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PHENOLIC CONSTITUENTS OF *CUCURBITA PEPO* L. CV `ESKANDRANI` (SUMMER SQUASH) FLOWERS

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في هذا البحث تم تحضير خلاصة الميثانول لأزهار نبات كيوكوربتا بيبو ل. (الكوسة الصيفى) وتم تجزئتها بالتتالى بالهكسان، خلات الإيثيل والبيوتانول. تم الفصل والتعرف على تسعة مركبات من مستخلص خلات الإيثيل منها مركب فلافونيدى جديد وهو ٥،٧ ثنائى هيدروكسى ٣،٦،٣` ثلاثى ميثوكسى فلافون (١)، بالإضافة الى ثمانية مركبات تم التعرف عليها كالآتى: ٣،٤ ثنائى هيدروكسى ميثيل بنزوات (٢) ، ٣،٤ ثنائى هيدروكسى حمض البنزويك (٣) ، أيزورامنتين (٤) ، كويرسيتين (٥) ، ميرستين (٦) ، أيزورامنتين ٤`-٠ بيتا جلوكوبيرانوزيد (٧) ، كويرسيتين ٤`-٠ بيتا جلوكوبيرانوزيد (٨)، كويرسيتين ٣،٤`-٠ بيتا ثنائى جلوكوبيرانوزيد (٩). والمركبات ٢،٤ ٩ فصل لأول مرة من النبات ماعدا مركب (٣) تم فصله من النبات سابقا". وقد تم التعرف على التركيب الدقيق لهذه المركبات بدراسة خواصها الطبيعية والكيميائية وباستخدام طرق التحليل الطيفى المختلفة بالإضافة إلى مقارنتها بالمراجع المختلفة أو بمثيلاتها من العينات القياسية. وقد أظهرت خلاصة الكحول الميثيلى وخلات الإيثيل تأثيرات كمضادات للميكروبات.

*One new flavonoid named 5,7-dihydroxy-3,6,3`-trimethoxyflavone (**1**), together with eight known phenolic compounds; 3,4-dihydroxy methyl benzoate (**2**), 3,4-dihydroxybenzoic acid (**3**), isorhamnetin (**4**), quercetin (**5**), myricetin (**6**), isorhamnetin-4`-O-β-D-glucopyranoside (**7**), quercetin-4`-O-β-D-glucopyranoside (**8**) and quercetin-3,4`-O-β-D-diglucopyranoside (**9**) were isolated from the flowers of Cucurbita pepo L. for the first time except **3** which was previously isolated from the plant. Their structures have been established on the basis of physical, chemical and spectroscopic methods in addition to comparison with literature data and/or authentic samples. The methanolic and ethyl acetate extracts were evaluated for their antimicrobial activity.*

INTRODUCTION

Summer Squash cv `Eskandrani` (الكوسة الصيفى) is the edible immature fruits of *Cucurbita pepo* L[1&2]. It is a herbaceous, monoecious, annual plant belongs to family Cucurbitaceae[3] and cultivated for human consumption (cooked flesh). Previous phytochemical studies on *Cucurbita* species led to the isolation of cucurbitacins[4-8], phenolic acids[9-11], phenolic glycosides[12&13], ascorbic acid conjugates[14], sterols[15-17], fatty acids[5&18] and flavonol (rhamnazin-3-rutinoside and isorhamentin-3-rutinoside-4`-rhamnoside)[19]. To the best of our knowledge, most of the reported chemical studies on *C. pepo* L. were carried out on lipids of squash seeds. Consulting the available literatures, few records could be traced dealing with *C. pepo* L. flowers concerning its phenolic constituents[19] so, it was of great interest to make comprehensive study on this plant. In this article we reported the isolation and characterization of one new flavonoid and other eight phenolic compounds from the soluble ethyl acetate fraction.

EXPERIMENTAL

General procedures

UV spectra were recorded in absolute MeOH on a Shimadzu 1601 UV/VIS spectrophotometer. Melting point was carried out using an Electrothermal 9100 Digital Melting Point apparatus (Electrothermal Engineering Ltd, Essex, England). The mass spectra (electro-spray ionization (ESI) and atmospheric pressure chemical ionization (APCI)) were performed on a Thermofinnigan

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LCQ DECA mass spectrometer coupled to an Agilent 1100 HPLC system equipped with a photodiode-array detector. 1D and 2D NMR spectra were measured on Bruker DRX 500 spectrometer (Bruker, Rheinstetten, Germany). Vacuum liquid chromatography (VLC) was carried out on silica gel 60 (0.04-0.063 mm, 500 g, Merck). Column chromatographic separations were performed over silica gel 60 (0.040-0.063 mm, Merck), Sephadex LH-20 (0.25-0.1 mm, Merck) and RP-18 columns (40-63 μm, Merck). TLC analyses were carried out on pre-coated silica gel F_{254} aluminium sheets and RP-18 F_{254S} plates (Merck). Compounds were detected by UV absorption at λ 254 and 366 nm followed by spraying with anisaldehyde/H_2SO_4 reagent and heating at 110°C for 1-2 min. The solvent systems used for TLC analyses were: chloromform-methanol (9.5:0.5, system I), chloromform-methanol (9:1, system II), chloromform-methanol (85:15, system III) and *n*-butanol-acetone-formic acid-water (60:17:8:15, system IV). Authentic flavonoids were obtained from Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt.

Plant material

The flowers of *Cucurbita pepo* L. cv `Eskandrani` (Summer Squash) were collected during April 2007 from plants cultivated in El-Galaa Village, Samalout, Minia, Egypt. The plant was kindely identified by Prof. Mohamed A. Farghali, Professor of Horticulture (Vegetable Crops), Faculty of Agriculture, Assiut University, Assiut, Egypt.

Extraction and isolation

The air-dried powdered flowers of *Cucurbita pepo* L. (0.65 kg) were extracted with methanol (3 L × 4) by percolation at room temperature. The combined extract was concentrated under reduced pressure to afford a dark yellowish brown residue (32.6 g). The latter was suspended in distilled water (100 ml) then partitioned with *n*-hexane (500 ml × 4), ethyl acetate (500 ml × 4) and *n*-butanol (500 ml × 3), successively. Each fraction was concentrated under reduced pressure to give *n*-hexane (12.5 g), ethyl acetate (8.1 g), and *n*-butanol (10.2 g) residues.

The ethyl acetate fraction was subjected to VLC using *n*-hexane-ethyl acetate and ethyl acetate-methanol gradients to obtain five group fractions: fraction CE-1 (1.3 g, eluted with *n*-hexane-ethyl acetate 50:50), fraction CE-2 (1.5 g, eluted with ethyl acetate), fraction CE-3 (1.6 g, eluted with ethyl acetate-methanol 75:25), fraction CE-4 (1.5 g, eluted with ethyl acetate-methanol 50:50) and fraction CE-5 (1.7 g, eluted with methanol). Fraction CE-1 was subjected to silica gel column chromatography (90.0 g, 30×1.5 cm) using chloroform-methanol 96:4 to afford compound **1** (5 mg). Fraction CE-2 was treated in the same manner as fraction CE-1 over silica gel column chromatography (90.0 g, 50×3 cm) using chloroform-methanol 92:8 to afford compounds **2** (11 mg) and **3** (7 mg). Fraction CE-3 was rechromatographed on Sephadex LH-20 column (100 g, 60×2 cm) using methanol-chloroform 90:10 as an eluent to obtain two main subfractions A (350 mg) and B (730 mg), each subfraction was rechromatographed over silica gel column chromatography (90.0 g, 50 × 3 cm) using chloroform-methanol 90:10 to afford compounds **4** (5 mg, subfraction A), **5** (16 mg, subfraction B) and **6** (14 mg, subfraction B). Fraction CE-4 was chromatographed on Sephadex LH-20 column (100 g, 60×2 cm) using methanol to give two main subfractions B-2-I (450 mg) and B-2-II (680 mg). Rechromatography of the subfraction B-2-I on RP-18 column (100 g, 30×1.5 cm) using methanol-water 60:40 afforded compound **7** (10 mg). Purification of the subfraction B-2-II on silica gel column (30 g, 30×1.5 cm) using chloroform-methanol (85:15) yielded compound **8** (19 mg). Fraction CE-5 was treated in the same manner as CE-4 to give two main subfractions CE-5-A (1.2 g) and CE-5-B (400 mg).

CE-5-B was chromatographed over RP-18 column (100 g, 30×1.5 cm) using methanol-water 50:50 to afford compound **9** (22 mg).

RESULTS

Compound 1: Yellow needles (MeOH); m.p. 162-164°C; R_f = 0.71 (system I); UV λ_{max} (MeOH) nm: 253, 271, 343; +NaOMe: 270, 326 sh, 358; +$AlCl_3$: 266, 280 sh, 375; +$AlCl_3$/HCl: 264, 284 sh, 372; +NaOAc: 263, 321, 371; +NaOAc/H_3BO_3: 257, 353. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.80 (1H, s, 5-O<u>H</u>), 9.90 (1H, s, 7-O<u>H</u>), 7.69 (1H, m, H-4′), 7.63 (1H, d, *J*= 2.2 Hz, H-2′), 7.56 (1H, dd, *J*= 2.2, 8.8 Hz, H-6′), 6.95 (1H, t, *J*= 8.2 Hz, H-5`),

6.58 (1H, s, H-8), 3.85 (3H, s, 3′-OC$\underline{H}_3$), 3.79 (3H, s, 3-OC$\underline{H}_3$) and 3.74 (3H, s, 6-OC$\underline{H}_3$). Negative-ion APCIMS *m/z* (rel. int.%): 343 $[M-H]^+$ $C_{18}H_{16}O_7$ (25), 301 $[M-3(CH_3-)-H]^+$, (53), 161 (20%), 687 $[2M-H]^+$ (17).

Compound 2: Brown residue; R_f = 0.65 (system I); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.41 (1H, d, *J*= 2.1 Hz, H-2), 7.39 (1H, dd, *J*= 2.1, 8.2 Hz, H-6), 6.78 (1H, d, *J*= 8.2 Hz, H-5) and 3.82 (3H, s, OC$\underline{H}_3$). Positive ESI-MS *m/z* (rel. int.%): 169 $[M+H]^+$ $C_8H_8O_4$ (85).

Compound 3: Brown residue; R_f = 0.63 (system II); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.32 (1H, s, COO$\underline{H}$), 9.66, 9.29 (each 1H, s, 3,4-O$\underline{H}$), 7.32 (1H, d, *J*= 2.2 Hz, H-2), 7.27 (1H, dd, *J*= 2.2, 8.2 Hz, H-6) and 6.77 (1H, d, *J*= 8.2 Hz, H-5). Positive ESI-MS *m/z* (rel. int.%): 155 $[M+H]^+$ $C_7H_6O_4$ (100).

Compound 4: Yellow powder; R_f = 0.60 (system II); UV λ_{max} (MeOH) nm: 254, 268 sh, 303 sh, 369; +NaOMe: 270, 327, 430; +$AlCl_3$: 262, 304 sh, 352, 427; +$AlCl_3$/HCl: 262, 303 sh, 356, 426; +NaOAc: 274, 325 sh, 391; +NaOAc/H_3BO_3: 257, 274 sh, 375. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.45 (1H, s, 5-O$\underline{H}$), 8.00 (1H, d, *J*= 2.2 Hz, H-2′), 7.67 (1H, dd, *J*= 2.2, 8.5 Hz, H-6′), 6.91 (1H, d, *J*= 8.5 Hz, H-5′), 6.45 (1H, d, *J*= 2.2 Hz, H-8), 6.17 (1H, d, *J*= 2.2 Hz, H-6) and 3.83 (3H, s, 3`-OCH_3). Positive-ion ESI-MS *m/z* (rel. int.%): 317 $[M+H]^+$ $C_{16}H_{12}O_7$ (100).

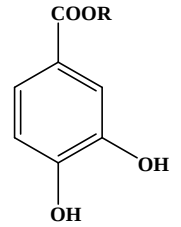
Compound 5: Yellow powder; R_f = 0.52 (system II); UV λ_{max} (MeOH) nm: 256, 268 sh, 299 sh, 369; +NaOMe: 274, 327, 400; +$AlCl_3$: 275, 304 sh, 332, 453; +$AlCl_3$/HCl: 265, 303 sh, 356, 426; +NaOAc: 274, 325 sh, 386; +NaOAc/H_3BO_3: 261, 298 sh, 385. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.47 (1H, s, 5-O$\underline{H}$), 7.66 (1H, d, *J*= 2.2 Hz, H-2′), 7.52 (1H, dd, *J*= 2.2, 8.5 Hz, H-2′), 6.87 (1H, d, *J*= 8.5 Hz, H-5′), 6.38 (1H, d, *J*= 1.9 Hz, H-8) and 6.16 (1H, d, *J*= 1.9 Hz, H-6). Positive-ion ESI-MS *m/z* (rel. int.%): 303 $[M+H]^+$ $C_{15}H_{10}O_7$ (100).

Compound 6: Yellow powder; R_f = 0.46 (system II); UV λ_{max} (MeOH) nm: 255, 273 sh, 304 sh, 375; +NaOMe: 262, 322, 426; +$AlCl_3$: 272, 314 sh, 452; +$AlCl_3$/HCl: 266, 309 sh, 365 sh, 428; +NaOAc: 271, 381; +NaOAc/H_3BO_3: 260, 306 sh, 389. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.48 (1H, s, 5-O$\underline{H}$), 10.76, 9.32, 9.20, 8.79 (each 1H, s, O$\underline{H}$ groups), 7.22 (2H, s, H-2′, 6`), 6.35 (1H, brs, H-8) and 6.16 (1H, brs, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1, Fig. 1. Positive-ion ESI-MS *m/z* (rel. int.%): 319 $[M+H]^+$ $C_{15}H_{10}O_8$ (100).

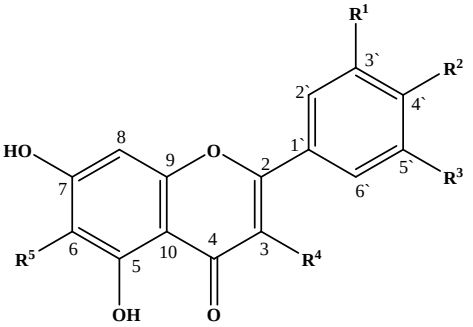
Table 1: ^{13}C-NMR data for compounds **6**, **8** and **9** (125 MHz in DMSO-d_6).

No.	**6**	**8**	**9**
2	146.8	146.8	155.4
3	135.8	136.5	133.8
4	175.7	176.1	177.5
5	160.7	160.8	161.7
6	98.1	98.3	98.8
7	163.8	164.1	164.3
8	93.2	93.6	93.7
9	156.0	156.3	156.4
10	102.9	103.2	104.1
1′	120.7	125.2	124.5
2`	107.1	115.2	115.5
3`	145.7	146.4	147.5
4`	134.8	146.0	146.1
5′	145.7	115.9	116.6
6′	107.1	119.6	121.1
		4`-Glc	**3-Glc**
1′′		101.4	100.7
2′′		73.3	74.1
3′′		76.0	76.5
4′′		69.8	70.0
5′′		77.3	77.6
6′′		60.8	61.0
			4`-Glc
1′′′			101.5
2′′′			73.8
3′′′			75.8
4′′′			69.7
5′′′			77.7
6′′′			60.7

Compound 7: Yellow powder; R_f = 0.60 (system II); UV λ_{max} (MeOH) nm: 254, 270 sh, 304 sh, 363; +NaOMe: 263, 400; +$AlCl_3$: 262, 304 sh, 342 sh, 425; +$AlCl_3$/HCl: 261, 303 sh, 346 sh, 420; +NaOAc: 274, 319 sh, 387; +NaOAc/H_3BO_3: 257, 274 sh, 366. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.40 (1H, s, 5-O$\underline{H}$), 7.78 (1H, brs, H-2′), 7.75 (1H, d, *J*= 8.5 Hz, H-6′), 7.25 (1H, d, *J*= 8.5 Hz, H-5′), 6.48 (1H,



Compound **2** R = CH_3
Compound **3** R = H



Compound **1** $R^1 = R^4 = R^5 = OCH_3$, $R^2 = R^3 = H$
Compound **4** $R^1 = OCH_3$, $R^2 = R^4 = OH$, $R^3 = R^5 = H$
Compound **5** $R^1 = R^2 = R^4 = OH$, $R^3 = R^5 = H$
Compound **6** $R^1 = R^2 = R^3 = R^4 = OH$, $R^5 = H$
Compound **7** $R^1 = OCH_3$, $R^4 = OH$, $R^3 = R^5 = H$, $R^2 = O$-Glc
Compound **8** $R^1 = R^4 = OH$, $R^3 = R^5 = H$, $R^2 = O$-Glc
Compound **9** $R^1 = OH$, $R^3 = R^5 = H$, $R^2 = R^4 = O$-Glc

Fig. 1: Structures of the isolated compounds.

brs, H-8), 6.18 (1H, brs, H-6), 3.82 (3H, s, 3`-OCH_3), 5.03 (1H, d, *J*= 7.00 Hz, H-1``) and 5.38-3.45 (m, sugar protons). Positive-ion ESI-MS *m/z* (rel. int.%): 479 $[M+H]^+$ $C_{22}H_{22}O_{12}$ (100), 317 [M-162 (hexose unit)+H]$^+$ (37).

Compound 8: Yellow powder; R_f = 0.68 (system III); UV λ_{max} (MeOH) nm: 255, 270 sh, 290 sh, 367; +NaOMe: 268, 412; +$AlCl_3$: 266, 304 sh, 424; +$AlCl_3$/HCl: 266, 303 sh, 356, 419; +NaOAc: 258, 325 sh, 384; +NaOAc/H_3BO_3: 257, 293 sh, 371. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.40 (1H, s, 5-OH), 7.69 (1H, dd, *J*= 2.2 Hz, H-2′), 7.60 (1H, dd, *J*= 2.2, 8.8 Hz, H-6′), 7.23 (1H, d, *J*= 8.8 Hz, H-5′), 6.43 (1H, d, *J*= 1.9 Hz, H-8), 6.18 (1H, d, *J*= 1.9 Hz, H-6), 4.84 (1H, d, *J*= 7.3 Hz, H-1″), 5.43-3.51 (m, sugar protons). ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1. Positive-ion ESI-MS *m/z* (rel. int.%): 465 $[M+H]^+$ $C_{21}H_{20}O_{12}$ (100), 303 [M-Glc+H]$^+$ (42).

Compound 9: Yellow powder; R_f = 0.43 (system III); UV λ_{max} (MeOH) nm: 254, 269 sh, 294 sh, 346; +NaOMe: 268, 391; +$AlCl_3$: 275, 300 sh, 403; +$AlCl_3$/HCl: 269, 303 sh, 346, 396; +NaOAc: 268, 323 sh, 370; +NaOAc/H_3BO_3: 255, 268 sh, 347. ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.51 (1H, s, 5-OH), 7.65 (1H, d, *J*= 2.2 Hz, H-2′), 7.62 (1H, dd, *J*= 2.2, 8.5 Hz, H-6′), 7.22 (1H, d, *J*= 8.5 Hz, H-5′), 6.44 (1H, d, *J*= 2.2 Hz, H-8), 6.21 (1H, d, *J*= 2.2 Hz, H-6), 5.51 (1H, d, *J*= 7.3 Hz, H-1``), 4.87 (1H, d, *J*= 7.3 Hz, H-1```), 5.51-3.25 (m, sugar protons). ^{13}C-NMR (125 MHz, DMSO-d_6) see Table 1. Positive-ion ESI-MS *m/z* (rel. int.%): 627 $[M+H]^+$ $C_{27}H_{30}O_{17}$ (100), 465 [M-162 (hexose unit)+H]$^+$ (35), 303 [M-162-162 (2 hexoses)+H]$^+$ (10).

Acid hydrolysis of compounds (7-9)[20]

A solution of the isolated glycoside (**7**-**9**) (each, 3 mg in 10 ml methanol) was treated with 3% H_2SO_4 (1.5 ml) and heated in water bath at 100°C for 1 hr. The aglycone was extracted with EtOAc, concentrated under reduced pressure, purified on Sephadex LH-20 column using methanol and identified by co-TLC with an authentic sample using solvent system II. The sugars in the aqueous layer were identified by co-TLC with authentic materials using solvent system IV.

Antimicrobial assay[21]

The procedure was described previously by Bonev *et al.*[21]. The antibacterial and antifungal activities were evaluated using the agar plate diffusion assay. Susceptibility discs (5.5 mm) were impregnated with solution at concentrations 25 and 50 mg/mL. The discs were dried and placed on agar plates inoculated with the test bacterial strains: *Bacillus cereus* (AUMC No. B-52), *Staphylococcus aureus* (AUMC No. B-54), *Escherichia coli* (AUMC No. B-53), *Pseudomonas aeruginosa* (AUMC No. B-73), and *Serratia marcescens* (AUMC No. B-55), and the fungal strains: *Candida albicans* (AUMC No. 418), *Geotrichum candidum* (AUMC No. 226), *Trichophyton rubrum* (AUMC No. 1804), *Fusarium oxysporum* (AUMC No. 5119), *Scopulariopsis brevicaulis* (AUMC No. 729), and *Aspergillus flavus* (AUMC No. 1276), all bacterial and fungal strains were obtained from Assiut University Mycology center. Each plate was inoculated with a single organism and the test was run in duplicates. The plates were incubated at 37°C and checked for inhibition zones after 24 hrs for bacteria and after 48 hrs for fungi. Chloroamphenicol and clotrimazole (25 mg/mL) were used as positive reference standards for antibacterial and antifungal activities, respectively.

DISCUSSION

The methanolic extract of *Cucurbita pepo* L. flowers was successively partitioned between *n*-hexane, ethyl acetate and *n*-butanol. The ethyl acetate soluble fraction through a series of different chromatographic fractionation techniques, afforded nine phenolic compounds **1-9** (Fig. 1).

Compound **1** was isolated as yellow needles. The negative APCIMS showed a pseudomolecular ion peak at *m/z* 343 (25%) $[M-H]^+$, which was compatable with the molecular formula $C_{18}H_{16}O_7$. The fragment ion peak at *m/z* 161 (20%) indicated typical fragmentation pattern of a 3-methoxyflavone[22] which was further confirmed by UV absorption bands at 253 and 343 nm[20&23]. ^{1}H-NMR spectum (Fig. 2) revealed the presence of six singlet signals at δ 3.74 (3H), 3.79 (3H), 3.85 (3H), 6.58 (1H), 9.90 (1H, s) and 12.80 (1H) were assigned to three methoxy groups, H-8 and 5,7-OH groups, respectively. Also ^{1}H-NMR showed four coupled protons in the aromatic region at δ 7.69 (1H, m, H-4`), 7.63 (1H, d, *J*= 2.2 Hz, H-2`), 7.56 (1H, d, *J*= 8.2, 2.2 Hz, H-6`) and 6.95 (1H, t, *J*= 8.2 Hz, H-5`) suggested the presence of unusual B-ring with 3`-methoxy group[22]. This pattern of B-ring was confirmed from the counter plot of COSY spectrum through cross-peaks ($^2J_{CH}$) from signals at δ 7.56 (H-6`) with the signals resonating at δ 6.95 (H-5`) and 7.69 (H-4`) confirming the assignment of these signals. The placement of the methoxy groups at C-3, C-6 and C-3` were established by the correlations observed in the HMBC spectrum (Figs. 2, 3). The protons of methoxy groups at δ 3.74, 3.79 and 3.85 showed correlations with C-3 (137.3), C-6 (δ 131.6) and C-3` (147.8), respectively. By comparison of the obtained spectral data, mass, ^{1}H-NMR, COSY and HMBC with literature data[20,24&25], compound **1** was identified as 5,7-dihydroxy-3,6,3`-trimethoxy flavone and its considered as a new compound.

ESIMS of compounds **2** and **3** showed $[M+H]^+$ at *m/z* 169 and 155, respectively. The ^{1}H-NMR spectral data exhibited typical pattern of 3,4-dihydroxybenzoic acid **2** and its methyl ester **3**[26&27].

The ESIMS spectra of compounds **4**, **5**, and **6** showed $[M+H]^+$ at *m/z* 317, 303 and 319 corresponding to the molecular formulae $C_{16}H_{12}O_7$, $C_{15}H_{10}O_7$ and $C_{15}H_{10}O_8$, respectively. By comparison of their UV and NMR spectral data with the published data[20&24], they were characterized as isorhamentin, quercetin and myricetin, respectively.

Compounds **7**, **8** and **9** displayed characteristic UV absorption maxima for flavonol skeleton[20&24]. The ^{1}H-NMR spectral

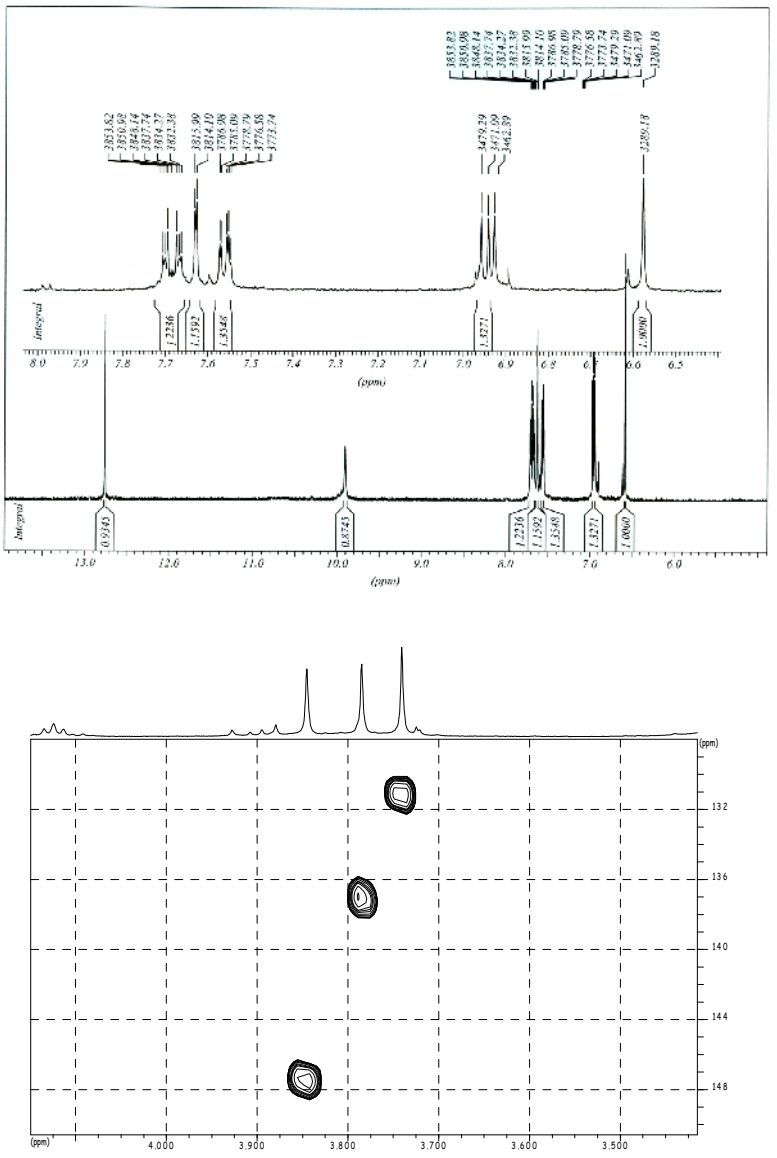


Fig. 2: [1]H-NMR and expanded HMBC spectra of compound **1**.

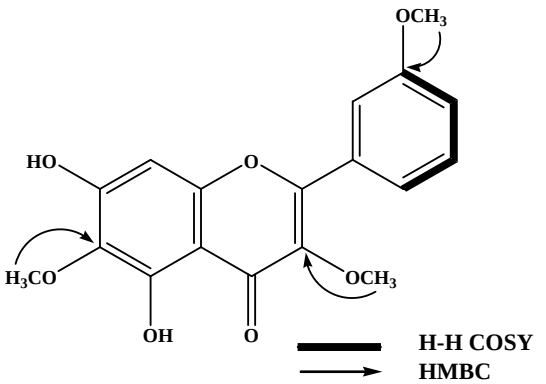


Fig. 3: Important 2D correlations of compound **1**.

data for these compounds also confirmed the presence of flavonol nucleus in these molecules[24].

ESIMS of compound **7** showed $[M+H]^+$ at *m/z* 479 corresponding to the molecular formula $C_{22}H_{22}O_{12}$ in addition to a prominent fragment peak at *m/z* 317 [M-162 (hexose unit)+H]$^+$ indicating its monoside nature. The UV spectral data suggested its structure to be 3,5,7-trihydroxyflavone derivative[24]. In the ^{1}H-NMR spectrum, signals for isorhamentin nucleus[24&28] and a β-glucose moiety were observed[29]. Considering the UV results the sugar moiety could be placed at C-4`. Acid hydrolysis[20] of **7** yielded isorhamentin and glucose (co-TLC with authentic samples). On the basis of these results and by comparing the spectral data with literature[20&24], compound **7** could be identified as isorhamentin 4′-*O*-β-D-glucopyranoside.

Compound **8** was isolated as yellow amorphous powder. It showed molecular weight at *m/z* 464 determined by the ESIMS which showed molecular ion peak at *m/z* 465 $[M+H]^+$, in addition to a prominent fragment peak at *m/z* 303 [M-162 (hexose unit)+H]$^+$ indicating its monoside nature. The UV spectral data of **8** suggested its structure to be flavonol with free hydroxyl groups at positions 3, 5, 7 and 3′[20]. ^{1}H- and ^{13}C-NMR spectral data exhibited a typical pattern for quercetin and a β-glucose moiety[29]. Depending on the UV and ^{13}C-NMR spectral data (Table 1) the glucose unit could be placed at C-4`. Acid hydrolysis[20] of **8** yielded quercetin and glucose (co-TLC with authentic samples). On the basis of these results and by comparison with literature[20,24&25], compound **8** could be identified as quercetin 4′-*O*-β-D-glucopyranoside.

The molecular formula of compound **9** was established as $C_{27}H_{30}O_{17}$ by ESI mass analysis that showed $[M+H]^+$ at *m/z* 627, it was 162 mass units more than **8**. The two significant fragment peaks at *m/z* 465 [M-162 (hexose unit)+H]$^+$, 303 [M-162-162 (2 hexoses)+H]$^+$ indicated **9** as quercetin bioside. The NMR spectral data were similar to those of **8**, except the presence of a further signal for anomeric proton at δ 5.51 (1H, d, *J*= 7.3 Hz) indicated the presence of additional glucose moiety. The ^{13}C-NMR spectrum displayed 15 carbon signals were attributed to quercetin skeleton[24&25] and 12 carbon signals in the region of sugars (see Table 1) their multiplicities were determined by DEPT experiment. The attachments of the sugar moieties to C-3 and C-4` were confirmed by the observed HMBC correlations; the anomeric proton signal at δ 5.51 (Glc H-1``) showed correlation with the carbon signal at δ 133.8 (C-3) indicating the *O*-glycosidic linkage at the 3-position of querectin. Also, the anomeric proton signal at δ 4.87 (Glc H-1```) showed correlation with the carbon signal at δ 146.1 (C-4`) confirming the attachment of the other glucose moiety at C-4`. Acid hydrolysis[20] of **9** gave querectin and glucose (co-TLC with authentic samples). Thus, **9** was identified as quercetin 3,4`-*O*-β-D-diglucopyranoside. Chemical and biological investigations of *n*-hexane and *n*-butanol fractions are in progress.

The antimicrobial evaluation (Table 2) of the methanolic and ethyl acetate extracts revealed that the methanolic extract showed mild antibacterial activity against *Serratia marcescens*, *Escherichia coli* and *Staphylococcus aureus*, while the ethyl acetate extract displayed moderate activity against *Serratia marcescens*, *Escherichia coli* and *Bacillus cereus* and potent activity against *Staphylococcus aureus*. None of the tested extracts showed antifungal activity except that the methanolic extract showed weak activity against *Aspergillus flavus*.

Table 2: Antimicrobial activity of different extracts of *Cucurbita pepo* L. cv `Eskandrani`

Extracts	Conc. mg/mL	Inhibition zone in mm					
		Bacterial strains					Fungal strain
		B. cereus (AUMC No. B-52)	*S. aureus* (AUMC No. B-54)	*E. coli* (AUMC No. B-53)	*P. aeruginosa* (AUMC No. B-73)	*S. marcescens* (AUMC No. B-55)	*A. flavus* (AUMC No. 1276)
Methanolic	25	0	0	11	0	11	0
	50	0	11	12	0	12	10 p.i.
Ethyl acetate	25	8	12	12	0	10	0
	50	12	14	14	0	12	0
Chloroamphenicol[a]	25	36	17	28	17	35	-
Clotriamazole[b]	25	-	-	-	-	-	26

AUMC: Assiut University Mycology Center
p.i.: partial inhibition.
[a]Positive control for antibacterial activity
[b]Positive control for antifungal activity.

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