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Comparison of flavonoid contents and antioxidant activities of *Vicia* species

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Abstract

A total of 27 accessions from ten *Vicia* species were investigated for flavonoid contents, total polyphenol contents, and DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2′-azinobis (3-ethylbenzothiazoline 6-sulfonic acid)] free radical-scavenging activities. The results revealed that NAC17 (*V. monantha*) and NAC14 (*V. hyrcanica*) had the highest total flavonoid content (1.42 ± 0.09 mg/g) and total polyphenol content [124.2 ± 0.5 μg/gallic acid equivalents (GAE) mg], respectively. Among four flavonoids, naringenin was detected at high concentrations in *Vicia* species. The DPPH and ABTS assays showed values in the range of 57.2 (IC_{50}) (NAC13, *V. faba*) to 6530.0 (IC_{50}) (NAC24, *V. sativa* subsp. *nigra*) and 19.1 μg/Trolox mg (NAC7, *V. cracca*) to 253.4 μg/Trolox mg (NAC13, *V. faba*), respectively. Among ten *Vicia* species, *V. monantha* and *V. hyrcanica* had the highest flavonoid content (1.31 ± 0.09 mg/g) and total polyphenol content (116.5 ± 2.0 μg/GAE mg), respectively. The highest antioxidant activity was detected in *V. faba*. These results will expand the flavonoid database and provide valuable information on *Vicia* species for the development of functional foods or feed-additive resources.

Keywords: ABTS; antioxidant activity; DPPH; flavonoids; *Vicia* species

Introduction

Legumes are a diverse and important angiosperm family, with more than 650 genera and 18,000 species. Legumes are the third largest family of higher plants and second only to grasses in agricultural importance (Young *et al.*, 2003). The genus *Vicia* belongs to the Hologalegina clade of the legume family, and includes a number of important food and forage crops (Wojciechowski, 2003). Numerous vetch species are frequently found in local floras, which contribute to the quality of pasture and meadow communities as well as soil fertility (Mikic *et al.*, 2009). Vetch species are rich in nitrogen compared with other forage legumes, such as *Medicago sativa* or pea, and are a quality source of forage and green manure, as their crude dry matter protein concentration is >200 g/kg (Mikic *et al.*, 2009).

Antioxidant compounds have received attention from natural product consumers and researchers due to their pharmacological properties. Antioxidants lower the oxidative stress caused by reactive oxygen species (ROS) (Nordberg and Arner, 2001). There is increasing interest in natural antioxidant products for use as medicines and food additives (Mossi *et al.*, 2004; Willcox *et al.*, 2004). Phytochemicals, such as polyphenols and carotenoids, are important because of their contributions to human health and their multiple biological effects, such as antioxidant, antimutagenic, anticarcinogenic and cytoprotective activities (Ajila and Prasada Rao, 2008).

Flavonoids provide colour to plants and attract pollinators and seed dispersers. They include antioxidants to protect plants against UV radiation, attract insects, act as

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signalling molecules to facilitate nitrogen fixation and defend against bacterial and fungal attack, as well as their bitter or astringent taste repels birds and other animals (Croteau *et al.*, 2000; Wildman, 2001; Winkel-Shirley, 2001, 2002). Several health beneficial properties of dietary flavonoids have been recognized in humans due to their antioxidant and antiproliferative effects, which protect the body from various pathologies, such as cancers and cardiovascular and inflammatory diseases (Middleton *et al.*, 2000; Nijveldt *et al.*, 2001).

Because of their widespread occurrence and chemical stability, flavonoids are accepted as taxonomic chemical markers for characterizing and classifying higher plants (Asen, 1984; Harborne and Turner, 1984; Van Sumere *et al.*, 1985). Webb and Harborne (1991) studied flavonoid aglycones from acid-hydrolysed leaf extracts, indicating that flavonoid data are meaningful at the sectional level and suggested that variations in the glycosidic type present in *Vicia* flavonoids could be interesting from a chemotaxonomic perspective.

The nutritional properties of other *Vicia* species have been reported (Alzueta *et al.*, 2001). The polyphenol contents and antioxidant activities of *V. faba* (Amarowicz *et al.*, 2004) and *V. sativa* (Amarowicz *et al.*, 2008) have also been reported. The present study was conducted to (1) screen for flavonoid content and total polyphenol content (TPC) as well as antioxidant capacities in the stems and leaves of 27 *Vicia* accessions, and (2) identify flavonoids and total polyphenols in the 27 accessions of ten *Vicia* species with the highest antioxidant activities.

Materials and methods

Plant materials and sample preparation

A total of 27 accessions from ten *Vicia* species in 13 countries were obtained from USDA-ARS (Supplementary Table S1, available online). Crude extracts from the 27 samples were produced using 7 g of oven-dried stems and leaves from each accession using an ASE-200 extractor (Dionex, Sunnyvale, CA, USA). Extractions were performed in 40 ml of 75% EtOH under nitrogen gas at 1500 psi and 70°C. The extracted samples were dried using an HT-4X vacuum concentrator (Genevac, Stone Ridge, NY, USA).

DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activities of the extracts were assessed using the method of Lee and Lee (2004) with some modifications. The DPPH solution (150 μl; 150 μM in anhydrous EtOH) was added to 100 μl of the sample solution. The mixture was shaken vigorously and left to stand at 25°C in the dark for 30 min. Absorbance at 517 nm was measured using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). Results are expressed as half maximal inhibitory concentration (IC_{50}) and compared with ascorbic acid used as the standard.

ABTS assay

ABTS [2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid)] free radical-scavenging activity was estimated using the method of Re *et al.* (1999) with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulphate, followed by overnight incubation in the dark at room temperature. The ABTS radical cation solution was diluted with methanol (MeOH) to obtain an absorbance of 0.7 ± 0.02 at 735 nm. The diluted ABTS radical cation solution (190 μl) was added to 10 μl of the sample solution. After 6 min, absorbance at 735 nm was determined using a spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). Trolox was used as the standard, and results are expressed in μg of Trolox equivalents/mg of dried sample.

Flavonoid assay

The flavonoid aglycone contents of the 27 *Vicia* accessions were investigated using high-performance liquid chromatography (HPLC; Thermo Scientific, Waltham, MA, USA) with a Hypersil ODS column (125 × 4 mm, 5 μm particles; Hewlett-Packard, Palo Alto, CA, USA). Flavonoid aglycone contents were estimated using the method of Olszewska (2007) with some modifications. The eggplant leaf and fruit extract (50 mg) was heated at 90°C for 2 h with 10 ml of 1 N hydrochloric acid, followed by shaking for 2 h with 10 ml of MeOH. The hydrolysate was diluted with MeOH to 25 ml using a volumetric flask and filtered through a PTFE syringe filter (13 mm, 0.45 μm pore; Whatman, Maidstone, Kent, UK). Detection was performed at 370 nm.

TPC assay

TPC was measured using the modified Folin–Ciocalteu method (Waterhouse, 2002). Folin–Ciocalteu reagent (100 μl) was added to 100 μl of the sample solution and reacted at room temperature for 3 min. After adding 100 μl of 2% sodium carbonate, the mixture was

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incubated at room temperature for 30 min. Absorbance was measured at 750 nm using an ELISA reader with distilled water as the blank. Total phenolic content is expressed as milligrams of gallic acid equivalents (GAE) per gram dried weight sample (μg/GAE mg of dry seed).

Statistical analyses

Duncan's multiple-range test was used to detect significant differences among the 27 *Vicia* accessions using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). Results of DPPH, expressed as IC_{50}, were converted to $1/IC_{50}$, before the clustering analysis and correlation analysis. Hierarchical clustering was performed using the R statistical software environment (http://www.r-project.org). Distances were calculated using complete linkage clustering and Pearson's correlation analysis. A P value <0.05 was considered to indicate significance.

Results

Flavonoid content and TPC in the 27 Vicia *accessions*

The distribution of flavonoids in the 27 accessions of ten *Vicia* species is presented in Table 1 and Supplementary Table S1 (available online). Among the flavonoids, myricetin, kaempferol, naringenin and isorhamnetin were detected in all the 27 accessions of *Vicia* species. The total flavonoid content (TFC) was 0.03–1.42 mg/g among the 27 *Vicia* accessions. Among these accessions, the TFC was the highest in NAC17 (*V. monantha*, 1.42 ± 0.09 mg/g). Similarly, NAC6 (*V. cracca*) exhibited the lowest polyphenol content (0.03 ± 0.01 mg/g). Of the four detected flavonoids, the content of naringenin was high in 22 *Vicia* accessions, but was not detected in the accessions NAC14, NAC15 and NAC16 (*V. hyrcanica*), and NAC19 and NAC20 (*V. peregrina*). The content of naringenin was 0.05–1.38 mg/g in 22 *Vicia* accessions. Naringenin was found at high levels in NAC17 (*V. monantha*, 1.42 ± 0.09 mg/g) and NAC18 (*V. monantha*, 1.10 ± 0.01 mg/g) accessions. Kaempferol was detected in only four accessions, namely NAC11, NAC12, NAC13 (*V. faba*) and NAC22 (*V. sativa*). The content of kaempferol in three accessions, NAC11, NAC12 and NAC13, was 0.04 mg/g, whereas the content in the NAC22 accession was 0.01 mg/g. The content of myricetin in the 27 *Vicia* accessions was 0.02–0.08 mg/g. Of the accessions, NAC2 (*V. articulata*, 0.08 ± 0.00 mg/g) had the highest myricetin content and NAC11 (*V. faba*, 0.02 ± 0.00 mg/g) had the lowest. Myricetin was not detected in eight accessions, namely NAC4, NAC5, NAC6, NAC7 (*V. cracca*), NAC21, NAC22 (*V. sativa*), NAC24 and NAC25 (*V. sativa* subsp. *nigra*). Among the 27 *Vicia* accessions, isorhamnetin was detected in 13 accessions. The content of isorhamnetin was high in NAC2 (0.14 ± 0.01 mg/g) and low in NAC20 (*V. peregrina*, 0.01 mg/g). The TPC in all the 27 accessions of *Vicia* species was found to be 15.9–124.2 μg/GAE mg, as determined by the Folin–Ciocalteu method (Fig. 1(b); Supplementary Table 1, available online). NAC14 (*V. hyrcanica*) had a significantly ($P < 0.05$) greater TPC (124.2 ± 0.5 μg/GAE mg) than the other accessions, whereas NAC7 (*V. cracca*) had the lowest TPC (15.9 ± 0.2 μg/GAE mg).

Antioxidant activities of the 27 Vicia *accessions*

DPPH and ABTS assays were performed using an ethanol extract to determine the antioxidant activities of the 27 *Vicia* accessions (Table 1; Supplementary Table S1, available online). The DPPH radical-scavenging activities of the 27 *Vicia* accessions were in the range of 57.2 to 6530.0 (IC_{50}). NAC13 (*V. faba*) had the highest DPPH radical-scavenging activity (57.2 ± 0.3, IC_{50}) and NAC24 (*V. sativa* subsp. *nigra*) had the lowest (6530.0 ± 15.3, IC_{50}). The antioxidant activities to scavenge ABTS free radicals in the 27 *Vicia* accessions were

Table 1. Descriptive statistics of flavonoid and total polyphenol contents, and antioxidant activities in the 27 *Vicia* accessions

	No. of accessions	Min	Max	Mean	SD	Skewness	Kurtosis
Myricetin (mg/g)	19	0.016	0.075	0.035	0.017	0.746	−0.238
Naringenin (mg/g)	22	0.030	1.380	0.342	0.393	1.454	1.069
Kaempferol (mg/g)	4	0.005	0.037	0.029	0.016	−1.995	3.983
Isorhamnetin (mg/g)	13	0.006	0.136	0.058	0.041	0.261	−0.894
TFC (mg/g)	27	0.027	1.421	0.334	0.378	1.577	1.678
TPC (μg/GAE mg)	27	15.9	124.2	39.2	37.3	1.707	1.085
DPPH (IC_{50})	27	57.2	6530.0	1631.1	1406.5	1.860	4.745
ABTS (μg/Trolox mg)	27	18.3	253.4	53.9	63.6	−0.239	−0.452

TFC, total flavonoid content; TPC, total polyphenol content; GAE, gallic acid equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, half maximal inhibitory concentration; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid).

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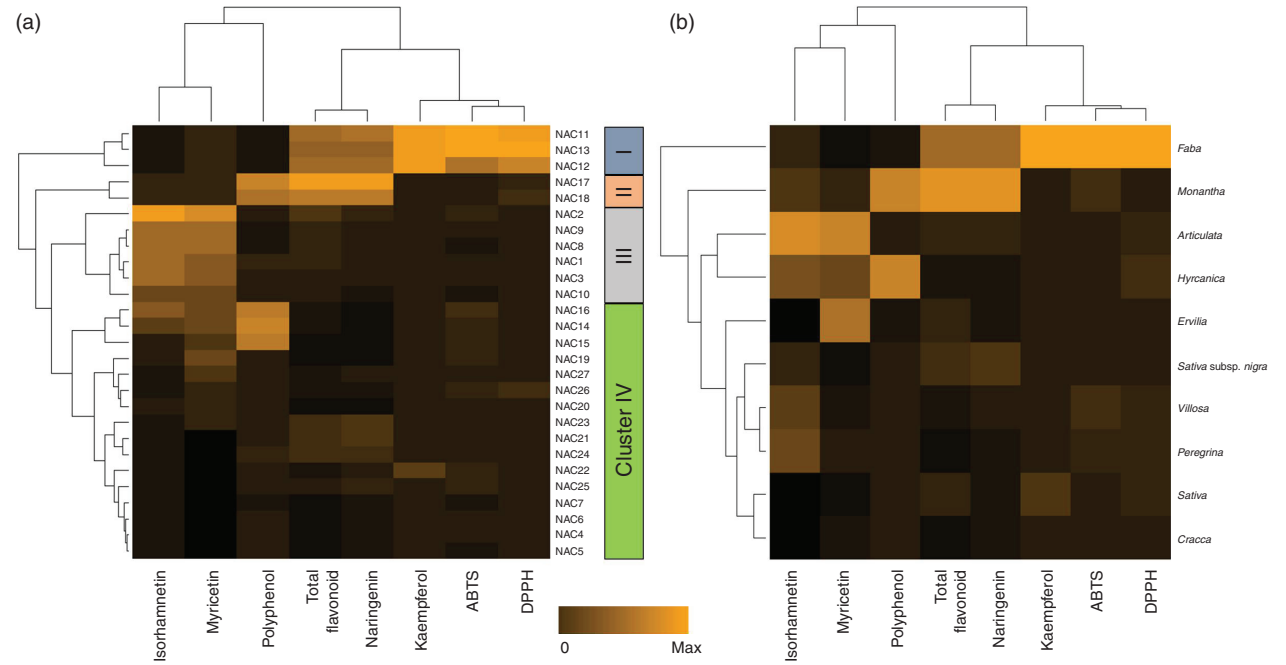

Fig. 1. Hierarchical clustering analysis of flavonoid and total polyphenol contents, and antioxidant activities in the 27 *Vicia* accessions (a) and ten *Vicia* species (b). ABTS, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

19.1–253.4 μg/Trolox mg. NAC13 (*V. faba*) had the highest ABTS free radical-scavenging activity (253.4 ± 4.6 μg/Trolox mg), and NAC7 (*V. cracca*) had the lowest (19.1 ± 0.6 μg/Trolox mg).

TFC, TPC and antioxidant activities of ten Vicia *species*

Table 2 presents the total flavonoid and polyphenol contents and antioxidant activities in the ethanol extracts of ten *Vicia* species. The TFCs of ten *Vicia* species were in the range of 0.04–1.31 mg/g. Among them, *V. monantha* had the highest TFC (1.31 ± 0.09 mg/g) and *V. cracca* had the lowest (0.04 ± 0.00 mg/g). Kaempferol was detected only in *V. faba* (0.04 ± 0.00 mg/g) and *V. sativa* (0.01 ± 0.00 mg/g). Naringenin content was in the range of 0.08–1.26 mg/g. *V. monantha* (1.26 ± 0.09 mg/g) had the highest naringenin content and *V. cracca* (0.04 ± 0.00 mg/g) had the lowest. However, naringenin was not detected in *V. hyrcanica* and *V. peregrina*. Myricetin and isorhamnetin contents were in the rage of 0.2–0.06 mg/g and 0.1–0.10 mg/g, respectively. The TPC was 18.3–116.5 μg/GAE mg in the ten *Vicia* species. Among them, *V. hyrcanica* had the highest TPC (116.5 ± 2.0 μg/GAE mg), whereas *V. ervilia* had the lowest (18.3 ± 1.0 μg/GAE mg). *V. faba* showed the highest antioxidant activities to scavenge DPPH and ABTS free radicals (63.7 ± 5.1, IC_{50} and 223.7 ± 14.7 μg/Trolox mg, respectively).

Clustering analysis

The 27 accessions of *Vicia* species were classified into four clusters according to their TFC, TPC and antioxidant activities (Fig. 1(a)). Cluster I contained NAC11, NAC12 and NAC13 with higher DPPH and ABTS free radical-scavenging activities and kaempferol contents than those of the other clusters. Cluster II consisted of NAC17 and NAC18 with higher TFC, TPC and naringenin content than those in the other clusters. Cluster III comprised six accessions with higher isorhamnetin and myricetin contents than those in the other clusters. Cluster IV comprised three sub-clusters, which were divided based on the TPC and the presence or absence of myricetin (Fig. 1(a)).

The ten *Vicia* species were also divided into four clusters (Fig. 1(b)). Cluster I contained only *V. faba* with a higher kaempferol content and antioxidant activity compared with those in the other clusters. Cluster II contained only *V. monantha*, which showed higher TPC, TPF and naringenin contents than those in the other clusters. Cluster III contained *V. articulata* and *V. hyrcanica*, which showed higher myricetin and isorhamnetin contents than those in the other clusters. Cluster IV

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Table 2. Flavonoid and total polyphenol contents, and antioxidant activities in ten *Vicia* species

Scientific name	Flavonoid aglycones (mg/g)					TPC (μg/GAE mg)	DPPH (IC_{50})	ABTS (μg/Trolox mg)	Cluster[a]
	Myricetin	Naringenin	Kaempferol	Isorhamnetin	TFC				
V. faba	0.02 ± 0.00a	0.87 ± 0.02d	0.04 ± 0.00b		0.92 ± 0.03c	18.5 ± 0.6a	63.7 ± 5.1c	223.7 ± 14.7c	I
V. monantha	0.02 ± 0.00ab	1.26 ± 0.09e		0.02 ± 0.00a	1.31 ± 0.09d	114.0 ± 2.9d	542.8 ± 109.1b	29.7 ± 2.5ab	II
V. articulata	0.06 ± 0.00e	0.16 ± 0.02c		0.1 ± 0.01d	0.33 ± 0.04b	25.0 ± 1.1c	2123.4 ± 878.4a	39.1 ± 2.1ab	III
V. hyrcanica				0.05 ± 0.01b	0.05 ± 0.01a	116.5 ± 2.0d	1822.2 ± 468.0a	47.0 ± 1.2b	
V. ervilia	0.05 ± 0.00d	0.11 ± 0.01b		0.08 ± 0.01c	0.25 ± 0.02b	18.3 ± 1.0a	2180.7 ± 77.2a	22.0 ± 1.1a	IV
V. sativa subsp. *nigra*	0.02 ± 0.00a	0.35 ± 0.01c			0.36 ± 0.02b	26.3 ± 1.2c	2943 ± 1799.6a	26.2 ± 0.9ab	
V. villosa	0.03 ± 0.00bc	0.08 ± 0.01ab		0.01 ± 0.00a	0.11 ± 0.01a	24.7 ± 1.1c	632.4 ± 262.9b	33.2 ± 4.3ab	
V. peregrina	0.03 ± 0.01c			0.01 ± 0.00a	0.04 ± 0.01a	21.8 ± 0.2abc	861.9 ± 8.4a	36.3 ± 3.4ab	
V. sativa		0.27 ± 0.06c	0.01 ± 0.00a		0.28 ± 0.07b	23.0 ± 1.0bc	2675.3 ± 1254.3a	36.2 ± 5.3ab	
V. cracca		0.04 ± 0.00a			0.04 ± 0.00a	20.5 ± 0.9ab	1803.7 ± 221.3a	23.1 ± 1.3a	

TFC, total flavonoid content; TPC, total polyphenol content; GAE, gallic acid equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC_{50}, half maximal inhibitory concentration; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid).
Values within a column with different letters were statistically significant ($P < 0.05$; Duncan's multiple range test).
[a]The clusters were obtained from the hierarchical clustering analysis (Fig. 1(b)).

comprised three sub-clusters, which were divided based on isorhamnetin and myricetin contents (Fig. 1(b)).

Correlation among the parameters of the 27 Vicia *accessions*

The correlations among flavonoids, TPC, DPPH and ABTS are given in Table 3. A negative correlation was detected between myricetin and naringenin ($r = -0.608$, $P < 0.05$) and myricetin and DPPH ($r = -0.526$, $P < 0.05$). The naringenin content was positively correlated with kaempferol ($r = 0.996$, $P < 0.01$), TFC ($r = 0.987$, $P < 0.01$), DPPH ($r = 0.563$, $P < 0.05$), ABTS ($r = 0.509$, $P < 0.05$) and TPC ($r = 0.702$, $P < 0.01$). The kaempferol content was positively correlated with TFC ($r = 0.996$, $P < 0.01$) and DPPH ($r = 0.972$, $P < 0.01$). TFC was positively correlated with antioxidant activities (DPPH, $r = 0.574$, $P < 0.01$; ABTS, $r = 0.507$, $P < 0.01$). DPPH and ABTS free radical-scavenging activities were positively correlated ($r = 0.979$, $P < 0.01$).

Discussion

We revealed that the TFCs of 27 accessions of ten *Vicia* species varied markedly. The flavonoids myricetin, kaempferol, isorhamnetin and naringenin were detected in all the 27 accessions of *Vicia* species. Campeol *et al.* (2003) reported that four *Vicia* species had various flavonoid patterns. In particular, kaempferol and quercetin were present in different quantities in the four species. In our results, the 27 *Vicia* accessions showed various flavonoid contents. Because the 27 *Vicia* accessions comprised ten *Vicia* species collected in 13 countries, we expected that they would have various flavonoid compositions. In addition, the characteristic flavonoid compositions of the 27 *Vicia* accessions of various origins may be of interest for developing pharmaceutical or nutraceutical products.

Flavonoids and total polyphenols are important secondary plant metabolites present at high levels in plants under stress (Koh *et al.*, 2009; Stanojevic *et al.*, 2009), as they play a role in reducing the oxidative stress caused by ROS (Patil and Jadhav, 2013). In this study, naringenin, kaempferol and TFC were positively correlated with DPPH free radical-scavenging activity, whereas myricetin was negatively correlated. TFC and naringenin were positively correlated with ABTS free radical-scavenging activity. Gee and Johnson (2001) and Malaveille *et al.* (1996) reported that myricetin, kaempferol and naringenin exert their effects by controlling the mechanisms of ROS production, which may be a

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Table 3. Correlations among flavonoids, total polyphenol and antioxidant activities of the 27 *Vicia* accessions

	Myricetin	Naringenin	Kaempferol	Isorhamnetin	TFC	DPPH	ABTS
Naringenin	−0.608*						
Kaempferol	−0.693	0.996**					
Isorhamnetin	0.920**	−0.116	–				
TFC	−0.391	0.987**	0.996**	0.122			
DPPH	−0.526*	0.563**	0.972*	−0.314	0.574**		
ABTS	−0.452	0.509*	0.894	0.208	0.507**	0.979**	
TPC	−0.178	0.702**	−0.869	−0.112	0.256	−0.168	−0.13

TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid); TPC, total polyphenol content.
Pearson's correlation coefficient: * $P < 0.05$, ** $P < 0.01$.

positive effect considering the importance of antioxidant agents during oxidative stress. Our results show similar correlations of flavonoids with antioxidant activities, with the exception of myricetin. Our finding of a correlation between myricetin and antioxidant activity was different from other studies, but may be related to the complex mixtures in the extracts, which have distinct activities (Mensor *et al.*, 2001; Hou *et al.*, 2003).

Six flavonoid aglycones (apigenin, myricetin, kaempferol, luteolin, quercetin and diosmetin) were present in the acid-hydrolysed leaf extracts of *Vicia* species (Webb and Harborne, 1991). Campeol *et al.* (2000) reported the presence of kaempferol and quercetin in three *Vicia* species, and Perrino and Maruca (1989) identified the flavonoids apigenin, kaempferol and quercetin in *Vicia* species. In particular, kaempferol was the most abundant flavonoid aglycone in *V. faba*. Although kaempferol was not the main flavonoid in our accessions, it was detected in four accessions, three of which were *V. faba*. Naringenin was the predominant flavonoid because we used leaves and stems, whereas kaempferol was found to be the most abundant flavonoid in previous reports. We also found that *Vicia* stems contain abundant naringenin; however, more detailed studies are needed to determine the flavonoid contents of *Vicia* species.

Naringenin (4′,5,7-trihydroxyflavanone) has several biological activities, such as antimicrobial, expectorant, antitussive, antiatherogenic, antifibrogenic, neuroprotective, anticomplementary, antimutagenic, antioxidant, anticancer, anti-inflammatory and inhibitory effects on asthma and acute lung injury (Choi *et al.*, 1994; Takahashi *et al.*, 1998; Lee *et al.*, 2001; Kumar *et al.*, 2003; Lee *et al.*, 2004). It also has antidiabetic, antiproliferative and anticancer activities (Lin and Chiou, 2009; Annadurai *et al.*, 2012). Naringenin feed supplementation increases egg production, reduces egg yolk cholesterol content, reduces serum cholesterol and triglyceride concentrations, and improves antioxidant activities (Lien *et al.*, 2008). Many studies have reported the presence of naringenin in various plants. Lee *et al.* (2007) reported that 0.20 mg/g of naringenin is present in citrus peels. The truffle (*Terfezia boudieri* Chatin) contains 0.25 μg/g of naringenin (Akyuz, 2013). The mean concentration of naringenin in cultivated *Astragalus membranaceus* is 49.0 μg/g (Jun *et al.*, 2012). Our *Vicia* accessions had high naringenin contents, which accounted for >70% of TFC (Supplementary Table S1, available online). In particular, NAC17 and NAC18 (*V. monantha*) had naringenin contents higher (1.38 ± 0.09 and 1.06 ± 0.02 mg/g, respectively) than those reported previously. This novel finding will facilitate the development of resources with high naringenin concentrations.

In conclusion, we found that the flavonoid patterns differed among the ten *Vicia* species (Table 2). The flavonoid patterns of *Vicia* species have been investigated using HPLC and UV spectrophotometry (Perrino and Maruca, 1989; Webb and Harborne, 1991; Campeol *et al.*, 2000; Campeol *et al.*, 2003). Those studies reported that flavonoid data are meaningful at the sectional level, and suggested that variations in glycosidic type present in *Vicia* flavonoids could be interesting from a chemotaxonomic perspective. These results indicate that the ten *Vicia* species are closely associated with their flavonoid contents, geographical origins and antioxidant activities. Although a broader investigation of *Vicia* species is needed to confirm the characterization, our results are a preliminary step in the investigation of the flavonoid contents of *Vicia* species.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S147926211500043X

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