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

Rwaida A. Al-Haidari a, Mai M. Al-Oqail b, *

a Department of Pharmacognosy, College of Pharmacy, Taibah University, Al Madinah Al Munawwarah 30078, Saudi Arabia
b Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia

A R T I C L E   I N F O
Article history:
Received 14 May 2020
Accepted 28 July 2020
Available online 3 August 2020

Keywords:
Cassia italica
Fabaceae
Diglyceride derivative
Daucosterol derivative
Antioxidant

A B S T R A C T
Two new benzoic acid derivatives: 1-p-hydroxy benzoyl-3-palmitoyl glycerol (1) and 6-p-hydroxy benzoyl daucosterol (2), along with scutellaren-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 have 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 and degenerative illnesses.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 (Molodin 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 (Abbas 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 ialtica (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 complaints, gout, biliary-ness, 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., 2012; 2014).

* Corresponding author.
E-mail address: maloqail@ksu.edu.sa (M.M. Al-Oqail).
Peer review under responsibility of King Saud University.
Khalaf et al. reported the isolation of tinnevellin; emodin, physcion, 2-methoxy-emodin-6-O-β-D-glucopyranoside, 1,6,8-trihydroxy-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’-bianthracen-9,9’-dione were separated from *C. italica* pods growing in Sudan (Yagi et al., 2013). Moreover, (22E)-3-β-hydroxycycloart-22-en-24-one, uvaol, β-sitosterol, daucosterol, emodin, methyl 3,4-dihydroxybenzoate, aloin, 4-hydroxypheny-O-β-D-glucopyranoside, and rutin were reported from aerial parts of Saudi *C. italica* (Mohamed, 2014). However, little available reports 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 ($lambda_{max}$ 255 and 366 nm) and spray reagent (anisaldehyde/H$_2$SO$_4$).

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 (Cl). The latter (Cl, 27.0 g) was subjected to SiO$_2$ VLC (silica gel vacuum liquid chromatography) using n-hexane, EtOAc, and MeOH to obtain 4 fractions; Cl-1 (6.9 g), Cl-2 (8.7 g), and Cl-3 (10.3 g).
respectively. The EtOAc (8.0 g) fraction was submitted to SiO₂ CC (column chromatography) (n-hexane:EtOAc gradient) to give nine subfractions: CIE-1-CIE-9. SiO₂ CC for CIE-4 eluting with n-hexane:EtOAc gradient afforded 1, which was purified on extraction tube (LiChrolut RP-18, H₂O:acetonitrile) to give 1 (17.2 mg). Subfraction CIE-5 was separated on SiO₂ CC (CHCl₃:MeOH gradient) to get 2 and RP-18 CC (H₂O: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 SiO₂ 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 SiO₂ CC (CHCl₃:MeOH gradient).

2.4. Spectral data

1-p-Hydroxy benzyol-3-palmitoyl glycerol (1): Yellow oil, [α]D + 35.2 (c 0.5, CHCl₃); IR (KBr) νmax: 2956, 3354, 1605, 1726 cm⁻¹; see NMR Table 1; HRESIMS m/z 451.3054 [M+H]⁺ (calcd for C₂₅H₄₂O₆, 451.3060).

6'-p-Hydroxy benzyol daucosterol (2): White amorphous powder; [α]D + 64.3 (c 0.3, CH₂OH); IR (KBr) νmax: 3425, 2964, 1716, 1662, 1085 cm⁻¹; see NMR Table 2; HRESIMS m/z 697.4683[M + H]⁺ (calcd for C₉₆H₁₆₀₂O₆, 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 CHCl₃ (each at room temperature for 2 h then neutralized utilizing HCl/MeOH). The obtained residue was subjected to TLC, fractions CIE-6 and CIE-7 CC (CHCl₃:MeOH gradient) was utilized for its purification to get nine subfractions. CIE-1-CIE-9. SiO₂ CC (CHCl₃:MeOH gradient) to get nine subfractions: CIE-1-CIE-9.

2.6. Acid hydrolysis of compound 2

To a solution of 2 (5 mg in 10 mL MeOH), 5 mL of H₂SO₄ (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 to TLC with authentic material (Mohamed, 2014).

Table 1

| No. | δC [mult., J (Hz)] | δC (mult.) | HMBC |
|-----|--------------------|------------|------|
| 1   | 4.22 dd (11.1, 43) | 66.2 CH₂  | 2, 3, 7' |
| 2   | 4.20 dd (11.1, 6.0) | 60.9 CH₂  | 3' |
| 3   | 4.07 m              | 69.3 CH   | 1, 3 |
| 4   | 3.18 dd (11.3, 45) | 65.4 CH₂  | 1, 2, 1' |
| 5   | 4.16 dd (11.3, 58) | 123.8 C   | -   |
| 6   | 3.72 d (8.5)       | 128.4 CH  | 3', 5', 4' |
| 7   | 4'                 | 158.5 C   | -   |
| 8   | 6.79 d (8.5)       | 115.2 CH  | 2', 6', 4' |
| 9   | 7'                 | 167.0 C   | -   |
| 10  | 4'-OH              | 9.61 s    | -   |
| 11  | 1'                 | 173.3 C   | -   |
| 12  | 2'                 | 2.29 t (6.8) | 33.5 CH  | 3' |
| 13  | 3'                 | 1.51 m    | 24.3 CH₂ | 1', 2' |
| 14  | (CH₃)₂CO           | 1.25-1.22 m | 0.86 CH₂ | -   |
| 15  | 14''               | 1.23 m    | 31.3 CH  | 15', 16' |
| 16  | 15''               | 1.26 m    | 22.1 CH₂ | 14', 16' |
| 17  | 16''               | 0.86 t (6.8) | 14.0 CH₁ | 14', 15' |

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 SiO₂ 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 C₂₅H₄₂O₂₆, 451.3060), correspondent to a formula C₂₅H₄₂O₂₆, which required six double bond equivalent. It had absorptions at 2956 (CH₃ aliphatic), 3354 (OH), 1605 (C = O aromatic), and 1726 (C = O) cm⁻¹ in the IR (Silverstein and Webster, 1998). The 13C and HSQC displayed signals for 26 carbons, containing 2 carboxyls
test, suggesting a steroidal nature of (Shanawany et al., 2015). It had a molecular formula C\(_{42}\)H\(_{64}\)O\(_8\) (calcd for C\(_{42}\)H\(_{65}\)O\(_8\), 697.4679). The IR spectrum displayed characteristic absorptions at 3425, 2964, 1716, 1662, and 1085 cm\(^{-1}\), and a doublet–doublet oxymethylene signal at 4.22 and 4.20 (H-1) and 4.18 and 4.16 (H-3) and a multiplet one of them for an oxymethylene (\(\delta_c\) 65.5, C-6'), eighteen methines, and six quaternary carbons, including an ester carbonyl (\(\delta_c\) 166.7, C-7') and oxygenated aromatic carbon (\(\delta_c\) 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 \(\delta_h\) 5.33 (H-6) and the methyl signals at \(\delta_h\) 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 \(\delta_c\) 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, H-21/C-17, H-21/C-17, H-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-C6 olefinic bond. Moreover, a doublet signal at \(\delta_h\) 4.22 (d, \(J = 7.7\) Hz, H-1'), having HSQC cross peak to the carbon at \(\delta_c\) 100.8 (C-1'), characterized the \(\beta\)-glucose moiety in 2. H-1' had cross peak in HMBC to the carbon at \(\delta_c\) 77.0 (C-3) established the connectivity of glucose moiety at C-3. Furthermore, signals relevant for \(\beta\)-hydroxy benzoyl moiety [\(\delta_h\) 6.81 (H-5', 3''), \(\delta_c\) 11.52, 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-H\(_2\)O\(_2\)]\(^{+}\). The HMBC correlation of H-6' to C-7'' signified the attachment of the \(\beta\)-hydroxy benzoyl moiety at C-6' of the glucose moiety. On the basis of these findings, 2 was assigned as \(\beta\)-6'-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
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* 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 work may also assist in validating the widely claimed ethnobotanical uses of the plant in folk and traditional medicine.

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 work 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.

**References**

Ahmad, F., Anwar, F., Hira, S., 2016. Review on medicinal importance of Fabaceae family. Pharmacology Online 3, 151–156.

Ahmed, S., Al-Rehaily, A.J., Alam, P., Alqahtani, A.S., Hidayatullah, S., Rehman, M.T., Siddiqui, N.A., 2019. Antidiabetic, antioxidant, molecular docking and HPTLC analysis of miquelianin isolated from *Euphorbia schimperi* C. Presl. Saudi Pharma. J. 27, 655–663.
Al-Said, M.S., 1993. Traditional medicinal plants of Saudi Arabia. AMJ Chinese Med. 2, 1–12.

Al-Yahya, M.A., Al-Meshal, I.A., Mossa, J.S., Al-Badr, A.A., Tariq, M., 1990. Saudi plants. In: A phytochemical and biological approach. King Saudi University, Riyadh, p. 349.

Araz, S.S., Abdel-Daim, M., Eldahshan, O.A., 2013. Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of Delonix regia leaves extract. Med. Chem. Res. 22, 4269–4277.

Collenette, S., 1999. Wild flowers of Saudi Arabia. King of Saudi Arabia: National Commission for Wild Life Conservation and Development (NCWCD) & Sheila Collenette, King Fahd National Library; p. 523.

Dai, Y., Kawo, A.H., Aliyu, R.M., 2012. Phytochemical screening and antibacterial activity of the leaf and root extracts of S. italica. Afr. J. Pharm. Pharmacol. 6, 914–918.

Dave, H., Ledwani, L., 2012. A review on anthraquinones isolated from Cassia species and their applications. Indian J. Nat. Prod. Resour. 3, 291–319.

El-Khashab, W.A., Osman, S.M., Gaara, A.H., ElToumy, S.A., Mohamed, T.K., Brouard, L., 2017. Phenolic metabolites, biological activities, and isolated compounds of Terminalia muelleri extract. Pharm. Biol. 55, 2277–2284.

Elsayed, N.H., Abu-Dooh, A.M., Elkhrisy, E.A.M., Mabry, T.J., 1992. Flavonoids of Viola odorata. Chromatographia. 31, 2187.

El-Shanawany, M.A., Sayed, H.M., Ibrahim, S.R.M., Fayed, M.A.A., 2015. Stigmastanol tetraacetoate, a new stigmastanol ester from the Egyptian Elephnis clarius. Drug Res. 65, 347–353.

Gololo, S.S., Mapfumari, N.S., Mogale, M.A., 2018. Comparative quantitative activities of the acetone extract of the roots of Senna italica. Nat. Prod. Res. 5, 347–353.

Dave, H., Ledwani, L., 2012. A review on anthraquinones isolated from Cassia species and their applications. Indian J. Nat. Prod. Resour. 3, 291–319.

El-Sayed, N.H., Abu-Dooh, A.M., Elkhrisy, E.A.M., Mabry, T.J., 1992. Flavonoids of Viola odorata. Chromatographia. 31, 2187.

Elsayed, N.H., Abu-Dooh, A.M., Elkhrisy, E.A.M., Mabry, T.J., 1992. Flavonoids of Viola odorata. Chromatographia. 31, 2187.

Nieva-Ecevarría, B., Goicoechea, E., Manzanos, M.J., Guillén, M.D., 2014. A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. Food Res. Int. 66, 379–387.

Salih, Y.B., El-Tigani, S., Ali, M., Elkhidir, I., Mohammed, A.M.A., 2013. Chemical and pharmacological properties of Nephrolepis micrantha. Vietnam J. Chem. 56, 187–190.

Azab, S.S., Abdel-Daim, M., Eldahshan, O.A., 2013. Phytochemical, cytotoxic, hepatoprotective and antioxidant properties of Delonix regia leaves extract. Med. Chem. Res. 22, 4269–4277.

Collenette, S., 1999. Wild flowers of Saudi Arabia. King of Saudi Arabia: National Commission for Wild Life Conservation and Development (NCWCD) & Sheila Collenette, King Fahd National Library; p. 523.

Dai, Y., Kawo, A.H., Aliyu, R.M., 2012. Phytochemical screening and antibacterial activity of the leaf and root extracts of S. italica. Afr. J. Pharm. Pharmacol. 6, 914–918.

Dave, H., Ledwani, L., 2012. A review on anthraquinones isolated from Cassia species and their applications. Indian J. Nat. Prod. Resour. 3, 291–319.

El-Khashab, W.A., Osman, S.M., Gaara, A.H., ElToumy, S.A., Mohamed, T.K., Brouard, L., 2017. Phenolic metabolites, biological activities, and isolated compounds of Terminalia muelleri extract. Pharm. Biol. 55, 2277–2284.

Elsayed, N.H., Abu-Dooh, A.M., Elkhrisy, E.A.M., Mabry, T.J., 1992. Flavonoids of Viola odorata. Chromatographia. 31, 2187.

El-Shanawany, M.A., Sayed, H.M., Ibrahim, S.R.M., Fayed, M.A.A., 2015. Stigmastanol tetraacetoate, a new stigmastanol ester from the Egyptian Elephnis clarius. Drug Res. 65, 347–353.