



## Bioactive Compounds from *Marrubium Vulgare* L. based on *in vitro* Antioxidant Activity

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### Abstract

*Marrubium vulgare* L. (Lamiaceae) used in the world a viable source of medically important. Six compounds included: flavonoids, triterpenoids, and phenolic acid, they were isolated from the leaf extract by methanolic solvent and were determined as luteolin-7-O-β-glucopyranoside (1), apigenin-7-O-β-glucopyranoside (2), oleanolic acid (3), β-sitosterol (4) luteolin-7-O-rutinoside (5), and rosmarinic acid (6). In the MTT assay, compounds 1-6 at 250 μg/mL concentration gave an absorbance value of 0.524, 0.455, 0.187, 0.088, 0.0362, and 0.241, respectively. The significant LPO inhibitory of the purifications afforded compounds 1, 2, and 5 by 66, 84, and 89 at 100 μg/mL concentration, respectively.

**Keywords:** *Marrubium vulgare* L., Flavonoids, Triterpenoids, Phenolic acid, Antioxidant.

### Introduction

*Marrubium vulgare* L. belongs to family (Lamiaceae). It is occurring widely in the tropics of North Africa, Central Asia, Western Asia, and South Europe [1].

They are ascribed as having great medicinal value. Which are rich in important secondary metabolites including tannins, saponins, diterpenoids, triterpenoids, polyphenol, flavonoids, and others [2]. Various of natural compounds are responsible many of physiological functions including antidiabetic [3], antioxidant, anti-inflammatory [4], antispasmodic [5], analgesic [6], hypoglycemic [7], antibioresistance, antibiogram, and antibacterial [8], and antihypertensive effects [9]. Methanolic extract of *Marrubium vulgare* L. showed the ability to biological activities based on *in vitro* systems because of the presence of flavonoids, diterpenes, and phenols [10].

Therefore, in this study, we report the purification of the methanolic extract and determined the structure elucidation. Also, we study bioactivity of pure compounds as a gauge to determine their antioxidant activity based on *in vitro* bio assays were investigated using MTT assay [3-(4, 5-dimethylthiazole-2-

yl)-2,5-diphenyltetrazolium bromide] [11, 12, 13] and (LPO) lipid peroxidation inhibitory assay [14, 15, 13, 16].

### Materials and Methods

#### Genera

This study was carried out at the University of Anbar, Rumadi, Iraq and Michigan State University, USA. All solvents and chemicals used in this study were of ACS reagent grade (Sigma-Aldrich).

The resulting spots on TLC plates were viewed under UV light at 254 and 366 nm; respectively, and sprayed with 10% H<sub>2</sub>SO<sub>4</sub>. Silica gel plates at 250 and 500 μm, (Analtech) were used for preparative (TLC).

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were with a Varian Unity plus spectrometer at 500 and 125 MHz. MTT, *tert*-butyl hydroquinone (TBHQ), vitamin C, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT). MTT and LPO assays were used to test the antioxidant activity by the universal micro plate reader (Bio-Tek) and turner fluorometer (Barnstead), respectively.

## Extraction and Purification

The dried leaves of *Marrubium vulgare* L.(150 g) grown in west Iraq, were extracted with methanol solvent (1L×3) over night and the combined extract evaporated to yield (8.35 g).The methanolic extract (1 g) was chromatographed on a C-18 column, and eluted with CHCl<sub>3</sub>/Me OH (1:9, 3:7, 5:5, 7:3, 9:1 v/v).

The fractions collected were A,76.8 mg; B, 140.4 mg; C,276.5mg; D,336.4 mg; E, 137.5 mg; and F, 32.4 mg. An aliquot of fraction B (100 mg) was purified by preparative TLC (CHCl<sub>3</sub>/Me OH, 200:1, v/v) to yield compounds 1(4.1) and 2 (5.3 mg).

We were yielded compounds 3 (7.4 mg) and 4 (5.3 mg) from Fraction E (120 mg) when used for silica gel eluted with CHCl<sub>3</sub>/Me OH (5:1, 1:1, v/v) as the mobile phases yielded three fractions G, 23.7 mg, H 79.3 mg and I, 17.0 mg and then by preparative TLC (CHCl<sub>3</sub>/Me OH) 10:1, v/v, were isolated from fraction H.C-18 column chromatography of fraction D (300 mg) using CHCl<sub>3</sub>/Me OH as the mobile phase (0:10,1:9, 3:7, 5:5, 7:3, 9:1, 10:0, v/v) as the mobile phases yielded seven fractions J, 35.4mg; K, 68.0mg; L, 89.7mg; M,50.2 mg; N, 38.5 mg; O,10.6 mg; and P, 7.6 mg. An aliquot of fraction M was purified by preparative TLC (CHCl<sub>3</sub>/Me OH/H<sub>2</sub>O, 5:4:0.1, v/v) to yield compound 5 (4.1 mg).Compound 6 (2.8 mg) was isolated from the fraction N by preparative TLC (CHCl<sub>3</sub>/Me OH, 5:1, v/v).The

<sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectral data for these compounds are given in Tables (1-3).

## Bioactive Compounds from the Leaves of *Marrubium vulgare* L.

Compound 1: (Luteolin-7-O-β-glucopyranoside) amorphous, yellow powder [17]

<sup>1</sup>H NMR and <sup>13</sup>C NMR in DMSO-d<sub>6</sub> (Table 1)  
Compound 2: (Apigenin-7-O-β-glucopyranoside) amorphous, yellow powder [17].

<sup>1</sup>H NMR and <sup>13</sup>C NMR in CD<sub>3</sub>OD (Table 1)  
Compound 3: (Oleanolic acid) white powder [18].

<sup>1</sup>H NMR and <sup>13</sup>C NMR in CD<sub>3</sub>OD (Table 2)  
Compound 4: (β-Sitosterol) white amorphous powder [19].

<sup>1</sup>H NMR and <sup>13</sup>C NMR in CD<sub>3</sub>Cl<sub>3</sub> (Table 2)  
Compound 5: (Luteolin-7-O-rutinoside) yellow amorphous powder [20].

<sup>1</sup>H NMR and <sup>13</sup>C NMR in DMSO-d<sub>6</sub> (Table 3)  
Compound 6: (Rosmarinic acid) yellow amorphous powder [21].

<sup>1</sup>H NMR and <sup>13</sup>C NMR in CD<sub>3</sub>OD (Table 3)  
2.4 Biological assays: The antioxidant activity of the isolated compounds (1-6) was measured *in vitro* by using MTT assay [11, 12, 13] and (LPO) lipid peroxidation inhibitory assay [13, 14, 15, 16].

**Table 1:** <sup>1</sup>H and <sup>13</sup>C spectral data for compound 1 and 2 at 500 and 125 MHz, respectively

Position	Compound 1		Compound 2	
	δH	δC	δH	Δc
1	-	-	-	-
2	-	164.4	-	166.7
3	6.75 s	103.1	6.66 s	104.1
4	-	181.7	-	184.0
5	-	161.1	-	159.0
6	6.42 (d, J=2)	99.7	6.50 (d, J=2)	101.2
7	-	163.0	-	164.9
8	6.78 (d, J=2)	94.7	6.82 (d, J=2)	96.1
9	-	156.8	-	162.6
10	-	105.5	-	107.1
1'	-	121.2	-	123.1
2'	7.41 (d, J=2)	113.6	7.88 (d, J=9)	129.4
3'	-	145.9	6.92 (d, J=9)	117.2
4'	-	150.0	-	162.9
5'	6.89 (d, J=8)	115.9	6.92 (d, J=7.5)	117.2
6'	7.48 (d, J=8.5, 2)	119.0	7.86 (d, J=9)	129.6
1glu.	5.05 (d, J=7.5)	99.6	5.06 (d, J=7.5)	101.7
2 glu.	3.28*	73.1	3.44	74.6
3 glu.	3.31*	76.1	3.43	77.9
4 glu.	3.18*	69.6	3.40	71.3
5 glu.	3.46*	77.3	3.50	78.4
6 glu.	3.48*, 3.70	60.5	3.70m, 3.92m	62.3

Table 2: <sup>1</sup>H and <sup>13</sup>C NMR spectral data for compound 3 and 4 at 500 and 125 MHz, respectively

Position	Compound 3		Compound 4	
	$\delta$ H	$\delta$ C	$\delta$ H	$\delta$ C
1	0.94*, 1.60	39.4	1.05*, 1.86*	37.2
2	1.56*	27.2	1.49*, 1.84*	32.0
3	3.14 (dd, $J=10.5,5$ )	79.1	3.54 (m, $J=11,4.5$ )	71.8
4	-	39.2	2.23 m, 2.29 m	42.3
5	$\alpha$ 0.70 (d, $J=10$ )	56.0	-	140.8
6	1.37, 1.53*	18.9	5.35 (d, $J=5.5$ )	121.7
7	1.30, 1.46	33.7	1.96* (2H)	31.9
8	-	34.0	1.43*	31.9
9	1.54*	49.0	0.92*	50.1
10	-	37.6	-	36.5
11	1.07, 1.84	24.0	1.05*, 1.50*	21.0
12	5.23 (t, $J=3$ )	123.0	1.16*, 1.99*	39.8
13	-	144.7	-	42.3
14	-	42.3	0.99*	56.8
15	1.04, 1.71*	28.1	1.08*, 1.55*	24.3
16	1.58, 1.96	23.6	1.84* (2H)	28.2
17	-	47.0	1.08*	56.1
18	2.80 (dd, $J=13.5,4$ )	42.0	0.68 (3H, s)	11.9
19	1.10, 1.63*	46.6	1.03 (3H, s)	19.4
20	-	31.2	1.34*	36.1
21	1.19, 1.33	34.3	0.90* (3H)	18.7
22	1.52*, 1.71*	33.2	1.05*, 1.34*	34.0
23	$\alpha$ 0.94 (3H, s)	28.4	1.16* (2H)	26.1
24	80.74 (3H,s)	16.0	0.92*	45.8
25	80.89 (3H, s)	15.5	1.25*	29.0
26	80.77 (3H,s)	17.3	0.83 (3H,d, $J=7$ )	19.8 I.
27	81.12 (3H,s)	26.3	0.84 (3H) (d, $J=7$ )	19.0 I
28	-	181.2	1.08*, 1.28*	23.1
29	$\alpha$ 0.87 (3H,s)	33.4	0.84 (3H, s)	12.0
30	80.90 (3H,s)	23.5	-	-

(\*) overlapped; (glu) glucose; (I) interchangeable

Table 3: <sup>1</sup>H and <sup>13</sup>C NMR spectral data for compound 5 and 6 at 500 and 125 MHz, respectively

Position	Compound 5		Compound 6	
	$\delta$ H	$\delta$ C	$\delta$ H	$\delta$ C
1	-	-	-	127.9
2	-	164.6	7.03 (d, $J=2$ )	115.1
3	6.74* s	103.1	-	146.7
4	-	181.9	-	149.4
5	-	161.2	6.76 (d, $J=8$ )	116.5
6	6.45 (d, $J=2$ )	99.1	6.91 (dd, $J=8,2$ )	122.9
7	-	162.9	7.48 (d, $J=16$ )	146.5
8	6.74* s	94.8	6.27 (d, $J=15.5$ )	115.7
9	-	156.9	-	169.2
10	-	105.4	-	-
1'	-	121.0	-	131.3
2'	7.39br s	113.5	6.76 (d, $J=2$ )	117.5
3'	-	145.8	-	146.0
4'	-	150.0	-	144.7
5'	6.89 (d, $J=8.5$ )	116.5	6.65 (d, $J=8$ )	116.2
6'	7.44 (dd, $J=8,2$ )	119.2	6.63 (dd, $J=8$ )	121.7
7'	-	-	2.92 (dd, $J=14,10$ )	38.9
8'	-	-	2.92, 3.08	77.8
9'	-	-	-	177.6
1 glu.	5.06 (d, $J=7.5$ )	99.7	-	-
2 glu.	3.23*	73.1	-	-
3 glu.	3.30*	76.9	-	-
4 glu.	3.15*	69.6	-	-
5 glu.	3.56 (t, $J=7.5$ )	75.6	-	-
6 glu.	3.43*, 3.83 (d, $J=10.5$ )	66.0	-	-
1 rham.	4.54 br s	100.5	-	-
2 rham.	3.63 (d, $J=2$ )	70.0	-	-
3 rham.	3.45*	70.7	-	-
4 rham.	3.18*	72.1	-	-
5 rham.	3.41*	68.0	-	-
6 rham.	1.06 (3H, d, $J=8$ )	17.9	-	-

(rham) rhamnose

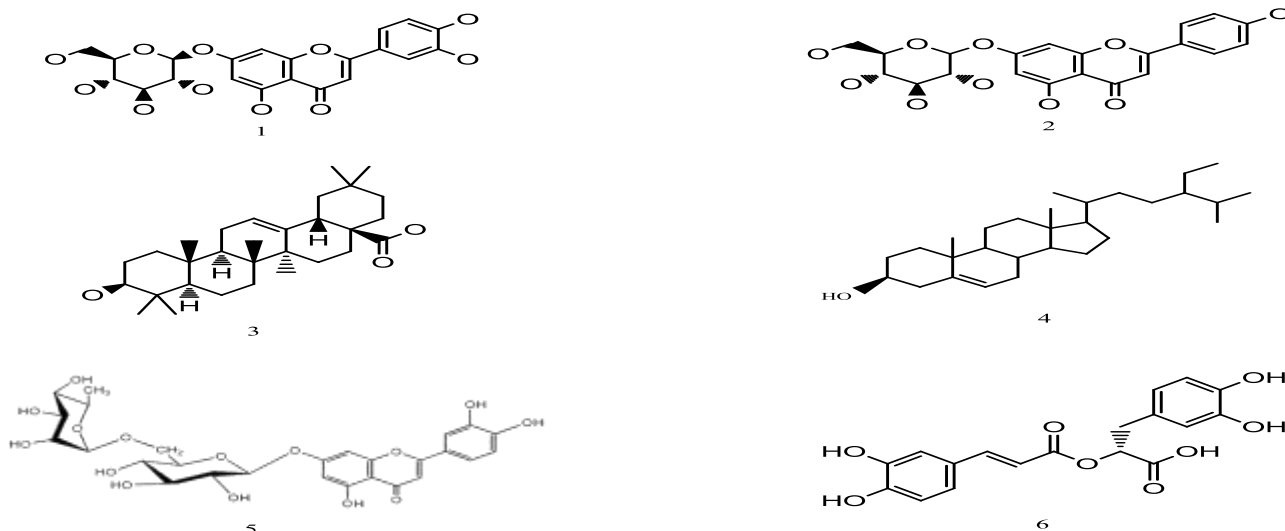


Figure 1: Structures of pure isolate compounds (1-6) from *Marrubium vulgare* L.

## Results and Discussion

The natural compounds in the leaves of *Marrubium vulgare* L. were extracted with methanol and purified by TLC profiles. Purification from extracts and afforded pure isolates Luteolin-7-O- $\beta$ -glucopyranoside (1), Apigenin-7-O- $\beta$ -glucopyranoside (2), Oleanolic acid (3),  $\beta$ -sitosterol (4) Luteolin-7-O-rutinoside (5), and Rosmarinic acid (6) by proton and carbon NMR spectral experiments (Figure 1).

The *in vitro* antioxidant assays used in our study to determine the ability of pure isolates as potential of antioxidants in *in vivo* conditions. The pure isolates were tested by using the assays of MTT and LPO to determine the antioxidant activity. We can discover the compounds that are competent of removing or reducing oxidative agents by using the MTT assay, while the assay of LPO assay discovers components, that are free radical scavengers [13, 16, 22]. In the MTT assay, compound 1 gave highest absorbance value reached 0.524 at 570 and at 250  $\mu$ g/m L

test concentration. It is a better activity than the positive controls, which were used by us. The results of compound 2, gave absorbance similar to that of TBHQ and vitamin C, which were used as positive controls in this test value by 0.455 at 250  $\mu$ g/mL concentration. At 250  $\mu$ g/m L concentration, compounds 3, 5, and 6 afford absorbance values of 0.187, 0.362, and 0.241, respectively.

While, compound 4, showed little activity as indicated by the poor absorbance value of 0.088 at 250  $\mu$ g/m L concentration (Figure 2). The LPO inhibitory activity of the pure isolated Compound 5 showed the highest LPO inhibition by 89% at 100  $\mu$ g/m L concentration, with compound 2 they are similar to that a positive controls, which were used in this assay. Compounds 1 and 6 showed moderate LPO inhibitory activity by 66% and 53%, respectively. Also, compounds 3 and 4 inhibited LPO by 24% and 40%, respectively (Figure 3).

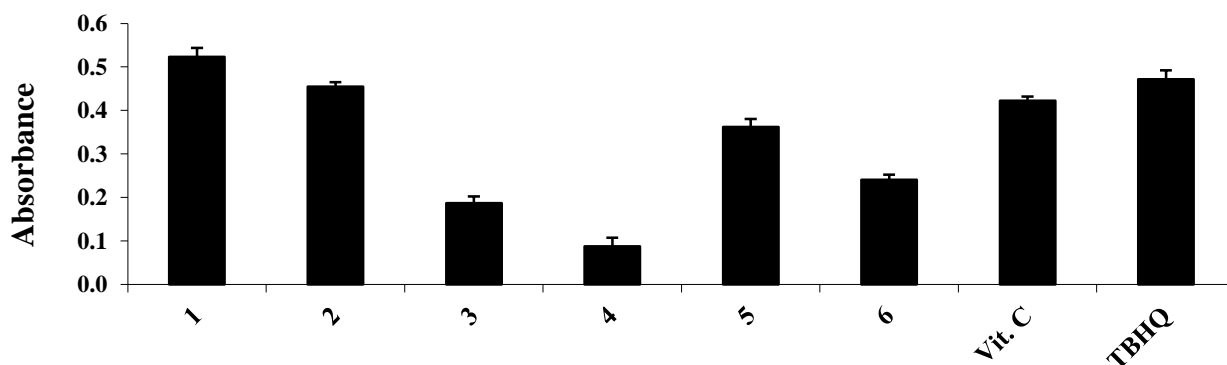


Figure 2: The values of absorbance at 570 nm isolated compounds 1-6 at 250  $\mu$ g/m L resulted after reaction with MTT at 37°C. Vitamin C, and TBHQ used as positive control tested at 25  $\mu$ g/m L. Vertical bars represent the standard deviation of each data point (n= 2)

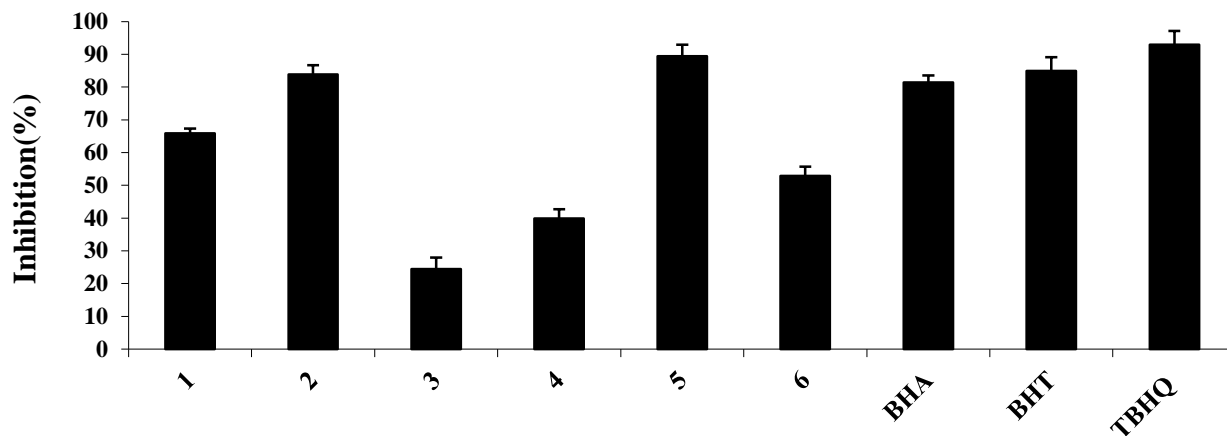


Figure 3: Lipid peroxidation (LPO) Inhibitory assay of isolated compounds 1-6 at 100 µg/mL and BHA, BHT, and TBHQ used as positive controls tested at 10 µg/mL. Vertical bars represent the standard deviation of each data point (n= 2)

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