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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Iridoid and phenylpropanoid glycosides from the roots of *Lantana montevidensis*

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Abstract A new iridoid glycoside; 6-*O*-*β*-D-xylopyranoside-shanzhiside methyl ester (**1**) along with six known compounds; shanzhiside methyl ester (**2**), lamalbid (**3**), geniposidic acid (**4**), theveside (**5**), verbascoside (**6**) and arenarioside (**7**) were isolated from the roots of *Lantana montevidensis*. The structures of the compounds were determined through 1D and 2D NMR spectroscopic data analysis, HRESIMS, electronic circular dichorism and UPLC-UV/MS method. The total extract, chloroformic (**F1**) and aqueous (**F2**) fractions together with the isolated compounds were tested for their antimicrobial, anti-protozoal, antiplasmodial, anti-inflammatory, monoamine oxidase inhibition and cell viability activities in addition to free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The phenylpropanoid compounds (**6** and **7**) resulted in a potent antioxidant activity. Total methanolic extract together with the aqueous fraction (**F2**) showed decrease in reactive oxidative stress with 57 and 66%, respectively, while the chloroformic fraction (**F1**), together with the total methanolic extract, showed a decrease in iNOS with IC_{50} values 5 and 30 µg/mL, respectively. Compounds **1**, **2**, **3**, **6**, and **7** showed inhibition in the reactive oxidative stress with values 50, 60, 57, 63, and 52%, respectively. Both **F1** and **F2** fractions demonstrated measurable inhibition of MCF-7 breast cancer cell growth, with IC_{50} value 0.3 mg/mL. Compounds **2** and **7** showed mild monoamine oxidase inhibition. None of the tested compounds showed antimicrobial, antiplasmodial or antiprotozoal activity.

Keywords *Lantana* · Iridoids · Phenylpropanoids · Anti-inflammatory · Anticancer · Reactive oxidative stress

Introduction

The Verbenaceae family is comparatively a large family of mainly tropical flowering plants. It comprises about 100 genera and 2000 species distributed in tropics and subtropics, mainly in temperate zone of Southern Hemisphere (Lawrence 1951; Datta 1970 and Core 1955). The Verbenaceae plants are well known for their uses in the traditional medicinal systems of various countries. Quite a number of the plants have been reported to contain bio-active phytochemicals with important pharmacological effects. Among these plants *Lantana montevidensis* (Spreng.) Briq, a plant that is popularly known as "chumbinho". It is a fast spreading, creeping, perennial herb that is native to Brazil and Uruguay. It was introduced as an ornamental plant and is considered to be invasive species in many parts of the world. The plant has various potential medicinal uses

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including treatment of fever, influenza, asthma, and bronchitis (Munir 1996; Nagao et al. 2002; Barreto et al. 2010; Montanari et al. 2011; Sousa et al. 2011). In South America, it is employed in native medicine for the treatment of broncho-pulmonary diseases, headaches and sunstroke (Munir 1996; Nagao et al. 2002; Barreto et al. 2010; Montanari et al. 2011; Sousa et al. 2011). The plant also was subjected to a number of phytochemical studies that included investigation of the volatile oils of the leaves and flowers (Makboul et al. 2013a), and isolation of pentacyclic triterpenoids and flavonoids together with the assessment of different leaf and root extracts and isolated compounds for their biological activities such as free radical scavenging, antimicrobial, antiprotozoal, anti-inflammatory, analgesic, and antipyretic activities (Makboul et al. 2013a, b; Makboul et al. 2014; Mohamed et al. 2016). In the current study, we report herein the isolation of one new iridoid glycoside; 6-*O*-*β*-D-xylopyranoside-shanzhiside methyl ester (**1**), along with six other known compounds: shanzhiside methyl ester (**2**), lamalbid (**3**), geniposidic acid (**4**), theveside (**5**), verbascoside (**6**), and arenarioside (**7**) from the methanolic extract of *L. montevidensis*. The antimicrobial, antiprotozoal, antiplasmodial, anti-inflammatory, anticancer, monoamine oxidase inhibition, and free radical scavenging activities were assessed. Mentioned in the Results and discussion section

Experimental

General procedures

Specific rotations were measured using a Rudolph Research AutoPol IV polarimeter at room temperature; UV spectra were recorded by a Hewlett-Packard 8452 A UV−Vis spectrometer; IR spectra were acquired using Bruker Tensor 27 and MIRacle ATR FT−IR spectrometers (Bruker Optics) and reported in wave number cm^{-1}. All NMR experiments were carried out on a Varian—Mercury AS 400 MHz NMR spectrometer (Varian Inc., USA). The chemical shifts values are reported in ppm (δ) and the coupling constants (J) are presented in Hertz. The spin multiplicities are given as singlet (s), doublet (d), triplet (t), q (quartet), and multiplet (m). High-resolution mass spectra were obtained using an HRESITOFMS spectrometer with the Analyst QS software for data acquisition and processing (Agilent Series 1100 SL, ESI source model #G1969A, Agilent Technologies, Palo Alto, CA, USA). The UPLC used for UPLC-UV/MS method was an Acquiry UPLC™ BEH C18 column (100 mm × 2.1 mm I.D., 1.7 µM) and was used on Waters Acquity UPLC™ system (Waters Corp.) that includes a binary solvent manager, sample manager, heated column compartment, photodiode array (PDA) detector, and single quadrupole detectors (SQD). Column chromatography was carried on Silica gel G60 (60–120 mesh, Merck, Darmstadt, Germany), Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). Analytical TLC was conducted on precoated aluminum sheets silica 60 F254, 0.25 mm (E-Merck, Darmstadt, Germany).

Plant material

The roots of *Lantana montevidensis* (Spreng.) Briq. were collected during the flowering stage in the period of February–November 2013 from the gardens of Assiut University, Assiut, Egypt. The plant was authenticated and identified by the late Prof. N. E. Keltawy, Professor of Ornamental, Horticulture, and Floriculture, Faculty of Agriculture, Assiut University, Assiut, Egypt. A voucher sample (No. 2009 LM) has been deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Extraction and isolation of constituents

The air-dried powdered roots of *L. montevidensis* (1.5 Kg) were exhaustively extracted using 70% MeOH (5L × 4) by maceration at room temperature. The methanolic extract was concentrated under reduced pressure to afford a dark brown viscous residue (112 g) which was suspended in distilled water (200 mL) and partitioned using $CHCl_3$ (1L × 4) to yield two major fractions, the chloroformic fraction **F1** (40 g) and aqueous fraction **F2** (70 g). About 40 g of the aqueous fraction was subjected to Diaion HP-20 CC which was eluted with H_2O then MeOH to yield two fractions F-2-1 (13.2 g) and F-2-2 (24.8 g), respectively. The methanolic subfraction (F-2-2) was subjected to VLC on silica gel (900 g) which was eluted using $CHCl_3$−MeOH mixture in the order of increasing polarities to yield three fractions FA-FC. FA (7.3 g) was subjected to silica gel (200 g) CC using EtOAc−MeOH as eluent in gradient elution. Fractions eluted with EtOAc−MeOH (8:2) were collected and concentrated to yield fraction FA-1 (1.2 g). Fraction FA-1 was furthermore purified using Sephadex LH-20 (100 g) CC using MeOH as eluent to yield two main fractions FA-1-1 which was collected and concentrated to yield compound **2** (50 mg) and fraction FA-1-2 (750 mg). Fraction FA-1-2 was subjected to silica gel (30 g) CC using $CHCl_3$−MeOH as eluent in gradient elution. The fractions eluted with $CHCl_3$−MeOH (8.5:1.5) were collected and concentrated to yield compound **6** (100 mg), while the fractions eluted with $CHCl_3$−MeOH (7.5:2.5) were collected and concentrated to yield compound **7** (33 mg). FB (6.3 g) was subjected to silica gel (200 g) CC using $CHCl_3$−MeOH as eluent in gradient elution. Fractions eluted with $CHCl_3$−MeOH (9:1) were collected and

concentrated to yield compound **4** (9 mg), while the fractions eluted with $CHCl_3$-MeOH (8.5:1.5) collected and concentrated to yield compound **3** and the fractions eluted with $CHCl_3$-MeOH (7.5:2.5) collected and concentrated to yield compound **1** (5 mg). About 1 g of FC was purified using sephadex LH 20 (100 g) CC using MeOH as eluent to afford compound **5** (7 mg).

6-O-β-D-xylopyranoside-shanzhiside methyl ester ***(1)***

Oily colourless residue; $[\alpha]_D^{20}$ −17.996 (c0.05, MeOH); UV (MeOH) λ_{max} 232 nm; IR(film) ν_{max} 3343, 2926, 1700, 1639, 1460 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz,): Aglycone: $\delta = 7.51$ (1H, d, $J = 1.2$ Hz, H-3), 5.77 (1H, d, $J = 2.4$ Hz, H-1), 4.53 (1H, t, $J = 4.4$ & 2.0 Hz, H-6), 3.89 (3H, s, OCH_3), 3.43 (1H, m, H-5), 2.80 (1H, dd, $J = 8.8$ & 2.4 Hz, H-9), 2.10 (1H, d, $J = 14.8$ Hz, H-7α), 1.91(1H, dd, $J = 14.8$ & 4.8 Hz, H-7β), 1.40 (3H, s, CH_3-10), Glucose: $\delta = 4.72$ (1H, d, $J = 7.6$ Hz, H-1'), 3.34–3.46 (4H, *m*, H-2"-H-5"), 4.01 (1H, d, $J = 2.4$ Hz, H-6'a), 3.75 (1H, *m*, H-6'b), Xylose: $\delta = 4.45$ (1H, d, $J = 7.6$ Hz, H-1"), 3.32–3.46 (3H, m, H-2"-H-5"), 3.98 (1H, d, $J = 2.4$ Hz, H-5"a), 3.31(1H, dd, $J = 4$ & 1.6 Hz, H-5"b); ^{13}C NMR (CD_3OD, 100 MHz,): $\delta = 168.8$ (C-11), 153.7 (C-3), 109.7 (C-4), 103.0 (C-1"), 99.9 (C-1'), 95.0 (C-1), 83.4 (C-6), 79.6 (C-8), 78.3 (C-3'), 78.0 (C-5'), 77.7 (C-3"), 74.9 (C-2"), 74.6 (C-2'), 71.6 (C-4"), 71.2 (C-4'), 62.8 (C-6'), 67.0 (C-5"), 52.6 (C-9), 51.7 (OCH_3), 46.0 (C-7), 40.5 (C-5), 25.6 (C-10) HRESIMS *m/z* 573.1554 $[M+Cl]^-$ (calcd. for $C_{22}H_{34}O_{15}Cl$, 573.1573).

Conformational analysis and ECD calculation

Conformational analysis of possible stereoisomer of compound **1** based on NMR data was performed with Schrödinger Macromodel 9.9 (Schrödinger, LLC, New York) employing the OPLS2005 (optimized potential for liquid simulations) force field in MeOH (Macro Model 9.9, Schrodinger LLC, 2012). Two conformers within a 2 kcal/mol energy window from the global minimum were selected for each stereoisomer, based on Boltzmann distribution as calculated from Schrödinger software. The output files for these conformers were prepared by Avogadro software for Gaussian 09 calculation, geometrical optimization, and energy calculation was applied on these conformers at B3LYP/6-31G (Frisch et al. 2010). Vibrational evaluation was done at the same level to confirm minimal excitation energy (denoted by wavelength in nm), rotatory strength dipole, and dipole length that were calculated in MeOH by TD-DFT/B3LYP/6-31G performed by the Gaussian 09 software package (Frisch et al. 2010). ECD curves were obtained in the SpecDis 1.62 program (Bruhn et al. 2014).

Determination of sugar configuration

The sugar composition and its configuration were determined according to the reported method by Wang and coworkers (Wang et al. 2012). In brief, 1 mg of compound **1** was hydrolyzed using 200 µL of 2 M HCl at 90 °C for 2 h. After hydrolysis, the reaction mixture was neutralized with 200 µL of 9M NH_4OH and dried with high purity N_2 gas. Monosaccharide standards, including D-(+)-xylose, L-(-)-xylose, L-rhamnose, D-(+)-glucose, and L-(-)-glucose together with the hydrolysis products of compound **1** were derivatized, respectively. The final products of each sample were analyzed by UHPLC-UV-MS. The mobile phase consisted of water with 0.05% formic acid (A) and acetonitrile/methanol/isopropanol (50:25:25, v/v) with 0.05% formic acid (B). Analysis was performed using gradient elution at a flow rate 0.3 mL/min: 14% B to 16.5% B in 22 min, and increasing B to 100% B in the following 0.5 min. The PDA detection wavelength was 254 nm and ESI source of SQD was used in positive mode. The configuration of the sugar unit was determined by comparing with reference standards, retention time and mass spectra.

Biological activities

Free radical scavenging assay

The free radical scavenging activity of compounds **1–7** was measured by spectrophotometric method (Qureshi et al. 2010). One milli litre of ethanolic solutions of the test compound (50 and 100 µg/mL) was mixed with 1 mL of the ethanolic solution of DPPH (200 µM) (SigmaAldrich, St. Louis, MO, USA). Similarly, 1 mL of ethanolic of ascorbic acid (50 and 100 µg/mL) was mixed with 1 mL DPPH solution and used as a positive control. One mL of ethanol alone was used as a blank. After mixing, all the solutions were incubated in dark for 20 min and absorbance was measured at 517 nm (Qureshi et al. 2010). The experiments were performed in triplicate and average percent scavenging activity was calculated as follows: Scavenging % = (Absorbance of control − Absorbance of test compound) / Absorbance of control

Reporter gene assay for the inhibition of NF-κB activity

Human chondrosarcoma (SW1353) cells were cultured in DMEM/F12 medium supplemented with 10% FBS, 100 U/mL penicillin G sodium, and 100 µg/mL streptomycin at 37 °C in an atmosphere of 5% CO_2 and 95% humidity. The cells were transfected with NF-κB luciferase plasmid construct were plated in 96-well plates at a density of 1.25×10^5 cells/well. After 24 h, cells were treated with the test compounds, and after incubating for 30 min,

phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) (70 ng/mL) was added and further incubated for 6–8 h. Luciferase activity was measured using a Luciferase Assay Kit (Promega, Madison, WI, USA). Percent decrease in luciferase activity was calculated relative to the vehicle control. Parthenolide (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control. (Ma et al. 2007)

Assay for the inhibition of iNOS activity

Mouse macrophages (RAW264.7) were cultured in phenol red-free RPMI medium supplemented with 10% bovine calf serum, 100 U/mL penicillin G sodium, and 100 µg/mL streptomycin. Cells were seeded in 96-well plates (1×10^5 cells/well) and incubated for 24 h for a confluency of ≥75%. The cells were treated with the test compounds, and after 30 min, lipopolysaccharide (LPS) (Sigma-Aldrich) (5 µg/mL) was added and further incubated for 24 h. The activity of iNOS was determined in terms of the concentration of NO by measuring the level of nitrite in the cell culture supernatant using Griess reagent (Sigma-Aldrich, St. Louis, MO, USA). Percent inhibition of nitrite production by the test compound was calculated in comparison to the vehicle control. IC_{50} values were obtained from dose−response curves. Parthenolide (Sigma-Aldrich, St. Louis, MO, USA) was used as the positive control (Zaki et al. 2013).

Assay for the inhibition of cellular oxidative stress

The cellular antioxidant activity was measured in HepG2 cells according to a method previously described (Wolfe and Rui 2007). The method measures the ability of test compounds to prevent intracellular generation of peroxyl radicals in response to 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP, Sigma-Aldrich), a generator of peroxyl radicals. The HepG2 cells were seeded in the wells of a 96-well plate at a density of 6×10^4 cells/well and incubated for 24 h. The medium was then removed and cells were washed with PBS before treating with the test compounds diluted in serum-free medium containing 25 µM 2′,7′ dichlorofluorescin diacetate (DCFH-DA, Invitrogen, Carlsbad, CA, USA) for 1 h. After removing the medium, the cells were treated with 600 µM ABAP and the plate was immediately placed on a SpectraMax plate reader for kinetic measurement every 5 min for 1 h (37 °C, excitation at 485 nm and emission at 538 nm). Quercetin (SigmaAldrich, St. Louis, MO, USA) was included as the positive control. The area under the curve (AUC) of fluorescence vs. time was used to calculate cellular antioxidant activity (CAA) units from the following equation: CAA unit = 100−[(AUC sample/AUC control) × 100].

Antimicrobial screening

The extracts and the isolated compounds were tested for antibacterial activity against *Staphylococcus aureus* (ATCC 29213), methicillin resistant *S. aureus* (ATCC 33591) (MRSA), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), *Mycobacterium intracellulare* (ATCC 23068) and antifungal activity against *Candida albicans* (ATCC 90028), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *Cryptococcus neoformans* (ATCC 90113), *Aspergillus fumigatus* (ATCC 204305). Ciprofloxacin and Amphotericin-B (ICN Biomedicals, Aurora, Ohio) were used as positive control for bacteria and fungi, respectively (Bharate et al. 2007).

Antiprotozoal screening

Leishmania donovani promastigote, *L. donovani* amastigote, *L. donovani* amastigote/THP1 cells and *Trypanosoma brucei brucei* strains were used for antiprotozoal screening. The in vitro anti-leishmanial and anti-trypanosomal assays were performed on cell cultures of *L. donovani* promastigotes, axenic amastigotes, THP1-amastigotes, and *Trypanosoma brucei* trypomastigotes by Alamar Blue assays, as described earlier (Makler and Hinrichs 1993). Difluoromethylornithine (DFMO, ICN Biomedicals, Aurora, Ohio) was used as a positive control.

Antiplasmodial screening

All isolates were tested against chloroquine-sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *P. falciparum* by measuring plasmodial LDH activity, as described earlier (Makler and Hinrichs 1993), in addition to the VERO mammalian cell line as an indicator of general cytotoxicity. The selectivity indices (SI), and the ratio of VERO IC_{50} to either D6 or W2 IC_{50}, were calculated. IC_{50} values were calculated using the XLfit software. Chloroquine (ICN Biomedicals, Aurora, Ohio) was used as a positive control.

Monoamine oxidase inhibition (MAOI) assay

Recombinant human MAO-A and MAO-B were used to investigate the effect of compounds using the kynuramine deamination assay adapted for 96-well plates (Samoylenko et al. 2010). The IC_{50} values were determined using range of concentrations (0.001 to 100 µM). All experiments were carried out in duplicate. Phenelzine, cloryline, and deprenyl (Sigma Chemical, St Louis, MO, USA) were used as positive controls.

Cell viability assay

MCF-7 breast cancer cells were maintained in exponential growth conditions in DMEM media supplemented with 10% FBS and 1% penicillin/streptomycin at 37 °C in a humid environment with 5% CO_2 until used for experiments. To measure compound effects on cell viability, 5×10^3 cells were plated in 90 µL of media in 96-well plates. Cells were allowed to attach overnight. The following day, 10 µL of a 10× compound stock as added to the cells in doses covering a 5–6 log range (0–100 µM for pure compounds and 0–1 mg/mL for whole extracts) and plates were incubated at 37 °C for 72 h. Twenty micro litre of an 3-(4,5-dimethylthiazol-2-yl)-5(3carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution (2 mg/mL in PBS; Promega, Madison, WI) supplemented with 5% Phenazine methosulphate was added and incubated for 2–4 hrs before absorbance was measured at 490 nm. Background was subtracted from the absorbance (compounds at each concentration plus media and MTS without cells), which were further normalized to % of untreated control. Cells were treated in triplicate. As applicable, IC_{50} values were determined by non-linear regression with GraphPad Prism software (La Jolla, CA).

Results and discussion

The aqueous fraction **F2** was subjected to a series of chromatographic separation steps to yield one new iridoidal glucoside (**1**) and six known compounds (**2–7**) (Fig. 1).

Compound **1** was obtained as a colorless oily residue. Its molecular formula was established as $C_{22}H_{34}O_{15}$ which is consistent with the molecular ion $[M+Cl]^-$ at *m/z* 573.1554 (calcd.: 573.1573) found in the negative mode HRESIMS spectrum and this was furthermore confirmed by spectral data. It was found to have a great similarity to compound **2** which was identified as shanzhiside methyl ester, except for the presence of an additional pentose sugar moiety. The structure of the compound and the placement of the two sugars were established using the available 1D (^{1}H- & ^{13}C-NMR) and 2D (HMQC, HMBC, ^{1}H-^{1}HCOSY & ROESY) NMR spectral data, in which all the data are given in the supplementary material Appendix 1(Figs. A1–A7). The ^{1}H- and ^{13}C-NMR spectra displayed signals characteristic for the iridoidal nucleus presented by an enol ether unit [δ_H 7.51, *d*, $J = 1.2$ Hz (H-3)/ δ_C 153.7 (C-3) & 109.7 (C-4)] with a carbomethoxy group [δ_H 3.80, s (OCH_3)/δ_C 51.7 (OCH_3) & δ_C 168.8 (C-11)], a dioxygenated methine [δ_H 5.77, *d*, $J = 2.4$ Hz (H-1)/δ_C 95.0 (C-1)], two aliphatic methines [δ_H 2.80, *dd*, $J = 8.8$ & 2.4 Hz (H-9)/δ_C 52.6 (C-9) and δ_H 3.43, *m* (H-5) / 40.5 (C-5)] and a methyl group [δ_H 1.40, s (H-10)/δ_C 25.6 (C-10)]. Also the spectra displayed two anomeric signals, one for the common glucose moiety attached to C-1 of the iridoidal skeleton at δ_H 4.72, *d*, $J = 7.6$ Hz (H-1′)/δ_C 99.9 (C-1′) and the other at δ_H 4.45, *d*, $J = 7.6$ (H-1″)/δ_C 103.0 (C-1″) was for the additional sugar unit, in which the sugar signals was found to be in agreement with those of xyloside (Breitmaier and Voelter 1990). The downfield shift of oxygenated methine proton H-6 to δ_H 4.53 and carbon C-6 to δ_C 83.4 highly recommends the placement of the xylose sugar moiety at C-6. This was further confirmed by the HMBC correlations (Fig. 2) which showed the presence of correlations between H-6 (δ_H 4.53) with C-1″ (δ_C 103.0), C-4 (δ_C 109.7), C-5 (δ_C 40.5), C-7 (δ_C 46.0), C-8 (δ_C 79.6) and C-9 (δ_C 52.6) and ^{1}H-^{1}H COSY correlations (Fig. 2) which showed correlations between H-6 (δ_H 4.53) with H-5 (δ_H 3.43, m), H-5 with H-9 (δ_H 2.80) and H-9 with H-1 (δ_H 5.77). The ROESY spectrum showed correlations between H-9 (δ_H 2.80, *dd*, $J = 8.8$ & 2.4 Hz) with H-5 (δ_H 3.43, m) and H-7β (δ_H 1.91, *dd*, $J = 14.8$ & 4.8 Hz) indicated that H-5 and H-9 are both β-oriented while correlations between CH_3-10 (δ_H 1.40, s) with H-7α (δ_H 2.10, *d*, $J = 14.8$ Hz), H-6 (δ_H 4.53, *t*, $J = 4.4$ & 2 Hz) and H-1 (δ_H 5.77, *d*, $J = 2.4$ Hz) supported that H-1, H-6, and CH_3-10 are all α-oriented. The stereochemistry of the compound was further more confirmed using the electronic circular dichroism in which the experimental ECD spectrum showed a peak of negative cotton effect at 250 nm and positive at 227 nm. The ECD spectrum (Fig. 3) of the possible stereoisomer of compound **1** was calculated to match with the experimental one. Also, the identity of the present sugar moieties as well as their absolute configurations was determined using UHPLC-UV/MS method. The hydrolysate of the compound **1** was treated following the reported procedures (Wang et al. 2012). From the chromatograms and mass spectra of the derivative of compound **1**, D-glucose and D-xylose was easily identified. By the comparison of the spectral data of compound **1** (Table 1) with the reported analogues (Dinda et al. 2007), compound **1** was identified as 6-*O*-β-D-xylopyranoside-shanzhiside methyl ester.

The known compounds were identified by analyzing the spectroscopic data (1D and 2D NMR) and comparing their data with those in the literature to be shanzhiside methyl ester (**2**) (Yue et al. 2013), lamalbid (**3**) (Kobayashi et al. 1986), geniposidic acid (**4**) (Güvenalp et al. 2006), theveside (Ford and Bendall 1980), verbascoside (**6**) (Akademir et al. 2004), and arenarioside (**7**) (Andary and Private 1985), respectively.

The phenolic constitute is considered one of the major group of compounds acting as primary antioxidants or free radical terminators and this activity was assessed for the isolated compounds (**1–7**) using the DPPH method. The DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH changing its color from deep violet into yellow color which was measured using UV spectrophotometer (Padmanabhan and Jangle 2012). All the

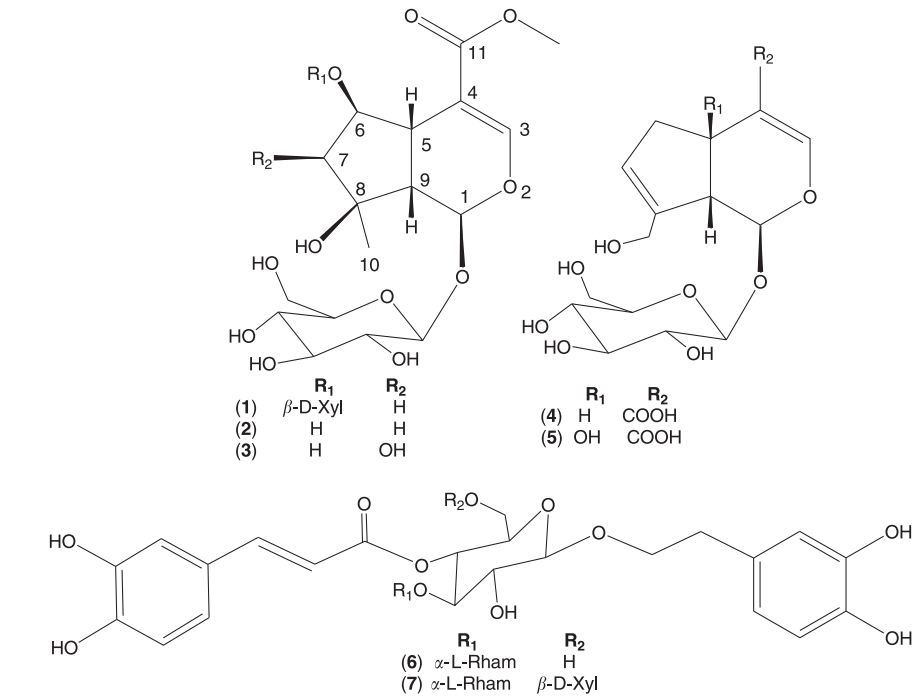

Fig. 1 List of isolated compounds

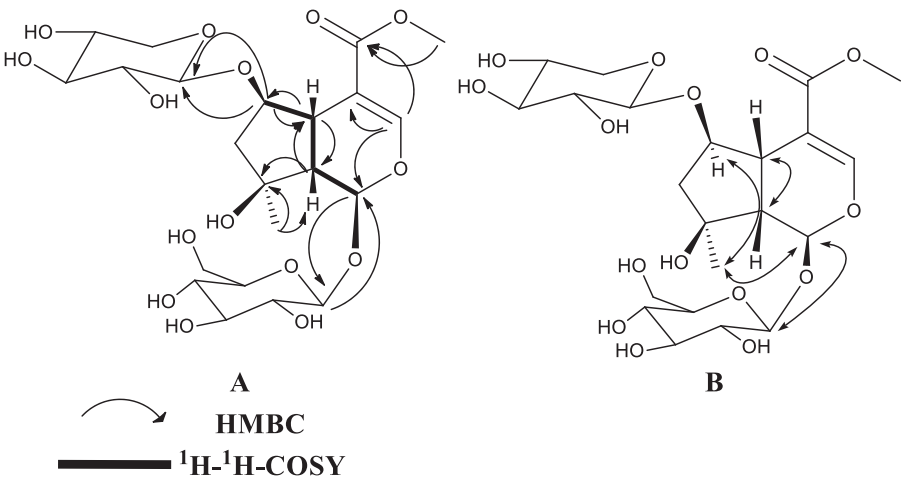

Fig. 2 **a** Important HMBC and ^{1}H-^{1}H COSY correlations of compound **1**. **b** Important ROESY correlations of compound **1**

isolated compounds were tested for radical-scavenging activity against DPPH organic free radicals. The results were exhibited in the form of percent of inhibition (Table 2), in which compounds **6** and **7** showed radical scavenging activity with value 100%, while the iridoid glycosides compounds **1–5** showed radical scavenging activity with values 73.80, 63.79, 66.56, 57.99, and 72.53% in concentration 100 µg/mL.

Inflammation is considered as a risk factor for several types of cancer, as well as a contributing factor in obesity and metabolic disorders. The activation of NF-κB in response to pro-inflammatory signals is associated with many diseases caused by unregulated inflammation. Since NF-κB is highly activated at the sites of inflammation in diverse diseases, the compounds that can suppress NF-κB activation have potential as anti-inflammatory agents (Tak and Firestein 2001). Excessive generation of nitric oxide (NO) and reactive oxygen species (ROS) also contributes significantly to the progress of inflammation and subsequent development of metabolic syndrome, characterized by obesity, diabetes, and cardiovascular disease. Inhibition of inducible nitric oxide synthase (iNOS) can reduce the intracellular NO production. Hence, NF-κB, iNOS, and ROS have been considered as important targets for inflammation (Ko and Auyeung 2013; Zhao et al. 2014). The total methanolic extract and the aqueous fraction **F2** showed a decrease in the oxidative stress ROS with values 57 and 66% respectively, while the chloroformic fraction

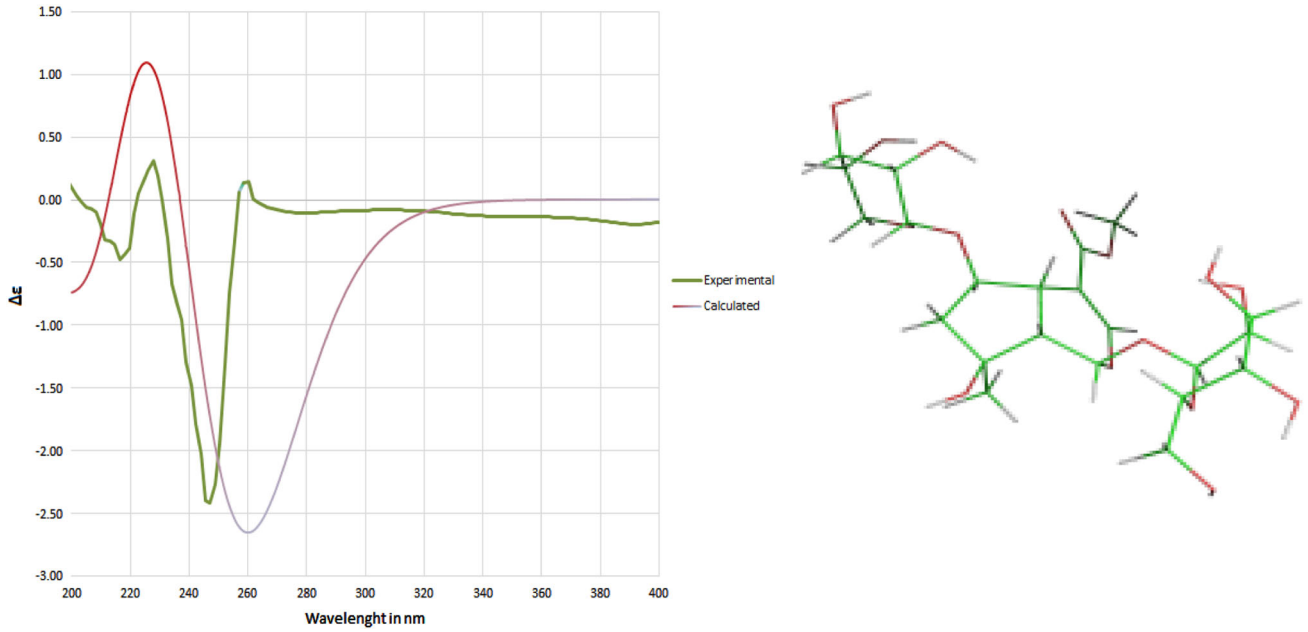

Fig. 3 The experimental and calculated ECD spectra of compound **1**

Table 1 ^{1}H- and ^{13}C-NMR spectral data for compound **1** (400 and 100 MHz) in CD_3OD

No.	δ_C	Type	δ_H
1	95.0	CH	5.77, d, $J = 2.4$ Hz
2	–	–	–
3	153.7	CH	7.51, d, $J = 1.2$ Hz
4	109.7	C	–
5	40.5	CH	3.43, m
6	83.4	CH	4.53, *t*, $J = 4.4$ & 2 Hz
7	46.0	CH_2	1.91, *dd*, $J = 14.8$ and 4.8 Hz, H-7β
			2.10, *d*, $J = 14.8$ Hz, H-7α
8	79.6	C	–
9	52.6	CH	2.80, *dd*, $J = 8.8$ & 2.4 Hz
10	25.6	CH_3	1.40, *s*
11	168.8	C	–
1'	99.9	CH	4.72, d, $J = 7.6$ Hz
2'	74.6	CH	3.45, m
3'	78.3	CH	3.46, m
4'	71.2	CH	3.40, m
5'	78.0	CH	3.34, m
6'	62.3	CH_2	4.01, d, J = 2.4 Hz
			3.75, m
1"	103.0	CH	4.45, d, $J = 7.6$ Hz
2"	74.9	CH	3.32, m
3"	77.7	CH	3.46, m
4"	71.6	CH	3.41, m
5"	67.0	CH_2	3.98, d, J = 2.4 Hz
			3.31, dd, J = 4 and 1.6 Hz
OCH_3	51.7	CH_3	3.80, s

Table 2 Free radical scavenging activity of the isolated metabolites **1–7**

Compounds	Antioxidant %	
	100 µg	50 µg
1	73.80%	54.50%
2	63.69%	45.42%
3	66.56%	50.69%
4	57.99%	37.45%
5	72.53	46.87%
6	100%	100%
7	100%	100%
Ascorbic acid	100%	100%

F1 didn't show any activity. Also, compounds **1**, **2**, **3**, **6**, and **7** decreased cellular oxidative stress by inhibiting ROS generation values 50, 60, 57, 63, and 52%, respectively, with comparison to the positive control quercetin which showed decrease in the oxidative stress with value 72%. Compounds **1–7**, as well as the aqueous fraction **F2** from which these compounds were isolated, did not show any inhibition of iNOS as they did not affect cellular nitric oxide levels in LPS treated macrophages. Furthermore, the increase in transcriptional activity of NF-κB in PMA-treated cells was also not suppressed by any of the tested compounds (**1–7**). Hence, these results indicated that their anti-inflammatory effect is not mediated by inhibition of iNOS or NF-κB activity. However, the total methanolic extract and the chloroformic fraction **F1** showed inhibition to the

iNOS with IC_{50} values 30 and 5 µg/mL, respectively, using Parthenolide as a positive control with IC_{50} value 0.26 µg/mL. This may be attributable to the high content of oleanolic acid, oleanonic acid, and 25-hydroxy oleanolic acid which were isolated before from the chloroformic fraction (**F1**) of the methanolic extract of *Lantana montevidensis* roots (Mohamed et al. 2016) and showed in this study inhibition in iNOS with IC_{50} values 6.9, 7.4, and 9.0 µg/mL in comparison to parthenolide which showed IC_{50} value 0.26 µg/mL. Also, oleanonic acid and 25-hydroxy oleanolic acid showed inhibition toward NF-κB with IC_{50} values 49 and 10, in comparison to parthenolide which showed IC_{50} value 1.0 µg/mL. All the potential anti-inflammatory activities of the extracts, compounds **1–7**, oleanolic acid, oleanonic acid, and 25-hydroxy oleanolic acid are listed in Table 3.

Both the chloroformic and aqueous fractions, **F1** and **F2** respectively, demonstrated measurable inhibition of MCF-7 breast cancer cell growth, with IC_{50} value 0.3 mg/mL (Fig. 4). None of the pure compounds inhibited cell growth

Table 3 Potential anti-inflammatory activity of the extracts and compounds **1**−**7**

Compounds	Stock conc. (µg/mL)	inhibition of iNOS IC_{50} (µg/mL)	inhibition of NF-κB IC_{50} (µg/mL)	inhibition of ROS generation* %
1	5	NA	NA	50
2	5	NA	NA	60
3	5	NA	NA	57
4	5	NT	NT	–
5	5	NT	NT	–
6	5	NA	NA	52
7	5	NA	NA	63
Oleanolic acid	5	6.9	NA	NA
Oleanonic acid	5	7.4	49	NA
25-Hydroxy oleanolic acid	5	9	10	NA
Total methanolic extract	20	30	NA	57
Chloroform Fr. (**F1**)	20	NA	NA	66
Aqueous Fr. (**F2**)	20	5	NA	NA
Parthenolide	–	0.26	1.0	NT
Quercetin	–	NT	NT	72

NA no activity, *NT* not tested

*at 125 µg/mL for pure and 500 µg/mL for extract

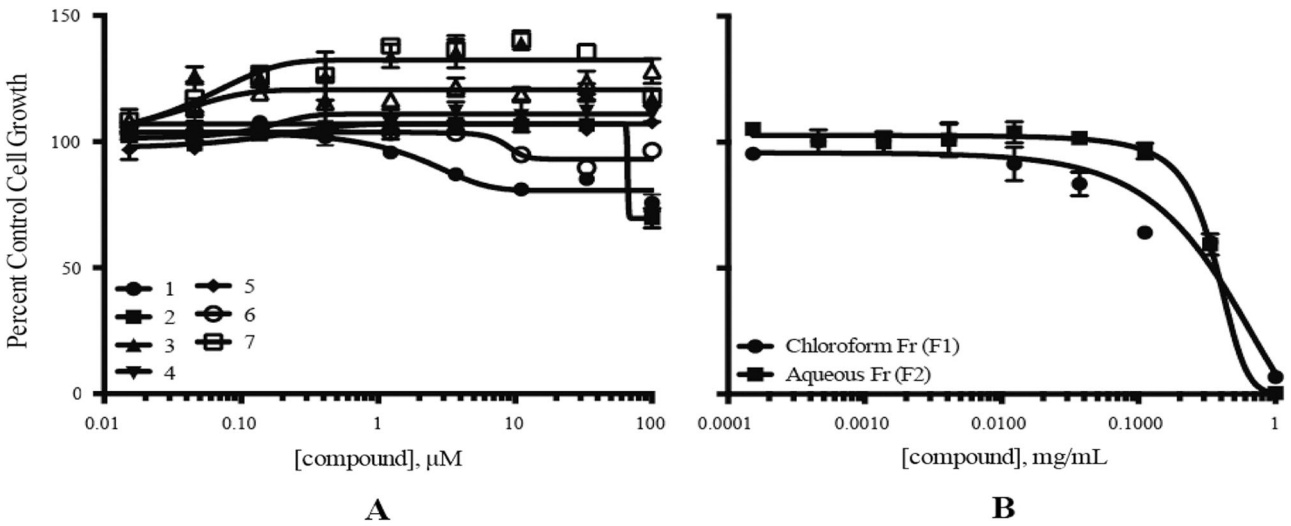

Fig. 4 Effect of pure compounds **1–7 a** and the chloroformic (**F1**) and aqueous (**F2**) fractions **b** on the viability of MCF-7 breast cancer cells

by 50% or more, and consequently IC_{50} values could not be determined.

Compounds **2** & **7** showed mild inhibition for monoamine oxidase. Compound **2** showed inhibition of MAO-A and B with IC_{50} values 39.48 and 27.77 μM, while compound **7** inhibited monoamine oxidase MAO-B with weak potency with IC_{50} value 65.02 μM using phenelzine as a reference with IC_{50} values 0.27 and 0.14 μM in inhibition of MAO-A and MAO-B, respectively. However compounds **3**, **5**, and **6** showed no inhibition to monoamine oxidase and compounds **1** and **4** were not tested due to lack of amount. More information is available in the supportive material appenddix 1 (Table A1).

None of the tested compounds showed any antimicrobial, antiplasmodial or antiprotozoal activities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interest.

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