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Effect of polyphenols from *Vicia faba* L on lipase activity and melanogenesis

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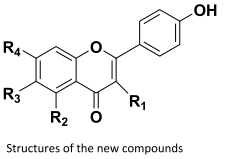
ABSTRACT

Two new flavonoid glycosides, kaempferol 3-*O*-*α*-L-rhamnopyranosyl (1→6) (3′′-acetyl)-*β*-D-galactopyranoside **1** and kaempferol 3-*O*-*α*-L-arabinopyranosyl-5-*O*-*α*-L-rhamnopyranoside **2**, along with six known ones **3–8** were isolated from the flowers of *Vicia faba* L. (Fabaceae). Methanol extract and the isolated compounds were tested against lipase and melanogenesis inhibition activities and resulted in that compound **2** showed 53 and 77% lipase inhibition activity in concentrations of 400 and 800 µg/mL, respectively. For melanogenesis, compounds **2**, **3** and **4** exhibited potent melanogenesis inhibition activity where the melanin content in melanoma cells was decreased to be about 57.5, 56 and 61%, respectively, with no obvious melanocytotoxicity. The rest of compounds showed weak to moderate activity. The results of melanogenesis inhibition activity of this study suggested the potential use of *Vicia faba* flowers as a skin-whitening agent and reveal the flowers to be a rich source of important phytochemicals with antilipase and melanogenesis inhibitory activity.



Structures of the new compounds

Vicia faba flowers

1 R_1= *O*-*α*-l-rhamnopyranosyl (1→6) (3′′-acetyl)-*β*-D-galactopyranosyl , R_2= OH, R_3= H, R_4= OH

2 R_1= *α*-l-arabinopyranosyl , R_2 = *O*-*α*-l-rhamnopyranoside, R_3= H, R_4= OH

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1. Introduction

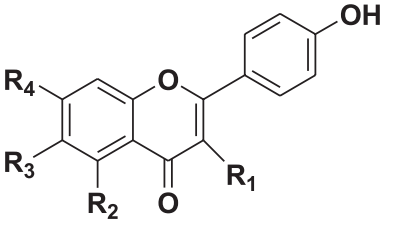
Vicia faba L. (broad bean) is a legume belonging to the plant family Fabaceae. It is an important winter crop in Mediterranean areas and is mostly a spring crop in other regions of Europe and South America and is one of the major plant food item for the Nile River populations. (Amarowicz and Pegg 2008). It is used in folk medicine as antihyperlipidimic to control cholesterol (Rabey et al. 1993; Mulvihill and Huff 2010; Bouchenak and Lamri-Senhadji 2013). For this reason, we were encouraged to search for bioactive compounds that have antilipase activity. The hydrolysis of triacylglycerols, and thus, its movement from the intestinal lumen into the body is stopped or minimised, the prevalence of obesity can be reduced (Han et al. 2005; Sharma et al. 2005). Also, in an effort to find a new whitening agent, we examined *Vicia faba* flowers and their isolated compounds with the aim of developing effective treatments against hyperpigmentation which occurs when an excess of melanin deposits in the skin. Common forms of hyperpigmentation are melasma, lentigo and age spots, (Briganti et al. 2003; Lim et al. 2009; Yamasaki et al. 2015). This study is the first of its kind to evaluate the ability of *Vicia faba* flowers and its isolated compounds to inhibit both lipase enzyme and melanogenesis in B16-F10 melanoma cell line.

2. Results and discussion

2.1. Structure elucidation of 1 and 2

Chemical study of the ethyl acetate fraction of *V. faba* afforded eight compounds (**1–8**) (Figure 1) of which six were identified by comparing their physicochemical and spectroscopic data with those reported in the literature as kaempferol 7-*O*-*α*-L-rhamnopyranoside **3**, (Veit and Pauli 1999), kaempferol 3-*O*-*α*-L-arabinopyranosyl-7-*O*-*α*-L-rhamnopyranoside **4** (Lawrence et al. 2003), kaempfrerol 3-*O*-rutinoside **5** (Petpiroon et al. 2015), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→3)-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside **6**, (Xu et al. 2009), kaempferol-3-*O*-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside **7**, (Keyume et al. 2006) and kaempferol 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranosyl-7-*O*-*α*-L-rhamnopyranoside **8.** (Jiaju et al. 2011).

Compound **1** was obtained as a yellow amorphous powder. The HR-TOF–MS spectrum showed a quasi-molecular ion peak at *m/z* 637.1245 $[M + H]^+$, calculated as (636.17) in



1	R_1= -*O*-*α*-l-rhamnopyranosyl- (1→6) (3´´-acetyl)- *β*-D-galactopyranosyl , R_2= OH, R_3= H, R_4= OH
2	R_1= *α*-l-arabinopyranosyl , R_2 = -*O*-*α*-l-rhamnopyranoside, R_3= H, R_4= OH 3
3	R_1= OH, R_2= OH, R_3= H, R_4= -*O*-*α*-L-rhamnopyranoside
4	R_1= -*O*- *α*- L -arabinopyranosyl , R_2= OH, R_3= H, R_4 = -*O*-*α*- L -rhamnopyranoside
5	R_1= -*O*-rutinoside , R_2= OH, R_3= H, R_4= OH
6	R_1= -*O*-*α*- L-rhamnopyranosyl-(1→3)-*β*- D- galactopyranosyl , R_2= OH, R_3= H, R_4= - *O*-*α*- L -rhamnopyranoside
7	R_1= -*O*-*β*-D-galactopyranosyl- , R_2= OH, R_3= H, R_4= -*O*-*α*- L -rhamnopyranoside
8	R_1= -*O*-*α*- L -rhamnopyranosyl (1→6)-*β*-D-galactopyranosyl-, R_2= OH, R_3= H, R_4= -*O*-*α*- L-rhamnopyranoside

Figure 1. Structures of compounds **1–8**.

accordance with the molecular formula $C_{29}H_{32}O_{16}$. It exhibited (UV) absorption at 230 and 254 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, two sugar moieties in addition to the presence of an acetyl moiety where a pair of doublets each is equivalent to two protons at δ_H 8.06, J = 8.9 Hz. (H-2′, H-6′) and at δ_H 6.89, J = 8.9 Hz. (H-3′, H-5′) is present, which are two features characteristic of a flavonol with phenolic group 4′. The spectrum showed also a signal at δ_H 1.90, 3H, suggesting the presence of an acetyl moiety. Analysis of chemical shifts and coupling constants in ^{1}H spectrum are in accordance with suggestion that the sugar residue is galactopyranosyl (Lambert et al. 1998). The anomeric protons showed characteristic doublets in the ^{1}H NMR spectrum at δ_H 5.12 for galactose with a doublet splitting of 7.2 Hz, indicating its β-configuration, and at δ_H 4.51 for rhamnose, with a doublet splitting of 1.2 Hz indicating its α-configuration (Markham et al. 1978; Hasegawa et al. 2008). Identification of the sugar residues was supported by GC/MS analysis of the alditole acetates obtained by mild acid hydrolysis (Leontein et al. 1978) as galactose and rhamnose. The ^{13}C NMR spectrum was in agreement with 3-substituted kaempferol moiety. Long-range correlations were observed in HMBC between the anomeric proton of galactose (δ_H 5.12) and the C-3 of flavonol (δ_C 135.5), confirming that the galactose was connected at this site, and between the anomeric proton of rhamnose (δ_H 4.51) and the C-6 of galactose (δ_C 68.6), verifying that rhamnose was connected at that site. Another HMBC correlation was also observed between H-3 of galactose (δ_H 3.53) and the carbonyl residue of the acetate moiety, confirming the site of its attachment. In addition to HMBC data, the sugar sequence was also confirmed to be (1→6) linkage from the NOESY experiment where the presence of inter-glycosidic NOE from the anomeric proton of rhamnose moiety (δ_H 4.51) to both H-6 of galactose (δ_H 3.80 and 3.42), which provides a very powerful means for determining the sugar sequence (Atta-ur-Rahman 2002). Also, 13C NMR downfield shift of C-6 of galactose residue to δ_C 68.6 confirmed the site of attachment to be at C-6 of galactose moiety where in the C-6 free analogue, C-6 usually at δ_C 61–62 (Markham et al. 1978).

Compound **2** was obtained as a yellow amorphous. The HR-LC-TOF MS spectrum showed a quasi-molecular ion peak at m/z 565.1508 $[M + H]^+$, calculated as (564.15), in accordance with the molecular formula $C_{26}H_{28}O_{14}$. It exhibited (UV) absorption at 230 and 280 nm. The ^{1}H-NMR spectrum of **2** was similar in its general features to that of **1** except for the presence of two 6-deoxy-hexose moieties attached to kaempferol; this was confirmed from the ^{13}C NMR spectrum, where the presence of eight methine carbon signals in addition to two anomeric carbon signals at δ_C 101.1 and 98.3, and one methyl carbon signal at δ_C 17.9, suggested the presence of two pentose moieties. One was confirmed to be rhamnose from both ^{1}H and ^{13}C NMR values at δ_H (5.55, brs., H-1‴, 1.25, 3H, d, J = 6.0, H-6‴) and at δ_C (98.3, C-1‴, 17.9, C-6‴), respectively. The other 6-deoxy-hexose moiety was confirmed from ^{1}H and ^{13}C NMR values to be β-D-arabinopyranosyl, where C-1″, C-2″, C-3″ and C-4″ of **2** were at δ_C 101.1, 71.5, 70.0 and 70.7, respectively, while in the α-L-arabinofuranosyl analogue, these values were shifted downward to about δ_C 107, 82, 76 and 85, respectively (Zhu et al. 2013). Identification of the sugar residues was supported by GC/MS analysis obtained by acid hydrolysis as arabinose and rhamnose. Long-range correlations were observed in HMBC between the anomeric proton of arabinose (δ_H 5.35) and the C-3 of flavonol (δ_C 133.8), confirming that it was the site of connection, and between the anomeric proton of rhamnose (δ_H 5.55) and the C-5 of flavonol (δ_C 160.8), clarifying that rhamnose was connected at that site.

2.2. Anti-lipase assay

Lipase inhibition activity of both methanol extract and the isolated compounds from *V. faba* L. flowers was shown in (Table 1). The results showed that methanol extract and compound **2** exhibit 66 and 81% for methanol extract, and 53 and 77% inhibition activity for compound **2** in concentrations of 400 and 800 µg/mL, respectively.

2.3. Melanogenesis inhibition assay

After establishing the structures, compounds (**1–8**) were investigated using B16-F10 melanoma cells to evaluate the inhibition of melanogenesis and cell viability at a final concentration of 20 µM using arbutin as a positive control at the same concentration. The effect of the compounds on cell viability refers to the cytotoxic effect of these compounds on melanocytes (melanocytotoxicity). The results of the assay are shown in (Table 2). Referring to cytotoxicity, the most active compounds with potent melanin synthesis inhibition and no obvious cytotoxicity were compounds **2**, **3** and **4** where the melanin content in melanoma cells was decreased to be about 57.5, 56 and 61%, respectively at a final concentration of 20 µM. Furthermore, these compounds are being safe to melanocytes reflected by the cell viability about 98, 102 and 100%, respectively, using MTT assay. For compound **2** and **3**, their inhibition activity was nearly

Table 1. Results of the *in vitro* assays for lipase inhibition activities of both methanol extract and isolated compounds of *V. faba* flowers.

Compound	Lipase inhibition %	
	Concentration (400 µg/mL)	Concentration (800 µg/mL)
1	33.60 ± 2.30	42.26 ± 3.41
2	52.98 ± 1.04	77.06 ± 1.25
3	12.52 ± 1.67	22.77 ± 2.08
4	4.78 ± 1.80	12.13 ± 2.70
5	1.96 ± 1.67	9.29 ± 0.80
6	7.09 ± 0.93	13.60 ± 1.93
7	15.53 ± 2.07	32.23 ± 0.60
8	5.37 ± 3.03	8.37 ± 2.49
Methanol extract	66.2 ± 2.41	81.18 ± 1.68
Positive control (Orlistat) (0.22 µg/mL)	95.94 ± 1.16	

Note: Data are shown as the mean ± SD ($n = 3$).

Table 2. Effects of the methanol extract and the isolated compounds on melanogenesis and cell proliferation of B16-F10 melanoma cells.

Compound	Melanin content %	Cell viability %
1	61.85 ± 7.75	84.80 ± 26.75
2	57.54 ± 6.80	97.99 ± 045
3	56.17 ± 0.26	101.98 ± 23.0
4	61.09 ± 4.54	100.81 ± 9.73
5	74.90 ± 24.07	98.23 ± 6.08
6	71.87 ± 27.82	82.25 ± 13.04
7	89.47 ± 0.85	77.20 ± 4.79
8	98.12 ± 7.39	88.49 ± 2.0
Methanol extract (160 µg/mL)	38.65 ± 5.79	100.78 ± 28.77
Methanol extract (40 µg/mL)	55.40 ± 3.77	92.80 ± 18.98
Positive control (Arbutin)	54.80 ± 3.46%	98.6 ± 19.37

Notes: The results are expressed as mean values ± SD ($n = 3$). Final concentration of the compounds and the positive control (arbutin) was 20 µM.

equal to that obtained with the positive control arbutin at the same concentration. For compound **1**, it has potent melanin synthesis inhibition but with a relative little effect on cell viability about 84% at a final concentration of 20 μM while for compounds **5** and **6**, the melanin content in melanoma cells decreased to about 75 and 72% at a concentration of 20 μM, reflect their good activity in inhibition of melanogenesis without affecting the cell viability as shown in (Table 2). Compounds **7** and **8** showed weak inhibition activity. All the compounds did not affect the viability of melanocytes; reflect their safety in inhibition of melanogenesis. The methanol extract showed about 60% inhibition of melanin at a final concentration of 160 μg/mL and by decreasing the concentration to 40 μg/mL, the extract suppressed the melanin content in the cells to about 45% without causing obvious cytotoxicity in both cases on melanocytes.

3. Conclusions

Two new flavonoid glycosides, along with six known ones, were isolated from the flowers of *Vicia faba*. The results indicated that compound **2** showed 53 and 77% lipase inhibition activity in concentrations of 400 and 800 μg/mL, respectively. Our study also showed that the new compounds **1** and **2** showed about 38 and 42.5% inhibition of melanogenesis at a final concentration of 20 μM. All the compounds did not affect the viability of melanocytes; reflect their safety in inhibition of melanogenesis. These results suggest the potential use of *Vicia faba* L, flowers as a lipase and a melanogenesis inhibitor and the possible use for it as a skin whitening agent.

Data analyses

Mean melanin production (μg/mL) and the percentage of control of melanogenesis are reported as the mean ± SD. Statistical significance was determined by Dunnett's multiple test $p < 0.05$ was considered statistically significant.

Supplementary material

Additional supporting information including the experimental section and the NMR/MS data of the new compounds can be found in the online version of this article at the publisher's website.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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