

http://www.aimspress.com/journal/agriculture

AIMS Agriculture and Food, 5(4): 635–648.

DOI: 10.3934/agrfood.2020.4.635

Received: 13 July 2020

Accepted: 13 September 2020 Published: 22 September 2020

Research article

Total phenolic, total flavonoid contents and antioxidant potential of Common Bean (*Phaseolus vulgaris* L.) in Vietnam

Pham Thi Thu Ha^{1,*}, Nguyen Thi Bao Tran¹, Nguyen Thi Ngoc Tram¹ and Vo Hoang Kha²

- ¹ Genomic Research Institute and Seed, Ton Duc Thang University, Ho Chi Minh City, Vietnam
- ² Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam
- * Correspondence: Email: phamthithuha@tdtu.edu.vn; Tel: +84933092584.

Abstract: In this study, the antioxidant properties of total phenolics and flavonoids extracts from different parts (leaves, pods, and seeds) of common bean were evaluated. Specifically, the highest total phenolic content was recorded with methanol extracts of pods (95.41 \pm 1.18 mg GAE/g), whereas methanolic extract of seeds contained the lowest content of phenols (6.87 \pm 1.45 mg GAE/g). The highest total flavonoid content was found in methanol extracts of leaves (44.59 \pm 2.15 mg RE/g). Meanwhile, the methanol extract of seeds and pods contained less flavonoid content (9.29 \pm 1.65 mg RE/g and 3.64 \pm 0.87 mg RE/g, respectively). The GC-MS analysis showed the presence of 29, 18 and 29 different plant compounds in methanol extracts of leaves, seeds and pods, respectively. The methanol extracts of leaves showed the highest antioxidant capacity with an inhibitory percentage of 48.74 \pm 0.32% at a concentration of 100 µg/mL and the EC₅₀ value of 137.4 µg/mL. The methanol extracts of seed had the lowest antioxidant capacity with an inhibitory percentage of 13.99 \pm 1.22% at a concentration of 100 µg/mL and the EC₅₀ value of 486.2 µg/mL. The results showed that the extract from leaves of common bean had the highest antioxidant activities as well as total contents of flavonoid in comparison with an extract from seeds and pods and the positive relationship between total flavonoids content and antioxidant activities in this plant.

Keywords: common bean; phenolic contents; flavonoid contents; antioxidant activity

1. Introduction

Nowadays, the development of chronic (e.g., renal failure, myocardial infarction, and heart failure) and neurogenerative (e.g., Parkinson's, multiple sclerosis, and Alzheimer's) diseases have

been attributed to oxidative stress as result of an imbalance between prooxidants and antioxidants [1]. Prooxidant refers to any endobiotic or xenobiotic that induces oxidative stress either by the generation of ROS or by inhibiting antioxidant systems. It can include all reactive, free radical containing molecules in cells or tissues [2]. Free radicals such as hydroxyl, singlet oxygen, nitric oxide, hydrogen peroxide, and superoxide radicals are produced as part of normal cellular function [3]. However, above physiological levels, free radicals have been shown to induce negative health effects such as carcinogenesis, aging DNA damage, and enzyme inactivation by attacking biological macromolecules [4].

To prevent the oxidation of molecules and cells by free radicals, the body has endogenous antioxidative systems through which it is able to quench free radicals and protect against oxidative stress [5]. Antioxidants might be categorized in multiple different ways like based on their activity, which they can be classified into two categories as enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants will be breaking down and be removing free radicals by converting dangerous oxidative products to hydrogen peroxide (H₂O₂) and then to water, the process had multistep and had the presence of cofactors such as copper, zinc, manganese, and iron. On the other hand, non-enzymatic antioxidants will interrupt free radical chain reactions [6]. However, in the immune system, antioxidants can deplete due to environmental pollutants, radiation, chemicals, toxins, deepfried foods, and spicy foods as well as physical stress, which induce changes in gene expression and formation of abnormal proteins [7]. Hence, the need is for sufficient dietary levels of antioxidants, to help protect the body against oxidative stress [8].

Phenolic compounds are natural compounds ubiquitous in plants and are the product of secondary plant metabolism [6]. They can be classified into various groups like phenolic acids, flavonoids, stilbenes, and lignans base on the presence of multiple phenolic groups that are associated with more or less complex structures [9]. Phenolic compounds are largely found in fruits, vegetables, cereals, olive, legumes, chocolate, and beverages, such as tea, coffee, and wine [9]. Although phenolics are primarily known for their antioxidative functions, they have also been shown to offer other beneficial health effects such as antidiabetic, anticancer, anti-inflammatory, cardioprotective, osteoprotective, neuroprotective, antiasthmatic, antihypertensive, antiageing, antiseptic, cerebrovascular protection, cholesterol-lowering, hepatoprotective, antifungal, antibacterial, and antiviral properties, specifically, their primary functions are as antioxidant [10]. According to previous studies, the presence of hydroxyl groups in the B-ring of flavonoids is responsible for their observed antioxidant properties through their donation of hydrogen atoms during free radical reactions [11]. Besides, phenolics is also a good source of antioxidants due to a number of different mechanisms such as free radical-scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelating, and acting as a substrate for radicals such as superoxide and hydroxyl [12].

The common bean (*Phaseolus vulgaris* L.) is a member of the legume family [13]. It is considered as important food resources due to their rich source of proteins, carbohydrates, dietary fiber, minerals, vitamins, phenolic acids, and flavonoids [14]. Many studies show that diets including common beans help reduce LDL-cholesterol while increasing HDL-cholesterol, thus helping reduce risks of cardiovascular diseases, obesity, and diabetes [15]. Moreover, previous researchers reported that common bean containing phenolics and showed high antioxidant activity by *in vitro* methods of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), and oxygen radical absorbance capacity (ORAC) [16–19]. Therefore, the objectives of the present study were to

determine the total phenolic and flavonoid contents as well as the antioxidant potential of common bean (*Phaseolus vulgaris* L.) in Vietnam.

2. Materials and methods

2.1. Plant material and extract preparation

Leaves, pods, and seeds of common bean (GRIS2) (Figure 1) were collected at Genomic Research Institute and Seed (GRIS), Ton Duc Thang University, Ho Chi Minh City, Vietnam. Before extraction, they were cleaned to eliminate soil and damaged seeds, dried and ground into a fine powder. The sample extract was extracted using the method according to [20]. The leaves, pods, and seeds extract were individually prepared in methanol (plant: solvent ratio [1: 10], w/v), and extracted for shaken overnight at 28 °C. The extract was then filtered through filter paper. The solvent was then removed by evaporation in a vacuum to obtain dry extracts. This process was repeated once. The extracts were stored at 4 °C in a refrigerator prior to use.

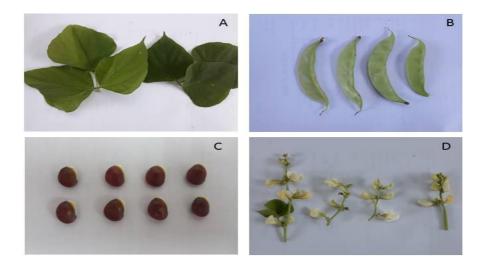


Figure 1. Common bean (*Phaseolus vulgaris* L.) (A) Leaves, (B) Pods, (C) Seeds, and (D) Flowers.

2.2. Total phenolic contents

The total phenolic content of each leaf, pods, and seeds extract was determined using the Folin-Ciocalteu reagent as described by the method of Singleton et al. [21] with slight modification. Approximately 0.5 mL of each extract was dissolved in methanol (100 µg/mL) was mixed with 2.5 mL of Folin-Ciocalteu reagent (0.2 N). This mixture was shaken well and was kept at room temperature for 5 min and then, 2 mL of sodium carbonate solution (75 g/L) was added. After 2 h of incubation in the dark, the absorbencies were measured at 760 nm against a water blank using a UV-Vis spectrophotometer. The same procedure was repeated by gallic acid solutions used as a standard for the calibration curve. The concentrations of the standard were set at 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL. The determination was performed in triplicate and the results were expressed as terms of gallic acid equivalent (mg of GAE/g of extract).

2.3. Total flavonoid contents

The total flavonoid content of each leaf, pods, and seeds extract was determined using the Dowd method as described by Sawadogo et al. [22] with slight modification. In brief, 2 mL of 2% AlCl₃ in methanol was mixed with 2 mL of each extract (100 µg/mL), shacked well, and hold for 10 minutes. Absorption was read at 415 nm against a blank sample consisting of 2 mL of methanol and 2 mL of each extract without AlCl₃ using a UV-vis spectrophotometer. The same procedure was repeated by rutin solutions used as a standard for the calibration curve. The concentrations of the standard were set at 0, 0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL. The determination was performed in triplicate and the results were expressed as terms of rutin equivalents (mg of RE/g of extract).

2.4. GC-MS analysis

Methanol extract from leaf, pod, and the seed of common bean was subjected to gas chromatography-mass spectrometry (GC-MS) analysis. The GC-MS was equipped with a DB-5MS column (30 m, 0.25 mm, and 0.25 μm) (Agilent Technologies, J & W Scientific Products, Folsom, CA, USA.). Helium gas was used as the carrier gas with a split ratio of 5:1. The temperature program was as follows: initial temperature of 50 °C without hold time and gradually increased to 300 °C at a rate of 10 °C/min for 20 min of hold time. The injector and detector temperatures were set to 300 °C and 320 °C, respectively. The mass spectra were scanned from 29 to 800 amu. The identification and characterization of chemical compounds in various crude extracts were based on the JEOL's GC-MS Mass Center System software, version 2.65a (JEOL Ltd., Tokyo, Japan).

2.5. Determination of free radical scavenging activity by DPPH method

The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay is one of the most commonly employed methods because it is simple, efficient, and inexpensive. DPPH radical scavenging method was used to evaluate the antioxidant properties of each leaf, pods, and seeds bean extract. The standard procedure for the DPPH assay was performed based on Bakasso et al. [23] with minor modifications. The samples were added of 1.5 mL DPPH solution (80 μg/mL) to 0.75 mL various concentrations (6.25, 12.5, 25, 50, and 100 μg/mL) of each extract. The solution was mixed vigorously and left to stand at room temperature for 30 minutes in the dark after which its absorbance was measured spectrophotometrically at 517nm, the analysis was done in triplicate. A positive control (ascorbic acid) was prepared in the same way as samples, while the blank solution by adding 0.75 mL methanol to 1.5 mL of DPPH (80 μg/mL solution). The absorbance of blank, positive control, and samples were recorded.

The percentage of inhibition can be calculated using the formula:

Inhibition (%) =
$$(AB - AA/AB) \times 100$$
 (1)

Where AB is the absorbance of the control and AA is the absorbance of the test. EC_{50} (µg/mL) was defined as the half-maximal effective concentration of the amount of sample necessary to decrease the absorbance of DPPH by 50%. It was obtained by interpolation from the linear regression analysis.

2.6. Statistical analysis

The results were expressed as mean \pm standard deviation of at least triplicate measurements. Analysis of variance (ANOVA) and Duncan's multiple range test were used for determining the significant differences at P < 0.05. All statistical analyses were carried out using the statistical program are SAS version 8.0 and Microsoft Excel 2010 software.

3. Results

3.1. Total phenolic and flavonoid contents

The content of total phenols in different extracts was presented in Table 1. The highest phenolic content was found in methanol extracts of pods (95.41 \pm 1.18 mg GAE/g). Whereas methanol extracts of seeds contained considerably least content of phenols (6.87 \pm 1.45 mg GAE/g).

The results of the total flavonoids contents determination of the examined plant extract are presented in Table 1. The highest flavonoid content was found in methanol extracts of leaves (44.59 ± 2.15 mg RE/g). Meanwhile, the methanol extract of seeds and pods contained less flavonoid content (9.29 ± 1.65 mg RE/g and 3.64 ± 0.87 mg RE/g, respectively).

Table 1. Total phenolic and total flavonoid contents of leaves, pods, and seeds of common bean.

Categories	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)
Leaves	$58.68 \pm 1.81^{\text{b}}$	44.59 ± 2.15^{a}
Pods	95.41 ± 1.18^{a}	$3.64 \pm 0.87^{\rm b}$
Seeds	$6.87 \pm 1.45^{\circ}$	9.29 ± 1.65^{b}

All data are mean \pm SD of triplicate (n = 3) analyses. Values with a different superscript in the same column differ significantly (P < 0.01).

3.2. GC-MS analysis

The chemical components in methanol extract from the leaves, seed, and pods of common bean were successfully analyzed using GC-MS in Figure 2. In total, 76 compounds were detected and presented in Table 2. Of these, the presence of 29, 18, and 29 various phytocompounds in methanol extract from the leaves, seed, and pods respectively. The methanol extract from pods presented the highest amount of phytocomponents compared to methanol extract from leaves and seed. The present study successfully identified the bioactive components present in methanol extract from leaves, pods, and seeds of the common bean by GC-MS included phenolics, flavonoids, fatty acids, amino acid, terpenoids, sterols, carbohydrates, alcohols, volatile oils, fatty acid ester, ester, amines, and others.

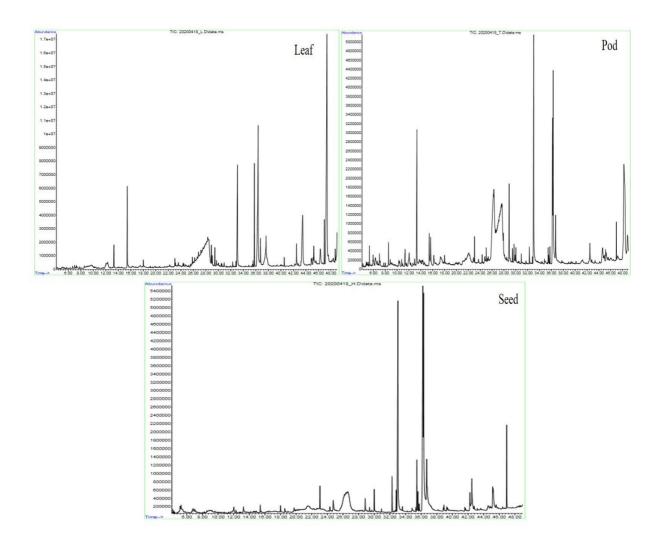


Figure 2. Mass Spectrometry of methanol extract from the leaves, seed, and pods of common bean.

Table 2. Chemical profile in methanol extract from the leaves, seed, and pods of common bean.

Peak number	Leaf	Pod	Seed	Chemical class
1	Desulphosinigrin	-	-	-
2	Butyrolactone	-	-	-
3	Cyclooctanone	-	-	-
4	Nanofin	-	-	-
5	Oxacyclododecan-2- one	-	-	-
6	8-Aminocaprylic acid	-	-	Amino acid lysine
7	Pebulate	-	-	Colorless oil
8	1,2- Benzenedimethanol	-	-	-
9	Bioallethrin	-	-	Ectoparasiticide

Continued on next page

Peak number	Leaf	Pod	Seed	Chemical class
10	Gibberellic acid	-	-	Pentacyclic diterpene
11	1,3,5-Triazin-2-amine, N-ethyl-4-methoxy-	-	-	-
12	Dodecanoic acid	-	-	Fatty acid
13	N-(2-Acetamido) iminodiacetic acid	-	-	Dicarboxylic acid
14	3-Hydroxy-β-damascone	-	-	-
15	Methoprene	-	-	Terpenoid
16	Gamolenic Acid	-	-	Fatty acid
17	γ-Tocopherol	-	-	Terpenoid
18	Phytol, acetate	-	-	Acyclic diterpene
19	Vitamin E	-	-	Terpenoid
20	Trilinolein	-	-	Fatty acid
21	Stigmasterol	-	-	Sterol
22	δ-Tocopherol, O- acetyl-	-	-	Terpenoid
23	Aspidofractinine-3-methanol, $(2\alpha,3\beta,5\alpha)$ -	-	-	Alcohol
24	Butyrolactone	-	-	Ester
25	Cholesterol, 7-oxo-	-	-	-
26	p-Menthane-1,2,3-triol	-	-	Terpenoid
27	trans-Isoeugenol	-	-	Volatile oil
28	2-n-Propylthiane	-	-	-
29	4-Nonene	-	-	-
30	-	1H-Pyrrole, 2,4-dimethyl-	-	Volatile oil
31	-	2-t-Butyl-5-propyl- [1,3]dioxolan-4-one	-	-
		l-Alanine, N-		
32	-	methoxycarbonyl-, butyl ester β-D-Glucopyranose, 1-	-	Amino acid ester
33	-	thio-,1-[N-hydroxy-5- (methylthio)pentanimidate]	-	Glycoside
34	-	2-Methyl-3-(methylthio)-1- propene	-	-
35	-	4-Cyclopentene-1,3-dione	-	-
36	-	(S)-(+)-2-Amino-3-methyl-1-butanol	-	Amino alcohol
37	-	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-	Phenolic

Continued on next page

Peak number	Leaf	Pod	Seed	Chemical class	
38	_	N-(N-Glycyl-glycyl)-		Amino acid	
		glycine		minio acia	
39	-	3-Methyladipic acid	-	Fatty acid	
40	-	Glycylsarcosine	-	-	
41	-	Clindamycin	-	Phenolic	
42	-	2-Propyl-tetrahydropyran- 3-ol	-	Alcohol	
43	-	dl-Lysine	-	Diamino acid	
44	-	5-Hydroxymethylfurfural	-	Carbohydrate	
45	-	Showdomycin	-	Phenolic	
46	_	Nitrosothymol	-	-	
47	-	β-D-Glucopyranoside, methyl	-	Carbohydrate	
		Acetic acid, 2,2'-			
48	-	[oxybis(2,1-	-	-	
		ethanediyloxy)]bis-			
49	_	Ingol 12-acetate	-	-	
		Acetamide, N-(4-ethoxy-3-			
50	-	hydroxyphenyl)-	-	-	
~ 1		1,2-Benzenedicarboxylic			
51	-	acid, butyl octyl ester	-	Ester	
~ 0		9,12-Octadecadienoic acid,			
52	-	methyl ester, (E,E)-	-	Fatty acid	
~~		9,12,15-Octadecatrienoic		-	
53	-	acid,(Z,Z,Z)-	-	Fatty acid	
54	_	Octadecanoic acid	_	Fatty acid	
55	_	α-Amyrin	-	Triterpene	
		3β-Myristoylolean-12-en-		F - 22	
56	-	16β-ol	-	-	
		E-11-Methyl-12-			
57	-	tetradecen-1-ol acetate	-	-	
58	_	HEPES	-	-	
59		Thymol			
		,	1H-Pyrrole, 2,4	_	
60	-	-	dimethyl-	Phenolic	
61	_	-	γ-Dodecalactone	Ester	
			Benzofuran, 2,3	_	
62	-	-	dihydro-	Phenolic	
			Pyridine, 1,2,3,6	_	
63			tetrahydro-1,2-	Pyridine	
			dimethyl-	1 jiidiile	
			•	Alkane	
64	-	-	Tridecane	hydrocarbon	

Continued on next page

Peak number	Leaf	Pod	Seed	Chemical class
65	-	-	Maltol	-
66	-	-	Isoglutamine	Gamma amino acid
67	-	-	Cycloate	Aliphatic amine
68	-	-	3-Butylindolizidine	Alkaloid
69	-	-	Dibutyl phthalate	Ester
70	-	-	Hexadecanoic acid, ethyl ester	Fatty acid ester
71	-	-	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid ester
72	-	-	10-Octadecenoic acid, methyl ester	Fatty acid ester
73	-	-	9,12-Octadecadienoic acid (Z,Z)-	Fatty acid
74	-	-	β-Sitosterol	Sterol
75	-	-	Bacteriochlorophyll- c-stearyl	-
76	-	-	Glycerol 1-stearate	Ester

3.3. Antioxidant properties by DPPH

In this study, the DPPH radical scavenging potential of methanol extracts from the leaves, seed, and pods of common bean and ascorbic acid were represented in Table 3 and EC $_{50}$ values result of different extracts were calculated by using concentration with mean percent inhibition of the DPPH radical curve of each different extracts also were presented in Table 3. From Table 3, all the extracts showed an inhibitory potential against DPPH free radical. The inhibitory percentages varied from $5.12 \pm 0.35\%$ for the seeds extract to $98.88 \pm 0.03\%$ for the vitamin C. The EC $_{50}$ values of the antioxidant capacity varied significantly (P < 0.01) from 23.31 µg/mL for the vitamin C to 486.2 µg/mL for the seeds extract. As it was known, the lower the EC $_{50}$ value the higher the antioxidant capacity of the plant extract. As can be seen from Table 3, the methanol extracts of leaves have the highest antioxidant capacity with inhibitory percentages are $48.74 \pm 0.32\%$ at a concentration of 100 µg/mL and EC $_{50}$ value was 137.4 µg/mL compared to the other extracts and 6 times lower than vitamin C. The methanol extracts of seed had the lowest antioxidant capacity with inhibitory percentages was $13.99 \pm 1.22\%$ at a concentration of 100 µg/mL and EC $_{50}$ value was 486.2 µg/mL compared to the other extracts and 21 times lower than vitamin C.

Table 3. Percentage inhibition of DPPH free radical scavenging activity and EC_{50} of ascorbic acid and plants extract common bean.

	DPPH inhibition (%)					EC ₅₀	
Treatment	6.25 μg/mL	12.5 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	Value (μg/mL)	
Seeds	5.12 ± 0.35^{d}	6.07 ± 1.27^{d}	7.42 ± 0.67^{d}	9.78 ± 0.44^{d}	13.99 ± 1.22^{d}	486.2	
Pods	14.19 ± 1.62^{c}	14.82 ± 1.28^{c}	16.94 ± 0.17^{c}	20.03 ± 0.69^{c}	$25.93 \pm 1.70^{\circ}$	290.3	
Leaves	45.60 ± 0.32^{a}	45.87 ± 0.59^{a}	46.20 ± 0.91^{b}	47.13 ± 0.05^{b}	48.74 ± 0.32^{b}	137.4	
Vitamin C	36.18 ± 1.00^{b}	40.00 ± 1.79^{b}	55.16 ± 2.52^{a}	71.79 ± 1.41^{a}	98.88 ± 0.03^{a}	23.31	

All data are mean \pm SD of triplicate (n = 3) analyses. Values with a different superscript in the same column differ significantly (P < 0.01).

4. Discussion

The consumption of common bean (*Phaseolus vulgaris* L.) has been greatly connected with many physiological and health-promoting effects such as the prevention of cardiovascular diseases, obesity, diabetes mellitus, and cancers [10]. The antioxidant properties of phenolic compounds lie in their ability to neutralize free radicals and the chelation of transition metals, thus they counteract the initiation and propagation of oxidative processes [24]. In the present study, total phenolic acid and total flavonoid contents and antioxidant activity in vitro were determined for methanol extracts of leaves, seeds, and pods of common bean.

In the present study, the total phenolic contents of seeds were 6.87 \pm 1.45 mg GAE/g (Table 1) whereas according to Yao et al. [13] reported that common bean contained 8.59 mg GAE/g total phenols, besides, according to Ombra et al. [25] reported that total phenolic content of the common bean in the range of 0.14–1.29 mg GAE/g. The total flavonoid content of seeds bean was 9.29 mg RE/g (Table 1) and was higher than the previously reported by Oomah et al. [26] for selected common bean in the range of 0.41–1.02 mg RE/g. The difference in the total contents of phenolic acid and flavonoid may be due to differences in the geographical region, environmental, climatic condition, and storage, and processing methods [27]. In addition, in the current study, the total contents of flavonoid and phenolic acid were different among leaves, pods, and seeds bean. These results showed that different levels of phenolic acids and flavonoids were influenced by the interaction between parts of plants. This finding is in agreement with that of Males et al. [28] who reported that I. candida contains higher phenolic compounds in leaves (1.031–1.423%) compared to stem (0.411-0.516%). Ghasemzadeh et al. [29] also recorded the total flavonoid and phenolic acid contents in the leaves were more than in the rhizomes, followed by contents in the stems. Elkhamlichia et al. [30] also confirmed that Calycotome villosa subsp. Intermedia had the total flavonoids contents in seeds. Previous studies by Ferry et al. [31] and Elattar and Virji [32] have shown that some flavonoids components such as quercetin, rutin had anticancer activities and were able to inhibit cancer cell growth. Therefore, the results of this study showed that flavonoids are important components of this plant.

Among the compounds discovered, several are reported as potential therapeutic agents. For instance, 9, 12-octadecadienoic acid, methyl ester is effective antihistamines, anti-coronary, insectifuge, and antieczemic [33]. Terpenoids have also been reported to exhibit antiplasmodial, antineoplastic, and antiviral activities [34]. Van Acker et al. [35] found other molecular parameters

related to electron distribution and structure, which correlate with the antioxidant action of vitamin E and its derivatives. Besides, flavonoids and phenolics are polyphenols that have been reported to possess great antioxidant properties due to the reducing ability of flavonoids when they play an important role in neutralizing free radicals and scavenging radicals or suppressing lipid peroxidation [26]. Vitamin P (Rutin) is a flavonol, it has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities [36–39], whereas showdomycin and clindamycin were known as an antibiotic [38].

Phenolic compounds restrain the formation of superoxide anion as well as the production of reactive oxygen species by inhibiting key enzymes such as protein kinase, xanthine oxidase, lipoxygenase, cyclooxygenase, S-transferase, glutathione, and NADH oxidase [26]. Moreover, in aqueous and lipophilic phases, these compounds also serve as hydrogen donating radical scavengers. The ability of flavonoids to complex with metal ions plays an important role in their antioxidant activity [24]. There is a specific relationship between flavonoid structures and their antioxidant activity as the larger the number of hydroxyl groups in the flavonoid nucleus, the greater would be the antioxidant activity [40].

According to [25] reported the common bean to have EC_{50} value in the range of 1570–55200 µg/mL. In this study, the EC_{50} value of seeds was 486.2 µg/mL whereas, the EC_{50} value of leaves was 137.4 µg/mL showed that the antioxidant activity of leaves bean higher than seeds (Table 3). Moreover, the total flavonoid contents of leaves bean also higher than seeds (Table 1). Hence, the different antioxidant activity might as well be due to the presence of phenolic compounds, especially the flavonoid contents in this plant. The antioxidant activity of phenolic compounds was based on several different mechanisms. It has the ability to scavenging of free radicals by single electron transfer and the hydrogen atoms in their hydroxyl groups, chelation of metal ions such as iron and copper, or inhibition of enzymes responsible for a free radical generation [26, 41].

From the determination of total phenolic contents, total flavonoid contents, and antioxidant activity in this study observed that the extracts of the pod bean showed the highest content of total phenolic; however, leaves bean although containing slightly fewer content of phenolic acid, exhibited higher total flavonoid content, and its highest the antioxidant capacity. The antioxidant capacity of phenolic compounds depends on the number and position of free OH groups [42], which means, the many free hydroxyl groups present in polyphenols, the higher their radical scavenging capacity. This reinforced the idea that the antioxidant potential could be linked strongly to the content of flavonoids in this plant.

5. Conclusions

Common bean could be a good source of natural antioxidants. In this study showed the methanol extracts from leaves, pods, and seeds of common bean exhibit good antioxidant ability on the DPPH radical scavenging potential, in which, the extracts of the leaves showed higher scavenging activities than the pods and seeds. Moreover, the total flavonoid content in extracts from leaves also higher than the pods and seed although the total phenolic acid content was found in extracts from extracts of pods higher than the leaves and seeds. Therefore, the results of this study showed that the positive relationship between total flavonoids content and antioxidant activities in this plant.

Conflict of interest

The authors declare that there is no conflict of interest.

References

- 1. Liu JK, Atamna H, Kuratsune H, et al. (2002) Delaying brain mitochondrial decay and aging with mitochondrial antioxidants and metabolites. In: Harman D. (Ed), *Increasing healthy life span: Conventional measures and slowing the innate aging process*. New York Academy of Sciences. 133–166.
- 2. Rahal A, Kumar A, Singh V, et al. (2014) Oxidative stress, prooxidants, and antioxidants: The interplay. *Bio Med Res Int* 2014: 1–19.
- 3. Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. *Ind J clin Biochem* 30: 11–26.
- 4. Rahman T, Hosen I, Islam MT, et al. (2012) Oxidative stress and human health. *Adv Biosci Biotechnol* 3: 997–1019.
- 5. Lobo V, Patil A, Phatak A, et al. (2010) Free radicals, antioxidants, and functional foods: Impact on human health. *Pharmacogn Rev* 4: 118–126.
- 6. Nimse SB, Pal D (2015) Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv* 5: 27986–28006.
- 7. Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* 5: 1142–1145.
- 8. Tan BL, Norhaizan ME, Liew WPP, et al. (2018) Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Front Pharmacol* 9: 1162.
- 9. Massimo DA, Carmela F, Roberta DB, et al. (2007) Polyphenols, dietary sources and bioavailability. *Ann-Ist Super Sanita* 43: 348–361.
- 10. Ganesan K, Xu B (2017) Polyphenol-Rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *Int J Mol Sci* 18: 2331.
- 11. Williams RJ, Spencer JP, Rice-Evans C (2004) Flavonoids: Antioxidants or signalling molecules? *Free Radical Biol Med* 36: 838–849.
- 12. Almokhtar AA, Ata SIE, Azab EA, et al. (2019) Oxidative stress and antioxidant mechanisms in human body. *J Appl Biotechnol Bioeng* 6: 43–47.
- 13. Yao Y, Cheng XZ, Wang LX, et al. (2011) Biological potential of sixteen legumes in china. *Int J Mol Sci* 12: 7048–7058.
- 14. Chávez-Mendoza C, Sánchez E (2017) Bioactive compounds from mexican varieties of the common bean (*Phaseolus vulgaris*): implications for health. *Molecules* 22: 1360.
- 15. Messina V (2014) Nutritional and health benefits of dried beans. *Am J Clin Nutr* 100: 437S–442S.
- 16. Apak R, Güdü K, Demirata B, et al. (2007) Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 12: 1496–1547.
- 17. Clarke G, Ting KN, Wiart C, et al. (2013) High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content

- indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. *Antioxidants* 2: 1–10.
- 18. Basu P, Maier C (2016) In vitro antioxidant activities and polyphenol contents of seven commercially available fruits. *Pharmacogn Res* 8: 258–264.
- 19. Frassinetti S, Gabriele M, Caltavuturo L, et al. (2015). Antimutagenic and antioxidant activity of a selected lectin-free common bean (*Phaseolus vulgaris* L.) in two cell-based models. *Plant Foods Hum Nutr* 70: 35–41.
- 20. Obeidat M, Shatnawi M, Al-alawi M, et al. (2012) Antimicrobial activity of crude extracts of some plant leaves. *Res J Microbiol* 7: 59–67.
- 21. Singleton VL, Orthofer R, Lamuela-Ravent & RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol* 299: 152–178.
- 22. Sawadogo WR, Meda A, Lamien CE, et al. (2006) Phenolic content and antioxidant activity of six acanthaceae from Burkina Faso. *J Biol Sci* 6: 249–252.
- 23. Bakasso S, Lamien-Meda A, Lamien CE, et al. (2008) Polyphenol contents and antioxidant activities of five Indigofera species (*Fabaceae*) from Burkina Faso. *Pak J Biol Sci* 11: 1429–1435.
- 24. Gulcin İ (2020) Antioxidants and antioxidant methods: an updated overview. *Arch Toxicol* 94: 651–715.
- 25. Ombra MN, d'Acierno A, Nazzaro F, et al. (2016) Phenolic composition and antioxidant and antiproliferative activities of the extracts of twelve common bean (*Phaseolus vulgaris* L.) endemic ecotypes of Southern Italy before and after cooking. *Oxid Med Cell Longevity* 2016.
- 26. Oomah BD, Cardador-Mart nez A, Loarca-Piña G (2005) Phenolics and antioxidative activities in common beans (*Phaseolus vulgaris* L). *J Sci Food Agric* 85: 935–942.
- 27. Alirezalu A, Salehi P, Ahmadi N, et al. (2018) Flavonoids profile and antioxidant activity in flowers and leaves of hawthorn species (*Crataegus* spp.) from different regions of Iran. *Int J Food Prop* 21: 452–470.
- 28. Males Z, Pilepic K, Petrovic L, et al. (2010) Quantitative analysis of phenolic compounds of *Inula candida* (L.) Cass. *Period Biol* 112: 307–310.
- 29. Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of malaysia young ginger (*Zingiber officinale Roscoe*). *Molecules* 15: 4324–4333.
- 30. Elkhamlichia A, Hajajia HE, Farajb H, et al. (2017) Phytochemical screening and evaluation of antioxidant and antibacterial activities of seeds and pods extracts of *Calycotome villosa* subsp. Intermedia. *J Appl Pharm Sci* 7: 192–198.
- 31. Ferry DR, Smith A, Malkhandi J, et al. (1996) Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clin Cancer Res* 2: 659–668.
- 32. Elattar TM, Virji AS (2000) The inhibitory effect of curcumin, genistein, quercetin and cisplatin on the growth of oral cancer cells *in vitro*. *Anticancer Res* 20: 1733–1738.
- 33. Krishnamoorthy K, Subramaniam P (2014) Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi Using GC-MS. *Int Scholarly Res Not* 2014.

- 34. Cox-Georgian D, Ramadoss N, Dona C, et al. (2019) Therapeutic and Medicinal Uses of Terpenes. In: Joshee N, Dhekney S, Parajuli P. (Eds), *Medicinal Plants*. Springer, Cham. 333–359.
- 35. Van Acker SA, Koymans LM, Bast A (1993) Molecular pharmacology of vitamin E: structural aspects of antioxidant activity. *Free Radical Biol Med* 15: 311–328.
- 36. Richetti SK, Rosemberg DB, Ventura-Lima J, et al. (2011) Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure. *Neurotoxicology* 32: 116–122.
- 37. Javed H, Khan MM, Ahmad A, et al. (2012) Rutin prevents cognitive impairments by ameliorating oxidative stress and neuroinflammation in rat model of sporadic dementia of Alzheimer type. *Neuroscience* 210: 340–352.
- 38. Ganeshpurkar A, Saluja AK. (2017) The Pharmacological Potential of Rutin. *Saudi Pharm J* 25: 149–164.
- 39. Enogieru AB, Haylett W, Hiss DC, et al. (2018) Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxid Med Cell Longevity* 2018.
- 40. Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013.
- 41. Mitra K, Uddin N (2014) Total phenolics, flavonoids, proanthrocyanidins, ascorbic acid contents and *in-vitro* antioxidant activities of newly developed isolated soya protein. *Discourse J Agric Food Sci* 2: 160–168.
- 42. Aryal S, Baniya MK, Danekhu K, et al. (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western nepal. *Plants* 8: 96.



© 2020 the Author(s), licensee AIMS Press. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0)