Med Chem Res
DOI 10.1007/s00044-017-1973-z

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Phytochemical characterization and biological activity of secondary metabolites from three *Limonium* species

Anastassiya V. Gadetskaya[1,2] · Shaymaa M. Mohamed[1,3] · Amer H. Tarawneh[4,5] · Nesma M. Mohamed[1,3] · Guoyi Ma[1] · Boris N. Ponomarev[6] · Galiya E. Zhusupova[2] · Charles L. Cantrell[7] · Stephen J. Cutler[4] · Samir A. Ross[1,4]

Received: 23 October 2016 / Accepted: 26 June 2017
© Springer Science+Business Media, LLC 2017

Abstract The comparative phytochemical constituents of three *Limonium* species *Limonium myrianthum*, *Limonium leptophyllum*, and *Limonium gmelinii* afforded a new compound (2*R*,3*S*)-2,3,4-trihydroxy-2-methylbutyl gallate (**1**) and twenty known compounds (**2–21**). Each species displayed different profiles in their phytochemical constituents. The isolated compounds (**1–21**) were evaluated for antifungal, antimalarial, and antitrypanosomal activities. Compound **1** showed good activity against chloroquine-resistant and chloroquine-sensitive strains of malaria, while compound **5** displayed moderate antimalarial activity. Compound **14** showed a significant activity against *Trypanosoma brucei*.

Keywords Plumbaginaceae · *Limonium* · Antifungal · Antimalarial · Antitrypanosomal

Electronic supplementary material The online version of this article (doi:10.1007/s00044-017-1973-z) contains supplementary material, which is available to authorized users.

✉ Samir A. Ross
sross@olemiss.edu

1 National Center for Natural Products Research, University of Mississippi, University, MS 38677, USA

2 School of Chemistry and Chemical Technology, Al-Farabi Kazakh National University, Almaty 050040, Kazakhstan

3 Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt

4 Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, USA

5 Department of Chemistry, Tafila Technical University, P.O. Box 179, Tafila, Jordan

6 Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

7 Natural Products Utilization Research Unit, USDA-ARS, University, MS 38677, USA

Introduction

Plant tissues typically contain a diverse complement of secondary metabolites (Keinänen et al. 1999 and Julkunen–Tiitto 1989) that provide protection against various biotic and abiotic hazards (Berenbaum 1995 and Wink 2003). Chemical similarities are commonly used to infer phylogenetic relationships among plant taxa (Downie and Palmer 1994). The genus *Limonium* Mill (Plumbaginaceae) includes over 300 species world-wide, of which 18 are found in Kazakhstan (Zhusupova 2007). Many of these *Limonium* species have been widely used in folk medicine as rich sources of bioactive compounds (Lin and Chou 2000; Tang et al. 2014; Yang et al. 1987; Liebezeit et al. 1999 and Rastogi et al. 2016). More recently, several species of the *Limonium* genus have been shown to have potential scientific, pharmacological, and medical uses (Murray et al. 2004; Aniya et al. 2002; Yuh-Chi et al. 2002 and Kandil et al. 2000). For example, *L. brasiliense* has anti-inflammatory and antibacterial properties (Murray et al. 2004), *L. wrightii* can be used to treat arthritis and fever (Aniya et al. 2002), *L. tetragonum* and *L. sinense* have antiviral properties (Yuh-Chi et al. 2002), and *L. axillare* and *L. californicum* have antibacterial and cytotoxic activity (Kandil et al. 2000). *L. gmelinii*, one of the *Limonium* species present in abundance throughout Kazakhstan, has been studied extensively for its potential pharmacological properties (Kozhamkulova et al. 2010; Korul'kina et al. 2004 and Zhusupova 2006). Another Limonium species

Published online: 12 July 2017

widely available in Kazakhstan, *L. myrianthum*, is an herbaceous plant with small violet flowers found in saline marshes throughout the country (Zhusupova 2007).

Previous phytochemical and biological studies on the *Limonium* genus demonstrated the presence of different classes of biologically active compounds, such as polysaccharides, tannins, alkaloids, flavonoids, terpenes, aliphatic compounds, amino acids, and minerals (Medini et al. 2014 and Gadetskaya et al. 2015).

As a part of our search for novel, plant-derived biological agents, the phytochemical constituents of *L. myrianthum* (Schrenk) Kuntze, *L.leptophyllum* (Schrenk) O. Kuntze, and *L. gmelinii* (Willd.) Kuntze were characterized. Herein, we report the isolation of a new natural compound from a *L. leptophyllum*, compound **1** (Fig. 1). Also found in this *Limonium* species are emodin (**2**), bergenin (**3**), and mahuannin B (**4**), 7-*O*-galloyl-D-sedoheptulose (**5**), arbutin (**6**), gallic acid-4-*O*-β-D-glucoside (**7**), myricetin (**8**), and myricetin-3-*O*-L-rhamnopyranoside (**9**). Compounds **8** and **9** were found in all investigated species. Another ten known compounds, 3,5,7,3′,4′,6′-hexahydroxyflavone (**10**), myricetin-3-*O*-L-arabinoside (**11**), myricetin-3-*O*-β-D-glucopyranoside (**12**), epigallocatechin-3-*O*-gallate (**13**), myricetin 3-*O*-(6″-galloylglucoside) (**14**), myricetin 3-*O*-(2′′-*O*-galloyl)-β-D-glucopyranoside (**15**), 1-galloyl-β-D-glucopyranoside (**16**), melitriose (**17**), β-sitosterol-3-glucoside (**18**), and β-sitosterol (**19**) were isolated from *L. myrianthum* (Fig. 1). While **11**, **12**, **14**, **15,** and **18** are reported for the first time from this species. Quercetin-3-*O*-α-L-rhamnopyranoside (**20**) and myricetin 3-*O*-(4″-*O*-galloyl)-α-rhamnopyranoside (**21**) were isolated only from *L. gmelinii* and were not found in the other investigated species. Compounds **14** and **15** were also found to be common to both *L. myrianthum* and *L. gmelinii*. In this study, all isolated compounds were evaluated for their antimalarial, antileishmanial, antimicrobial, and antitrypanosomal activities.

Materials and methods

Plant material and Extraction

The aerial part of the *L. leptophyllum* (Schrenk) O. Kuntze plant was collected near Balkhash Lake during its flowering stage on 24th July 2004, and identified by the Botanical Garden at the Institute of Botany and Phytointroduction in Almaty, Kazakhstan. The voucher specimen number of the sampled plant is 6351/18. The aerial part was air-dried followed by grinding in a Willey–Mill plant grinder. The ground plant material (260 g) was extracted at room temperature using methylene chloride (1.5 L) and ethanol (1.9 L), which provided 6.7 g and 10.5 g, of the crude extracts, respectively. Further extraction with water (2.3 L) provided 16.2 g of extract after lyophilization.

The *L. myrianthum* (Schrenk) Kuntze plant was collected from South Kazakhstan in August 2010 during its flowering stage and identified at the Biological Department at

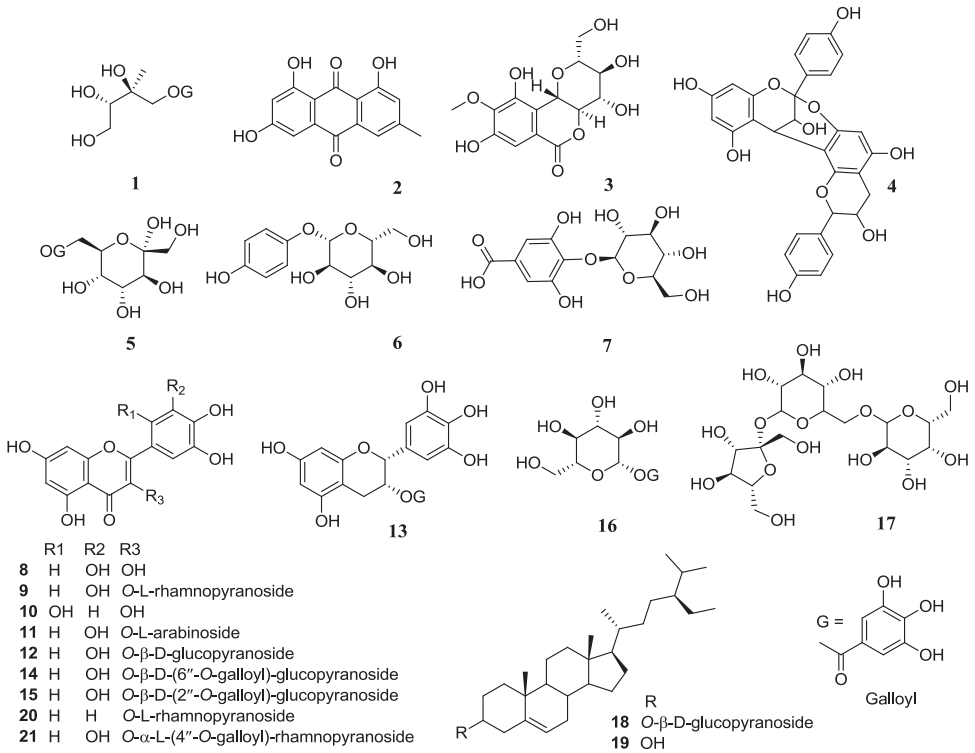

Fig. 1 Chemical structures of isolated compounds (**1–21**)

Al-Farabi Kazakh National University. A voucher specimen of the aerial part (6351) and the roots (359) of the plants were deposited at the Herbarium of the Institute of Botany and Phytointroduction in Almaty, Kazakhstan. Ground plant materials from the aerial part (180 g) and roots (300 g) were separately and sequentially extracted by maceration at room temperature using three solvents of different polarities, hexane (0.6 L), acetone (0.9 L), and methanol (1.0 L). The crude methanol extract from the aerial part of the plant (1.57 g) and acetone (23.40 g) and methanol (38.42 g) extract from the roots were subjected to further fractionation.

The aerial part of the *L. gmelinii* (Willd.) Kuntze plant was collected during its flowering period (September 2012) in the Almaty region of Kazakhstan and identified at the Institute of Botany and Phytointroduction in Almaty, Kazakhstan with the voucher specimen number 154958. Air-dried ground plant material (900 g) was extracted with methanol (3.1 L), which provided 58 g of the crude extract. Twenty grams of concentrated extract were dissolved in 100 ml water and successively partitioned by liquid-liquid extraction (3 times) with ethyl acetate (200 ml each). The ethyl acetate extract was 3.2 g.

Isolation

The ethanolic extract of the aerial part of *L. leptophyllum* (4.5 g) was subjected to Diaion HP-20 column chromatography fractionation to give two main fractions, an aqueous fraction (A) (2.14 g) and a methanolic fraction (B) (2.3 g). The Diaion aqueous fraction (A) (2.14 g) was subjected to MN-polyamide-SC-6 (85 g) column chromatography which was eluted with water then with water-methanol systems gradually decreasing in polarity to give seven subfractions (A1–A7). A1 (130 mg) was subjected to Sephadex LH-20 (25 g) column chromatography which was eluted with methanol to give compound **6** (30 mg). A3 (90 mg) was subjected to Sephadex LH-20 (15 g) column chromatography which was eluted with methanol to produce compound **5** (12 mg). A4 (70 mg) was subjected to Sephadex LH-20 (15 g) column chromatography which was eluted with methanol to give compound **7** (5 mg). A6 (150 mg) was further purified using Sephadex LH-20 (25 g) column chromatography which was eluted with methanol-water (4:1) to give compounds **8** and **9** (60 mg). The Diaion methanolic fraction (B) (2.3 g) was subjected to MN-polyamide-SC-6 (100 g) column chromatography which was eluted with water then with water-methanol systems gradually decreasing in polarity to give five subfractions (B1–-B5). B1 (240 mg) was subjected to Sephadex LH-20 (25 g) column chromatography which was eluted with methanol to produce compound **1** (70 mg). B3 (120 mg) was subjected to Solid Phase Extraction (SPE) Silica gel (5 g) column chromatography which was eluted with dichloromethane-methanol 9:1 to give compound **2** (6.5 mg). B4 (60 mg) was subjected to SPE Silica gel (5 g) column chromatography which was eluted with dichloromethane-methanol 14:1 to produce compound **3** (20 mg). B5 (700 mg) was subjected to SPE RP-C18 (10 g) column chromatography which was eluted with water-methanol gradient elution to produce compound **4** (30 mg).

The acetone extract of *L. myrianthum* root (21.89 g) was chromatographically fractionated on a silica gel column stepwise from dichloromethane to methanol, yielding seven fractions: dichloromethane (C1), 4:1 dichloromethane-methanol (C2), 3:1 dichloromethane-methanol (C3), 7:3 dichloromethane-methanol (C4), 6:4 dichloromethane-methanol (C5), 1:1 dichloromethane-methanol (C6), and methanol (C7). C2 (220 mg) was subsequently chromatographed on a silica gel column initially with hexane and stepwise elution to ethyl acetate then to methanol yielding 59 subfractions (C2/1–59). The subfractions C2/23–24 (25.2 mg) were rechromatographed using Sephadex LH-20 eluted with methanol to furnish compound **19** (25 mg). The subfractions C2/46-59 (599 mg) were rechromatographed using SPE chromatography under vacuum eluted with chloroform–methanol (5, 10, 15, 20, 30, 40, 50, 100%) to furnish compound **18** (18.3 mg). C3 (3.1 g) was rechromatographed using High-Performance Flash Chromatography with silica gel cartridges (KP-SIL, 10 g, 40–63 μm, 60 Å, Biotage SNAP), eluted with 100% dichloromethane, dichloromethane-methanol (10, 20, 40, 50%) and 100% methanol yielding 38 subfractions (C3/1–38). Subfractions C3/25–26 were identified as compound **9** (100 mg). The combined subfractions C3/16-18 (910 mg) were rechromatographed on Sephadex LH- 20 eluted with methanol to yield compound **15** (297 mg), compound **11** (66 mg) and compound **13** (133 mg). C4 (1 g) was chromatographed on Sephadex LH-20 eluted with methanol (100%) to yield compound **10** (201 mg). C5 (1 g) was rechromatographed by Sephadex LH-20 column elution methanol yielding compound **17** (158 mg).

The concentrated methanol extract obtained from the *L. myrianthum* root (1.24 g) was fractionated on a Sephadex LH-20 column eluted with methanol to yield 80 fractions (D1-80). Fractions D13-19 yielded compound **16** (38 mg) and fractions D26-28 yielded compound **8** (21 mg).

The concentrated methanol extract obtained from the aerial part of *L. myrianthum* (1.57 g) was fractionated on silica gel using ethyl acetate and methanol with increasing solvent polarity yielding 91 fractions (E1–91). Fractions E35–36 (24.1 mg) were combined and rechromatographed to furnish compounds **12** and **14**.

The ethyl acetate extract of the aerial part of the *L. gmelinii* plant (1 g) was chromatographed over a

polyamide column with chloroform, chloroform:ethanol, ethanol, acetone with a gradient of water (30, 50, 70%), and methanol. A total of 702 (F1-702) fractions were collected, with subsequent combining and rechromatography. Combined fractions F109-124 (26 mg) led to the identification of compound **20**. Compound **9** (72 mg) was found in chromatographically identical fractions F133–152. Compounds **21** and **15** were isolated from fractions F169–224, with a total weight of 38 mg. Fractions F225–292 (18 mg), with the same R_f on the TLC, were identified as compound **8**. Compound **14** (23 mg) was isolated from fractions F365–435.

*(2R,3S)-2,3,4-trihydroxy-2-methylbutyl 3,4,5-trihydroxybenzoate (**1**)*

Oily colorless residue; $[\alpha]^{25}_{D}$–9.446 (*c* 0.01, MeOH); UV (MeOH) λ_{max} 264.2 nm; ^{1}H NMR (CD_3OD, 400 MHz,): δ = 7.09 (2H, s, H-2, H-6), 4.16 (1H, d, J = 11.2 Hz, H-1′), 4.23 (1H, d, J = 11.6 Hz, H-1′), 3.72 (1H, dd, J = 8, 3.2 Hz, H-3′), 3.85 (1H, dd, J = 11.2, 3.2 Hz, H-4′), 3.61 (1H, m, H-4′), 1.20 (3H, s, H-5′); ^{13}C NMR (CD_3OD, 100 MHz,): δ = 168.4 (>C=O, C-7), 146.2 (Ph(C), C-5, C-3), 139.7 (Ph(C), C-4), 121.3 (q-Ar(C), C-1), 110.2 (Ar(CH), C-2, C-6), 75.8 (Sec-OH(CH), C-3′), 74.2 (t-OH(C), C-2′), 70.0 (CH_2O, C-1′), 63.6 (CH_2OH, C-4′), 19.7 (CH_3, C-5′); HRESIMS *m/z*: 287.0766 $[M-H]^-$ (calcd. for $C_{12}H_{16}O_8$ 288.0845).

General experimental procedures

^{1}H and ^{13}C NMR spectra were obtained on a Bruker model AMX 400 NMR spectrometer with standard pulse sequences, operating at 400 MHz in ^{1}H and 100 MHz in ^{13}C. The chemical shift values were reported in parts per million units (p.p.m.) and trimethylsilane or known solvent shifts, used as internal chemical shift references. Coupling constants were recorded in Hertz (Hz). Standard pulse sequences were used for COSY, HMQC, HMBC, TOCSY, NOESY, and DEPT. High-resolution mass spectra were measured on a Micromass Q-Tof Micro mass spectrometer with a lock spray source. Column chromatography was carried out on silica gel (70–230 mesh, Merck) and Sephadex LH-20 (Mitsubishi Kagaku, Tokyo, Japan). TLC (silica gel 60 F254) was used to monitor fractions from column chromatography. Visualization of the TLC plates was achieved with a UV lamp (l = 254 and 365 nm) and anisaldehyde/acid spray reagent (methanol-acetic acid-anisaldehyde-sulfuric acid, 85:9:1:5). All chemicals used were purchased from Sigma–Aldrich (St. Louis, MO, USA). CD-spectra were measured on a JASCO J-715 spectrometer.

Alkaline hydrolysis

Five miligram of compound **1** was dissolved in 10% alcoholic KOH (2 ml) and left overnight at room temperature. The reaction mixture was dried and the residue redissolved in water and extracted with ethyl acetate (5 ml × 3). The combined ethyl acetate fractions were dried to produce 2-methylbutane-1,2,3,4-tetraol (2 mg) (Fontana et al. 1999).

In vitro antimicrobial assay

Compounds **1–21** were tested for antimicrobial activity against a panel of microorganisms obtained from the American Type Culture Collection (Manassas, VA, USA) and included the fungi *Candida albicans ATCC 90028, Candida glabrata ATCC 90030, Candida krusei ATCC 6258, C. neoformans ATCC 90113, Aspergillus fumigatus ATCC 204305*, the bacteria *Staphylococcus aureus ATCC 29213*, methicillin-resistant *S. aureus ATCC 33591 (MRSA), Escherichia coli ATCC 35218, Pseudomonas aeruginosa ATCC 27853*, and *Mycobacterium intracellulare ATCC 23068*. The bioassays were performed as described previously (Ma et al. 2004 and Bharate et al. 2007). Ciprofloxacin and amphotericin B were used as positive drug controls.

In vitro antimalarial assay

Isolated compounds **1–21** were tested for antimalarial activity, which was determined in vitro against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) strains of *Plasmodium falciparum* by a colorimetric assay that determines the parasitic lactate dehydrogenase activity, as described earlier (Hamid et al. 2004). Chloroquine was used for positive drug control.

In vitro antileishmanial assay

Compounds **1–21** were tested for antileishmanial activity. Promastigote culture of *Leishmanial donovani* was grown in RPMI medium with 10% fetal bovine serum (FBS) with pH 7.4 at 26°C. Axenic amastigote culture of *L. donovani* was grown in RPMI medium with 10% FBS with pH 5.5 at 37 °C in 5% CO_2 incubator. The antileishmanial activity of the compounds was tested in vitro against the promastigote, axenic amastigote and macrophage internalized amastigote forms of the *L. donovani* parasite. Promastigotes and axenic amastigotes were based on alamar blue based florometric growth analysis. Differentiated THP1 cells have been used as macrophages in macrophage internalized amastigotes. The macrophage internalized amastigote method was based on parasites rescued and transformation assays (Jain et al. 2012). Pentamidine and amphotericin B were used as

positive drug controls (Ma et al. 2004 and Hamid et al. 2004).

In vitro antitrypanosomal assay

All isolated compounds (**1–21**) were tested for antitrypanosomal activity. Blood-stage forms of *Trypanosoma brucei brucei* were grown in IMDM medium supplemented with 10% FBS. The culture was maintained at 37 °C in a 5% CO_2 incubator. The antitrypanosomal screening assay was based on Alamar blue fluorometric growth analysis at a concentration range of 10–0.4 µg/ml. Active compounds were further screened at a concentration range of 10–0.0032 µg/ml. Pentamidine and difluoromethylornithine were used as positive drug controls (Manda et al. 2014). IC_{50} and IC_{90} values were computed from a dose response growth inhibition curve by XLfit version 5.2.2.

Conformational analysis and electronic circular dichroism (ECD) calculation

Conformational analysis of compound **1** was performed with Schrödinger Macromodel 9.9 (Schrödinger, LLC, New York), employing the OPLS2005 (optimized potential for liquid simulations) force field in methanol (http://www.schrodinger.com/productpage/14/11/). Two conformers within a 2 kcal/mol energy window from the global minimum were selected, based on Boltzmann distribution calculated from Schrödinger software (http://www.schrodinger.com/productpage/14/11/). The output files for these conformers were prepared for Gaussian 09 calculation by Avogadro software. Geometrical optimization and energy were then calculated using the B3LYP/6-31G method in the Gaussian 09 software. Vibrational evaluation was done at the same level to confirm minimal excitation energy (denoted by wavelength in nm), rotatory strength dipole, and dipole length, which were calculated in methanol by TD-DFT/ B3LYP/ 6-31G performed by the Gaussian 09 software package (http://avogadro.openmolecules.net/). ECD curves were obtained in the SpecDis 1.62 program (Bruhn et al. 2013).

Results and discussion

In this study, the acetone extract from the roots of *L. myrianthum* was subjected to multiple chromatographic fractionations over silica gel yielding 3,5,7,3′,4′,6′-hexahydroxyflavone (**10**) (Shi et al. 2008), myricetin-3-*O*-L-arabinoside (**11**) (Kadota et al. 1990), myricetin-3-*O*-L-rhamnopyranoside (**9**) (Chung et al. 2004), epigallocatechin-3-*O*-gallate (**13**) (Yang et al. 2003), myricetin 3-*O*-(2″-*O*-galloyl)-β-D-glucopyranoside (**15**) (Akdemir et al. 2001), melitriose (**17**) (Seibel et al. 2006), β-sitosterol-3-glucoside (**18**) (Chadwick et al. 2004), and β-sitosterol (**19**) (Zhang et al. 2006) (Fig. 1). Myricetin (**8**) (Medini et al. 2014) and 1-galloyl-β-D-glucose (**16**) (Pawlowska et al. 2006) were isolated from methanol extract of the roots after fractionation over Sephadex LH-20. In addition, two myricetin derivatives were isolated from the methanol extract of the aerial part of the plant: myricetin-3-*O*-β-D-glucopyranoside (**12**) (Scharbert et al. 2004) and myricetin 3-*O*-(6″-galloylglucoside) (**14**) (Braca et al. 2003).

An ethanolic extract of the aerial part of *L. leptophyllum* was fractionated using different chromatographic columns to yield a new compound, (2*R*,3*S*)-2,3,4-trihydroxy-2-methylbutyl gallate (**1**), along with emodin (**2**) (Guo et al. 2011), bergenin (**3**) (Nazir et al. 2011), mahuannin B (**4**) (Calzada et al. 1999), 7-*O*-galloyl-D-sedoheptulose (**5**) (Yokozawa et al. 2009), arbutin (**6**) (Miguel et al. 2014), and gallic acid-4-*O*-β-D-glucoside (**7**) (Pawlowska et al. 2006), myricetin (**8**) (Medini et al. 2014), and myricetin-3-*O*-L-rhamnopyranoside (**9**) (Chung et al. 2004).

An ethyl acetate extract of the aerial part of *L. gmelinii*, obtained after consistent liquid–liquid extraction with ethyl acetate and water, was subjected to fractionation yielding six compounds, **8**, **9**, **14**, **15**, along with quercetin-3-*O*-α-L-rhamnopyranoside (**20**) (Kong et al. 2014) and myricetin 3-*O*-(4″-*O*-galloyl)-α-rhamnopyranoside (**21**) (Lin and Chou 2000).

Compound **1** was obtained as colorless oil. The HRESIMS spectrum of **1** showed a molecular ion $[M-H]^-$ peak at *m/z* 287.0766, compatible with the molecular formula $C_{12}H_{13}O_8$. The ^{1}H NMR spectrum of **1** showed two-proton singlets at δ_H 7.09 (s, 2H) assigned to meta-coupled H-2 and H-6, respectively. Two protons at δ_H 4.16 (d, J = 11.2 Hz, 1H) and δ_H 4.23(d, J = 11.6 Hz, 1H) were ascribed to oxygenated methylene H_2-1′. Two one-proton doublets at δ_H 3.85 (dd, J = 11.2, 3.2 Hz, 1H) and 3.61 (1H, m) were assigned to oxygenated methylene H_2-4′ and a three-proton broad signal at δ_H 1.20 (s, 3H) attributed to the tertiary C-5′ methyl protons. Compound **1** also showed one proton signal (H-3′) at δ_H 3.72 (dd, J = 8, 3.1 Hz, 1H). The ^{13}C NMR spectrum of **1** exhibited signals for ester carbon at δ_C 168.4 (C-7) and another 6 aromatic carbon signals including δ_C 121.3 (C-1), δ_C 110.2 (C-2, C-6), along with three phenolic groups with features characteristic of a gallic acid moiety at δ_C 146.2 (C-3, C-5) and δ_C 139.7 (C-4). In addition, the ^{13}C NMR spectrum of the compound **1** revealed signals characteristic of the polyol moiety: oxygenated methylene carbon at δ_C 70.0 (C-1′) and 63.6 (C-4′), tertiary oxygenated carbon at δ_C 74.2 (C-2′) and secondary oxygenated carbon at δ_C 75.8 (C-3′). HMBC correlations allowed for the complete assignments (Fig. 2). The proton at δ_H 7.09 (2H, s, H-2 and H-6) showed a strong correlation

with the carbons at δ_C 121.3 (C-1), δ_C 139.7 (C-4), δ_C 146.2 (C-3 and C-5) and δ_C 168.4 (C-7). The two proton signals of H_2-1′ at δ_H 4.16 (d, J = 11.2 Hz, 1H) and δ_H 4.23(d, J = 11.6 Hz, 1 H) showed strong correlation with signals at δ_C 19.7 (C-5′), δ_C 74.2 (C-2′), and δ_C 168.4 (C-7) (Table 1). Accordingly, the structure of **1** was defined as 2,3,4-trihydroxy-2-methyl-butyl gallate.

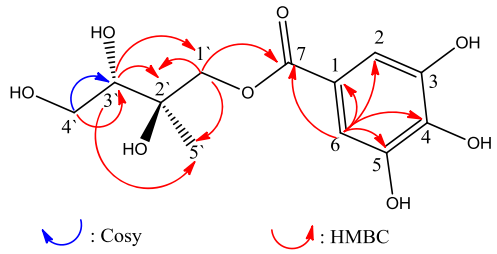

Fig. 2 Chemical structure and the HMBC and COSY correlations of the isolated new compound (**1**)

Table 1 400 MHz ^{1}H-NMR and 100 MHz ^{13}C-NMR data of compounds **1**[a]

No.	**1**(in CD_3OD) δ_H (mult; J in Hz)	δc
1	–	121.3
2	7.09 (2H, s)	110.2
3	–	146.2
4	–	139.7
5	–	146.2
6	7.09 (2H, s)	110.2
7	–	168.4
1′	4.16 (1H, d, J = 11.2), 4.23 (1H, d, J = 11.6)	70.0
2′	–	74.2
3′	3.72 (1H, dd, J = 8; 3.2)	75.8
4′	3.85 (1H, dd, J = 11.2; 3.2), 3.61 (1H, m)	63.6
5′	1.20 (3H, s)	19.7

[a] The δ_H and δ_C values were recorded using solvent signals (CD_3OD: δ_H 3.31/δ_C 49.0 for **1**) as references. Signal assignments were based on the results of ^{1}H–^{1}H COSY, HMQC, and HMBC experiments

Hydrolysis of compound **1** led to the obtaining of 2-methylbutane-1,2,3,4-tetraol. The optical rotation of the alcohol part was measured at a concentration of 0.01 mg/ml in methanol the $[\alpha]_D^{25}$ value (−9.446) matches with (2*R*,3*S*)-2-methylbutane-1,2,3,4-tetraol mentioned in the literature (Ghosh et al. 2012). The absolute configuration of the C-2′ and C-3′ positions was further confirmed by comparison of experimental ECD spectrum with calculated values for the enantiomers. The 1D NOESY NMR experiment showed no correlation between the methyl (C-5′) and the proton resonating at δ_H 3.72 (H-3′) (supplementary Figure 1), which indicates that the substitutes in positions 2′ and 3′ are either 2*R*,3*S* or 2*S*,3*R* enantiomers. The stereochemistry of compound **1** was assigned as 2*R*,3*S* based on the positive CD maxima at 245 and 298 nm (Fig. 3). The B3LYP/6-31 G simulated ECD spectrum for 2*R*,3*S* enantiomer, generated from 40 excited states using Gaussian band shapes for the peaks, had peaks at 240 and 290 nm. The calculated ECD of the 2*R*,3*S* enantiomer showed excellent agreement with the experimental data. On the basis of the above discussion, the structure of **1** has been characterized as (2*R*,3*S*)-2,3,4-trihydroxy-2-methylbutyl gallate, which represents a new gallic acid ester.

The chemical structures of the known compounds were confirmed by comparison of their spectroscopic properties with published data. The isolated compounds **1–21** (Fig. 1) were evaluated for their antibacterial, antifungal, antimalarial, antitrypanosomal, and antileishmanial activities. The antibacterial activity was tested against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli, Pseudomonas aeruginosa*, and *Mycobacterium*

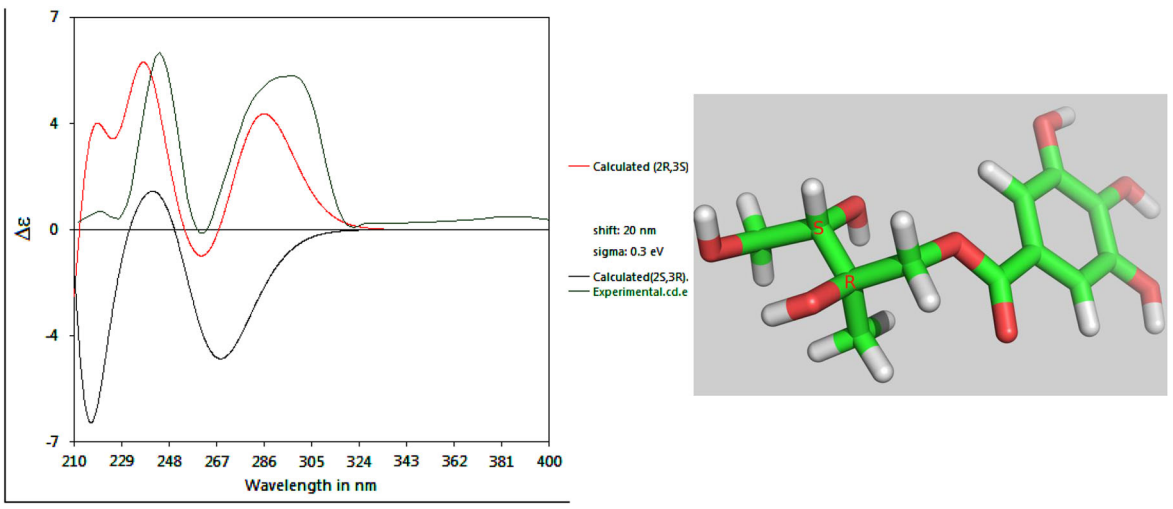

Fig. 3 The experimental spectrum of the compound 1compared to the calculated ECD spectra of 2*R*,3*S* and 2*S*,3*R* - enantiomers

intracellulare. None of these compounds showed in vitro antibacterial and antileishmanial activities. The antifungal activity was evaluated against a panel of pathogenic fungi (*Candida albicans, C. glabrata, C. krusei, Cryptococcus neoformans*, and *Aspergillus fumigatus*) associated with opportunistic infections. Compounds **10** and **13** exhibited average activity against *Candida glabrata* with IC_{50} values of 21.46 and 10.82 μM, respectively. The IC_{50} value for the positive drug control amphotericin B was 0.30 μM. Furthermore, all compounds were tested against *P. falciparum* D6 and W2 strains. Activity was found only for compounds **1** and **5**. These compounds showed promising action against both chloroquine-resistant (*P. falciparum* W2) and chloroquine-sensitive strains of malaria (*P. falciparum* D6). Compound **1** displayed the most potent activity with IC_{50} values of 1.097 and 0.583 μM, for D6 and W2, respectively. Structurally-related compound **5** showed moderate activity with IC_{50} values of 3.947 and 3.657 μM, for D6 and W2, respectively. Compound **14** revealed significant activity against *Trypanosoma brucei* with an IC_{50} value of 13.16 μM. Compounds **10, 13**, and **16** exhibited moderate antitrypanosomal activity compared to the positive control, with IC_{50} values of 25.04, 16.41, and 25.13 μM respectively. The IC_{50} value for the positive drug control α-difluoromethylornithine was 13.07 μM.

Acknowledgements We are grateful to the Government of the Republic of Kazakhstan and the National Center for Natural Products Research (NCNPR), University of Mississippi, School of Pharmacy, USA for financial support. Also we are grateful to Dr. Melissa Jacob, Dr. Shabana Khan, and Dr. Babu Tekwani for performing antibacterial, antifungal, antimalarial, and antileishmanial bioassays.

Compliance with ethical standards

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

References

Akdemir ZŞ, Tatlı İİ, Saracoğlu I, İsmailoğlu UB, Şahin-Erdemli I, Çalış İ (2001) Polyphenolic compounds from *Geranium pratense* and their free radical scavenging activities. Phytochemistry 56:189–193

Aniya Y, Miyagi C, Nakandakari A, Kamiya S, Imaizumi N, Ichiba T (2002) Free radical scavenging action of the medicinal herb *Limonium wrightii* from the Okinawa islands. Phytomedicine 9:239–244

Berenbaum MR (1995) The chemistry of defense: theory and practice. Proc Natl Acad Sci USA 92:2–8

Bharate SB, Khan SI, Yunus NA, Chauthe SK, Jacob MR, Tekwani BL, Khan IA, Singh IP (2007) Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg Med Chem 15:87–96

Braca A, Politi M, Sanogo R, Sanou H, Morelli I, Pizza C, De Tommasi N (2003) Chemical composition and antioxidant activity of phenolic compounds from wild and cultivated *Sclerocarya birrea* (Anacardiaceae) leaves. J Agric Food Chem 51:6689–6695

Bruhn T, Schaumlöffel A, Hemberger Y, Bringmann G (2013) SpecDis: Quantifying the Comparison of Calculated and Experimental Electronic Circular Dichroism Spectra. Chirality 25:243–249

Calzada F, Cerda-García-Rojas CM, Meckes M, Cedillo-Rivera R, Bye R, Mata R (1999) Geranins A and B, new antiprotozoal A-type proanthocyanidins from *Geranium niveum*. J Nat Prod 62:705–709

Chadwick LR, Nikolic D, Burdette JE, Overk CR, Bolton JL, van Breemen RB, Pauli GF (2004) Estrogens and Congeners from Spent Hops (*Humulus lupulus*). J Nat Prod 67:2024–2032

Chung SK, Kim YC, Takaya Y, Terashima K, Niwa M (2004) Novel flavonol glycoside, 7-O-methyl mearnsitrin, from *Sageretia theezans* and its antioxidant effect. J Agric Food Chem 52:4664–4668

Downie SR, Palmer JD (1994) Phylogenetic relationships using restriction site variation of the chloroplast DNA inverted repeat. In: H-D Behnke, TJ Mabry (eds) Caryophyllales: Evolution and Systematics. Springer-Verlag, Berlin, Heidelberg, p 223–233

Fontana G, Savona G, Rodriguez BN, De La Torre MA (1999) Unusual 6'-fatty acid esters of (24S)-24-ethylcholesta-5, 25-dien-3β-yl β-d-glucopyranoside from Teucrium fruticans. Phytochemistry 50:283–285

Gadetskaya AV, Tarawneh AH, Zhusupova GE, Gemejiyeva NG, Cantrell CL, Cutler SJ, Ross SA (2015) Sulfated phenolic compounds from *Limonium caspium*: Isolation, structural elucidation, and biological evaluation. Fitoterapia 104:80–85

Gaussian 09, Revision B.01, Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, Nakatsuji H, Caricato M, Li X, Hratchian HP, Izmaylov AF, Bloino J, Zheng G, Sonnenberg JL, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Vreven T, Montgomery JA, Peralta JE, Ogliaro, Jr. F, Bearpark M, Heyd JJ, Brothers E, Kudin KN, Staroverov VN, Kobayashi R, Normand J, Raghavachari K, Rendell A, Burant JC, Iyengar SS, Tomasi J, Cossi M, Rega N, Millam JM, Klene M, Knox JE, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Martin RL, Morokuma K, Zakrzewski VG, Voth GA, Salvador P, Dannenberg JJ, Dapprich S, Daniels AD, Farkas O, Foresman JB, Ortiz JV, Cioslowski J, and Fox DJ, Gaussian, Inc., Wallingford CT, 2010

Ghosh SK, Butler MS, Lear MJ (2012) Synthesis of 2-C-methylerythritols and 2-C-methylthreitols via enantiodivergent Sharpless dihydroxylation of trisubstituted olefins. Tetrahedron Lett 53:2706–2708

Guo S, Feng B, Zhu R, Ma J, Wang W (2011) Preparative isolation of three anthraquinones from *Rumex japonicus* by high-speed counter-current chromatography. Molecules 16:1201–1210

Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P (2004) Comparison of alamar blue and MTT assays for high through-put screening. Toxicol In Vitro 18:703–710

Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR (2012) Avogadro: An advanced semantic chemical editor, visualization, and analysis platform. J Cheminf 4:17

Jain SK, Sahu R, Walker LA, Tekwani BL (2012) A parasite rescue and transformation assay for antileishmanial screening against intracellular *Leishmania donovani* amastigotes in THP1 human acute monocytic leukemia cell line. J Visualized Exp 70:e4054

Julkunen-Tiitto R (1989) Phenolic constituents of *Salix*: a chemotaxonomic survey of further Finnish species. Phytochemistry 28:2115–2125

Kadota S, Takamori Y, Nyein KN, Kikuchi T, Tanaka K, Ekimoto H (1990) Constituents of the leaves of *Woodfordia fruticosa* Kurz. I. Isolation, structure, and proton and carbon-13 nuclear magnetic resonance signal assignments of woodfruticosin (woodfordin C), an inhibitor of deoxyribonucleic acid topoisomerase II. Chem Pharm Bull 38:2687–2697

Kandil FE, Ahmed KM, Hussieny HA, Soliman AM (2000) A new flavonoid from *Limonium axillare*. Arch Pharm 333:275–277

Keinänen M, Julkunen-Tiitto R, Rousi M, Tahvanainen J (1999) Taxonomic implications of phenolic variation in leaves of birch (*Betula* L.) species. Biochem Syst Ecol 27:243–254

Kong NN, Fang ST, Wang JH, Wang ZH, Xia CH (2014) Two new flavonoid glycosides from the halophyte *Limonium franchetii*. J Asian Nat Prod Res 16:370–375

Korul'kina LM, Shul'ts EE, Zhusupova GE, Abilov ZhA, Erzhanov KB, Chaudri MI (2004) Biologically active compounds from *Limonium gmelinii* and *L. popovii* I. Chem Nat Compd 40:465–471

Kozhamkulova ZhA, Radwan MM, Zhusupova GE, Abilov ZhA, Rahadilova SN, Ross SA (2010) Gmelinoside I, a new flavonol glycoside from *Limonium gmelinii*. Nat Prod Commun 5:1061–1062

Liebezeit G, Künnemann TD, Gad G (1999) Biotechnological potential of North Sea salt marsh plants – a review of traditional knowledge. J Biotechnol 70:77–84

Lin LC, Chou CJ (2000) Flavonoids and phenolics from *Limonium sinense*. Planta Med 66:382–383

Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, Walker LA, Khan IA (2004) Antimicrobial and antileishmanial activities of hypocrellins A and B. Antimicrob Agents Chemother 48:4450–4452

Macro Model 9.9, Schrodinger LLC, 2012. (http://www.schrodinger.com/productpage/14/11/) Accessed 2 Feb 2016

Manda S, Khan SI, Jain SK, Mohammed S, Tekwani BL, Khan IA, Vishwakarma RA, Bharate SB (2014) Synthesis, antileishmanial and antitrypanosomal activities of N-substituted tetrahydro-β-carbolines. Bioorg Med Chem Lett 24:3247–3250

Medini F, Fellah H, Ksouri R, Abdelly C (2014) Total phenolic, flavonoid and tannin contents and antioxidant and antimicrobial activities of organic extracts of shoots of the plant *Limonium delicatulum*. Journal of Taibah University for Science 8:216–224

Miguel MG, Faleiro ML, Guerreiro AC, Antunes MD (2014) *Arbutus unedo* L.: Chemical and biological properties. Molecules 19:15799–15823

Murray AP, Rodriguez S, Frontera MA, Tomas MA, Mulet MC (2004) Antioxidant metabolites from *Limonium brasiliense* (Boiss.) Kuntze. Zeitschrift für Naturforschung 59:477–480

Nazir N, Koul S, Qurishi MA, Najar MH, Zargar MI (2011) Evaluation of antioxidant and antimicrobial activities of Bergenin and its derivatives obtained by chemoenzymatic synthesis. Eur J Med Chem 46:2415–2420

Pawlowska AM, De Leo M, Braca A (2006) Phenolics of *Arbutus unedo* L.(Ericaceae) fruits: identification of anthocyanins and gallic acid derivatives. J Agric Food Chem 54:10234–10238

Rastogi S, Pandey MM, Rawat AKS (2016) Traditional herbs: a remedy for cardiovascular disorders. Phytomedicine 23:1082–1089

Scharbert S, Holzmann N, Hofmann T (2004) Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. J Agric Food Chem 52:3498–3508

Seibel J, Moraru R, Götze S, Buchholz K, Na'amnieh S, Pawlowski A, Hecht HJ (2006) Synthesis of sucrose analogues and the mechanism of action of *Bacillus subtilis* fructosyltransferase (levansucrase). Carbohydr Res 341:2335–2349

Shi S, Zhao Y, Zhou H, Zhang Y, Jiang X, Huang K (2008) Identification of antioxidants from *Taraxacum mongolicum* by high-performance liquid chromatography–diode array detection–radical-scavenging detection–electrospray ionization mass spectrometry and nuclear magnetic resonance experiments. J Chromatogr A 1209:145–152

Tang XH, Yu F, Liu J, Gao J, Yan LF, Dong MM (2014) Isolation and identification of anti-tumor polysaccharide LSP21 from *Limonium sinense* (Girard) Kuntze. Int J Biol Macromol 70:138–142

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3–19

Yang LL, Chang CC, Chen LG, Wang CC (2003) Antitumor principle constituents of *Myrica rubra* var. *acuminata*. J Agric Food Chem 51:2974–2979

Yang LL, Yen KY, Kiso Y, Hikino H (1987) Antihepatotoxic actions of Formosan plant drugs. J Ethnopharmacol 19:103–110

Yokozawa T, Park CH, Noh JS, Tanaka T, Cho EJ (2009) Novel action of 7-O-galloyl-D-sedoheptulose isolated from Corni Fructus as a hypertriglyceridaemic agent. J Pharm Pharmacol 61:653–661

Yuh-Chi K, Lie-Chwen L, Wei-Jern T, Cheng-Jen C, Szu-Hao K, Yen-Hui H (2002) Samarangenin B from *Limonium sinense* suppresses herpes simplex virus type 1 replication in vero cells by regulation of viral macromolecular synthesis. Antimicrob Agents Chemother 46:2854–2864

Zhang X, Cambrai A, Miesch M, Roussi S, Raul F, Aoude-Werner D, Marchioni E (2006) Separation of Δ5-and Δ7-phytosterols by adsorption chromatography and semipreparative reversed phase high-performance liquid chromatography for quantitative analysis of phytosterols in foods. J Agric Food Chem 54:1196–1202

Zhusupova GE (2006) Amino-acid and mineral composition of substances from the aerial part and roots of *Limonium gmelinii*. Chem Nat Compd 42:123–124

Zhusupova GE (2007) Chemical composition of *Limonium Mill* genus plants and creation of preparations on its basis. Dissertation, Al-Farabi Kazakh National University