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ORIGINAL ARTICLE

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM MOROCCAN FRESH FAVA BEANS PODS (*VICIA FABA L.*)

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ABSTRACT

Background. In Morocco, fava beans are widely used as a main meal or as an ingredient in various traditional recipes, in the form of fresh ripe seeds or dry seeds. In the past, the tender skin of bean pods was also used in certain specific dishes, thus diversifying the diet. However, the peels of the tender bean pods are currently less or not used and considered waste. In Moroccan, fava bean pods peels, traditionally used in food in the past, are today considered as waste. The valorization of fresh fava bean pods could revitalize the use of the specific dishes and diversify the diet. For this reason, the research aimed to assess the nutritional values and biological compounds of the whole fresh fava bean pods (*Vicia faba L.*).
Objective. Evaluate the content of nutrients, total phenolic, flavonoids and tannin contents and antioxidant activity in different extracts of the tender pods of the fava bean (*Vicia faba L.*).
Material and methods. The proximate composition and minerals were determined using AOAC methods. The total phenolic compounds by the *Folin-Ciocalteu* reagent, the total flavonoids were analyzed using aluminum chloride colorimetric method, the tannins by method of vanillin in an acidic medium and the antioxidant activity was evaluated by DPPH method.
Results. The results show that the fresh fava bean pods have a moisture content of 87.31 ± 0.25%, ash 4.67 ± 1.03, and protein 29.11 ± 3.20 g/100 g. The legume samples also contain potassium (1946.8±4.61), phosphorus (483.8 ± 3.14), and calcium (399.6 ±2.25) mg/100 g of dry matter representing at last 40-50% of the RDI. The content of the different extracts of (Vicia faba L) varied from 49.5 to 594.4 mg GAE/ g for the total phenols, from 0.7 mg to 3.4 mg QE/g for flavonoids, and from 4.9 mg to 73.91 mg TAE/g dry weight for tannins. The evaluation of the antioxidant activity in the various extracts revealed a better activity in the methanolic extract (IC50=491.2 µg/mL) compared to others extracts: the MeOH/water extract (IC50=606.61 µg/mL), DCM/ MeOH extract (IC50 = 642.67 µg/mL) and DCM extract below of 50%.
Conclusions. This study shows that fava bean pods, traditionally used in food, are rich in macro and micronutrients and bioactive substances, which demonstrates their potential contribution to human food and nutritional security.

Key words: *Vicia faba, chemical composition, antioxidant activity, total phenolic, flavonoids, mineral analysis*

INTRODUCTION

Legumes are an imperative food supply or bear an important function in traditional diets in many regions of the world [8]. They are served an excellent source of proteins especially lysine and threonine which have high levels, two essential amino acids that are deficient in cereal proteins [1, 31] and they are a good alternative to expensive protein from meat and fish. These nutritional properties make legumes a good supplement that improves the protein and nutritional quality of cereal-based products [10, 15], They are also a source of many nutrients, including starch, dietary fiber, essential fatty acids, vitamins, and trace elements. Moreover, legumes are also considered to be an important source of many secondary metabolites such as phenolic antioxidants [8, 16, 28].

Several studies have reported the content of Fava beans in anti-nutritional components that interfere with

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minerals absorption [10, 14, 32] and whose inhibitory effect is inactivated by cooking or autoclaving[22, 24].

Fava beans are reported with effects on health as they reduce blood sugar and cholesterol and prevent heart disease [21, 25, 33], renal, hepatic dysfunction and eye diseases [17, 19], cancers, and *Parkinson's* diseases [6, 13, 29]. In all these studies, the analyses focused on the fresh or dry seed of the fava bean.

The fava bean (*Vicia faba L.*) is one of the oldest plants in the world which belongs to the Leguminosae family [13, 30]. It is an important winter crop in Mediterranean regions (spring). It has been considered one of the most important plant foods for the people of the Nile [12, 26] and is popular food widely consumed in the Middle East, North Africa, and South America [24, 31]. Indeed, cooked beans are considered one of the most famous dishes over the years in Egypt [14, 18].

In Morocco, fava beans are widely used as a main meal or as an ingredient in various traditional recipes, in the form of fresh ripe seeds or dry seeds. In the past, the tender skin of bean pods was also used in certain specific dishes, thus diversifying the diet. However, the peels of the tender bean pods are currently less or not used and considered waste.

The objective of the present study, therefore, was to evaluate the content of nutrients and bioactive substances in the tender pods of the whole fava bean (*Vicia faba L.*) consumed in Morocco.

MATERIAL AND METHODS

Sampling

The immature fresh pods of fava bean (Vicia faba L) were collected from ten fields of the rural areas (elmechrek and krdid) of the province of Sidi Bennour (Morocco) in spring 2020. Each sample of 1 kg, taken at random from different points of each field. All samples are transported on the same day in airtight bags to the Biotechnology, Biochemistry and Nutrition laboratory of the Faculty of Sciences, at Chouaib Doukkali University in El Jadida city. They are mixed to have a homogeneous sample. A mass of this raw sample was used for moisture measurement and the remaining amount was carefully washed with tap water to remove attached dust particles, then with distilled water and deionized water. They were then sliced and dried in an oven (SM400, MEMMERT, Germany) at 45°C until constant weight. The pool is then ground into a fine powder and stored in airtight containers.

Preparation of crude extract

Maceration for 72 hours, of the powder of the sample, was carried out for the extraction of the phenolic compounds, by different solvents: (MeOH) methanol, (MeOH / H2O) methanol / water (70% / 30%), (DCM / MeOH) dichloromethane / methanol

a)
b)

Figure 1. The tender pods (a) of (*Vicia faba L*) and in traditional recipes (b)

(50/50) and (DCM) dichloromethane. The four extracts were filtered and the solvent removed by rotary evaporation under pressure and at 45°C and then stored at -4°C until analysis.

Proximate analysis

The moisture and ash content were determined according to AOAC [2]. The total protein content was determined by the Kjeldahl method according to AOAC procedures [4] and calculated by multiplying the total nitrogen content N (%) by the coefficient 6.25.The crude fat content was determined using the Soxhlet extraction technique according to AOAC [5]. The total carbohydrates (including fibre) were calculated by difference [Total carbohydrates (g/100 g) = 100 - (g fat + g protein + g ash)] [5]. Total energy was calculated according to the following equations:

Total energy (kJ) = 17 x (crude protein (g) + total carbohydrate (g)) + 37 x (crude fat (g)).

The mineral content is determined according to AOAC 985.01 method [3], using inductively coupled plasma ICP-AES ULTIMA 2C HORIBA technique. One gram of the plant powder was weighted into a 30-mL glazed porcelain crucible placed into a cool muffle furnace and muffle at 500°C for 2 hours then cooled and added with 3.0 mL of $HN0_3$ (1+1). The sample is heated on a hot plate at 100-120°C until dry. The crucible is placed back into the muffle furnace and muffle at 500°C oven for 1 additional hour and afterward removed from the muffle furnace, cooled, and added with 10 mL HC1 (1+1), and then the sample is transferred to a 50 mL volumetric flask, and diluted to volume with deionized water and well mixed.

The operating parameters were as follows: Radiofrequency power: 1200 W; Detector type: High dynamic range detector (HDD); Nebulizer: Meinhard Type, Plasma gas flow: 14 l/min; Auxiliary gas flow: 3 l/min; sample time delay: 30 sec; Integration time: 5 sec).

Determination of total phenolic content:

The assessment of phenolic compounds is carried out by the spectrophotometric technique using the *Folin-Ciocalteu* reagent [20] (JENWAY 6300 spectrophotometer). The sample concentration in total phenolic compounds is deduced from a standard curve using known gallic acid concentration solutions. The results are expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Determination of total flavonoids content

The determination of the total flavonoids in the extracts was carried out using the spectrophotometer method described by *Dehpour* et al. [11], and the results are expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/g dw).

Determination of tannins content

The tannins are determined in the extracts by the spectrophotometer method of vanillin in an acidic medium [7]. The results were expressed as the equivalent of tannic acid in milligrams per gram of dry weight (mg TAE/g dw).

UV-Visible spectrophotometer analysis

The UV-visible spectra of the various extracts were obtained after extraction of the sample powders with MeOH / H_2O, MeOH, MeOH / DCM, and DCM (0.1 g/10 ml). The acquisition of spectral data on the UV-visible wavelength (200 - 800 nm) was carried out with solutions of the same dilution using a UV-Visible Shimadzu UV-2450 spectrometer (Kyoto, Japan).

DPPH free radical-scavenging activity

The free radical scavenging potentials of methanolic extracts were evaluated using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method described by *Sánchez-Moreno* et al. [27], using a UV spectrophotometer (Jenway 6300, USA).

The DPPH free radical scavenging activity (AA) in (%) was calculated using the following formula:

$$(AA)\ (\%) = [(A_{blank} - A_{sample})/A_{blank}] \times 100.$$

where:

A_{blank} - is the absorbance of the control reaction (containing all reagents except the test compound)

A_{sample} - is the absorbance of the test compound.

The concentration of extract ensuring 50% inhibition (IC50) was calculated from the graph plotted of the inhibition capacity (AA) in (%) as a function of the different concentrations of the extracts with as standards, ascorbic acid and butylated hydroxytoluene (BHT), using Graph Pad Prism 8.0.1 Software.

Statistical analysis was performed by analysis of variance (ANOVA) and differences between means were determined by *Tukey* test using SPSS software version 26. The significant difference threshold is set at $p<0.05$.

RESULTS

Proximate analysis

Table 1 shows the results of the proximate composition. The table shows that the fresh bean pod samples contain 87.31 ± 0.25% of moisture, 4.67 ± 1.03 g /100 g ash, 29.11 ± 3.20 g/100 g of protein, and 1.2 ± 0.05 g/100 g of lipids.

Table 1. Proximate composition of faba bean pods

Proximate	Content
Moisture %	87.31±0.25
Ash (g/100 g)	4.67±1.03
Proteins (g/100 g)	29.11±3.20
Fat (g/100 g)	1.2± 0.05
Total carbohydrate (g/100 g)	65.02±1.42
Energy (kJ)	1644.61

The analysis of the mineral composition (Table 2) shows that the fresh pods of (*Vicia faba L*) have a high content in particular of potassium (1946.8 ± 4.61 mg/100 g), phosphorus (483.8 ± 3.14 mg/100 g), sodium 430.9 ± 8.73, calcium (399.6 ± 2.25 mg/100 g), magnesium (301.0 ± 2.35 mg/100 g) and a considerable content of manganese, iron and copper. These content are considerable as they contribute to the recommended daily intake (RDI) representing 40% for Ca, 45.5% for protein, 48% for phosphorus, 51.2% for potassium, 70.6% for Cu, 71.7% for Mg, 82% for iron, 70% for sodium of RDI [35].

Table 2. Mineral composition of fresh faba bean pods

Element	Content (mg/100 g dry matter)
Ca	399.6±2.25
P	483.8±3.14
K	1946.8±4.61
Mg	301.0±2.35
Na	430.9±8.73
S	212,.±9.75
Fe	6.6±0.09
Mn	13.1±0.21
Cu	1.2±0.04

The results concerning the yield of the various extracts expressed as a percentage relative to 100 g of dry weight are presented in Table 3. The extract with methanol/ water revealed the highest yield (48.10%) followed by the methanol extract (19.55%) then methanol/dichloromethane 16.5% and finally that was obtained with dichloromethane (9.75%) extracts.

Total phenolic content

Table 3 shows the results concerning the total phenolic content in the fresh pods of (Vicia faba L.) extracts. The methanol/water extract reveals the highest levels of total phenolic compounds (594.4 mg GAE/g), followed by methanol extracts, that of methanol/dichloromethane, and finally dichloromethane extracts (49.5 mg GAE / g).

Total flavonoids content

The total flavonoids contents in the fresh pods of (*Vicia faba L.*) ranged from 0.7 to 7.2 mg QE/g in the methanol/water extract and the methanol extract respectively. Also, the total flavonoids content in the methanol extract was significantly higher than the other extracts.

The tannins content

Table 3 also presents the tannin content in the various extracts of fresh bean pods (*Vicia faba L.*) analyzed. The results show that the tannins contents are ranging from 4.9 mg TAE/g to 73.91 mg TAE /g in the methanol/water and the methanol extracts, respectively.

The highest content in tannins (73.91 mg TAE/g) is found in the methanol extract followed by methanol/Dichloromethane extract (46.7 mg TAE/g) with no significant difference between the other extracts.

The study carried out by *Baginsky* et al. [8] showed that condensed tannin content in immature seeds of fava bean ranged from 309 to 958 mg catechin equivalent per kilogram.

UV-Visible spectrophotometric analysis

Figure 2 shows the UV-visible spectra of the extracts obtained by MeOH/H_2O, MeOH, MeOH / DCM, and DCM with the characteristics presented in Table 3. The spectra of the four extracts are characterized by the high absorbance intensities in the region 260 - 280 nm, commonly attributed to phenolic and aromatic compounds rich in unsaturated groups of C = C, C = O, and N = N [36]. From this analysis, it appears that the contents of the total phenolic compounds in

Table 3. Content of total phenolic compounds and flavonoids and tannins in different extracts of immature Faba bean pods

Extract	Total phenolic compounds	Total flavonoids	Tannins	Extraction yield (%)
MeOH/water	594.4±18.3	0.7±0.1	4.9±0.2	48.10
MeOH	189.1±5.3[a]	7.2±1.2[a]	73.91±35.1[8]a	19.55
MeOH/DCM	81.4±2.7[a]	3.4±0.1[a]	46.7±0.[9]b	16.5
DCM	49.5±21.5[b]	0.9±0.1[b]	5.9±2.6[b]	9.75

Data presented as means ± standard deviation from the triplicate analysis.

[a] Significant when MeOH/water was compared to the other extracts at $p < 0.05$.

[b] Not Significant when MeOH/water was compared to the other extracts at $p < 0.05$.

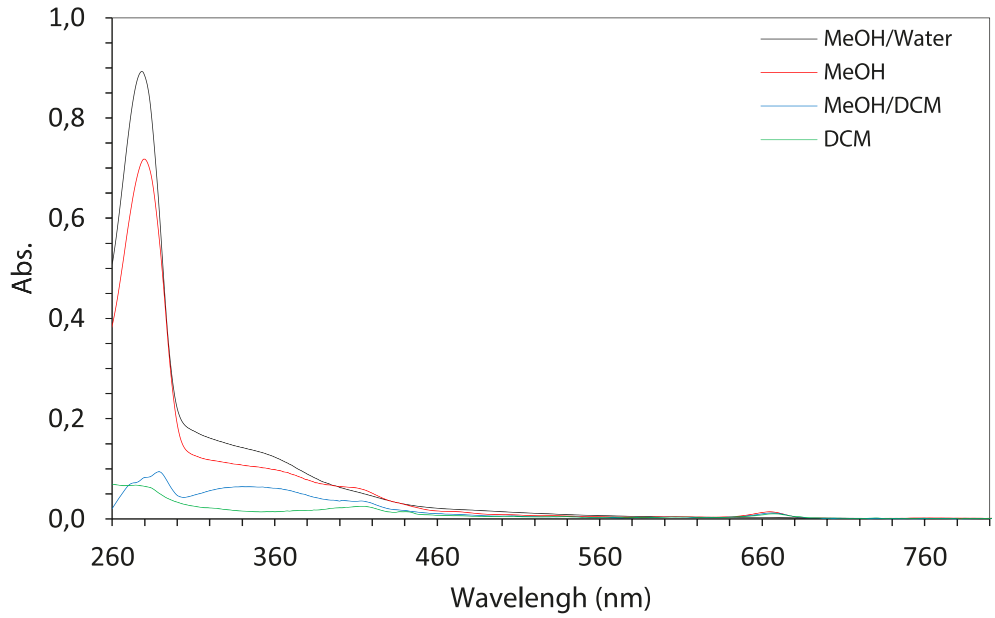

Figure 2. UV-visible spectra of the diluted extracts obtained by MeOH / H_2O, MeOH, MeOH/ DCM, and DCM

the extracts are in agreement with the absorbance intensities observed for all spectra at 285 nm.

DPPH free radical scavenging activity

The scavenging profiles of the extracts and standards ascorbic acid and BHT are presented in Figure 3. The figure shows that all extracts have free radical scavenging potential. The methanol extract was the most active while the dichloromethane extract was the least.

As shown in Table 4, for all the extracts, the IC50 values are significantly different from each other. Also, the IC50 value of the MeOH extract was significantly lower than all the standards. The best activity was recorded for the methanol extract with an IC50 value of 491.2 µg/mL followed by that of the MeOH / water extract and the DCM / MeOH extract with IC50 values of 606.61 µg/mL and 642.67 µg/mL respectively, while the inhibition percentage of the DCM extract of the 2 mg/mL concentration does not exceed 23.53%. Compared to two standards samples, ascorbic acid showed better activity (IC50 value=117.05 µg/mL followed by BHT (IC50 = 235.42 µg/mL).

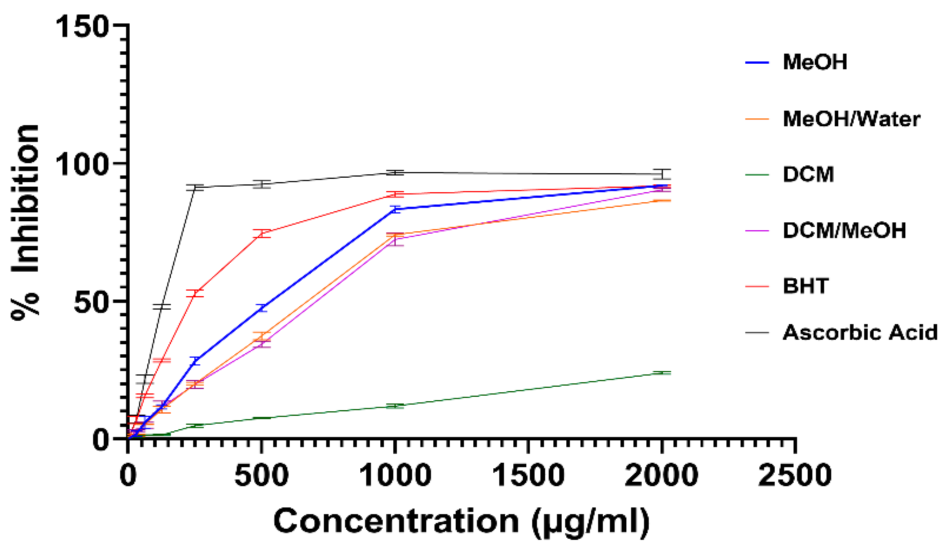

Figure 3. Antiradical activity of extracts of immature pods of (*Vicia faba L*) (each value represents the average of three determinations ± SD).

Table 4. IC50 values for the extracts as well as ascorbic acid and BHT

Extract / standard	IC 50 (μg/ml)
MeOH**	491.2 ± 10.81
MeOH/Water*	606.61 ± 2.36
DCM	ND
DCM/MeOH*	642.67± 26.57
BHT**	235.42± 1.79
Ascorbic acid**	117.05 ±1.51

*Significant level $p < 0.05$, **Significant level $p < 0.01$
ND - not determined

DISCUSSION

This work focused on the evaluation of the nutritional and biological value of fresh pods of (Vicia faba L), including their bark, as traditionally used in recipes consumed by the Moroccan population. The analyzes carried out in this study revealed considerable nutrient contents in a 100 g portion of this legume which can meet a proportion ranging from 40 to 100% of the recommended dietary intake. The study also showed that the nutrient contents found are higher or in line with the values reported for legumes in other studies.

Indeed, the ash, protein and lipid contents obtained in this study are similar to those reported by *Millar* et al. on shelled/split beans (3.40 ± 0.09 g/100 g; 28vg/100 g; 1.57 ± 0.11 g/100 g) dw respectively [23].

The fresh bean pods analyzed contain also a high content of energy (1644.61 kJ) and carbohydrate (65.02 g/100 g). The carbohydrate content found in the present study is similar to that reported by *Berrios* et al. [9] for lentils and chickpeas (62.49–65.7 g/100 g dw).

The minerals values found here were comparable to those previously published by *Millar* et al. [23] for the potassium, iron, calcium, and magnesium contents in the split fava beans. On the other hand, the contents of Ca, K, Mg, Na reported in the present study are higher than those found in the bean seed by *Hacıseferoğulları* et al. [17].

The total phenolic compounds content found in this study samples were higher than those reported previously by Millar *et al.* [23] and *Baginsky* et al. [8] in the split beans as well as in other species of the same family, like common beans (*Phaseolus vulgaris L.*) [34].

Several studies have analyzed flavonoids and reported different contents in legumes. Among these *El-Feky* et al. [13] studies have found 16 flavonoids in the ethyl acetate fraction of the bean peels and in the whole fraction a content of 12.30 mg/g dry weight. Other legumes like common beans (*Phaseolus vulgaris L)* were reported with a content of total flavonoids of 252 mg CE/100 g dw as shown by *Yang* et al. [34].

CONCLUSIONS

The results obtained show that fresh bean pods represent an important nutritional source of protein, energy and trace elements. These bean pods in addition to diversifying diets, their nutritional value testifies to their contribution to meet the RDI and at the same time to fight against nutrient deficiencies and associated malnutrition. They also contain bioactive compounds, in particular polyphenols which confer considerable natural antioxidant power to these legumes.

The nutrients and biological values found in this study are determined in the two parts of the studied legume, namely, the bean kernel and the peels. These parts are used separately or together in traditional Moroccan recipes. Indeed, the peels are used as the main vegetable of traditional recipes which could diversify the diet of the local populations because they are added with other ingredients according to the recipes. By throwing away this part of legumes as is the case today and viewing them as waste, the nutritional and functional values found in this study are also diminished or lost. It is recommended that all these qualities be considered and valued by the revitalization of traditional recipes using them to fight against their abandonment and waste.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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