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Extraction and evaluation of the anti-inflammatory activity of six compounds of *marrubium vulgare* L.

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Marrubium vulgare L. belonging to (Lamiaceae) family. It is characterized by its high content of medically important compounds. Recent study was to investigate therapeutic properties of the leaves of this plant *in vitro* anti-inflammatory activity of the pure isolates by using the methanolic solvent. We can identified six compounds based on NMR data, including [1] luteolin-7-O-β-glucopyranoside, [2] apigenin-7-O-β-glucopyranoside, [3] oleanolic acid, [4] β-sitosterol, [5] luteolin-7-O-rutinoside, and [6] rosmarinic acid. This compounds were checked for their biological activites on cyclooxygenase enzymes. The pure isolates 1, 3, and 5 inhibited COX-1 by 65, 50, and 76%, respectively. In addition, the pure isolates 2, 5, and 6 inhibited COX-2 enzyme by 65, 64, and 59, respectively at 25 µg/mL concentration. This is the first study were extraction and isolation of compounds 1, 2, and 5 from this plant and tested for anti-inflammatory activity by their COX-1 and COX-2 enzyme assays.

Keywords:*Marrubium vulgare* L., Cyclooxygenase inhibitory, Triterpenoids, Phenolics, Flavonoidsglycosides.

INTRODUCTION

The Kingdom of plant is a valuable source of recovery medication for various diseases. Today, many studies have been directed towards identifying plant extracts that can cure or prevent the occurrence of various diseases and determine their effective natural components (Shsrms and Thokchom, 2014). The World Health Organization has shown that 80% of the world's population uses natural extracts for their Health care (Das et al., 2014). Wherefore, the W.H.O launched in 2014 the Traditional Medicine Strategy until 2023. Which aims to develop action plans to strengthen the role of traditional medicine in protecting human health (W.H.O., 2017).

Marrubium vulgare L. (Lamiaceae) an important medicinal plant that grows in different regions of the world. Itis grown in the South Europe, Central and western Asia, and North Africa (Audrius et al., 2012).

The antioxidant (Neamah et al., 2017; Amri et al., 2017), hypoglycemic (Elberry et al., 2015), analgesic (De-Souza et al., 2009), vasodilator (ElBardai et al., 2003), antibioresistance (Khadidja et al., 2016), and antibacterial effects (Khadidja et al., 2016; Masoodi et al., 2008), antihepatotoxic, anticancer, anti-diabetic, antimicrobial, anti-inflammatory, anti-nociceptive, antihypertensive, antispasmodic, and gastroprotective activity (Lodhi et al., 2017). These are the most important of therapeutic properties. As a result of the diversity of secondary metabolic compounds contained in this plant including flavonoids, saponins, terpenoids, tannins, sterols (Rigano et al., 2006;Kahlouche-Riachi et al., 2015), phenylpropanoids (Calis et al., 1992; Lodhi et al.,

2017), and diterpenes, and phenylethanoid glycosides (Lodhi et al., 2017).

The results of previous studies have demonstrated the ability of methanol solution to extract Bioactive compounds from the leaves of *Marrubium vulgare* L. (Matkowski and Piotrowska, 2006). Also, The compounds extracted from the methanol solution were able to inhibitory of LPO assay and showed high absorption values for the MTT assay (Neamah et al., 2017).

In this study, determination of pure compounds extracted from the leaves of *Marrubium vulgare* L.by using NMR, and investigation of their pure isolates in inhibition of COX-1 and COX-2 enzymes.

MATERIALS AND METHODS

General:

The chemicals materials and all solvents used in this study were of ACS reagent grade (Sigma-Aldrich). Thin layer chromatography technique was used for isolation and purification compounds (250 and 500) µm silica gel plates (Analtech), and viewed this plates under ultraviolet light at 254 and 366 nm, and sprayed with the solution of sulfuric acid (%10). NMR spectra were recorded on VRX instruments (1H) at 500 MHz and (13C) at 125 MHz. The positive control including aspirin, ibuprofen, and naproxen in this assays were bought from chemical company (Sigma-Aldrich). The nonsteroidl anti-inflammatory drug Celebrex sample was provided by Ibn Sina Hospital in Baghdad, Iraq.

Plant material:

The leaves of *Marribum vulgare* L. were obtained from Haditha in the western Iraqiabout 150 mil. northwest of Baghdad. It was identified by Dr. Nasrallah, A. Y. Professor of medicinal plants, Faculty of Agriculture, University of Baghdad.The drying was at room temperature.

Extraction and Purification:

We took a sample of *Marrubium vulgare* L. leaves, methanol solution (1L×3) was added to the dried sample (150 g.), and the combined extract evaporated to yield (8.35 g). methanolic extract 1g. was chromatographed on column of C-18, and eluted with MeOH/CHCl3 (9:1, 7:3, 5:5, 3:7, 1:9 v/v). Six fractions collected were A, B, C, D, E, and F, the weight of each fraction was 76.8mg, 140.4 mg, 276.5 mg, 336.4 mg, 137.5 mg, and 32.4 mg, respectively. An aliquot of fraction B (100 mg) was purified by preparative TLC (MeOH/CHCl3,1:200, 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 MeOH/CHCl3 (1:5, 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 (MeOH/CHCl3) 1:10, v/v, were isolated from fraction H. C-18 column chromatography of fraction D (300 mg) using MeOH/CHCl3 as the mobile phase (10:0,9:1, 7:3, 5:5, 3:7, 1:9, 0:10, v/v) as the mobile phases yielded seven fractions J, 35.4 mg; K, 68.0 mg; 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 (MeOH/CHCl3/H2O, 4:5:0.1, v/v) to yield compound 5 (4.1 mg). Compound 6 (2.8 mg) was isolated from the fraction N by preparative TLC (MeOH/CHCl3, 1:5, v/v). The NMR spectra data of these compounds are available in tables (1-6).

Pure isolates from *Marrubium vulgare* L. Leaves

Compound 1

yellow amorphous powder.

The spectral data of 1H and 13C NMR (DMSO-d6) shown in table 1, which is identical withthose Luteolin-7-O-β-glucopyranoside (Zeliha et al., 2003).

Table 1: ^{1}H and ^{13}C spectral data for ompound 1 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
1	-	-	2'	7.41 (d, *J*=2)	113.6
2	-	164.4	3'	-	145.9
3	6.75 s	103.1	4'	-	150.0
4	-	181.7	5'	6.89 (d, *J*=8)	115.9
5	-	161.1	6'	7.48 (d, *J*=8.5, 2)	119.0
6	6.42 (d, *J*=2)	99.7	1g.	5.05 (d, *J*=7.5)	99.6
7	-	163.0	2 g.	3.28*	73.1
8	6.78 (d, *J*=2)	94.7	3 g.	3.31*	76.1
9	-	156.8	4 g.	3.18*	69.6
10	-	105.5	5 g.	3.46*	77.3
1'	-	121.2	6 g.	3.48* , 3.70	60.5

Compound 2

yellow amorphous powder.

The spectral data of 1H and 13C NMR (CD3OD) shown in table 2, which is identical with those Apigenin-7-O-β-glucopyranoside (Zeliha et al., 2003).

Table 2: ^{1}H and ^{13}C spectral data for compound2 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
1	-	-	2'	7.88 (d,*J*=9)	129.4
2	-	166.7	3'	6.92 (d,*J*=9)	117.2
3	6.66 s	104.1	4'	-	162.9
4	-	184.0	5'	6.92 (d, *J*=7.5)	117.2
5	-	159.0	6'	7.86 (d,*J*=9)	129.6
6	6.50 (d, *J*=2)	101.2	1g.	5.06 (d,*J*=7.5)	101.7
7	-	164.9	2 g.	3.44	74.6
8	6.82 (d, *J*=2)	96.1	3 g.	3.43	77.9
9	-	162.6	4 g.	3.40	71.3
10	-	107.1	5 g.	3.50	78.4
1'	-	123.1	6 g.	3.70 m, 3.92 m	62.3

Compound 3

white powder.

The spectral data of 1H and 13C NMR (CD3OD) shown in table 3, which is identical with those Oleanolic acid (Catharina et al., 2013).

Table 3: ^{1}H and ^{13}C spectral data for compound3 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
1	0.94*, 1.60	39.4	16	1.58, 1.96	23.6
2	1.56*	27.2	17	-	47.0
3	3.14 (dd, *J*=10.5,5)	79.1	18	2.80(dd, *J*=13.5,4)	42.0
4	-	39.2	19	1.10, 1.63*	46.6
5	α0.70(d, *J*=10)	56.0	20	-	31.2
6	1.37, 1.53*	18.9	21	1.19, 1.33	34.3
7	1.30, 1.46	33.7	22	1.52*, 1.71*	33.2
8	-	34.0	23	α0.94 (3H, s)	28.4
9	1.54*	49.0	24	β0.74 (3H,s)	16.0
10	-	37.6	25	β0.89 (3H, s)	15.5
11	1.07, 1.84	24.0	26	β0.77 (3H,s)	17.3
12	5.23 (t, *J*=3)	123.0	27	β1.12 (3H,s)	26.3
13	-	144.7	28	-	181.2
14	-	42.3	29	0.99*	56.8
15	1.04, 1.71*	28.1	30	1.08*, 1.55*	24.3

Compound 4

white amorphous powder.

The spectral data of 1H and 13C NMR (CD3Cl3) shown in table 4, which is identical with those β-Sitosterol (Kyun et al., 2008).

Table 4: ^{1}H and ^{13}C spectral data for compound 4 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
1	1.05*, 1.86*	37.2	16	1.84* (2H)	28.2
2	1.49*, 1.84*	32.0	17	1.08*	56.1
3	3.54 (m, *J*=11,4.5)	71.8	18	0.68 (3H, s)	11.9
4	2.23 m,2.29 m	42.3	19	1.03 (3H, s)	19.4
5	-	140.8	20	1.34*	36.1
6	5.35 (d, *J*=5.5)	121.7	21	0.90* (3H)	18.7
7	1.96* (2H)	31.9	22	1.05*, 1.34*	34.0
8	1.43*	31.9	23	1.16* (2H)	26.1
9	0.92*	50.1	24	0.92*	45.8
10	-	36.5	25	1.25*	29.0
11	1.05*, 1.50*	21.0	26	0.83 (3H,d,*J*=7)	19.8 I.
12	1.16*, 1.99*	39.8	27	0.84 (3H) (d,*J*=7)	19.0 I
13	-	42.3	28	1.08*, 1.28*	23.1
14	0.99*	56.8	29	0.84 (3H, s)	12.0
15	1.08*, 1.55*	24.3			

Compound 5

yellow amorphous powder.

The spectral data of ^{1}H and ^{13}C NMR (DMSO-d_6) shown in table 5, which is identical with those Luteolin-7-O-rutinoside (Abeer, 2011).

Table 5: ^{1}H and ^{13}C spectral data for compound 5 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
2	-	164.6	6'	7.44 (dd, *J=8,2*)	119.2
3	6.74* s	103.1	1 g.	5.06 (d, *J=7.5*)	99.7
4	-	181.9	2 g.	3.23*	73.1
5	-	161.2	3 g.	3.30*	76.9
6	6.45 (d, *J=2*)	99.1	4 g.	3.15*	69.6
7	-	162.9	5 g.	3.56 (t, *J=7.5*)	75.6
8	6.74* s	94.8	6 g.	3.43*, 3.83 (d, *J=10.5*)	66.0
9	-	156.9	1 rh.	4.54 br s	100.5
10	-	105.4	2 rh.	3.63 (d, *J=2*)	70.0
1'	-	121.0	3 rh.	3.45*	70.7
2'	7.39 br s	113.5	4 rh.	3.18*	72.1
3'	-	145.8	5 rh.	3.41*	68.0
4'	-	150.0	6 rh.	1.06 (3H, d, *J=8*)	17.9
5'	6.89 (d, *J=8.5*)	116.5			

Compound 6

yellow amorphous powder.

The spectral data of ^{1}H and ^{13}C NMR (CD3OD) shown in table 6, which is identical with those Rosmarinic acid (Nawal et al., 2011).

Table 6: ^{1}H and ^{13}C spectral data for compound6 at 500 and 125 MHz, respectiveiy.

C	^{1}H	^{13}C	C	^{1}H	^{13}C
1	131.3	-	1'	127.9	-
2	117.5	6.76 (d, *J=2*)	2'	115.1	7.03 (d, *J=2*)
3	146.0	-	3'	146.7	-
4	144.7	-	4'	149.4	-
5	116.2	6.65 (d, *J=8*)	5'	116.5	6.76 (d, *J=8*)
6	121.7	6.63 (dd, *J=8*)	6'	122.9	6.91 (dd, *J=8,2*)
7	38.9	2.92 (dd, *J=14,10*)	7'	146.5	7.48 (d, *J=16*)
8	77.8	2.92, 3.08	8'	115.7	6.27 (d, *J=15.5*)
9	177.6	-	9'	169.2	-

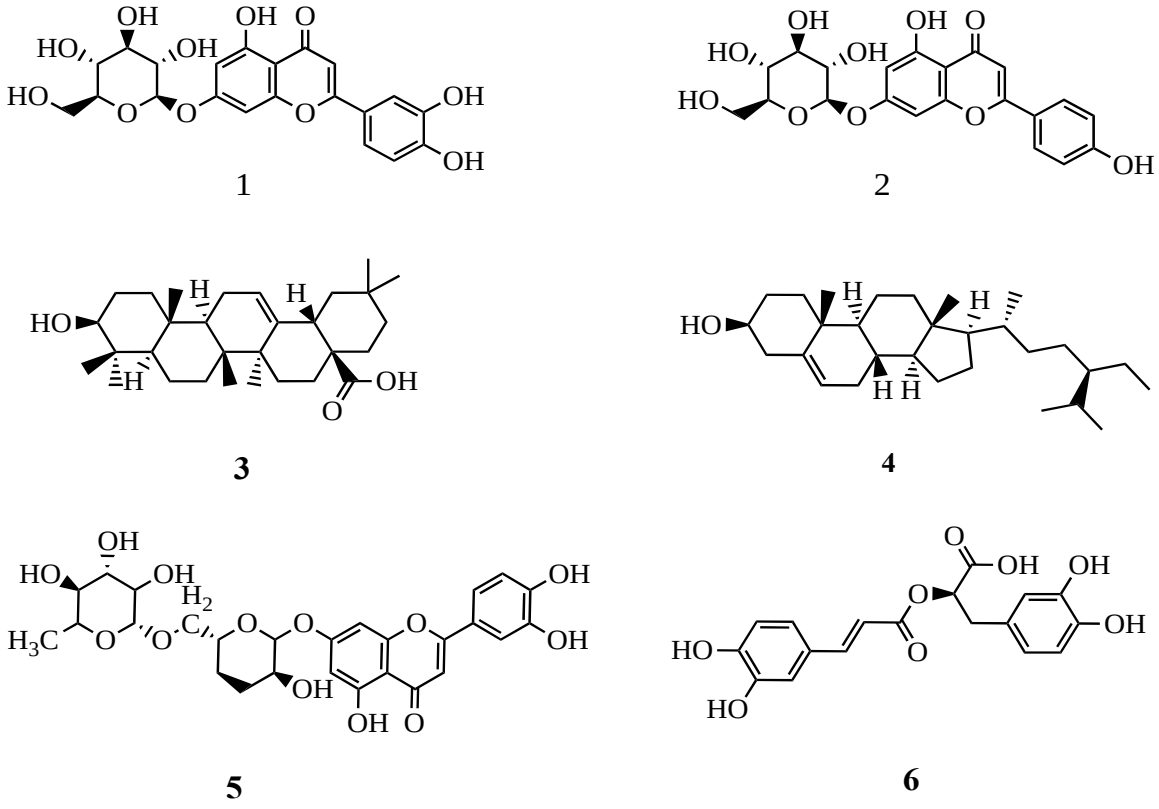

Figure 1. Structures of six pure compounds extracted from *Marrubium vulgare* L. leaves.

Biological assays:

The anti-inflammatory activity was determined by the use of COX-1 and COX-2 Enzymes Inhibitory Assay. The pure isolates 1–6 were tested at 50 µg/mL concentrations, respectively. The positive controls, commercial Celebrex, Aspirin, Ibuprofen, and Naproxen were tested at 1, 108, 12, and 25 µg/mL, respectively. after following the published method (Liu et al., 2013; Dissanayake et al., 2017a; Dissanayake et al., 2017b ; Zhang et al., 2017).

RESULTSAND DISCUSSION

The anti-inflammatory activity of *Marrubium vulgare* L. leaves extract was extracted with MeOH solvent and purified by TLC profiles. The pure isolates to afforded from this plant were Luteolin-7-O-β-glucopyranoside, Apigenin-7-O-β-glucopyranoside, Oleanolic acid, β-sitosterol, Luteolin-7-O-rutinoside, and Rosmarinic acid. The structural formula for six compounds are shown in (Figure 1).

In this study, we used *in vitro* anti-inflammatory assays, so that we can determine the ability of these pure compounds in inhibiting the enzymes that cause by them *in vivo* conditions (Zhang et al., 2016; Dissanayake et al., 2017b). COX-1 and COX-2 enzymes assays were tested on these compounds to determine the anti-inflammatory activity. in this methods, we can discover the compounds that are competent in potential inhibition of COX-1 and COX-2 enzymes assay, that is causing many diseases because partakes in gene regulations and this health beneficial trait (Liu et al., 2013; Dissanayake et al., 2017b; Zhang et al., 2017).

In the COX-1 assay, compound 5 gave the highest inhibitory activity reached 76% at 50 µg/mL, concentration. It is a better activity than the positive controls Celebrex and Naproxen, but it is similar than Aspirin. Which was used by us. At 50 µg/mL concentration compound 5, showed high COX-1 inhibition by 65 at 50 µg/mL concentration. The results of compounds 2, 3, 4, and 6 afford percentage of inhibition by 45, 50, 43, and 45, respectively, (Figure 2).

The results showed COX-2 enzyme inhibition activity of the pure isolates Compounds 2, and 5 showed the highest COX-2 inhibition by 65 and 64% at 50 µg/mL concentration. They are similar to that a positive controls Ibuprofen and Naproxen. But, this compounds were better of Celebrex. Which was used in this assay. Compounds 1, 3, 4, and 6 inhibited COX-2 enzyme by 38, 49, 42, and 58%, respectively, (Figure3).

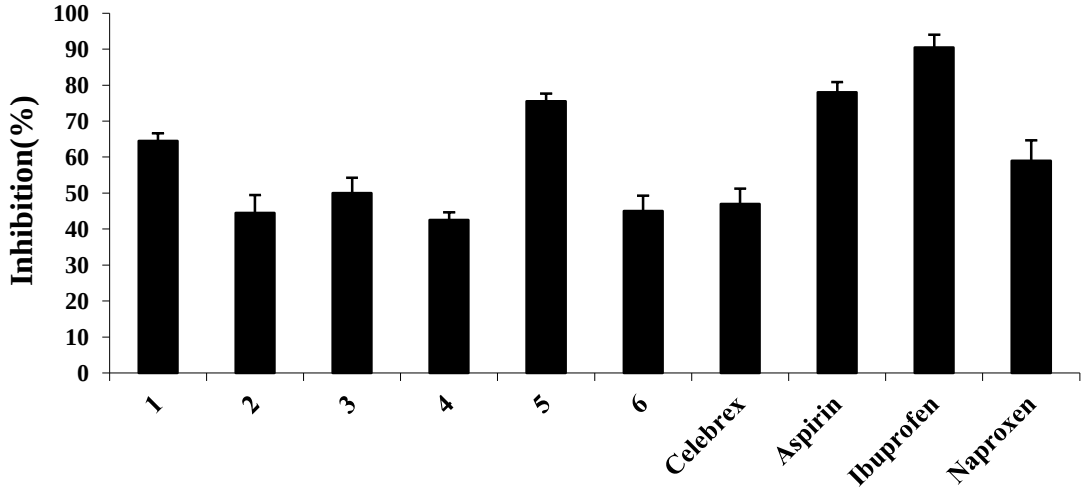

Figure 2. Inhibition of COX-1 enzyme by six compounds at 50 µg/mL and commercial Celebrex, Aspirin, Ibuprofen, and Naproxen used as positive controls tested at 1, 108, 12, and 15 µg/mL, respectively. The standard error of the mean was represented for n=2.

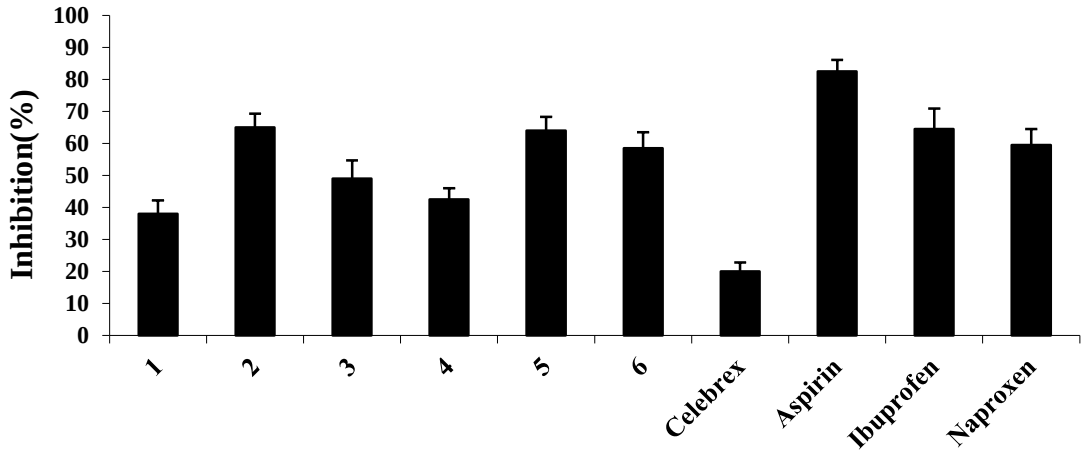

Figure 3. Inhibition of COX-2 enzyme by six compounds at 50 µg/mL and commercial Celebrex, Aspirin, Ibuprofen, and Naproxen used as positive controls tested at 1, 108, 12, and 15 µg/mL, respectively. The standard error of the mean was represented for n=2.

CONCLUSION

In this article, we assay based on *in vitro* anti-inflammatory activity.The results showed that the leaves of this plant contain important compounds that inhibit COX enzymes because of the ability of compounds in these extracts to inhibit the formation of hormones such as prostaglandins and peroxasalandine, which contribute to the production of inflammatory intmediators.The results of the bio-assay proved these extracts were highly effective in inhibiting COX-1 and COX-2 enzymes, which confirmed the presence of significant therapeutic benefits for this plant.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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