

## FLAVONOIDAL CONTENT OF *SOLANUM UNGUICULATUM* (A.) RICH.

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### ABSTRACT

Phytochemical investigation of chloroformic and ethyl acetate extracts of *Solanum unguiculatum* (A.) Rich. (Family Solanaceae) afforded chrysoeriol 7-methyl ether, quercetin 3-methyl ether, myricetin 3,4'-dimethyl ether, quercetin 3-O- $\beta$ -galactoside, kaempferol 3-O-rutinoside and quercetin 3-O- $\alpha$ -rhamnosyl (1  $\rightarrow$  2)  $\beta$ -galactoside. Structure of these compounds have been determined on bases of spectral methods; UV, MS and NMR as well as comparison with literature values.

### INTRODUCTION

Methylated flavonoids, free or as glycosides were found to have cytotoxic properties<sup>(1)</sup>. Some *Solanum* species have been reported to contain methylated flavonoids<sup>(2,3)</sup>. Thus, the present investigation was under taken to disclose the flavonoid compounds in *Solanum unguiculatum* (A.) Rich. Although many other *Solanum* species have been studied and shown to contain different types of flavonoids<sup>(2,4)</sup> Yet, nothing has been reported in the literature about flavonoidal content of *Solanum unguiculatum*.

*Solanum unguiculatum* (A.) Rich. is a small, wild, strongly branching perennial shrub that grow in several places including Yemen<sup>(5)</sup> and Egypt<sup>(6)</sup>. An infusion prepared from its fruiting tops is used in folk medicine as a contraceptive.

Surveys of *Solanum* species have shown that 3 and 3,7-glycosides of the flavonols quercetin and kaempferol are predominant, while quercetin and kaempferol 3-methyl ethers as well as 8-hydroxy - myricetin 3,7,4'-trimethyl ether, luteolin and 8-hydroxy - chrysoeriol 7-dimethyl ether are of restricted occurrence<sup>(3,7,8)</sup>.

Also, quercetin 3-glycosides and an unspecified quercetin 3-diglucoside have been reported from *Solanum nigrum*<sup>(9)</sup>.

In the present communication, isolation and structure elucidation of chrysoeriol 7-methyl ether, quercetin 3-methyl ether, myricetin 3,4'-dimethyl ether, quercetin 3-O- $\beta$ -galactoside as well as kaempferol 3-O-rutinoside and quercetin 3-O- $\alpha$ -rhamnosyl (1 $\rightarrow$ 2)- $\beta$ -galactoside have been described.

### EXPERIMENTAL

#### General Experimental procedures :

UV spectra were obtained on a Varian CARY 2290 spectrophotometer in MeOH. <sup>1</sup>HNMR chemical shifts were measured in CDCl<sub>3</sub> (for aglycones) and in DMSO-d<sub>6</sub> (for the glycosides) using TMS as an internal

standard at 90 MHz with a Varian XL300 (Darmstadt, Germany). FabMS spectra were recorded with an MS 2500 high-resolution spectrometer (Kratos, Manchester, UK) with 70 eV, an ion source temperature of 180°C and a direct inlet. Precoated thin - layer chromatographic (TLC) plates (coated with silica gel 60 F254; the thickness of the layer was 0.25 mm) were obtained from Merck (Darmstadt, Germany).

Solvent systems for PC (Whatman No. 1) : 1. H<sub>2</sub>O; 2. HOAc (HOAc-H<sub>2</sub>O, 3:17); 3. BAW (n. BuOH - HOAc-H<sub>2</sub>O, 4:1:5, top layer); 4. C<sub>6</sub>H<sub>6</sub>-n-BuOH-pyridine-H<sub>2</sub>O (1:5:3:3, top layer). Solvent systems 2 and 3 were used for PPC on Whatman paper 3 mm while solvent systems 3 and 4 were used in sugar analysis.

#### Plant Material.

*Solanum unguiculatum* was collected in the fruiting stage from road sides and from hill sides in the Sana'a region (Wadi Zahr) in Yemen in May 1991. Identity of the plant was confirmed by Professor M. El-Monastery, Faculty of Science, El-Azhar University, Egypt. A certified specimen has been deposited at the Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Egypt. The herb was air - dried and powdered before extraction.

#### Extraction and Purification.

Air-dried and ground plant material (250 g) were extracted with 3000 ml of 90% aqu. EtOH at room temperature. The filtered extract was concentrated under reduced pressure and the residue was freeze-dried to give dark brown residue (40g). Following suspension of the residue in water (500 ml) containing 1% methanol and the suspension was partitioned into n-hexane, chloroform, ethyl acetate and water soluble fractions to yield 7, 4, 5 and 19.6 g respectively. The fractions were examined by chromatography using TLC and PC to determine the presence or absence of flavonoids. Both CHCl<sub>3</sub> and EtOAc fractions showed the presence of yellow pigments. The CHCl<sub>3</sub> fraction (4 g) was subjected to CC (100g, silica gel, 1.5 x 50 cm)



using  $\text{CHCl}_3$  as eluent and the polarity was increased gradually with MeOH. Fractions eluted with 1% MeOH contained one major compound. On evaporation and crystallization from MeOH, compound 1 (20 mg) was obtained. Fractions eluted with 8% MeOH afforded a mixture of two flavonoidal substances (2 and 3). These two substances were separated by repeated column and preparative TLC with  $\text{CHCl}_3$  - MeOH (95 : 5) to afford compound 2 (36 mg) and compound 3 (23 mg).

The dried EtOAc fraction (5 g) was chromatographed on silica gel column (200 g, 2.5 x 50 cm) using  $\text{CHCl}_3$  and increasing proportions of MeOH. Fractions eluted with 10% MeOH contained one compound, crystallized from MeOH as brown amorphous powder (100 mg, compound 4). Fractions eluted with 15% MeOH contained one major compound and two minor spots, on crystallization from MeOH, pale yellow granules (60 mg, compound 5) was obtained. Fractions eluted with 25% MeOH afforded mixture of substances 5 and 6). These substances were separated by PPC (Whatman 3 mm) paper developed by ascending mode in solvent system 3 and the resulting bands were cut out and eluted with MeOH. Flavonoid solutions thus obtained were filtered, concentrated and passed through small silica gel column using MeOH as solvent. The individual eluates were dried *in vacuo* and repeatedly crystallized from MeOH to afford compound 5 (30 mg) and compound 6 (70 mg).

#### General procedure for the hydrolysis of O-glycosides

Normal acid hydrolyses were carried out in 2M aqu. HCl at 100°C for 2hr. Controlled acid hydrolyses were carried out in 0.1 M aqu. HCl at 100°C for 30 min. Flavonoidal aglycones were extracted from the resulting sugar-flavonoid mixtures with EtOAc. The sugar-containing aqu. fractions were cochromatographed with standard sugars on (Whatman 1mm) paper using solvent system 4. Sugar spots were detected with alkaline  $\text{AgNO}_3$  (10).

Chrysoeriol 7-methyl ether (1): MW 314, negative FABMS  $[\text{M}-\text{H}]^-$  313 calculated for  $\text{C}_{17}\text{H}_{14}\text{O}_6$ ; colors on paper, purple-green (with UV), yellow-green (with UV/ $\text{NH}_3$ ); Rfs 0.88 (BAW), 0.79 (HOAc). UV data  $\lambda_{\text{max}}$  (nm) : MeOH : 255, 273, 295 sh, 347; NaOMe: 246 sh, 264, 303 sh 390;  $\text{AlCl}_3$  : 266 sh, 279, 304, 358, 390;  $\text{AlCl}_3$ -HCl : 266 sh, 279, 304, 357, 390; NaOAc : 260, 266 sh, 297 sh, 395; NaOAc- $\text{H}_3\text{BO}_3$  : 253 sh, 271, 292 sh, 350.  $^1\text{H NMR}$  :  $\delta$  6.18 (d, J = 2.5 Hz, 6-H); 6.47 (d, J = 2.5 Hz, 8-H); 6.72 (s, 3-H); 6.88 (d, J = 8 Hz, 5'-H); 7.49 (m, 2'-H, 6'-H); 3.92 (6H, s, two methoxyls).

Quercetin 3-methyl ether (2): MW 316, -ve FABMS  $(\text{M}-1)^-$  315 calculated for  $\text{C}_{16}\text{H}_{12}\text{O}_7$ ; Rfs 0.84

(BAW), 0.75 (HOAc). UV  $\lambda_{\text{max}}$  (nm) : MeOH : 255 sh, 292 sh, 354; NaOMe : 265, 325, 396;  $\text{AlCl}_3$  : 270, 418;  $\text{AlCl}_3$ -HCl : 264, 296 sh, 364 sh, 396; NaOAc 266, 275 sh, 319, 378; NaOAc- $\text{H}_3\text{BO}_3$  : 260, 380.  $^1\text{H NMR}$  :  $\delta$  6.24 (d, J = 2.5 Hz, 6-H); 6.3 (d, J = 2.5 Hz, 8-H), 7.52 (d, J = 2Hz, 2'-H); 6.72 (d, J = 8.5 Hz, 5'-H); 7.44 (dd, J = 2, 8.5 Hz, 6'-H); 3.7 (3H, s, one methoxyl).

Myricetin 3,4'-dimethyl ether (3) : MW 356, -ve FABMS  $(\text{M}-1)^-$  355 calc. for  $\text{C}_{17}\text{H}_{14}\text{O}_8$ ; colors on paper purple-green with UV, unchanged with UV/ $\text{NH}_3$ ; Rfs 0.80 (BAW), 0.69 (HOAc). UV data  $\lambda_{\text{max}}$  (nm): MeOH : 274, 308 sh, 323, 359; NaOMe : 266, 305, 336 sh, 374;  $\text{AlCl}_3$  : 283, 312, 351, 421;  $\text{AlCl}_3$ -HCl : 283, 312, 420; NaOAc : 286, 308 sh, 326, 360; NaOAc- $\text{H}_3\text{BO}_3$  : 273, 308 sh, 324, 356.  $^1\text{H NMR}$ :  $\delta$  6.18 (2H each as d, J = 2.5 Hz, 6-H and 8-H); 7.3 (2H each as s, 2'-H and 6'H); 3.81 (3H, s, methoxyl) and 3.38 (3H, s, methoxyl).

Quercetin 3-O- $\beta$ -galactoside (4): MW 464, -ve FABMS  $(\text{M}-1)^-$  463 calc. for  $\text{C}_{21}\text{H}_{20}\text{O}_{12}$ ; colors on paper purple-green with UV, unchanged with UV/ $\text{NH}_3$ ; Rfs 0.78 (BAW), 0.64 (HOAc). UV data  $\lambda_{\text{max}}$  (nm): MeOH : 257, 265 sh, 292 sh, 359; NaOMe : 265, 325, 400;  $\text{AlCl}_3$  : 266, 300, 364, 420;  $\text{AlCl}_3$ -HCl : 264, 296 sh, 364 sh, 396; NaOAc : 266, 270 sh, 319 sh, 378; NaOAc- $\text{H}_3\text{BO}_3$  : 260, 380. Acid hydrolysis gave galactose and quercetin,  $^1\text{H NMR}$  of the aglycone:  $\delta$  6.24 (d, J = 2.5 Hz, 6-H); 6.44 (d, J = 2.5 Hz, 8-H); 6.84 (d, J = 8 Hz, 5'-H); 7.56 (d, J = 2.5 Hz, 2'-H), 7.64 (dd, J = 2.5 and 8 Hz, 6'-H); galactose moiety :  $\delta$  5.32 (d, J = 8 Hz, 1-H); 3.2 - 3.8 (m, galactose protons overlapped with hydroxyl protons).

Kaempferol 3-O-rutinoside (5): MW 594, -ve FABMS  $(\text{M}-1)^-$  593 calc. for  $\text{C}_{27}\text{H}_{30}\text{O}_{15}$ ; Rfs 0.83 (HOAc), 0.53 (BAW). UV data  $\lambda_{\text{max}}$  (nm) : MeOH: 265, 350; NaOMe : 245 sh, 269, 325 sh, 400;  $\text{AlCl}_3$ : 273, 302, 350, 397;  $\text{AlCl}_3$  - HCl : 273, 303, 345, 395; NaOAc: 270, 305, 355, 387; NaOAc -  $\text{H}_3\text{BO}_3$  : 265, 282 sh, 342. Normal acid hydrolysis gave rhamnose, glucose (co-PC) and kaempferol (co-PC, UV absorption and  $^1\text{H NMR}$ ).  $^1\text{H NMR}$  of the aglycone :  $\delta$  8 (2H, d, J = 7 Hz, 2'-H and 6'-H); 6.9 (2H, d, J = 7 Hz, 3'-H and 5'-H), 6 (d, J = 2.5 Hz, 6-H), 6.2 (d, J = 2.5 Hz, 8-H); sugar moieties :  $\delta$  4 (d, J = 8 Hz, 1-H glucosyl); 3.2 - 3.6 (m, sugar protons overlapped by hydroxyl protons); 5 (d, J = 2 Hz, 1-H rhamnosyl); 1.12 (d, J=6 Hz, Me-rhamnosyl).

Quercetin 3-O- $\alpha$ -rhamnosyl (1  $\rightarrow$  2)- $\beta$ -galactoside (6) : MW 610, -ve FABMS  $(\text{M}-1)^-$  609; Rfs 0.53 (HOAc), 0.55 (BAW). UV  $\lambda_{\text{max}}$  (nm) MeOH : 255, 267, 360; NaOMe : 270, 330, 403;  $\text{AlCl}_3$  : 266,



300, 364, 420;  $\text{AlCl}_3\text{-HCl}$ : 266, 300, 365, 419;  $\text{NaOAc}$ : 255, 270, 378;  $\text{NaOAc-H}_3\text{BO}_3$ : 261, 280. Normal acid hydrolysis gave rhamnose, galactose and quercetin (co-PC).  $^1\text{HNMR}$  of the aglycone:  $\delta$  6.24 (d,  $J = 2.5$  Hz, 6-H); 6.44 (d,  $J = 2.5$  Hz, 8-H); 6.84 (d,  $J = 8$  Hz, 5'-H); 7.56 (d,  $J = 2.5$  Hz, 2'-H), 7.64 (dd,  $J = 2.5$  and 8 Hz, 6'-H); galactose moiety:  $\delta$  5.32 (d,  $J = 8$  Hz, 1-H); 3.2 - 3.8 (m, galactose protons overlapped with hydroxyl protons); 5.03 (d,  $J = 2$  Hz, 1-H rhamnosyl); 1.02 (d,  $J = 6$  Hz, Me -rhamnosyl).

## RESULTS AND DISCUSSION

Column chromatography of the chloroform extract of *S. unguiculatum* afforded compounds 1-3, while compounds 4-6 were isolated from the ethyl acetate fraction by applying a combination of CC on silica gel followed by preparative TLC and Paper Chromatography.

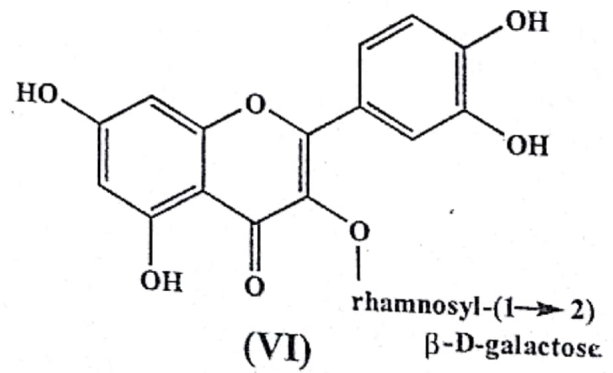
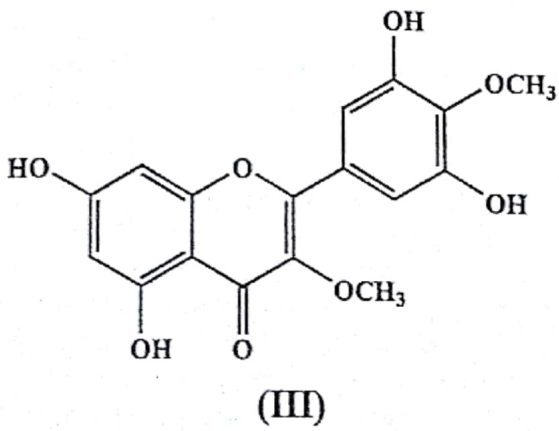
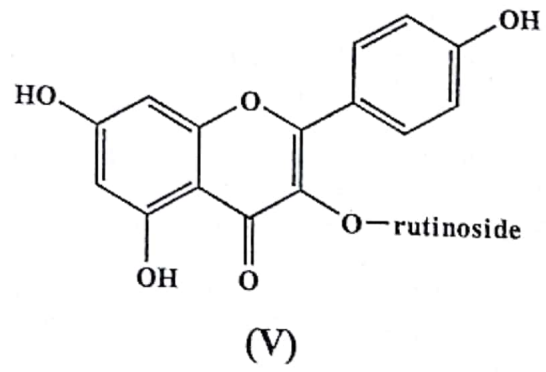
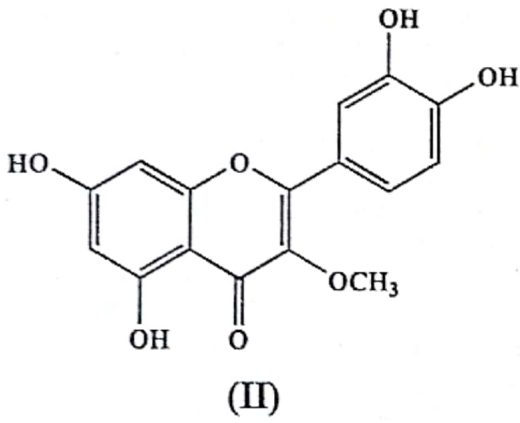
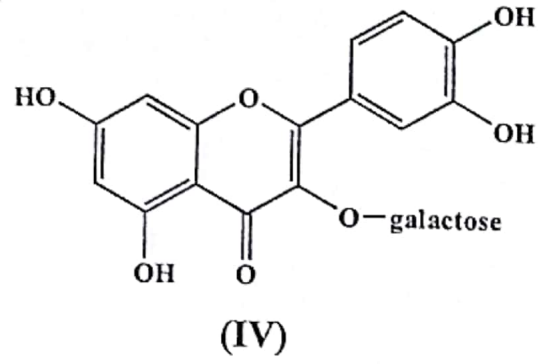
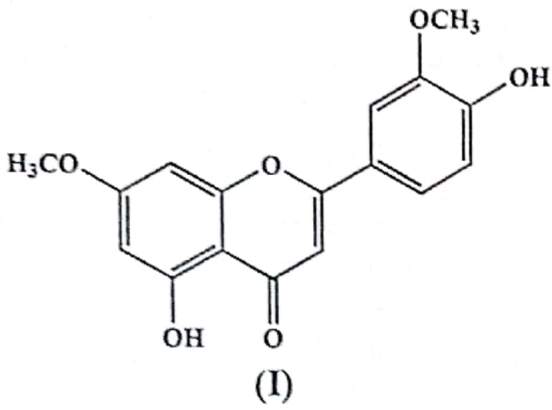
**Compound 1**, the MS of compound 1 exhibited a molecular ion at  $m/e$  314 indicating a dimethoxy dihydroxy flavone  $\text{C}_{17}\text{H}_{14}\text{O}_6$ . Band I of the UV spectrum of 1 in MeOH appeared at 347 nm in the range of flavones. A 3',4'-dioxxygenation pattern in the B-ring was indicated by the two UV peaks at 255 and 273 nm (11). The large bathochromic shift of band I (43 nm) with an increase in the intensity in NaOMe relative to MeOH signified the presence of unsubstituted hydroxyl group at C-4', as did the color change from purple to yellow observed when the compound on paper, was fumed with ammonia in UV light. The complete acid stability of the  $\text{AlCl}_3$  complex showed that the B-ring lacked an O-dihydroxyl group (11 - 13) and thus a substituted hydroxy function must be located at C-3'. The  $\text{AlCl}_3/\text{HCl}$  spectrum of compound 1 typically consists of four major absorption peaks indicated the presence of a free hydroxyl group at C-5(11). Failure of Band II to show a bathochromic shift in NaOAc relative to MeOH indicated the substitution of the C-7 hydroxyl group. The  $^1\text{HNMR}$  spectrum of compound 1 was in accordance with the proposed structure and revealed a pair of meta coupled aromatic protons doublets ( $J = 2.5$  Hz) localized at  $\delta$  6.18 and 6.47 were assigned to 6-H and 8-H, olefinic proton singlet(s) localized at  $\delta$  6.72 was assigned to 3-H, one ortho coupled aromatic proton doublet ( $J = 8$  Hz) localized at  $\delta$  6.88 was assigned to 5'-H, two aromatic protons multiplet localized at  $\delta$  7.49 were assigned to 2'-H and 6'-H. In addition, six protons as singlet (s) at 3.92 assigned to 3' and 7 methoxyl groups. These data suggested that compound 1 is dimethoxy, dihydroxy flavone(3,14). Evidently, compound 1 was confirmed to be chrysoeriol 7 - methyl ether (structure I) by comparing its UV and  $^1\text{HNMR}$  spectra with those reported data for chrysoeriol (3,14).

**Compound 2**, exhibited chromatographic and UV spectral properties similar to those of quercetin 3-

methyl ether(11). Its MW was determined to be 316 by negative FABMS  $[\text{M-H}]^-$  315. The  $^1\text{HNMR}$  spectrum of compound 2 proved the presence of a pair of meta coupled aromatic protons doublets ( $J = 2.5$  Hz) at  $\delta$  6.24 and 6.3 were assigned to 6-H and 8-H; one ortho coupled aromatic proton doublet ( $J = 8.5$  Hz) assigned to 5'-H, another two aromatic protons at  $\delta$  7.52 meta coupled (d,  $J = 2$  Hz, 2'-H) and at  $\delta$  7.44 ortho and meta coupled (dd,  $J = 8.5$  Hz and 2 Hz, 6'-H). In addition to a singlet at  $\delta$  3.7 for three protons due to 3- $\text{OCH}_3$ , thus confirming the structure as quercetin 3-methyl ether. (structure II)

**Compound 3**, was obtained as yellow amorphous powder which exhibited a MW of 356 (-ve FABMS, M-1 355) suggesting a dimethoxy tetra-hydroxy flavone  $\text{C}_{17}\text{H}_{14}\text{O}_8$ . The  $^1\text{HNMR}$  spectrum of compound 3 in  $\text{CDCl}_3$ , displayed two singlets at  $\delta$  3.38 (3 protons) and 3.81 (3 protons) for two methoxyl groups and a doublet at  $\delta$  6.18 (2H, d,  $J = 2.5$  Hz) assigned for 6-H and 8-H). The 2' and 6' protons appeared as a singlet at  $\delta$  7.3 typical for a symmetrically substituted myricetin type B-ring. Band I of the UV spectrum of compound 3 in MeOH was at 359 nm as expected for a 3-O-substituted flavonol. The dark purple color of the compound on paper in UV light was further evidence for 3-O-substitution and indicated a free 5-hydroxyl which was confirmed by a Band I shift of (61 nm) in  $\text{AlCl}_3/\text{HCl}$  relative to MeOH spectrum(11). The bathochromic shift of Band II in NaOAc indicated free 7-hydroxyl group. Substitution of 4'-hydroxyl group as shown by the dark color of the compound on paper in UV light + $\text{NH}_3$  and the small magnitude of the Band I bathochromic in NaOMe (11). The above data for compound 3 were in accord with 3,5,7,3',4',5'-oxygenation pattern with methoxyl group at 3 and 4' since the C-5 hydroxyl group was free and the B-ring symmetrically substituted from the NMR data. Compound 3 is therefore myricetin 3,4'-dimethyl ether (structure III) and confirmed by comparison with literature data (3).

**Compound 4**, was obtained as a brown amorphous powder, which exhibited a MW464 (-ve FABMS). It was recognized as a quercetin 3-O-galactoside from acid hydrolysis, its chromatographic behaviour and UV spectral analysis(4). Acid hydrolysis of compound 4 gave quercetin and galactose. In the  $^1\text{HNMR}$  spectrum of compound 4, the presence of one sugar moiety was evidenced by the one proton signal at  $\delta$  5.32 (d,  $J = 8\text{Hz}$ ) assignable to the anomeric  $\beta$ -galactoside proton. Comparing the FABMS, UV and  $^1\text{HNMR}$  spectral data of compound 4 with literature (15) indicated that they were identical. Therefore, compound 4 is quercetin 3-O- $\beta$ -galactoside (structure IV).





Compound 5, was isolated as a pale yellow granules with MW 594 as shown by negative FABMS. It was recognized as a kaempferol 3-O-diglycosides from acid hydrolysis, its chromatographic behaviour and UV spectral analysis (11). Acid hydrolysis of compound 5 yielded kaempferol, rhamnose and glucose

Mild acid hydrolysis gave the intermediate kaempferol 3-O- $\beta$ -glucoside (co-PC and UV) suggesting that compound 5 is a kaempferol 3-O-rhamnosyl glucoside. In the  $^1\text{H-NMR}$  spectrum of compound 5, the presence of two anomeric sugar proton signals at  $\delta$  4 (d,  $J = 8$  Hz) assignable to the 1-H- $\beta$ -glucosyl proton and at  $\delta$  5 (d,  $J = 2$  Hz) assignable to the 1-H- $\alpha$ -rhamnosyl proton. The remaining signals in this spectrum were in agreement with the proposed structure. Finally, the structure of compound 5 was proved to be kaempferol 3-O-rhamnoglucoside through comparison of its UV spectral data with those reported for kaempferol 3-O-rhamnoglucoside (11) (structure V).

Compound 6, was obtained as a brown amorphous powder which exhibited a MW 610 (-ve FABMS). It was recognized as a quercetin 3-O-diglycoside from acid hydrolysis, its chromatographic behaviour and UV spectral analysis (12). Thus acid hydrolysis of compound 6 gave quercetin, rhamnose and galactose. Partial acid hydrolysis gave intermediate quercetin 3-O- $\beta$ -galactoside (co-PC and UV absorption). In the  $^1\text{H-NMR}$  spectrum of compound 6, the presence of two sugar moieties was evidenced by the two proton signals at  $\delta$  5.03 (d,  $J = 2$  Hz) assignable to the anomeric  $\alpha$ -rhamnose proton and at 5.62 (d,  $J = 8$  Hz) assignable to the anomeric  $\beta$ -galactoside proton. The recognizable down field shift ( $\Delta\delta = 0.3$  ppm) which was detected on comparing the chemical shifts of the anomeric galactoside proton signal in the spectrum of compound 6 with those of quercetin 3-galactoside (4) proved that the terminal  $\alpha$ -rhamnose moiety is attached to C-2 of the inner  $\beta$ -galactoside moiety, consequently compound 6 was identified as quercetin 3-O- $\alpha$ -rhamnosyl (1  $\rightarrow$  2)  $\beta$ -D-galactoside. The remaining signals in this spectrum are in close agreement with the proposal structure of compound 6.

A final proof of the proposed structure of quercetin 3-O- $\alpha$ -rhamnosyl (1  $\rightarrow$  2)  $\beta$ -D-galactoside was the identity for the spectral data of compound 6 with those reported for quercetin 3-O- $\alpha$ -rhamnosyl (1  $\rightarrow$  2)  $\beta$ -D-galactoside (4) (structure VI).

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## المحتويات الفلافونيدية لنبات سولانوم انجيكولاتم (أ.رش).

فوقية عبد الله عباس

قسم العقاقير - كلية الصيدلة - جامعة الملك سعود - المملكة العربية السعودية

نبات سولانوم انجيكولاتم (أ.رش) من النباتات دائمة الخضرة شائعة الانتشار في منطقة وادي زهر بمدينة صنعاء باليمن . وفي دراسات سابقة تم فصل العديد من القلوينيدات الاستيرولية وقد أثبت الفحص الكيميائي الأولى وجود العديد من المركبات الفلافونيدية بالاضافة إلى المركبات السابق ذكرها . في هذا البحث تم فصل ٧-ميثيل كريسزول ، ٣-ميثيل كوارسيتين ، ٣ ، ٤ ثنائي ميثيل الميرستين ، ٣ جلكتوسيد كوارسيتين ، ٣-روتينوسيد الكمفيرول ، ٣-رمنوسيد (١ ← ٢) جلكتوسيد الكوارسيتين وتم التعرف على هذه المواد باستخدام طرق التحليل الطيفية والطبيعية المختلفة.