SHORT REPORT

A C G publications

Rec. Nat. Prod. 5:3 (2011) 238-241

records of natural products

Lignans and an Abundant flavone glycoside with Free-Radical Scavenging Activity from the Roots of the Endemic Species *Stachys mialhesi* de Noé

Souheila Laggoune[1], Ignacio Brouard[2], Francisco Leon[2], Claude-Alain Calliste[3], Jean-luc Duroux[3], Jaime Bermejo[2], Zahia Kabouche[1] and Ahmed Kabouche[1*]

[1]*Laboratoire d'Obtention de Substances Thérapeutiques (L.O.S.T), Université Mentouri - Constantine, Route de Ain El Bey, 25000 Constantine, Algérie*

[2]*Instituto de Productos Naturales y Agrobiologı´a-C.S.I.C.-Instituto Universitario de Bio-Orga´nica ''Antonio Gonza´lez'', Universidad de La Laguna, Av. Astrofı´sico F. Sa´nchez 3, 38206 La Laguna, Tenerife, Spain*

[3]*Laboratoire de Biophysique, UPRES EA-1085, Biomolécules et cibles cellulaires tumorales Faculté´ de Pharmacie, 2 rue du Dr. Marcland, 87025 Limoges Cedex, France*

(Received September 19, 2010; Revised October 5, 2011; Accepted December 10, 2011)

Abstract: Two sterols, stigmasterol (**1**) and β-sitosterol (**2**), two lignans, (+)-sesamin (**3**), new for the genus *Stachys* and (±)-paulownia (**4**), new in the Lamiaceae family, reported for the second time from a natural source, and one acetylated flavone glycoside isoscutellarein-7-*O*-(2"-*O*-6"'-*O*-acetyl-*β*-D-allopyranosyl-*β*-D-glucopyranoside (**5**), were isolated from the roots of *Stachys mialhesi* de Noé. Surprisingly, 3g of compound **5** are gathered from 5g of roots extract. The structures of compounds **1-5** were established on the basis of physical and spectroscopic analysis, and by comparison with the literature data. The free-radical-scavenging property of compound **5** was evaluated by the use of the ESR method in order to visualize the inhibition of the 1,1-diphenyl-2-1picrylhydrazyl (DPPH) free radical.

Keywords: *Stachys mialhesi;* Lamiaceae; lignans, free radical scavenging activity; ESR.

[*] Corresponding author: E-Mail: ahkabouche@yahoo.fr . Phone/fax : 213-31-818859

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 03/15/2011 EISSN: 1307-6167

1. Plant Source

The roots of *Stachys mialhesi* de Noé were collected on April 2005 at Djebel El-Ouahch Constantine (North Eastern Algerian) [1].The voucher specimen was identified by Professor Gérard De Bélair (University Badji-Mokhtar, Annaba) and was deposited at the Musée botanique de la Ville d'Angers (France) under the reference MBAng2005.10

2. Previous Studies

There is no study on the metabolites of this species in the liteature.

3. Present Study

Dried and finely powdered roots (180 g) of *Stachys mialhesi* were extracted with acetone by the use of a Soxhlet apparatus for two weeks. After removal of the solvent at 40 °C, the residue (5g) was column chromatographed on a silica gel column eluting with a gradient of cyclohexane/ethyl acetate with increasing polarity, then with methanol. Fraction F2 (50 mg) obtained from cyclohexane 94% was subjected to preparative silica gel TLC eluted with hexane/ethyl acetate (95:05), leading to compound **1** which was identified as stigmasterol (20 mg) [2] and compound **2** which was identified as β-sitosterol (24 mg) [2]. Fraction F3 (20 mg), obtained from cyclohexane 90% was further subjected to a Si-gel column eluting with hexane/ethyl acetate (40/10) to afford compound **3** (6 mg) which was identified as (+) sesamin [3,4], reported for the first time from *Stachys* genus. Fraction F4 (15 mg), obtained from cyclohexane 86 % was subjected to preparative silica gel TLC, and eluted with hexane/ethyl acetate (40:10) leading to compound **4** (5 mg) which was identified as (±)-paulownia [5], reported for the first time from Lamiaceae family as the second time from a natural source. Isoscutellarein-7-*O*-(2"-*O*-6"'-*O*-acetyl-*β*-D-allopyranosyl-*β*-D-glucopyranoside **5** [6,7] precipitated in all fractions starting from cyclohexane 76%. Surprisingly, compound **5** was found in all fractions with important amounts. Compounds **1-5** (Figure 1) were identified by the use of ^{1}H- and ^{13}C-NMR, DEPT, and two dimensional NMR experiments, COSY, HMQC, and HMBC in addition to UV spectroscopy.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Scavenging Test. The antioxidant activity of compound **5** was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH, from Sigma) free radical [8] using ESR (Electron Spin Resonance) spectroscopy [9]. Reaction mixtures contained 100 ml test samples and 100 ml DPPH ethanolic solution (5 x 10^{-4} M). Inhibition ratio was determined by comparison with a water-treated control group. ESR spectra were obtained with a Bruker ESP300E spectrometer using micro-sampling pipettes at room temperature under the following conditions: modulation frequency, 100 kHz; modulation amplitude, 0.197 mT; scanning field, 349.7 mT; receiver gain, 1.25 x 10^{5}; sweep time, 11 s; microwave power, 4 mW; microwave frequency, 9.78 GHz. All spectra were recorded at 3 min after homogenization by agitation. The inhibition percentage was calculated by using the double integral of the signal (Eq. 1):

$$\text{Inhibition ratio} = \frac{\text{ref-extract}}{\text{ref-bg}} \qquad \text{Eq. 1}$$

Where ref is the reference signal (DPPH + water), extract is the test signal, bg is the background signal. The data were the means of five measurements.

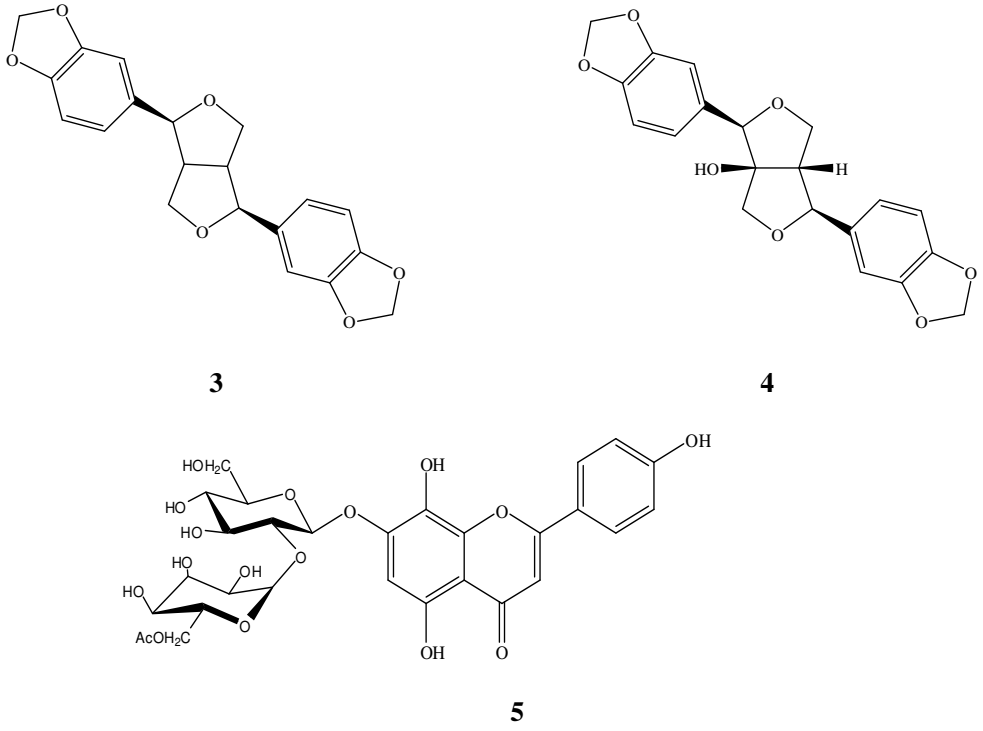

Figure 1. Isolated from the roots of *Stachys mialhesi* de Noé

Oxygen free radicals are involved in the pathophysiology of many diseases, such as inflammation, ischemic heart diseases, cancer, and many more. Possibly these plants possess good antioxidant properties due to the presence of polyphenolic compounds [10].
The free radical scavenging capacity of compound **5** was therefore evaluated.

The results showed the concentration of DPPH which gave 50% inhibition (IC_{50}). By comparison, the value of IC_{50} of vitamin E was, under the same experimental conditions, 0.025 mg/mL. Quercetin IC_{50} values were used as a reference [10]. In the DPPH test, quercetin IC_{50} was 0.012 mg/mL. The IC_{50} value 0.066 mg/mL was the result of a pure molecule **5**.
Many plants which contain flavonoids are known to possess good antioxidant activity by comparison with reference molecules, such as vitamin E.

In this study, it was expected that *S. mialhesi* de Noé might be a source of natural antioxidants. The performed test confirmed that antioxidant potential of the plant, representing by an abundant flavonoid glycoside (**5**) in its root extract with a fairly high IC_{50} value (0.066 ± 0.002 (mg/mL) to scavenge DPPH free radical.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/RNP

References

[1] P. Quezel and S. Santa (1962). Nouvelle Flore de l'Algérie et des Régions Désertiques Méridionales. C.N.R.S Paris, 2, 815-816.

[2] T. Akihisa, N. Shimizu, P. Ghosh, S. Thakur, F.U. Rosenstein, T. Tamura and T. Matsumoto (1987). Sterols of the Cucurbitaceaei *Phytochemistry* **26,** 1693-1700.

[3] J.K. Alvarez (2007). Aislamieto, purification e identification de sesamina a partir de lodos de microfiltrado en la fabricacion del aceite virgin de *Sesamum indicum* L. (ajonjoli), *Rev. Col. Cienc. Quim. Farm*. **36,** 5-10.

[4] Y. Li, M. Ye, H.W. Liu, X.H. Ji and Y.N. Yan (2000). Identification and determination of (+)-sesamin in *Semen cuscutae* by capillary GC and GC-MS, *Chinese Chem. Lett.* **11,** 1076-1076.

[5] S.R. Angel, I. Choi and F.S. Tham (2008). Stereoselective synthesis of 3-alkyl-2-aryltetrahydrofuran-4-ols: total synthesis of (±)-paulownin, *J. Org. Chem*, **73,** 6268-6278.

[6] A. Lenherr, M.F. Lahloub and O. Sticher (1984). Three flavonoid glycosides containing acetylated allose from *Stachys recta*, *Phytochemistry* **23,** 2343-2345.

[7] A. Lenherr and T.J. Mabry (1987). Acetylated allose-containing flavonoid glucosides from *Stachys anisochila, Phytochemistry* **26,** 1185-1188.

[8] J. Fatimi, J.F. Lagorce, J.L. Duroux, M.L. Chabernaud, J. Buxeraud and C. Raby (1993). 1,4,5-trialkylimidazole system anti-inflammatory properties of substitued derivatives, *Chem. & Pharm. Bul.* **42,** 698-701.

[9] F. Yamaguchi, T. Ariga, Y. Yoshimura and H. Nakazawa (2000). Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind, *J. Agric. & Food Chem.* **48,** 2320-2325.

[10] C.A. Rice-Evans, N.J. Miller and G. Paganga (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids, *Free Rad. Biol. & Med.* **20,** 933-956.