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PHENOLIC COMPOSITION AND ANTIOXIDANT ACTIVITY OF RAW SEEDS, GREEN SEEDS AND SPROUTS OF TEN FABA BEAN (*VICIA FABA*) CULTIVARS CONSUMED IN EGYPT

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ABSTRACT

Faba beans are widely consumed in Egypt as a vegetable by many ways. A study was conducted to determine the levels of phenolic compounds and antioxidant properties of raw seeds, green seeds and sprouts of ten faba bean cultivars commonly consumed in Egypt. Nubaria 2, Sakha 2 and Nubaria 1 cultivars showed higher amounts of phenolic compounds and exhibited the highest antioxidant activities (DPPH and FRAP). Four flavonoid constituents, myricetin, daidzein, apigenin and quercetin, were detected in tested faba bean seeds using HPLC with variable concentrations. For all tested cultivars, the mean values of total phenolics, tannins, total flavonoids, DPPH and FRAP in green seeds reached 342%, 485%, 267%, 439% and 136% relative to those found in raw seeds, respectively. The study concludes that the green faba bean seeds afforded an important source and good adequate level of antioxidants for daily inclusion in the human diet.

KEYWORDS: **Phenolics, Antioxidant activity, Cultivars tannins, Green seeds, Sprouts, *Vicia faba*.**

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INTRODUCTION

Faba bean (*Vicia faba* L.) is the most cultivated leguminous species in the world[1]. Legumes are important component of traditional diets of several regions all over the world as they are low in fat, rich in protein, dietary fiber and a variety of micronutrients and phytochemicals [2]. Egypt is one of the largest consumers of pulses in the world. Faba beans are a common staple food in the Egyptian diet, eaten by rich and poor alike. The most popular dishes of faba bean in Egypt are Medamis (stewed beans), Falafel (deep fried cotyledon paste with some vegetables and spices), Bissara (cotyledon paste poured onto plates) and Nabet soup (boiled germinated beans) [3]. Leguminous seeds, including faba bean belong to plant foods that are generally rich in phenolic compounds and possess high antioxidant capacity, which may be beneficial in the prevention of several health-related conditions like coronary and cardiovascular diseases, obesity, diabetes, inflammation and cancer [4,5]. Phenolic compounds have antioxidant activity, which delays the oxidation of various ‘‘important for life” compounds by inhibiting the initiation or propagation of oxidising chain reactions. Natural antioxidants endogenous to food of plant origin can scavenge reactive oxygen and nitrogen species (RONS); evidence suggests that these may be of great importance in preventing the onset of oxidative diseases in the human body [6,7]. Dry faba beans are widely known for their fiber, mineral and protein contents; however, its nutraceutical value is yet to gain as much attention in the prevention of chronic diseases [8]. Seeds of different faba beans cultivars are widely consumed in Egypt as a vegetable by many ways using dry seeds or green seeds or sprouts. There have been several studies of phenolic composition in dry faba bean seeds as this is the main means of consumption in the world [9,10]. But, however, much less is known about the nutritional value of green seeds, which is considered as one way the faba beans are generally consumed in Egypt. Recently, differences in the phenolic composition and antioxidant activity between *V. faba* L. cultivars were observed by Chaieb et al [11]. and Baginsky et al [12]. Sprouts are believed to be rich in health-promoting phytochemicals compare with their mature counterparts. Germination (sprouting) has been suggested as an inexpensive and effective way to improve the quality of legumes by increasing the levels of health-promoting antioxidants and decreasing of some antinutritional factors [13]. Cevallos-Casals and Cisneros-Zevallos [14] examined 13 edible seeds for the levels of phenolic compounds and the antioxidant activity at different germination states (dormant, imbibed and 7d sprouts). They found that germinated edible seeds are an excellent source of dietary phenolic antioxidants. On the contrary, Al-Numair et al [9] reported that, for two faba bean and three white bean cultivars, phenolic content decreased significantly throughout the sprouting periods up to 6 days. In order to demonstrate the health benefits of consuming faba beans with different ways, our objective was to determine the levels of different phenolic compounds, antioxidant properties and individualized flavonoid constituents by HPLC of raw seeds, green seeds and sprouts of ten faba bean cultivars commonly consumed in Egypt.

MATERIALS AND METHODS

(i) Chemicals and reagents

Flavonoid standards, including apigenin, isorhamnetin, kaempferol, luteolin, myricetin were purchased from Fluka (Bucks, Switzerland), quercetin, and neringenin were purchased from Sigma Chemical Co. (St. Louis, USA). Gallic acid, 1-1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were acquired from Sigma Chemical Co. (St. Louis, MO, U.S.A). Folin-Ciocalteu´s reagent, methanol, hydrochloric acid, acetic acid, acetonitrile, sodium nitrite, aluminum chloride, sodium hydroxide, sodium acetate, ferric chloride, potassium dihydrogen phosphate,

butylated hydroxyanisole (BHA) and polyvinylpolypyrrolidone (PVPP) were purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade or higher.

(i) Source and preparation of faba bean seeds

Raw faba bean seeds, which are the seeds of mature bean plants, of ten faba bean (*V. faba*) cultivars namely Giza 3, Giza 716, Giza 843, Nubaria 1, Nubaria 2, Sakha 1, Sakha 2, Sakha 3, Sakha 4, and Misr 1 were obtained from the Institute of Legumes in the Agriculture Research Centre, Giza, Egypt.

A germination test was conducted on ten faba bean cultivars. Seeds surface-sterilized with sodium hypochlorite (0.1%, w/v) for 2min, washed under running tap water for 5 min followed by distilled water and stored for further use. Five ml of distilled water were placed in a 9-cm plastic Petri dish lined with one Whatman No. 1 filter paper; ten seeds were placed on the paper. Petri dishes were kept in a growth chamber at 25 °C for three days (3d sprouts). Four replicates were maintained for each cultivar in a completely randomized manner. In all cultivars, 3d sprouts of each cultivar were stored in separate plastic bags at -20 °C prior to analyses. Seeds of ten tested cultivars were grown in green house of National Research Centre, Giza, Egypt. Mature green seeds were harvested for fresh consumption at the same physiological stage in three replicates of fifty plants each. Each replicate employed the same cultural practices, such as irrigation, fertilization, weeds and disease management, as are commonly used for *V. faba* cultivars in Egypt. Green pods with fully developed seeds were hand-harvested. In all cultivars, faba bean seeds of each replicate were stored in separate plastic bags at -20 ^{0}C prior to analyses.

(i) Seed extraction

Raw seeds, green seeds and sprouts, were collected, dried by a freeze-dryer (77500-00 M, Lab Conco Co, Mo, USA), finely grounded. Whole seed samples were initially crushed in a traditional stone mill and finally grounded with an analytic mill (IKA A11 basic; IKA Werke GmbH & Co. KG, Staufen, Germany). About one gram of powdered sample was accurately weighed, and shacked with 250 ml of 80% ethanol for 12 hrs, using shaking incubator at room temperature. Solids were separated by centrifugation and filtration. Extracts were performed in triplicate for each individual cultivar, and subjected to determine antioxidant activity, total phenolics, tannins and total flavonoids.

(i) Determination of total phenolics tannins and flavonoids content

Total phenolic content was determined using Folin-Ciocalteu´s reagent [15] with some modifications. Seed extract (1 ml) was mixed with 1.5 ml of deionised water followed by 0.25 ml of Folin-Ciocalteu´s reagent and allowed to react 6 min. Then 2.5 ml of sodium carbonate 7% was added and allowed to stand for 1 hr, then absorption at 765 nm was measured. Measurements were calibrated to a standard curve of prepared gallic acid solution, and the total phenolic was expressed as mg gallic acid equivalent (GAE)/ g of sample on a dry weight basis. Total tannin were determined in the extracts by a modification of the Folin-Ciocalteu´s method using polyvinylpolypyrrolidone (PVPP) to separate tannin phenols from non-tannin phenols [16]. About 100 mg of PVPP was added to 1ml sample extract diluted with 1ml water and left 15 min at 4^{o}C. After centrifugation, PVPP forms a precipitate with tannins, and the supernatant has only simple phenols. Simple phenols were determined using the Folin-Ciocalteu's reagent as previously mentioned. The difference between total and simple phenol values represents the tannin content, expressed as mg gallic acid equivalent (GAE)/ g of dried seeds. The flavonoid contents were measured using a colorimetric assay according to Zhishen et al[17]. A known volume (1 ml) of the extracts or standard solutions of quercetin was added to a 10 ml volumetric flask. Distilled water was added to make a volume of 5 ml. At zero time, 0.3 ml of 5% w/v sodium nitrite was added to the flask. After 5 min, 0.6 ml of 10% (w/v) $AlCl_3$ was added and, after 6 min, 2 ml of 1M NaOH were added to the mixture, followed by the addition of 2.1 ml distilled water. Absorbance

was read at 510 nm against the blank (water) and flavonoid content was expressed as mg quercetin equivalents (QE)/ g of dried seeds.

(i) Determination of antioxidant activity

The DPPH assay was done according to the method of Brand-Williams et al[18] with some modifications. The stock solution was prepared by dissolving 24 mg 1, 1-diphenyl-2-picrylhydrazyl (DPPH) with 100 ml methanol and then stored at −20 °C until needed. The working solution was obtained by mixing 10 ml stock solution with 45 ml methanol to obtain an absorbance of 1.1±0.02 units at 515 nm using the spectrophotometer. Seed extracts (750 µl) were allowed to react with 1500 µl of the DPPH solution for 5 min in the dark. Then the absorbance was taken at 515 nm. The standard curve was linear between 25 and 800 µmol Trolox. Results are expressed in µmol Trolox/100g dry matter. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve. Ferric reducing antioxidant power assay (FRAP) was done according to Benzie and Strain[19] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3O_2Na{\cdot}3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3{\cdot}6H_2O$ solution and then warmed at 37 °C before using. Seed extracts (150µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 µM Trolox. Results are expressed in µmol Trolox/ 100g dry matter. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

(i) Analysis of flavonoid composition by HPLC

Flavonoid constituents were extracted according to McKeehen et al[20]. Ground seeds were prepared as follows: 40 ml of 62.5% aqueous methanol containing BHA (2g/ l) was added to 0.5 g dried sample material. To this, 10 ml of 6 N HCl was added to give a total volume of 50 ml. The extraction mixture was heated to 90 °C on a steam water bath and refluxed for 2 hrs, allowed to cool in the refrigerator, diluted to 100 ml with methanol and sonicated for 5 min to form the final extract. HPLC separation was carried out at a flow rate of 1ml/min and a temperature of 30 ^{O}C using a Shimadzu HPLC (Japan) with diode array detection and a supelcosil C_{18} column (250 x 4.6 mm). The mobile phase was a linear gradient with O-phosphoric acid 0.25% (A)-acetonitrile (B) for 42 min starting with A:B (95:5) for 2 min, changing to A:B (90:10) for 5 min, A:B (85:15) for 3 min, A:B (80:20) for 13 min, A:B (70:30) for 5 min, A:B (50:50) for 4 min with equilibrating for 10 min. Detection was measured at 340 nm. Flavonoid standards, including apigenin, isorhamnetin, kaempferol, luteolin, myricetin, quercetin and neringenin. All the standards were dissolved in methanol to a concentration of 1 mg/10ml and were stored in darkness at -20 °C.

(i) Statistical analysis

Analyses were performed in triplicate and the data are presented as means. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Stat graphics Plus Version 5.1.

RESULTS

1. Total phenolics

Total phenolics content in raw seeds, green seeds and 3d sprouts of ten faba bean cultivars are summarized in Table 1. Total phenolics in tested faba bean cultivars were significantly different and greatly influenced by seed form. Among test cultivars, phenolics in raw, green and sprouts are in the range 8.38 -14.01 mg GAE/g, 20.89- 46.58 mg GAE/g and 8.72-13.61 mg GAE/g, respectively. The greatest accumulation of phenolic compounds occurred in the green seeds. Since, the mean value of phenolics in green seeds for all tested cultivars was 35.6 mg GAE/g compared with 11.43 and

10.42 mg GAE/g for sprouts and raw seeds, respectively. Total phenolic content in the tested cultivars was significantly differed. In the raw seeds, the higher values were observed for Nubaria 2, followed by Misr 1 and Nubaria 1. But, in the green seeds, the phenolic contents of faba bean cultivars decreased as following Sakha 2 > Nubaria 2> Sakha 3> Sakha 4. Whereas, in the sprouts, Nubaria 1 and Nubaria 2 exhibited the high levels of total phenolics of all cultivars studied.

2. Tannins and flavonoids

As shown in Table 1, levels of tannins and total flavonoids presented a high variability according to seed cultivars and seed forms. The results indicated that the greatest accumulation of tannins and flavonoids occurred in green seeds as compare with raw seeds or sprouts. Tannins in green seeds ranged from 9.5 to 20.98 mg GAE/g, in raw seeds ranged from 2.07 to 5.06 mg GAE/g and in sprouts ranged from 3.36 to 6.67 mg GAE/g. Whereas, total flavonoids in green seeds ranged from 4.27 to 13.20 mg QE/g, in raw seeds ranged from 2.73 to 4.73 mg QE/g and in sprouts ranged from 3.13 to 5.47 mg QE/g. Significant differences in the contents of tannins and flavonoids between tested cultivars were detected. In raw seeds and green seeds, Nubaria 2 and Sakha 2 cultivars accumulated high amounts of tannins and flavonoids. Whereas, in sprouts, the greatest accumulation of tannins and flavonoids occurred in Nubaria 1 and Nubaria 2 cultivars.

Table 1

Phenolic compounds in raw, green and sprouts of different faba bean cultivars.

Cultivars	Total phenolics (mg GAE/g DW)			Tannins (mg GAE/g DW)			Total flavonoids (mg QE/g DW)		
	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout
Giza 3	8.38^{dB}	36.20^{bA}	8.72^{eB}	2.56^{abB}	17.04^{abA}	3.36^{dB}	2.80^{cC}	9.07^{cA}	4.93^{abB}
Giza 716	9.31^{cdC}	20.89^{cA}	11.26^{bcB}	2.37^{abC}	9.50^{cA}	5.19^{bB}	3.47^{bcB}	4.27^{eA}	3.13^{dB}
Giza 843	9.64^{cdB}	33.51^{bA}	9.85^{dB}	2.55^{abB}	13.24^{bcA}	4.30^{cB}	2.73^{cC}	8.53^{cA}	3.60^{bcdB}
Nubaria 1	10.95^{bcC}	33.15^{bA}	13.61^{aB}	4.33^{abB}	17.28^{abA}	6.46^{aB}	3.87^{abcC}	7.87^{cA}	5.27^{aB}
Nubaria 2	14.01^{aB}	44.65^{aA}	13.33^{aB}	5.06^{aB}	20.25^{aA}	6.67^{aB}	4.73^{aB}	12.80^{abA}	5.47^{aB}
Sakha 1	9.64^{cdC}	23.33^{cA}	11.73^{bcB}	3.79^{abC}	13.21^{bcA}	6.03^{aB}	3.20^{cB}	5.87^{dA}	3.47^{bcdB}
Sakha 2	10.93^{bcB}	46.58^{aA}	11.35^{bcB}	4.02^{abB}	20.98^{aA}	6.26^{aB}	4.40^{abB}	13.20^{aA}	3.47^{bcdB}
Sakha 3	9.49^{cdB}	42.88^{aA}	10.33^{cdB}	3.30^{abB}	19.30^{abA}	4.39^{cB}	2.80^{cC}	12.40^{abA}	4.73^{abcB}
Sakha 4	9.81^{cdC}	41.21^{aA}	12.24^{bB}	3.52^{abC}	19.59^{aA}	6.4^{aB}	3.47^{bcB}	11.07^{bA}	3.47^{bcdB}
Misr 1	12.03^{bB}	33.61^{bA}	11.84^{bcB}	2.07^{bC}	12.58^{bcA}	6.30^{aB}	3.53^{bcB}	8.53^{cA}	3.33^{cdB}

**GAE: gallic acid equivalent; ** QE: Quercetin equivalent. The same small letters in a column are not significantly different (p< o.o5). The same capital letters in a row are not significantly different (p<o.o5).*

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3. Antioxidant activity

The antioxidant activity of raw, green and sprouts for ten faba bean cultivars were assayed using DPPH and FRAP (Table 2). Significant variations in antioxidant activities were observed between cultivars and between seed forms. The DPPH values showed a more changeable behavior than FRAP values. Among tested cultivars, DPPH and FRAP values were in the range 2196- 5604 and 2333- 3391 µmol Trolox/100 g for raw seeds, 5400 - 26880 and 3539 - 3852 µmol Trolox/100 g, for green seeds as well as 2520-5448 and 2639-3377 µmol Trolox/100 g for sprouts, respectively. Green seeds exhibited the highest DPPH and FRAP values (mean values14724 and 3792 µmol Trolox/100 g, respectively) followed by sprouts (mean values 4096 and 2947 µmol Trolox/100 g, respectively) and finally raw seeds (mean values 3358 and 2789 µmol Trolox/100 g, respectively). Between raw seeds, the highest values of DPPH and FRAP occurred by Nubaria 2 cultivar. This cultivar had 155 % more DPPH activity than the Giza 3, the cultivar with the lowest value of DPPH activity and had 45 % more FRAP activity than the Giza 843, the cultivar with the lowest value of FRAP activity. In the green seeds, Sakha 2 showed the highest DPPH antioxidant activity, while, in sprouts, Nubaria 1 and Nubaria 2 cultivars had

the highest FRAP and DPPH activities of all cultivars studied.

Table 2
Antioxidant activity (µmol Trolox/100 g DW) of raw, green and sprouts of different faba bean cultivars.

Cultivars	DPPH			FRAP		
	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout
Giza 3	2196^{cB}	14880^{bcA}	2520^{dB}	2509^{bB}	3834^{aA}	2747^{cB}
Giza 716	2256^{cC}	6240^{cA}	3720^{cB}	2671^{bB}	3539^{cA}	2891^{bcB}
Giza 843	2256^{cC}	7080^{cA}	3720^{cB}	2333^{bC}	3802^{aA}	2743^{cB}
Nubaria 1	3792^{bC}	12840^{bcA}	5232^{abB}	2923^{bC}	3809^{aA}	3377^{aAB}
Nubaria 2	5604^{aB}	21120^{abA}	5448^{aB}	3391^{aB}	3852^{aA}	3344^{aB}
Sakha 1	3336^{bcB}	5400^{cA}	4260^{bcB}	2711^{bB}	3704^{bA}	2819^{bcB}
Sakha 2	3624^{bB}	26880^{aA}	3600^{cB}	2866^{bB}	3845^{aA}	2639^{cB}
Sakha 3	3300^{bcB}	17160^{abcA}	4140^{bcB}	2768^{bB}	3852^{aA}	2902^{bcB}
Sakha 4	3036^{bcB}	21000^{abA}	3888^{cB}	2765^{bC}	3852^{aA}	3186^{abB}
Misr 1	4176^{bB}	14640^{bcA}	4428^{abcB}	2956^{bB}	3830^{aA}	2819^{bcB}

The same small letters in a column are not significantly different (p< o.o5).
The same capital letters in a row are not significantly different (p< o.o5).

4. Flavonoid constituents

Four flavonoids constituents, myricetin, daidzein, apigenin and quercetine, were detected in faba bean seeds (Table 3). Myricetin and daidzein found to be the main flavonoid components in raw seeds, green seeds and sprouts. Myricetin varied from

Table 3
Flavonoid constituents (µg/g DW) of raw, green and sprouts of different cultivars.

Cultivars	Myricetin			Daidzein			Apigenin			Quercetine		
	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout	Raw seed	Green seed	Sprout
Giza 3	5.42± 0.41	3.86± 0.32	7.83± 0.44	1.22± 0.08	1.16± 0.03	7.39± 0.43	0. 85± 0.01	0. 54± 0.01	1.50± 0.01	0. 56± 0.01	0. 61± 0.01	4.15± 0.12
Giza 716	4.58± 0.21	3.38± 0.23	7.60± 0.43	2.67± 0.04	1.44± 0.02	7.23± 0.32	1.99± 0.01	ND	2.42± 0.03	1.35± 0.01	0. 52± 0.02	3.61± 0.02
Giza 843	4.32± 0.11	3.39± 0.08	12.6± 0.60	2.29± 0.02	1.84± 0.02	7.69± 0.04	0.71± 0.01	ND	2.12± 0.02	2.36± 0.04	ND	6.44± 0.19
Nubaria 1	5.08± 0.15	3.59± 0.06	8.70± 0.32	5.08± 0.18	3.59± 0.09	9.28± 0.72	1.63± 0.03	1.07± 0.01	2.96± 0.02	0. 70± 0.01	1.28± 0.02	1.94± 0.01
Nubaria 2	4.58± 0.15	4.08± 0.09	12.78± 0.40	1.18± 0.08	2.48± 0.07	8.88± 0.56	1.81± 0.01	1.16± 0.02	2.84± 0.04	1.04± 0.02	0. 83± 0.02	5.39± 0.18
Sakha 1	4.14± 0.08	3.82± 0.09	6.12± 0.29	1.81± 0.05	1.45± 0.03	4.95± 0.16	ND	0. 53± 0.01	ND	0. 84± 0.02	0. 72± 0.02	7.24± 0.81
Sakha 2	4.34± 0.11	4.36± 0.98	14.22± 0.73	2.10± 0.03	2.43± 0.01	9.75± 0.65	0. 66± 0.00	ND	3.08± 0.05	0. 81± 0.01	0. 68± 0.01	4.31± 0.31
Sakha 3	3.65± 0.06	2.61± 0.04	7.18± 0.49	1.13± 0.01	1.78± 0.02	5.27± 0.60	0. 40± 0.01	ND	1.55± 0.03	0. 56± 0.01	0. 86± 0.01	5.97± 0.22
Sakha 4	2.84± 0.03	3.52± 0.04	6.37± 0.20	1.84± 0.02	1.38± 0.01	7.16± 0.54	0. 80± 0.01	0. 55± 0.01	1.32± 0.02	1.05± 0.03	0. 99± 0.02	2.37± 0.18
Misr 1	3.81± 0.23	4.27± 0.11	8.11± 0.33	1.19± 0.01	2.88± 0.01	7.46± 0.34	0. 29± 0.00	0. 68± 0.02	1.08± 0.01	2.78± 0.04	3.15± 0.40	11.10± 0.92

Values are means of three samples; ND: not identified.

2.84 to 5.42 ug/g in raw seeds, from 2.61 to 4.27 ug/g in green seeds and from 6.12 to 12.78 ug/g in sprouts. While, daidzein ranged from 2.1 to 5.08 ug/g in raw seeds, and from 11.6 to 3.59 ug/g in green seeds. Apigenin and quercetin appeared in small quantities in raw seeds or green seeds. For the same cultivar, the highest values for myricetin, daidzein, apigenin and quercetin were observed for the sprouts as compared with green seeds or raw seeds. Among studied cultivars, concentrations of flavonoid constituents showed considerable variations. Nubaria 1, Sakha 2 and Nubaria 2 seeds tended to contain higher concentrations of myricetin, daidzein and apigenin than the other tested cultivars. Whereas, Misr 1 recorded the highest concentration of quercetin constituent

DISCUSSION

Significant differences between faba bean cultivars were equally detected for all tested phenolic compounds and antioxidant activity, among the same seed form. Variations in phenolic content and antioxidant activity between faba beans cultivars were previously observed by Chaieb et al[11] and Ismael et al[10] Our results about the levels of total phenolic and tannin contents are comparable with those obtained by Baginsky et al[12] . They found that, among ten *V*. *faba* L. cultivars, total phenolics ranged from 817 to 1337 mg gallic acid equivalent per kilogram and condensed tannin content ranged from 309 to 958 mg (+)-catechin equivalent per kilogram. The differences in the concentration of phenolic compounds and antioxidant activity, among tested cultivars are likely to primarily reflect genotypic variation [21]. For all tested seeds, Nubaria 2, Sakha 2 and Nubaria 1cultivars tended to contain higher amounts of tested phenolic compounds and better antioxidant activity. The large amount of variation observed among the ten cultivars is promising for future efforts to develop and improve strains of faba beans in Egypt for maximal phenolic content and antioxidant activity. A few studies have been performed on the antioxidant activity of faba beans [11]. The results of this study indicated that the seeds extract of faba bean was found to display a good antioxidant effect. The superiority of DPPH and FRAP antioxidant activities of faba bean in comparison with other legumes such as peas, lentils and soybean were observed by Lin & lai [21] Lopez-Amoros et al[22]. , and Sreeramulu et al[23]. Also, Chaieb et al[11] reported that faba bean extracts had a higher capacity of scavenging the DPPH radical than synthetic antioxidants (BHT or TBHQ) and pure phenolic constituents as quercetin, catechin, rutin and gallic acid.

Presence of myricetin, apigenin and quercetin in faba bean seeds is consistent with others studies that have reported that in faba beans, the flavonols are mainly myricetin, kaempferol and quercetin, both free aglycones and glycosides [24,25]. Also, Nubaria 1, Sakha 2 and Nubaria 2 seeds tended to contain higher concentrations of myricetin, daidzein and apigenin than the other tested cultivars. The observed differences in the concentration of flavonoid compounds among cultivars are likely to primarily reflect genotype variations [12]. From our results, it can be observed that sprouts tended to accumulate more phenolics, tannins and flavonoids content and antioxidant activity than raw seeds. An increase of total phenols and antioxidant activity after germination was reported by Cevallos-Casals & Cisneros-Zevallos [14] for selected seeds included mungbean, alfalfa, fava, fenugreek, mustard, wheat, broccoli, sunflower, soybean, radish, kale, lentil and onion. Duenas et al[26] reported that germination caused significant changes in the phenolic composition (increasing) due mainly to endogenous enzymes' activation and the complex biochemical metabolism of seeds during this process. The obtained results indicated that, for all faba bean cultivars, greatest accumulation of all tested phenolic compounds and antioxidant activity occurred in green seeds as compare with either raw seeds or sprouts. The mean value of green seeds showed 242%, 385%, 167%, 439% and 360% increases in total phenolics, tannins, total flavonoids, DPPH and FRAP relative to these found in raw seeds, respectively. In line to these results, Toma´s-Barbera´n et al[25] reported that

the variations in phenolic constituents in faba bean seeds depended on the stage of maturity. There is a lack of information about phenolic composition and antioxidant activity of green seeds, which is considered as one way the faba beans are generally consumed in Egypt. Our results revealed that the green seeds are a good source of phenolics, the bioactive compounds with health promoting antioxidants.

CONCLUSION

This research is a contribution to the determine of the phenolic contents, antioxidant activity and individualized flavonoid constituents by HPLC for raw seeds, green seeds and sprouts of ten Egyptian faba bean cultivars. Significant differences between tested cultivars were detected for total phenolics, tannins and total flavonoids contents as well as for the values of DPPH and FRAP antioxidant activities. The greatest accumulation of all tested phenolic compounds and the highest antioxidant activities occurred in green seeds as compare with either raw seeds or sprouts. In this study it was found that the green faba bean seeds are a good source of bioactive compounds in Egyptian diet with health-promoting antioxidants.

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