ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/332254241

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

**Article** *in* Journal of Global Pharma Technology · September 2017

CITATIONS
0

READS
164

**1 author:**

Ismail Ahmed
University of Anbar
**9** PUBLICATIONS **0** CITATIONS

SEE PROFILE

**Some of the authors of this publication are also working on these related projects:**



Evaluation of Three Cultivars Performance of Sorghum Under Different Zinc Concentration Spraying View project



RESPONSE OF GROWTH AND YIELD OF MUNG BEAN (VIGNA RADIATA L.) TO FOLIAR APPLICATION WITH B1 VITAMIN (THIAMIN AND BORON) View project

All content following this page was uploaded by Ismail Ahmed on 07 April 2019.

The user has requested enhancement of the downloaded file.

ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at www.jgpt.co.in

RESEARCH ARTICLE

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

**Shamil I. Neamah[1*], Ismail A. Sarhan[2], Oqba N. Al-Shaye'a[1, 3]**

[1]*Center of Desert Studies, University of Anbar, Ramadi, Anbar, Iraq.*

[2]*College of Agriculture, University of Anbar, Ramadi, Anbar, Iraq.*

[3]*College of Pharmaceutical, University of Anbar, Ramadi, Anbar, Iraq.*

***Corresponding Author: Shamil Ismail Neamah**

## 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-ß-glucopyranoside (1), apigenin-7-O-ß-glucopyranoside (2), oleanolic acid (3), ß-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% $H_2SO_4$. Silica gel plates at 250 and 500µm, (Analtech) were used for preparative (TLC).

$^1$H-NMR and $^{13}$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.

©2009-2017, JGPT. All Rights Reserved

## 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 chromate graphed on a C-18 column, and eluted with $CHCl_3$/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 ($CHCl_3$/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 $CHCl_3$/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 ($CHCl_3$/Me OH) 10:1, v/v, were isolated from fraction H.C-18 column chromatography of fraction D (300 mg) using $CHCl_3$/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 ($CHCl_3$/Me OH/$H_2O$, 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 ($CHCl_3$/Me OH, 5:1, v/v).The $^1$H-NMR and $^{13}$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-ß-glucopyranoside) amorphous, yellow powder [17]

$^1$H NMR and $^{13}$C NMR in DMSO-$d_6$ (Table 1)
Compound 2: (Apigenin-7-O-ß-glucopyranoside) amorphous, yellow powder [17].

$^1$H NMR and $^{13}$C NMR in CD3OD (Table 1)
Compound 3: (Oleanolic acid) white powder [18].

$^1$H NMR and $^{13}$C NMR in CD3OD (Table 2)
Compound 4: (ß-Sitosterol) white amorphous powder [19].

$^1$H NMR and $^{13}$C NMR in $CD3Cl_3$ (Table 2)
Compound 5: (Luteolin-7-O-rutinoside) yellow amorphous powder [20].

$^1$H NMR and $^{13}$C NMR in DMSO-$d_6$ (Table 3)
Compound 6: (Rosmarinic acid) yellow amorphous powder [21].

$^1$H NMR and $^{13}$C NMR in CD3OD (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: $^1$H and $^{13}$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 |

©2009-2017, JGPT. All Rights Reserved 

**Table 2: $^{1}H$ and $^{13}C$ NMR spectral data for compound 3 and 4 at 500 and 125 MHz, respectively**

| Position | Compound 3 | | Compound 4 | |
|---|---|---|---|---|
| | δH | δC | δH | δC |
| 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 | α0.70 (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 | α0.94 (3H, s) | 28.4 | 1.16* (2H) | 26.1 |
| 24 | ß0.74 (3H,s) | 16.0 | 0.92* | 45.8 |
| 25 | ß0.89 (3H, s) | 15.5 | 1.25* | 29.0 |
| 26 | ß0.77 (3H,s) | 17.3 | 0.83 (3H,d,*J*=7) | 19.8 I. |
| 27 | ß1.12 (3H,s) | 26.3 | 0.84 (3H) (d,*J*=7) | 19.0 I |
| 28 | - | 181.2 | 1.08*, 1.28* | 23.1 |
| 29 | α0.87 (3H,s) | 33.4 | 0.84 (3H, s) | 12.0 |
| 30 | ß0.90 (3H,s) | 23.5 | - | - |

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

**Table 3: $^{1}H$ and $^{13}C$ NMR spectral data for compound 5 and 6 at 500 and 125 MHz, respectively**

| Position | Compound 5 | | Compound 6 | |
|---|---|---|---|---|
| | δH | δC | δH | δC |
| 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) rhammnose

©2009-2017, JGPT. All Rights Reserved 

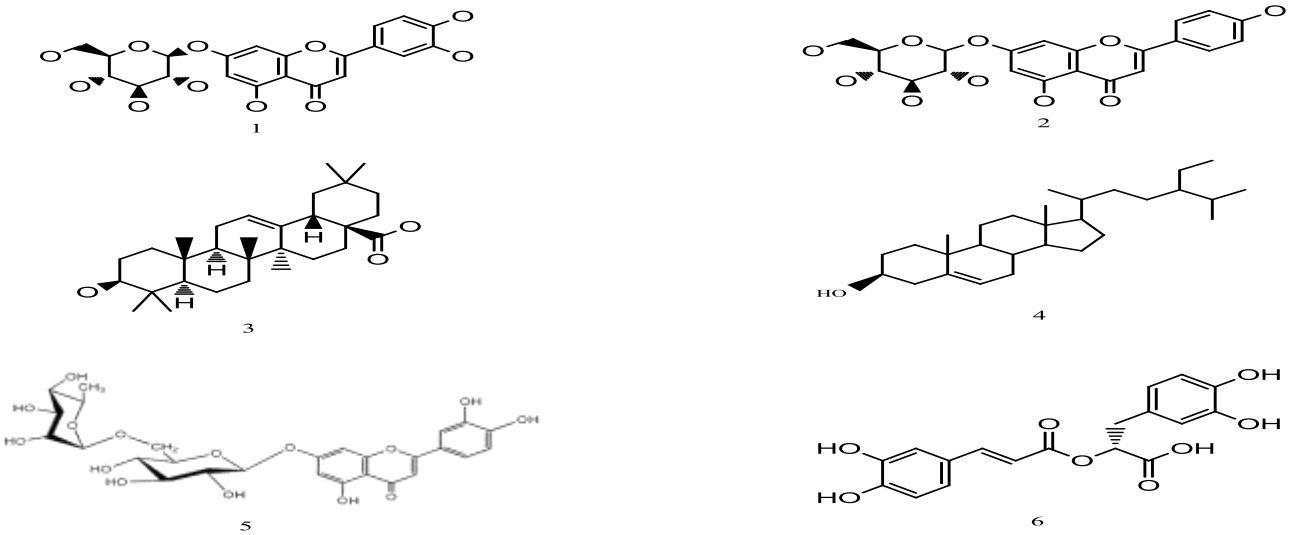

**Figure 1: Structures of pure isolate compounds (1-6) from*Marrubium vulgare* L.**

## Results and Discussion

The natural compounds in the leaves of *Marrubium vulgare* L. were extracted with methanol and purificated by TLC profils. Purification from extracts and afforded pure isolatesLuteolin-7-O-ß-glucopyranoside (1), Apigenin-7-O-ß-glucopyranoside (2), Oleanolic acid (3), ß-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 compelent 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, compound1 gave highest absorbance value reached 0.524 at 570 and at 250 µg/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 µg/mL concentration. At 250 µg/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 µg/m L concentration (Figure 2).The LPO inhibitory activity of the pure isolated Compound 5 showed the highest LPO inhibition by 89% at 100 µg/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).

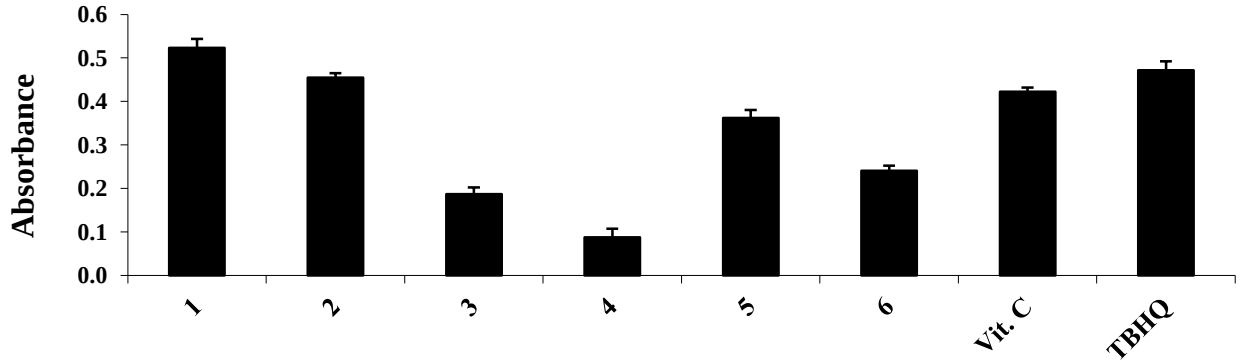


**Figure 2: The values of absorbance at 570 nm isolated compounds 1-6 at 250 µg/m L resulted after reaction with MTT at 37°C.Vitamin C, and TBHQ used as positive control stested at 25 µg/m L. Vertical bars represent the standard deviation of each data point (n= 2)**

©2009-2017, JGPT. All Rights Reserved 

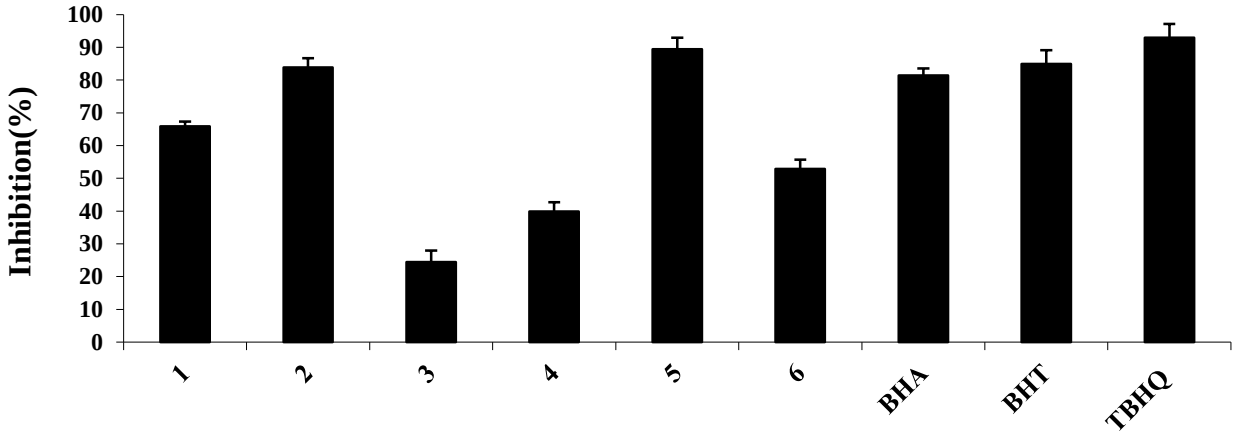


**Figure 3: Lipid peroxidation (LPO) Inhibitory assay of isolated compounds 1-6 at100 µg/mL and BHA, BHT, and TBHQ used as positive controlstested at 10 µg/m L. Vertical bars represent the standard deviation of each data point (n= 2)**

## References


1. Audrius P, Petras RV, Sofia S, Pieter DW, Teris AVB (2012) Isolation, identification and activity of natural antioxidants from horehound (Marrubium vulgareL.) cultivated in Lithuania, Food Chemistry, 130: 695–701.
2. Trease, Evans (1999) Pharmacognosy. 14 th ed. Harcourt publisher. LTD. U.K.
3. Boudjelal A, Henchiri C, Siracusa L, Sari M, Ruberto G (2012) Compositional analysis and in vivo anti-diabetic activity of wild Algerian Marrubium vulgare L. infusion.Fitoterapia.83(2): 286-292.
4. Nabil G, Leila H, Haoues B, Massoud, H, Asma D, Asma A, Sidi MO (2016) Evaluation of In vitro Antioxidant and In vivo Anti-inflammatory Potential of White Horehound (Marrubium vulgare L) Leaves, International Journal of Pharmaceutical Sciences Review and Research,41(1): 252-259.
5. Zaabat N, Hay AE, Michalet S, Darbour N, Bayet C, Skandrani I, Chekir-Ghedira L, Akkal S, MG Dijoux-Franca (2011)Antioxidant and antigenotoxic properties of compounds isolated from Marrubiumdeserti de Noe, Food and Chemical Toxicology, 49: 3328–3335.
6. De-Souza M M, De JRA, Cechinel-Filho V (2009) Analgesic profile of hydro alcoholic extract obtained from Marrubium vulgare. Phytomedicine. 5(2):103-107.
7. Jorge V, Francisco A, Adrian TS, Alejandra AC, Marisa EC, Irene JH, Angelica FF, amuel ES, AR Ortiz (2012) Acute hypoglycemic effect of ethanolic extracts from Marrubium vulgare. Phytopharmacology. 3(1): 54-60.
8. Khadidja SL, Amina K, Nourdine N, Aicha TM (2016) The in vitro evaluation of antbacterial effect of Marrubium vulgare L. leaf extract grown in Algeria, CRBS.1(1): 13-20.
9. El-Bardai S, Lyoussi B, Wibo M, Morel N (2004) Comparative Study of the antihypertensive activity of Marrubiumvulgare and of the dihydropyridine calcium antagonist amlodipine in spontaneously hypertensive rat, Clinical and Experimental Hypertension, 26(6):465-474.
10. Matkowski A, Piotrowska M,(2006)Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae, Fitoterapia.,77:346-353.
11. Liu Y, Nair MG(2010)An efficient and economical MTT assay for determining the antioxidant activity of compounds and extracts, Journal of Natural Products, 77:1193-1195
12. Liu Y, Nair MG (2012) Curcuma longa and Curcuma mangga leaves exhibit functional food property, Food Chemistry, 135: 634–640.
13. Zhang CR, Aldosari SA, Vidyasagar PSPV, Nair KM, Nair MG (2013) Antioxidant and anti-inflammatory assays confirm bioactive compounds in Ajwa date fruit, Journal of Agriculture and Food Chemistry, (61):5834-5840.
14. Roger HC, Yunbao Muraleedharan GN (2012) Organic ginger Zingiberofficinale inhibits COX-1 and -2 enzymes and lipid peroxidation Journal de la Societe Ouest-Africaine de Chimie,33:44- 51.
15. Ali SI, Chuan-RuiZ, Amal AM, Farouk KE, Ahmed KH, Maimona AK, Muraleedharan GN(2013)Major Constituents of Boswelliacarteri Resin Exhibit Cyclooxygenase Enzyme Inhibition and Anti proliferative Activity, Natural Product Communications, 8(10):1365-1366.
16. Zhang CR, Robert ES, Muraleedharan GN (2013) Antioxidant and Anti-inflammatory


©2009-2017, JGPT. All Rights Reserved 

compounds in the popular landscape plant Berberisthunbergii var. atropurea, Natural Product Communications, 8(2): 165-168.

17. Zeliha SA, I. Irem T., Erdal B., Ikhlas A. K (2003)Antioxidant Flavonoids from Verbascumsalviifolium Boiss, FABAD Journal of Pharmacy Sciences, 28:71-75.
18. Catharina EF, Thabata SS, Marcelo DMVF, Maria ACK (2013) Triterpene esters: natural products from Dorsteniaarifolia (Moraceae), Molecules,18:4247-4256.
19. Kyun L, Min AK, Seung YL, Jong KH, Jei HL, Kang RL (2008) Phytochemical Constituents of Schizonepetatenuifolia Briquet, Natural Product Sciences, 14 (2): 100-106.
20. Abeer T (2011) Flavonoidal content of Vangueriainfausta extract grown in Egypt: investigation of its antioxidant activity,International Research Journal and Pharmacy, 2 (3):157-161.
21. NawalA, Shagufta P, Itrat F, Muhammad N, Ajaz H(2011) Antioxidant, Anti-Glycation and Anti-Inflammatory Activities of Phenolic Constituents from ordiasinensis,Molecules,16: 10214-10226.
22. Chuan-Rui Z, Saleh AA, Polana SPVV, Paraj S, Muraleedharan GN (2015) Health-benefits of date fruits produced in Saudi Arabia based on in vitro antioxidant, anti-inflammatory and human tumor cell proliferation inhibitory assays, Journal of the Saudi Society of Agricultural Sciences.

©2009-2017, JGPT. All Rights Reserved 

**©2009-2017, JGPT. All Rights Reserved** 

View publication stats