Two New Prenylated Isoflavonoids from *Erinacea anthyllis* with Antioxidant and Antibacterial Activities

Mouffouk, Soumia; Marcourt, Laurence; Benkhaled, Mohammed; Boudiaf, Kaouthar; Wolfender, Jean-Luc; Haba, Hamada

How to cite

MOUFFOUK, Soumia et al. Two New Prenylated Isoflavonoids from *Erinacea anthyllis* with Antioxidant and Antibacterial Activities. In: Natural Product Communications, 2017, vol. 12, n° 7, p. 1065–1068. doi: 10.1177/1934578X1701200716

This publication URL: https://archive-ouverte.unige.ch/unige:116652

Publication DOI: 10.1177/1934578X1701200716

NPC Natural Product Communications

2017
Vol. 12
No. 7
1065 - 1068

Two New Prenylated Isoflavonoids from *Erinacea anthyllis* with Antioxidant and Antibacterial Activities

Soumia Mouffouk[a], Laurence Marcourt[b], Mohammed Benkhaled[a], Kaouthar Boudiaf[c], Jean-Luc Wolfender[b] and Hamada Haba[a,*]

[a]*Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences de la Matière, Université de Batna-1, Algérie*

[b]*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Rue Michel-Servet, CH-1211 Geneva 4, Switzerland*

[c]*Département de Biologie, Faculté des Sciences de la Nature et de la Vie, Université de Batna-2, Algérie*

haba.hamada@yahoo.fr

Received: February 20th, 2017; Accepted: April 12th, 2017

Two new prenylated isoflavonoids, namely Erinasone A and Erinasone B along with 19 known secondary metabolites, including twelve isoflavonoids, three polyphenols, one flavonol, two flavanones and three steroids, were isolated from the whole plant (roots and aerial parts) of *Erinacea anthyllis.* Structures of all isolated compounds were elucidated by spectroscopic analysis, including 1D and 2D NMR (^{1}H, ^{13}C, COSY, HSQC, TOCSY, HMBC and NOESY), mass spectrometry (ESI-MS), UV-Vis, measurement of optical rotation $[\alpha]_D$ and by comparison with the literature data. The total phenolic and flavonoid contents were determined in this study. Furthermore, the antioxidant and antibacterial activities of the EtOAc and *n*-BuOH extracts of *E. anthyllis* were evaluated. These extracts exhibited moderate antibacterial and antioxidant activities. Their IC_{50} values were approximately 0.04 mg/mL (*n*-BuOH) and 0.037 mg/mL (EtOAc). Consequently, *Erinacea anthyllis* is a rich source of polyphenolic compounds particularly isoflavonoids used as chemotaxonomic markers for the subfamily Papilionoideae of the family Fabaceae.

Keywords: *Erinacea anthyllis*, Fabaceae, Prenylated isoflavonoids, Antioxidant activity, Antimicrobial activity, NMR.

The genus *Erinacea* belonging to the subfamily Papilionoideae of the family Fabaceae and the tribe Genisteae, is represented by a single species named *Erinacea anthyllis* Link or *Erinacea pungen* [1]. *E. anthyllis* is a shrub with purplish blue flowers that is found mainly in the Pyrenees Orientales in France, Spain, Algeria, Tunisia and Corsica [2]. In Algeria, this species is used in traditional medicine to treat rheumatic diseases [3] while in the Siroua region of Morocco it is utilized as honey source [4]. The present work describes the isolation and structural determination of two new prenylated isoflavonoids **1** and **2** (Figure 1), together with 19 known compounds from the EtOAc and *n*-BuOH extracts of *E. anthyllis*. Structures of all the isolated metabolites **1-21** were established mainly by 1D and 2D NMR and mass spectrometry ESI-MS experiments, and by comparison with the literature data. Moreover, the total phenolic and flavonoid contents were established. In addition, the antioxidant and the antimicrobial activities of the crude extracts (EtOAc and *n*-BuOH) were determined.

All the isolated compounds **1-21** (Figure S1) were obtained from the EtOAc and *n*-BuOH extracts of *E. anthyllis* by the use of different chromatographic methods including vacuum liquid chromatography (VLC), column chromatography (CC) and TLC. The known compounds were identified as β-sitosterol (**3**) [5], erythrinin D (**4**) and (±)-erythrinin F (**9**) [6], sitoindoside II (**5**) [7], vomifoliol (**6**) [8], diadzein (**7**) [9], genistein (**8**) [10], alpinumisoflavone (**10**) [11], ammopiptanine B (**11**) [12], liquiritigenin (**12**) [13], erysubin-A (**13**) and erysubin-B (**14**) [14], 7-hydroxytremetone (**15**) [15], 7,4'-dihydroxy-3'-methoxyflavanone (**16**) [16], genistein-8-*C*-glucoside (**17**) [17], daucosterol (**18**) [18], orobol-8-*C*-β-*D*-glucopyranoside (**19**) [19], (6*S*, 9*R*)-roseoside (**20**) [20] and isokaempferide (**21**) [21].

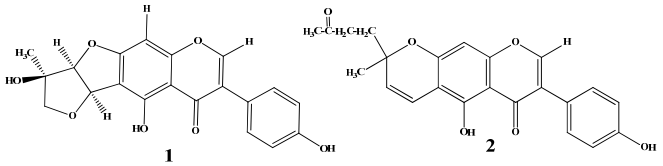

Figure 1: Structures of new compounds **1** and **2**.

Compound **1** was obtained as an amorphous white powder soluble in acetone. The molecular formula was determined as $C_{20}H_{16}O_7$ by HRESIMS (*m/z* 367.0785 $[M–H]^-$; calcd for $C_{20}H_{15}O_7$, 367.0818), which indicated 13 degrees of unsaturation.

The UV spectrum of **1** displayed the characteristic absorption maxima of an isoflavone chromophore at 262 and 332 nm [6,14]. The analysis of ^{1}H NMR, HSQC and COSY spectra (Table 1) of compound **1** indicated the presence of one proton singlet at δ_H/δ_C 8.22/154.2 assignable to the proton H-2 of an isoflavone skeleton [22], four aromatic protons (A_2X_2-System) at δ_H 6.91 (2H, d, J = 8.4 Hz) and 7.47 (2H, d, J = 8.4 Hz) which were attributed to the protons H-3'/H-5' and H-2'/H-6' of a *p*-disubstituted ring B [23], a singlet aromatic proton at δ_H 6.44 potentially positioned on ring A, two doublets of oxymethine protons (δ_H/δ_C 5.82/79.3 and 4.84/92.2 for H-1"/C-1" and H-2"/C-2", respectively), two non-equivalent protons of an oxymethylene (δ_H/δ_C 3.31, 3.52/73.3, CH_2-4"), a methyl singlet (δ_H/δ_C 1.42/24.3, CH_3-5") and three hydroxyl protons (δ_H 4.23, 8.55 and 13.58 for OH-3", -4' and -5, respectively). The singlet aromatic proton was located at C-8 position of ring A based on HMBC correlations from H-8 to carbons C-6 (δ_C 106.9), C-10 (δ_C 109.3), C-7 (δ_C 167.7) and C-9 (δ_C 159.9), from H-2 to C-9 and from the characteristic chelated phenolic hydroxyl group at the C-5 position (δ_H 13.58) to C-5 (δ_C 160.0), C-6 and C-10. The chemical

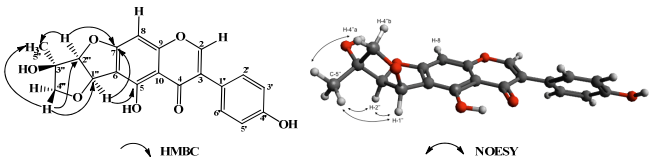

Figure 2: Pertinent HMBC and NOESY correlations for **1**.

Table 1: ^{13}C and ^{1}H NMR data of compounds **1** (in Acetone-d_6) and **2** (in DMSO-d_6).

Atom	1		Atom	2	
	δ_C	δ_H (m, *J* in Hz)		δ_C	δ_H (m, *J* in Hz)
2	154.2	8.22 (s)	2	153.9	8.36 (s)
3	123.7	-	3	120.6	-
4	181.8	-	4	180.1	-
5-OH	160.0	13.58 (s)	5-OH	155.6	13.38 (s)
6	106.9	-	6	104.0	-
7	167.7	-	7	157.7	-
8	89.3	6.44	8	94.1	6.47 (s)
9	159.9	-	9	156.4	-
10	109.3	-	10	105.1	-
1'	122.3	-	1'	122.1	-
2'	130.9	7.47 (d, 8.4)	2'	129.8	7.38 (d, 8.6)
3'	115.6	6.91 (d, 8.4)	3'	114.7	6.82 (d, 8.6)
4'-OH	158.1	8.55 (s)	4'-OH	157.1	9.58 (s)
5'	115.6	6.91 (d, 8.4)	5'	114.8	6.82 (d, 8.6)
6'	130.9	7.47 (d, 8.4)	6'	129.8	7.38 (d, 8.6)
1"	79.3	5.82 (d, 5.7)	1"	114.9	6.67 (d, 10.1)
2"	92.2	4.84 (d, 5.7)	2"	127.2	5.73 (d, 10.1)
3"-OH	77.7	4.23 (s)	3"	79.7	-
4"a	73.3	3.52 (d, 8.5)	1"'	34.2	1.92 (t, 8.0)
4"b		3.31 (d, 8.5)	2"'	37.2	2.54 (t, 8.0)
5"	24.3	1.42 (s)	3"'	207.2	-
			4"'	29.4	2.08 (s)
			5"'	26.2	1.40 (s)

shift at δ_H 8.55 was assigned to the hydroxyl proton OH-4' as this proton correlated with the carbons C-3'/5' in its HMBC spectrum. The presence of 13 degrees of unsaturation according to molecular formula, the fact that an isoflavone skeleton account for 11 degrees of unsaturation and the lack of additional NMR signals characteristic of double bonds in **1** suggested that it remained two degrees of unsaturation corresponding to two supplementary cycles. Based on the HMBC correlations (Figure 2) from H-1" to C-5 and C-7, from H-2" to C-7, from the methyl H-5" to C-2", C-3" (δ_C 77.7) and C-4" and from H-4" to C-1" and C-2", the presence of two furan rings for **1** was suggested. The first one being fused to C-6 and C-7 of ring A and the second one fused to C-1" and C-2" of the first furan moiety (Figure 2). Complete assignment of protons and carbons of **1** (Table 1) was achieved with COSY, HSQC, HMBC and NOESY experiments. The NOESY correlations (Figure 2) from methyl H-5" to both H-1" and H-2" indicated the relative configuration of **1** (all these protons were in the same side of the molecule whereas the hydroxyl was in the opposite side). Thus, compound **1** was elucidated to be Erinasone A.

Compound **2** was obtained as a brown oil. The ESI^+ mass spectrum of **2** gave pseudomolecular ion peaks at *m/z* 393 $[M + H]^+$ and 785 $[2M + H]^+$, indicating a molecular mass M = 392. The molecular formula was confirmed as $C_{23}H_{20}O_6$ by HRESIMS (*m/z* 393.1343; calcd for $C_{23}H_{21}O_6$, 393.1338). **2** also displayed typical UV absorption bands of an isoflavone at 226, 285 and 340 nm [6,14]. The ^{1}H NMR of **2** (Table 1) was similar to that of **1** for the aglycone moiety with signals of aromatic rings at δ_H 8.36 (s, H-2) for the ring C, 6.82 (2H, d, *J* = 8.6 Hz, H-3'/H-5') and 7.38 (2H, d, *J* = 8.6 Hz, H-2'/H-6') for the ring B and 6.47 (s, H-8) for the ring A, and two downfield protons at δ_H 9.58 and 13.71 attributed to the protons of hydroxyl groups OH-4' and OH-5, respectively. The main differences between **1** and **2** were the lack of oxymethine and oxymethylene signals and the appearance of two coupled olefinic protons signals at δ_H/δ_C 5.73/127.2 and 6.67/114.9 (H-1" and H-2", respectively), two coupled methylene signals at δ_H/δ_C 1.92/34.2 and

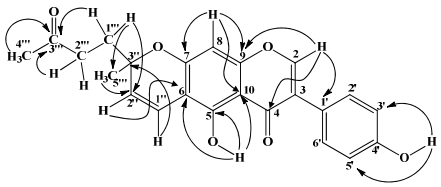

Figure 3: Key HMBC correlations for **2**.

2.54/37.2 (H-1"' and H-2"', respectively), a methyl signal at δ_H/δ_C 1.40/26.2 (H-5"') and an acetyl signal at δ_H/δ_C 2.08/29.4 (H-4"'). The double bond was located at C-6 based on the HMBC correlations between H-1" and carbons at δ_C 104.0 (C-6), 155.6 (C-5) and 157.7 (C-7). Furthermore, the HMBC spectrum revealed correlations (Figure 3) from H-1" and H-2" to methylene C-1"', the methyl C-5"' and a quaternary carbon at δ_C 79.7 (C-3"). The acetyl was linked to the methylene H-2"' as it correlated with the carbonyl ketone at δ_C 207.2. Analyses of COSY, HSQC, and HMBC experiments allowed assignments of all protons and carbons of compound **2** (Table 1). These structural features confirmed that **2** was a derivative of alpinumisoflavone [11]. Moreover, the configuration at chiral carbon C-3" remained unclarified. Based on the above data, the structure of **2** was characterized as Erinasone B.

The total phenolic and flavonoid contents of the EtOAc and *n*-BuOH extracts were carried out according to the calibration curve established with gallic acid and quercetin, respectively. The total phenolic and flavonoid contents of the ethyl acetate extract (26.13 ± 0.02 µg GAE/mg extract and 16.80 ± 0.007 µg QE/mg extract, respectively), were higher than the *n*-butanol extract (25.42 ± 0.004 µg GAE/mg extract and 12.53 ± 0.01 µg QE/mg extract, respectively).

The antioxidant activity of the EtOAc and *n*-BuOH extracts as well as the standard antioxidants (ascorbic acid and BHT) against the free radical DPPH indicated that EtOAc and *n*-BuOH extracts reacted positively with the DPPH radical as antioxidant. Both extracts possessed a moderate antioxidant activity. Their inhibitory concentrations at 50% (IC_{50}) were approximately 0.04 mg/mL (*n*-BuOH) and 0.037 mg/mL (EtOAc), but relatively low compared to ascorbic acid and BHT which exhibiting values of 0.0031 and 0.0059 mg/mL, respectively (Figure S2 and Table S1).

The FRAP assay was used to evaluate the reduction of iron in the presence of an antioxidant. The power reducing ability was calculated from the curve of the regression equation determined by the ascorbic acid. The obtained values were expressed in microgram of ascorbic acid equivalents per mg of extract (Table S1). The data indicated that the *n*-BuOH extract was more active than the EtOAc extract. The phosphomolybdate assay of the crude extracts was evaluated by the transformation of Mo (VI) to Mo (V) to obtain a phosphomolybdenum complex, which can be followed spectrophotometrically. In this study, the antioxidant power of EtOAc and *n*-BuOH extracts was expressed as µg of ascorbic acid equivalents per mg of the dried extract (µg AAE/mg extract). The results displayed that the antioxidant power of the EtOAc extract was higher than the *n*-BuOH extract (Table S1). The antioxidant activity of this plant evaluated by different methods can be attributed to the presence of flavonoids and polyphenolic compounds, which explain their ability to capture free radicals and metal complex [24].

The antibacterial activity of EtOAc and *n*-BuOH extracts was determined against four strains of microorganisms. The mean of the zones of inhibition were calculated and the results were presented in (Table S2). The results of the antibacterial activity revealed a sensibility only against Gram positive strain *Staphylococcus aureus*

and penicillin as a positive control with MIC values at 0.25 and 1 g/mL, respectively. The antibacterial activity of the EtOAc was higher than *n*-BuOH extracts. These findings can be explained by the presence of isoflavonoids which are known for their antibacterial activity [25].

Phytochemical investigation of *Erinacea anthyllis* allowed the isolation and characterization of two new prenylated isoflavonoids **1** and **2** and nineteen known compounds **3**-**21,** including twelve isoflavonoids, three polyphenols, one flavonol, two flavanones and three steroids. It is very important to indicate that all these compounds were isolated for the first time from the *Erinacea* genus. In this study, the total phenolic and flavonoid contents of EtOAc and *n*-BuOH extracts were determined. Furthermore, the antioxidant activity was evaluated by three different methods: DPPH radical scavenging, ferric reducing power and phospho-molybdate assay, and the antibacterial activity was also carried out against four strains. The obtained results indicated that both extracts (EtOAc and *n*-BuOH) showed moderate antibacterial and antioxidant activities. Moreover, this investigation revealed that isoflavonoids are major constituents of *E. anthyllis* which could be used as specific chemotaxonomic markers for the subfamily Papilionoideae. In addition, the occurrence of isoflavonoids in *E. anthyllis* known for their anti-inflammatory activity could explain the traditional use of this plant against the rheumatism disease [26,27].

Experimental

General: UV-Vis measurements were obtained on Beckman DU-600 spectrophotometer (Beckman, California, USA). IR spectra were recorded using a Shimadzu model IR-470 spectrometer (Shimadzu, Ontario, Canada) and FT-IR Bruker Tensor 27 spectrophotometer (Bruker, Wissembourg, France). The HR-ESI-MS analyses were performed on a Micromass-LCT Premier Time of Flight mass spectrometer (Waters, Milford, MA, USA). ^{1}H and ^{13}C spectra were measured in acetone-d_6, $CDCl_3$, CD_3OD or DMSO-d_6 using a Varian/Agilent Inova spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C (Varian, Palo Alto, CA, USA). Optical rotations were measured in methanol solution on a Perkin-Elmer 241 polarimeter (Perkin-Elmer, California, USA). Column chromatographies were carried out using Merck Kieselgel 60 (320–400 mesh), Merck Lobar Lichroprep RP-18 (40 × 63 μm), Polyamide SC6, and Sephadex LH-20. Analytical and preparative (1 mm thickness) TLCs were carried on Silica gel (Kieselgel 60 F_{254}, Merck), RP-18 (Kieselgel 60 F_{254s}) plates (Merck, Darmstadt, Germany), and developed by spraying with 50 % sulfuric acid reagent followed by heating.

Plant material: The plant material of *E. anthyllis* was collected in May 2013 in the Aures region (high mountains of Bellezma, Algeria) and was identified by Prof. Bachir Oudjehih, Agronomic Insitute of the University of Batna-1. A voucher specimen is kept under the number 690/LCCE.

Extraction and isolation: The powder of the whole plant *Erinacea anthyllis* (1500 g) was macerated twice (15 L × 2, each 48 h) with EtOH/H_2O (70:30) at room temperature. After filtration, the filtrate was concentrated and submitted to liquid–liquid fractioning using solvents successively petroleum ether, EtOAc and *n*-BuOH (each solvent, 125 mL × 5) to give 1 g of petroleum ether, 10.7 g of EtOAc and 50 g of *n*-BuOH extracts.

The EtOAc extract (10.7 g) was separated over a VLC carried on silica gel eluting with PE/EtOAc (100:0 to 0:100) and EtOAc/MeOH (100:0 to 0:100) to give nine fractions (F1–F9).

Fraction F5 (2260 mg) was subjected to polyamide column chromatography with the solvent system toluene/MeOH (100:0 to 0:100) to yield 13 sub-fractions (F5-1 to F5-13). The grouped three sub-fractions F5-5,6,7 (301.7 mg) were applied to purification on a silica gel CC using $CHCl_3$/EtOAc (100:0 to 30:70) to give **1** (2 mg). Sub-fractions F5-2,3 (415.6 mg) were combined and purified on a silica gel CC eluting with PE/EtOAc to afford 12 sub-fractions (F5-A to F5-L). Sub-frations F5-J (16.9 mg), F5-D (21.9 mg) and F5-F (69.1 mg) were purified by precipitation using hexane, acetone and MeOH respectively, to provide **2** (4.6 mg), **3** (6 mg) and **4** (3.1 mg). Sub-fraction F5-K (61.4 mg) was precipitated in MeOH to isolate **5** (2 mg) and the filtrate was submitted on a preparative TLC RP-18 in MeOH/H_2O (7:3) to afford **6** (4.9 mg). Sub-fraction F5-4 (250.8 mg) was separated over silica gel CC eluting with PE/EtOAc to yield **7** (3.0 mg). Sub-fraction F5-10 (373 mg) was applied to silica gel CC using PE/EtOAc to allow the isolation of **8** (7.8 mg), **9** (2.5 mg) and **10** (5.8 mg).

Fraction F3 (740.4 mg) of the VLC was applied to silica gel CC using PE/EtOAc (100:0 to 50:50) to obtain 16 sub-fractions (F3-1 to F3-16). Sub-fraction F3-6 (57.4 mg) was precipitated in petroleum ether to give **11** (52.7 mg). Preparative TLC of sub-fraction F3-8 (45.3 mg), developed with PE/EtOAc (7:3), afforded **12** (2.9 mg).

Fraction F4 (1826.3 mg) of the VLC was submitted to CC over silica gel, eluted with a gradient of solvents PE/EtOAc (100:0 to 30:70) to provide 15 sub-fractions (F4-1 to F4-15). Sub-fraction F4-12 (31.5 mg) was separated on TLC RP-18, to allow the isolation of **13** (8.3 mg) and **14** (8.5 mg). Sub-fraction F4-14 (182.3 mg) was purified on silica gel CC using $CHCl_3$/MeOH to give **15** (6.2 mg). Sub-fraction F4-6 (66.5 mg) was chromatographed on silica gel CC eluting with PE/EtOAc, and followed by TLC RP-18 in MeOH/H_2O (8:2) to yield **16** (6.1 mg) and **17** (4 mg).

The *n*-BuOH extract (14 g) was separated over a VLC (RP-18). Elution was performed with H_2O/MeOH (100:0 to 0:100) to give eleven fractions (F1–F11).

Fraction F2 (694.9 mg) of the VLC was fractionated on silica gel CC eluting with $CHCl_3$/MeOH to obtain 7 sub-fractions (F2-1 to F2-7). Sub-fraction F2-1 (22.3 mg) was precipitated in MeOH to provide **18** (7.2 mg).

Fraction F3 (2456.2 mg) was separated on CC of polyamide using H_2O/MeOH (100:0 to 0:100) to provide 11 sub-fractions (F3-1 to F3-11). Sub-fraction F3-5 (214.7 mg) was separated on silica gel CC with $CHCl_3$/MeOH (100:0 to 50:50) to give **19** (7.5 mg) and **20** (5.3 mg). Sub-fraction F3-1 (382.7 mg) was purified on silica gel CC using CH_2Cl_2/acetone (100:0 to 0:100) and MeOH/acetone (0-100 to 100-0) to afford **21** (3.4 mg).

Erinasone A (**1**)
Amorphous white powder.
$[\alpha]_D^{20}$ –24.6 (*c* 0.142, MeOH).
UV-Vis (MeOH) λ_{max} nm (log ε): 214 (3.84), 262 (3.93), 332 (2.55).
^{1}H NMR (500 MHz, Acetone-d_6): (Table 1).
^{13}C NMR (125 MHz, Acetone-d_6): (Table 1).
(–) HR-ESI-MS *m/z*: 367.0785 $[M - H]^-$ (calcd $C_{20}H_{15}O_7$, 367.0818), and 735.1729 $[2M - H]^-$; (+) HR-ESI-MS *m/z*: 369.1005 $[M + H]^+$ (calcd $C_{20}H_{17}O_7$, 369.0974).

Erinasone B (**2**)
Brown oil.
$[\alpha]_D^{20}$ +30.5 (*c* 0.292, MeOH).
UV-Vis (MeOH) λ_{max} nm (log ε): 226 (3.55), 285 (3.75), 340 (2.64).

^{1}H NMR (500 MHz, DMSO-d_6): (Table 1).
^{13}C NMR (125 MHz, DMSO-d_6): (Table 1).
(–) HR-ESI-MS *m/z*: 391.1208 $[M - H]^-$ (calcd $C_{23}H_{19}O_6$, 391.1182); (+) HR-ESI-MS *m/z*: 393.1343 $[M+H]^+$ (calcd for $C_{23}H_{21}O_6$, 393.1338).

Determination of the total phenolic content *(more details in the supplementary data)**:*** The total phenolic content of the extracts obtained from *E. anthyllis* was estimated by the Folin-Ciocalteu method.

Determination of the total flavonoid content *(more details in the supplementary data)**:*** The total flavonoid content of the extracts (*n*-BuOH and EtOAc) was evaluated by the method of the aluminum trichloride.

Antioxidant activity *(more details in the supplementary data):* The evaluation of the antioxidant activity of the crude extracts (EtOAc and *n*-BuOH) was carried out by three different methods including DPPH radical scavenging, iron reduction power test (FRAP) and phosphomolybdate assay (PPM).

Antibacterial activity *(more details in the supplementary data)**:*** The antibacterial activity of the EtOAc and *n*-BuOH extracts was estimated by the agar disk diffusion assay against four bacterial strains.

Supplementary data: Details on determination of the total phenolic and flavonoid contents, antioxidant and antibacterial assays, ^{1}H NMR, HSQC, HMBC, NOESY and ESI-MS spectra for compounds **1** and **2**, and structures of isolated compounds (**1-21)** are available online.

Acknowledgments - The authors thank the DGRSDT-Algeria for providing a research grant (PNR Project 8/u05/853) and School of Pharmaceutical Sciences, EPGL, University of Geneva, Geneva, Switzerland.

References

[1] Adanson M. (**1763**) Familles des Plantes. II partie. (Ed). Vincent, Paris, France, 1-640.
[2] Maire R. (**1952-1987**) Flore de l'Afrique du Nord. Vol. ***16***, (Ed). Le chevalier, Paris.
[3] M'hirit O, Blerot P. (**1999**) Plantes aromatiques et médicinales dans les hauts atlas-Le grand livre de la forêt marocaine. (Ed). Mardaga, France, 1-280.
[4] Birouk A. (**2009**) Consultant national en biodiversité et ressources phytogénétiques, Projet FAO/TCP/MOR/3201, Maroc, 1-34.
[5] Nes WD, Norton RA, Benson M. (**1992**) Carbon-13 NMR studies on sitosterol biosynthesized from [^{13}C]mevalonates. *Phytochemistry*, ***31***, 805-811.
[6] Wang F, Li X-L, Wei G-Z, Ren F-C, Liu J-K. (**2013**) New isoflavonoids from *Erythrina arborescens* and structure revision of anagyroid isoflavone A. *Natural Products and Bioprospecting*, ***3***, 238-242.
[7] Luo X-D, Wu S-H, Ma Y-B, Wu D-G. (**2001**) Chemical constituents from *Walsura yunnanensis*. *Acta Botanica Yunnanica*, ***23***, 515-520.
[8] Hammami S, Ben Jannet H, Bergaoui A, Ciavatta L, Cimino G, Mighri Z. (**2004**) Isolation and structure elucidation of a flavanone, a flavanone glycoside and vomifoliol from *Echiochilon fruticosum* growing in Tunisia. *Molecules*, ***9***, 602-608.
[9] Kaufman PB, Duke LA, Brielmann H, Boik J, Hoyt JE. (**1997**) A comparative survey of leguminous plants as sources of the isoflavones, genistein and daidzein: Implications for human nutrition and health. *Journal of Alternative and Complementary Medicine*, ***3***, 7-12.
[10] Almahy HA, Alhassan NI. (**2011**) Studies on the chemical constituents of the leaves of *Ficus bengalensis* and their antimicrobial activity. *Journal of Science and Technology*, ***12***, 118-124.
[11] Huang K-F, Hsu C-J. (**2001**) Constituents of stem bark of *Erythrina arborescens*. *Journal of Chinese Medical*, ***12***, 61-67.
[12] Tiana XM, Chena SZ, Tang L, Tu PF. (**2008**) Three new isoflavonoids from the aerial parts of *Ammopiptanthus mongolicus*. *Helvetica chimica Acta*, ***91***, 1015-1022.
[13] Yahara S, Ogata T, Saijo R, Konishi R, Yamahara J, Miyahara K, Nohara T. (**1989**) Isoflavan and related compounds from *Dalbergia odorifera*. *Journal of Chemical and Pharmaceutical Bulletin*, ***37***, 979-987.
[14] Tanaka H, Tanaka T, Etoh H, Watanabe N, Ahmad M, Qurashi I, Khan MR. (**1998**) Two new isoflavones from *Erythrina suberosa* var. *glabrescences. Heterocycles*, ***48***, 2661-2667.
[15] *Bohlmann F*, Grenz M. (**1970**) Neue isopentenyl-acetophenon-derivate aus *Helianthella uniflora*. *Chemische Berichte*, ***103***, *90*-96.
[16] Recourt K, Schripsema J, Kijne JW, Van Brussel AAN, Lugtenberg BJJ. (**1991**) Inoculation of *Vicia sativa* subsp, *nigra* roots with *rhizobium* leguminosarum biovar *viciae* results in release of *nod* gene activating flavanones and chalcones. *Journal of Plant Molecular Biology, **16**, 841-852.*
[17] Van Rensen, I, Veit M, Wray V, Czygan F-C. (**1995**) Genistein-*C*-Glucosides from *Genista cinerea*. *Natural Product Letters*, ***6***, 203-207.
[18] Yoo JS, Ahn EM, Bang MH, Song MC, Yang HJ, Kim DH, Lee DY, Chung HG, Jeong TS, Lee KT. (**2006**) Steroids from the aerial parts of *Artemisia princeps* Pampanini. *Korean Journal* of Medicinal *Crop Science, **14***, 273-277.
[19] Sato S, Hiroe K, Kumazawa T, Jun-Ichi, O. (**2006**) Total synthesis of two isoflavone *C*-glycosides: genistein and orobol 8-*C*-β-D-glucopyranosides. *Carbohydrate Research*, ***341***, 1091-1095.
[20] Yoshikawa M, Shimada H, Saka M, Yoshizumi S, Yamahara J, Matsuda H. (**1997**) Medicinal foodstuffs. V.[1)] Moroheiya. (1): Absolute stereostructures of corchoionosides A, B, and C, histamin release inhibitors from the leaves of Vietnamese *Corchorus olitorius* L. (Tiliaceae). *Chemical and Pharmaceutical Bulletin*, ***45***, 46-69.
[21] Dreyer DL. (**1978**) Kaempferol methyl ethers from flowers of *Dodonaea viscosa*. *Revista Latinoamericana Quimica*, ***9***, 97-98.
[22] Markham KR, Mabry TJ. (**1975**) Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids. In: *The Flavonoids*. Harborne J, Mabry TJ, Mabry H (Ed). Chapman and Hall, London, 45-77.
[23] Mabry TJ, Markham KR, Thomas MB. (**1970**) *The Systematic Identification of Flavonoids*. (Ed). Springer, Berlin, Germany.
[24] Joseph B, Raj SJ. (**2010**) Phytopharmacological properties of *Ficus racemosa* Linn an overview. *International Journal of Pharmaceutical Sciences Review and Research*, ***3***, 134-138.
[25] Dhayakaran RPA, Neethirajana S, Xue J, Shi J. (**2015**) Characterization of antimicrobial efficacy of soy isoflavones against pathogenic biofilms. *LWT-Food Science and Technology*, ***63***, 59-865.
[26] Sudhadevi PK. (**1999**) Folkloric plant remedies for rheumatism. *Ancient Science of Life, **18***, 264-265.
[27] Yankep E, Njamen D, Fotsing MT, Fomum ZT, Mbanya JC, Giner RM, Recio MC, Máñez S, Ríos JL. (**2003**) Griffonianone D, an isoflavone with anti-inflammatory activity from the root bark of *Millettia griffoniana*. *Journal of Natural Products*, ***66***, 1288-1290.