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Phenolic constituents of *Kancolla* seeds

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Abstract

Chemical investigation of *Kancolla* seeds revealed the presence of an array of polyphenols which were dominated by kaempferol and quercetin glycosides. Also isolated was a glucoside of vanillic acid. This is the first report of quercetin 3-*O*-β-D-apiofuranosyl-(1‴→∼2″)-*O*-[a-L-rhamnopyranosyl-(1⁗→6″)]-∼3-D-galactopyranoside-3′,4′-dimethyl ether. All structures were elucidated by chemical and spectroscopic methods, namely NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC), mass data (FAB–MS, HRFAB–MS, GC–MS), IR and UV.
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Keywords: NMR; Mass data; Chenopodiaceae; Flavonols; *Kancolla*; Quinoa

1. Introduction

Kancolla is a sweet variety of *Chenopodium quinoa* (quinoa), used as a food plant, principally in the same way as wheat and rice. Quinoa is known as a pseudo-cereal, recently rediscovered by agricultural researchers of industrialized societies (Schlick & Bubenheim, 1993). It is a highly nutritious food and the main edible parts are the seeds (Koziol, 1992). They contain bitter-tasting constituents (chiefly water-soluble saponins) located in the outer layers of the seed coat. Because of this, they need to be washed or milled to remove the seed coat. The increased demand for quinoa has led investigators to produce several cultivars, selected and bred for their tolerance to heat and cold, resistance to disease, and for other desirable characteristics (sweet taste). Perhaps the oldest and most widespread of the new varieties is *Kancolla* (Popenoe, King, Leòn, & Sumarkaunowsk, 1989). The aim of this work is the isolation and characterization of the intact phenolic compounds present in the whole flour of kancolla. Their occurrence is interesting for nutritional properties and for chemotaxonomical purposes. A broad range of phenolic compounds occurs in food products, especially from plant material, in which they contribute to the organoleptic properties, i.e. astringency, beer hazes, specific (dis)coloration and off-flavours. The effects of dietary phenolic compounds are of great current interest due to their antioxidative, cardiovascular protective, antiallergic, anti-inflammatory, antiviral and anticarcinogenic activities (Di Carlo, Mascolo, Izzo, & Capasso, 1999). Usually they differ in the aglycone, in the saccharide moieties, and also in the substitution pattern of sugars (Agrawal, 1989). The taste and pharmacological properties are linked to the whole molecule (Shin, Kim, Shin, & Kim, 1995). Therefore, the identification of the intact flavonoid glycosides may imply a benefit that is beyond basic nutrition and may increase interest in this under-exploited food plant.

2. Materials and methods

2.1. Material

The plant material was collected in Peru in April 1999 and identified by Dr. S.E. Jacobsen of the International Potato Centre (CIP), Lima, Peru. A sample used has been deposited in the Herbarium Neapolitanum of the Dipartimento di Biologia Vegetale Università degli

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Studi "Federico II" of Naples. The collection number was NAP # A. C. 002.

2.2. Extraction and isolation

The whole flour from the seeds (709 g) was extracted with MeOH. The MeOH extract (49.03 g) was partitioned between BuOH and H_20. The buthanol extract (26.2 g) was evaporated and defatted with $CHC1_3$. The residue fraction (10 g) was chromatographed on a Sephadex LH-20 column (100×5 cm), with MeOH as eluent. Fractions (9 ml) were collected and checked by TLC [Si-gel plates in *n*-BuOH/HOAc/H_2O (60:15:25)]. Fractions 34–71 (4.5 g), containing the crude glycosidic mixture, were further separated by DCCC using *n*-BuOH/Me_2CO/H_2O (60:12:28) as stationary phase and *n*-BuOH/Me_2CO/H_2O (14:12:74) as descending phase. DCCC fractions 85–202 (365.0 mg), containing the crude glycosidic mixture, were chromatographed by reversed-phase HPLC with MeOH/H_20 (40:60) at a flow rate of 2.2 ml/min to yield pure compounds **1** (5.5 mg; R_f, 0.49; R_t, 5.0 min), **2** (238.9 mg; R_f, 0.53; R_t, 19.2 min), **3** (10.0 mg; R_f, 0.49; R_t, 19.5 min), **4** (4.7 mg; R_f, 0.5; R_t, 18.6 min), **5** (36.4 mg; R_f, 0.69; R_t, 4.0 min), **6** (3.7 mg; R_f, 0.63; R_t, 6.5 min).

2.3. Droplet counter current chromatography (DCCC)

DCCC separation was performed on a Bruchi apparatus equipped with 300 tubes.

2.4. High-performance liquid chromatography (HPLC)

HPLC separations were performed on a Hewlett-Packard HP 1050 series apparatus with a Varian RI-4 refractive index detector, equipped with a Waters μ-Bondapack C-18 column (7.8×300 mm).

2.5. Identification

All structures were elucidated by chemical and spectroscopic methods NMR (^{1}H, ^{13}C, COSY, HMQC, HMBC), mass data (FAB–MS, HRFAB–MS, GC–MS), IR and UV.

2.5.1. *Quercetin-O-fi-D-apiofuranosyl-(1‴→2″)-O-[a-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside-3′,4′-dimethyl ether (1)*

IRν^{OH} (cm^{-1}): 1653, 3379. The FAB–MS spectrum gave a quasi-molecular anion at *m/z* 769 $[M-H]^-$, fragment ions at *m/z* 329 $[aglycone–H]^-$, *m/z* 623 $[M-H-146]^-$ and *m/z* 491 $[M-H-146-132]^-$. HRFAB–MS found, 769.22695 *m/z*, calcd for *m/z* $C_{33}H_{40}O_{20}$ 770.68524. The chromatographic analysis of the methanolysis products showed the presence of apiose, galactose and rhamnose in the ratio 1:1:1. ^{1}H and ^{13}C NMR see (Table 1).

Table 1
NMR data of compound **1** recorded in CD_3OD

Position	^{1}H	DEPT	^{13}C
2		C	158.6
3		C	135.5
4		C	179.4
5		C	163.2
6	6.21 *d* (1.9)	CH	99.8
7		C	166.2
8	6.41 *d* (1.9)	CH	94.2
9		C	158.6
10		C	105.6
1′		C	122.2
2′	7.81 *d* (2.0)	CH	110.4
3′		CH	149.2
4′		C	152.6
5′	6.79 *d* (8.4)	CH	119.6
6′	7.15 *d* (8.4)	CH	120.3
OCH_3	3.86	OCH_3	54.1
OCH_3	3.78	OCH_3	57.7
Galactose			
1″	5.40 *d* (7.5)	CH	102.2
2″	3.95 *dd* (7.5, 9.7)	CH	78.5
3″	3.72 *dd* (7.5, 3.5)	CH	75.5
4″	3.48 *dd* (3.5, 1.5)	CH	70.7
5″	3.42 *ddd* (1.5, 5.0, 7.0)	CH	75.4
6″a	3.47 *dd* (12.0, 7.0)	CH_2	
6″b	3.74 *dd* (12.0, 5.0)		67.5
Ramnose			
1⁗	4.55 *d* (1.5)	CH	101.5
2⁗	3.54 *dd* (3.5, 1.5)	CH	72.5
3⁗	3.76 *dd* (9.5, 3.5)	CH	72.2
4⁗	3.30 *t* (9.5)	CH	74.0
5⁗	3.37 *dq* (9.5, 6.0)	CH	69.8
6⁗	1.21 *d* (6.0)	CH_3	17.8
Apiose			
1‴	5.48 *d* (1.5)	CH	110.9
2‴	4.06 *d* (1.5)	CH	77.2
3‴		C	80.7
4‴a	3.82 *d* (10)	CH_2	75.5
4‴b	4.05 *d* (10)		
5‴a	3.61 *s*	CH_2	66.3
5‴b	3.61 *s*		

2.5.2. *Kaempferol 3-O-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside (mauritianin) (2)*

Irν^{OH} (cm^{-1}): 1653, 3379. The FAB–MS spectrum gave a quasi-molecular anion at *m/z* 739 $[M-H]^-$, fragment ions at *m/z* $[aglycone–H]^-$, *m/z* 593 $[M-H-146]^-$ and *m/z* 447 $[M-H-146-146]^-$. HRFAB–MS found, 739.2164 *m/z*, calcd for *m/z* $C_{33}H_{40}O_{19}$ 740.6593. The chromatographic analysis of the methanolysis products showed the presence of

galactose and rhamnose in the ratio 1:2. ^{1}H NMR (CD_3OD): δ 6.18 (1H, d, $J=1.9$ Hz, H-6), δ 6.36 (1H, d, $J=1.9$ Hz, H-8), δ 8.09 (2H, d, 20 $J=8.4$ Hz, H-2′,6′), δ 6.93 (2H, d, $J=8.4$ Hz, H-3′,5′), δ 5.63 (1H, d, $J=7.5$ Hz, galactose anomeric proton), δ 5.25 (1 H, d, $J=1.5$ Hz, rhamnose anomeric $proton_{linked\ at\ Gal\text{-}2}$) and δ 4.55 (1 H, d, $J=1.5$ Hz, rhamnose anomeric $proton_{linked\ at\ Gal\text{-}6}$). ^{13}C NMR (CD_3OD): 158.4 (C-2), 134.3 (C-3), 179.2 (C-4), 161.3 (C-5), 100.0 (C-6), 165.8 (C-7), 95.2 (C-8), 163.1 (C-9), 105.8 (C-10), 123.1 (C-1′), 132.2 (C-2′), 116.2 (C-3′), 158.6 (C-4′), 116.2 (C-5′), 132.2 (C-6′), 102.5 (Gal-1), 77.7 (Gal-2), 75.7 (Gal-3), 70.8 (Gal-4), 75.4 (Gal-5), 67.4 (Gal-6), 101.8 ($Ram_{linked\ at\ Gal\text{-}2}$-1), 72.3 ($Ram_{linked\ at\ Gal\text{-}2}$-2), 72.4 ($Ram_{linked\ at\ Gal\text{-}2}$-3), 74.1 ($Ram_{linked\ at\ Gal\text{-}2}$-4), 69.9 ($Ram_{linked\ at\ Gal\text{-}2}$-5), 17.5 ($Ram_{linked\ at\ Gal\text{-}2}$-6), 100.9 ($Ram_{linked\ at\ Gal\text{-}6}$-1), 72.4 ($Ram_{linked\ at\ Gal\text{-}6}$-2), 72.1 ($Ram_{linked\ at\ Gal\text{-}6}$-3), 73.9 ($Ram_{linked\ at\ Gal\text{-}6}$-4), 69.8 ($Ram_{linked\ at\ Gal\text{-}6}$-5), 17.9 ($Ram_{linked\ at\ Gal\text{-}6}$-6).

2.5.3. *Kaempferol 3-O-β-D-apiofuranosyl(1‴→2″-O-[α-L-rhamnopyranosyl(1⁗→6″]-β-D-galactopyranoside (3)*

$IR\nu^{OH}$ (cm^{-1}): 1653, 3379. The FAB–MS spectrum gave a quasi-molecular anion at *m/z* 741 $[M-H]^-$, fragment ions at *m/z* 285 $[aglycone–H]^-$ and *m/z 595* $[M-H-146]^-$. HRFAB–MS found, 741.6319 *m/z*, calcd for $C_{32}H_{38}O_{20}$ *m/z* 742.1957. The chromatographic analysis of the methanolysis products showed the presence of galactose, apiose and rhamnose in the ratio 1:1:1. ^{1}H NMR (CD_3OD): δ 6.21 (1H, d, $J=1.9$ Hz, H-6), δ 6.41 (1H, d, $J=1.9$ Hz, H-8), δ 8.08 (2H, d, $J=8.4$ Hz, H-2′,6′), δ 6.92 (2H, d, $J=8.4$ Hz, H-3′,5′), δ 5.40 (1H, d, *J 7.5* Hz, galactose anomeric proton), 6 5.48 (1H, d, $J=1.5$ Hz, apiose anomeric proton) and δ 4.55 (1H, d, $J=1.5$ Hz, rhainnose anomeric proton). ^{13}C NMR (CD_3OD): 158.4 (C-2), 134.5 (C-3), 179.4 (C-4), 161.2 (C-5), 100.0 (C-6), 165.5 (C-7), 94.3 (C-8), 163.1 (C-9), 105.8 (C-10), 123.1 (C-1′), 132.2 (C-2′), 116.2 (C-3′), 158.6 (C-4′), 116.2 (C-5′), 132.2 (C-6′), 102.5 (Gal-1), 77.7 (Gal-2), 75.7 (Gal-3), 70.8 (Gal-4), 75.4 (Gal-5), 67.4 (Gal-6), 100.9 (Ram-1), 72.5 (Ram-2), 72.2 (Ram-3), 74.0 (Ram-4), 69.7 (Ram-5), 17.7 (Ram-6), 110.8 (Ap-1), 77.1 (Ap-2), 80.8 (Ap-3), 75.4 (Ap-4), 66.3 (Ap-5).

2.5.4. *Quercetin 3-O-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside (4)*

$IR\nu^{OH}$ (cm^{-1}): 1653, 3379. The FAR–MS spectrum gave a quasi-molecular anion at *m/z* 755 $[M-H]^-$, fragment ions at *m/z* 301 [aglycone–H], *m/z* 609 $[M-H-146]^-$ and *m/z* 463 $[M-H-146-146]^-$. HRFAB–MS found, 755.2111 *m/z,* calcd for *m/z* $C_{33}H_{40}0_{20}$ 756.6587. The chromatographic analysis of the methanolysis products showed the presence of galactose and rhamnose in the ratio 1:2. ^{1}H NMR (CD_3OD): δ 6.19 (1H, d, $J=1.8$ Hz, H-6), δ 6.38 (IH, d, $J=1.8$ Hz, H-8), δ 7.70 (1H, d, $J=1.8$ Hz, H-2′), δ 6.89 (1H, d, $J=8.4$ Hz, H-5′), 6 7.58 (1H, dd, $J=8.4$, 1.8 Hz, H-6′), δ 5.65 (1H, d, $J=7.5$ Hz, galactose anomeric proton), δ 5.24 (1H, d, $J=1.5$ Hz, rhamnose anomeric $proton_{linked\ at\ Gal.2}$) and 64.58 (1H, d, $J=1.5$ Hz, rhamnose anomeric $proton_{linked\ at\ GAL\text{-}6}$). ^{13}C NMR (CD_3OD): 157.9 (C-2), 134.3 (C-3), 179.0 (C-4), 162.8 (C-5), 99.3 (C-6), 165.8 (C-7), 95.5 (C-8), 157.8 (C-9), 105.4 (C-10), 123.3 (C-1′), 116.1 (C-2′), 145.5 (C-3′), 149.4 (C-4′), 117.2 (C-5′), 122.9 (C-6′), 102.6 (Gal-1), 77.6 (Gal-2), 75.7 (Gal-3), 70.8 (Gal-4), 75.2 (Gal-5), 66.9 (Gal-6), 101.8 ($Ram_{linked\ at\ Ga1\text{-}2}$-1), 72.1 ($Ram_{linked\ at\ Ga1\text{-}2}$-2), 72.4 ($Ram_{linked\ at\ Ga1\text{-}2}$-3), 74.1 ($Ram_{linked\ at\ Ga1\text{-}2}$-4), 69.9 10 ($Ram_{linked\ at\ Ga1\text{-}2}$-5), 17.5 ($Ram_{linked\ at\ Ga1\text{-}2}$-6), 101.0 ($Ram_{linked\ at\ Ga1\text{-}6}$-1), 72.4 ($Ram_{linked\ at\ Ga1\text{-}6}$-2), 72.0 ($Ram_{linked\ at\ Ga1\text{-}6}$-3), 73.9 ($Ram_{linked\ at\ Ga1\text{-}6}$-4), 69.7 ($Ram_{linked\ at\ Ga1\text{-}6}$-5), 17.8 ($Ram_{linked\ at\ Ga1\text{-}6}$-6).

2.5.5. *Kaempferol 3-O-β-D-glucuronic acid (5)*

$IR\nu^{OH}$ (cm^{-1}): 1653, 3379. The FAB–MS spectrum gave a quasi-molecular anion at *m/z* 461 $[M-H]^-$ and fragment ion at *m/z* 284 $[M-H-177]^-$. HRFAB–MS found, 461.36045 *m/z,* calcd for $C_{21}H_{18}O_{12}$ *m/z* 462.07983. The chromatographic analysis of the methanolysis products showed the presence of glucuromc acid moiety. ^{1}H NMR (CD_3OD): 6 6.21 (1H, d, $J=1.9$ Hz, H-6), 6 6.42 (1H, d, $J=1.9$ Hz, H-8), 68.11 (2H, d, $J=8.4$ Hz, H-2′,6′), 66.91 (2H, d, $J=8.4$ Hz, H3′,5′) and 6 4.51 (1H, d, $J=7.5$ Hz, glucuronic acid anomeric proton). ^{13}C NMR (CD_3OD): 158.5 (C-2), 135.6 (C-3), 179.5 (C-4), 161.5 (C-5), 100.0 (C-6), 166.2 (C-7), 94.9 (C-8), 163.0 (C-9), 105.7 (C-10), 122.7 (C-1′), 132.4 (C-2′), 116.2 (C-3′), 159.0 (C-4′), 116.2 (C-5′), 132.2 (C-6′), 104.9 (GlcA-1), 75.1 (GlcA-2), 77.9 (GlcA-3), 73.7 (GlcA-4), 77.6 (GlcA-5), 176.1 (GlcA-6).

2.5.6. *Vanillic acid glucosyl ester (6)*

UV λ_{max} (MeOH): 202.5, 221.5, 265.9 mm IR^{KBr} (cm^{-1}): 1653. $[\alpha]_D^{25}=-16.5$ (MeOH, *c* 0.07).

The FAB–MS spectrum gave a quasi-molecular anion at *m/z* 329 $[M-H]^-$ and *m/z* 167 $[M-H-162]^-$. HRFAB–MS found, 330.09509 *m/z*, calcd for $C_{14}H_{18}0_9$ *m/z* 330.28730. The chromatographic analysis of the methanolysis products showed the presence of glucose moiety. ^{1}H NMR (CD_3OD): δ 7.60 (IH, d, $J=1.2$ Hz, H-2), δ 6.87 (1H, d, $J=8.4$ Hz, H-5), δ 7.66 (1H, dd, $J=8.4$, 1.2 Hz, H-6), δ 3.93 (3H, s, OCH_3) and δ 5.71 (1H, d, $J=8.4$ Hz, glucose anomeric proton). ^{13}C NMR (CD_3OD): 121.2 (C-1), 113.8 (C-2), 149.0 (C-3), 152.8 (C-4), 116.1 (C-5), 10 125.8 (C-6), 166.8 (C-7), 56.4 (OCH_3), 96.0 (Glc-1), 74.0 (Glc-2), 78.8 (Gbc-3), 71.1 (Glc-4), 78.1 (Glc-5), 62.3 (Glc-6).

2.6. Methanolysis of compounds **1–6**

Each fraction (1.0 mg) was heated in a vial for 24 h at 800 in MeOH–HCl 2% (2 ml). After MeOH and HCl distillation in a N_2 stream, Ag_2CO_3 and MeOH were added until CO_2 production stopped. The centrifugate was dried over P_2O_5. The resulting monosaccharides were treated with TRLSIL-Z (Pierce) and analysed by GC–MS. Retention times were identical to those of the authentic trimethylsilylated sugars.

2.7. Acid hydrolysis of compounds **1–6**: monosaccharide composition

A solution of each compound (1 mg) was refluxed for 1 h in 6% HC1 (3 ml) and extracted with EtOAc. The resulting aglycones were identified by their ^{1}H NMR.

2.8. NMR

The NMR spectra were obtained in CD_3OD with a Brüker AMX 500 spectrometer. The DEPT experiments were performed with a pulse of 135° to obtain positive signals for CH and CH_3 and negative signals for CH_2 an average CH coupling constant of 135 Hz was assumed. Two dimensional homonuclear proton chemical shift correlation (COSY) experiments were measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set ($t_1 \times t_2$) of 1024×1024 points for a spectral width of 1165 Hz (relaxation delay 1 s). The data matrix was processed using an unshifted sine bell window function, followed by transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and Fl dimension, 1.13 Hz for point). The 2D NOESY (two-dimensional nuclear Overhauser effect) experiment was performed in the phase-sensitive mode (PT). The spectral width (t_2) was 1002 Hz; 512 experiments of 80 scans each (relaxation delay 1.5 s, mixing time 300 ms) were acquired in 2 K data points. ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) experiments were performed according to the procedure of Martin and Crouch (1991), using an initial BIRD pulse to suppress ^{1}H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in the ^{1}H dimension was 2994.05 Hz; 256 experiments of 240 scans each (relaxation delay = 1.5 s, delay after BIRD pulse = 0.4 s, fixed delay $t_1 = 3.3$ ms) were acquired in 1 K points. A sine square function was applied in the t_2 dimension, and a trapezoidal window was applied in the t_1 dimension ($TM_1 = 0.03$ Hz, $TM_2 = 0.6$ Hz) before Fourier transformation (digital resolution in F_2 dimension = 2.994 Hz/point). ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to the methods of Bax, Aszavalos, Dinya, and Sudo (1986) and Martin and Crouch (1991). The data processing was identical to that used for the HMQC experiment, and the 20 final digital resolution was 2.25 Hz/point.

2.9. FAB–MS

FAB–MS (recorded in a glycerol matrix) was measured on a Pro spec Fisons mass spectrometer.

2.10. HRFAB–MS analysis

HRFAB–MS spectra were recorded in a glycerol matrix on a VG Autospec instrument; GC–MS was run using a Hewlett-Packard 5890 gas chromatograph equipped with an HP-5 column (25 m×0.2 mm i.d. 0.33 μm film), fitted with an HP 5970B mass detector and an HP 59970 MS Chemstation.

2.11. FTIR analysis

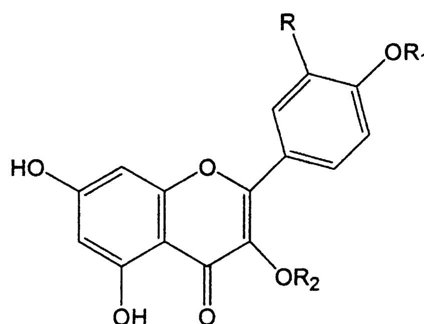
The FTIR spectra were obtained on a Brüker IFS-48 spectrophotometer using a KBr matrix.

2.12. UV analysis

UV spectra were obtained on a Beckman DU7O spectrophotometer in MeOH solution.

3. Results and discussion

The flour of *Kancolla* grains was extracted with CH_3OH. The methanolic extracts were partitioned into a mixture of *n*-BuOH and H_2O to afford the BuOH-soluble portion, which was subjected to Sephadex LH-20 chromatography, followed by DCCC, to give phenol derivatives. The fractions were checked by TLC, and fractions 85–202, containing the crude glycosidic mixture, were submitted to reversed-phase HPLC to afford almost pure compounds **1–6** (Fig. 1). The structures were determined by IR, UV, negative ion FAB–MS mass spectra, and by 1D-NMR (^{1}H–^{13}C and ^{13}C-DEPT) and 2D-NMR (COSY, ROESY, HMQC, HMBC). Particularly, the known phenols **2–6** were identified by direct comparison of their physical properties with those reported previously for these compounds as *kaempferol 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside (mauritianin)* (**2**) (De Simone, Dini, Pizza, Saturnino, & Schettino, 1990), *kaempferol 3-*O*-β-D-apiofuranosyl(1‴→2″)-*O*-[α-L-rhamnopyranosyl(1⁗→6″)]-β-D-galactopyranoside* (**3**) (De Simone et al., 1990), *quercetin 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside* (**4**) (Rastrelli,



1 R= OCH_3, R_1= CH_3 , R_2 = 3-*O*-β-D-apiofuranosyl(1‴→2″)-*O*-[α-L-rhamnopyranosyl -(1⁗→6″)]-β-D- galactopyranoside
2 R= H, R_1= H, R_2 = 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside
3 R= H, R_1= H, R_2 = 3-*O*-β-D-apiofuranosyl(1‴→2″)-*O*-[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D- galactopyranoside
4 R= OH, R_1= H, R_2 = 3-*O*-(2,6-di-α-L-rhamnopyranosyl)-β-D-galactopyranoside
5 R= H, R_1= H, R_2 = 3-*O*-β-D-glucuronopyranoside

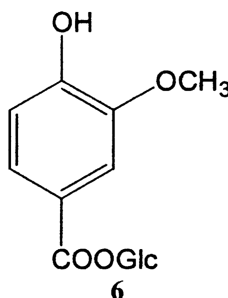


Fig. 1. Chemical structures of compounds **1–6**.

De Simone, Schettino, & Dini, 1996), *kaempferol 3-*O*-β-D-glucuronic acid* (**5**) (Plumb, Price & Williamson, 1999), *vanillic acid glucosyl ester* (**6**) (Sakushima, Coskun, & Maoka, 1995), although this is the first report of their presence in *Kancolla*. For compounds **2–5** the antioxidant activity has been already reported (Plumb et al., 1999; Zhu et al., 2001).

3.1. *Quercetin-*O*-β-D-apiofuranosyl-(1‴→2″)-*O*-[α-L-rhamnopyranosyl-(1‴→6″)]-β-D-galactopyranoside-3′,4′-dimethyl ether **1***

Acid hydrolysis of **1** released quercetin 3′,4′-dimethyl ether, galactose and rhamnose, identified by TLC on cellulose plates compared with authentic samples. The IR spectrum (KBr) showed a strong absorption band at 1653 cm^{-1} for a chelated carbonyl group and an intense broad band centred at 3379. The FAB–MS spectrum gave a quasi-molecular anion at *m*/*z* 769 $[M-H]^-$. The fragment at *m*/*z* 329 corresponded to the deprotonated aglycone $[A-H]^-$, thus indicating a quercetin derivative. Fragment ions occurred at *m*/*z* 623 $[M-H-146]^-$ and at *m*/*z* 491 $[M-H-146-132]^-$, corresponding to independent losses of the terminal rhamnose and the apiose, respectively. The HRFAB–MS spectrum gave 769.22695 *m*/*z*, calcd for $C_{33}H_{40}O_{20}$ *m*/*z* 770.68524. The chromatographic analysis of the methanolysis products showed the presence of galactose, apiose and rhamnose in the ratio 1:1:1. The ^1H NMR and ^{13}C NMR (Table 1) data of the aglycone corresponded well to the shifts for quercetin 3′,4′-dimethylether (Ogasawara, Matsubara, & Hideyo, 2001), the only significant difference being those referred to C-2 and C-3. These shifts are analogous to those reported when the 3-hydroxy group is glycosylated in a flavonol glycoside (Shin et al., 1995). The complete structure of **1** was elucidated by 1D- and 2D-NMR experiments at 500 MHz. A careful analysis of the ^1H–^1H COSY spectrum, combined with the NOESY data, secured the assignments of the spin system for each sugar belonging to the triglycoside moiety. The HMQC spectrum correlated the ^1H resonances with those of the corresponding carbons. Three anomeric protons were easily identified in the spectra of **1**. They resonated at δ 5.48 (1H, d, J = 1.5 Hz, H-1 of apiose), δ 5.40 (1H, d, J = 7.5 Hz, H-1 of galactose), δ 4.55 (1H, d, J = 1.5 Hz, H-1 of rhamnose) and correlated to carbons at δ 110.9, 102.2 and 101.5, respectively. For the assigned aglycone and sugar values see Table 1.

Information about the sequence of the trisaccharide chain was deduced from HMBC experiments. Key correlation peaks were observed between the anomeric proton of galactose (δ 5.40) and C-3 of the quercetin 3′,4′-dimethylether (δ 135.5), between the anomeric proton of apiose (δ 5.48) and C-2 of galactose (δ 78.5), and between the anomeric proton of rhamnose (δ 4.55) and C-6 of galactose (δ 67.5). Chemical shifts, multiplicity of the signals, absolute values of the coupling constants and their magnitude in the ^{1}H NMR spectrum, as well as ^{13}C NMR data (Table 1), indicated the β configuration at the anomeric positions for galactopyranosyl *(J* = 7.5 Hz) and apiofuranosyl *(J* = 1.5 Hz) units and the α configuration for the rhamnopyranosyl unit *(J* = 1.5 Hz). These data suggested that the structure of 1 was quercetin 3-*O*-β-D-apiofliranosyl-(1‴→2″)-*O*-[α -L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside-3′,4′-dimethy1 ether (Fig. 1).

4. Conclusion

Five flavonol glycosides and a vanillic acid glucosyl ester were found in the sample of *Kancolla* seeds and obtained by MeOH extract. The quercetin 3-*O*-β-D-apiofuranosyl-(1‴→2″)-*O*-[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside-3′,4′-dimethyl ether (**1**) was isolated for the first time in quinoa varieties.

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