Full Length Research Paper

Alkaloids and flavonoids from the air dried aerial parts of *Citrullus colocynthis*

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Accepted 5 April, 2012

From the air dried aerial parts of *Citrullus colocynthis*, two new alkaloids namely, 2-(nonan-8-one)-(1H)-4-quinolone and 2-(Nonan-8-one) 4-methoxy-quinoline were isolated. Two flavonoids, (2S)-3', 4' methylenedioxy -5, 7-dimethoxy flavan and hispidulin 7- (6 – E – β coumaroyl – β – D glucopyranoside) were also extracted and identified. The structures were established by conventional methods of analysis and confirmed by UV, IR, ¹H and ¹³CNMR, and mass spectra.

Key words: Citrullus colocynthis, cucurbitaceae, new quinoline, quinolone alkaloids, flavonoids.

INTRODUCTION

Citrullus colocynthis (L.) is an important medicinal plant belonging to the family cucurbitaceae. It is an annual herb widely distributed in Mediterranean strip (Tackholm, 1974). The plant is prostrate, herbaceous characterized by 5-angled stems and coiled tendrils, leaves alternate, palmately 5-lobed or divided, exstipulate and flowers are unisexual and solitary (Täckholm, 1974; Chaudhary and Al-jowaid, 1999). Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan et al., 2009). Several active chemical constituents of C. colocynthis plant were recorded. They are grouped as alkaloids, flavonoids, saponins, tannins, carbohydrates, glycosides and essential oils. Plant based natural constituents can be derived from any part of the plant like stems, leaves, flowers, roots, fruits and seeds (Gordon and David, 2001). A number of plant secondary metabolites including flavonoids and curcubitacins have previously been reported from C. colocynthis (Maatooq et al., 1997; Seger et al., 2005). Adam et al. (2001) reported the bitter substances (colocynthin and colocythetin) and cucurbitacins A, B, C, D and E (α – elatrin); cucurbitacines E, I, J, K and L (Sturm et al., 2009), cucurbitacin glycosides (Hatam et al., 1989; Abbas et al., 2006). The cucurbitacins are of great interest because of the wide range of biological activities exhibited in plants and animals. They are predominantly found in the Cucurbitaceae family. A number of chemical constituents have been investigated for their cytotoxic, heptoprotective, cardiovascular and antidiabetic effects and antioxidant activity of cucurbitacins B and I (Jayaprakasam et al., 2003) and the glucosides of cucurbitacin I and L (Abbas et al., 2006).

Sunil et al. (2008) studied antioxidant and free radical scavenging potential of C. colocynthis methanolic fruit extract. C. colocynthis is also one of the plants belonging to family cucurbitaceae. It is a fruit commonly known as bitter apple. It is a native plant of North Africa, being common throughout Morocco, Egypt and Sudan (Al-Ghamdi et al., 2009). It has been used in herbal treatment of diabetes (Karim et al., 2011), edema, bacterial infection and cancer. The aqueous pulp extract of fruit is used for kidney, liver functions treatment (Rahbar and Nabipour, 2010). The phenolic compounds isolated from plants are of great interest due to their antioxidative and anticarcinogenic activity. They play a very important role in absorbing and neutralizing free radicals. They contain not only minerals and primary metabolite but also a diverse array of secondary metabolite with antioxidant potential (Chanda et al., 2011).

In the present investigation of *C. colocynthis*, we report the isolation and structural elucidation of two new alkaloids 1, 2 and two flavonoids 3, 4 that has not been previously investigated with respect to its alkaloid and flavonoid constituents.

		1		2		
	¹ H	¹³ C	НМВС	¹ H	¹³ C	НМВС
1	-	-	-	-	-	-
2	-	125.0 ^s		-	119.95	
3	6.21 ^s	100.3 ^d	C-5, C-9, C-10	6.62 ^s	99.8 ^d	C-1, C-4, C-5, C-10
4	-	178.9 ^s		-	164.2 ^s	
5	8.35 ^{brd}	125.3 ^d	C-4, C-7, C-10	8.18 ^{brd}	121.5 ^d	C-7, C-9
6	7.32 ^{brt}	123.5 ^d		7.42 ^{brt}	124.8 ^d	
7	7.58 ^{dt}	131.7 ^d		7.63 ^{dt}	129.7 ^d	C-8, C-9
8	7.72 ^{brd}	118.3 ^d	C-5, C-9, C-10	7.97 ^{brd}	128.2 ^d	
9	-	154.7 ^s		-	149.6 ^s	
10	-	140.5 ^s		-	162.3 ^s	
OMe	-	-		4.05 ^s	55.6 ^q	C-4, C-10
NMe	-	-		-	-	
1′	2.63 ^t	34.3 ^t		2.85 ^t	39.6 ^t	C-3, C-4
2′	1.70 ^{brs}	28.8 ^t		1.8 ^m	23.7 ^t	
3′	1.50 ^{brs}	28.8 ^t		1.6 ^m	30.1	
4′	1.20 ^{brs}	28.8 ^t		1.28 ^m	29.0	
5′	1.20 ^{brs}	28.8 ^t		1.30 ^m	29.3	
6′	1.20 ^{brs}	28.8 ^t		1.32 ^m	29.9	
7′	2.38 ^t	43.6 ^t		2.42 ^t	43.7	
8′	-	210.5 ^s		-	209.5	
9′	2.1 ^s	29.8 ^q	C-6 [′] , C-7 [′] , C-9 [′]	2.18 ^s	29.4	C-7 [′] , C-8 [′]
10 [/]	-	-		-	-	

Table 1. NMR data of compounds 1 and 2.

MATERIALS AND METHODS

General procedures

UV shift reagents were prepared according to standard procedures (Mabry et al., 1970; Markham, 1982). IR: Perkin - Elmer Model 983 in CHCl₃. ¹H and ¹³CNMR in Bruker AC 200L instrument 200 and 50.32 MHz and for the 2D exp. 500 and 125 MHz, respectively with TMS as int. standard. HRMS: VG Zab Spec GC – MS spectrometer.

Plant materials

The aerial parts of C. colocynthis were collected in June 2010 from plants growing wild in the Mediterranean Coastal Strip, Egypt. The plant identify was verified by plant taxonomist. A voucher specimen has been deposited at the Herbarium of the Botany Department, Faculty of Science, Cairo University. The collected plant samples of C. colocythis were washed with distilled water and then air dried for two weeks. The dried samples were homogenized with electrical grinder and finally stored in airtight bottles before analysis.

Extraction and isolation

Dried and powdered aerial parts (1 kg) were extracted with MeOH and the extract evaporated to dryness under reduced pressure. The residue (2.5 g) was acidified with 0.1 N H₂SO₄ and extracted with CHCl₃. The acid soluble part was basified with NH₄OH (Balbaa et al., 1981) and extracted with CHCl₃. The CHCl₃ extract (1.3 g) was fractionated on a basic AL_2O_3 column (5 x 70 cm) eluting with hexane (fraction A), followed by a gradient of CH₂Cl₂ and ethyl

acetate up to 100% (fraction B). Fraction A had vielded compound 1 (75 mg) and fraction B has compound 2 (35 mg). Dried aerial parts of C. colocynthis (500 g) were extracted with MeOH for 6 h. The collected methanol (MeOH) extracts were concentrated to dryness under reduced pressure. The residue (4.5 g) was partitioned between 1 : 1 EtOAc / $H_2O.$ The EtOAc layer was concentrated to give residue (3.4 g) and then applied to silica gel column eluted with n - hexane - EtOAc (1 : 1). The fraction was applied to a porous polymer Diaion HP - 20 (H₂O - MeOH gradient) followed by MPLC silica gel, (CH₂Cl₂ - MeOH gradient) to give compound 3 (35 mg) and compound 4 (25 mg).

Identification of compounds

Compound (1): 2-(Nonan-8-one)-(1H)-4-quinolone: UV $\lambda \frac{MeOH}{max}$ nm (log ζ): 322 (3.8), 320(3.8), 236(4.2), 216(4.5). IR V^{CHCI}₃ (cm⁻¹): 2925, 2850, 1710, 1640, 1595, 1560, 1510, 1460, 1420, 1360, 1190, 1160, 1120, 990, 840, 760. ¹H and ¹³CNMR (CDCl₃) are tabulated in Table 1. MS m/z (rel.int.): 385 [M]⁺ (14), 270 [M-Me]⁺

(8), 242 [M-COMe]⁺ (47), 228 [242-CH₂]⁺ (49), 214 [228-CH₂]⁺ (20), 200 [214-CH₂]⁺ (11), 186 [200-CH₂]⁺ (36), 172 [186-CH₂]⁺ (78), 159 [M-C₈H₁₄O]⁺ (100), 130 (37), 77 (7).HR-MS m/z:285.1832. calc. for C₁₈H₂₃NO₂ 285. 1829.

Compound (2): 2-(Nonan-8-one) 4-methoxy-quinoline: UV MeOH

 $λ_{max}$ nm (log ζ): 312 (sh), 314 (2.8), 300 (2.9), 228 (4.5). IR V

^{CHCl}₃ (cm⁻¹): 2930, 2850, 1705, 1595, 1565, 1510, 1450, 1430, 1370, 1190, 1150, 1140, 1000, 980, 840, 760. ¹H and ¹³CNMR (CDCl₃) resulted as shown in Table 1. MS m/z (rel. int.) 299 [M]⁺ (40), 284

Compound	2	4	
Carbon	3		
2	77.7	164.5	
3	29.5	102.7	
4	19.3	182.4	
5	158.5	152.7	
6	91.4	132.6	
7	159.4	156.3	
8	93.4	94.4	
9	156.8	152.2	
10	103.0	106.0	
1′	135.6	121.2	
2′, 6′	106.7, 119.6	126.6	
3/, 5/	108.1, 147.2	115.9	
4′	147.8	161.5	
OMe	55.4	60.6	
Glucosyl			
1″		100.1	
2″		73.2	
3″		76.6	
4″		70.4	
5″		74.1	
6″		63.7	
Coumaroyl			
1‴		125.0	
2′′′′, 6′′′		130.0	
3 ^{///} , 5 ^{///}		116.2	
4‴		159.8	
7′′′		113.7	
8′′′′		145.2	
9///		166.7	

 Table 2. ¹³CNMR spectral data for compounds 3 and 4 (400)
MHz, CDCl₃).

 $\begin{array}{l} [\text{M-Me}]^{+} \ (14), \ 256 \ [284-\text{CO}]^{+} \ (58), \ 242 \ [256-\text{CH}_2]^{+} \ (66), \ 228 \ [242-\text{CH}_2]^{+} \ (2S), \ 214 \ [228-\text{CH}_2]^{+}, \ 200[214-\text{CH}_2]^{+} \ (74), \ 186 \ [200-\text{CH}_2]^{+} \ (89), \ 173 \ [\text{M-C}_8\text{H}_{14}\text{O}]^{+} \ (100), \ 149 \ (47), \ 130 \ (65), \ 115 \ (18), \ 102 \ (15), \end{array}$ 83 (8), 71 (17). HR-MS m/z: 299. 1978 [M]⁺, calc. for C₁₉H₂₅NO₂ 299.1985.

Compound (3): (2S)- 3¹, 4¹ - Methylenedioxy-5, 7-dimethoxy MeOH flavan: UV λ max nm (log ζ): 283 (3.83), 230 (5.22). IR V^{CHCI}₃ (cm $^{1}):$ 3060, 2940, 2840, 1620, 1595, 1495, 1440, 1250, 1200, 1140, 1100. $^{1}\mathrm{HNMR}$ (400 MHz, CDCl_3) $^{13}\mathrm{CNMR}$ (400 MHz, CDCl_3) Table 2. HR-MS m/z: 314. 1145 (M⁺. calc. for C₁₈H₁₈O₅; 314. 1154). MS m/z (rel. int.): 314 [M]⁺, (100), 283 (8), 166(9), 148 (59), 147 (26), 138 (14).

Compound (4): Hispidulin 7-(6-E-P-coumaroyl-β-D-

glucopyranoside): UV λ_{max}^{MeOH} nm (log ζ): 338(4.46), 275(4.26),

230(4.23); IR V^{CHCI}₃ (cm⁻¹): 3350, 1700, 1660, 1600, 1560, 1510, 1495, 1300, 1260, 1189, 1080. ¹HNMR (400 MHz, CDCl₃), ¹³CNMR (400 MHz, CDCl₃) Table 2. HR-MS m/z: 608. 1573 (M⁺. calc. for C31H28O13; 608. 1529). MS m/z (rel. int.): 300(100), 285 (68), 257 (63), 167(12), 164(13), 147(24), 139(15), 119(22), 69(50).

RESULTS AND DISCUSSION

Two new alkaloids (compounds 1 and 2) were isolated from the aerial parts of C. colocynthis. The UV spectra for compound 1 showed typical 4-quinolone peaks at 332, 320, 236 and 216 nm. The IR spectrum showed aromatic signals at 1595 and 1510 cm⁻¹. The ¹HNMR spectra of compound 1 showed quinolone structure with the broad doublet. Also the ¹HNMR spectrum showed the signals for the aliphatic side chain as given in Table 1. The placement of the side chain was decided by HMBC experiment for compounds 1 and 2. ¹³CNMR (Table 1). From the HMBC experiments the structure of compound 1 was assigned as 2-(nonan-8-one)-(1H)-4 guinolone. HR-MS indicated the molecular formula C₁₈H₂₃NO₂ (m/z 285, 1832). Thus, compound 1 was assigned as 2-(nonan-8-one)-(1H)-4-quinolone which was not previously isolated from the aerial parts of C. colocynthis (Figure 1). Compound 2: The molecular formula of the second new compound 2 as C19H25NO2 calculated from its HR-MS (m/z 299, 1978); indicated eight degrees of unsaturation, two of which were accounted for the bicyclic ring system, one for the carbonyl group on the side chain and the remaining five for the double bonds. The ¹HNMR spectrum showed the chemical shift of the aromatic proton H-5 in a fairly lower field at δ 8.18 (1 H, brd, J = 8 Hz, H-5). The chemical shift of the methoxy group at δ 4.05 an O-methyl rather than an N-methyl group correlated with the aromatic character of the ring system (Table 1). The IR spectrum showed the side chain carbonyl group at 1705 cm⁻¹ and aromatic signals at 1595, 1565, 1510 cm⁻¹. The UV spectrum showed the presence of conjugated aromatic system with the maxima at 312 (sh), 300 and 228 nm. The correlation of the protons and carbons were followed from the HMBC spectrum and unambiguous assignment of the molecule (Table 1). The spectral data showed the structure of compound 2 as 2-(nonan-8-one)-4-methoxy-guinoline (Figure 1).

Furthermore, two flavonoids were isolated from aerial parts of the plant. These were identified as $(2S)-3^{1}$, 4^{1} methylenedioxy-5, 7-dimethoxy flavan (Figure 2, compound 3) and hispidulin 7-(6-E-P-coumaroyl-B-Dglucopyranoside) (Figure 2, compound 4). The molecular formula of compound 3 was determined as C₁₈H₁₈O₅ on the basis of HR-MS. The broad band decoupled ¹³CNMR spectrum showed 18 carbon signals. The IR spectrum showed absorption bands at 1595 and 1495 cm⁻¹ which were assignable to an aromatic ring. The aforementioned data and UV spectrum (λ max) at 230 and 283 nm (sh) suggested aflavan nature for compound 3, which was further evidenced by the analysis of the ¹HNMR and ¹H-¹H COSY spectra. The ¹HNMR spectrum showed proton signals at δ 6.07 and 6.11. Also, the proton signals were at δ 6.76, 6.87 and 6.92. The H-2, H₂-3 and H₂-4 signals were at (δ 4.87), (δ 1.96, 2.13) and (δ 2.61, 2.73), respectively. Additionally, the ¹HNMR spectrum showed two methoxy signlets at δ 3.74, 3.78 and one





Figure 1. Alkaloids (compounds 1 and 2).

methylenedioxy signal at δ 5.94. The ¹³CNMR spectra revealed C₆-C₃ signals at δ 19.3 (C-4), 29.5 (C-3), 77.7 (C-2), 91.4 (C-6), 93.4 (C-8), 103.0 (C-10), 156.2 (C-9), 158.5, 159.4 (C-5,7) and C₆ signals at δ 106.7 (C-2), 108.1 (C-5[']), 119.6 (C-6[']), 135.6 (C-1[']), 147.2, 147.8 (C- $3^{\prime},4^{\prime}$). In addition, two methoxyl signals were found at δ 55.3 (C-OMe) and 55.4 (C-OMe) and one methylenedioxy signal was at δ 101.0 (Table 2, Figure 2). Atypical fragmentation at m/z 148 from MS spectrum suggested one methlendioxy unit in ring B. The H-2 proton formed a doublet of doublets with trans coupling ³J (10.5 Hz) and cis coupling ³J (1.8 Hz) suggesting that this methine proton must be axial. The absolute configuration at position 2 was determined as 2S by comparing the negative cotton effect at 281 nm in CD experiment with the authentic (2S)-4-hydroxy-5, 7, 3'-trimethoxyflavan. From the aforementioned evidence, compound 3 was determined to be $(2S)-3^{1},4^{1}$ -methylenedioxy -5, 7dimethoxyflavan.

The molecular formula of compound 4 was determined as $C_{31}H_{28}O_{13}$ on the basis of HR-MS. The IR spectrum revealed hydroxyl (3350 cm⁻¹), conjugated ester (1700 cm⁻¹), α , β -unsaturated carbonyl (1660 cm⁻¹), and aromatic absorptions (1600, 1560 and 1500 cm⁻¹). The

UV spectrum in methanol exhibited absorptions (λ max) at 338 and 275 nm (sh) and 230 (sh). The ¹HNMR, ¹H-¹H COSY, ¹³CNMR and HMBC spectra displayed characteristic signals for the flavone, glucose and P-coumaroyl moieties.

The ¹HNMR and ¹H-¹H COSY spectra showed a methoxyl group δ 3.66(6-OMe), two singlet protons at δ 6.90 (1H, s, H-3), 7.08 (1H, s, H-8), three para substituted aromatic protons at δ 7.00 (2H, d, J = 8.4, H-3[\],5[\]), 8.02 (2H, d, J = 8.4, H-2^{\,},6[\]) and a hydrogen bonded hydroxyl group at δ 13.06 (1H, br, s, 5-OH). From the aforementioned data, the flavone moiety was suggested as hispidulin. Moreover, glucose moiety signals were found at δ 5.30 (1H, d, J = 8.4, H-1^{\(\)}), 3.39 (1H, m, H-4^{\(\)}), 3.50 (2H, m, H-2¹, 3¹), 3.96 (1H, br, t, J = 8.4, H-5¹), 4.30 $(1H, dd, J = 11.2, 7.6, H-6^{\circ})$ a) and 4.57 (1H, br, d, J = 11.2, H-6["] b). The anomeric hydrogen signal at δ 5.30</sup> (1H, d, J = 8.4 Hz, H-1^{\left)}) supported the β -pyranoside configuration. Compound 4 (Table 2) revealed extra ¹HNMR signals at δ 7.33 (2H, d, J = 8.4, H-2¹¹, 6¹¹), 6.66 (2H, d, J = 8.4, H-3¹¹, 5¹¹), 6.36 (1H, d, J = 15.6, H-8¹¹), 7.54 (1H, d, J = 15.6, H-7¹¹) and extra ¹³CNMR signals at δ 125.0, 130.0, 116.2, 159.8, 113.7, 145.2 and 166.7. These extra NMR signals gave the evidences of the



Figure 2. Flavonoids (compounds 3 and 4).

existence of an E-P-coumaroyl group in compound 4. The downed field shifted H-6^{$\$} resonance suggested that the E-P-coumaroyl moiety was adjacent to C-6^{\\}. Finally, through HMBC results, the attachment positions were determined. The methoxyl signal at δ 3.66 (s, 6 - OMe) showed at ³J correlation to a carbon at δ 132.6 (C-6) suggesting the methoxl group was connected to C-6 position in hispidulin moiety. The anomeric proton of the glucose unit (δ 5.30, H-1^{\(\)}) showed a ³J correlation to a carbon of hispidulin (δ 157.6, C-7) indicating the attachment of the glucoside group at position C-7. Further, a ³J interaction between H-6^{$"} of the glucose (\delta$ </sup> 4.30, H-6^(h)) and the P-coumaroyl carbonyl carbon (δ 166.7, C-9^(N)) suggested the attachment of the Pcoumaroyl ester at C-6["] of the glucose moiety (Figure 2).</sup> From the aforementioned evidences, the structure of compound 4 was determined to be hispidulin 7- (6-E-Pcoumaroyl- β -D-glucopyranoside).

ACKNOWLEDGEMENT

This research was supported by a grant from the Research Center of the Center for Female Scientific and Medical Colleges, King Saud University.

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