



## Original article

# Selective cytotoxic effects on human breast carcinoma of new methoxylated flavonoids from *Euryops arabicus* grown in Saudi Arabia



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## ABSTRACT

The chloroform–methanol extract of *Euryops arabicus*, collected from Saudi provenance, yielded a new kaurane diterpene (**1**) and seven methoxylated flavones (**2–8**), two of which are new (**2** and **3**). Structures of the compounds were elucidated through interpretation of spectral data of NMR, MS and comparison with literature values. All compounds were evaluated for their anti-tumor activities, employing four different cancer cell lines (WI-38, VERO, HepG2 and MCF-7), ABTS free radical scavenging and immunomodulatory effects. All metabolites had considerable antioxidant and immunestimulatory effects. All compounds showed anticancer activity with IC<sub>50</sub> in range 10–125 μM, whilst **2** and **6** showed significant anti-proliferative activity against HepG2 (IC<sub>50</sub> = 20 and 15 μM) and MCF-7 (IC<sub>50</sub> = 15 and 10 μM), respectively. This effect was attributed to significant S-phase cell cycle arrest.

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## 1. Introduction

Cancer is one of the leading causes of death in the world [1,2]. The International Agency for Research on Cancer reported that, more than 7 million people died from cancer in 2008 and it was anticipated that it is going to be more or less triplicate by the year 2030 [3]. The treatment of cancer was recently designed by two major approaches aiming at discovering potent antitumor metabolites; bio-chemical and targeted-based. The former gained a significant attention in the recent two decades, which led to discovery of several antitumor agents [4–7].

Asteraceae is a big family of shrubs and herbaceous plants contains about 1100 genera and 25,000 species, widely distributed in the tropical and subtropical regions. It is economically important as a source of food, such as lettuce and artichokes, cooking oils, sweetening agents, and tea infusions [8,9]. Among 100 species of the genus *Euryops*, only *Euryops arabicus* is known in Saudi Arabia [8]. Up to date, Seco-furoermophilanes, furoermophilanes, eremophilanolides, and flavonoids were identified from the genus *Euryops* [10].

The current results showed the proving of the pharmacological mechanism of known class of natural products as a lead of anticancer drug with significant safety. It was clear from the results that compounds **2** and **6** showed potent anti-proliferative activity against HepG2 (IC<sub>50</sub> were 20 ± 0.35 and 15 ± 1.21 μM) and MCF-7 (IC<sub>50</sub> were 15 ± 0.35 and 10 ± 0.14 μM) respectively. The anti-proliferative activity was attributed to significant S-phase cell cycle arrest employing the 5-FU as a positive control.

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## 2. Results and discussion

### 2.1. Chemistry

In the course of our projects on the isolation of bioactive metabolites from higher plants [11,12], the herbs of *E. arabicus* were collected from Al-Taeif area (Saudi Arabia, April 2011) and extracted with a mixture of  $\text{CHCl}_3$ :MeOH (1:1, 4 L). Successive fractionation of the total extract on NP-Silica column, preparative thin layer chromatography (PTLC), followed by Sephadex LH-20, yielded a new kaurane diterpene (18,19-dihydroxy-kaura-16-en-3-one, **1**) and two new flavones; 7-hydroxy-3',4',6,8-tetramethoxyflavone (**2**) and 3',8-dihydroxy-4',5',6,7-tetramethoxyflavone (**3**) (NMR spectra, S1). In addition to these compounds, five known flavones: 5,6-dihydroxy-3',4',6,7-tetramethoxyflavone (**4**), 5-hydroxy-4',6,7,8-tetramethoxyflavone (**5**), 5-hydroxy-3',4',7-trimethoxyflavone (**6**), 5-hydroxy-3',4',6,7,8-pentamethoxyflavone (**7**), and 5,8-dihydroxy-3',4',6,7-tetramethoxyflavone (**8**) (Fig. 1), were isolated and characterized in comparison with the published data [13–18].

Compound **1** was isolated as white amorphous solid;  $[\alpha]_D^{20} = +70$  (c 0.066,  $\text{CHCl}_3$ ); its molecular formula was established to be  $\text{C}_{20}\text{H}_{30}\text{O}_3$ , based on HRESIMS (positive-ion mode),  $m/z = 319.2256$   $[\text{M} + \text{H}]^+$ . The  $^{13}\text{C}$  NMR spectral data ( $^1\text{H}$  decoupled and DEPT) of **1** showed 20 resonances attributable to one methyl, three methine, eleven methylenes and five non-protonated carbons (Table 1).

Two of the six elements of unsaturation, as indicated by the molecular formula of **1**, could be attributed to one carbon–carbon double bond (Table 1) and a carbonyl group ( $\nu_{\text{max}}$  1694); thus the molecule is tetracyclic. As the  $^1\text{H}$  and  $^{13}\text{C}$  NMR data enabled all but two of the hydrogen atoms within **1** to be accounted for, it was evident that the remaining two protons were present as part of hydroxyl functions, this deduction was supported by IR absorption at  $\nu_{\text{max}}$  3387  $\text{cm}^{-1}$ . After association of all the protons with directly bonded carbons via 2D NMR (HSQC) spectral measurements, it was possible to deduce the planer structure of **1** by interpretation of the  $^1\text{H}$ – $^1\text{H}$  COESY and  $^1\text{H}$ – $^{13}\text{C}$  HMBC spectra (Fig. 2).

From the  $^1\text{H}$ – $^1\text{H}$  COESY spectrum of **1**, a spin system between H<sub>2</sub>-1 and H<sub>2</sub>-2 was observed. Long-range C–H (HMBC) correlations observed between H-1<sub>b</sub> ( $\delta_{\text{H}}$  2.65, dd,  $J = 14.4, 1.8$ ) and, C-2 ( $\delta_{\text{C}}$  44.6 ppm), C-3 ( $\delta_{\text{C}}$  213.9 ppm), C-9 ( $\delta_{\text{C}}$  54.9 ppm), C-10 ( $\delta_{\text{C}}$  43.8 ppm) and C-20 ( $\delta_{\text{C}}$  19.3 ppm); between the resonances of H<sub>2</sub>-

**Table 1**

$^1\text{H}$  [ $\text{CDCl}_3$ , 600 MHz] and  $^{13}\text{C}$  NMR [ $\text{CDCl}_3$ , 150 MHz] NMR spectral data of **1**<sup>a</sup>

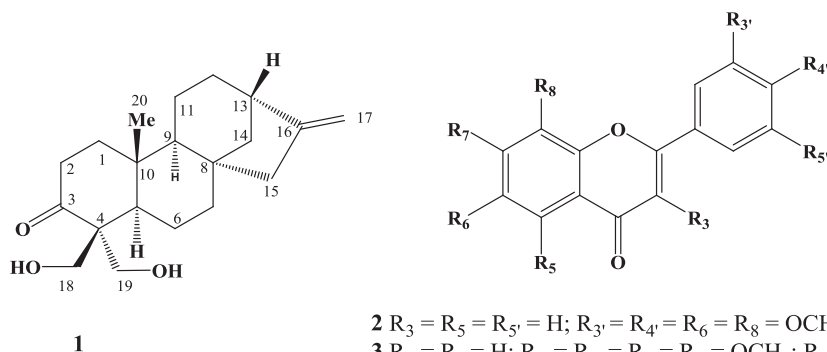
P.	$\delta_{\text{C}}^b$	$\delta_{\text{H}}^c$	HMBC
1 <sub>a</sub>	55.8	2.65 (dd, 14.4, 1.8)	C-2, C-3, C-9, C-10, 2 C-20
1 <sub>b</sub>		2.37 (d, 14.4)	
2 <sub>a</sub>	44.6	2.57 (dd, 13.2, 1.8)	
2 <sub>b</sub>		1.95 (d, 13.2)	
3	213.9		
4	46.8		
5	50.2	1.59 (dd, 6.6, 3.6)	C-3, C-4, C-10, 2 C-20
6 <sub>a</sub>	20.8	1.73 (m)	C-5, C-7, C-8, C-10
6 <sub>b</sub>		1.46 (m)	
7 <sub>a</sub>	32.7	1.62 (m)	
7 <sub>b</sub>		1.51 (m)	C-5, C-6, C-8, C-9
8	43.9		
9	54.9	1.33 (br d, 7.8)	C-7, C-8, C-10, C-11, C-12, C-15, C-20
10	43.8		
11 <sub>a</sub>	18.5	1.68 (m)	
11 <sub>b</sub>		1.44 (m)	
12	40.4	1.55 (m)	
13	43.5	2.66 (m)	C-8, C-11, C-12, C-15
14 <sub>a</sub>	39.1	1.87 (br d, 7.8)	
14 <sub>b</sub>		1.14 (m)	
15	48.5	2.09 (br s)	C-8, C-9, C-13, 2 C-14
16	154.8		
17 <sub>a</sub>	103.6	4.82 (s)	C-13, C-15, C-16
17 <sub>b</sub>		4.76 (s)	
18 <sub>a</sub>	70.5	3.87 (d, 10.8)	C-3, C-4, C-5
18 <sub>b</sub>		3.52 (d, 10.8)	
19 <sub>a</sub>	65.0	3.78 (d, 10.8)	C-3, C-5
19 <sub>b</sub>		3.74 (d, 10.8)	
20	19.3	1.07 (s)	C-1, C-9, C-10

<sup>a</sup> All assignments are based on 1D and 2D measurements (HMBC, HSQC, COESY).

<sup>b</sup> Implied multiplicities were determined by DEPT (C = s, CH = d, CH<sub>2</sub> = t).

<sup>c</sup>  $J$  in Hz.

18 ( $\delta_{\text{H}}$  3.87, d,  $J = 10.8$ ;  $\delta_{\text{H}}$  3.52, d,  $J = 10.8$ ) and those of C-3 ( $\delta_{\text{C}}$  213.9 ppm), C-4 ( $\delta_{\text{C}}$  46.8 ppm), and C-5 ( $\delta_{\text{C}}$  50.2 ppm); between H-5 ( $\delta_{\text{H}}$  1.59, dd,  $J = 6.6, 3.6$ ) and C-3 ( $\delta_{\text{C}}$  213.9 ppm), C-4 ( $\delta_{\text{C}}$  46.8 ppm), C-10 ( $\delta_{\text{C}}$  43.8 ppm) and C-20 ( $\delta_{\text{C}}$  19.3 ppm), this led to closing of ring A. A  $^1\text{H}$ – $^1\text{H}$  spin system between H<sub>2</sub>-7 ( $\delta_{\text{H}}$  1.62, m;  $\delta_{\text{H}}$  1.51, m) and H<sub>2</sub>-6 ( $\delta_{\text{H}}$  1.73, m;  $\delta_{\text{H}}$  1.64, m) which further correlated to H-5 ( $\delta_{\text{H}}$  1.59, dd,  $J = 6.6, 3.6$ ) were observed. Long-range C–H correlations were observed between H-9 ( $\delta_{\text{H}}$  1.33, br d,  $J = 7.8, 3.6$ ) and, C-7 ( $\delta_{\text{C}}$  32.7 ppm), C-8 ( $\delta_{\text{C}}$  43.9 ppm), C-10 ( $\delta_{\text{C}}$  43.8 ppm) and C-20 ( $\delta_{\text{C}}$  19.3 ppm); between the resonances of H<sub>2</sub>-7<sub>b</sub> ( $\delta_{\text{H}}$  1.51, m) and those of C-5 ( $\delta_{\text{C}}$  50.2 ppm), C-6 ( $\delta_{\text{C}}$  20.8 ppm), C-8 ( $\delta_{\text{C}}$  43.9 ppm), and C-9



- 2 R<sub>3</sub> = R<sub>5</sub> = R<sub>5'</sub> = H; R<sub>3'</sub> = R<sub>4'</sub> = R<sub>6</sub> = R<sub>8</sub> = OCH<sub>3</sub>; R<sub>7</sub> = OH  
 3 R<sub>3</sub> = R<sub>5</sub> = H; R<sub>4'</sub> = R<sub>5'</sub> = R<sub>6</sub> = R<sub>7</sub> = OCH<sub>3</sub>; R<sub>3'</sub> = R<sub>8</sub> = OH  
 4 R<sub>3'</sub> = R<sub>5'</sub> = H; R<sub>3</sub> = R<sub>4'</sub> = R<sub>6</sub> = R<sub>7</sub> = R<sub>8</sub> = OCH<sub>3</sub>; R<sub>5</sub> = OH  
 5 R<sub>3</sub> = R<sub>8</sub> = H; R<sub>3'</sub> = R<sub>4'</sub> = R<sub>5'</sub> = R<sub>6</sub> = R<sub>7</sub>; R<sub>5</sub> = OH  
 6 R<sub>3</sub> = R<sub>5'</sub> = R<sub>6</sub> = R<sub>8</sub> = H; R<sub>3'</sub> = R<sub>4'</sub> = R<sub>7</sub> = OCH<sub>3</sub>; R<sub>5</sub> = OH  
 7 R<sub>3</sub> = R<sub>6</sub> = H; R<sub>3'</sub> = R<sub>4'</sub> = R<sub>5'</sub> = R<sub>7</sub> = R<sub>8</sub> = OCH<sub>3</sub>; R<sub>5</sub> = OH  
 8 R<sub>3</sub> = R<sub>5'</sub> = R<sub>8</sub> = H; R<sub>3'</sub> = R<sub>4'</sub> = R<sub>6</sub> = R<sub>7</sub> = OCH<sub>3</sub>; R<sub>5</sub> = R<sub>8</sub> = OH

**Fig. 1.** Chemical structures of **1–8** (1).

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