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Original Research Article

New flavonoid glycoside from *Vicia faba* L.

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ABSTRACT

Kaempferol 4´-*O*-α-rhamnopyranosyl-3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside (**1**), a new flavonoid glycoside, along with eight known ones (**2-9**) were isolated from the flowers of *Vicia faba* L. (Leguminosae) which were identified as kaempferol 3-*O*-α-arabinopyranosyl-5-*O*-α-rhamnopyranoside (**2**), kaempferol 7-*O*-α-rhamnopyranoside (**3**), kaempferol 3-*O*-α-arabinopyranosyl-7-*O*-α-rhamnopyranoside (**4**), kaempferol 3-*O*-rutinoside (**5**), kaempferol 3-*O*-α-rhamnopyranosyl-(1→3)-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**6**), kaempferol-3-*O*-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**7**), kaempferol-3-*O*-α-rhamnopyranosyl(1→6)-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**8**) in addition to a quercetin derivative; quercitin-3´-*O*-α-rhamnopyranosyl-4´-(3´´-acetyl)-β-galactopyranoside (**9**). Structure elucidation of the new compound was established based on 1D and 2D NMR analyses including ^{1}H-, ^{13}C-NMR, and HMBC experiments, in addition to HR-TOF-MS spectrometry using matrix assisted laser desorption ionization (MALDI).

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1. Introduction

Vicia faba L. (broad bean) is a legume belonging to the plant family *Leguminosae*. It is an important winter crop in Mediterranean areas and is mostly a spring crop in other regions of Europe and South America and is one of the major plant food items for the Nile River populations (Amarowicz and Pegg 2008). It is used in folk medicine by Indian people, where it is cultivated as a major crop, as antihyperlipidemic to control cholesterol (Rabey et al., 1993; Bouchenak and Lamri-Senhadji 2013; Mulvihill and Huff 2010) clover, lupins, green beans and peas, peanuts, soybeans, dry beans, broad beans, dry peas, chickpeas, and lentils.
It was utilized since long time as a drug to treat kidney, liver and ocular perceiver diseases (Duc, 1997; Crepon et al., 2010; Kopke and Nemecek, 2010).
It also has effective biological protection against diabetic complications due to its high contents of dietary fiber (57.46%), carbohydrate (18.93%) and protein (13.81%) versus low fat content (<1%) contributing to low energy value139.24 kcal/100 g (Faiza et al., 2018) and anticancer agent. Peptides obtained from *Vicia faba* were tested as antiglycation, antioxidants and antitumor activity against different cell lines (Abudukadeer et al., 2020).
Many phytochemical reports have focused on the *V. faba* seed, as it contains a high number of bioactive compounds such as proteins (protease inhibitors, α-amylases, lectins), glycosides (α-galactosides, vicine, and convicine), tannins, saponins, and alkaloids (Piotr et al., 2014).
In contrast to the *V. faba* seed, little research has focused on the phytochemistry of *V. faba* flower, except a study that isolated the tyramine, jasmonic acid, and some flavonoid monoside derivatives from *V. faba* flowers (Kapinová et al., 2015), in addition to epicuticle wax esters (Wynne et al., 1999).

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2. Experimental

2.1 Plant material

Flowers of *V. faba* L. were collected in full maturation stage (March 2019) from upper Egypt about 500 Km far from Cairo with the geographical coordinates of 26.209°N,32.768°E where a dry and a very hot weather is available. The plant was identified and authenticated by Prof. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University Egypt. For further authentication, a voucher specimen (V-25) was deposited at the Laboratory Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

2.2 Extraction and isolation

Air-dried *V. faba* flowers (120 g) were extracted three times with methanol (5 L of each) at room temperature to yield the methanol extract (16 g), which was suspended in distilled water and partitioned between chloroform, ethyl acetate and *n*-butanol (1 L each) to give the chloroform fraction (3.5 g), ethyl acetate fraction (4 g), an *n*-butanol fraction (5.5 g), and the remaining aqueous fraction (2.5 g). The ethyl acetate fraction (4 g) was sub-fractionated on a silica gel column (350 g) using CHCl3-MeOH gradient elution (25%, 50%, 75% and 100%; 2 L each). The fraction eluted by 50% methanol (2.1 g) was further separated by chromatography on an ODS, (octadecyl-silica) (100 g ODS) column (80 × 200 mm; Cosmosil 140 C18 PREP, Nacalai Tesque, Kyoto, Japan) using six mobile phase systems of MeOH-H2O (10%, 25%, 40%, 50%, 70% and 90% v/v; elution volume: 1.5 L of each) to give six corresponding fractions. The fraction eluted with 40% MeOH (850 mg) was further chromatographed by column chromatography on silica gel (100 g) and eluted on a stepwise gradient of $CHCl_3$-MeOH (ratios of 90:10, 85:15, 80:20, 70:30 and 1:1; v/v elution volume: 200 ml each) to give five corresponding sub fractions. The sub fraction eluted with 90:10 was re chromatographed on silica gel (50 g) and eluted by a stepwise gradient of $CHCl_3$-MeOH ratios of 95:5 to give compound (**1**) (20 mg), 90:10 resulting in isolation of compound (**2**) (12 mg), 85:15 resulting in isolation of compound (**3**) (10 mg), 80:20 resulting in isolation of compound (**4**) (13 mg) and 70:30 to give compound (**5**) (11 mg). The sub fraction eluted with 85:15 was re chromatographed on silica gel (50 g) and eluted by a stepwise gradient of $CHCl_3$-MeOH ratios of; 95:5 to give compound (**6**) (9 mg), 90:10 resulting in isolation of compound (**7**) (12 mg). The sub fraction eluted with 80:20 was re chromatographed on silica gel (50 g) and eluted by a stepwise gradient of $CHCl_3$-MeOH ratios of; 95:5 to give compound (**8**) (6 mg). The sub fraction eluted with 70:30 was re chromatographed on silica gel (50 g) and eluted by a stepwise gradient of $CHCl_3$-MeOH ratios of 90:10 to give compound (**9**) (4 mg). All compounds were isolated in single pure forms and subsequently characterized.

2.3 Apparatus

^1H, ^{13}C NMR and 2D spectra of the isolated compounds were recorded using a Bruker DRX 600 NMR spectrometer (Bruker Daltonics, Billerica, MA) using TMS as an internal standard for chemical shifts. Chemical shifts (δ) were expressed in ppm and the coupling constants (*J*) in Hz. The solvent peaks at δH 2.50 in the ^1H-NMR spectra and δC 39.52 in ^{13}C-NMR spectra, respectively were used as internal references downfield of tetramethylsilane (TMS) at 0 ppm. Spectral widths were 9008 Hz (26 K acquisition points) and 37878 Hz (26 K acquisition points) for ^1H-and ^{13}C-NMR, respectively. Mass was determined using LC-MS-IT-TOF (Shimadzu, Tokyo, Japan). The instrument was fitted with an Inertsil ODS-3, 5 µm, 4.6 × 150 mm column (GL Science, Tokyo, Japan), using a mobile phase composed of solvents A (water) and B (methanol). The total flow rate was 0.5 mL/min. Based on the previous result of HPLC-PDA analysis. The wavelengths used for monitoring of separation of fraction were set between 230 and at 280 nm. HPLC analysis of compound 1 was carried out using Inertsil ODS-3 (5 µm, 4.6 X 150 mm, ambient temp.) with flow rate 0.5 mL/min using an isocratic elution of Water-Methanol (50:50). The wavelengths were set at 230 nm (a) and at 280 nm (b). Rt value of 9.6 min. Dimethylsulfoxide (DMSO) and other organic solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sephadex LH-20 was purchased from GE Healthcare (Uppsala, Sweden). Silica gel (75-120 mesh) and RP-18 silica gel (38-63 µm) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Thin-layer chromatography (TLC) silica gel 60 F254 was purchased from Merck (Darmstadt, Germany). The developed chromatograms were visualized under 254 nm UV light and the spots were made visible by spraying with vanillin/H_2SO_4 reagent before warming in an oven preheated to 110 °C for 5 min.

3. Results and Discussion

3.1. Structure elucidation of (**1**)

Chemical study of the ethyl acetate fraction of *V. faba* afforded eight compounds (**1-8**), (Fig. 1) of which seven were identified by comparing their physicochemical and spectroscopic data with those reported in the literature as kaempferol 3-*O*-α-arabinopyranosyl-5-*O*-α-rhamnopyranoside (**2**) (Ahmed et al., 2017), kaempferol 7-*O*-α-rhamnopyranoside (**3**), (Veit and Pauli 1999), kaempferol 3-*O*-α-arabinopyranosyl-7-*O*-α-rhamnopyranoside (**4**) (Lawrence et al., 2003), kaempfrerol 3-*O*-rutinoside (**5**) (Petpiroon et al., 2015), kaempferol 3-*O*-α-rhamnopyranosyl-(1→3)-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**6**), (Xu et al., 2009), kaempferol-3-*O*-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**7**), (Ablajan et al., 2006) and kaempferol 3-*O*-α-rhamnopyranosyl (1→6)-β-galactopyranosyl-7-*O*-α-rhamnopyranoside (**8**) (Jiaju et al., 2011) and quercitin-3´-*O*-α-rhamnopyranosyl-4´-(3´´-acetyl)-β-galactopyranoside **(9)** (Susanne et al., 2015). Compound (**1**) (Table 1, Fig. 1) was obtained as a yellow amorphous powder.

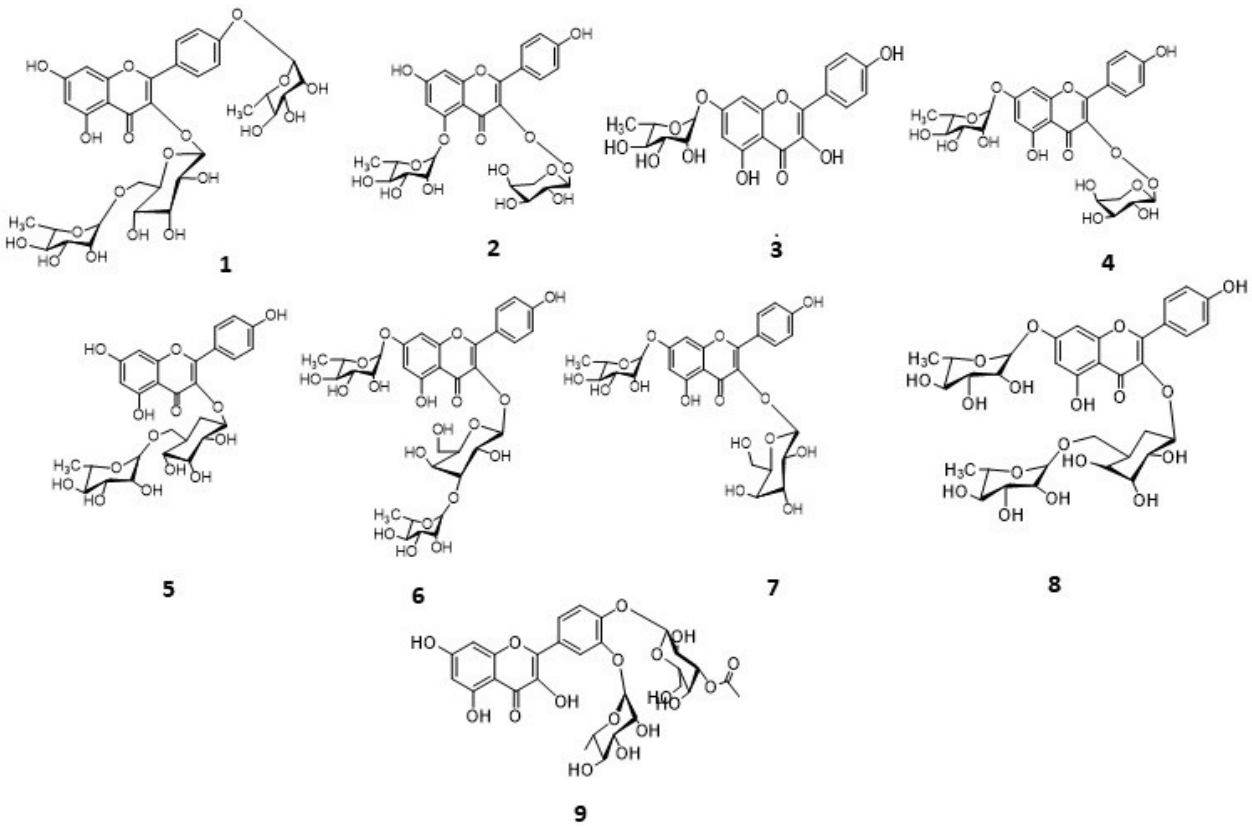

Fig. 1. Molecular structure of compounds (**1-9**).

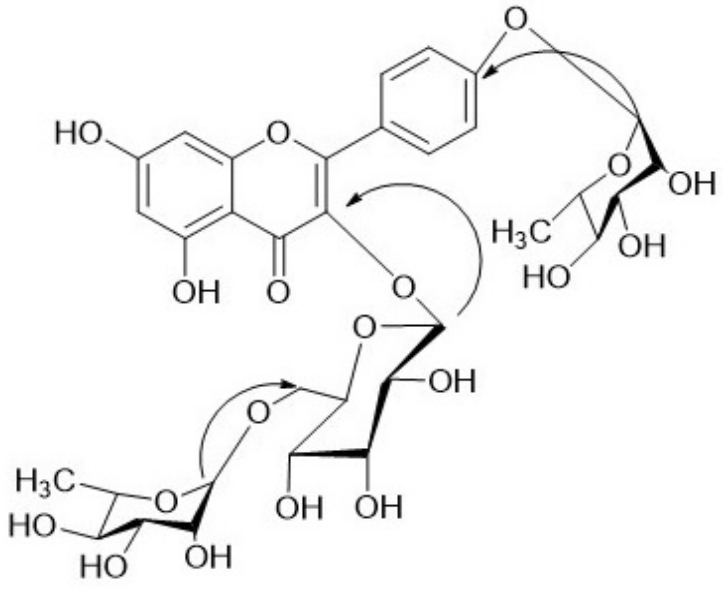

Fig. 2. Key HMBC correlation of compound (**1**).

Table 1

^{13}C (150 MHz, DMSO-*d6*),^{1}H NMR (600 MHz, DMSO-*d6*) and HMBC correlations assignments for (**1**) recorded in DMSO-*d6*.

	^{13}C NMR (δ, mult.)	**^{1}H NMR** [δ, mult., J (Hz)]	**HMBC**		**^{13}C NMR** (δ, mult.)	**^{1}H NMR** [δ, mult., J (Hz)]	**HMBC**
2	156.0, C	-		C-3- Rha (1→ 6) Glu.			
3	133.5, C	-		1‴	100.0, CH	4.25,d (1.8)	2‴, 3‴, 5‴
4	177.6, C	-		2‴	70.1, CH[a]	3.44, m	1‴
5	160.1, C	-		3‴	70.2, CH	3.81, m	2‴
6	99.3, CH	6.45,d (2.4)	5, 7, 8, 10,	4‴	71.8, CH[a]	3.43, m	3‴, 5‴
7	161.63, C	-		5‴	69.8, CH[b]	4.40,t (6.6)	4‴
8	94.6, CH	6.78,d (2.4)	6, 9, 10	6‴	17.9, CH_3	1.13,3H,d (6.6)	5‴
9	157.1, C	-	-	C-4´ Rha.			
10	105.6, C	-		1⁗	100.7, CH	5.55,br.s	5, 2⁗, 3⁗, 5⁗
1´	120.7, C	-		2⁗	71.4, CH[a]	3.44, m	1⁗
2´,6´	131.0, CH	8.11, 2H, d (7.2)	2, 3´, 4´, 5´	3⁗	72.3, CH	3.81, m	2⁗
3´,5´	116.1, CH	6.88, 2H, d (7.2)	4´, 2´, 6´	4⁗	71.1, CH[a]	3.43, m	3⁗, 5⁗
4´	161.65, C	-		5⁗	71.6, CH[b]	4.42,t (6.0)	4⁗
C-3 Glu.				6⁗	17.7, CH_3	1.06,3H,d (6.0)	5⁗
1''	101.8, CH	5.36, d (7.2)	3				
2''	71.9, CH	3.64, m	1''				
3''	73.6, CH	3.53, dd (9.6, 3.6)	2'', 6''				
4''	70.6, CHa	3.64, m	3''				
5''	72.9, CH	3.63, m	4''				
6''	68.2, CH2	3.80, m,	1''				
		3.57, m					

[a,b] Assignments bearing the same superscript may be reversed.

The HR-TOF-MS spectrum using Matrix assisted laser desorption ionization (MALDI) showed a quasi-molecular ion peak at m/z 741.2227 $[M + H]^+$, calculated as (740.2164) per the molecular formula $C_{33}H_{40}O_{19}$. It exhibited (UV) absorption at 266 and 347 nm. The structure of (**1**) was elucidated by 1-D and 2-D NMR spectroscopy, including ^{1}H, ^{13}C, and HMBC experiments, as well as HR-LC-TOF-MS. The ^{1}H NMR spectrum of (**1**) indicated the presence of a kaempferol moiety and three sugar moieties where a pair of doublets each is equivalent to two protons at δH 8.11, *J*=7.2 Hz. (H-2′, H-6′) and at δH 6.88, *J*=7.2 Hz. (H-3′, H-5′) is present, which are two features characteristic of a flavonol with phenolic group 4′.The anomeric protons showed characteristic TOF-doublets in the ^{1}H NMR spectrum at δH 5.36 for glucose with a doublet splitting of 7.2 Hz, indicating its β-configuration (Lambert et al., 1998) and at δH 5.55 for rhamnose, with a doublet splitting

of 1.8 Hz indicating its α-configuration, in addition to a third anomeric proton for another rhamnose, at δH 4.25 with a broad singlet indicating its α-configuration (Markham et al., 1978; Hasegawa et al., 2008). The ^{1}H NMR spectrum (600 MHz, DMSO-d*6*) was in agreement with 3,4´ substituted kaempferol moiety where the presence of two free hydroxyl groups at C-5 and C-7 which were confirmed from the two proton chemical shifts at δH 10.17 and δH 12.57 revealed the glycosylation to be at C-4´. Long-range correlations were observed in HMBC (Fig. 2) between the anomeric proton of glucose (δH 5.36) and the C-3 of flavonol (δC 133.5), confirming that the glucose was connected at this site, and between the anomeric proton of rhamnose (δH 5.55) and the C-6 of glucose (δC 68.2), verifying that rhamnose was connected at that site. Another long range correlation between H-6 of glucose (δH 3.80, m) and the anomeric carbon of rhamnose (δC 100.0) was noticed which is an additional evidence of substitution at this site. The site of attachment was also confirmed from the downfield shift of C-6 of glucose residue to δC 68.2, where in the free analogue, C-6 signal usually located at δC 61-62 (Markham et al., 1978). An important long range correlation is noticed between the anomeric proton of rhamnose (δH 5.55, br.s) and the C-4´ of kaempferol (δC 161.63) which is an evidence of substitution at C-4´ of the aglycone. From the above mentioned data, compound (**1**) could be assigned as kaempferol 4´-*O*-α-rhamnopyranosyl-3-*O*-α-rhamnopyranosyl-(1→6)-β-glucopyranoside which is a new flavonoid glycoside.

4. Concluding remarks

A new flavonoid glycoside, along with eight known ones were isolated from the flowers of *Vicia faba* L. (Leguminosae), which affords a plenty of compounds belonging to that class of high potential biological activities for further investigation.

Conflict of interest

The authors declare that there is no conflict of interest.

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Supplementary material

Additional supporting information including the experimental section and the NMR/MS spectral data of the new characterized compounds can be found in the online version of this article at the publisher's website (http://tpr.iau-shahrood.ac.ir/).

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