Optimal extraction method of Phenolics from root of the *Euphorbia condylocarpa M. Beib*

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The plants of the Euphorbiaceae contain acrid, milky or colourless juice.Chemical data are available for several genera, especially Eubhorbia, where more than 120 species have been investigated. A survey of this 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 0- 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 the *Euphorbia condylocarpa M. Beib* has important applications in folk medicine for treatment the cancer, costiveness, emollient and migrant [2]. Furthermore, the studies in 1970 on the *Euphorbia condylocarpa M. Beib* demonstrated the presence of phytochemicals such as Flavonoids, tetracyclic triterpenoids and Trifolin in different parts of the plant [3-5]. The the purpose of this study is to phytochemically analyze of the root of the *Euphorbia condylocarpa M. Beib* as an relatively unknown plant in phytochemical research and also to apply the Emerson reaction as an organic reaction to optimize the extraction conditions in phytochemical researches for the first time. In this study, the optimised conditions for extraction of phenolics from the root of the *Euphorbia condylocarpa M. Beib* have been investigated via Emerson reaction as following:

The root of *Euphorbia condylocarpa* was collected in July 2008 in ñSarshiveò region in Irainian Kurdistan. The dried powder of the root of the *Euphorbia condylocarpa* (150 g) was lyophilized with n-hexan, and then extracted with a mixture of ethanol and water. After filtration and enrichment, it was made up to volume using 90% EtOH in 25ml flask (solvent A). 5 mL of solvent A was poured into a flask (100 mL) and 40 mL EtOH 90% was added then made up to volume with doubly distilled water (solvent

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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 $K_3[Fe(CN)_3]$ 2% was added to decanter and shaken for 5 min. 25 mL CH_3Cl was added to extract the oxidized phenolic compounds (3 times). The extracted layers was transeferred to a 100 mL flask then made up volume (solvent C). The absorbance of solvent C was measured at 455 nm and 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 absorbent of solvent C at 455 nm, b, is the weight of the dried sample (g), $E^{1\%}_{1cm}$, is the absorbance of 1% solvent of standard Arbutin in a 1cm cell at 455 nm, V_1 and V_2 are dilution factors or the volume of the flask containing solvent A and final dilution for solvent C.

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

Using the optimal conditions, 200 g dried powder of the root of the *E.condylocarpa* was lyophilized with n-hexan and extracted with EtOH 80% in 60°C for 6h using soxhlet extractor apparatus. After filtration and enrichment, the concentrated product divided in two parts, A and B. Part A was again extracted using EtOAc. The EtOAc extract (5g) was impregnated with 3g silica gel and loaded on column chromatography (100 cm × 2.5 cm) containing silica gel G-60. The column was eluted with n-hexan (100%), n-hexan: EtOAc (9:1, 1:1), EtOAc (100%), n-hexan:MeOH (9:1, 1:1) and MeOH (100%). Three widely distributed Flavonoids **1-3** identified in n-hexan: MeOH (80%). Part B also loaded on column chromatography using silica gel as stationary phase. Elution was performed with a mixture of MeOH:$CHCl_3$ (1:9) with increasing polarity. The solvent from the eluate was evaporated under vacuum and recrystallised. In addition, for further puriýcation, again the 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, ŭ, J/Hz): 12.52 (s, OH-5), 10.81 (s, OH-7), 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, ŭ, J/Hz): 12.99 (s, OH-5), 10.12 (s, OH-7,OH-3',OH-4'), 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), 6.30 (AX, δA = 6.43, H-6/ δX = 6.16, H-8, J = 1.32): ^{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, ŭ, J/Hz): 6.16 (J = 2.01, d, H-8), 7.25 (J = 2.01, d, H-6), 6.92 (J = 2.01, d, H-3'), 6.36 (J = 2.21, d, H-6'), 6.44 (J = 2.01-8.42, dd, H-5'), 12.61 (OH-5), 10.66 (OH-7), 9.74 (OH-3), 9.4 (OH-2' and OH-4'); ^{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) an 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 (400MHz, DMSO, ŭ, J/Hz): 11.88 (s, OH-5), 9.51 (s, OH-4'), 7.17 (J = 8.0, d, H-2',H-6'), 6.64 (J = 8.0, d, H-3', H-5'), 5.94 ($J_{H6/H8}$ = 2.0-2.5, d, H-8), 4.97 ($J_{H6/H8}$ = 2.0-2.5, d, H-6), 5.13 ($J_{H2/H3A}$ = 4.5, dd, H-2), 4.70 ($J_{H3A/H3B}$ = 12.0, dd, $J_{H2/H3A}$ = 4.5, H-3A), 4.8 ($J_{H3A/H3B}$ = 12.0, dd, $J_{H2/H3A}$ = 3.0, H-3B).

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