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Original article

New benzoic acid derivatives from *Cassia italica* growing in Saudi Arabia and their antioxidant activity

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ABSTRACT

Two new benzoic acid derivatives: 1-*p*-hydroxy benzoyl-3-palmitoyl glycerol (**1**) and 6 -*p*-hydroxy benzoyl daucosterol (**2**), along with scutellarein-6-methyl ether (**3**), quercetin (**4**), and rutin (**5**) had been separated from *Cassia italica* (Fabaceae) aerial parts from EtOAc fraction. Their characterisation was accomplished by various spectroscopic techniques and by comparing with the published data. The Ethyl acetate (EtOAc) fraction and compounds **1**–**5** had been assessed for their antioxidant potential utilizing DPPH assay. They had significant antioxidant capacities with activity ranged from 19.7 to 95.8%, in comparison to butylated hydroxyanisole (BHA) (93.8%). These findings could provide a further evidence to support the traditional use of *C. italica* for the treatment of chronic or degenerative illnesses.

1. Introduction

Antioxidants possess a significant role in the defense system of the body towards deleterious reactive oxygen species (ROS) (Salah et al., 1995). Increase the intake of antioxidants could assist to maintain the physiological function of the body systems (Van Acker et al., 1996). They are classified into natural and synthetic antioxidants (Gupta and Sharma, 2006). In spite of the fact that antioxidants obtained from synthetic sources are vastly used but there are many published researches pointing out an obvious relation among the long-term use of these antioxidants and several health problems: skin allergies, GIT complications, and raised the cancer's risk (Lourenço et al., 2019). It was stated that natural antioxidants are more efficient, powerful, and safer than synthetic antioxidants (Tavasalkar et al., 2012). Thus, the studies have been intensified for discovering non-toxic and effective natural metabolites with antioxidant potential (Gupta and Sharma, 2006). Plants are of a remarkable value due to their bioactive metabolites which have nutritional and medicinal benefits (Hussain et al., 2012; Mansoor et al., 2016). Fabaceae plants range from perennial and annual herbs to vines, shrubs, trees and aquatic plants, which are distributed in temperate, tropical, and aquatic regions (Molares and Ladio, 2012). *Astragalus*; *Acacia*, *Indigofera*, *Crotalaria*, and *Mimosa* are largest genera (Ahmad et al., 2016). Many plants of these genera are known to possess antioxidant activities such as *Cicer arietinum* (Wagh et al., 2012); *Cajanus cajan* (Rao and Sresty, 2000); *Pterocarpus marsupium* (Tippani et al., 2010); *Pseudopiptadenia contorta* (Moreira et al., 2005); *Delonix regia* (Azab et al., 2013); *Arachis hypogaea* (Jiang et al., 2014), and *Acacia arabica* (Sundaram and Mitra, 2007). *Cassia* genus comprises 600 species (Dave and Ledwani, 2012). *Cassia italic* (Eshrinq) is abundantly growing in Saudi Arabia. In Saudi traditional medicine, it is used to treat skin infections, constipation, and oedema (Al-Yahya et al., 1990). Its leaves or plants decoction is utilized as expectorant and laxative as well as purgative (Al-Said, 1993). Additionally, it is used for various intestinal and rheumatic complains, gout, biliousness, ringworm, and urinary infections (Al-Said, 1993; An anthraquinone derivative from Cassia italica, 1994). It was reported to possess a wide array of bioactivities: antioxidant, antibacterial, CNS depressant, hepatoprotective, hypoglycaemic, antiviral, anti-inflammatory, and anticancer (Dabai et al., 2012; Masoko et al., 2012; Mohamed, 2014). This plant is considered to be a wealthy pool of various secondary metabolites (An anthraquinone derivative from Cassia italica, 1994; Dabai et al., 2012; Mohamed, 2014; Elsayed et al., 1992). Interestingly, Gololo et al. stated that the geographical location has an influence on the accumulation of phytochemicals in of *C. italica* (Gololo et al.,

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2018). Khalaf et al. reported the isolation of tinnevellin; emodin, physcion, 2-methoxy-emodin-6-O-β-D-glucopyranoside, 1,6,8-tri hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene, and rutin from *C. italica* growing in Egypt (Khalaf et al., 2019). 10,10'-Chrysophanol bianthrone, chrysophanol, 1,1,8,8'-tetrahydroxy-6′-methoxy-3,3'-dimethyl-10,10'-bianthracen-9,9'-dione, physcion, and 1,1,8,8'-tetrahydroxy-7'-methoxy-3,3'-dimethyl-10,10'-bian thracen-9,9'-dione were separated from *C. italica* pods growing in Sudan (Yagi et al., 2013). Moreover, (22E)-3-β-hydroxycycloart-2 2-en-24-one, uvaol, β-sitosterol, daucosterol, emodin, methyl 3,4-dihydroxybenzoate, aloin, 4-hydroxypheny-O-β-D-glucopyrano side, and rutin were repoted from aerial parts of Saudi *C. italica* (Mohamed, 2014). However, little available repots on phytoconstituents of *C. italica* growing in Saudi Arabia. In the present study, two new (**1** and **2**) and three known metabolites (**3**–**5**) were separated and characterized from *C. italica* aerial parts (Fig. 1). The isolated metabolites **1**–**5** and EtOAc fraction were assessed for their antioxidant capacities.

2. Materials and methods

2.1. Experimental

Optical rotations were estimated with a Perkin-Elmer polarimeter (Model 341LC). Infrared-400 Shimadzu spectrophotometer was utilized to get IR (infrared) spectra. HRESIMS (high resolution electrospray ionization mass spectrometry) was recorded on a LTQ Orbitrap. GCMS Clarus 500 was used for GCMS (gas chromatography mass spectrometry) analysis (Perkin Elmer). NMR data were recorded on 600 and 850 BRUKER Unity INOVA. Chromatographic analysis was performed on RP-18 (reversed phase-18), sephadex LH-20, and SiO_2 60. TLC SiO_2 60 F_{254} plates were used for TLC (thin layer chromatography) analysis. Purification of compounds was achieved using a six mL extraction tube LiChrolut RP-18 solid phase. Detection of compounds was done using UV (ultraviolet) absorption (λ_{max} 255 and 366 nm) and spray reagent (anisaldehyde/H_2SO_4).

2.2. Plant material

In April 2017, *C. italica* aerial parts were collected from Gabal Al-Ateeq, Al Madinah Al Munawwarah (24°24′37.5″N 39°32′34.0″ E). The plant taxonomy was done based on its morphological characteristics and library database (Collenette, 1999) and confirmed by a taxonomist at the Department of pharmaceutical Chemistry, Taibah University. A specimen (CI-2017-1) was archived at the Pharmacognosy and Pharmaceutical Chemistry Department herbarium.

2.3. Extraction and isolation

The powdered aerial parts (300 g) were extracted with MeOH (2.5 L × 5) and the extracts were evaporated to give total extract (CI). The latter (CI, 27.0 g) was subjected to SiO_2 VLC (silica gel vacuum liquid chromatography) using *n*-hexane, EtOAc, and MeOH to obtain 4 fractions; CI-1 (6.9 g), CI-2 (8.7 g), and CI-3 (10.3 g),

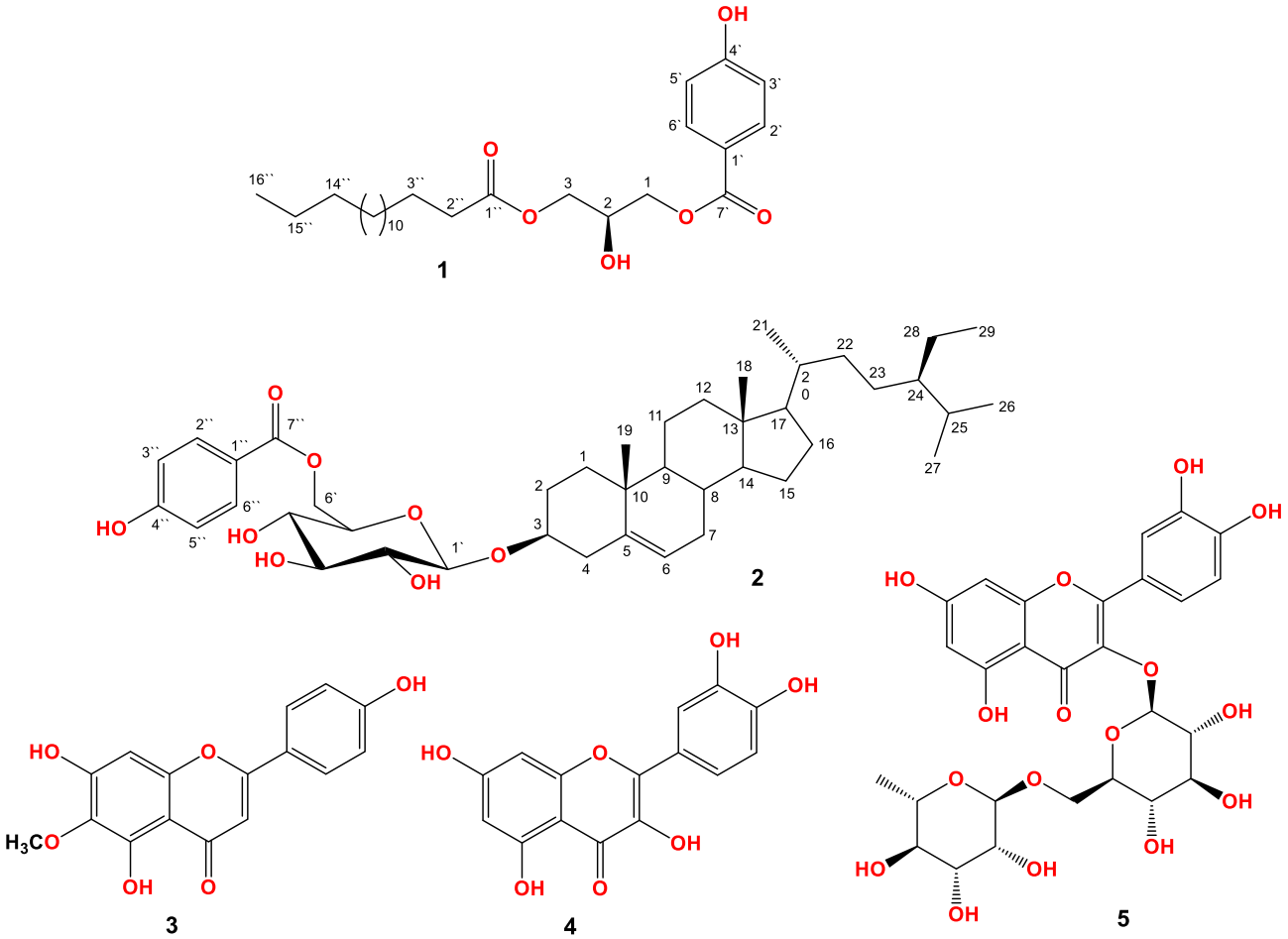

Fig. 1. Chemical structures of isolated compounds **1**–**5** from *Cassia italica*.

respectively. The EtOAC (8.0 g) fraction was submitted to SiO_2 CC (column chromatography) (*n*-hexane:EtOAc gradient) to give nine subfractions: CIE-1:CIE-9. SiO_2 CC for CIE-4 eluting with *n*-hexane: EtOAc gradient afforded **1**, which was purified on extraction tube (LiChrolut RP-18, H_2O:acetonitrile) to give **1** (17.2 mg). Subfraction CIE-5 was separated on SiO_2 CC ($CHCl_3$:MeOH gradient) to get **2** and RP-18 CC (H_2O:MeOH gradient) was utilized for its purification, giving **2** (23.8 mg). Based on TLC, fractions CIE-6 and CIE-7 were gathered and chromatographed on sephadex LH-20 using MeOH to give **3** and **4** which were further subjected to repeated SiO_2 CC (CHCl3-MeOH gradient) to get **3** (7.9 mg) and **4** (15 mg). Compound **5** (26.9 mg) was obtained from CIE-8 using SiO_2 CC ($CHCl_3$:MeOH gradient).

2.4. Spectral data

1-*p*-Hydroxy benzoyl-3-palmitoyl glycerol (**1**): Yellow oil, $[\alpha]_D$ + 35.2 (*c* 0.5, $CHCl_3$); IR (KBr) γ_{max}: 2956, 3354, 1605, 1726 cm^{-1}; see NMR Table 1; HRESIMS *m/z* 451.3054 $[M+H]^+$ (calcd for $C_{26}H_{43}O_6$, 451.3060).

6'-*p*-Hydroxy benzoyl daucosterol **(2)**: White amorphous powder; $[\alpha]_D$ + 64.3 (*c* 0.3, CH_3OH); IR (KBr) γ_{max}: 3425, 2964, 1716, 1662, 1085 cm^{-1}; see NMR Table 2; HRESIMS *m/z* 697.4683[M +H]$^+$ (calcd for $C_{42}H_{65}O_8$, 697.4679).

2.5. Alkaline hydrolysis of compound 1

Compound **1** solution (5 mg, KOH/MeOH 3%, 4 mL) had been left at room temperature for 2 h then neutralized utilizing HCl/MeOH (1 N). The solution was extracted three times with $CHCl_3$ (each 15 mL) and then concentrated. The obtained residue was chromatographed on a SiO_2 CC using *n*-hexane:EtOAc (99:1–80:20) to furnish palmitic acid methyl ester, which was specified by GCMS (Khedr et al., 2018).

2.6. Acid hydrolysis of compound 2

To a solution of **2** (5 mg in 10 mL MeOH), 5 mL of H_2SO_4 (5%) was added and refluxed for 3 h on WB (water bath). The solution was then extracted with EtOAc and then concentrated. The obtained residue was subjected sephadex LH-20 (MeOH). The aglycone and *p*-hydroxy benzoic acid were specified using co-TLC along with authentic. The sugar in the aqueous layer was identified by co-PC with authentic material (Mohamed, 2014).

Table 1
NMR spectral data of compound **1** (DMSO d_6, 850 and 214 Hz).

No.	δ_H [mult., *J* (Hz)]	δ_C (mult.)	HMBC
1	4.22 dd (11.1, 4.3) 4.20 dd (11.1, 6.0)	66.2 CH_2	2, 3, 7'
2	4.07 m	69.3 CH	1, 3
3	4.18 dd (11.3, 4.5) 4.16 dd (11.3, 5.8)	65.4 CH_2	1, 2, 1''
1'	–	123.8 C	–
2', 6'	7.32 d (8.5)	128.4 CH	3', 5', 4'
4'	–	158.5 C	
3', 5'	6.79 d (8.5)	115.2 CH	2', 6', 4'
7'	–	167.0 C	
4'–OH	9.61 s	–	3', 5'
1''	–	173.3 C	–
2''	2.29 t (6.8)	33.5 CH_2	3''
3''	1.51 m	24.5 CH_2	1'', 2''
$(CH_2)_{10}$	1.25–1.22 m	0.86 CH_2	
14''	1.23 m	31.3 CH_2	15'', 16''
15''	1.26 m	22.1 CH_2	14'', 16''
16''	0.86 t (6.8)	14.0 CH_3	14'', 15''

Table 2
NMR spectral data of compound **2** (DMSO d_6, 850 and 214 MHz).

No.	δ_H [mult., *J* (Hz)]	δ_C	HMBC
1	2.36 m, 2.12 m	36.9 CH_2	2, 3, 5, 6
2	1.52 m, 1.26 m	29.2 CH_2	1, 3, 10
3	3.46 m	77.0 CH	1', 4
4	1.96 m, 1.12 m	38.3 CH_2	3, 5, 6
5	–	140.5 C	–
6	5.33 t (2.6)	121.3 CH	4, 7, 8
7	1.92 m, 1.78 m	31.3 CH_2	5, 6, 9, 14
8	1.81 m, 1.46 m	31.5 CH	6, 13, 14
9	0.88 m	49.7 CH	7, 11, 19
10	–	31.4 C	–
11	1.47 m, 1.38 m	20.7 CH_2	9, 12
12	1.76 m, 0.98 m	36.3 CH_2	13, 17
13	–	41.9 C	–
14	0.97 m	56.2 CH	9, 16, 18
15	1.03 m	23.9 CH_2	14, 17
16	1.78 m	27.9 CH_2	14, 17
17	1.08 m	55.5 CH	14, 16
18	0.65 s	11.7 CH_3	12, 13, 14, 17
19	0.96 s	19.2 CH_3	1, 5, 9, 10
20	1.33 m	35.5 CH	17, 21
21	0.90 d (6.8)	19.0 CH_3	17, 20, 22
22	1.28 m, 0.99 m	33.5 CH_2	17, 21
23	1.13 m	25.5 CH_2	20, 28
24	0.91 m	45.2 CH	26, 27, 29
25	1.62 m	28.8 CH	24, 26, 27
26	0.80 d (6.8)	18.7 CH_3	24, 25, 27
27	0.82 d (6.8)	19.8 CH_3	24, 26, 27
28	1.18 m	22.7 CH_2	23, 24, 25, 29
29	0.83 t (7.2)	11.8 CH_3	24, 28
1'	4.22 d (7.7)	100.8 CH	3, 2', 3'
2'	2.90 m	73.5 CH	1', 4'
3'	3.13 m	76.8 CH	1', 2', 5'
4'	3.02 m	70.2 CH	2'
5'	3.07 m	76.8 CH	3', 4'
6'	4.03 dd (11.2, 4.3) 3.89 dd (11.2, 6.3)	65.5 CH_2	4', 7''
1''	–	121.2 C	–
2'', 6''	7.78 d (8.5)	131.6 CH	3'', 5'', 4'', 7''
3'', 5''	6.81 d (8.5)	115.2 CH	1'', 2'', 6'', 4''
4''	–	161.2 C	–
7''	–	166.7 C	–

2.7. Antioxidant activity

2,2-Diphenylpicrylhydrazyl (DPPH) assay was utilized to estimate the antioxidant activity of the EtOAc fraction and isolated compounds using butylated hydroxyanisole (BHA) (standard) as previously outlined (Ahmed et al., 2019).

2.8. Statistical analysis

The results were demonstrated as mean ± SD. Significance was calculated using One-way ANOVA followed by Tukey Kramer test. $p < 0.05$ was assigned as statistically significant.

3. Results and discussion

Extensive chromatographic separation of the EtOAc fraction obtained from *C. italica*, using RP-18, sephadex LH-20, and SiO_2 60 CC led to the separation of two new (**1** and **2**) and three known compounds (**3**–**5**) (Fig. 1).

Compound **1** was separated as yellow oil. Its HRESIMS revealed a pseudomolecular peak at *m/z* 451.3054 $[M+H]^+$ (calcd for $C_{26}H_{42}O_6$, 451.3060), correspondent to a formula $C_{26}H_{42}O_6$, which required six double bond equivalent. It had absorptions at 2956 (C-H aliphatic), 3354 (OH), 1605 (C = C aromatic), and 1726 (C = O) cm^{-1} in the IR (Silverstein and Webster, 1998). The ^{13}C and HSQC displayed signals for 26 carbons, containing 2 carbonyls

[δ_C 167.0 (C-7‘) and 173.3 (C-1‘‘)], two oxymethylenes, oxymethine, five aromatic carbons, and one oxygen-bonded aromatic carbon (Table 1). The two doublet–doublet oxymethylene signals at δ_H 4.22 and 4.20 (H-1) and 4.18 and 4.16 (H-3) and a multiplet oxymethine at δ_H 4.07 (H-2) indicated that **1** was a 1,3-diglyceride derivative (Salvo et al., 2017; Nieva-Echevarría et al., 2014). These signals having cross peaks in HSQC to the carbons at δ_C 66.2, 65.4, and 69.3, respectively (Kumar et al., 2011). This assignment was assured by the COSY cross peak of H-3 and H-1/H-2 and cross peaks in HMBC of H-1/C-2 and C-3 and H-3/C-1 and C-2 (Fig. 2). The NMR spectra for **1** revealed signals at δ_H 6.79/δ_C 115.2 (C-5‘, 3‘), 7.32 (C-6‘, 2‘)/128.4 (C-6‘, 2‘), 9.61 (4‘–OH), 158.5 (C-4‘), 123.8 (C-1‘), and 167.0 (C-7‘) reflected the existence of *p*-hydroxy benzoyl moiety in **1** (Van et al., 2018). The HMBC cross peaks of H-5‘ and H-3‘/C-6‘, C-4‘, and C-2‘, H-6‘ and H-2‘/C-4‘, C-3‘, and C-5‘, and 4‘–OH/C-5‘ and C-3‘ proved this moiety (Fig. 2). The cross peak of H-1 to C-7‘ established the connection of the *p*-hydroxy benzoyl moiety to C-1 of glycerol (Table 1). The ^{13}C and ^{1}H NMR revealed signals at δ_H 2.29 (H-2‘‘)/δ_C 33.5 (C-2‘‘), 173.3 (C-1‘‘), 1.25–1.22 $(CH_2)_n$/29.1–28.5 $(CH_2)_n$, and 0.86 (H-16‘‘)/14.0 (C-16‘‘), characterizing a fatty acyl moiety in **1**, which was secured by the HMBC and COSY correlations. The connectivity of this moiety at C-3 of glycerol was proved by the HMBC cross peak of H-3 to the carbon at δ_C 173.3 (C-1‘‘). This moiety was specified to be consisted of C16 on the basis of alkaline hydrolysis which afford methyl ester of palmitic acid that was established by GCMS (Machida et al., 1997). Therefore, **1** was assigned as 1-*p*-hydroxy benzoyl-3-palmitoyl glycerol and considered a new metabolite.

Compound **2** was separated as white amorphous powder with $[\alpha]_D$ + 64.3 (*c* 0.3, CH_3OH) and gave positive Lieberman Burchard test, suggesting a steroidal nature of **2** (Tieh and Chang, 1980; El-Shanawany et al., 2015). It had a molecular formula $C_{42}H_{64}O_8$ based on the HRESIMS molecular peak at *m/z* 697.4683 $[M+H]^+$ (calcd for $C_{42}H_{65}O_8$, 697.4679). The IR spectrum displayed characteristic absorptions at 3425, 2964, 1716, 1662, and 1085 cm^{-1}, indicating the existence of hydroxyl, C-H aliphatic, ester carbonyl, C=C, and C—O—C functionalities, respectively (Silverstein and Webster, 1998). The HSQC and ^{13}C spectra of **2** exhibited resonances for forty two carbons: six methyls, twelve methylenes, one of them for an oxymethylene (δ_C 65.5, C-6‘), eighteen methines, and six quaternary carbons, including an ester carbonyl (δ_C 166.7, C-7‘‘) and oxygenated aromatic carbon (δ_C 161.2, C-4‘‘) (Table 2). The extensive analysis of NMR spectra indicated that **2** was a daucosterol derivative (Peshin and Kar, 2017). This was established by the characteristic signals for a *tri*-substituted olefinic bond at δ_H 5.33 (H-6) and the methyl signals at δ_H 0.96 (H-19), 0.65 (s, H-18), 0.90 (H-21), 0.82 (H-27), 0.80 (d, *J* = 6.8 Hz, H-26), and 0.83 (H-29), correlating to the carbons at δ_C 121.3, 19.2, 11.7, 19.0, 19.8, 18.7, and 11.8, respectively in the HSQC (Table 2). This was confirmed by the observed HMBC cross peaks of H-19/ C-9, C-1, C-5, and C-10, H-18/C-14, C-12, C-13, and C-17, H-21/C-17, C-20, and C-22, H-27 and H-26/C-25 and C-24, and H-29/C-28 and C-24 (Fig. 2). In addition, the cross peaks of H-6/ C-7, C-4, and C-8 and H-4 and H-8/C-6 in HMBC established the C5-C-6 olefinic bond. Moreover, a doublet signal at δ_H 4.22 (d, *J* = 7.7 Hz, H-1‘), having HSQC cross peak to the carbon at δ_C 100.8 (C-1‘), characterized the β-glucose moiety in **2**. H-1‘ had cross peak in HMBC to the carbon at δ_C 77.0 (C-3) established the connectivity of glucose moiety at C-3. Furthermore, signals relevant for *p*-hydroxy benzoyl moiety [δ_H 6.81 (H-5‘‘, 3‘‘)/δC 11.5.2, 7.78 (H-6‘‘, 2‘‘)/131.6, 121.2 (C-1‘‘), 161.2 (C-4‘‘), and 166.7 (C-7‘‘)] were observed (Salib et al., 2011). This was secured by the observed HMBC cross peaks of H-2‘‘ and H-6‘‘/C-3‘‘, C-5‘‘, C-4‘‘, and C-7‘‘ and H-3‘‘ and H-5‘‘/C-1‘‘, C-2‘‘, C-6‘‘, and C-4‘‘. This was assured by the observed fragment peak at *m/z* 576.8593 $[M+H\text{-}C_7H_5O_2]^+$. The HMBC correlation of H-6‘ to C-7‘‘ signified the attachment of the *p*-hydroxy benzoyl moiety at C-6‘ of the glucose moiety. On the basis of these findings, **2** was assigned as 6‘-*p*-hydroxy benzoyl daucosterol and found to be a new natural product.

The known metabolites; scutellarein-6-methyl ether (**3**), quercetin (**4**), and rutin (**5**) (Harborne, 1994) were specified by comparing their data (Table 3) to those previously reported as well as co-TLC along authentic samples.

DPPH assay was utilized to assess the antioxidant capacities of phenolic metabolites. They are considered as safe natural antioxidants, as they delayed the progression many diseases by protecting the body from free radicals (El-Kashak et al., 2017). Thus, the antioxidant capacities of the EtOAc fraction and the isolated compounds were assessed using DPPH (Fig. 3). Compounds **1**–**5** and

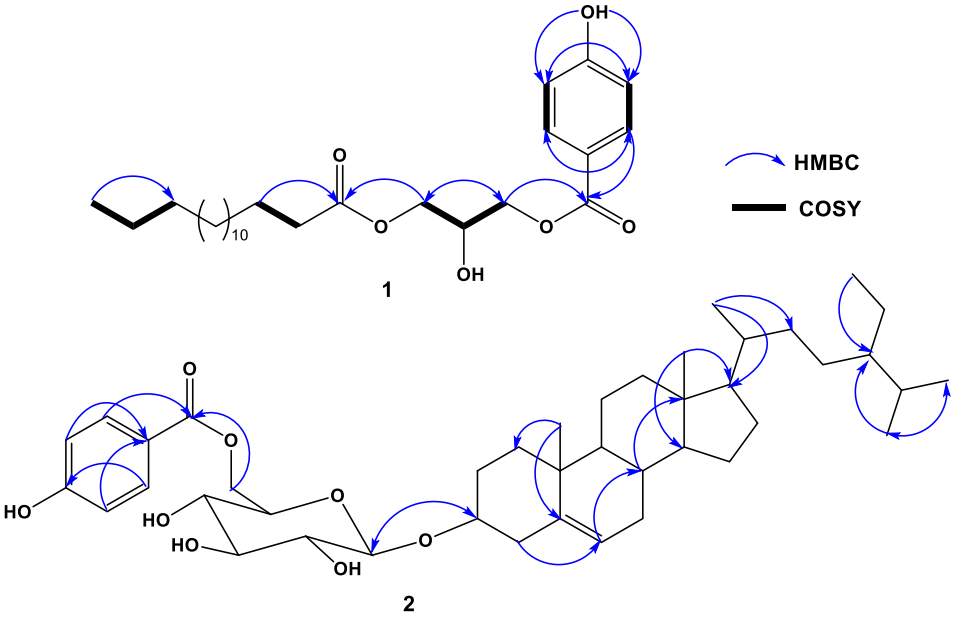

Fig. 2. Some key ^{1}H–^{1}H COSY (-) and HMBC (→) correlations of compounds **1** and **2**.

Table 3
NMR spectral data of compound **3–5** in DMSO d_6.

	3[a]		**4**[b]		**5**[b]	
No.	**δ_H [mult., *J* (Hz)]**	**δ_C (mult.)**	**δ_H [mult., *J* (Hz)]**	**δ_C (mult.)**	**δ_H [mult., *J* (Hz)]**	**δ_C (mult.)**
2	–	163.8 C	–	147.7 C	–	156.6 C
3	6.59 s	102.4 CH	–	135.7 C	–	133.2 C
4	–	182.1 C	–	175.8 C	–	177.3 C
5	–	145.2 C	–	160.7 C	–	156.4 C
6	–	131.4 C	6.17 d (2.4)	98.1 CH	6.20 d (2.4)	98.6 CH
7	–	152.5 C	–	163.8 C	–	161.2 C
8	6.78 s	94.3 CH	6.40 d (2.4)	93.3 CH	6.39 d (2.4)	93.6 CH
9	–	152.8 C	–	156.1 C	–	156.4 CH
10	–	105.7 C	–	103.0 C	–	104.0 C
1'	–	121.3 C	–	121.9 C	–	121.5 C
2'	7.93 d (8.5)	128.5 CH	7.66 d (2.4)	115.0 CH	7.55 d (2.4)	115.2 CH
3'	6.93 d (8.5)	115.9 CH	–	145.0 CH	–	144.7 C
4'	–	161.2 C	–	148.5C	–	148.4 C
5'	6.93 d (8.5)	115.9 CH	6.87 d (8.4)	115.6 CH	6.84 d (8.4)	116.2 CH
6'	7.93 d (8.5)	128.5 CH	7.53 dd (8.4, 2.4)	119.9 CH	7.54 dd (8.4, 2.4)	121.1 CH
1''	–	–	–	–	5.34 d (7.6)	101.1 CH
2''	–	–	–	–	3.05–5.33 (m)	74.0 CH
3''	–	–	–	–		75.8 CH
4''	–	–	–	–		70.0 CH
5''	–	–	–	–		76.3 CH
6''	–	–	–	–		68.2 CH_2
1'''	–	–	–	–	4.38 d (1.2)	100.7 CH
2'''	–	–	–	–	3.05–5.33 (m)	70.5 CH
3'''	–	–	–	–		70.5 CH
4'''	–	–	–	–		71.8 CH
5'''	–	–	–	–		68.2 CH
6'''	–	–	–	–	0.99 d (6.0)	17.7 CH_3
6-OCH_3	3.75 s	60.0 CH_3	–	–	–	–
5-OH	13.07 s	–	12.45 s	–	12.61 s	–

[a] Measured at 850 and 214 Hz.
[b] Measured at 600 and 150 Hz.

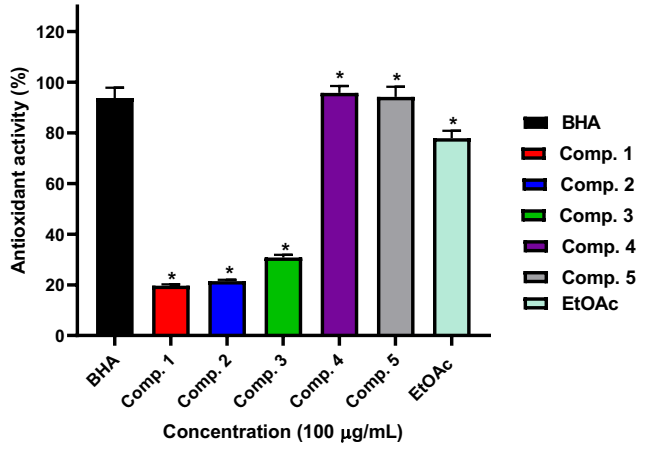

Fig. 3. Antioxidant activity of isolated compounds (**1–5**) and EtOAc extract of *C. italica* by DPPH method. * Compared to BHA (Positive control); (one-way ANOVA followed by Tukey Kramer). Data are the mean ± SE. (n = 3).

EtOAc fraction exhibited significant antioxidant potentials with antioxidant activities 19.7, 21.5, 30.7, 95.8, 94.2, and 77.9%, respectively at 100 µg/mL, compared to BHA (93.8%). Our results are in a good agreement with a number of publications that reported on the antioxidant activities of extracts of *C. italica* from different geographical locations which were attributed to the presence of various classes of phenolic metabolites. The ethyl acetate and *n*-butanol extracts of aerial parts of the Egyptian *C. italica* possessed antioxidant capacity using ABTS assay (Khalaf et al., 2019; Madkour et al., 2017). It is noteworthy that the extracts of the roots of the southern African *C. italica* exhibited antioxidant activity in the DPPH assay (Masoko et al., 2010; Mokgotho et al., 2013). Jothi et al reported that the different fractions of aerial parts of the Indian *C. italica* ssp *micrantha* displayed antioxidant potential using different assays such as DPPH, ABTS, superoxide, and reducing power assays (Jothi et al., 2015). It is noteworthy that these highest activity of quercetin (**4**) and rutin (**5**) are in a good agreement with the formerly stated results (Yang et al., 2008; DPPH-Scavenging activities and structure-activity relationships of phenolic compounds, 2010).

4. Conclusions

C. italica is a rich source of diverse natural compounds with variable pharmacological properties. Chromatographic fractionation of *C. italica* afforded two new (**1** and **2**) and three known compounds (**3–5**). Their structures elicitation was carried out by various spectral techniques. Compounds **1–5** had promising antioxidant activity in DPPH assay. The antioxidant capacities of isolated metabolites merit further concern for the use of this plant in various disorders related to oxidative stress. Also, this wok may also assist in validating the widely claimed ethnobotanical uses of the plant in folk and traditional medicine.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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