International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html

Flavonoids from Euphorbia *condylocarpa* roots

Govan Fakhir Hassan[a], Muhammad Abdussamad Omer[a], Shahram Babadoust[b]* Darin Dldar Najat[a]

[a]*Soran Ishik College, Soran, Kurdistan Regional Government, Iraq*
[b]*Young Researchers Club, I. A. U, Sanandaj, Iran*

Abstract

During this study, the extraction and characterization of flavonoids from the root of *Euphorbia condylocarpa* has been studied and some widely distributed phenolics of Apigenin, Isorhamnetin-3-rutinoside, Kaempferol-3-O-rutinoside and Quercetin 3-O-glucoside are reported for the first time from the genus.

Key words: *Euphorbia condulocarpa*, Apigenin, Isorhamnetin-3-rutinoside, Kaempferol-3-O-rutinoside and Quercetin 3-O-glucoside

Full length article Received: 04-07-2014 Revised: 17-07-2014 Accepted: 25-07-2014 Available online: 31-07-2014
**Corresponding Author*, e-mail: shahram.babadoust.D@gmail.com

1. Introduction

Nowadays, heterocyclic compounds, especially those containing nitrogen, oxygen and sulfur moieties attract the interest of many researchers, in which during the last decades, an increasing number of scientific findings about their highly efficient synthetic methods and effects of them on health and human life have been reported [1-8]. Among the mentioned category, flavonoids are the secondary heterocyclic metabolites that are widespread in different plants. In some cases they present as flavonoid aglycones in plants which are including one or more sugar molecules attached to the flavon nuclei as O-glycoside or C-glycoside [9]. Flavonoid glycosides and free aglycones are involved in interactions of plants with microorganisms, both pathogenic and symbiotic, [10-12] and therefore have a wide spectrum of antimicrobial and pharmacological activities [13, 14].
The family *Euphorbiaceae* is rich in antioxidant flavonoids, particularly flavones and flavonols, which have been identified from several genera. Furthermore, the previous studies in 1970 on *Euphorbia condylocarpa* demonstrated the presence of phytochemicals such as flavonoids, tetracyclic triterpenoids, and trifolin in different parts of the plant [15-17]. In continuation of our study on different aspects of heterocycles [18] we investigated the extraction and characterization of some Phenolics as pharmacological bioactive agents from the root of the *Euphorbia condylocarpa* as an important medicinal plant in Kurdish folk medicine.

2. Material and Methods

All the chemicals and reagents used in the present study were purchased from Aldrich. The NMR spectra were obtained for ^{13}C-NMR (100 MHz) and 1H-NMR (400MHz) using Bruker- Avance spectrometer.

2.1. Plant material.

Euphorbia condylocarpa M. bieb roots shown in figure 1 was collected was collected in July 2012 in Haji Omran region in Kurdistan of Iraq. The Voucher specimen was deposited at the Kurdistan natural recourses office.

2.2. Extraction and Isolation.

According our previous study on the optimal extraction conditions of phenolics from the root of *E. Condylocarpa*, we applied the optimum conditions during this research [19]. Therefore, for extraction of phenolics from the plant 500g dried powder of the root of *E.condylocarpa* was lyophilized with n-hexane and extracted with 80% EtOH in 60^{o}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 (10g) was impregnated with 3g silica gel and loaded on a column chromatograph (80cm × 2.5cm) containing silica gel G-60. The column was eluted with an increasing polarity gradient of n-hexane (100%), n-hexane–EtOAc (9:1, 1:1), EtOAc (100%), n-hexane–MeOH (9:1, 1:1), and MeOH (100%). A widely distributed phenolic (I) was 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 elute was evaporated under vacuum and for further purification, a column of Sephadex LH-20 was used to give known flavonoids II, III and IV, Figure 2. The structures of separated compounds were elucidated using ^{1}H-NMR and ^{13}C-NMR and comparing with references and authentic samples [20-25].
Apigenin (I): ^{1}HNMR (DMSO-d_6, 400 MHz, δ(ppm)) δ 12.94 (s, 1H, exchanges with D_2O, OH on C-5), 10.78 (br s, 1H, exchanges with D_2O, OH on C-7), 10.33 (br s, 1H, exchanges with D_2O, OH on C-4′), 7.46 (AB, 4H, δA = 7.92

(H-3′ and H-5′) and δB = 6.95 (H-2′ and H-6′), 3JAB = 9.53), 6.72 (s, 1H, H-3), 6.2820 (AX, 2H, δA = 6.43 (H-6) and δX = 6.14 (H-8), 4JAX = 1.59).

^{13}C NMR (DMSO-d_6, 100 MHz, δ(ppm)) δ 181.64 (C-4), 164.01 (C-7 or C-2), 163.61 (C-2 or C-7), 161.35 (C-5), 161.05 (C-4′), 157.19 (C-9), 128.35 (C-2′ and C-5′), 121.07 (C-1′), 115.84 (C-3′ and C-5′), 103.60 (C-10), 102.72 (C-3), 98.73 (C-6), 93.85 (C-8).

Isorhamnetin-3-rutinoside(II): ^{1}HNMR (DMSO-d_6, 400 MHz, δ(ppm)) 12.56 (s, OH-5); 9.81 (s, OH-4); 7.51 (dd, J= 2.5, 8.5 Hz, H-6'); 6.44 (d, J= 2 Hz, H-8);5.44 (d, J= 7 Hz, H-1"); 3.84 (s, OCH3-3'); 0.95 (d, J= 6 Hz, CH3-6"), 10.91(s, OH-7); 7.86 (d, J= 2.5 Hz, H-2'); 6.91 (d, J= 8.5 Hz, H-5'); 6.21 (d, J= 2 Hz, H-6);4.42 (s, H-1'"); 3.06-3.7 (m, glu, rhe)

^{13}C NMR (DMSO-d6, 100 MHz, δ(ppm)) 177.4 (C-2); 147.2 (C-4′); 113.6 (C-2′); 94.1(C-8); 70.9/70.6 (C-2‴,C-3‴);17.9(C-6‴),164.2(C-7);133.3(C-3);104.3(C-10);76.7(C-3′);161.4(C-5);122.6(C-6′);101.4(C-1″); 76.2(C-5″); 68.6(C-5‴); 156.8(C-2, C-8); 121.4(C-1′); 101.2(C-1‴); 72.1(C-4‴); 67.2(6″); 149.6 (C-3′); 115.6, (C-5′); 99.0 (C-6); 56.0 (OCH_3).

Kaempferol-3-O-rutinoside(III): ^{1}H-NMR (CD_3OD, 400 MHz, δ(ppm)): 8.06 (d, 2H, J2',3' = J6',5' = 9.0 Hz, H-2', 6'), 6.88 (d, 2H, J3',2' = J5',6' = 8.7 Hz, H-3', 5'),6.40 (d, 1H, JJ8,6 = 2.4 Hz, H-8), 6.25 (d, 1H, JJ6,8 = 2.3 Hz, H-6), 5.12 (d, 1H, J",2" = 7.3 Hz, H-1"), 4.57 (d, 1H, J1",2" = 6.1Hz, H-l'"), 3.80 (dd, 1H, J6a,6b = 1Hz, J6a,5 = 9.6Hz, H-6a), 3.61 (dd, 1H, J2'",1'" = 1.7 Hz, J2'",3'" = 3.4 Hz, H-2'"), 3.53 (dd, 1H, J6b, 6a = 3.6Hz, J6b,5 = 9,5 Hz, H-6b),3.20-3.40 (m, 7H, H-2", 3", 4",5",3" ,4" ,5'"), 1.12 (d, 3H, J6",y' = 6.1 Hz, Rham-CH_3).

^{13}C-NMR (CD_3OD, 100 MHz, δ(ppm)): 179.4(C-4), 166.3(C-7), 163.1(C-s), 161.6(C-4'),159.7(C-9), 158.7(C-2), 135.7(C-3), 132.6(C-2 ,6'), 123.0(C-l'), 116.4(C-3, 5), 105.8(C-10),104.7(C-l'"), 102.6(C-1"), 100.3(C-6), 95.2(C-8), 78.1(C-3'"), 77.3(C-s'"), 75.9(C-2'"), 74.1(C-4"), 72.5(C-3"), 72.3(C-2"), 71.6(C-4'"), 70.0(C-5"), 68.8(C-6'"), 18. 1(C-6").

Quercetin3-O-glucoside(IV): ^{1}H-NMR(CD_3OD,400MHz, δ(ppm)): 6.10(1H, d, J= 2.0 Hz, H-6), 6.26(1H, d, J= 2.0 Hz, H-8), 6.85(1H, d, J= 8.0Hz, H-5′), 7.57(1H, dd, J = 2.0, 7.5 Hz, H-6′), 7.70(1H, d, J = 2.0 Hz, H-2′), 5.10(1H, d, J = 7.7 Hz, H-1″), 3.30-3.80(6H, m, H-2″- H-6″).

^{13}C-NMR (CD_3OD, 100 MHz, δ (ppm)): 158.0(C-2), 135.1(C-3), 178.9(C-4), 163.2(C-5), 101.6(C-6), 167.3(C-7), 95.4(C-8), 158.6 (C-9), 105.2(C-10), 123.0(C-1′), 116.2(C-2′), 145.9(C-3′), 149.5(C-4′), 117.4(C-5′), 122.7(C-6′), 101.4(Glc-1″), 74.3(Glc-2″), 76.8(Glc-3″), 70.3(Glc-4″), 77.5(Glc-5″), 61.3(Glc-6″).

3. Results and Discussion

In this research, the root of the *Euphorbia condylocarpa* was phytochemically analyzed and some pharmacological bio active phenolics were extracted, that can somewhat support the rumor indicating the healing effect of root of the plant for treatment of cancer in Kurdistan folk medicine.

The isolated compounds from the ethyl acetate and ethanol 80% extracts of the root of the *Euphorbia condylocarpa* revealed the presence of flavonoids Apigenin(I), Isorhamnetin-3-rutinoside(II), Kaempferol-3-O-rutinoside(III) and Quercetin 3-O-glucoside(IV). Previously the presence of Quercetin(V), Luteolin(VI), Morin(VII) and Naringin(VIII), [19] and also flavanon-7-glucoside, trifoline and tetracyclic triterpenoids, [26] were reported from *Euphorbia condylocarpa*. Because of the constant use of this plant in Kurdish folk medicine, here some important properties of extracted phenolics from the plant are discussed.

Apigenin presents predominantly in herbal medicines and foods. It is a naturally occurring plant flavone (4', 5, 7,-trihydroxyflavone) abundantly present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. Apigenin has a variety of pharmacological activities, including antioxidant, anti-tumour, anti-inflammatory, anti-bacterial, anti-proliferative, oxygenase inhibitor induces apoptosis. It has protective effect on radiation-induced chromosomal damage in human lymphocytes [27, 28].

Isorhamnetin-3-rutinoside showed various biological activity and bioavailability because of the sugar moiety. The antiproliferative activities of quercetin 3-O-glucoside have been reported on some cancer cell lines including colon, breast, hepatocellular, and lung cancer. It showed the most potent growth inhibition [29]. Kaempferol-3-O-rutinoside scavenging ability against the hydroxyl radical was demonstrated. Hydroxyl radicals are among the strongest free radicals; with damaging effects on living cells. They produce other kinds of cell-damaging free radicals and oxidizing agents, which can attack DNA to cause strand scission. In biochemical systems, superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions such as iron or copper by UV photolysis [30, 31-33].

It is reported that the quercetin 3-O-glucoside can inhibit in vitro absorption of cyanidin 3-glucoside. Research into the bioactivity of quercetin derivatives and its impact on human health is still at the developing stage. It is common knowledge that metabolic modification of quercetin derivatives alters their antioxidant properties. Ample investigations have confirmed a beneficial effect of quercetin derivatives, but the exact mechanism of their action is still unresolved. Simple derivatives of quercetin mono-glycosides such as 3-O-glucoside and 3-O-rhamnoside as well as diglycoside–rutin, have been best investigated to date. A human body needs these substances to absorb and use vitamin C. Investigators have also found that quercetin 3-O-glucoside and rutin contribute to the relaxation of smooth muscles in mammals [34, 35].

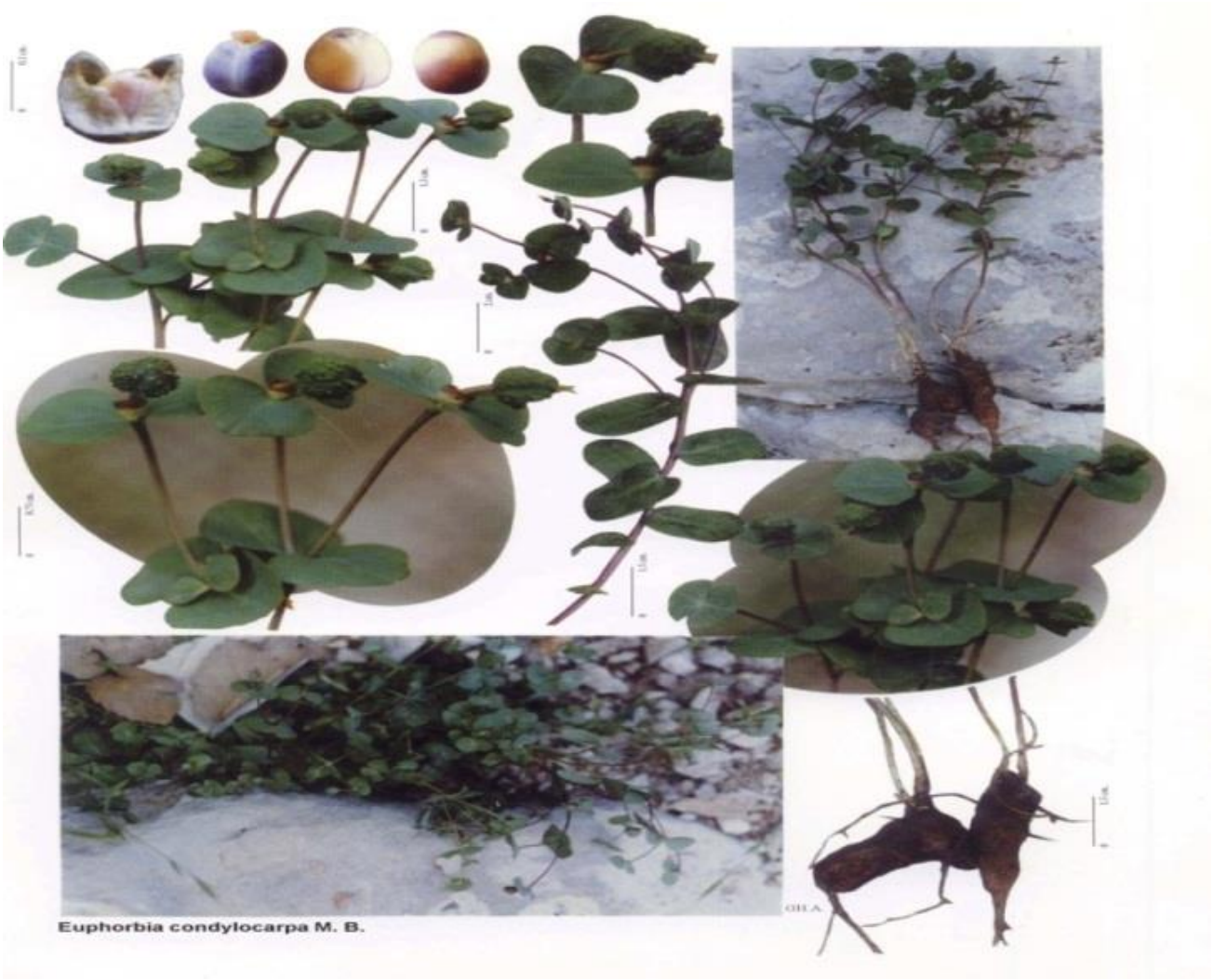
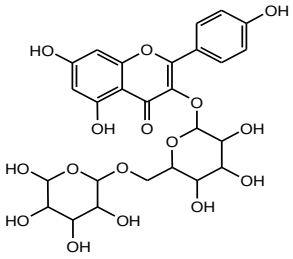
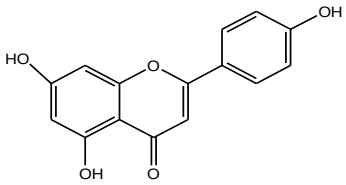


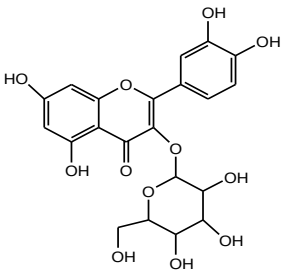
Figure1. Photograph of the *Euphorbia condylocarpa M. Bieb*



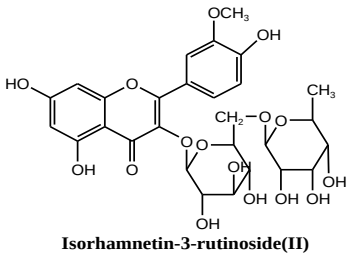
Kaempferol-3-O-rutinoside(III)



5,7,4'-trihydroxyflavone(I)



Quercetin 3-O-glucoside(IV)



Isorhamnetin-3-rutinoside(II)

Figure2. Widely distributed flavonoids from the root of *Euphorbea condylocarpa M. Bieb*

4. Conclusions

In this research, the root of the *Euphorbia condylocarpa* as an approximately unknown plant in folk medicine was phytochemically analyzed and some antioxidant bio active phenolics as Apigenin, Isorhamnetin-3-rutinoside, Kaempferol-3-O-rutinoside and Quercetin 3-O-glucoside were extracted and identified, that can somewhat support the rumor indicating the healing effect of root of the plant for treatment of cancer in Kurdistan folk medicine.

Acknowledgments

We are thankful from Soran Ishik College for support of this work.

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