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A Flavonol Triglycoside from *Ecballium elaterium* (Cucurbitaceae)

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ABSTRACT

Quercetin 3-O-β-(2^{G}-O-β-xylopyranosyl-6^{G}-O-α-rhamnopyranosyl) glucopyranoside a flavonol triglycoside was isolated for the first time from the butanolic extract of aerial parts of Ecballium elaterium (L.) with rutin, its structures were established by spectroscopic analysis including Ultraviolet (UV), Infra-red (IR), ^{1}H, Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR), 2D-NMR (COSY, HMQC, HMBC and NOESY) and MS-FAB.

Keywords*:* Flavonol triglycoside, *Ecballium elaterium* (L.), Cucurbitaceae

INTRODUCTION

Ecballium elaterium (L.) is a species belonging to Cucurbitaceae family [1]. It is used in the Mediterranean region as a medicinal plant for the treatment of fever, cancer, liver disorders, jaundice, constipation, hypertension, dropsy, rheumatic diseases and fungicidal [2-9]. In Algeria it is known as «Fakous el hamir» where the fruit juice is still used in herbal folk remedy for the treatment of jaundice by nasal instillation while root are used externally by rubbing for painful articular conditions. All the parts of the plant are toxic at low doses particularly the root and undiluted fruit juice which are violent purgative, diuretic and emetic [10]. The major biological and pharmacological effects of the plant are attributed to cucurbitacin compounds [11-20] and previous phytochemical studies of the plant revealed the presence of flavonoid glycosides [21,22].

The goal of this study was to investigate chemical content of the butanolic extract obtained from the methanolic extract of aerial parts of *E. elaterium*; because it was found that, nothing was reported dealing with phytochemical study of the plant from Algeria. Herein we report the isolation and structure elucidation of two flavonol glycosides identified as quercetin 3-*O*-β-(2^{G}-*O*-β-xylopyranosyl-6^{G}-*O*-α-rhamnopyranosyl) glucopyranoside 1 and rutin 2.

To our knowledge, compound 1 (Figure 1) is identified for the first time from Cucurbitaceae family. These two compounds were fully characterized along by MS, IR, UV, ^{1}H and ^{13}C NMR spectroscopy, 2D NMR experiments and chromatographic behavior.

MATERIALS AND METHODS

General procedure

NMR spectra were recorded with either a Brucker DRX500 (500 MHz) or Brucker AVANCE DPX (250 MHz). Chemical shifts are shown in δ values (*ppm*). The signals of the deuterated solvent DMSO were taken as the reference (2.50 ppm in ^{1}H NMR and 39.9 ppm in ^{13}C NMR). FAB-MS spectra were carried out on Joel JMS-AX 500, 70 eV. Ultra violet absorption spectrums were recorded on Thermo Scientific Evolution 300UV-Vis spectrophotometer. IR spectroscopy was performed using the KBr disc method on Shumadzu 8201 PC infrared Fourier transformation spectrometer. Column chromatography was carried using Polyamide SC6 and Sephadex LH20. Chemicals are of analytical reagent grade and TLC cellulose plates (0.1 mm) were purchased from Macherey-Nagel (Germany).

Plant materials

The aerial parts of *Ecballium elaterium* (L.) A. Rich, was collected in the flowering period on June 2014 from Ain Samara location, Constantine (East Algeria). The plant was identified by Dr. H. Laouar, faculty of Sciences, University of Setif, Algeria. A voucher specimen is deposited in our laboratory, Chemistry Department, Mentouri-Constantine University.

Extraction and isolation

Air-dried powdered aerial parts (0.8 kg) were extracted 3 times with a solution of 80% methanol during 48 h. The combined extract was evaporated under reduced pressure and temperature and then the residue was dissolved in hot distilled water (300 ml). After filtration, the aqueous extract was successively extracted by solvents with increasing polarities and evaporated under reduced pressure to yield: Ethyl acetate (7 g) and 1-butanol (19 g).

The 1-butanol extract (10 g) was subjected to a Polyamide SC6 column chromatography, the elution was carried out with a gradient of water to MeOH, to give 70 fractions of 150 ml each. Similar fractions were combined according to their TLC (cellulose) properties using 15% HOAc (S1) and BAW (1-butanol-acetic acid-water, 4:1:5, upper phase, S2) as eluent to give fifteen main fractions.

Fractions eluted by 10% MeOH/ H_2O and gave positive reaction to $AlCl_3$ solution were combined and purified over a Sephadex LH20 column using MeOH-H_2O (7:3). A total of five fractions were collected, each of about 50 ml, fractions 2 and 4 afforded compounds 1 (30 mg) and 2 (12 mg) respectively.

Acid hydrolysis

A solution of each isolated glycosides (~5 mg in 5 ml methanol) was treated with 2 M HCl (5 ml) and heated at 100°C in boiling water bath for 1 h. The aglycone was extracted with EtOAc and concentrated under reduced pressure. The identification of aglycone was carried using UV spectra and by co-TLC with an authentic sample. The sugars in the aqueous layer were identified by co-TLC with authentic samples on silica gel TLC impregnated with 0.2 M NaH_2PO_4 using acetone-water (9:1) as eluent. The detection of sugar spots was visualized by aniline malonate reagent.

Isolated and identified compounds

Quercetin 3-*O*-β-(2^{G}-*O*-β-xylopyranosyl-6^{G}-*O*-α-rhamnopyranosyl)glucopyranoside (1)

Green-yellow amorphous powder (Methanol), R_f=0.74 (S1), 0.30 (S2); UV-Visible, λ_{max} (nm): MeOH 360 299sh 258, +NaOH 398 327 270, +$AlCl_3$ 430 305sh 274, +HCl 398 360sh 305sh 270, +NaOAc 396 330sh 269, +H_3BO_3 380 295sh 263; IR (KBr) ν_{max} (cm^{-1}): 3418 (O-H stretching), 2928, 2864 (CH_2 stretching), 1728 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz, δ, ppm, *J*/Hz): 12.62 (1H, *s*, 5-OH), 7.58 (1H, *dd*, *J*=8.4, 2.1Hz, H-6), 7.50 (1H, *d*, *J*=2.1Hz, H-2), 6.83 (1H, *d*, *J*=8.4Hz, H-5), 6.35 (1H, *d*, *J*=1.7Hz, H-8), 6.15 (1H, *d*, *J*=1.7Hz, H-6); *β*-Glc: 5.58 (1H, *d*, *J*=7.4Hz, Glc H-1), 3.47 (1H, Glc H-2), 3.2 (1H, Glc H-3), 3.5 (1H, Glc H-4), 3.3 (1H, Glc H-5), 3.7 (1H, Glc H-6a), 3.4 (1H, H-6b); *β*-Xyl: 4.56 (1H, *d*, *J*=7.0Hz, Xyl H-1), 3.05 (1H, Xyl H-2), 3.35 (1H, Xyl H-3), 3.2 (1H, Xyl H-4), 3.67 (1H, Xyl H-5a), 3.15 (1H, Xyl H-5b); *α*-Rha: 4.34 (1H, *br. s*, Rha H-1), 3.45 (1H, Rha H-2), 3.15 (1H, Rha H-3), 3.25 (1H, Rha H-4), 3.3 (1H, Rha H-5), 0.95 (3H, *d*, *J*=6.1Hz, Rha H-6); ^{13}C-NMR (DMSO-d_6, 125 MHz, δ, ppm): 156.36 (C-2), 132.87 (C-3), 177.28 (C-4), 161.21 (C-5), 98.22 (C-6), 164.43 (C-7), 93.56 (C-8), 155.78 (C-9), 103.73 (C-10), 121.83 (C-1), 116.06 (C-2'), 144.88 (C-3), 148.52 (C-4'), 115.3 (C-5), 121.15 (C-6'); *β*-Glc: 98.75 (Glc C-1), 81.56 (Glc C-2), 76.72 (Glc C-3), 69.61 (Glc C-4), 75.88 (Glc C-5), 66.40 (Glc C-6); *β*-Xyl: 104.35 (Xyl C-1), 73.88 (Xyl C-2), 75.99 (Xyl C-3), 69.38 (Xyl C-4), 65.52 (Xyl C-5); *α*-Rha:100.5 (Rha C-1), 70.31 (Rha C-2), 70.57 (Rha C-3), 71.87 (Rha C-4), 68.21 (Rha C-5), 17.66 (Rha C-6); MS (FAB$^+$): 765.26 [M+Na]$^+$, 743.24 [M+H]$^+$, 611.24 [M+H-Xyl]$^+$, 463.13 [M+H-Xyl-Rha]$^+$, 302.04 [M+H-Xyl-Rham-Glc]$^+$.

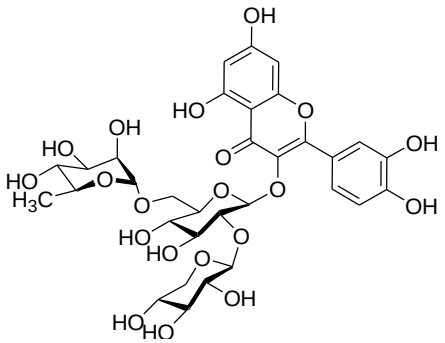

Figure 1: Structure of compound 1

Quercetin 3-*O*-β-(6-*O*-α-rhamnopyranosyl)glucopyranoside (2)

Yellow powder (Methanol), R_f=0.53 (S1), 0.50 (S2); UV-Visible, λ_{max} (nm): MeOH 360 300sh 266sh 258, +NaOH 408 329 271, +$AlCl_3$ 432 300sh 274, +HCl 400 360sh 300sh 269, +NaOAc 394 323 272, +H_3BO_3 380 299sh 263; ^{1}H-NMR (DMSO-d_6, 250 MHz, δ, ppm, *J*/Hz): 7.55 (1H, *dd*, *J*=8.5, 2Hz, H-6'), 7.54 (1H, *d*, *J*=2Hz, H-2), 6.85 (1H, *d*, *J*=8.5Hz, H-5), 6.40 (1H, *d*, *J*=2Hz, H-8), 6.20 (1H, *d*, *J*=2Hz, H-6); *β*-Glc: 5.34 (1H, *d*, *J*=7.4Hz, Glc H-1); *α*-rha: 4.38 (1H, *d*, *J*=1Hz, Rha H-1), 0.98 (3H, *d*, *J*=6.1Hz, Rha H-6), 3-4 ppm (*m*, 10H, overlapping signals); ^{13}C-NMR (DMSO-d_6, 62 MHz, δ, ppm): 157.04 (C-2), 133.66 (C-3), 177.76 (C-4), 161.6 (C-5), 99.10 (C-6), 164.5 (C-7), 94.0 (C-8) 156.83 (C-9), 104.35 (C-10), 122.01 (C-1), 116.64 (C-2), 145.15 (C-3), 148.81 (C-4), 115.63 (C-5), 121.56 (C-6); *β*-Glc: 101.54 (Glc C-1), 74.47 (Glc C-2), 76.80 (Glc C-3), 70.76 (Glc C-4), 76.27 (Glc C-5), 67.38 (Glc C-6); *α*-Rha: 101.15 (Rha C-1), 70.39 (Rha C-2), 70.93 (Rha C-3), 72.21 (Rha C-4), 68.66 (Rha C-5), 18.14 (Rha C-6).

RESULTS AND DISCUSSION

Compound 1 was obtained as a green-yellow amorphous powder with the expected behavior for a flavonol triglycoside and shown to be a quercetin glycoside since acid hydrolysis with 2 N HCl afforded quercetin, D-glucose, D-xylose and L-rhamnose. The positive ion FAB mass spectrum of compound showed a [M+H]$^+$ peak *m/z* 743 consistent with a molecular formula $C_{32}H_{38}O_{20}$ for the glycoside and the sodium complex [M+Na]$^+$ at *m/z* 765. The signals at *m/z* 611, 463 and 302 indicated the successive elimination of xylose, rhamnose and glucose respectively. The UV spectrum and its change after addition of shift reagents suggested the presence of free hydroxyl groups at C5, C7, C3 and C4' [21-24], these findings supported a 3-glycosylated quercetin.

The ^{1}H-NMR spectrum showed signals due to five kinds of aromatic protons [δ7.58 (1H, *dd*, *J*=8.4, 2.1, H-6'), δ7.50 (*d*, *J*=2.1, H-2'), δ6.83 (*d*, *J*=8.4, H-5'), δ6.35 (*d*, *J*=1.7, H-8), δ6.15 (*d*, *J*=1.7, H-6)] assignable to the quercetin skeleton and three anomeric signals [δ5.58(1H, *d*, *J*=7.4, Glc H-1), 4.56 (1H, *d*, *J*=7, Xyl H-1), 4.34 (1H, *br.s*, Rha H-1)]. The high field position of H-1 of *β*-xylose (δ4.56) and H-1 of α-rhamnose (δ4.34) indicated that they were terminal sugars and the chemical shift of the signal δ 0.95 (3H, *d*, *J*=6.1 Hz, Rha H-6) for methyl of rhamnose confirmed the presence of rutinosyl moiety [25].

The ^{13}C-NMR spectrum and its DEPT experiences displayed fifteen carbon signals that were attributed to quercetin nucleus and seventeen carbon signals in the region of sugars including three anomeric signals at δ104.35, 100.5 and 98.75 that gave cross peak with anomeric protons in HMQC spectrum. The ^{13}C-NMR shifts of the three sugars are consistent with those corresponding to β-D-glucopyranosyl, β-D-xylopyranosyl and α-L-rhamnopyranosyl moiety [26]. The deshielding of the ^{13}C signals of C-2 (δ81.56) and C-6 (δ66.40) of the glucose unit indicated the glycosylation at C-2 and C-6 respectively. The identity of the three sugars and their sequence were determined by the exhaustive analysis of 2D-NMR spectral data (COSY, HMQC, NOESY and HMBC). In the HMBC spectrum, the anomeric proton signal at δ4.56 (Xyl H-1) showed correlation with the carbon signal at δ81.56 (Glc C-2) confirmed the interglycosidic linkage xylosyl (1→2) glucose. Furthermore, the correlation between the anomeric proton signal at δ4.34 (Rha H-1) and the carbon signal at δ66.40 (Glc C-6) confirmed the interglycosidic linkage rhamnosyl (1→6) glucose. The chemical shifts of the other sugar protons were determined for the first time by the analysis of 2D-NMR spectra (COSY, HMQC, HMBC and NOESY) and its multiplicities are not mentioned because all signals overlap with solvent signal.

From the above evidences compound 1 was established as quercetin 3-*O*-[2-*O*-β -D-xylopyranosyl-6-*O*-α-L-rhamnopyranosyl]-β-D-glucopyranoside or quercetin 3-*O*-[2^{G}-xylosylrutinoside] which is isolated here for the first time from Cucurbitaceae family. It was previously reported only one time from the leaves of *Actinidia arguta* (Actinidiaceae) [27].

Compound 2 was identified as rutin by UV, ^{1}H, ^{13}C-NMR and chromatographic behavior [23,24,28,29]. Acid hydrolysis gave quercetin, glucose and rhamnose, which were identified by co-TLC with authentic samples [24].

CONCLUSION

From the survey of the literature to the best of our knowledge, this is the first report of the isolation of compound 1 from *E. elaterium* and from Cucurbitaceae family and the second isolation from a natural source. In addition, the chemical shifts of glycosidic protons are attributed and their MS spectrum is given for the first time.

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REFERENCES

[1] P. Quezel, S. Santa, CNRS, Paris, **1963**.
[2] C. Kavalci, P. Durukan, Y. Çevik, M. Özer, *Akademik Acil Tip Dergisi.*, **2007**, 5(3), 39-40.
[3] R.W. Rust, B.E. Vaissiere, P. Westrich, *Apidologie.*, **2003**, 34, 29-42.
[4] G. Kloutsos, D.G. Balatsouras, A.C. Kaberos, D. Kandiloros, E. Ferekidis, C. Economou, *Laryngoscope.*, **2001**, 111(9), 1652-1655.
[5] I. Touihri, O. Kallech-Ziri, A. Boulila, S. Fatnassi, N. Marrakchi, J. Luis, B. Hanchi, *Arabian J. Chem.*, **2015**.
[6] K.T. Chan, F.Y. Meng, Q. Li, T.C. Ho, T.S. Lam, Y. To, *Cancer Lett*., **2010**, 294, 118-124.
[7] K.P. Latté, *Phytother.*, **2009**, 30(3), 148-154.
[8] E.E. Mazokopakis, C.M. Karefilakis, I.K. Starakis, *J. Altern. Compl. Med*., **2009**, 15(12), 1273-1274.
[9] C. Uslu, R.M. Karasen, F. Sahin, S. Taysi, F. Akcay, *Int. J. Pediatr. Otorhinolaryngol.*, **2006**, 70, 515-518.
[10] L. Boulous, References Publication Inc. Michigan, USA, **1983**.
[11] R. Abou-Khalil, A. Jraij, J. Magdalou, N. Ouaini, D. Tome, H. Greige-Gerges, *J. Photochem. Photobiol. B*., **2009**, 95(3), 189-195.
[12] E. Attard, M. Brincar, A. Cuschieri, *Fitoterapia.*, **2005**, 76, 439-441.
[13] J. Sun, M.A. Blaskovich, R. Jove, S.K. Livingston, D. Coppola, S.M. Sebti, *Oncogene.*, **2005**, 24, 3236-3245.
[14] B. Jayaprakasam, N.P. Seeram, M.G. Nair, *Cancer Lett*., **2003**, 189, 11-16.
[15] M.A. Blaskovich, J. Sun, A. Cantor, J. Turkson, R. Jove, S.M. Sebti, *Cancer Res*., **2003**, 63, 1270-1279.
[16] G.A. Everaldo, S.S. Anthony, *Fitoterapia.*, **2001**, 72, 46-53.
[17] A. Ahmad, M. Manuel, J. Jose, A. Jose, C. Dolores, G. Andres, N. Concepcion, *Planta Med.*, **1999**, 65, 673.
[18] K.L. Duncan, M.D. Duncan, M.C. Alley, E.A. Sausville, *Biochem. Pharmacol*., **1996**, 52, 1553-1560.
[19] E. Yesilada, S. Tanaka, E. Sezik, M. Tabata, *J. Nat. Prod*., **1988**, 51, 504-508.
[20] S.I. Balbaa, A.Y. Zaki, S.M. EL-Zalabani, *Egypt. J. Pharm. Sci*., **1979**, 20, 221.
[21] I. El Sayed, Zeinab, H. W. Badr, *J. Appl. Sci. Res.*, **2012**, 8(2), 1252-1258.
[22] M. Krause Baranowska, W. Cisowski, *Herba Polonica*, **1995**, 41(1), 5-10.
[23] J.B. Harborne, T.J. Mabry, H. Mabry, Chapman and Hall, London, **1975**.
[24] K.R. Markham, Academic Press, London, **1982**.
[25] T.J. Mabry, K.R. Markham, M.B. Thomas, Springer-Verlag, **1970**.
[26] P.K. Agrawal, Elsevier, Amsterdam, **1989**.
[27] F.W. Rosemary, *Phytochemistry.*, **1991**, 30(7), 2443-2444.
[28] T.J. Mabry, K.R. Markham, M.B. Thomas, **1996**.
[29] J.B. Harborne, T.J. Mabry, Chapman and Hall, London, **1982**.