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OPTIMAL EXTRACTION METHOD OF PHENOLICS FROM THE ROOT OF *Euphorbia condylocarpa*

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The plants of Euphorbiaceae contain acrid, milky, or colorless juice. Chemical data are available for several genera, especially Euphorbia, where more than 120 species have been investigated. A survey of the data showed that the triterpenoids, followed by flavonoids and alkaloids, are the main classes of substances of interest to phytochemists. However, the presence of other substances, e.g., coumarins, cyanogenic glucosides, and tannins, are also reported. The family Euphorbiaceae is rich in flavonoids, particularly flavones and flavonols, which have been identified from several genera. They occur both as *O*- and C-glycosides and as methyl ethers. Flavanones also occur, but in relatively few plants. The flavonoids were detected in different parts of the plant other than the roots [1]. The root of *Euphorbia condylocarpa* M. Bieb. has important applications in folk medicine for the treatment of the cancer, costiveness, and migraine and as an emollient [2]. Furthermore, the studies in 1970 on *Euphorbia condylocarpa* demonstrated the presence of phytochemicals such as flavonoids, tetracyclic triterpenoids, and trifolin in different parts of the plant [3–5]. The purpose of this study is to phytochemically analyze the root of *Euphorbia condylocarpa* as a relatively unknown plant in phytochemical research and also to apply the Emerson reaction as an organic reaction to optimize the extraction conditions in phytochemical research for the first time. In this study, the optimized conditions for extraction of phenolics from the root of *Euphorbia condylocarpa* have been investigated via the Emerson reaction.

The root of *Euphorbia condylocarpa* was collected in July 2008 in the "Sarshive" region in Iranian Kurdistan. The dried powder of the root of *Euphorbia condylocarpa* (150 g) was lyophilized with *n*-hexane, and then extracted with a mixture of ethanol and water. After filtration and enrichment, it was made up to volume using 90% EtOH in a 25 mL flask (solvent A). 5 mL of solvent A was poured into a flask (100 mL), and 40 mL 90% EtOH was added, then made up to volume with doubly distilled water (solvent B). A mixture of 5 mL solvent B, 45 mL doubly distilled water, 1 mL 3.5% NH_3 (aq.), and 1 mL 2% 4-AAP was poured into a decanter (100 mL) and vigorously shaken. 4 mL 2% $K_3[Fe(CN)_3]$ was added to the decanter and the mixture shaken for 5 min. 25 mL CH_3Cl was added to extract the oxidized phenolic compounds (3 times). The extracted layer was transferred to a 100 mL flask, then made up to volume (solvent C). The absorbance of solvent C was measured at 455 nm, and the percentage of phenolics was measured as following:

$$\text{Percentage of phenolics} = 100\,[E \times V_1 \times V_2] / [E^{1\%}_{1cm} \times b \times y_1 \times y_2],$$

where E is the absorbance of solvent C at 455 nm, b is the weight of the dried sample (g), $E^{1\%}_{1cm}$ is the absorbance of a 1% solvent of standard arbutin in a 1 cm cell at 455 nm, and V_1 and V_2 are the dilution factors or the volume of the flask containing solvent A and final dilution for solvent C.

Therefore, the optimized conditions were obtained at temperature 60°C, time of extraction 6 h, EtOH concentration 80%, and ratio of dried powder to volume of solvent 1:10 (w/v).

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Using the optimal conditions, 200 g dried powder of the root of *E. condylocarpa* was lyophilized with *n*-hexane and extracted with 80% EtOH at 60°C for 6 h using a Soxhlet extractor apparatus. After filtration and enrichment, the concentrated product was divided in two parts, A and B. Part A was again extracted using EtOAc. The EtOAc extract (5 g) was impregnated with 3 g silica gel and loaded on a column chromatograph (100 cm × 2.5 cm) containing silica gel G-60. The column was eluted with *n*-hexane (100%), *n*-hexane–EtOAc (9:1, 1:1), EtOAc (100%), *n*-hexane–MeOH (9:1, 1:1), and MeOH (100%). Three widely distributed flavonoids **1–3** were identified in *n*-hexane: MeOH (80%). Part B was also loaded on a column chromatograph using silica gel as stationary phase. Elution was performed with a mixture of $CHCl_3$–MeOH (9:1) with increasing polarity. The solvent from the eluate was evaporated under vacuum and recrystallized. In addition, for further purification, a column of Sephadex LH-20 was used to give flavonoid **4**.

Quercetin (1). UV (MeOH, λ_{max}, nm): 374, 256. PMR (400 MHz, DMSO-d_6, δ, J/Hz): 12.52 (s, 5-OH), 10.81 (s, 7-OH), 9.40 (s, OH on C-3, C-3′ and C-4′), 7.71 (d, J = 2.65, H-2′), 7.57 (dd, J = 2.65, 7.94, H-5′), 6.91 (d, J = 7.94, H-6′), 6.44 (d, J = 2.65, H-6), 6.22 (d, J = 2.65, H-8). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 175.74 (C-4), 163.78 (C-7), 160.02 (C-5), 156.08 (C-9), 147.60 (C-3′) an 146.69 (C-2), 144.95 (C-4′), 135.64 (C-1′), 121.87 (C-3), 119.89 (C-6′), 115.51 (C-5′), 114.96 (C-2′), 102.91 (C-10), 98.08 (C-6), 93.26 (C-8).

Luteolin (2). UV (MeOH, λ_{max}, nm): 354, 253. PMR (400 MHz, DMSO-d_6, δ, J/Hz): 12.99 (s, 5-OH), 10.12 (s, 7, 3′, 4′-OH), 7.42 (dd, J = 2.21, 8.81, H-6′), 7.41 (d, J = 2.21, H-2′), 6.90 (d, J = 8.81, H-5′), 6.29 (s, H-3), 6.43 (d, J = 1.32, H-6), 6.16 (d, J = 1.32, H-8). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 181.56 (C-4), 164.08 (C-7), 163.78 (C-2), 161.37 (C-5), 157.18 (C-9), 149.61 (C-4′), 145.64 (C-3′), 121.37 (C-1′), 118.90 (C-6′), 115.90 (C-5′), 113.24 (C-2′), 103.57 (C-10), 102.75 (C-3), 98.73 (C-6), 93.74 (C-8).

Morin (3). UV (MeOH, λ_{max}, nm): 377, 263. PMR (400 MHz, DMSO, δ, J/Hz): 6.16 (d, J = 2.01, H-8), 7.25 (d, J = 2.01, H-6), 6.92 (d, J = 2.01, H-3′), 6.36 (d, J = 2.21, H-6′), 6.44 (dd, J = 2.01, 8.42, H-5′), 12.61 (5-OH), 10.66 (7-OH), 9.74 (3-OH), 9.4 (2′, 4′-OH). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 176.7 (C-4), 163.78 (C-7), 156.23 (C-5), 156.8 (C-2′), 165.71 (C-3), 160.75 (C-4′), 93.44 (C-6), 98.12 (C-8), 103.29 (C-3′), 106.67 (C-10), 109.73 (C-5′), 131.38 (C-6′), 136.69 (C-3), 149.09 (C-2), 160.74 (C-9).

Naringin (4). UV (MeOH, λ_{max}, nm): 282, 326. PMR (400 MHz, DMSO, δ, J/Hz): 11.88 (s, 5-OH), 9.51 (s, 4′-OH), 7.17 (d, J = 8.0, H-2′, 6′), 6.64 (d, J = 8.0, H-3′, 5′), 5.94 (d, $J_{H6/H8}$ = 2.0–2.5, H-8), 4.97 (d, J = 2.0–2.5, H-6), 5.13 (d, J = 4.5, H-2), 4.70 (dd, J = 12.0, J = 4.5, H-3A), 4.8 (dd, J = 12.0, J = 3.0, H-3B).

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