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Chapter

Antioxidant Activity of Faba Bean Extracts

Wojciech Rybiński, Magdalena Karamać, Katarzyna Sulewska and Ryszard Amarowicz

Abstract

Phenolic compounds were extracted from seeds of 22 cultivars of faba bean (*Vicia faba* L.) by using 80% (v/v) aqueous acetone. The total phenolic compound and condensed tannins contents of the extracts and their antioxidant activity were determined using the Folin-Ciocalteu's phenol reagent, vanillin/HCl method, and ABTS and FRAP assays, respectively. The content of total phenolic compounds ranged from 40.7 to 66.1 mg/g extract and from 326 to 574 mg/100 g seeds. Contents of condensed tannins ranged from 2.40 to 49.9 mg/g extract and from 22.2 (FAB) to 365 mg/100 seeds. The extracts and seeds were characterized by Trolox equivalent antioxidant capacity (TEAC) values ranging from 0.550 (FAB 443) to 1.030 mmol Trolox/g extract (FAB 187) and from 4.85 (FAB 318) to 9.81 mmol Trolox/100 g seeds (FAB 187). Ferric-reducing antioxidant power (FRAP) values varied from 0.595 (FAB 443) to 0.908 mmol Fe^{2+}/g extract (FAB 5023) and from 4.61 (FAB 297) to 7.90 mmol Fe^{2+}/100 g seeds (FAB 187). The total phenolic content of faba bean extract was correlated with the results of the ABTS (r = 0.864) and FRAP (r = 0.862) assays. The coefficients of correlations between the contents of condensed tannins and ABTS and FRAP results were 0.543 and 0.862. We also noted a correlation between results of ABTS and FRAP assays (r = 0.795).

Keywords: faba bean, *Vicia faba* L., phenolic compounds, antioxidant activity, ABTS assay, DPPH assay

1. Introduction

Phenolic compounds of plant origin can inhibit or delay the oxidation of nutrients present in food products. In the human organism, natural antioxidants can protect lipids, proteins, and DNA against reactive oxygen and nitrogen species (ROS, RNS) [1, 2]. Results of numerous research groups demonstrate the protective effect of consuming phenolic-rich grains, legumes, oilseeds, fruits, berries, and nuts against several chronic diseases [3–7].

In human nutrition, legumes are an important source of proteins, starch, oligosaccharides (prebiotics), dietary fibers, vitamins, and minerals [8, 9]. As a rich source of natural antioxidants, legumes can play an important role in cardio and cancer protection [5, 10–15].

Faba bean (*Vicia faba*) is a species of Fabaceae family. It is native to South America, North Africa, and southwest and south Asia and is extensively cultivated elsewhere. Similar to other legumes, faba bean seeds contain phenolic compounds

[16] including condensed tannins [17–19]. The antioxidant potential of faba bean was determined using DPPH, FRAP, and ORAC assays [17, 20, 21]. Very high antioxidant capacity of *Vicia faba* sprouts was confirmed by Okumura et al. [22].

2. Experimental

2.1 Material

Plant material consisted of a collection of 22 faba bean accessions derived from Syria, Morocco, Tunisia, Sudan, Egypt, Yemen, Israel, Georgia, Azerbaijan, Tajikistan, Mongolia, Afghanistan, India, Australia, and Poland (**Table 1**). Information about seed coat color and 100 seed weight was provided on the basis of results obtained in a field experiment conducted in Cerekwica (Poland, 51°55′N, 17°21′E).

Description of faba bean seeds is reported in **Table 1**.

2.2 Chemicals

Ferrous chloride, sodium persulfate, ferrous chloride, 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),

No.	Accession code	Country of origin	Seeds coat color	Weight of 100 seeds (g)
1	FAB 337	Syria	Dark	47.2
2	FAB 443	Syria	Bright	76.6
3	FAB 5023	Morocco	Bright	45.8
4	FAB 5019	Morocco	Bright	52.1
5	FAB 6440	Tunisia	Bright	68.0
6	FAB 6441	Tunisia	Bright	65.0
7	FAB 225	Sudan	Bright	64.7
8	FAB 297	Sudan	Bright	45.0
9	FAB 6474	Egypt	Bright	27.4
10	FAB 219	Yemen	Bright	57.0
11	FAB 6318	Israel	Bright	81.5
12	FAB 344	Israel	Dark	46.8
13	FAB 604	Georgia	Bright	66.3
14	FAB 294	Azerbaijan	Bright	43.4
15	FAB 354	Tajikistan	Bright	40.0
16	FAB 202	Mongolia	Bright	68.4
17	FAB 144	Afghanistan	Dark	65.5
18	FAB 187	Afghanistan	Bright	30.9
19	FAB 250	India	Bright	38.6
20	FAB 446	India	Bright	41.1
21	FAB 7077	Australia	Bright	72.5
22	Martin	Poland	Bright	55.8

Table 1.
Description of faba bean seeds.

6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), Folin–Ciocalteu's phenol reagent, and (+)-catechin were purchased from Sigma (Poznań, Poland). Methanol, acetone, and hexane were obtained from P.O.Ch. Company (Gliwice, Poland).

2.3 Extraction

Phenolic compounds were extracted from grounded and defatted with hexane faba bean seed by using 80% acetone (v/v) at a solid to solvent ratio of 1:10 (w/v), for 15 min at 50°C [23]. Extraction was carried out in flasks placed in a shaking water bath (Elpan 357, Wrocław, Poland). Acetone from the combined extract was evaporated using a Büchi rotary evaporator. The sample was then freeze-dried.

2.4 Determination of total phenolic compound contents

The content of total phenolic compounds in the extracts was determined using the Folin–Ciocalteu's phenol reagent [24]. The results were expressed as (+)-catechin equivalents per g of the extract and per 100 g of seeds.

2.5 Determination of condensed tannins content

The content of condensed tannins was determined using a vanillin/HCl colorimetric method [25]. The results obtained were reported as mg catechin equivalent per g extract and 100 g of seeds.

2.6 ABTS assay

The Trolox equivalent antioxidant capacity (TEAC) was determined using the method of Re et al. [26]. The results obtained were reported as mmol Trolox equivalents per g extract and 100 g of seeds.

2.7 FRAP assay

The ferric-reducing antioxidant power (FRAP) assay was performed as previously described by Benzie and Strain [27]. The results obtained were reported as mmol Fe^{2+} equivalents per g extract and 100 g of seeds.

2.8 Statistical analysis

The results are reported as a mean value of three determinations ± standard deviation. The Pearson correlation was used to determine the relation between results of total phenolics, condensed tannins, TEAC, and FRAP values. Moreover the principal component analysis (PCA) and hierarchical cluster analysis (HCA) with Ward's method using Euclidean distances were conducted. Statistical and chemometric analyses of data were performed using the Statistica software (Windows software package 8.0).

3. Results and discussion

3.1 Content of total phenolics and condensed tannins

The content of total phenolics in the extracts was determined using the Folin–Ciocalteu's phenol reagent. The results were expressed as (+)-catechin equivalents per g of the extract or 100 g seeds. The results are reported in **Tables 2** and **3**. The content of total phenolics ranged from 40.7 (FAB 219) to 66.1 mg/g extract (FAB 187) and from 326 (FAB 219) to 574 mg/100 g seeds (FAB 5019).

The content of condensed tannins in the extracts was determined using the vanillin/HCl colorimetric method. The results ranged from 2.40 (FAB 297) to 49.9 mg/g extract (FAB 225) and from 22.2 (FAB 297) to 441 mg/100 seeds (FAB 5019) (**Tables 1** and **3**).

The results obtained in this research confirm the fact that faba bean is a rich source of phenolic compounds as well as condensed tannins. A lower content of total phenolic compounds was previously reported for extracts of pea [28], white bean [29], broad bean [23, 30] lupin [31], and grass pea [32]. A similarly high content of total phenolic compounds was previously reported for red lentil [33], green lentil [34], red bean [35], and adzuki bean [36].

The presence of condensed tannins in faba bean seeds determined with the vanillin method was reported by Amarowicz et al. [37], Baginsky et al. [38], Amarowicz and Shahidi [39], and Zduńczyk et al. [17]. In a research conducted by

No	Total phenolics[a] (mg/g extract)	Condensed tannins[b] (mg/g extract)	TEAC (mmol TE/g extract)	FRAP (mmol Fe^{2+}/g extract)
1	51.5 ± 0.4	26.4 ± 0.7	0.656 ± 0.014	0.673 ± 0.014
2	42.1 ± 1.0	10.9 ± 0.9	0.550 ± 0.016	0.535 ± 0.010
3	61.2 ± 0.5	25.9 ± 1.4	0.860 ± 0.011	0.908 ± 0.012
4	62.8 ± 2.4	48.2 ± 1.3	0.854 ± 0.022	0.753 ± 0.008
5	60.5 ± 1.6	43.2 ± 1.8	0.775 ± 0.023	0.742 ± 0.011
6	47.6 ± 0.2	21.5 ± 1.1	0.563 ± 0.008	0.634 ± 014
7	60.2 ± 0.7	49.9 ± 0.7	0.886 ± 0.017	0.715 ± 0.015
8	46.9 ± 0.9	2.40 ± 0.72	0.710 ± 0.018	0.572 ± 0.013
9	41.8 ± 0.4	8.77 ± 1.06	0.669 ± 0.014	0.636 ± 0.009
10	40.7 ± 1.4	4.32 ± 0.78	0.661 ± 0.012	0.603 ± 0.016
11	45.5 ± 0.3	4.15 ± 0.69	0.592 ± 0.011	0.651 ± 0.014
12	55.0 ± 1.6	22.1 ± 1.6	0.826 ± 0.022	0.734 ± 0.012
13	59.1 ± 1.1	21.3 ± 1.5	0.806 ± 0.020	0.807 ± 0.011
14	46.8 ± 1.0	35.3 ± 2.1	0.691 ± 0.004	0.671 ± 0.011
15	61.1 ± 1.4	49.1 ± 1.4	0.844 ± 0.017	0.824 ± 0.014
16	51.7 ± 1.5	28.2 ± 1.3	0.841 ± 0.024	0.703 ± 0.012
17	46.3 ± 1.5	23.8 ± 1.4	0.721 ± 0.014	0.588 ± 0.008
18	66.1 ± 1.9	32.5 ± 1.3	1.035 ± 0.014	0.833 ± 0.009
19	47.9 ± 1.0	14.4 ± 0.8	0.631 ± 0.011	0.718 ± 0.013
20	48.8 ± 1.7	47.8 ± 2.3	0.694 ± 0.009	0.675 ± 0.010
21	48.7 ± 0.6	34.2 ± 1.5	0.631 ± 0.010	0.694 ± 0.015
22	65.7 ± 1.9	39.9 ± 1.1	0.894 ± 0.015	0.807 ± 0.007

[a]*Catechin equivalents.*
[b]*Catechin equivalents.*

Table 2.
Contents of total phenolic compounds and condensed tannins and antioxidant activity of faba bean extracts. Results are reported per g of extract.

No	Total phenolics[a] (mg/100 g seeds)	Condensed tannins[b] (mg/100 g seeds)	TEAC (mmol TE/100 g seeds)	FRAP (mmol Fe^{2+}/100 g seeds)
1	484 ± 4	247 ± 6	6.16 ± 0.13	6.32 ± 0.13
2	421 ± 10	109 ± 19	5.50 ± 0.16	5.35 ± 0.10
3	524 ± 5	222 ± 12	7.37 ± 0.09	7.78 ± 0.10
4	574 ± 22	441 ± 12	7.80 ± 0.20	6.88 ± 0.08
5	509 ± 13	362 ± 15	6.51 ± 0.20	6.23 ± 0.09
6	425 ± 2	192 ± 9	5.82 ± 0.07	5.66 ± 0.13
7	539 ± 6	447 ± 6	7.94 ± 0.15	6.40 ± 0.13
8	378 ± 7	22.2 ± 1.0	5.72 ± 0.15	4.61 ± 0.10
9	395 ± 4	82.8 ± 9.9	6.31 ± 0.13	6.01 ± 0.08
10	326 ± 11	34.5 ± 7.5	5.28 ± 0.10	4.82 ± 0.13
11	373 ± 2	34.0 ± 4.9	4.85 ± 0.09	5.34 ± 0.11
12	475 ± 13	191 ± 14	7.13 ± 0.19	6.34 ± 0.10
13	559 ± 9	201 ± 14	7.63 ± 0.19	7.64 ± 0.10
14	410 ± 9	309 ± 18	6.04 ± 0.03	5.85 ± 0.12
15	561 ± 13	451 ± 13	7.75 ± 0.16	7.57 ± 0.11
16	486 ± 14	265 ± 12	7.91 ± 0.22	6.61 ± 0.08
17	421 ± 14	217 ± 12	6.56 ± 0.13	5.34 ± 0.08
18	626 ± 18	308 ± 13	9.81 ± 0.14	7.90 ± 0.12
19	418 ± 9	126 ± 7	5.52 ± 0.09	6.28 ± 0.09
20	482 ± 17	471 ± 23	6.85 ± 0.09	6.66 ± 0.15
21	464 ± 6	326 ± 14	6.01 ± 0.10	6.60 ± 0.07
22	602 ± 17	365 ± 10	8.20 ± 0.14	7.40 ± 0.10

[a]*Catechin equivalents.*
[b]*Catechin equivalents.*

Table 3.
Contents of total phenolic compounds and condensed tannins and antioxidant activity of faba bean seeds. Results are reported per 100 g of seeds.

Luo et al. [40], the content of condensed tannins in faba bean extracts ranged from 0.9 to 1.9 g of gallic acid equivalents/100 g extract. In this research, the authors used the Folin-Ciocalteu's phenol reagent to determine the content of tannins precipitated from the extract by using polypyrrolidone (PVPP). Amarowicz and Shahidi [39] identified gallate procyanidin dimer and three gallate procyanidins in faba bean extract by using HPLC-DAD-MS. The content of the abovementioned compounds was 689, 89.8, 28.6, and 18.3 μg/g extract. Five procyanidin dimmers and three procyanidin trimers were determined in faba bean extract using an UHPLC-ESI-QTOF-MS method [41]. The presence of procyanidin B1, B2, B3, B4, C1, and C2 has been reported by De Pascual-Teresa et al. [42].

3.2 Antioxidant activity

Antioxidant properties of the extracts were investigated using ABTS and FRAP assays. The extracts and seeds were characterized by the Trolox equivalent antioxidant capacity (TEAC) values ranging from 0.550 (FAB 443) to 1.030 mmol Trolox/g extract (FAB 187) and from 4.85 (FAB 318) to 9.81 mmol Trolox/100 g seeds (FAB 187). Ferric-reducing antioxidant power (FRAP) values varied from 0.595 (FAB 443) to 0.908 mmol Fe^{2+}/g extract (FAB 5023) and from 4.61 (FAB 297) to 7.90 mmol Fe^{2+}/100 g seeds (FAB 187).

The results of ABTS assay obtained in this study for faba bean extracts were much higher than those reported before for extracts of grass pea (0.017–0.037 mmol Trolox/g) [32], cow pea (0.285–0.665 mmol Trolox/g) [43], white bean (0.0270–0.043 mmol Trolox/g) [29], mung bean (0.021–0.031 mmol Trolox/g) [35], and lupin (0.260–0.620 mmol Trolox/g) [31]. The results of FRAP assay were also much higher than those reported for extracts of grass pea (0.045–0.120 mmol Fe^{2+}/g), [32], cow pea (0.487–1.566 mmol Fe^{2+}/g) [43], white bean (0.066–0.089 mmol Fe^{2+}/g) [29], and lupin 0.046–0.064 [31].

3.3 Statistical analysis

In our study, for the first time, a correlation was calculated between the content of phenolic compounds in the faba bean extracts and their antioxidant activity. The

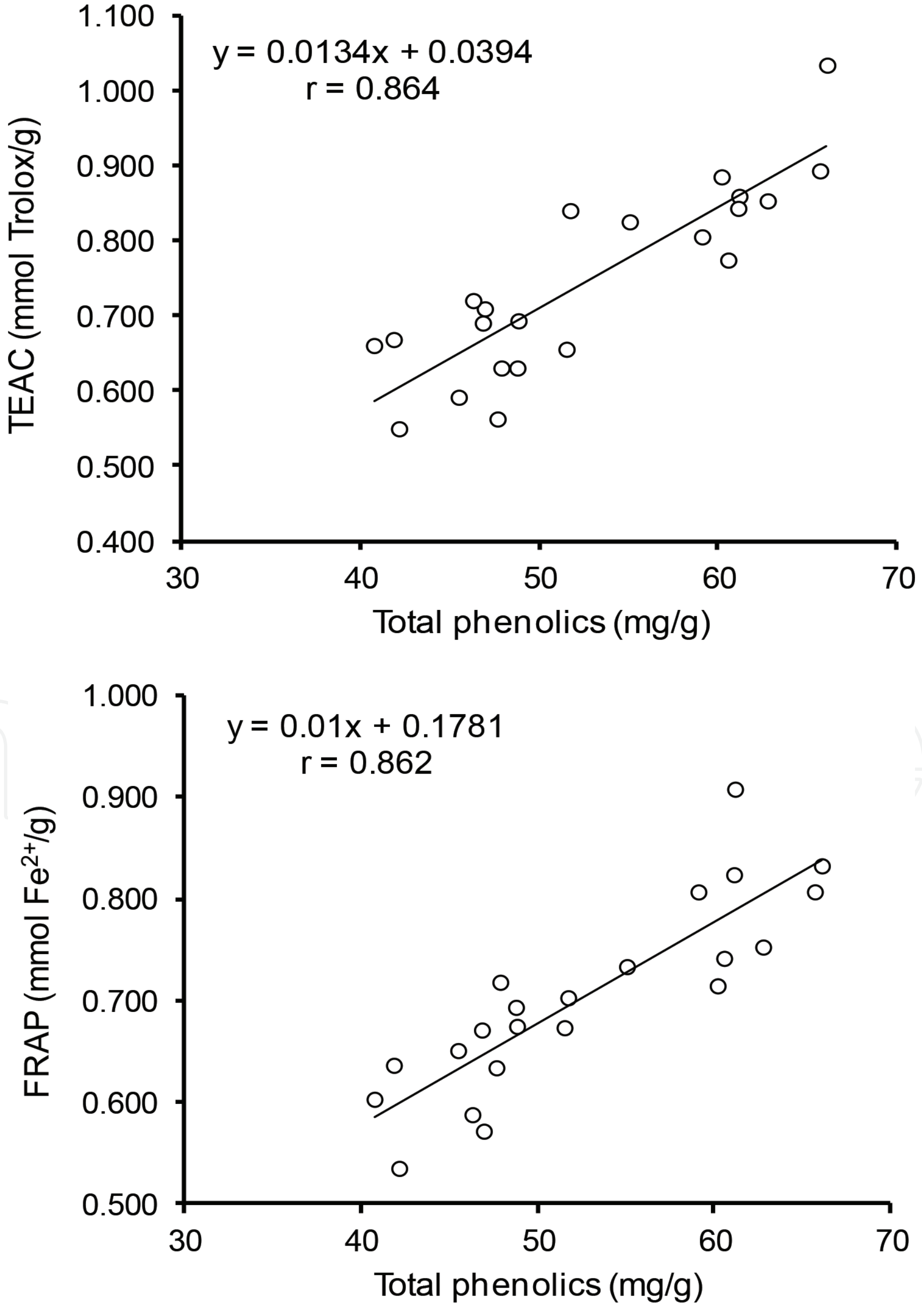

Figure 1.
Correlation between the total phenolic contents and the results of ABTS and FRAP assays.

coefficients of correlation between the total phenolic content and the results of the ABTS and FRAP assays were 0.864 and 0.862, respectively (**Figure 1**). The correlations between the content of condensed tannins and results of ABTS and FRAP assays were weaker and characterized by r = 0.543 and r = 0.528 (**Figure 2**). A correlation was also observed between the results of both assays (r = 0.795) (**Figure 3**).

In our previous study, we noted a correlation between the total phenolic content and ABTS and FRAP results determined for red bean (r = 0.997 and 0.997, respectively) [35], white (r = 0.480 and 0.850, respectively) [29], and grass pea (r = 0.881 and 0.781, respectively) [32]. Statistically significant correlations between the content of total phenolics and TEAC as well as between the content of condensed tannins and TEAC (r = 0.857 and 0.787, respectively) were reported for extracts obtained from seeds of faba bean, broad bean, adzuki bean, red bean, pea, red lentil, and green lentil [44].

In the principal component analysis (PCA) (**Figure 4**), the two first components accounted for 93.6% of the total variability between the faba bean cultivars. The analysis includes the content of total phenolic compounds and condensed tannins

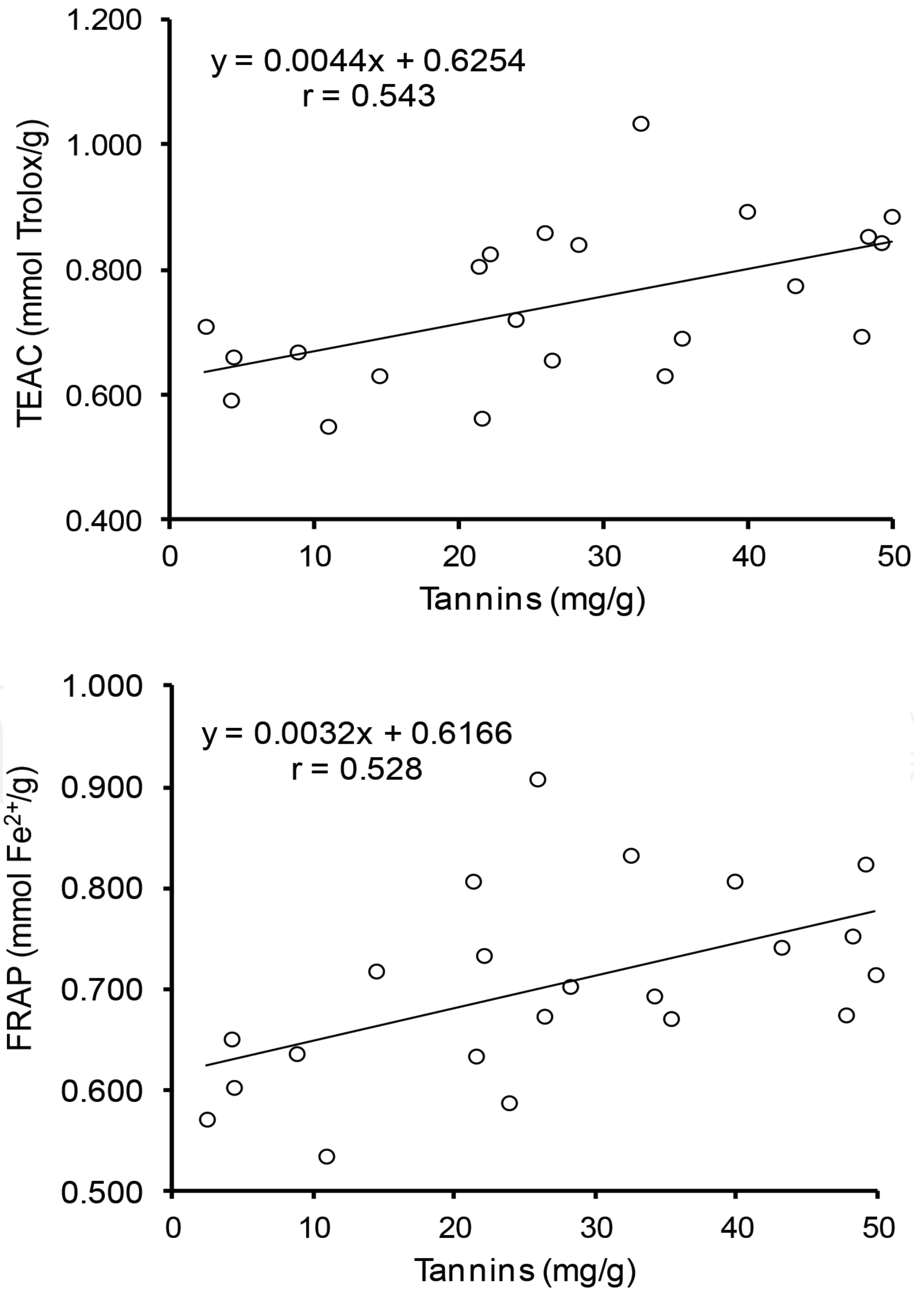

Figure 2.
Correlation between the content of tannins and the results of ABTS and FRAP assays.

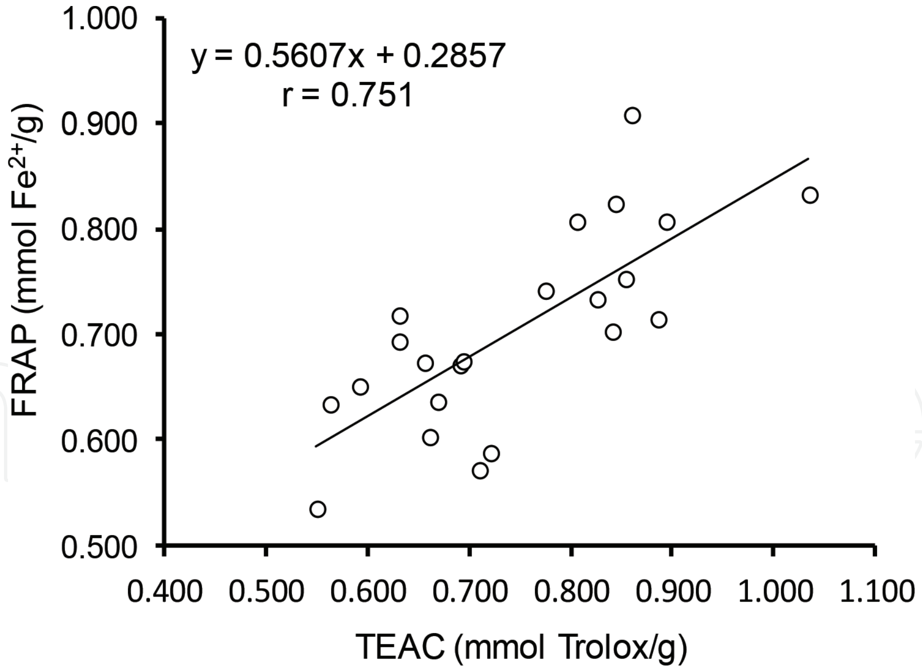

Figure 3.
Correlation between the results of FRAP and ABTS assays.

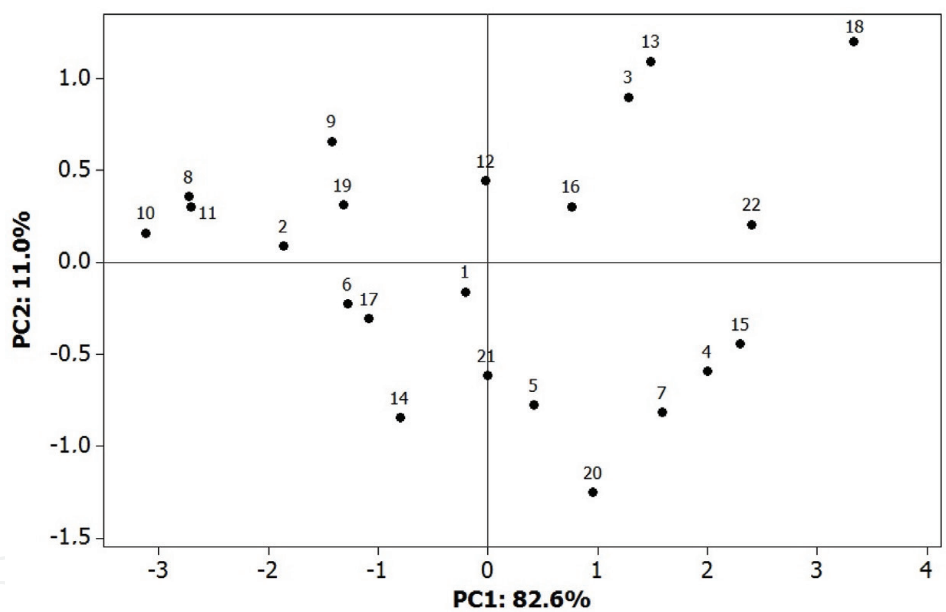

Figure 4.
Results of the principal component analysis (PCA).

in seeds as well as their antioxidant potential determined using ABTS and FRAP methods. A considerable variability in terms of the analyzed traits expressed jointly with the grates Mahalanobis distance was recorded for sample 18 from Afghanistan (FAB 187) and samples 8, 10, and 11 from Sudan (FAB 297), Yemen (FAB 219), and Israel (FAB 6318). The close clustering was observed for seeds from Sudan, Yemen, and Afghanistan and for seeds from Morocco, Sudan, and Tajikistan (sample 4, 7, and 15; FAB 5019, FAB 225, and FAB 354).

The hierarchical cluster analysis (**Figure 5**) showed several pairs of faba bean accessions (e.g., FAB 337 and FAB 202, FAB 5023 and FAB 604). Two main clusters were observed. The first contained 16 accessions, whereas the second contained only 6. The presence of similar pairs of faba bean accessions from different countries confirms the limitation of the hierarchical cluster analysis in the discrimination of the geographical origin of samples.

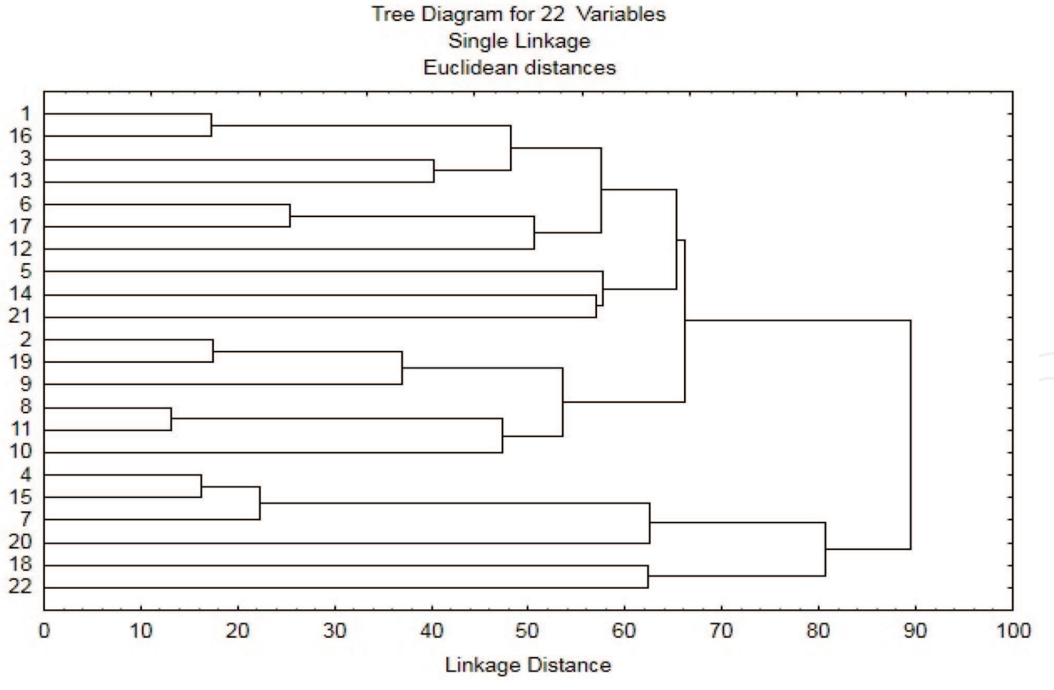

Figure 5.
The hierarchical cluster analysis.

4. Conclusions

The extracts obtained from faba bean were characterized by a high content of phenolic compounds and condensed tannins. Their antioxidant potential was higher than that reported previously for the extracts of other legume seeds. The content of total phenolics and condensed tannins in the faba bean extracts strongly influenced the antioxidant activity of extracts determined using ABTS and FRAP assays.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Wojciech Rybiński[1], Magdalena Karamać[2], Katarzyna Sulewska[2] and Ryszard Amarowicz[2*]

1 Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

2 Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland

*Address all correspondence to: r.amarowicz@pan.olsztyn.pl

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