PharmacologyOnLine December 30, 2014

Archives • 2014 • vol.3 • 141-144

PHYTOCHEMICAL INVESTIGATION AND ANTIPROLIFERATIVE ACTIVITY OF *LIMONIASTRUM FEEI* (GIRARD) BATT.

Sihem Boudermine[1], Nicola Malafronte[2*], Noureddine Bghidja[1], Samir Benayech[1], Andrea Esposito[2], Salvatore Velotto[3], Lorella Severino[4]

[1]Department of Chemistry, Research Unit, Development of Natural Resources, Bioactive Molecules and Physiochemical and Biological Analysis,University Mentouri Constantine, Algeria
[2]Department of Pharmacy, University of Salerno, Giovanni Paolo II, 84084 Fisciano (SA), Italy
[3] Department of Agriculture, University of Napoli, Federico II, via Università 100, 80059 Portici, Napoli, Italy
[4]Department of Veterinary Medicine and Animal Production, Division of Toxicology, University of Napoli Federico II via Delpino 1, 80137, Napoli, Italy

[*]nmalafronte@unisa.it

Abstract

In the present work we reported the phytochemical analysis of *n*-buthanol extract of *Limoniastrum feei* (Girard) Batt. a plant belonging to Plumbaginaceae family. A new phenolic compound (1) together with six know compounds were isolated. Their structures were elucidated by 1D and 2D NMR experiments including 1D TOCSY, DQF-COSY, HSQC, and HMBC spectroscopy, as well as ESI-MS analysis. The antiproliferative activity of the extracts was evaluated.

Keywords: Limoniastrum feii; Plumbaginaceae; Phenolic compounds, NMR

Introduction

The aerial parts of *Limoniastrum feei* (*Plumbaginaceae*) were used in Sahara folk medicine for treating gastrointestinal tract ailments, fever, icterus and various diseases. *Limoniastrum feei* (*plumbaginaceae*) is traditionally used to treat gastric disorders, prepared by decoction in water, took one cup of tea for each day. The plant is native to the southeast of Algeria (Saoura, region of Bechar) and Northern Africa [1,2]. The other uses of *Limoniastrum feei* are as an antibacterial for the treatment of bronchitis and for stomach infections [3]. Previous phytochemical studies reported the presence of poliphenols, flavonoids, and saponins [4]. In our ongoing research for new bioactive compounds from mediteranean medicinal plants [5-7] the whole plant of *Limoniastrum feei*, has been studied. Herein, we described the isolation and the structural identification by spectroscopic and spectrometric techniques of a new compound together with six known compounds. Finally the antiproliferative activity of *n*-BuOH extract has been evaluated.

Material and Methods

Plant Material

Limoniastrum feei (Girad) Batt. (Plumbaginaceae) whole plant, was collected in April 2012 during the flowering period in the region of Bechar, southwest of Algeria.

Chemicals

All reagents for cell culture were from Hy-Clone (Euroclone, Paignton Devon, U.K.); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] and 6-mercaptopurine (6-MP) were from Sigma Chemicals (Milan, Italy). *n*-Hexane, *n*-Butanol chloroform, methanol, hydrochloric acid and glacial acetic acid were purchased from VWR (Milano-Italy).

Extraction and isolation

The whole plant of *Limoniastrum feei* (800 g) was macerated with 80% ethanolic solution. The hydro-alcoholic solution was concentrated under reduced pressure to dryness and was successively extracted with $CHCl_3$, EtOAc and *n*-BuOH for three times for each solvent. Part of *n*-BuOH extract (10 g) was fractionated by a Silica gel 60 (Merck 200-400 mesh) column chromatography eluted with a isocratic system EtOAc:CH_3COOH:H_2O (2:1:1). Fractions of 50 ml were collected, analyzed by TLC and pooled into seven fractions (A–G). Fraction C (92 mg) was purified by RP-HPLC using MeOH–H_2O (28:72) to give gallic acid (3.0mg, t_R=5 min), compound 1 (1.5 mg, t_R=7 min), myrciaphenone A (2.0mg, t_R=14 min) and epigallocatechin (1.0mg, t_R=18 min). Fraction D (279 mg) was purified by RP-HPLC using MeOH–H_2O (25:75) to give catechin (2.0mg, t_R=11 min), salidroside (2.0mg, t_R=14min), epicatechin(5.3 mg, t_R=18 min) and epigallocatechin (3.9 mg, t_R=23 min). Fraction F (502.1 mg) was purified by RP-HPLC using MeOH–H_2O (3:7) to give myrciaphenone A (2.2 mg, t_R=12 min) and myricetin-3-*O*-rhamnoside (2.2 mg, t_R=45 min).

Experimental procedures

Column chromatography was performed over silica gel (63-200 µm, Merck, Darmstadt, Germany); TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck, Darmstadt, Germany); compounds were detected by $Ce(SO_4)_2/H_2SO_4$ (Sigma-Aldrich, Milano, Italy) solution. HPLC separations were conducted on a Shimadzu LC-20AT Prominence system equipped with a Shimadzu RID-10A refractive index detector, and with a Waters µ-Bondapak C18 column (Waters, Milford, MA). NMR experiments were performed on a Bruker DRX-600 spectrometer at 300 K [8,9]. The NMR data were processed on a Silicon Graphic Indigo2 Workstation using UXNMR software**.** HRESIMS spectra were acquired in the positive ion mode on a Q-TOF premier spectrometer equipped with a nanoelectrospray ion source (Waters-Milford, MA, USA).

Antiproliferative assay

J774.A1, murine monocyte/macrophage, WEHI-164, murine fibrosarcoma, and HEK-293, human epithelial kidney cells were grown as reported previously [10]. All reagents for cell culture were from Hy-Clone (Euroclone, Paignton Devon, U.K.); MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2H-tetrazolium bromide] and 6-mercaptopurine (6-MP) were from Sigma Chemicals (Milan, Italy). J774.Al, WEHI-164, and HEK-293 (3.4 x 10^4 cells) were plated on 96-well microtiter plates and allowed to adhere at 37°C in 5% CO_2 and 95% air for 2 h. Thereafter, the medium was replaced with 50 µL of fresh medium and a 75 µL aliquot of 1:4 serial dilution of each test compound was added and then the cells incubated for 72 h. In some experiments, serial dilutions of 6-MP were added. The cell viability was assessed through an MTT conversion assay [11,12]. The optical density (OD) of each well was measured with a microplate

spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-MP [10] was calculated as: % dead cells=100-(OD treated/OD control) x 100. Table 1 shows the results obtained expressed as an IC_{50} value (µM), the concentration that inhibited cell growth by 50% as compared to the control.

Results and Discussion

The ethanolic-aqueous extract (80%) of *L. feei* whole plant, was successively partitioned with chloroform, ethyl acetate and *n*-butanol. Fractionation and purification of *n*-butanol extracts by combination of chromatographic techniques led to the isolation of one new compound **1** and six known compounds.

Compound **1** NMR and MS data demonstrated the molecular formula $C_{14}H_{20}O_{11}S$ (HRESIMS at *m/z* 395.3589 $[M-H]^-$), which suggested the presence of a sulfate group in the molecule. Acid hydrolysis of **1** followed by treatment with $BaCl_2$, confirmed the presence of a sulfate group.

The ESI-MS spectrum in negative ion mode showed the $[M-H]^-$ ion peak at *m/z* 395, and fragments in MS/MS analysis at *m/z* 241 $[M-H-C_8H_{10}O_2]^-$), and at *m/z* 97 corresponding to the sulfate group for the loss of sugar moiety. The ^1H NMR spectrum (Table 1) showed signals of three aromatic protons [δ_H 7.22 (H, d, *J* = 1.0 Hz, H-2), 6.76 (1H, d, *J* = 8.0, H-6), 6.79 (1H, br d, *J* = 8.0, H-6)], and of four methylene protons at [δ_H 2.84 2H, br t, *J* = 6.7 Hz), 4.04 (1H, m), 3.75 (1H, m)]. The ^1H NMR spectrum also showed the presence of a β-glucopyranosyl unit (δ_H 4.34, 1H, d, *J* = 7.5 Hz). A combination of 1D-TOCSY, COSY and HSQC experiments provided evidence for the presence in the molecule of the segments H-1'-H_2-6', and H-7-H-8, and H-2-H-6. The HMBC spectrum exhibited correlations between the proton signal at δ_H 2.84 and the carbons at δ_C 135.2 (C-1) and 146.0 (C-3) confirming the position of 4-hydroxyphenylethanol structure [13]. The location of glucopyranose was obtained on the basis of the HMBC correlations between the proton signal at δ_H 4.34 (H-1') and the carbon signal at 70.0 (C-1') ppm, and between the proton signals at δ 4.04 and 3.75 (H_2-8) and the carbon signal at 102.9 ppm. The position of the sulfate group was established at C-6 of glucose unit on the basis of the downfield shifts of the H_2-6' (δ_H 4.38, dd, J=12.0, 2.5 Hz; and 4.20, dd, J=12.0, 5.0 Hz) and of C-6' δc 67.0, consistent with the presence of an ester moiety.

Thus compound 1 was established as new 3,4-diihydroxyphenylthanol-8'-*O*-β-D-(6-sulpho)-glucopyranoside. The six known compounds were identified as gallic acid, myrciaphenone A [14] epigallocatechin [14], epicatechin [14], myricetin-3-*O*-rhamnoside [14], catechin [14] and salidroside [15]. All the structures were established by detailed NMR and MS analyses and comparison with literature data.

The antiproliferative activity of *n*-BuOH extract was evaluated against the J774.A1, WEHI-164, and HEK-293 cell line. This extract is active at IC_{50} = 27 µg/mL.

References

1. Maire R, Flore de L'Afrique du Nord: Maroc, Algerie,Tunisie, Tripolitaine, Cyrenaique et Sahara 1953; Vol. **5**, Paul Lechevaller: Paris.
2. Cheriti A. Rapport du Projet CRSTRA, Plantes Medicinales de la Region de Bechar (Ethnopharmacologie studies), 2000; Bechar: Algeria.
3. Belboukhari N. and A. Cheriti A. Antimicrobial Activity of Aerial Part Crude Extracts from *Limoniastrum feei.* Asian Journal of Plant Science 2005; 4: 496-498.
4. Rahamani S, Belboukhari N and Cheriti A. Phytochemical Investigation of Bioactive Extract from Endemic Medicinal Plant *Limoniastrum feei* (Girard) Batt (Plumbaginaceae). Asian Journal of Chemistry 2014; 26 (2): 365-368
5. De Tommasi N, Autore G, Bellino A, Pinto A, Pizza C, Sorrentino R, Venturella P. Antiproliferative triterpene saponins from Trevesia palmata. Journal of Natural Products 2000; 63: 308-314.
6. Aquino R, Peluso G, De Tommasi, N, De Simone F, Pizza, C. New polyoxypregnane ester derivatives from Leptadenia hastate. Journal of Natural Products 1996; 59: 555-564.
7. Braca A, Prieto JM, De Tommasi N, Tomè, F, Morelli, I. Furostanol saponins and quercetin glycosides from the leaves of Helleborus viridis L. Phytochemistry 2004; 65: 2921-2928.
8. Dal Piaz F, Cotugno R, Lepore, L. Vassallo A ,Malafronte N, Lauro G, Bifulco G, Belisario M A, De Tommasi N. Chemical proteomics reveals HSP70 1A as a target for the anticancer diterpene oridonin in Jurkat cells. Journal of Proteomics 2013; 82: 14-26..
9. Dal Piaz F, Vassallo A ,Temraz A, Cotugno R, Belisario MA, Bifulco G, Chini MG, Pisano C, De Tommasi N. Braca, A. Chemical-Biological Study Reveals C9-type Iridoids as Novel Heat Shock Protein 90 (Hsp90) Inhibitors. J Med Chem. 2013; 28: 56(4):1583-95.
10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assay. J. Immunol. Methods 1983; 65: 55-63.
11. Opipari AWJ, Hu HM, Yabkowitz R, Dixit YM. The A20 zinc finger protein protects cell from tumor necrosis factor cytoxicity. J. Biol. Chem. 1992; 267: 12424-7.
12. Dal Piaz F, Tosco A, Eletto D, Piccinelli AL, Moltedo O, Franceschelli S, Sbardella G, Remondelli P, Rastrelli L, Vesci L, Pisano C, De Tommasi N. The identification of a novel natural activator of p300 histone acetyltranferase provides new insights into the modulation mechanism of this enzyme. Chem.Bio.Chem 2010; 11: 818-827.

13. Maldini M, Montoro P, Piacente S, Pizza C. Phenolic compounds from *Bursera simaruba* Sarg. bark: phytochemical investigation and quantitative analysis by tandem mass spectrometry. Phytochemistry 2009; 70: 641-649.
14. Chaabi M; Beghidja N; Benayache S; Lobstein A. Activity-guided isolation of antioxidant principles from Limoniastrum feei (Girard) Batt Zeitschrift fur Naturforschung. C, Journal of biosciences 2008; 63: 801-7.
15. Morimura K, Gatayama A, Tsukimata R, MatsunamiK, Otsuka H, Hirata E, Shinzato T, Aramoto M, Takeda Y. 5-O glucosyldihydroflavones from the leaves of Helicia cochinchinensis. Phytochemistry 2006; 67(24): 2681-5

Table 1: NMR data of compound **1** (CD_3OD, 600 MHz)[a]

	1	
position	δ_H	δ_C
1		135.2
2	7.22 *d* (1.0)	119.0
3	-	146.0
4	-	147.9
5	6.76 *d* (8.0)	117.0
6	6.79 br *d* (8.0)	125.0
7	2.84 *t* (6.7)	35.8
8	4.04, *m*	70.0
	3.75, *m*	
1'	4.34, *d* (7.5)	102.9
2'	3.26 *dd* (8.5, 9.0)	74.1
3'	3.41 *t* (9.5)	76.7
4'	3.39 *t* (9.5)	70.4
5’	3.53 m	74.9
6’	4.38 *dd* (12.0, 2.5)	67.0

[a]*J* values are in parentheses and reported in Hz;
chemical shifts are given in ppm;
assignments were confirmed by COSY, HSQC,HMBC

Fig.1: Compound **1**