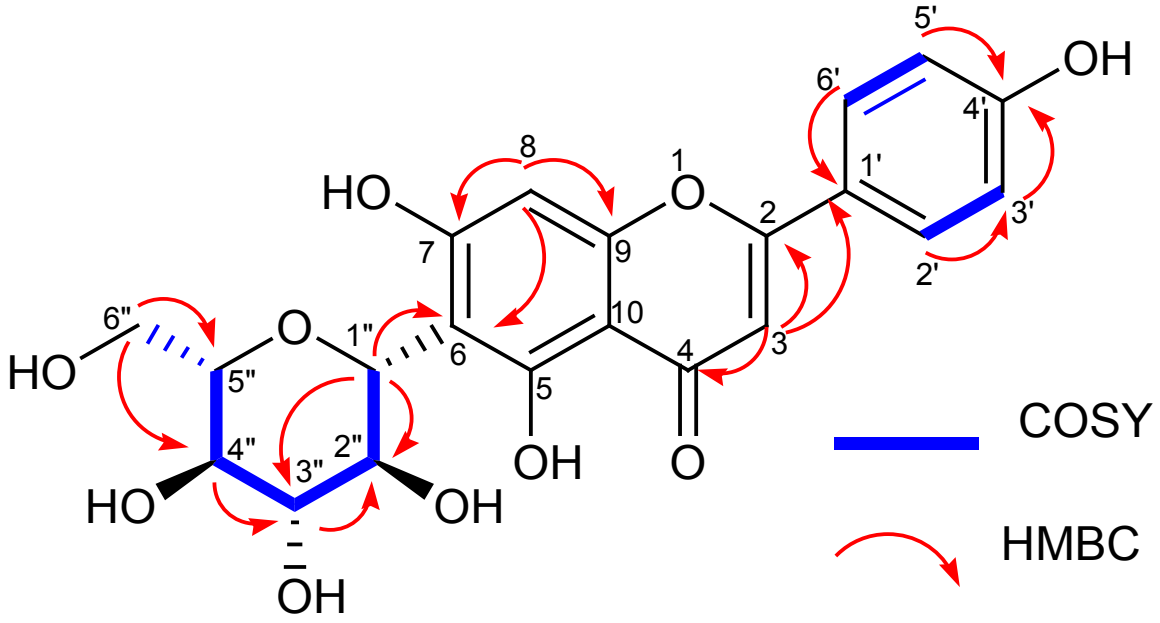

FIGURE 2 - Chemical structure and Key HMBC and 1H-1H COSY correlations of isovitexin.

TABLE I - NMR spectral data of isolated compound (isovitexin)

Carbon position	^{13}C, δ (ppm)	^{1}H, δ, ppm (mult., *J* (Hz))	13C / 1H HSQC
2	164.3		-C-
3	102.4	6.44, 1H *s*	-CH-
4	182.9		-C-
5	162.0		-C-
6	110.6		-C-
7	161.7		-C-
8	97.4	6.27, 1H, *s*	-CH-
9	159.4		-C-
10	102.6		-C-
1’	122.5		-C-
2’	129.1	7.78, 1H, *d*, 8.8	-CH-
3’	117.5	6.86, 1H, *d*, 8.8	-CH-
4’	165.3		-C-
5’	117.5	6.86, 1H, *d*, 8.8	-CH-
6’	129.1	7.78, 1H, *d*, 8.8	-CH-
1’’	75.7	4.89, 1H, *d*, 9.9	-CH-
2’’	72.2	4.35, 1H, *dd*, 9.8, 9.0	-CH-
3’’	80.7	3.47, 1H, *dd*, 9.1	-CH-
4’’	71.5	3.54, 1H, *dd*, 9.2, 3.9	-CH-
5’’	82.3	3.39, 1H, *ddd*, 9.7, 4.6, 2.4	-CH-
6’’	62.6	3.76, 1H, *dd*, 12.0, 4.7 3.84, 1H, *dd*, 11.9, 2.2	$-CH_2-$

^{1}H and ^{13}C spectra were recorded at 600 and 150 MHz in CD_3OD respectively. Data here were comparable with NMR spectra of isovitexin isolated from *Vitex grandifolia* (Bello *et al.*, 2019)

Antidiabetic activity of MEP and its CC fractions

The methanol extract of *P. santalinoides* (100 and 200 mg/kg) significantly reduced the FBG of alloxan-induced diabetic rats, when compared with the negative control (Table II). This result agrees with previous reports of blood glucose-lowering activity by *P. santalinoides* (Okwuosa *et al*., 2011; Aja *et al*., 2015; Aba, Udechukwu, 2018; Madubuike, Anaga, Asuzu, 2020b). Similarly, fractions, F1, F3, F4 and F9 caused a significant ($p < 0.05$) decrease in the FBG of the treated rats when compared with the control. However, F4 exhibited the highest antidiabetic activity (69.4%, against 13.2%, 52.3% and 23.2% by F1, F3 and F9, respectively, 6 h post-treatment). Fractions F7 and F8 significantly ($p < 0.05$) increased the FBG in the treated rats, whereas the antidiabetic activity of fractions F2, F5, F6 and F10 did not differ significantly ($p < 0.05$) when compared with

the control (Table III). Fraction F4 was therefore selected for further separation.

Antidiabetic screening of the sub-fractions showed that F4.3 caused the highest effect, evoking 57.7 % antidiabetic activity, 6 h post-treatment (Table IV). Following further separation and testing, F4.3.2 (a yellowish amorphous compound) exhibited significant ($p < 0.05$) antidiabetic activity by lowering the FBG of diabetic rats from 241.7 ± 3.8 mg/dl before treatment to 85.7 ± 3.5 mg/dl, 6 h after treatment. The antidiabetic activity of the F4.3.2 (64.5%) tested at 5 mg/kg is comparable to that of 2 mg/kg of glibenclamide (reference drug), which caused a 69.9% antidiabetic activity, by lowering the FBG of diabetic rats from 232.3 ± 04.3 mg/dl at 0 h to 70.0 ± 0.02.5 6 h after treatment (Table V).

Alloxan monohydrate used to induce diabetes in the present study causes hyperglycemia through selective destruction of the insulin-secreting beta cells of the islets of Langerhans (Dewanjee *et al*., 2008; Ezeja, Anaga, Asuzu, 2015). This leads to poor glucose utilization by tissues and a persistent rise in blood glucose above the normal physiological range (El-Missiry, El-Gindy, 2000; Ezeigbo, Asuzu, 2012). The overall effect of antidiabetic agents is the lowering of raised blood glucose levels (Lakey, Burridge, Shapiro, 2003). In the present study, MEP and its antidiabetic constituent exhibited significant ($p < 0.05$) antihyperglycemic activity in alloxan-induced diabetic rats. This is an indication of the antidiabetic potential of *P. santalinoides*.

Previous studies have shown that isovitexin, which is the antidiabetic principle isolated from MEP exhibits diverse biological activities which include antioxidant, anti-inflammatory, anti-cancer, anti-hyperalgesia, neuroprotective and hypoglycemic effects (Yao *et al*., 2011). It is reported that vitexin possesses cardioprotective potential (Yao *et al*., 2011). This suggests that its isomer, isovitexin which shares similar pharmacological activities with vitexin may also protect the heart against diabetes-induced cardiovascular disease.

Administered orally, isovitexin significantly reduced postprandial blood glucose both in sucrose-loaded normoglycemic mice and sucrose-induced diabetic rats, thus demonstrating a potential role in the treatment of diabetes mellitus (Yang, He, Lu, 2014). Several studies agree that isovitexin exerts a significant inhibitory effect on α-glucosidase, a key enzyme in carbohydrate digestion which is located in the intestinal cell brush-border surface membrane (Shibano *et al*., 2008; Choo *et al*., 2012; Yang, He, Lu, 2014; Miao *et al*., 2016). Besides α-glucosidase inhibition, Yang, He, and Lu (2014) reported α-amylase inhibition as well as direct interaction with starch by isovitexin. There is also documented evidence of significant antioxidant activity of isovitexin in thiobarbituric acid (TBA), DPPH and ferric-reducing antioxidant power (FRAP) assays (Zhang *et al*., 2014). The antidiabetic activity exhibited by MEP in this study could therefore be attributed to its content of isovitexin, since inhibition of α-glucosidase and α-amylase, as well as enhancing antioxidant defense system constitute possible mechanisms through which *P. santalinoides* may have mediated its antidiabetic activity. Glibenclamide, the reference drug used in this study is a sulfonylurea and causes hypoglycemia through stimulation of insulin secretion (Bakirel *et al*., 2008; Distefano, Watanabe, 2010). It is also possible that in the present study, MEP and its fractions achieved a blood glucose-lowering effect by enhancing the pancreatic secretion of insulin from the existing residual beta cells of the islet.

TABLE II - Effect of graded doses of MEP on FBG of alloxan-induced diabetic rats

Groups/Treatment	Fasting blood glucose (mg/dl)				*P* – value (6 h)
	0 h	1 h	3 h	6 h	
1. Water (5ml/kg)	286.8 ±7.8	274.0 ±13.0	256.8 ±10.0	230.8 ±8.5	-
2. Glibenclamide (2mg/kg)	262.2 ±6.9	162.4 ±1.2*	97.8 ±3.4*	68.0 ±8.5*	0.000

TABLE II - Effect of graded doses of MEP on FBG of alloxan-induced diabetic rats

Groups/Treatment	Fasting blood glucose (mg/dl)				*P* – value (6 h)
	0 h	1 h	3 h	6 h	
3. MEP (50 mg/kg)	312.2 ±7.7	284.6 ±5.1	258.2 ±7.4	222.8 ±9.9	0.051
4. MEP (100 mg/kg)	242.8 ±8.4	206.2 ±4.6*	153.0 ±6.3*	119.2 ±5.9*	0.001
5. MEP (200 mg/kg)	336.2 ±9.1	293.8 ±3.3	211.8 ±4.5*	129.2 ±8.3*	0.000

* $p < 0.05$ when compared with the control (using one-way analysis of variance)

MEP = Methanol extract of *Pterocarpus santalinoides*

FBG = Fasting blood glucose

TABLE III - Antidiabetic activity of MEP fractions

Treatment	Fasting blood glucose (mg/dl)		*P-value* (6h)	Antidiabetic activity (%)
	0 h	6 h		
Water (5 ml/kg)	306.0 ± 15.0	366.7 ± 17.6	-	-
F1 (50 mg/kg)	326.3 ± 30.2	283.3 ± 11.0*	0.030	13.2
F2 ″ ″	237.0 ± 40.5	221.0 ± 36.1	0.104	06.8
F3 ″ ″	297.0 ± 39.6	141.7 ± 14.3*	0.000	52.3
F4 ″ ″	238.7 ± 17.1	73.0 ± 04.7*	0.000	69.4
F5 ″ ″	222.0 ± 28.9	227.0 ± 12.2	0.207	-02.3
F6 ″ ″	227.7 ± 14.1	225.3 ± 34.9	0.213	01.1
F7 ″ ″	255.3 ± 18.2	335.0 ± 39.1*	0.031	-31.2
F8 ″ ″	221.7 ± 06.2	277.7 ± 19.8*	0.022	-22.0
F9 ″ ″	321.7 ± 39.6	242.7 ± 22.2*	0.002	23.2
F10 ″ ″	299.3 ± 38.9	270.7 ± 44.9	0.094	09.6
Glibenclamide (2 mg/kg)	278.0 ± 33.6	26.3 ± 10.6*	0.000	90.5

* $p < 0.05$ when compared with the control (using one-way analysis of variance)

MEP = Methanol extract of *Pterocarpus santalinoides*

FBG = Fasting blood glucose

TABLE IV - Effect of MEP sub-fractions on alloxan-induced diabetic rats

Treatments	Fasting blood glucose (mg/dl) 0 h	6 h	*P* – value (6h)	Antidiabetic activity (%)
Water (5 ml/kg)	230.7 ± 17.6	237.0 ± 28.6	-	-
F4.1 (10 mg/kg)	237.7 ± 39.6	193.3 ± 44.9	0.227	18.7
F4.2 (10 mg/kg)	260.3 ± 39.4	226.3 ± 25.8	0.790	13.1
F4.3 (10 mg/kg)	223.0 ± 06.4	94.3 ± 03.8*	0.001	57.7
F4.4 (10 mg/kg)	270.3 ± 02.3	272.0 ± 02.1	0.327	-00.6
Glibenclamide (2 mg/kg)	226.3 ± 02.7	67.3 ± 02.4*	0.000	70.3

* $p < 0.05$ when compared with control (using one-way analysis of variance)

MEP = Methanol extract of *Pterocarpus santalinoides*

TABLE V - Effect of semi-purified compounds from MEP on alloxan-induced diabetic rats

Treatment	Fasting blood glucose (mg/dl)		*P* – value (6h)	Antidiabetic activity (%)
	0 h	6 h		
Water (5 ml/kg)	275.0 ± 32.4	270.3 ± 14.1	-	-
F4.3.1 (5 mg/kg)	263.7 ± 35.2	203.0 ± 45.3	0.061	23.0
F4.3.2 (5 mg/kg)	241.7 ± 03.8	85.7 ± 03.5*	0.000	64.5
F4.3.3 (5 mg/kg)	251.7 ± 13.2	254.0 ± 16.3	0.619	-00.9
Glibenclamide (2 mg/kg)	232.3 ± 04.3	70.0 ± 02.5*	0.000	69.9

* $p < 0.05$ when compared with control (using one-way analysis of variance)

MEP = Methanol extract of *Pterocarpus santalinoides*

CONCLUSION

In summary, isovitexin, an apigenin 6-C glucoside, was isolated for the first time from the aerial part of *Pterocarpus santalinoides*. The compound significantly ($p < 0.05$) reduced the FBG of alloxan-induced diabetic rats, evoking a 64.5 % antidiabetic activity. The results of this study, therefore, validate the folkloric use of the plant for treating diabetes mellitus and provide insights for lead identification of new antidiabetic molecules.

CONFLICTS OF INTEREST

The authors declare that they have no competing interest

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