



Flavonoids from ethyl acetate extract of *Echinops spinosus* (Asteraceae)

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ABSTRACT

The aim of the present study was the phytochemical investigation of the ethyl acetate (EtOAc) soluble parts of the aqueous-MeOH extract of the aerial parts of *Echinops spinosus* L. (Asteraceae). This work resulted in the isolation and identification of three known compounds, Apigenin(1), Apigenin-7-O-β-glucopyranoside (cosmosiin) (2) and apigenin-7-β-D-O-(6''-O-E-p-coumaroyl)-glucopyranoside (3). The structures of the compounds were determined on the basis of extensive spectroscopic analysis, including UV, 1D and 2D NMR and comparison with the related known compounds. All these compounds are isolated from this species for the first time.

Key words: Apigenin, Apigenin derivatives, *Echinops spinosus*, Asteraceae.

INTRODUCTION

Plants from the Asteraceae family are distributed throughout the world and commonly used in treatment of various diseases [1-2]. The genus *Echinops* (Asteraceae), consisting in about 120 species of perennials, annuals, and biennials [3-4]. Species of this genus are predominately distributed in Eastern and Southern Europe, Tropical and North Africa and Asia [5]. *Echinops* plant was reported to possess variety of compounds belonging to various classes like: alkaloids, flavonoids, terpenoids, lipids, steroids and polyacetylenes [6-8].

Echinops spinosus is a perennial herb growing 1 meter and more and locally named Tassekra. As a medicinal plant, it was frequently employed in folk medicine as an abortifacient and a diuretic and for blood circulation, diabetes, gastric pain, indigestion and spasmodic problems [9]. *Echinops spinosus* contains quinoleic alkaloid, sesquiterpenoids: echinopine A and B and flavonoids derived from the apigenol and the chrysoeriol [10-11]. The purpose of the present work was the isolation and the structural elucidation of the constituents of ethyl acetate extract of the aerial parts of *Echinops spinosus*.

MATERIALS AND METHODS

Plant material

The aerial parts of *Echinops spinosus* L. were collected during the flowering phase in April 2009 in the North East of Algeria. The specimen was further identified by Dr. Gérard De Bélaïr, Université Badji Mokhtar, Annaba, Algeria on the basis of Quezel and Santa [12]. A voucher specimen has been deposited in the Herbarium of the Unit Research VARENBIOMOL of University frères Mentouri, Constantine (ESA04/09).

Extraction and isolation

The air-dried aerial parts of *Echinops spinosus* (1.5 kg) were macerated at room temperature with (MeOH/H₂O; 80:20; v/v) for 48 h. The hydromethanolic extract was concentrated and dissolved in H₂O (800 ml) under magnetic stirring. The resulting solution was filtered and successively extracted with CHCl₃, EtOAc and *n*-butanol. The organic layers were dried with Na₂SO₄, giving after removal of solvents under reduced pressure the following extracts: chloroform (3.0 g), EtOAc (4.0 g) and *n*-butanol (22.0 g). The EtOAc extract (3g) was fractionated by column chromatography (CC) on silica gel (70-230 mesh), eluting with CHCl₃/MeOH in gradient polarity to obtain 16 fractions (F1-F16), by combining the eluates on the basis of TLC analysis. The fractions F1 (14.5mg) and F6 (18mg) obtained as white powder, were washed successively with methanol to give two compounds identified as apigenin (**1**) and apigenin 7-*O*-glucoside (**2**). Fraction F8 (30 mg) was submitted to preparative TLC on silica gel GF254 eluting with (CHCl₃/MeOH, 8:2) to give apigenin-7-β-D-O-(6''-O-E-p-coumaroyl)-glucopyranoside (**3**). The structures of compounds were established using UV, 1D and 2D NMR as well as comparison with literature data.

RESULTS AND DISCUSSION

The phytochemical investigation of the EtOAc extract of *Echinops spinosus* led to the isolation of three flavonoids (**1-3**) using chromatographic methods than identified on the basis of their UV and NMR spectral data and comparison with literature data. All compounds were isolated for the first time from the aerial parts of this plant.

Compound (1): UV (λ_{\max} nm, MeOH): 268, 335; +NaOH: 274, 324,392 with hyperchromic effect; +NaOAc: 276, 306, 380; +NaOAc +H₃BO₃: 269, 338; +AlCl₃: 274, 300, 348, 382; +AlCl₃+HCl: 274, 300, 334, 380. ¹H-NMR (500 MHz, MeOH-d₄, δ /ppm, *J*/Hz): 7.7 (2H, *d*, *J*=8.5, H-2'/H-6'), 6.8 (2H, *d*, *J*=8.5, H-3'/H-5'), 6.4 (1H, *s*, H-3), 6.1 (1H, *brs*, H-8), 5.8 (1H, *brs*, H-6). This compound was identified as **Apigenin** [13].

Compound (2): UV (λ_{\max} nm, MeOH): 269,336; +NaOH: 268, 388 with hyperchromic effect; +NaOAc: 269, 386; +NaOAc+H₃BO₃: 269,340; +AlCl₃: 272, 299, 345, 381; +AlCl₃+HCl: 276, 298, 341, 380. ¹H NMR (500 MHz, MeOH-d₄, δ /ppm, *J*/Hz):7.86 (2H, *d*, *J*=8.6, H-2'/H-6'), 6.88 (2H, *d*, *J*=8.6, H-3'/H-5'), 6.82 (1H, *s*, H-3), 6.63 (1H,*brs*, H-8), 6.50 (1H,*brs*, H-6), 5.08 (1H, *d*, *J*=7.7, anomeric proton of glucosyle), 3.93(1H, *brd*, *J*=12 Hz, H-6''a); 3.72 (1H, *dd*, *J*=11.5; 5.5, H-6''b); 3.66 to 3.38 the other protons of the glucosyle. This compound was identified as **Apigenin 7-O-glucopyranoside** [14].

Compound (3): ¹H NMR (500 MHz, DMSO-d₆, δ /ppm, *J*/Hz): flavonoid moiety: 12.99 (1H, *s*, OH-5),7.94 (2H, *d*, *J*=8.8, H-2'/H-6'), 6.93 (2H, *d*, *J*=8.8, H-3'/H-5'), 6.82 (1H, *s*, H-3), 6.81 (1H, *d*, *J*=2.0, H-8), 6.47 (1H, *d*, *J*=2.0, H-6). Glucopyranoside moiety: 5.16 (1H, *d*, *J*=7.5,anomeric proton of glucosyle), 4.46 (1H, *brd*, *J*=10.4, H-6''a), 4.16 (1H, *dd*, *J*=10.4; 6.9,H-6''b), 3.52 to 3.90 the other protons of the glucoside deduced from COSY spectrum; P-(E)-coumaroyl: 7.36 (2H, *d*, *J*=8.6, H-2'''/H-6'''), 7.49 (1H, *d*, *J*=15.9, H-β), 6.67 (2H, *d*, *J*=8.6, H-3'''/H-5'''), 6.33(1H,*d*, *J*=15.9, H-α) deduced from COSY spectrum.¹³C NMR (125 MHz, DMSO-d₆, δ_c /ppm): 164.7 (C-2), 103.5 (C-3), 182.4 (C-4), 161.9 (C-5), 99.9 (C-6), 163.1 (C-7), 95.1 (C-8), 157.4 (C-9), 105.8 (C-10), 121.3 (C-1'), 129.0 (C-2'/C-6'), 116.1 (C-3'/C-5'), 161.6 (C-4'), 114.1 (C-α), 145.4 (C-β), 125.3 (C-1'''), 130.6 (C-2''/C-6'''), 116.2 (C-3''/C-5''') deduced from HSQC and HMBC spectra.This one was identified as **Apigenin-7-β-D-O-(6''-O-E-p-coumaroyl)-glucopyranoside** [15].

CONCLUSION

The present study relates to the phytochemical investigation of the EtOAc extract of the aqueous-methanolic extract obtained from the aerial parts of *Echinops spinosus*. This contribution led to the isolation of three known flavonoids named as *apigenin*, *apigenin7-O-β-glucopyranoside* and *apigenin-7-β-D-O-(6''-O-E-p-coumaroyl)-glucopyranoside*. All compounds are reported for the first time from this plant.

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