



## Antioxidant Compounds from *Euphorbia schimperiana* scheele in Aseer Region, Saudi Arabia

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### ABSTRACT

From the whole part of *Euphorbia schimperiana* scheele, four compounds were isolated (**1-4**) and identified by different spectral techniques. The isolated compounds included one triterpene 3 $\beta$ -Cycloartenol (**1**) and three phenolic compounds Chrysin (**2**), Quercetin- 7-O- $\beta$ -D-glucuronoside (**3**) and 3-Methyl- Quercetin-7-O- $\beta$ -D-glucuronoside (**4**). The structure elucidation of the isolated compounds was done by spectroscopic tools (mass spectrometry and nuclear magnetic resonance NMR spectroscopy). The antioxidant activities were evaluated for three extracts of *Euphorbia* species (*E. schimperiana*, *E. peplus*, *E. cuneata*) as well as fractions and pure compounds of *E. schimperiana* by DPPH (1,1-diphenyle-2-picryl-hydrazyl) assay.

**Keywords:** *Euphorbia schimperiana*, antioxidant activities, triterpene, phenolic compounds, DPPH scavenging

### INTRODUCTION

**E**uphorbiaceae family is very diverse in range, composed of a wide variety of plants ranging from large woody trees to simple weeds that grow prostrate to the ground. The family composed of over 315 genera and nearly 8,000 species. The major genus is *Euphorbia* L. with over 2000 species found in the tropical and subtropical regions as well as in the temperate zones worldwide<sup>1-2</sup>. Due to the variety habitat of *Euphorbia* plants, a wide range of unusual secondary metabolites such as terpenoids, phenolic derivatives and alkaloid compounds were reported<sup>3</sup>. Therefore, some of these plants have been used as traditional medicines to treat various diseases for over one thousand years. The research has shown that some Euphorbiaceae are actually potent as medicinal plants and their extracts have been isolated and patented as modern drugs<sup>4</sup>. There are some reports of toxicity in *Euphorbia* species where toxic substances originate from the milky sap. Severe pain and inflammation can result from contact with the eyes, nose, mouth and even skin<sup>5</sup>. The toxic constituents of *Euphorbia* species were considered to be a kind of specific diterpenes, globally called phorboids, which comprise tiglane, ingenane and daphnane diterpene derivatives<sup>6-7</sup>. So studies of various *Euphorbia* species include the roots, seeds, latex, lactiferous tubes, stem wood, stem barks, leaves, and whole plants have been reported<sup>8-12</sup>. Many diterpenoids, triterpenoids and flavonoids have been isolated from Euphorbiaceae plants with interesting bioactivities<sup>8</sup>. A number of interesting biological activity were also reported such as cytotoxic<sup>9,10</sup> and antiviral activities<sup>11-12</sup>. In the present study, four compounds were isolated from *Euphorbiaschimperiana* grown in southern area of Saudi Arabia. Compounds **3**

and **4** were not previously isolated from *E. schimperiana*. The antioxidant activity of *E. peplus*, *E. cuneata* and *E. schimperiana* extracts were evaluated as well as fractions and pure compounds of *E. schimperiana* using DPPH (1,1-diphenyle-2-picryl-hydrazyl) assay.

### MATERIALS AND METHODS

#### Equipment

Rotary evaporator (BUCHI Rotavapor R-200, Germany). NMR (<sup>1</sup>H 500.13 MHz, <sup>13</sup>C: 125.0 MHz and 2D NMR spectra) were measured by Bruker Avance II 500 MHz spectrometer (King Khalid University, Saudi Arabia and Max Planck Institute, Jena, Germany). ESI-MS were run on a Finnigan LCQ Advantage Max (Thermo Electron Corporation) at Micro-analytical Unit, National Research Centre, Cairo, Egypt.

UV spectra were record on UV-1800 Spectrophotometer (SHIMADZU). Column chromatography was used for fractionation and purification employing silica gel (60 mesh, Fluka, Germany), Sephadex LH-20 (Sigma-Aldrich) and polyamide 6 (Fluka, Germany). TLC was performed on precoated silica gel plates (thickness 0.2mm, 0.5x10cm, Fluka, Germany) using several solvent systems.

The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour, as well as spraying with 5% sulphuric acid/methanol.

Semi-preparative HPLC purification was conducted using a preparative HPLC system from Shimadzu corporation, Japan with a LiChroprep-RP semi-preparative column (250 x 8 mm, 5  $\mu$ m). The separation was achieved using an isocratic elution with 25% acetonitrile/water.



## Plant Material

Samples from the aerial part of *Euphorbia schimperiana* Scheele, were collected from Abha City and Alssoda Resort in February 2011.

The specimen of the plant was deposited in the Herbarium of Biology Department, College of Science, King Khalid University.

## Extraction of *Euphorbia* Species

70 gm of *E. schimperiana*, *E. peplus* and *E. cuneate* were extracted with 80 % methanol and the methanolic extracts were concentrated under reduced pressure at 45°C. The plant extracts were subjected to antioxidant activities.

## Extraction and Fractionation of *E. schimperiana*

The air-dried plant (2 kg) was exhaustively extracted with 80% methanol (8.0 liters).

The methanol extract was concentrated under reduced pressure at temperature of 45°C.

The methanol extract (215.0 gm) was partitioned by using petroleum ether, ethyl acetate and n-butanol/water. (TLC) showed the presence of different compounds in each fraction.

The ethyl acetate fraction (12.5 gm) was subjected to column chromatography on silica gel and successively eluted with hexane, hexane/ethyl acetate, ethyl acetate, and ethyl acetate/methanol. The fractions eluted with hexane/ethyl acetate (9.5:0.5) offered one major compound with two minors (52.0 mg).

Therefore, this fraction was further subjected to column chromatography on silica gel, eluted with hexane and hexane/ethyl acetate to afford pure compound **1** (12.0 mg). Further fractions eluted with ethyl acetate afford two sub-fractions of (83.0 mg) and (123.7 mg).

The first fraction (83.0 mg) was purified on Sephadex LH-20 column using methanol as eluent to afford semi-pure compound **2**, which purified on a silica gel column, to yield the pure compound **2** (15.0 mg). The second fraction (123.7 mg) was purified on a silica gel column followed by a Sephadex LH-20 column to afford (7.4 mg) of the pure compound **3**. The *n*-butanol fraction (26.87 gm) was subjected to silica gel column and eluted with chloroform then chloroform/methanol. The fraction eluted with chloroform/methanol (8:2) affords major impure compounds.

The first fraction (3.43 gm) was subjected on polyamide material and eluted with water then water/methanol. The selected semi pure fraction (515.4 mg) was loaded on a Sephadex LH-20 column and eluted with methanol as mobile phase to afford mixture of three compounds (112.7 mg). Further purification on silica gel column followed by Sephadex LH-20 affords two major compounds (85.5 mg). The final purification was performed on semi-preparative HPLC column (250 x 8.0 mm, 5 µm) using isocratic elution with 25% acetonitrile/water to afford pure **4** (25.0 mg).

## Spectroscopic Data

### Compound 1

3β-Cycloartenol, (C<sub>30</sub>H<sub>50</sub>O): EIMS m/z (100%): 426 [M<sup>+</sup>, 30], 40 [M<sup>+</sup>-H<sub>2</sub>O] (40), 259 (11), 109 (49), 95(62). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (CDCl<sub>3</sub>) Table 1.

### Compound 2

Chrysin (C<sub>15</sub>H<sub>10</sub>O<sub>4</sub>): EIMS: 254 m/z (100%): 254 [M<sup>+</sup>, 100], 227[M<sup>+</sup>-CO](15),152 [M<sup>+</sup>-C<sub>8</sub>H<sub>6</sub>] (18), 102 [M<sup>+</sup>-C<sub>7</sub>H<sub>4</sub>O<sub>4</sub>] (5). UV: (MeOH: 272, 316 nm), (MeOH + NaOMe: 277, 360 nm), (MeOH + AlCl<sub>3</sub>:280, 327,383 nm), (MeOH + AlCl<sub>3</sub> + HCl: 280, 327, 388 nm), (MeOH + NaOAc: 276, 355 nm), (MeOH + NaOAc + H<sub>3</sub>BO<sub>3</sub>: 274, 315 nm). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (CD<sub>3</sub>CO CD<sub>3</sub>) Table 1.

**Table 1:** <sup>1</sup>H- and <sup>13</sup>C-NMR Spectroscopic data of compounds 1 and 2

Compound 1			Compound 2		
Position	<sup>1</sup> H-NMR	<sup>13</sup> C-NMR	Position	<sup>1</sup> H-NMR	<sup>13</sup> C-NMR
1a,1b	1.21 – 1.41	30.40	2	-----	156.01
2a,2b	1.41-1.50	29.91	3	6.7 s	106.28
3	3.3 (dd, J=3.0,11.0 Hz)	78.8	4	-----	183.09
4	-----	40.49	5	12.84 (OH)	163.18
5	1.25	47.12	6	6.1 (d, J=1.5)	99.79
6a,6b	0.88-1.45	21.10	7	12.84 (OH)	164.66
7a,7b	0.92-1.21	26.05	8	6.5 (d, J=1.5 Hz)	94.91
8	1.5	47.99	9	-----	158.96
9	-----	20.02	10	-----	105.58
10	-----	26.02	1'	-----	132.72
11a,11b	1.03-1.81	26.49	2'	7.9 (d, J=7 Hz)	127.34
12	1.62	31.97	3'	7.59 m	130.14
13	-----	45.30	4'	7.59 m	133.08



14	-----	48.81	5'	7.59 m	130.14
15	1.21	32.91	6'	7.9 (d, J=7Hz)	127.34
16	1.21-1.84	28.15			
17	1.60	52.30			
18	0.98	18.04			
19a,19b	0.3,0.54-(d, J=4.1 Hz)	29.71			
20		35.89			
21	0.86 d	18.21			
22a,22b	1.26-1.56	36.34			
23a,23b	1.87-1.86	24.95			
24	5.12	125.2			
25	-----	130.9			
26	1.58	25.75			
27	1.64	17.64			
28	0.88 s	19.31			
29	0.86 s	25.44			
30	0.81 s	14.01			

**Table 2:** <sup>1</sup>H- and <sup>13</sup>C-NMR spectroscopic data of compounds 3 and 4.

Compound 3			Compound 4		
Position	<sup>1</sup> H-NMR	<sup>13</sup> C-NMR	Position	<sup>1</sup> H-NMR	<sup>13</sup> C-NMR
2	-----	159.0	2		157.4
3	-----	137.0	3		134.0
4	-----	176.4	4		179.0
5	-----	161.2	5		164.0
6	6.18, (d, J=2.0 Hz)	99.9	6	6.19 s	100.5
7	-----	163.0	7		163.0
8	6.37, (d, J=2.0 Hz)	94.7	8	6.39 s	94.8
9	-----	158.5	9		157.3
10	-----	104.3	10		104.5
1'	-----	122.8	1'		122.5
2'	7.88, (d, J=2.0Hz)	116.2	2'	7.51, (d, J=2.0 Hz)	116.8
3'	-----	146.0	3'		145.7
4'	-----	148.5	4'		148.5
5'	6.76, (d, J=8.5 Hz)	116.1	5'	6.83, (d, J=8.3 Hz)	116.1
6'	7.39, (dd, J=8.5, 2.0 Hz)	122.7	6'	7.56, (dd, J=8.3-2.0Hz)	122.7
1''	5.28-(d, J=7.5 Hz)	100.2	1''	5.47, (d, J=7.5 Hz)	102.2
2''	3.33 m	75.7	2''	3.30 m	74.6
3''	3.35 m	78.2	3''	3.70 m	77.1
4''	3.40 m	73.5	4''	3.40 m	72.2
5''	3.31 m	77.8	5''	3.32 m	76.4
6''	-----	Not detected	6''		170.0
			OCH <sub>3</sub>	3.60 s	53.0

**Compound 3**

Quercetin- 7- O-β-D-glucuronide, (C<sub>21</sub>H<sub>18</sub>O<sub>13</sub>): ESI-MS

(-ve) m/z (100%): 477.07 [M -1], <sup>1</sup>H-NMR (CD<sub>3</sub>OD) and <sup>13</sup>C-NMR (CD<sub>3</sub>OD) Table 2.

**Compound 4**

3-Methyl-Quercetin-7-O-β-D-glucuronide, (C<sub>22</sub>H<sub>20</sub>O<sub>13</sub>):

ESI-MS (-ve) m/z (100%): 491 [M-1], 477 [M-1-CH<sub>3</sub>]. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>) Table 2.

**Antioxidant Activities**

Evaluation of antioxidant activities of the plants extract of the three *euphorbia*: *E. schimperiana*, *E. peplus*, *E. cuneata* and fractions with the isolated pure compounds of *E. schimperiana* were tested by chemical methods

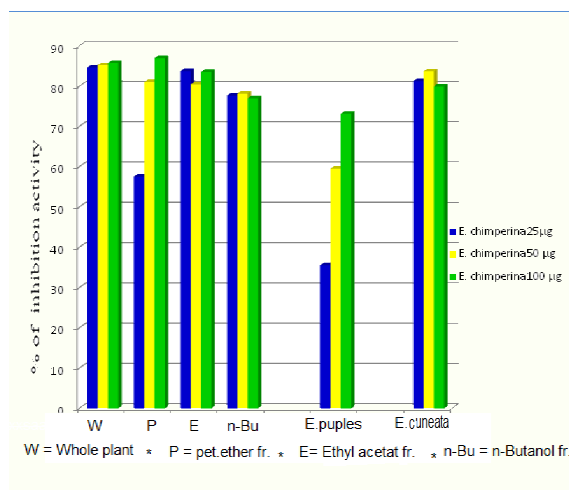


using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. 1 ml of methanolic solution of varying concentration samples (25, 50 and 100  $\mu\text{g}/\text{ml}$ ) were added to 1 ml of methanol solution of DPPH (60 $\mu\text{M}$ ). The prepared solutions were mixed and left for 30 min at room temperature. The optical density was measured at 520 nm using a spectrophotometer (UV-1650PC, Shimadzu, Japan). The percent scavenging effect was determined by comparing the absorbance of the solution containing the test sample to that of a control solution without the test sample taking the corresponding blanks. Mean of three measurements for each compound/fractions was calculated according to the method of Matsushige.<sup>13</sup>

## RESULTS AND DISCUSSION

### The DPPH Free Radical Scavenging Assay

The plant extracts showed antioxidant activities with different inhibition, where *E. schimperiana* had the highest inhibition at range of  $\approx 83\%$  followed by *E. cuneata* at 80% for all concentrations while *E. peplus* showed different inhibition according to the used concentrations at 71%, 59%, 35% for 100 $\mu\text{g}$ , 50 $\mu\text{g}$  and 25 $\mu\text{g}$  respectively (Figure 1).



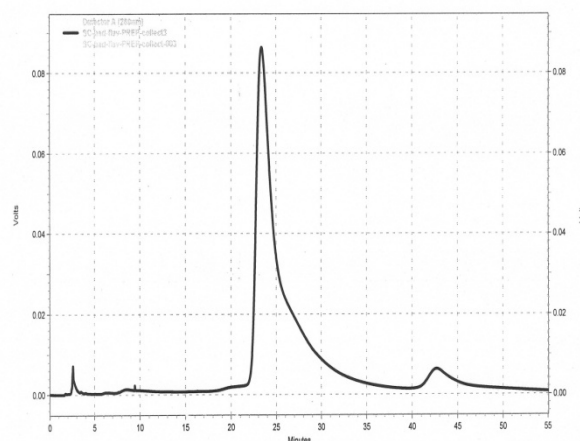
**Figure 1:** The antioxidant activity of the plant extracts (*E. schimperiana*, *E. peplus*, *E. cuneata* and fractions of *E. schimperiana*

The *E. schimperiana* fractions namely: petroleum ether, ethyl acetate, and *n*-butanol exhibited to a similar antioxidant activities as the whole plant extract which encourage the authors to identify their chemical constituents.

### Isolation of Compounds

Compound **1** was isolated from ethyl acetate fraction by successive column chromatography on silica gel using hexane, hexane/ethyl acetate in the order of increasing polarity. Where compounds **2** and **3** were purified from two sub-fractions of ethyl acetate by silica gel column followed by size exclusions on Sephadex LH-20. While compound **4** was obtained from *n*-butanol fraction by successive columns on silica gel, polyamide and Sephadex

LH-20 to afford semi pure compound followed by semi-preparative HPLC column (Figure 2) (see experimental part for details).



**Figure 2:** HPLC chromatogram of compound 4.

### Structure Determination

#### Compound 1

Compound **1** gave a pink colour with Libermann-Burchardt test, suggested the possibility of triterpene moiety. EIMS showed a molecular ion at  $m/z$  426 and the presence of 30 carbons in the  $^{13}\text{C}$ -NMR spectrum, suggested a molecular formula of  $\text{C}_{30}\text{H}_{50}\text{O}$ . The  $^1\text{H}$ -NMR spectrum revealed to seven signals in aliphatic protons which characteristic for methyl groups. The AB spin doublets of two methylene protons at  $\delta_{\text{H}}$  0.3 and  $\delta_{\text{H}}$  0.54 (both d,  $J = 4.1$  Hz) is characteristic for [cyclopropane] of cycloartane triterpene. The olefinic proton at  $\delta_{\text{H}}$  5.12 (t,  $J = 7.0$  Hz, 1H-24) and a signal of hydroxyl methine proton at  $\delta_{\text{H}}$  3.3 (dd,  $J = 3.0, 11.0$  Hz, H-3) confirmed the presence of triterpene moiety.  $^{13}\text{C}$ -NMR (125 MHz,  $\text{CDCl}_3$ ) confirmed the presence of seven methyl groups observed in aliphatic carbons, and olefinic carbons appeared at  $\delta$  125.2 (C-24),  $\delta$  130.9 (C-25) with hydroxymethine at  $\delta$  78.25 (C-3) and the characteristic signal of cyclopropane at  $\delta$  29.3 (C-19). The full assignments for the carbon and proton signals were determined by COSY, HSQC and HMBC experiments. The above finding results and assignments of the molecular ion peak at  $m/z$  426 confirmed the proposed structure of 3 $\beta$ -Cycloartenol which showed a complete agreement with the reported data of compound which isolated previously from *E. schimperiana*<sup>14</sup>.

#### Compound 2

Compound **2** is a yellow amorphous powder, showed yellow fluorescence color on TLC under UV. It acquires a deep yellow color after spraying TLC by 5%  $\text{H}_2\text{SO}_4/\text{MeOH}$ , which suggest the presence of flavonoid skeleton, which was confirmed by different flavonoid spraying reagent (Aluminum chloride and Ferric chloride). The UV spectral data showed two major absorption bands in MeOH at 316 nm and at 272 nm for band I and band II, typical for a flavonoid nucleus<sup>15</sup> and the shift reagents assumed the

chrysin moiety. The  $^1\text{H-NMR}$  spectrum exhibited a flavonoid pattern where the aromatic proton reveals five signals. The chemical shift at  $\delta$  7.9 (d,  $J = 7.0$ , 2H) was attributed to H-2', H-6' and the signal at  $\delta$  7.59 (m, 3H) attributed to H-3', H-4' and H-5' which suggested no substitution in ring B of the flavonoid. The signal at  $\delta$  12.84 ppm was assigned to the C-5 and C-7 hydroxyl groups. The  $^{13}\text{C-NMR}$  spectrum confirmed the chrysin moiety by the presence of signals of two carbons in the area of double bonds at  $\delta$  156.01, 106.28 corresponding to C-2, C-3 respectively, signals of B-ring carbons appeared as follow: signal at  $\delta$  133.08 (C-4'),  $\delta$  127.34 corresponding to (C-2'/C-6'), signal at  $\delta$  130.14 corresponding to (C-3'/C-5') confirming that there is no substitution on the B ring of the flavone. The Mass spectrometry (EIMS) showed the molecular ion peak  $m/z$  at 254, corresponding to chrysin structure.

### Compound 3

This compound showed the flavonoid behavior on TLC with different spraying reagent. The  $^1\text{H-NMR}$  spectrum exhibited AB spin system of ring A at  $\delta$  6.18 and  $\delta$  6.37. The protons of ring B exhibited the chemical shift at  $\delta$  6.76, 7.39 and 7.88 and the coupling constants (Table 2) suggested the orthodihydroxy substitution in ring B. The presence of anomeric proton at  $\delta$  5.28 suggested one sugar moiety with  $\beta$ -configuration at the anomer center ( $J = 7.5$ , H-1").  $^{13}\text{C-NMR}$  spectrum confirmed the presence of sugar moiety by the presence of anomeric carbon at  $\delta$  100.2 with the other sugar carbons in the range of  $\delta$  73.5 to 78.2. HMBC correlations of H-1"/C-7 confirmed the attachment of the sugar moiety at C-7 of the aglycone. The absence of hydroxyl methylene carbons in the  $^{13}\text{C}$ -data suggested the presence of glucuronic acid moiety which confirmed by the complete agreement with the published data of glucuronic acid<sup>16</sup>. The ESIMS (negative mode) at  $m/z$  477 confirmed the molecular formula of  $\text{C}_{21}\text{H}_{18}\text{O}_{13}$ . From the above data, we assumed the structure of compound **3** is Quercetin-7-O- $\beta$ -D-glucuronoside which is isolated for the first time from *Euphorbia* species. The literature showed that compound **3** was isolated previously from *Verbascum lychnitis*<sup>17</sup> and from Madagascanian *Uncarina* species<sup>18</sup>.

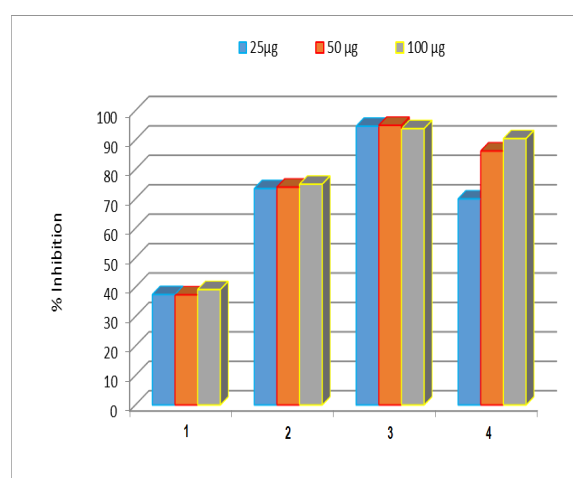
### Compound 4

The UV spectra in methanol and in shift reagents showed typical characteristic absorption spectra for flavanol. Comparison of  $^1\text{H}$  and  $^{13}\text{C-NMR}$  data of compound **4** showed similarity to compound **3** except the exchange of the chemical shift of H-2' and H-6' positions.  $^1\text{H-NMR}$  signal at  $\delta$  3.60 with the  $^{13}\text{C}$ - at  $\delta$  53.0 indicated to the presence of methoxy group at C-3 of the flavonoid structure.  $^1\text{H-}^1\text{H}$  COSY and HMQC confirmed the connectivity for the flavonoid coupled protons and hydrogen's-carbons correlation respectively. The presence of anomeric proton at  $\delta$  5.47, d,  $J = 7.5$  Hz with anomeric carbon at  $\delta$  102.2 indicated to one sugar moiety. The missing of the hydroxyl methylene  $\text{Sp}^3$  carbon shifts of the common sugars (glucose, galactose,

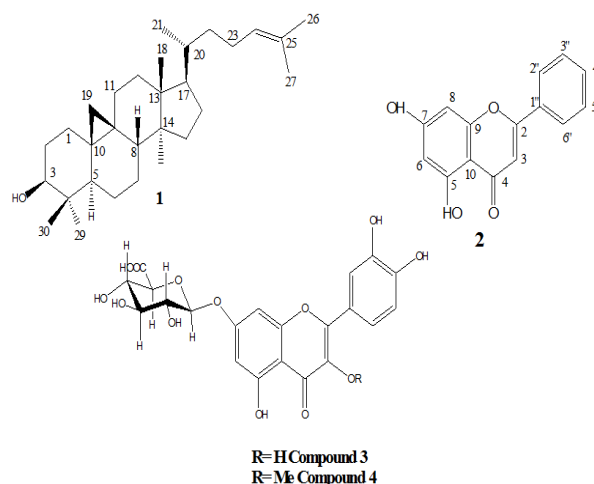
xylose, arabinose) and the presence of carbonyl carbon at  $\delta$  170.0 suggested glucuronic acid moiety. Compound **4** exhibited the ESIMS at  $m/z$  491 with 14 units more than compound **3**. So, the assumed structure of compound **4** is 3-Methyl-Quercetin-7-O- $\beta$ -D-glucuronoside which is isolated for the first time from *Euphorbia* genus and identified by UV only from *Viguier series*<sup>19</sup>. It is the first time to isolate compound **4** from *E. schimperiana* and it was synthesized previously by Deniset<sup>20</sup>.

### Antioxidant Activity of the Pure Compounds

The antioxidant activities of the pure compounds revealed that compounds **3** and **4** have the highest activity in different concentrations (25  $\mu\text{g}$ , 50  $\mu\text{g}$  and 100  $\mu\text{g}$ ) while compound **2** showed a medium activity and compound **1** has the lowest activity (Figure 3).



**Figure 3:** The antioxidant activity of the pure isolated compounds of *E. schimperiana*



Compounds 1-4 isolated from *E. schimperiana*

### CONCLUSION

The antioxidant activities of *E. schimperiana* and their fractions showed the highest activities compared to *E. Peplus* and *E. cuneata*. Furthermore, the antioxidant activities of the pure compounds revealed that compound (**3**) has a high antioxidant activity which could be useful *in Vivo* study. The structure elucidation by modern spectroscopic tools (MS and NMR spectroscopy)

of the isolated compounds confirmed their structures as: 3 $\beta$ -cycloartenol(**1**), Chrysin(**2**), Qurecetin-7-O- $\beta$ -D-glucuronoside(**3**) and 3-Methyl-Qurecetin-7-O- $\beta$ -D-glucuronoside(**4**). According to our best knowledge, Compounds (**3**) and (**4**) have not been previously isolated from *E. schimperiana*.

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