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Artículo Científico

Variation of the seed and foliar phenolic contents of five wild forms of common bean (*Phaseolus vulgaris* L.)

Variación del contenido fenólico foliar y de semillas de cinco formas silvestres de frijol común (*Phaseolus vulgaris* L.)

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Abstract
The objective of the present study was to evaluate the seed and foliar phenolic composition from populations of wild *Phaseolus vulgaris* of Durango, Mexico. Seeds and leaves extracts were analyzed by HPLC-DAD, and UV-visible spectrophotometry. Analysis of variance were used to determine the capacity of the phenolic contents to discriminate between samples, the data were submitted to principal component analysis (PCA) and cluster analysis. The population with the highest content of phenolic compounds in both seed and leaves was the wild population of Nuevo Ideal and those that accumulated the lowest content of these compounds were Nombre de Dios and Canatlán. A total of 37 phenolic compounds in both seed and leaves from wild common bean were identified by HPLC–DAD. Wild species are important for the conservation of biodiversity, and for the genetic improvement of new varieties. Likewise, they could be used as forage, food or medicine, due to the high content of phytochemicals in seeds and leaf tissue, therefore, the information generated is relevant to knowledge about the characterization and diversity of wild *Phaseolus* species.

Keywords: **phytochemicals, wild forms, spectroscopy, *Phaseolus vulgaris*, HPLC-DAD, phenolic composition.**

Resumen

El objetivo del presente estudio fue evaluar la composición fenólica de hojas y de semillas de poblaciones silvestres de *Phaseolus vulgaris* de Durango, México. Los extractos de semillas y hojas se prepararon y analizaron mediante HPLC-DAD y espectrofotometría UV-visible. Se utilizó análisis de varianza para determinar la capacidad de los contenidos fenólicos para discriminar entre muestras. Los datos se sometieron a análisis de componentes principales (PCA) y análisis de agrupamiento. La población con mayor contenido de compuestos fenólicos tanto en semilla como en hoja es la población silvestre de Nuevo Ideal y las que acumularon menor contenido de estos compuestos fueron Nombre de Dios y Canatlán. Mediante HPLC-DAD se identificaron un total de 37 compuestos fenólicos tanto en semilla como en hojas de frijol común silvestre. Las especies silvestres son importantes para la conservación de la biodiversidad, y para el mejoramiento genético. Asimismo, podrían usarse como forraje, alimento o medicina, debido al alto contenido de fitoquímicos en tejido foliar y semillas. Por lo tanto, la información generada es relevante para el conocimiento sobre la caracterización y diversidad de especies silvestres de *Phaseolus*.

***Palabras clave*: fitoquímicos, formas silvestres, espectroscopia, *Phaseolus vulgaris*, HPLC-DAD, composición fenólica.**

1. Introduction

Legumes are important sources of nutrients and bioactive compounds. Depending on the genotype, bean seeds also contain a wide variety of polyphenolic compounds, which have prospective health benefits. These may include flavonoids such as anthocyanins, flavonoids, proanthocyanidins, and tannins, as well as a wide range of phenolic acids (Ganesan and Xu, 2017; Yang *et al*., 2018). Phenolic compounds contribute to the taste, smell and color of foods, and their bioactive role as antioxidants is associated with the prevention of cardiovascular and neurodegenerative diseases, diabetes, and cancer (Bhuyan and Basu, 2017).

The analysis of the foliar phenolic composition of common bean has received less attention than that of seeds, probably because humans do not consume the leaves. However, as leaves of common bean are used as forage for livestock, it is important to determine their phenolic composition because phenolics have nutraceutical properties (Mueller *et al*., 2019). This may improve not just animal health, but the meat quality could be enhanced, and even organic waste improves soil structure.

All the previous studies worked just with domesticated beans and were focused on plant phenolic contents. These reports registered a variation according to changes in the environmental growth conditions (Del Valle *et al*., 2015), plant age (Bystricka *et al*., 2014), and genotypes (Yusnawan *et al*., 2018). The state of Durango has physiographic and climatic diversity, due to this diversity wild species of common bean develop with variable phenotypes. In the last ten years the state has occupied the second place with the highest production of this legume at the national level. Wild species represent important information for the improvement and development of new varieties, so it is necessary to protect and conserve this plant genetic resource.

Hence, it is important to determine the seed and foliar phenolic contents among varieties and wild forms of common bean growing in different environmental conditions. There are few reports on the variation in foliar phenolic content of domesticated common bean (Reyes *et al*., 2014). The leaf tissue and seeds come from different municipalities with different altitudes, El Mezquital located at a low elevation, Súchil and Nombre de Dios at mid elevation, and Canatlán and Nuevo Ideal at high elevations. In Mexico there are few previous reports on the phenolic contents of wild forms of *P. vulgaris*. The objective of this study was to determine and compare the phenolic content of seeds and leaf tissue of five wild forms of common bean from Durango, Mexico.

2. Materials and methods

2.1 Materials

2.1.1 Plant Materials

Seeds (Fig. 1) and leaves of five wild forms of common bean were collected from their natural populations in Durango, Mexico (Table 1) during september - december 2019. The leaves and seeds were dried in a botanical drier at 40 °C to constant weight, ground in a domestic blender, and stored in paper bags in dark at room temperature.

Table 1. Collection data for five wild common beans (*Phaseolus vulgaris*) from Durango, Mexico.
Tabla 1. Datos de recolección de cinco frijoles silvestres (*Phaseolus vulgaris*) de Durango, México.

Localities	Latitude N	Longitude W	Altitude (m)	Weather	Tmax/Tmin* (°C)
El Mezquital	23° 26′ 48.1′′	104° 21′ 49.5′′	1400	Temperate subhumid	42.4/-0.2
Súchil	23° 39′24.7′′	104° 02′ 20.9′′	1963	Temperate	35.7/-6.4
Nombre de Dios	24° 04′ 71′′	104°14′ 23′′	1877	Temperate	36.7/-3.5
Canatlán	24° 51′03.4′′	104°51′44.8′′	2039	Semicold	35.1/-7.3
Nuevo Ideal	24° 45′11.9′′	105° 00′ 05.6′′	2037	Temperate	36.2/-5.0

*Data obtained from CONAGUA (2019).

Figure 1. Localities provenance and pictures of the seeds of five populations of wild common beans (*Phaseolus vulgaris*) from Durango, Mexico.

Figura 1. Localidades de procedencia y fotografías del grano de cinco poblaciones de frijol común silvestre (*Phaseolus vulgaris*) de Durango, México.

2.2 Methods

2.2.1 Preparation of extracts

Phenolic extracts were prepared according to Kim and Lee (2002). One gram of dry ground samples was macerated in 10 mL of 80 % methanol (v/v) for 24 hours, in the dark at room temperature. The extracts were centrifuged at 1,013 g, in a BOECO centrifuge U-32R, 230-240V, 50/60 Hz, for ten minutes at room temperature and the supernatants were separated. The pellets were reextracted and macerated in 10 mL of 20 % methanol (v/v) for one hour and centrifuged under the same conditions. Both solvents from the same sample were combined and formed the total extract, which was concentrated to dryness (by evaporation) and resuspended in 80 % methanol. For the HPLC-DAD analysis of both foliar and seed samples, total extracts were concentrated to dryness (by evaporation), and dissolved in 3 mL of methanol; these were the phenolic enriched extracts.

2.2.2 Spectrometric analysis of Phenolic compounds

The equipment used was the Multiskan Go spectrophotometer; high-quality monochromatorbased UV/VIS spectrophotometer. It is used in spectral scanning, endpoint and kinetic analysis. It measures absorbance in the 200–1000 nm wavelength range and it appropriate to 96- or 384-well plates.

Total Phenolic content (TPC)

The total phenolic concentrations were determined according to Falleh *et al*. (2011), from a standard curve constructed with six concentrations of gallic acid (Abs 760nm = 4.4875 [gallic acid] + 0.0368, correlation coefficient r = 0.9946). Total phenolics concentrations were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE / 100 g DW).

Flavonoid contents (FC)

The flavonoid contents were determined according to Lauranson and LebGreton (1993), from a standard curve constructed with six concentrations of quercetin (Abs 420 nm = 6.7545 [quercetin] + 0.081, correlation coefficient r = 0.997). Flavonoid contents were expressed as milligrams of quercetin equivalents per 100 grams of dry weight (mg QE / 100 g DW).

Total condensed tannins (TCT)

The contents of condensed tannins were determined according to Julkunen (1985), from a standard curve constructed with seven concentrations of epicatechin (Abs 510 nm = 0.0113 [epicatechin] – 0.0055 r = 0.9982). The contents were expressed as milligrams of epicatechin equivalents per gram of dry weight (mg EC / 100 g DW).

Anthocyanins (AC)

As reference, Monomeric anthocyanins were determined with the differential pH method described by Giusti and Wrolstad (2001), Abs 510 nm y Abs 700 nm using the molecular weight (449.2) and the molar absorption (26.900) of cyanidin-3-O-glucoside as reference. The values were expressed as milligrams of cyanidin-3-O-glucoside per dry extract (mg C3OG / 100 g DE).

2.3 HPLC-DAD analysis

Aliquots (100 uL) of foliar and seeds phenolic enriched extracts (50 mg/mL) were analyzed in a Perkin Elmer® Flexar™ PDA Plus™ detector, according to the gradient method reported by da Graça and Markham (2007). Chromatographic conditions: Mobile phase: A: acidified water (phosphoric acid) y B: acetonitrile, 20 uL of sample was injected, with a flow of 0.8 uL/min. Column: Brownlee™ SPP C18, 100 x 2.1 mm, 2.7 µm at 45 °C. Chromatograms were registered at 265 and 340 nm. Spectral data of all picks were recorded between 200 and 400 nm using diode array detection (DAD).

2.4 Experimental design and data analysis

The data of phenolic contents were subjected to an analysis of variance ($p \leq 0.05$) and means were compared with the Tukey test. To determine the capacity of the phenolic contents to discriminate between samples, the data were submitted to principal component analysis (PCA) and cluster analysis, XLSTAT v 2021.2.2 (Addinsoft 2024).

3. Results and discussion

3.1 Spectrometric analysis of phenolic compounds

3.1.1 Phenolic compounds in seeds

The seeds accumulated different pigments in the coat (Fig. 1). In common bean seeds, pigments are predominantly phenolics (Capistrán *et al*., 2019; Rodriguez *et al*., 2021). The coloration of the common bean seed is related to the P (pigment) gene and, in the case of the white coloration in the coat seed, it is due to a recessive phenotype of all the races that have been domesticated of this species along its history. Moreover, the same pigmentation of the seed has been used as a quantitative trait genetic marker (McClean *et al*., 2018).

Total Phenolic content

Significant variations were found in the content of secondary metabolites in seeds (Table 2). The locality of Nuevo Ideal found higher TPC (269.9 mg GAE/ 100 g DW), and El Mezquital TPC 173.67 mg GAE / 100 g DW, in the locality Nombre de Dios the lowest amount was found of (133.39 mg GAE / 100 g DW). The main beneficial health effect of phenolics is their antioxidant activity (Khang *et al*., 2016; Singh *et al*., 2017; Carbas *et al*., 2020), have reported values of total phenolics in common beans of 117 mg EAG/100 g to 440 mg EAG/100 g.

Flavonoid contents

The bean populations that presented the highest content of flavonoids was from the locality of Nuevo Ideal (77.66 mg QE / 100 g DW) and the lowest amount was found in the town of El Mezquital (50.33 mg QE / 100 g DW). Previous studies have reported that black bean varieties are characterized by having the highest flavonoid values compared to light varieties (Armendáriz *et al*., 2019). The nutraceutical properties attributed to the bean have been mainly related to this type of bioactive compounds. The seeds from the locality of Nuevo Ideal is a variegated seed with dark colors, between black and grey. It has been reported that anthocyanins are responsible for the red, black and red pigmentation, blue in bean grains, these pigments are generally located in the seed coat of the grain (Dzomba *et al*., 2013; Harlen and Jati, 2018). According to Heredia (2017), the consumption of common beans has adequate concentrations of flavonoids, in addition, when compared with other legumes (Chickpea 124.59 μg QE / g DW, lentils 17.13 μg QE / g DW) it has a greater amount of this element. Likewise, when grain consumption is complete, there is a considerable biological effect of flavonoids in legumes, showing anticancer and anti-inflammatory properties (Maleki *et al*., 2019). Within the group of flavonoids, anthocyanins are the most important type, which are found in pigmented beans and are responsible for the antioxidant capacity.

Total condensed tannins

The concentration of tannins in the wild common bean populations evaluated showed values in a range of 51.35 mg EC / 100 g DW to 75.17 mg ECE / 100 g DW. Tannins are compounds that can have a beneficial or adverse nutritional effect and are the predominant phenolic compounds in legumes. These can interact with macronutrients, especially with proteins (Zhang *et al*., 2014).

Anthocyanins

The wild forms of common bean studied showed anthocyanin concentrations in a range from 0.38 mg C3OG / 100 g DW to 3.12 mg C3OG / 100 g DE. The highest content of anthocyanins was found in the seeds from El Mezquital (3.120 mg C3OG / 100 g) and Nuevo Ideal (1.73 mg C3OG / 100 g), seeds with dark colors in the case of those from El Mezquital and those from New Ideal with marbled colour. Dark coloured beans are considered a good source of anthocyanins. (Mojica *et al*., 2017; Harlen 2018).

Table 2. Contents of total phenolics, flavonoids, condensed tannins, and anthocyanins of seeds of five wild forms of common bean (*Phaseolus vulgaris*) from Durango, Mexico.

Tabla 2. Contenidos de fenoles totales, flavonoides, taninos condensados y antocianinas de semillas de cinco formas silvestres de frijol común (*Phaseolus vulgaris*) de Durango, México.

Localities	**Total phenolics[1]**	**Flavonoids[2]**	**Condensed tannins[3]**	**Anthocyanins[4]**
El Mezquital	173.67 ± 10.39 [b]	50.33 ± 6.81 [b]	74.30 ± 1.27 [a]	3.12 ± 0.11 [a]
Súchil	160.74 ± 7.50 [c]	69.81 ± 5.75 [ab]	71.58 ± 3.33 [ab]	1.14 ± 0.41 [c]
Nombre de Dios	133.39 ± 5.64 [c]	76.56 ± 6.32 [a]	68.69 ± 1.40 [b]	0.85 ± 0.11 [c]
Canatlán	154.20 ± 1.63 [c]	55.80 ± 9.27 [ab]	51.35 ± 1.93 [c]	0.38 ± 0.10 [d]
Nuevo Ideal	269.90 ± 2.95 [a]	77.66 ± 8.58 [a]	75.17 ± 0.66 [a]	1.73 ± 0.18 [b]
Pr > F(Model)	0.0.14	0.048	<0.0001	<0.0001
Significant	Yes	Yes	Yes	Yes

[1]: Expressed in mg GAE / 100 g DW, [2]: mg QE / 100 g DW, [3]: mg EC / 100 g DW, [4]: mg C3OG / 100 g DW. Different uppercase letters indicate significant differences between five populations ($p \leq 0.05$) (Fisher's test), media ± SD, n = 3.

In this study, the seeds with the highest pigmentation (dark colors) were those from Nuevo Ideal and El Mezquital, likely contain a diverse range of beneficial phenolic compounds, contributing to their higher antioxidant activity, followed by those from Súchil, and the lighter-colored seeds from Canatlán and Nombre de Dios, may limit their health benefits in comparison to more pigmented alternatives. According to the literature, the seeds with the highest pigmentation, in this case those from Nuevo Ideal and El Mezquital, presented the highest levels and the one with the lowest phenolic content was the seed from Nombre de Dios, the lightest-colored seed included in this study.

Variation in phenolic composition and antioxidant activities between common bean cultivars and their processed products has also been widely studied, for example, Akon e*t al*., 2011; Suárez *et al*., 2015; Aquino *et al*., 2016. The phenolic profile can vary not just between cultivated varieties, but also between wild species, which can manifest similarities in composition. However, differences could occur due to factors like pigmentation (color) and their environmental adaptation, which influences their biochemical pathways. For instance, colored bean varieties often have higher levels of specific phenolic compounds compared to lighter colored ones due to the presence of additional pigments like anthocyanins. (Kleintop *et al*., 2016; Murube *et al*., 2021; Campa *et al*., 2023). The significant differences among varieties highlight the importance of cultivar selection in breeding programs aimed at enhancing the nutritional quality of common beans. (Assefa *et al*., 2019).

3.2 Phenolic compounds in leaves

Total Phenolic content

Regarding the content of phenolic compounds in leaves, significant variations were found (Table 3). The locality of Canatlán found higher TPC (828.98 mg GAE / 100 g DW), and in the locality of Nombre de Dios the lowest content was found (145.27 mg GAE / 100 g DW).

Flavonoid contents

The highest concentration in flavonoids was found in leaves population from El Mezquital (212.68 mg QE / 100 g DW) and the leaves populations of Nombre de Dios (48.45 mg QE / 100 g DW) and Súchil (49.57 mg EC / 100 g DW) were the ones that showed the lowest concentration. Some environmental factors such as ultraviolet radiation, low temperatures, droughts, water stress, among others, have been shown to induce the accumulation of anthocyanins in plants (Harlen *et al*., 2018; Maleki *et al*., 2019).

Total condensed tannins

The concentration of tannins in the leaves of the wild common bean evaluated showed values in a range of 5.20 mg EC / 100g DW to 10.91 mg ECE /100 g DW. The locality of Canatlán was the one with the highest concentration of condensed tannins (10.911 mg ECE / 100 g DW) and the town of Nuevo Ideal with the lowest amount (5.20 mg ECE / 100 g DW).

Anthocyanins

The concentration of anthocyanins in the leaves of the wild common bean evaluated showed values in a range of 3.89 mg C3OG / 100 g DE to11.54 mg C3OG / 100 g DE. The locality of Nuevo Ideal along with that of Súchil presented higher levels than the rest of the analyzed leaves (11.54 and 10.09 mg C3OG / 100 g DE) respectively, the lowest value was found in the locality Nombre de Dios 3.89 mg C3OG / 100 g DE.

In this study the leaves were taken in the vegetative phase. At this stage, the leaves are usually more tender and softer. Young leaves, in particular, are rich in nutrients and bioactive compounds, such as phenolic compounds, which play important roles in defense against pests and diseases, as well as in the regulation of physiological processes. (Chávez & Sánchez 2017; Gaafar *et al*., 2020; Šamec *et al.*, 2021). The leaves in which the greatest amount of phenolic compounds were obtained were those from Nuevo Ideal, El Mezquital and Canatlán and in which the least amount of these compounds was found is in the sample from Nombre de Dios, this coincides with what was found in the seeds analyzed in said study. The studies reported by Torche *et al*. (2018) have shown that young common bean leaves can be a valuable source of these compounds, which makes their study very significant in agrobiology and nutrition. The content of phenolic compounds in wild common bean leaves has been little explored when compared to the studies that exist on the content of these same compounds in its seeds.

Table 3. Contents of total phenolics, flavonoids, condensed tannins, and anthocyanins of leaves of five wild forms of common bean (*Phaseolus vulgaris*) from Durango, Mexico.
Tabla 3. Contenido de fenoles totales, flavonoides, taninos condensados y antocianinas de hojas de cinco formas silvestres de frijol común (*Phaseolus vulgaris*) de Durango, México.

Localities	Total phenolics[1]	Flavonoids[2]	Condensed tannins[3]	Anthocyanins[4]
El Mezquital	550.08±18.18 [a]	212.68 ± 14.27 [a]	7.53 ± 0.34 [b]	7.02 ± 0.50 [b]
Súchil	276.85 ± 3.97 [d]	49.57 ± 3.49 [d]	5.51 ± 0.20 [d]	10.09 ± 1.76 [a]
Nombre de Dios	145.27 ± 13.78 [e]	48.45 ± 8.78 [d]	6.68 ± 0.15 [c]	3.89 ± 1.71 [c]
Canatlán	828.98 ± 22.52 [a]	94.17 ± 2.89 [c]	10.91 ± 0.14 [a]	5.76 ± 0.35 [bc]
Nuevo Ideal	398.45 ± 8.23 [c]	124.87 ± 3.06 [b]	5.20 ± 0.14 [d]	11.54 ± 2.32 [a]
Pr > F(Model)	<0.0001	<0.0001	<0.0001	0.186
Significant	Yes	Yes	Yes	No

[1] Expressed in mg GAE / 100 g DW, [2] In mg QE / 100 g DW, [3] In mg EC / 100 g DW, [4] In mg C3OG / 100 g DW. Different uppercase letters indicate significant differences between five populations ($p \leq 0.05$) (Fisher's test), media ± SD (n = 3), DE (Dry extract), DW (Dry weight).

3.3 PCA and cluster analysis

Principal component analysis (Fig. 2A) showed that principal component 1 (PC1) explained 95.58 % of the total variance. The first three components explain 99.99 %. The variable with the greatest contribution expressed by the PC1 was condensed tannins, with 95.58 %, PC2, flavonoids and total phenols, with 3.06 % of the variance, and the PC3 was anthocyanins with 1.55 %. The dendrogram of Fig. 2B indicates the grouping of the populations based on their similarity of contents of phenolic compounds, which revealed two groups, one for seeds and the other for leaves, within the group of leaves a greater similarity between the populations of Canatlán and Nuevo Ideal and within the group of seeds a greater similarity between the populations of Canatlán and El Mezquital and Nombre de Dios and Nuevo Ideal.

It has been reported that the pigments in seeds are attributed to phenolic compounds. A similar study conducted by Armendáriz, *et al*. (2019) informed a phenol concentration ranges from 19.75 mg to 221.48 mg gallic acid equivalents / g extract, in bean varieties grown with traditional methods in

Oaxaca, Mexico. However, the values obtained in our study range from 133.39 to 269.90 mg gallic acid equivalents/ g seed extracts.

The correlation between seed color and phenolic content may be crucial for understanding how these characteristics affect seed quality and potentially their survival and adaptation in different environmental conditions (Arteaga, 2021). Additionally, it is relevant to consider the role that these compounds may have not only in the physical properties of seeds and leaves but also in antioxidant properties and, therefore, in plant health (Colina, 2016; Bedoya & Maldonado, 2022; Sahu *et al*., 2022).

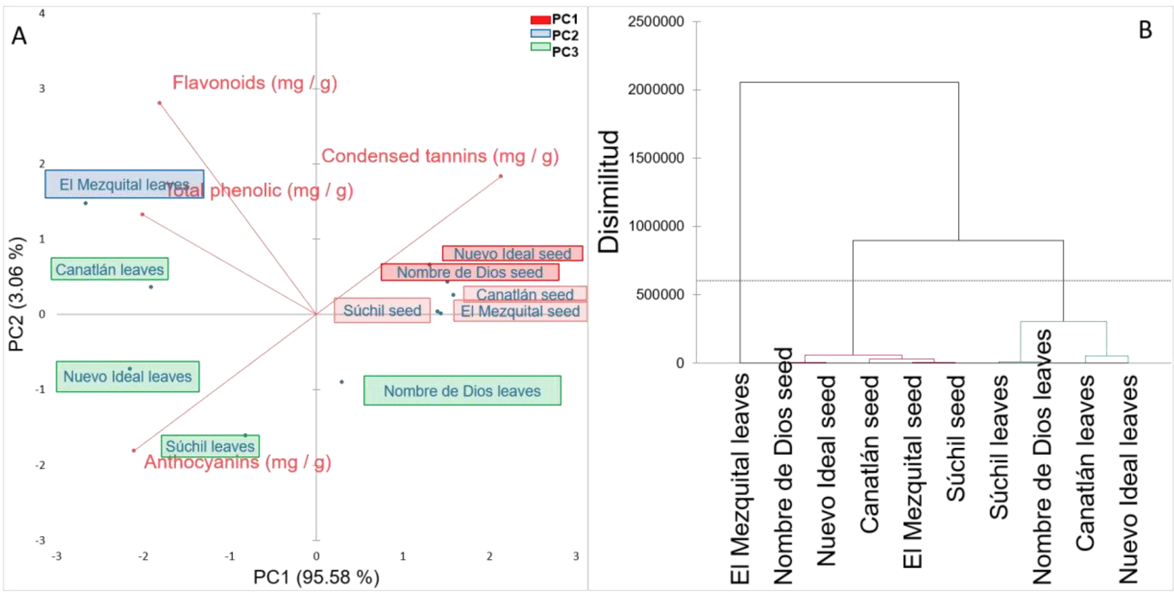

Figure 2. A) Results of a principal components analysis and B) dendrogram resulting from the comparison of the different phenolic contents of seeds and leaves of five wild populations of common bean (*Phaseolus vulgaris*) from Durango, Mexico.

Figura 2. A) Resultados del análisis de componentes principales y B) dendrograma resultante de la comparación de los diferentes contenidos fenólicos de semillas y hojas de cinco poblaciones silvestres de frijol común (*Phaseolus vulgaris*) de Durango, México.

3.4 HPLC-DAD

The results of the HPLC-DAD analysis of the leaves and seeds of wild forms of common beans revealed this species is a rich source of phenolic compounds (Fig. 3). A total of 37 compounds were identified from wild common beans with this technique. Flavonols and phenolic acid were the major phenolic compounds in seeds (Table 4). The results are similar with those published by Reyes *et al*. (2014) and Capistrán *et al*. (2019), who also found 27 phenolics in the leaves of *P. vulgaris*, the major compounds were flavonols and phenolic acid.

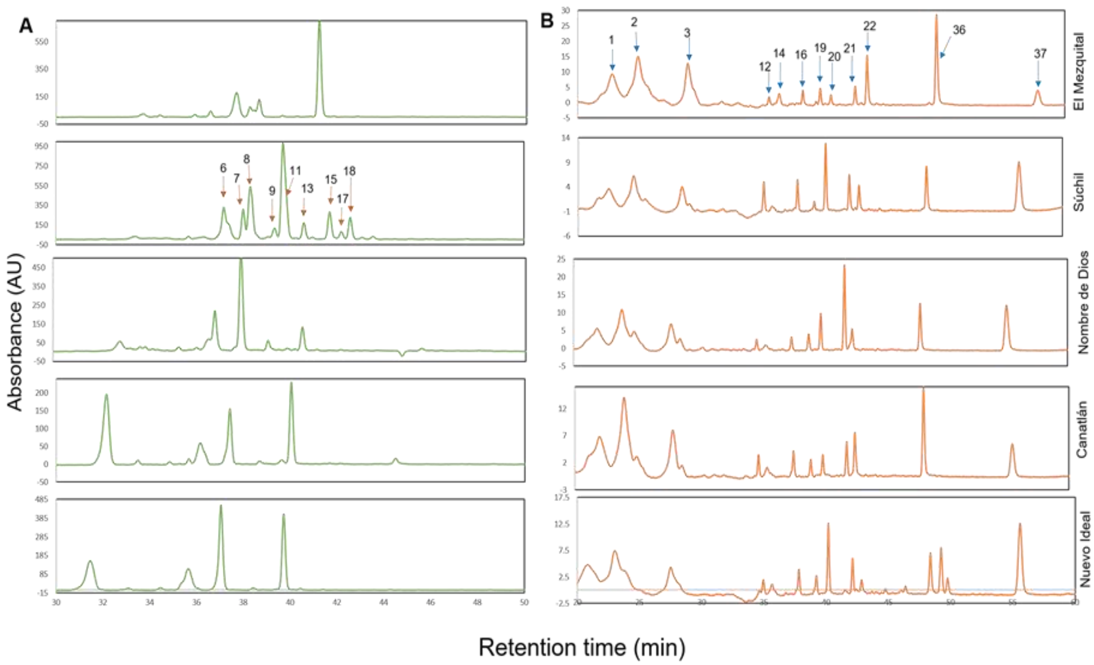

Figure 3. HPLC-DAD UV chromatograms of the leaves (A, registered at 265 nm) and seeds (B, registered at 340 nm) of wild forms of common bean from different regions of provenance of Durango, Mexico.
Figura 3. Cromatogramas UV HPLC-DAD de las hojas (A, registradas a 265 nm) y semillas (B, registradas a 340 nm) de formas silvestres de frijol común de diferentes regiones de procedencia de Durango, México.

The appearance of several absorption peaks, this fine structure reflects not only the different conformations such systems may assume, but also electronic transitions between the different vibrational energy levels possible for each electronic state. In the Table 4 are displayed the retention time and spectral data for each compound present in leaves and seeds. There are compounds that were observed both in leaves and seeds, but of the total of compounds eight of them were only found in seeds at the retention times of 36.6 min (Aromatic acid), 33.29 min (Flavonols), 35.15 min (Flavonols), 46.37 min (Dihydroflavonoids), 48.38 min (Flavonols), 49.35 min (Dihydroflavonoids), 55.36 min (Flavonols) and 57.35 min (Flavonols) and three were only found in leaves, at the retention times 34.58 min (Dihydroflavonols), 36.46 min (Flavonols) and 40.33 min (Flavonols). Chromatograms and UV spectra of some of the major compounds are shown in Fig. 4.

Table 4. Phenolic compounds found in leaves and seeds of wild forms of common bean from Durango, Mexico.
Tabla 4. Compuestos fenólicos encontrados en hojas y semillas de formas silvestres de frijol común de Durango, México.

Number of compund	Retention time (min)	λ_{max}	Type of phenolic compound	Type of sample
1	21.663 ± 0.33	325	Aromatic acid	seeds
2	22.32 ± 0.00	240sh-296sh-325	Aromatic acid	seeds
3	22.82 ± 0.00	245sh-296sh-323	Aromatic acid	seeds
4	23.44 ± 0.39	239sh-296sh.323	Aromatic acid	seeds
5	24.54 ± 0.50	325	Aromatic acid	seeds
6	26.39 ± 0.43	274	Aromatic acid	Leaves and seeds
7	27.58 ± 0.33	293sh-308	Aromatic acid	Leaves and seeds
8	28.67 ± 0.33	293sh-308	Aromatic acid	Leaves and seeds
9	31.55 ± 0.33	289-324sh	Dihydroflavonoids	Leaves and seeds
10	31.60 ± 0.00	293sh-308	Aromatic acid	Seeds
11	32.48 ± 0.31	239sh-293sh-323	Aromatic acid	Leaves and seeds
12	33.29 ± 0.00	249-263sh-298sh344	Flavonols	Seeds
13	33.45 ± 0.23	239sh-293sh-323	Aromatic acid	Leaves and seeds
14	34.58 ± 0.22	233sh-274-320sh	Dihydroflavonoids	Leaves
15	34.60 ± 0.00	288-334sh	Dihydroflavonoids	Leaves and seeds
16	35.14 ± 0.09	254-263sh-294sh348	Flavonols	Seeds
17	35.69 ± 0.12	293sh-308	Aromatic acid	Leaves and seeds
18	36.4 ± 0.00	274-329	Flavones	Leaves and seeds
19	36.46 ± 0.18	251-266sh-346	Flavonols	Leaves
20	37.00 ± 0.00	254sh-268-290sh-350	Flavones	Leaves and seeds
21	37.52 ± 0.27	255-266sh-294sh355	Flavonols	Leaves and seeds
22	38.42 ± 0.26	262-292sh-315sh-342	Flavonols	Leaves and seeds
23	38.60 ± 0.00	253-266sh-294sh352	Flavonols	Leaves and seeds
24	39.30 ± 0.00	252-262sh-290sh318sh-352	Flavonols	Leaves and seeds
25	39.50 ± 0.30	238sh- 270-295sh330	Flavonols	Leaves and seeds
26	40.10 ± 0.00	262-292sh-315sh-342	Flavonols	Leaves and seeds
27	40.33 ± 0.27	254-263sh-294sh348	Flavonols	Leaves

28	41.70 ± 0.31	276	Aromatic acid	Leaves and seeds
29	41.80 ± 0.00	255-295sh-372	Flavonols	Leaves and seeds
30	42.21 ± 0.10	233sh-273-320sh	Aromatic acid	Leaves and seeds
31	42.70 ± 0.17	265-290sh-320sh-346	Flavonols	Leaves and seeds
32	43.62 ± 0.16	255-263sh-297sh-357	Flavonols	Leaves and seeds
33	46.37 ± 0.05	289-326sh	Dihydroflavonoids	Seeds
34	48.38 ± 0.25	240-292sh-330	Flavonols	Seeds
35	49.35 ± 0.12	289-326sh	Dihydroflavonoids	Seeds
36	55.36 ± 0.28	248-262sh-318sh-346	Flavonols	Seeds
37	57.35 ± 0.64	230sh-248-312sh327sh-360	Flavonols	Seeds

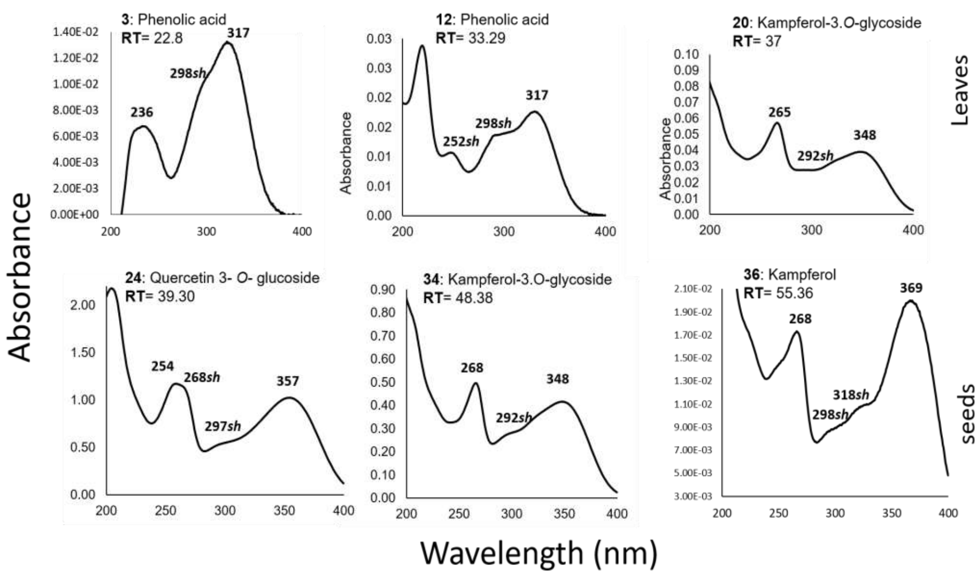

Figure 4. HPLC-DAD UV spectra (registered between 200 and 400 nm) of some phenolic compounds found in the methanol extract of leaves and seeds of wild forms of common bean from Durango, Mexico.

Figura 4. Espectros UV HPLC-DAD (registrados entre 200 y 400 nm) de algunos compuestos fenólicos encontrados en el extracto metanólico de hojas y semillas de formas silvestres de frijol común de Durango, México.

Phenolic compounds are an important group of secondary metabolites found in various plants, including the wild forms of common bean (*Phaseolus vulgaris*) (Espinoza *et al*., 2016; Capistrán, 2019). In the methanolic extract of leaves and seeds of these varieties, several phenolic compounds are usually identified, which may include simple phenolics, flavonoids, tannins, and phenolic acids. This study primarily found phenolic acids, known for their antioxidant properties (García-Díaz, 2016; Claros, 2021; Pérez *et al*., 2020), and flavonols, such as quercetin and kaempferol, which have anti-inflammatory and antioxidant effects (Capistrán, 2019).

4. Conclusions

The results provide valuable insight into the variability in phytochemical content among different forms of wild beans, which could have significant implications for their use in breeding programs. The differences in anthocyanin, flavonoid, total phenolic, and tannin content between seeds and leaves suggest that each part of the plant may have different functions and benefits in terms of health and adaptation. The finding that the seeds from Nuevo Ideal exhibit the highest contents of total phenolic compounds (TPC), flavonoids (FC), condensed tannins (TCT), and anthocyanins (AC) is particularly relevant, as it indicates that this variety may have greater nutraceutical potential. On the other hand, the fact that Canatlán shows higher contents of TPC and TCT in the leaf tissue suggests that this population could be explored for phytochemical applications in health products or as antioxidant agents.

The variability in phenolic compound levels in populations growing at different altitudes also opens the door for future studies on how environmental conditions affect the biosynthesis of these metabolites. This could lead to a better understanding of plant adaptations to their environments and how the cultivation of wild beans can be optimized to maximize their phytochemical content. It would be interesting to consider the possibility of correlating this data with agroecological factors, such as water availability, sunlight, and soil nutrients, to have a more comprehensive overview. Additionally, investigating how these compounds affect resistance to pests and diseases could be key to ensuring the sustainability of wild bean populations in the future.

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Conflict of interest

This article was prepared for academic and scientific purposes only, noting that there is no conflict of interest in the publication of these results.

5. References

Addinsoft, (2021). XLSTAT statistical and data analysis solution. New York, USA. https://www.xlstat.com

Akond, A. G. M., Khandaker, L., Berthold, J., Gates, L., Peters, K., Delong, H., & Hossain, K. (2011). Anthocyanin, total polyphenols and antioxidant activity of common bean. *American Journal of Food Technology*, 6: 385-394. https://doi.org/10.3923/ajft.2011.385.394

Armendáriz-Fernández, K. V., Herrera-Hernández, I. M., Muñoz-Márquez, E., & Sánchez, E. (2019). Characterization of Bioactive Compounds, Mineral Content, and Antioxidant Activity in Bean Varieties Grown with Traditional Methods in Oaxaca, Mexico. *Antioxidants* 8(1): 26. https://doi.org/10.3390/antiox8010026

Aquino-Bolaños, E. N., García-Díaz, Y. D., Chavez-Servia, J. L., Carrillo-Rodríguez, J. C., Vera-Guzman, A. M. and Heredia-Garcia, E. (2017). Anthocyanin, polyphenol, and flavonoid contents and antioxidant activity in Mexican common bean (*Phaseolus vulgaris* L.) landraces. *Emirates Journal of Food & Agriculture* 28(8): 581-588. https://ejfa.me/index.php/journal/article/view/1178

Arteaga-Castillo, S. M. (2021). Cultivos para el cambio climático: selección y caracterización de variedades de judía (*Phaseolus vulgaris* L.) y *Phaseolus lunatus* tolerantes a la sequía y salinidad (Doctoral dissertation, Universitat Politècnica de València). https://doi.org/10.4995/Thesis/10251/168450

Assefa, T., Assibi, M. A., Brown, A. V., Cannon, E. K., Rubyogo, J. C., Rao, I. M., & Cannon, S. B. (2019). A review of breeding objectives, genomic resources, and marker-assisted methods in common bean (*Phaseolus vulgaris* L.). *Molecular Breeding*, 39, 1-23. https://doi.org/10.1007/s11032-018-0920-0

Bhuyan, D. J., & Basu, A. (2017). Phenolic compounds potential health benefits and toxicity. In Utilisation of bioactive compounds from agricultural and food production waste (pp. 27-59). CRC Press. https://goo.su/CQkqX

Bedoya, R. A., & Maldonado, M. E. (2022). Características nutricionales y antioxidantes de la especie de frijol petaco (*Phaseolus coccineus*). *Revista chilena de nutrición*, 49(1): 34-42. https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1388583

Bystricka, J., Musilova, J., Tomas, J., Vollmannova, A., Lachman, J.,& Kavalcova, P. (2014). Changes of Polyphenolic Substances in the Anatomical Parts of Buckwheat (*Fagopyrum esculentum* Moench.) during Its Growth Phases. *Foods* 3(4): 558-568. https://doi.org/10.3390/foods3040558

Campa, A., Rodríguez-Madrera, R., Jurado, M., García-Fernández, C., Suárez-Valles, B., & Ferreira, J. J. (2023). Genome-wide association study for the extractable phenolic profile and coat color of common bean seeds (*Phaseolus vulgaris* L.). *BMC Plant Biology*, 23(1): 158. https://doi.org/10.1186/s12870-023-04177-z

Carbas, B., Machado, N., Oppolzer, D., Ferreira, L., Queiroz, M., Brites, C., Rosa, E.A., & Barros, A. I. (2020). Nutrients, antinutrients, phenolic composition, and antioxidant activity of common bean cultivars and their potential for food applications. *Antioxidants*, 9(2): 186. https://doi.org/10.3390/antiox9020186

Capistrán-Carabarin, A., Aquino-Bolaños. E. N., García-Díaz Y. D., Chávez-Servia, J. L., Vera-Guzmán, A. M., & Carrillo-Rodríguez, J. C. (2019). Complementarity in Phenolic Compounds and the Antioxidant Activities of *Phaseolus coccineus* L. and *P. vulgaris L.* Landraces. *Foods* 8(8): 295. https://doi.org/10.3390/foods8080295

Chávez-Mendoza, C., & Sánchez, E. (2017). Bioactive compounds from Mexican varieties of the common bean *(Phaseolus vulgaris*): Implications for health. *Molecules*, 22(8): 1360. https://doi.org/10.3390/molecules22081360

Claros-Osorio, P. D. P. (2021). Evaluacion de la capacidad antioxidante total y contenido de polifenoles totales del *Phaseolus vulgaris* “Frijol”. http://hdl.handle.net/20.500.14067/5297

Colina-Ramos, A. C. (2016). Análisis fitoquímico, determinación cualitativa y cuantitativa de flavonoides y taninos, actividad antioxidante, antimicrobiana de las hojas de

"Muehlenbeckia hastulata (JE Sm) IM Johnst" de la zona de Yucay (Cusco). [Tesis. Universidad Nacional Mayor de San Marcos]. https://goo.su/ed9KUg

da Graça, C. M., Markham, K. R. (2007). Structure information from HPLC and on-line measured absorption spectra: Flavones, Flavonols and Phenolic Acids. Imprensa da Universidade de Coimbra/Coimbra University Press. http://dx.doi.org/10.14195/978-989-26-0480-0

Del Valle, J. C., Buide, M. L., Casimiro-Soriguer, I., Whittall, J. B., & Narbona, E. (2015). On flavonoid accumulation in different plant parts: variation patterns among individuals and populations in the shore campion (*Silene littorae*). *Front Plant Sci* 6: 939. https://doi.org/10.3389/fpls.2015.00939

Dzomba, P., Togarepi, E., & Mupa, M. (2013). Anthocyanin content and antioxidant activities of common bean species (*Phaseolus vulgaris* L.) grown in Mashonaland Central, Zimbabwe. *African Journal of Agricultural Research*, 8(25): 3330-3333. https://goo.su/1XaGg0C

Espinoza-García, N., Martínez-Martínez, R., Chávez-Servia, J. L., Vera-Guzmán, A. M., Carrillo-Rodríguez, J. C., Heredia-García, E., & Velasco-Velasco, V. A. (2016). Contenido de minerales en semilla de poblaciones nativas de frijol común (*Phaseolus vulgaris* L.). *Revista fitotecnia mexicana,* 39(3): 215-223. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-73802016000300215&lng=es

Falleh, H., Ksouri, R., Medini, F., Guyot, S., Abdelly, C., & Magné, C. (2011). Antioxidant activity and phenolic composition of the medicinal and edible halophyte *Mesembryanthemum edule* L. *Industrial Crops and Products* 34(1): 1066-1071. https://doi.org/10.1016/j.indcrop.2011.03.018

Gaafar, A. A., Ali, S. I., El-Shawadfy, M. A., Salama, Z. A., Sękara, A., Ulrichs, C., & Abdelhamid, M. T. (2020). Ascorbic acid induces the increase of secondary metabolites, antioxidant activity, growth, and productivity of the common bean under water stress conditions. *Plants*, 9(5), 627. https://doi.org/10.3390/plants9050627

Ganesan, K., & Xu, B. (2017). Polyphenol-rich dry common beans (*Phaseolus vulgaris* L.) and their health benefits. *International Journal of Molecular Sciences*, 18(11): 2331. https://doi.org/10.3390/ijms18112331

García-Díaz, Y.D. (2016). Compuestos fenólicos y actividad antioxidante en testa y grano de 54 poblaciones nativas de frijol común (*Phaseolus vulgaris* L.) (Tesis Maestría en Ciencias Alimentarias, Universidad Veracruzana). https://www.uv.mx/mca/files/2018/01/L.-N.-Yatzil-Denih-Garcia-Diaz.pdf

Giusti, M. M., & Wrolstad, R. E. (2001). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *CPFAC* 0(1): 1-13. https://doi.org/10.1002/0471142913.faf0102s00

Harlen, W. C., & Jati, I. R.A.P. (2018). Antioxidant activity of anthocyanins in common legume grains. In: Watson, R.R., Preedy, V.R., Zibadi, S. (Eds.) *Polyphenols: Mechanisms of action in human health and disease.* Academic Press. pp. 81-92. https://doi.org/10.1016/B978-0-12-813006-3.00008-8

Heredia, R. L. (2017). Caracterización fisicoquímica y de compuestos bioactivos del frijol tepari (*Phaseolus acutifolius* gray) cultivado en Nuevo León, México. [Tesis de Maestría] Universidad Autónoma de Nuevo León. http://eprints.uanl.mx/id/eprint/14362

Julkunen-Tiitto, R. (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem.* 33(2): 213-217. https://doi.org/10.1021/jf00062a013

Khang, D. T., Dung, T. N., Elzaawely, A. A., & Xuan, T. D. (2016). Phenolic profiles and antioxidant activity of germinated legumes. *Foods*, 5(2): 27. https://doi.org/10.3390/foods5020027

Kleintop, A. E., Myers, J. R., Echeverria, D., Thompson, H. J., & Brick, M. A. (2016). Total phenolic content and associated phenotypic traits in a diverse collection of snap bean cultivars. *Journal* *of the American Society for Horticultural Science*, 141(1): 3-11. https://doi.org/10.21273/JASHS.141.1.3

Kim, D. O., & Lee, C. Y. (2002). Extraction and isolation of polyphenolics. In: Wrolstad, R.E. (Ed.) *Current Protocols in Food Analytical Chemistry*. John Wiley & Sons: New York. https://doi.org/10.1002/0471142913.fai0102s06

Lauranson-Broyer, J., & Lebreton, P. (1993). Flavonoids and morphological traits of needles, as markers of natural hybridization between *Pinus uncinata* Ram and *Pinus sylvestris* L. *Biochem. Syst. Ecol*. 21(2): 241-247. https://doi.org/10.1016/0305-1978(93)90041-O

Maleki, S. J., Crespo, J. F., & Cabanillas, B. (2019). Anti-inflammatory effects of flavonoids. *Food chemistry*, 299: 125124. https://doi.org/10.1016/j.foodchem.2019.125124

McClean, P. E., Bett, K. E., Stonehouse, R., Lee, R., Pflieger, S., Moghaddam, S. M., Geffroy, V., Miklas, P., & Mamidi, S. (2018). White seed color in common bean (*Phaseolus vulgaris*) results from convergent evolution in the P (*pigment*) gene. *New Phytologist*, 219(3): 1112-1123. https://doi.org/10.1111/nph.15259

Mojica, L., Berhow, M., & González de Mejia, E. (2017). Black bean anthocyanin-rich extracts as food colorants: Physicochemical stability and antidiabetes potential. *Food chemistry*, 229: 628-639. https://doi.org/10.1016/j.foodchem.2017.02.124

Mueller-Harvey, I., Bee, G., Dohme-Meier, F., Hoste, H., Karonen, M., Kölliker, R., Waghorn, G. C.… *et.al.* (2019). Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. *Crop Science*, 59(3): 861-885. https://doi.org/10.2135/cropsci2017.06.0369

Murube, E., Beleggia, R., Pacetti, D., Nartea, A., Frascarelli, G., Lanzavecchia, G., Bellucci, E., Nanni, L., Gioia, T., Marciello, U., Esposito, S., Foresi, G., Logozzo, G., Frega, G. N., Bitocchi, E., & Papa, R. (2021). Characterization of nutritional quality traits of a common bean germplasm collection. *Foods*, 10(7): 1572. https://doi.org/10.3390/foods10071572

Pérez-Perez, L.M., Del Toro Sánchez, C. L., Sánchez Chavez, E., González Vega, R. I., Reyes Díaz, A., Borboa Flores, J., … Flores-Cordova, M.A. (2019). Bioaccesibilidad de compuestos antioxidantes de diferentes variedades de frijol (*Phaseolus vulgaris* L.) en México, mediante un sistema gastrointestinal in vitro. *Biotecnia* 22(1): 117–125. https://doi.org/10.18633/biotecnia.v22i1.1159

Reyes-Martínez, A., Almaraz-Abarca, N., Gallardo-Velazquez, T., González-Elizondo, M.S., Herrera-Arrieta, Y., Pajarito-Ravelo, A., Alanís-Bañuelos, R. E., & Torres-Morán, M. I. (2014). Evaluation of foliar phenols of 25 Mexican varieties of common bean (*Phaseolus vulgaris* L.) as antioxidants and varietal markers. *Natural Product Research* 28(23): 2158-2162. https://doi.org/10.1080/14786419.2014.930855

Rodríguez-Madrera, R., Campa-Negrillo, A., Suárez-Valles, B., & Ferreira-Fernández, J.J. (2021). Phenolic content and antioxidant activity in seeds of common bean (*Phaseolus vulgaris* L.). Foods. 2021 Apr 15;10(4):864. https://doi.org/10.3390/foods10040864

Sahu, P. K., Cervera-Mata, A., Chakradhari, S., Singh Patel, K., Towett, E. K., Quesada-Granados, J. J., Martín-Ramos, P., & Rufián-Henares, J. A. (2022). Seeds as potential sources of phenolic compounds and minerals for the Indian population. *Molecules*, 27(10): 3184. https://doi.org/10.3390/molecules27103184

Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., & Salopek-Sondi, B. (2021). The Role of Polyphenols in Abiotic Stress Response: The Influence of Molecular Structure. *Plants*, 10(1): 118. https://doi.org/10.3390/plants10010118

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2017). Phenolic composition and antioxidant potential of grain legume seeds: A review. *Food research international,* 101: 1-16. http://dx.doi.org/10.1016/j.foodres.2017.09.026

Suárez-Martínez, S.E., Ferriz-Martínez, R. A., Campos-Vega, R., Elton-Puente, J.E., de la Torre-Carbot, K., & García-Gasca, T. (2016). Bean seeds: leading nutraceutical source for human health. *CyTA-Journal of Food* 14(1): 131-137. https://doi.org/10.1080/19476337.2015.1063548

Torche, Y., Blair, M., Saida, C. (2018). Biochemical, physiological and phenological genetic analysis in common bean (*Phaseolus vulgaris* L.) under salt stress. *Ann Agric Sci* 63(2): 153–161. https://doi.org/10.1016/j.aoas.2018.10.002

Yang, Q. Q., Gan, R. Y., Ge, Y. Y., Zhang, D., & Corke, H. (2018). Polyphenols in common beans (*Phaseolus* vulgaris L.): Chemistry, analysis, and factors affecting composition. *Comprehensive Reviews in Food Science and Food Safety*, 17(6): 1518-1539. https://doi.org/10.1111/1541-4337.12391

Yusnawan, E., Nugrahaeni, N., Utomo, J.S. (2018). Changes of Phenolic Contents and Antioxidant Activity in Soybean Seeds Harvested from Phakopsora pachyrhizi Infected Crops. *J. Biol. Edu.* 10(2): 369-378. https://doi.org/10.15294/biosaintifika.v10i2.14481

Zhang, H., Yu, D., Sun, J., Liu, X., Jiang, L., Guo, H., & Ren, F. (2014). Interaction of plant phenols with food macronutrients: charcaterisation and nutritional-physiological conse-quences. *Nutrition research reviews*, 27(1): 1-15. https://doi.org/10.1017/S095442241300019X