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Evaluation of phenolic compounds and antioxidant capacity in *Mucuna pruriens* and *Cajanus cajan* pods extracts

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ABSTRACT

The seeds of *Mucuna pruriens* (Mp) and *Cajanus cajan* (Cc) legumes are known to be rich in phenolic compounds (PC) that have various beneficial properties. However, there is limited knowledge on the composition of their pods (Mp-p and Cc-p, respectively), which are considered as agricultural waste. This study investigated the extraction of PC with antioxidant capacity (AOC) from in Mp-p and Cc-p using different solvents and the decoction method. The results showed that 50 % ethanol and 100 % methanol were the most effective solvents for extracting PCs with AOC from Mp-p and Cc-p, respectively. The decoction method was also found to be efficient for extracting PCs from Mp-p. The major PC detected in Mp-p extracts were caffeic acid and chlorogenic acid, while quercetin was found in high amounts in Cc-p extracts. Moreover, the combination of Density Functional Theory and the quantum theory of atom in molecules approaches allowed to provide a detailed understanding of the relationship between the PC detected in the extracts and their antioxidant activity. The results suggest that both Mp-p and Cc-p are viable sources of natural antioxidants with potential health benefits.

1. Introduction

Agricultural activity produces substantial amounts of wastes which are mainly disposed of in landfills. This waste accumulation not only poses a significant environmental risk but also represents a vast untapped reserve of bioactive compounds. Several studies have demonstrated the presence of valuable bioactive compounds, particularly phenolic compounds (PC), in agricultural wastes (Nguyen, 2017; Skendi et al., 2022). These compunds are known for their ability to scavenge free radicals, which can cause cellular damage and contribute to the development of various chronic diseases (Zhang et al., 2020). Furthermore, the harmful effects of free radicals, coupled with the toxic effects exerted by synthetic antioxidants used as food preservatives, have promoted the search for natural and sustainable sources of antioxidant compounds (Gasmi et al., 2022).

Legumes constitute a large group of flowering plants belonging to the Fabaceae family, which are cultivated in different parts of the world. *Mucuna pruriens* (Mp) and *Cajanus cajan* (Cc) also known as Velvet bean and Pigeon pea, respectively, are legumes common in tropical and subtropical regions around the world including the Northeastern region of Argentine (Dilipkumar et al., 2011; Sathiyanarayanan & Arulmozhi, 2007). Mp and Cc seeds are used as a source of nutrition for both humans and animals. Importantly, the seeds, stems, and roots of Mp and Cc contain bioactive compounds including tannins, polyphenols, and alkaloids, which contribute to their therapeutic potential (Green et al., 2003; Lampariello et al., 2012).

Several studies have reported that Mp and Cc exhibit potent antioxidant, anti-inflammation, hypoglycemic (Njemuwa et al., 2019; Rachsee et al., 2021), anti-microbial, hypocholesterolemia, hepatoprotective, anticancer, and nephroprotective activities with potential applications in both medicine and the health food industry (Jiao et al., 2020; Prasob-on & Suthira, 2018). Moreover, Mp has showed to improve male fertility with no toxicity on male reproductive system (Choowong-in et al., 2021). Since ancient times, both legumes are widely acknowledged by the traditional folk medicine for the treatment of several human diseases such as respiratory infections, reproductive system infections, dysentery, diabetes, hepatitis, measles, jaundice, ulcers, sores, and menstrual disorders (Kong et al., 2011; Vo et al., 2020).

The processing of the Mp and Cc seeds for the production of flours or their use in food recipes generates a significant amount of waste in form

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of pods (Mp-p and Cc-p, respectively) or other by-products. The pods are recognized as non-starch lignocellulosic biomass material that also contains PCs, whose concentration increases as the plant reaches maturity while the tannin content decreases (Chikagwa-Malunga et al., 2009).

Currently, there is limited information available regarding the bioactive components in Mp-p and Cc-p, which limits their utilization. Consecuently, a substantial, renewable and low-cost natural resource remains untapped. This contrasts with the rising demand for eco-sustainable utilization of natural matrices and the search of by-products for the development of novel food products, specifically those containing antioxidant compounds.

Hence, this study was designed to investigate the extraction of PCs from Mp-p and Cc-p using different solvents and assess their antioxidant capacities (AOC). Additionally, to better understand the relationship between the PCs detected in selected extracts and their AOC a theoretical study using Density Functional Theory (DFT) and the quantum theory of atom in molecules (QTAIM) was performed (Bader, 1990). The results of this study will provide a baseline data related to the extraction of PCs with AOC from these agricultural wastes which unlock their potential for several applications.

2. Materials and methods

2.1. Samples

Pods of *Mucuna pruriens* (Mp-p) and *Cajanus cajan* (Cc-p) were obtained from Estación Experimental “El Sombrero-Corrientes” (Instituto Nacional de Tecnología Agropecuaria-INTA) Argentina. These samples were ground in an electric mill (Decalab Fbr, Argentine) and subsequently sieved through were collected between 18 ASMT (1000 μm) and 100 ASMT (140 μm).

2.2. Extraction techniques

Preparation of Macerates: ground pods dispersions (10 g/ 100 mL) were prepared in different solvent mixtures: water, 50 % methanol, 100 % methanol, 50 % ethanol, 100 % ethanol. Mixtures were macerated for 24 h at room temperature.

Preparation of Decoctions: 2 g of ground pods were added to 100 mL and boiled for 15 min. Then, all the resultant dispersions were centrifuged (Sorvall ST 8R, Thermo Scientific, Germany) at 3000 rpm for 25 min, and filtered. The solvent was evaporated using a rotary evaporator (RE100-Pro, DragonLab, China) and a freeze-dryer (Christ alpha 1–4 LD-2; Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). The dried extracts were stored at 4 °C until used. The extraction yield was expressed as plant material percentage.

2.3. Analysis of phenolic compounds

2.3.1. Total phenolic content (TPC)

The TPC was performed by the colorimetric method, as described by Singleton and Rossi (1965), with some modifications. Five mg of extract was re-dissolved in 1 mL of extraction medium. Ten μL of sample was mixed with 50 μL of Folin-Ciocalteu reactive and 190 μL of water. After 1 min, 150 μL of 20 % aqueous solution of Na_2CO_3 were added. The mixture was kept for 2 h in the dark at room temperature. Three hundred μL of each reaction mixture were transferred to a 96-well microplate. The absorbance was measured at 760 nm in a microplate reader (Multiskan GO, Thermo Scientific, Finland). Gallic acid (2 mg/mL) was used for constructing the standard curve, and the results were expressed as mg of gallic acid equivalents (GAE)/ g dry extract.

2.3.2. Total ortho-diphenol content (TODC)

TODC was determined as described by Bendini et al. (2003), with some modifications. Briefly, 5 mg of extract was re-dissolved in 1 mL of extraction medium, then and 0.1 mL of it was mixed with 1 mL of a 5 % solution sodium molybdate in 50 % ethanol. After incubation in the dark for 15 min, 300 μL of each reaction mixture were transferred to a 96-well microplate and the absorbance was measured at 370 nm in a microplate reader (Multiskan GO, Thermo Scientific, Finland). The results were expressed as mg of caffeic acid equivalents (CAE)/ g of dry extract using the prepared standard curve.

2.3.3. Total flavonoids content (TFC)

The TFC was estimated by using aluminum chloride colorimetric method as described by Wang et al. (2019) with some modifications. Briefly, 5 mg of extract was re-dissolved in 1 mL of extraction medium. The assay was conducted by mixing 100 μL of each sample extract, 140 μL of methanol, 60 μL of 8 mM aluminum trichloride solution. The mixture was incubated in the dark at room temperature for 30 min, and the absorbance was measured at 425 nm using a microplate reader (Multiskan GO, Thermo Scientific, Finland). A calibration curve of quercetin (24 μg/mL) was prepared and the TFC was expressed as mg quercetin equivalent (QE)/ g dry extract.

2.4. Antioxidant assays

2.4.1. DPPH radical scavenging assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method was determined as reported by Brand-Williams et al. (1995) with some modifications. A DPPH working solution (6.10^{-5} M) was prepared from a DPPH stock solution (6.10^{-4} M) in methanol. Both solutions were stored at 4 °C until used. Five different concentrations of extracts were tested (from 0.625 to 10 mg/mL). Twenty-five μL of each extract concentration solution were mixed with 975 μL of DDPH solution. After shaking, 300 μL of each reaction mixture were transferred to a 96-well microplate, and the absorbance was measured at 516 nm in a microplate reader ($t = 0$ h) (Multiskan GO, Thermo Scientific, Finland). The reaction was kept in the darkness for 4 h at room temperature and once the reaction was finished, the absorbance was measured again. The DPPH concentration remaining in the reaction medium was calculated from a calibration curve ($0.75–9.10^{-5}$ M). The percentage of remaining DPPH was then plotted against the extract concentration to obtain the antioxidant capacity necessary (expressed in μg/mL) to decrease the initial DPPH concentration by 50 %, that is, the EC_{50} value.

2.4.2. Ferric reducing antioxidant power (FRAP) assay

Antioxidant capacity of samples was analyzed by using FRAP assay (Benzie & Strain, 1996). The working FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution and 20 mM $FeCI_3{\cdot}6H_2O$ in a ratio of 10:1:1. Forty μL of each extract were mixed with 1160 μL of FRAP reagent and after shaking, 300 μL of each reaction mixture were transferred to a 96-well microplate. The changed in absorbance was measured at 593 nm using a microplate reader (Multiskan GO, Thermo Scientific, Finland), after 90 min of incubation at 37 °C. Sample AOC was determined by generating a FRAP standard curve using a series of Trolox standard solutions (0.00–0.40 mg/mL). Results were expressed as mmol Trolox equivalent (TE) / g dry extract.

2.5. HPLC determination of individual polyphenols

The extracts were prepared as previously described for TPC and filtrated through 0.45 μm Nylon membrane before injection into HPLC Shimadzu LC-10/20 A (Tokyo, Japan). The mobile phase was composed of acetic acid 1 % (A) and methanol:acetic acid (99:1) (B) (Zhang et al., 2008). The flow rate was 0.8 mL min^{-1} and the elution gradient program was set as follows: 0 to 10 min: 10 % to 26 % B; 10 to 25 min: 26 % to 40 % B; 25 to 45 min: 40 % to 65 % B; 45 to 55 min: 65 % to 95 % B; 55 to 58 min: 95 % to 10 % B; and 58 to 60 min: 10 % B. A Hypersil ODS column 250 × 4.6 mm, 5 μm particle size (Thermo Scientific, Walthman, MA, USA) was used to separate compounds and a UV–Visible DAD detector Shimadzu, SPD-20 MA (Tokyo, Japan) was set at 280 nm. Standard solutions of catechin, quercetin and gallic, chlorogenic and caffeic acids, were injected in the same conditions. Retention time (r_t) and UV spectra were used to evaluate the compounds and quantification was made by comparison to calibrations curves of the standard compounds.

2.6. *Statistical analysis*

All experiments were carried out in triplicates, and data were reported as mean ± standard deviation. The differences of mean values among solvent systems were determined using analysis of variance (ANOVA) followed by LSD tests at the significant level of $p < 0.05$. Correlation analysis was performed between TPC, TODC, TFC, and antioxidant activity of sample extracts using Pearson's correlations coefficient. All statistical analyses were conducted using Infostat software (Balzarini et al., 2017).

2.7. *Computational methods*

The PC geometries were optimized at the B3LYP/6–311++G(d,p) level, and verified as stationary points via frequency calculations. Solvent effects were taken into account by full optimizations using the SMD-continuum solvation method. Restricted and unrestricted calculation schemes were applied to closed-shell and open-shell systems, respectively.

The radical scavenging activities of selected PCs were determinate by evaluating the AOC via three mechanisms: hydrogen atom transfer (HAT), electron transfer-proton transfer (SET-PT), and sequential proton loss-electron transfer (SPLET).

The thermodynamic parameters such as bond dissociation enthalpy (BDE), adiabatic ionization potential (IP), proton dissociation enthalpy (PDE), proton affinity (PA), and electron-transfer enthalpy (ETE) were calculated to explore these mechanisms, following the literature (Leopoldini et al., 2011). All calculations were performed with Gaussian 09 suite of programs (Frisch et al., 2009). The topological QTAIM analysis, including the population of atomic spin densities (Bader, 1990), was carried out using the AIMALL program. Further theoretical background and computational details can be found in the Supporting Information.

3. Results and discussion

The extraction of solid samples using organic solvents is widely employed for the recovery of PCs from agricultural waste. The optimal solvent selection and condition is typically varied for different plant matrices analyzed. To our knowledge, there is no information in the literature regarding the extraction conditions for the samples under study. Therefore, we have tested the extraction efficiency of water, methanol and ethanol and their mixtures (50 %) as solvents (Vijayalaxmi et al., 2015). In addition, the extraction by decoction method was evaluated because it is commonly used extraction method in herbal preparation such as tisanes, tinctures and similar solutions (Isla et al., 2021).

3.1. *Extraction yields*

The extraction yields of each sample obtained with different solvents are shown in Table 1. The results indicated that the yields of the extracts from Mp-p were higher than those from Cc-p. The extract yields from Mp-p were about 6 % by using water, 50 % ethanol and 50 % methanol ($p < 0.05$), while lower values (< 2 %) were obtained with pure organic solvents. Similar yield (6.57 %) was obtained from roasted seeds of *M. pruriens* by continuous extraction with a Soxhlet by using 80 % ethanol as solvent (Rachsee et al., 2021).

The extract yields from Cc-p were about 3 % by using water, 50 % ethanol and 50 % methanol ($p < 0.05$). Again, the use of pure organic solvents led to the lowest extract yields, especially with 100 % ethanol (0.77 %). Overall, the use of water and hydroalcoholic mixtures resulted in higher yields compared to pure organic solvents, aligning with previous studies (Vijayalaxmi et al., 2015).

Table 1
Extraction yield, total phenolic (TPC), *ortho*-diphenols (TODC) and flavonoids (TFC) contents of the Mp-p and Cc-p extracts using different solvents.

Sample extract	Extraction yield (%)	TPC (mg GAE/g)	TODC (mg CAE/g)	TFC (mg QE/g)
Mp-p				
H_2O	6.05±0.35b	175.35 ± 8.76b	103.93 ± 5.37b	31.69 ± 0.66c
50% MeOH	6.01±0.14b	155.56 ± 7.17bc	103.96 ± 5.20b	42.79 ± 0.52ab
100% MeOH	1.33±0.21c	140.31 ± 7.30c	92.65 ± 4.63 b	45.34 ± 1.22a
50% EtOH	5.45±0.07b	251.21 ± 6.17a	132.85 ± 6.64a	43.07 ± 0.50ab
100% EtOH	0.77±0.02c	78.99 ± 3.49d	50.56 ± 2.53 c	27.71 ± 0.52d
H_2O 100°	12.51 ±0.71a	263.90 ± 4.19a	137.02 ± 6.85a	40.97 ± 0.92b
Cc-p				
H_2O	3.05±0.07b	39.79 ± 2.02e	15.20 ± 0.76c	25.55 ± 1.28 d
50% MeOH	2.75±0.07b	67.43 ± 3.37cd	36.81 ± 0.91b	43.69 ± 2.18bc
100% MeOH	1.95±0.07c	87.20 ± 4.36a	45.81 ± 2.29a	57.94 ± 2.90a
50% EtOH	3.10±0.42b	72.49 ± 3.62bc	39.98 ± 2.00b	46.07 ± 2.30ab
100% EtOH	0.77±0.02d	58.58 ± 2.93d	32.87 ± 1.64c	29.04 ± 1.45c
H_2O 100°	7.91±0.28a	81.01 ± 0.83ab	41.05 ± 2.05a	55.89 ± 2.79ab

Data were expressed as mean standard deviation ($n = 3$). Means with the same letters within a column do not differ significantly ($p > 0.05$) for each species.

The decoction method resulted in the highest yields (12.5 % for Mp-p and 7.9 % for Cc-p). The extract yields for Mp-p and Cc-p yields were nearly double those of the aqueous extracts obtained at room temperature. These results agree with that (10.68 % ± 0.04 %) of the *M pruriens* seed extract obtained using water at 80–95 °C for 30 min (Iamsaard et al., 2020). A higher temperature can help to soften plant tissues and weaken the interaction between the phenol and the protein or polysaccharide, which facilitates the extraction of PCs from plant material. However, care must be taken when using high temperatures because these can promote the oxidation of PCs, reducing their bioactivity (Antony & Farid, 2022).

3.2. *Total phenolic contents*

The recovery of PCs was significantly varied depending on the type and ratios of the solvents used and the vegetal matrix (Table 1). The highest TPC value for Mp-p extracts obtained at room temperature was achieved using 50 % ethanol (251.21 mg GAE/g). The TPC values decreased in the following order $H_2O > 50$ % methanol > 100 % methanol > 100 % ethanol. For *M. pruriens* leaves extracts obtained with ethanol and methanol using Soxhlet equipment for 36 h, the reported TPC were of 155.89 and 72.11 mg GAE/g, respectively, falling within the range of TPC values obtained for Mp-p extracts (Njemuwa et al., 2019).

The TPC values of Cc-p extracts were lower than those of Mp-p extracts. Among the different solvents used, the highest TPC value for Cc-p was obtained with 100 % methanol (87.20 mg GAE/g). Notably, a significant amount of TPC was also achieved with 50 % ethanol (72.49 mg GAE/g). However, the TPC values decreased for the extracts obtained with 50 % methanol, and further with 100 % ethanol and H_2O. In a related study, Gerrano et al. (2022) reported lower levels of TPC

(13.50–20.42 mg GAE/g) in *C. cajan* seed extracts obtained with 50 % methanol using a sonication bath for 1 h.

The decoction extracts of Mp-p and Cc-p yielded higher TPC values (almost twice as high) compared to the extracts obtained with water at room temperature.

3.3. *Total ortho-diphenol content*

Ortho-diphenol compounds have shown to improve radical stability by forming an intramolecular hydrogen bond between the hydrogen and phenoxyl radicals (Vella et al., 2019). TODC of the pods extracted by using different solvents and decoction method are presented in Table 1.

The highest TODC value for Mp-p was obtained in 50 % ethanol extract (132.85 mg CAE/g). In contrast, the lowest TODC value was observed in the 100 % ethanol extract. The TODC values were found to be lower in extracts obtained using H_2O, 50 and 100 % methanol, and there was no significant difference among them ($p > 0.05$).

The Cc-p extract obtained with 100 % methanol showed the highest TODC value (45.81 mg CAE/g). The TODC exhibited a slight decrease in extracts obtained with 50 % ethanol and 50 % methanol. Furthermore, the extract obtained with 100 % ethanol and water showed the lowest TODC values.

Therefore, using 100 % ethanol resulted in a low recovery of TPC and TODC from both pods. This outcome may be attributed to the selective capability of pure ethanol, which predominantly extracts hydrophobic PCs, while hydrophilic ones remain in the plant matrices. Conversely, an ethanol-water mixture is more effective in extracting a wider range of PCs from different plant material, as was observed for Mp-p.

Regarding the solvent used for the extraction, the TODC values followed a similar trend as the TPC values. Notably, a strong linear correlation was found between the TPC and TODC values for the extract of both pods obtained at room temperature with the different solvents, as evidenced by high R^2 values of 0.92 and 0.94 for Mp-p and Cc-p, respectively (See Fig. S1 in the Supporting Information).

The decoction extracts of Mp-p and Cc-p showed TODC values (137.02 and 41.05 mg CAE/g, respectively) as high as those of 50 % ethanol and 100 % methanol extracts, respectively, and this difference was not statistically significant ($p < 0.05$).

In the extracts obtained from Mp-p at room temperature, TODC values represented between 52 and 67% of TPC, while for Cc-p extracts, those levels varied from 38 % to 56 %. The TODC values for Mp-p and Cc-p decoction extracts were 52 and 50% of the TPC values, respectively. These findings suggest that *ortho*-diphenols are relatively abundant PCs in the extracts under study. To the best of our knowledge, TODC in seeds, leaves and pods of Mp and Cc has not been reported in the literature and, because of these compounds are highly antioxidant, their presence in the pods is encouraging (Vella et al., 2019).

3.4. *Total flavonoid contents*

The TFC of samples extracted by different solvents are shown in Table 1. For Mp-p, the highest levels of TFC were achieved using 100 % methanol, 50 % ethanol and 50 % methanol. There were no significant differences observed between these three solvents ($p < 0.05$), indicating their effectiveness in extracting flavonoid compounds from Mp-p. Interestingly, a similar trend was observed for the Cc-p extracts, with the highest TFC value obtained using 100 % methanol, and a slight decrease in the value for the 50 % ethanol and 50 % methanol extracts. These findings are in agreement with previous studies that have demonstrated methanol to be the most suitable solvent for extraction flavonoid from plant matrices (Chirinos et al., 2007). The lowest TFC values were found in 100 % ethanol and water extracts for both pods.

On the other hand, the decoction extracts showed higher TFC values than the water extracts, indicating that the heat treatment may enhanced the release of flavonoid from the pods. For Mp-p decoction extract, the TFC (40.97 mg QE/g) was lower than that obtained with 100 % methanol and 50 % ethanol, while for Cc-p decoction extract, the TFC was as high as that of the 100 % methanol and 50 % ethanol extracts. Vo et al. (2020) reported TPC (14.24-33.33 mg GAE /g) and TFC (8.42–23.54 mg QE/g) values for *C. cajan* roots extracts using hot water (98 °C ± 2 °C for 2 h), 50 % and 95 % ethanol (for 48 h). Such values were lower than those for Cc-p extracts obtained using similar solvents. This clearly demonstrates that the phenolic concentration in the extracts varies depending on the part of the plant and the extraction conditions.

3.5. *Antioxidant capacity*

The DPPH radical scavenging activity and the FRAP assays are commonly used to measure the AOC of a sample. The DPPH assay measures the ability of a compound to scavenge free radical by donating hydrogen atoms, while FRAP assay measures the ability of a compound to reduce the ferric ion to ferrous ions. The AOC estimated by means of the DPPH and FRAP assays for the Mp-p and Cc-p extracts are displayed in Fig. 1.

The AOC values exhibited variations depending on the plant matrix and solvent utilized, as depicted in Fig. 1. Among the Mp-p extracts, those obtained with 50 % ethanol (5.90 mg/mL) and H_2O (7.32 mg/mL) displayed the lowest EC_{50} values, ($p > 0.05$), indicating a stronger antioxidant capacity. Furthermore, the EC_{50} values followed an increasing trend in the order of 50 % methanol < 100 % methanol < 100% ethanol for Mp-p extracts. It is worth noting that the crude methanol extract of *M. pruriens* leaves was reported to have an EC_{50} value of 19.63 µg/mL, which aligns with the values observed for Mp-p 100 % methanol extract (Ferdous et al., 2021).

The Cc-p extract obtained with 100 % methanol demonstrated the greatest radical scavenging capacity, with an EC_{50} value of 6.82 mg/mL. In comparison to the other extracts, this extract displayed a significantly higher AOC. The EC_{50} values increased following the order 50 % methanol < 50 % ethanol < 100 % ethanol < water.

The Mp-p decoction extract showed a low value of EC_{50} that was not significantly different from the values obtained from the 50 % ethanol and H_2O extracts ($p > 0.05$). In contrast, the EC_{50} value for Cc-p decoction extract was higher than that of 100 % methanol extract, but notably lower than that obtained with water at room temperature.

Significant differences in the AOC, assessed by the FRAP assay, were observed between the extracts obtained from the two matrices. Particularly, the Mp-p (123.43–29.28 mmol TE/g) displayed a significantly higher AOC than the Cc-p extract (40.30–6.79 mmolTE/g).

The Mp-p extracts obtained with 50 % ethanol and water exhibited the highest AOC. Whereas the FRAP values decreased for 50 % and 100% methanol extract. The 100 % ethanol extract exhibited the lowest value of FRAP indicating a lower antioxidant activity compared with the other extracts.

For Cc-p, the highest AOC was observed for 100 % methanol extract (40.30 ± 2.02 mmol TE/g). The AOC of Cc-p decreased with the solvent polarity, with the 50 % methanol extract showing higher AOC than 50 % ethanol and 100 % ethanol extracts. In contrast to the results observed for Mp-p, the lowest value of FRAP was measured in Cc-p aqueous extract.

The Mp-p decoction extract showed AOC that was as high as that of the 50 % ethanol and water extracts. The AOC of Cc-p decoction extract was lower than that obtained with 100% methanol.

Overall, the increase of the FRAP values and the decrease of EC_{50} values follow the same trend relative to the solvent used. These findings suggest that active compounds in Mp-p are likely to be soluble in a mixture of water and ethanol, as well as in water alone, while their solubility in pure ethanol is limited. The active compounds in Cc-p are more soluble in methanol. The highest AOC was observed in the Cc-p 100 % methanol extract, and the AOC significantly decreased with decreasing solvent polarity. This indicate that the active compounds in Cc-p are more polar in nature and thus more soluble in polar solvents such as methanol. In addition, the results demonstrate that the high

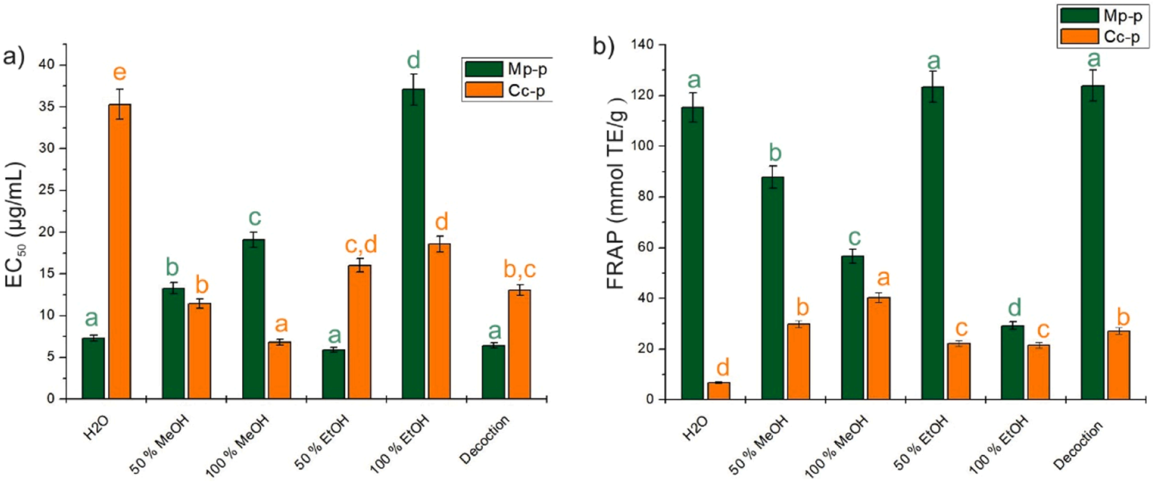

Fig. 1. Antioxidant capacity evaluated through of a) DPPH and b) FRAP assays in Mp-p and Cc-p extracts obtained with different solvents and decoction method. Results are expressed as mean ± standard deviation of three means ($n = 3$). Different letters denote significant differences ($p < 0.05$).

temperatures in short times implemented by decoction method, does not significantly affect the AOC of the compounds.

3.6. Correlation between TPC, TODC and TFC with antioxidant activity

Overall, the extracts with higher AOC showed higher TPC and, hence higher TODC. However, there was not clear relationship between AOC and TFC. In the literature, some authors have found correlation between these parameters while others did not (Sulaiman et al., 2011). To evaluate the relationship among AOC with TPC, TODC and TFC, a Pearson correlation test was performed, and *r*-values are presented in Table 2.

The results revealed a significant negative correlation ($p < 0.05$) between TPC and TODC with DPPH (EC_{50}) for the extracts of both pods. However, the correlation between TFC and DPPH (EC_{50}) was moderately negative for Cc-p and weak for Mp-p, with a *r*-value of −0.5131, but it was not statistically significant ($p > 0.05$).

Strong positive correlations ($r > 0.91$) were observed between TPC and TODC with FRAP for both legume pods. Furthermore, a moderate positive correlation was found between TFC and FRAP for Cc-p extracts. However, in the case of Mp-p extracts, only a weak positive correlation was observed between these parameters, which was not statistically significant ($p > 0.05$). Therefore, the consistency of the relationship between TFC and FRAP may be lower in Mp-p extracts compared to Cc-p extracts.

Comparing both legume pods, the relationships between TFC and AOC evaluated by both assays, showed the greatest differences in correlations. The results suggest that the PC present in Mp-p and Cc-p extracts significantly contribute to their AOC. However, the influence of other compounds and factors on these properties cannot be ruled out.

3.7. Quantitative RP-HPLC analysis

Based on the above results, PCs were detected in 50 % ethanol of Mp-p and 100 % methanol Cc-p extracts, as well as in decoction extracts. The sample peaks in the chromatogram were tentatively identified as gallic acid, chlorogenic acid, caffeic acid, catechin and quercetin by comparing their retention time with those of the corresponding standards (Fig. S2 and Table S1 in the Supporting Information). Table 3 summarize the quantification of all identified peaks.

The results indicated that the major phenolic acids present in the Mp-p extracts are chlorogenic acid and caffeic acid. The concentration of chlorogenic acid was almost twice higher in the 50 % ethanol extract (31.3834 mg/100 mg) compared to the decoction extract (14.0299 mg/100 mg). Conversely, the concentration of caffeic acid in the decoction extract (11.2628 mg/100 mg) was higher than in ethanoic one (7.1943 mg/100 mg). Gallic acid was detected in a small amount in both the decoction and 50 % ethanol Mp-p extracts, while catechin and quercetin were not quantifiable in either Mp-p extracts.

Chlorogenic acid was also detected in significant amount in Cc-p extracts. The content of chlorogenic acid in both 100% methanol (5.3991 mg/100 mg) and decoction extracts (6.8574 mg/100 mg), did not show significant difference ($p > 0.05$). The Cc-p decoction extract was found to contain caffeic acid and gallic acid. The Cc-p 100% methanol extract had a lower concentration of caffeic acid than decoction extract, and gallic acid was not quantifiable in it.

Quercetin was detected in high concentration (6.1059 ± 0.0132 mg/100 mg) in Cc-p 100 % methanol extract, while its presence was significantly reduced (0.3370 ± 0.0071 mg/100 mg) in the Cc-p decoction. These findings support a previous study that identified quercetin as one of the most abundant PC in methanolic extracts of *C. cajan* pod surfaces (Green et al., 2003). The results suggest that methanol is more effective in extracting quercetin than the decoction method, but it should also be considered that flavonoids are sensitive to heat (Antony & Farid, 2022).

Chlorogenic acid is a natural phenolic compound found in various plant species and is structurally an ester of caffeic acid and quinic acid. Dawidowicz and Typek (2010) showed that high-temperature water extraction can cause chlorogenic acid hydrolysis, leading to changes in the extract's chemical composition. As a result, the decrease in chlorogenic acid concentration and increase in caffeic acid in the Mp-p decoction extract compared to the ethanolic extract may not only be due to solvent efficiency but also the transformation of chlorogenic acid at high temperatures. Hence, it is important to consider the possibility of chemical transformations occurring during the extraction process.

The similar high AOC of 50 % ethanol and decoction Mp-p extracts ($p < 0.05$) suggests that both caffeic acid and chlorogenic acid have comparable antioxidant capacity. Moreover, the higher amount of

Table 2
Pearson's correlation test (*r*-values) between total phenolic content (TPC), *ortho*-diphenols content (TODC), total flavonoid content (TFC) and antioxidant activities (obtained from DPPH and FRAP assays) for Mp-p and Cc-p extracts.

Sample	DPPH			FRAP		
	TPC	TODC	TFC	TPC	TODC	TFC
Mp-p	−0.8939*	−0.9518*	−0.5131	0.9115*	0.9132*	0.3079
Cc-p	−0.9439*	−0.9699*	−0.8573	0.9407*	0.9192*	0.8889*

[*] Correlation is significant at $p < 0.05$.

Table 3
Concentration of major PCs detected in Mp-p and Cc-c extracted by selected solvent and decoction methods. Results are expressed in mg /100 mg dry extract.

Sample	Extraction method	Gallic acid	Chlorogenic acid	Caffeic acid	Catechin	Quercetin
Mp-p	50 % EtOH	0.6178 ± 0.0505 ^{b}	31.3834 ± 2.7442 ^{a}	7.1943 ± 1.3651 ^{b}	*ND	*ND
	Decotion	2.6753 ± 0.0897 ^{a}	14.0299 ± 0.8040 ^{b}	11.2628 ± 0.4525 ^{a}	*ND	*ND
Cc-p	100 % MeOH	*ND	5.3991 ± 0.0031 ^{b}	0.6779 ± 0.0019 ^{b}	*ND	6.1059 ± 0.0132 ^{a}
	Decoction	0.0663 ± 0.0042	6.8574 ± 0.0666 ^{a}	1.6788 ± 0.0073 ^{a}	*ND	0.3370 ± 0.0071 ^{b}

$^{a.b.c.d}$. different superscripts indicate significant differences for the same compound between samples.

* ND: not detected.

quercetin in Cc-p 100 % methanol extract resulted in the highest AOC among all extracts. To gain a better understanding of the antioxidant activity of the phenolic compounds detected in Mp-p and Cc-p, a theoretical study was conducted.

3.8. Computational chemistry calculations

Thermodynamic parameters related to HAT, SPLET, and SET-PT mechanisms calculated for caffeic acid (CA), chlorogenic acid (CGA) and quercetin (QE) are shown in Table 4. Fig. 2 depicts the molecular graph with the spin density distribution of the most stable phenoxyl radicals of CA, CGA and QE (the remain radicals are depicted in the Supporting Information). Additionally, the contour plot of the Laplacian of electron density for anionic species of CA and CGA were computed and compared (Fig. 2c).

The results disclosed that the 4-O• radical of CA and CGA exhibit lower BDE than their 3-O• counterpart, implying that the 4-O• radical possesses a higher reactivity towards radical scavenging. For QE, the 3-O• radical showed the lowest BDE value.

The calculated IP values for all three compounds were significantly higher than the corresponding BDE values, indicating that the studied compounds are not inclined towards the SET-PT mechanism.

The PA values of the three compounds were considerably lower than their BDE and IP values, indicating that the SPLET pathway may be a more likely route for them in methanol. In the case of CA and CGA, the 4-OH position exhibited the lowest PA values, while for QE, the lowest value was observed at the 7-OH position. Therefore, these positions are more susceptible to proton extraction than others, which is consistent with previous studies conducted in water (Marković & Tošović, 2016).

CA and CGA exhibited remarkably similar values for all thermodynamic descriptors. Additionally, we examined the topological pattern and spin density distribution on the radicals CA-4-O• and CGA-4-O• and found them to be comparable. The spin density was primarily located on their catechol and acrylic groups, and the charge density distribution on their CA-4-O• and CGA-4-O• species showed similar patterns in those regions (Fig. 2b).

The CA-4-O^- and CGA-4-O^- species displayed a bcp between the anionic O4 and HO-3 group (Fig. 2c). The values of r_b (0.024 au) and $\tilde{N}^2 r_b$ (0.090 au) at these bcps are consistent with the characteristic of a weak H-bond interaction, and the atomic charge on O4 was similar for both species. Thus, CA and CGA and their derivative species, exhibited similar thermodynamic properties and electronic structures.

Table 4
Thermodynamic parameters (in kcal/mol) of antioxidative mechanisms for CA, CGA and QE in methanol.

	position	HAT	SET-PT		SPLET	
		BDE	IP	PDE	PA	ETE
CA	3-OH	80.6	111.9	9.1	35.4	85.6
	4-OH	76.0		4.5	28.3	88.1
CGA	3-OH	80.8	113.0	8.2	35.2	86.0
	4-OH	76.4		3.8	28.1	88.7
QE	3-OH	69.2	108.9	0.7	24.7	84.9
	5-OH	79.9		11.4	27.2	93.1
	7-OH	78.1		9.6	22.0	96.5
	3′-OH	70.9		2.4	23.8	87.4
	4′-OH	70.8		2.3	24.1	87.0

Based on our findings, it can be inferred that the shikimic moiety in CGA has a negligible impact on its radical scavenging activity. This suggests that the observed activity of CGA is mainly attributable to the presence of the catechol moiety, which is also present in CA. Therefore, despite the Mp-p decoction extract having a lower concentration of CGA compared to the ethanolic extract, the higher content of CA in the decoction extract might have compensated for the reduced CGA levels. This could potentially account for the similar antioxidant activity observed in both extracts.

On the other hand, the thermodynamic parameters revealed that QE exhibits slightly higher radical scavenging power compared CA and CGA. Furthermore, the spin density distribution on the 3-O• radical of QE (Fig. 2b) was more extensive than that on CA and CGA. The large amount of QE detected in Cc-p methanolic extract could be associated with its high antioxidant activity, which was comparable to that of decoction and ethanolic Mp-p extracts. Conversely, the levels of QE and other PCs were lower in the Cc-p decoction extract, which is consistent with the lowest observed antioxidant activity.

Although diverse compounds and factors contribute to the antioxidant activity of the analyzed extracts, the theoretical analysis of the principal compounds detected in the extracts can provide a satisfactory explanation for their antioxidant activity.

4. Conclusions

This study investigated the effectiveness of different solvents (water, methanol, ethanol, and 50 % methanol and 50 % ethanol mixture) and the decoction method for recovering of PCs with AOC from Mp-p and Cc-p, both agricultural by-products of the legume family.

The results revealed that the effectiveness of different solvents varied depending on the plant material, with 50 % ethanol and 100 % methanol showing the highest suitability for the extraction of PCs from Mp-p and Cc-p, respectively, at room temperature. The decoction method was also found to be effective in recovering a significant amount of PCs with AOC from Mp-p, suggesting its potential as a viable alternative method.

Overall, extracts with higher PC content and TODC showed higher AOC. The Mp-p ethanoic and decoction extracts were rich in chlorogenic and caffeic acid, while Cc-p methanolic extract contained a larger amount of the flavonoid quercetin.

Furthermore, the combination of DFT and QTAIM approaches proved valuable in understanding the relationship between the major compounds detected in the extracts and their antioxidant activity.

Our findings demonstrate the potential of Mp-p and Cc-p as natural sources of PCs with antioxidant properties, providing a promising basis for further research into their applications in the development of functional foods, nutraceuticals, and other natural antioxidant products.

CRediT authorship contribution statement

Beatriz I. Avalos: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Gonzalo A. Ojeda:** Investigation, Methodology, Writing – review & editing. **Erica D. Spinnenhirn:** Conceptualization, Investigation, Methodology, Software. **Belén A. Acevedo:** Formal analysis, Funding acquisition, Writing – review &

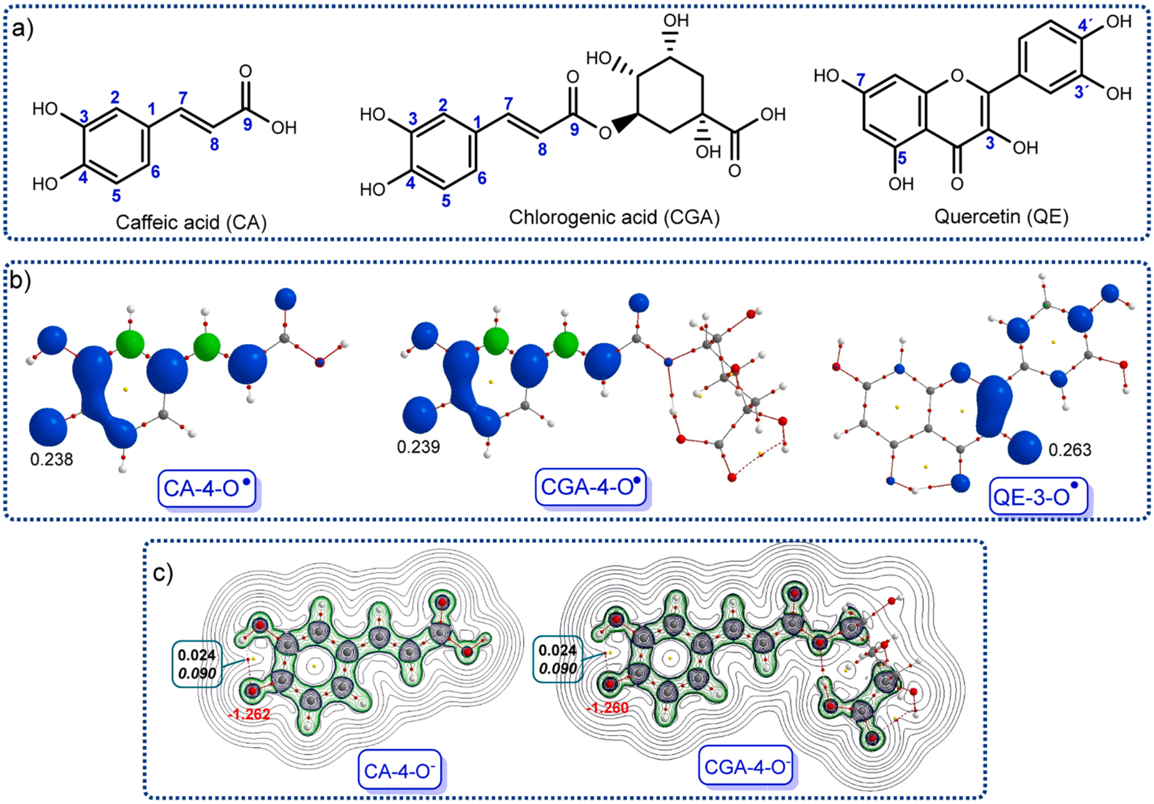

Fig. 2. (a) Structural formula of CA, CGA and QE with atom labeling. (b) Molecular graph for the most stable radical stems of CA, CGA and QE, overlaid with their respective spin density distribution and atomic spin density values in au. c) Contour plots of $-\tilde{N}^{2}r$ superimposed on the molecular graph of CA-4-O^{-} and CGA-4-O-. Blue and green lines depict regions of local charge density depletion and concentration, respectively. The values at the bcps, listed from top to bottom, include electron density (r_b), and the Laplacian of electron density ($\tilde{N}^{2}r_b$) in au. The atomic charge on the O4 atom is given in *e*.

editing. **Margarita M. Vallejos:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100503.

References

Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Appl. Sci., 12*(4), 2107. https://doi.org/10.3390/app12042107

Bader, R. F. W. (1990). *Atoms in molecules. A quantum theory*. London Oxford: Science Publications, Clarendon Press.

Balzarini, M.G., González, L., Tablada, M., Casanoves, F., Di Rienzo, J.A., & Robledo, C. W. (2017). InfoStat. In A. Universidad Nacional de Córdoba (Ed.).

Bendini, A., Bonoli, M., Cerretani, L., Biguzzi, B., Lercker, G., & Toschi, T. G. (2003). Liquid-liquid and solid-phase extractions of phenols from virgin olive oil and their separation by chromatographic and electrophoretic methods. *Journal of Chromatography A, 985*(1–2), 425–433. https://doi.org/10.1016/s0021-9673(02)01460-7

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology, 28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chikagwa-Malunga, S. K., Adesogan, A. T., Sollenberger, L. E., Badinga, L. K., Szabo, N. J., & Littell, R. C. (2009). Nutritional characterization of *Mucuna pruriens*: 1. Effect of maturity on the nutritional quality of botanical fractions and the whole plant. *Animal Feed Science and Technology, 148*(1), 34–50. https://doi.org/10.1016/j.anifeedsci.2008.03.004

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. *Separation and Purification Technology, 55*(2), 217–225. https://doi.org/10.1016/j.seppur.2006.12.005

Choowong-in, P., Sattayasai, J., Boonchoong, P., Poodendaen, C., Wu, A. T. H., Tangsrisakda, N., et al. (2021). Protective effects of Thai *Mucuna pruriens* (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. *Journal of Traditional and Complementary Medicine*. https://doi.org/10.1016/j.jtcme.2021.12.001

Dawidowicz, A. L., & Typek, R. (2010). Thermal stability of 5-o-caffeoylquinic acid in aqueous solutions at different heating conditions. *Journal of Agricultural and Food Chemistry, 58*(24), 12578–12584. https://doi.org/10.1021/jf103373t

Dilipkumar, P., Pragya, M., Neetu, S., & Ashoke, K. G (2011). Biological activities and medicinal properties of *Cajanus cajan* (L) Millsp. *Journal of Advanced Pharmaceutical Technology & Research, 2*, 207–214. https://doi.org/10.4103/2231-4040.90874

Ferdous, S. E., Rahman, M., Ahmed, F., & Muhit, M. A. (2021). Phytochemical analysis, antioxidant and cytotoxic activities of *Mucuna pruriens* leaves. *Dhaka University Journal of Pharmaceutical Sciences, 20*(1), 103–109. https://doi.org/10.3329/dujps.v20i1.54038

Frisch, M.J.T., G.W., Schlegel, H.B., Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A., Jr., Peralta, J.E., Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K.N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, N.J., Klene, M., Knox, J.E., Cross, J.B., Bakken, V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli, C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V. G., Voth, G.A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Ö., Foresman, J.B., Ortiz, J.V., Cioslowski, J., Fox, D.J. (2009). Gaussian 09, revision D.01. In. Wallingford CT: Gaussian, Inc.

Gasmi, A., Mujawdiya, P. K., Noor, S., Lysiuk, R., Darmohray, R., Piscopo, S., et al. (2022). Polyphenols in metabolic diseases. *Molecules, 27*(19), 6280. https://doi.org/10.3390/molecules27196280

Gerrano, A. S., Moalafi, A., Seepe, H. A., Amoo, S., & Shimelis, H. (2022). Nutritional and phytochemical compositions and their interrelationship in succulent pods of pigeonpea (*Cajanus cajan* [L.] Millsp.). *Heliyon, 8*(3), e09078. https://doi.org/10.1016/j.heliyon.2022.e09078

Green, P. W., Stevenson, P. C., Simmonds, M. S., & Sharma, H. C. (2003). Phenolic compounds on the pod-surface of pigeonpea, *Cajanus cajan*, mediate feeding behavior of Helicoverpa armigera larvae. *Journal of Chemical Ecology, 29*(4), 811–821. https://doi.org/10.1023/a:1022971430463

Iamsaard, S., Arun, S., Burawat, J., Yannasithinon, S., Tongpan, S., Bunsueb, S., et al. (2020). Evaluation of antioxidant capacity and reproductive toxicity of aqueous extract of Thai *Mucuna pruriens* seeds. *Journal of Integrative Medicine, 18*(3), 265–273. https://doi.org/10.1016/j.joim.2020.03.003

Isla, M. I., Ezquer, M. E., Leal, M., Moreno, M. A., & Zampini, I. C. (2021). Flower beverages of native medicinal plants from Argentina (Acacia caven, Geoffroea decorticans and Larrea divaricata) as antioxidant and anti-inflammatory. *Journal of Ethnopharmacology, 281*, Article 114490. https://doi.org/10.1016/j.jep.2021.114490

Jiao, J., Gai, Q. Y., Wang, X., Liu, J., Lu, Y., Wang, Z. Y., et al. (2020). Effective production of phenolic compounds with health benefits in pigeon pea [*Cajanus cajan* (L.) Millsp.] hairy root cultures. *Journal of Agricultural and Food Chemistry, 68*(31), 8350–8361. https://doi.org/10.1021/acs.jafc.0c02600

Kong, Y., Wei, Z. F., Fu, Y. J., Gu, C. B., Zhao, C. J., Yao, X. H., et al. (2011). Negative-pressure cavitation extraction of cajaninstilbene acid and pinostrobin from pigeon pea [*Cajanus cajan* (L.) Millsp.] leaves and evaluation of antioxidant activity. *Food Chemistry, 128*(3), 596–605. https://doi.org/10.1016/j.foodchem.2011.02.079

Lampariello, L. R., Cortelazzo, A., Guerranti, R., Sticozzi, C., & Valacchi, G. (2012). The magic velvet bean of *Mucuna pruriens*. *Journal of Traditional and Complementary Medicine, 2*(4), 331–339. https://doi.org/10.1016/S2225-4110(16)30119-5

Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry, 125*(2), 288–306. https://doi.org/10.1016/j.foodchem.2010.08.012

Marković, S., & Tošović, J. (2016). Comparative study of the antioxidative activities of caffeoylquinic and caffeic acids. *Food Chemistry, 210*, 585–592. https://doi.org/10.1016/j.foodchem.2016.05.019

Nguyen, V. T. (2017). *Recovering bioactive compounds from agricultural wastes*. West Sussex, UK: John Wiley & Sons, Ltd.

Njemuwa, N. N., Dickson, N. U., Elekwa Elizabeth, A., Uchenna, R. M., & Ogbonnaya, C. N. (2019). Evaluation of the antioxidant and anti-diabetic effect of Mucuna puriens extract. *European Journal of Medicinal Plants, 27*, 1–9. https://doi.org/10.9734/ejmp/2019/v27i230110

Prasob-on, R., & Suthira, M. (2018). Total phenolic content and tyrosinase inhibitory potential of extracts from *Cajanus cajan* (L.) Millsp. *Pharmacognosy Journal, 10*(6s), 109–112. https://doi.org/10.5530/pj.2018.6s.21

Rachsee, A., Chiranthanut, N., Kunnaja, P., Sireeratawong, S., Khonsung, P., Chansakaow, S., et al. (2021). *Mucuna pruriens* (L.) DC. seed extract inhibits lipopolysaccharide-induced inflammatory responses in BV2 microglial cells. *Journal of Ethnopharmacology, 267*, Article 113518. https://doi.org/10.1016/j.jep.2020.113518

Sathiyanarayanan, L., & Arulmozhi, S. (2007). *Mucuna pruriens* Linn. - a comprehensive review. *Pharmacognosy Reviews*, (1), 157–162.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*(3), 144–158. https://doi.org/10.5344/ajev.1965.16.3.144

Skendi, A., Irakli, M., Chatzopoulou, P., Bouloumpasi, E., & Biliaderis, C. G. (2022). Phenolic extracts from solid wastes of the aromatic plant essential oil industry: Potential uses in food applications. *Food Chemistry Advances, 1*, Article 100065. https://doi.org/10.1016/j.focha.2022.100065

Sulaiman, S. F., Sajak, A. A. B., Ooi, K. L., Supriatno, & Seow, E. M (2011). Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *Journal of Food Composition and Analysis, 24*(4), 506–515. https://doi.org/10.1016/j.jfca.2011.01.020

Vella, F. M., Cautela, D., & Laratta, B. (2019). Characterization of polyphenolic compounds in cantaloupe melon by-products. *oods, 8*(6), 196. https://doi.org/10.3390/foods8060196

Vijayalaxmi, S., Jayalakshmi, S. K., & Sreeramulu, K. (2015). Polyphenols from different agricultural residues: Extraction, identification and their antioxidant properties. *Journal of Food Science and Technology, 52*, 2761–2769. https://doi.org/10.1007/s13197-014-1295-9

Vo, T. L., Yang, N. C., Yang, S. E., Chen, C. L., Wu, C. H., & Song, T. Y. (2020). Effects of *Cajanus cajan* (L.) millsp. roots extracts on the antioxidant and anti-inflammatory activities. *Chinese Journal of Physiology, 63*(3), 137–148. https://doi.org/10.4103/cjp.cjp_88_19

Wang, W., Li, J., Zhang, H., Wang, X., Fan, J., & Zhang, X. (2019). Phenolic compounds and bioactivity evaluation of aqueous and methanol extracts of Allium mongolicum Regel. *Food Science & Nutrition, 7*(2), 779–787. https://doi.org/10.1002/fsn3.926

Zhang, W., Han, F., He, J., & Duan, C. (2008). HPLC-DAD-ESI-MS/MS analysis and antioxidant activities of nonanthocyanin phenolics in mulberry (*Morus alba L.*). *Journal of Food Science, 73*(6), C512–C518. https://doi.org/10.1111/j.1750-3841.2008.00854.x

Zhang, Y., Meenu, M., Yu, H., & Xu, B. (2020). An Investigation on phenolic and antioxidant capacity of under-utilized food legumes consumed in China. *Foods, 9*(4), 438. https://doi.org/10.3390/foods9040438