Scientia Agropecuaria

Web page: http://revistas.unitru.edu.pe/index.php/scientiaagrop

Facultad de Ciencias Agropecuarias

Universidad Nacional de Trujillo

RESEARCH ARTICLE

Extraction of flavonoids from *Mucuna pruriens* seeds by ultrasound: Evaluation of their antioxidant properties and toxicity in *Caenorhabditis elegans*

Luis A. Ramírez-Contreras[1]; Luis M. Anaya-Esparza[2]; Efigenia Montalvo-González[3]; Sergio Sánchez-Enríquez[4]; Jorge Manuel Silva-Jara[5]; Ángel Eduardo Rubio-Castillo[6]; Leonardo Hernández-Hernández[7]; Gabriela Camargo-Hernández[4]*

[1] Doctorado en Biociencias, Centro Universitario de Los Altos, Universidad de Guadalajara, Av. Rafael Casilla Aceves 1200, Tepatitlán de Morelos 47600, México.

[2] Centro de Estudios para la Agricultura, la Alimentación y la Crisis Climática, Centro Universitario de Los Altos, Universidad de Guadalajara, Av. Rafael Casillas Aceves No. 1200, 47620, Tepatitlán de Morelos, Jalisco, México.

[3] Laboratorio Integral de Investigación en Alimentos, Tecnológico Nacional de México/Instituto Tecnológico de Tepic. Av. Tecnológico 2595 Fracc. Lagos del Country, Tepic 63175, México.

[4] Instituto de Investigación en Ciencias Médicas, Centro Universitario de Los Altos, Universidad de Guadalajara, Av. Rafael Casilla Aceves 1200, Tepatitlán de Morelos 47600, México.

[5] Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara. Blvd. Gral. Marcelino García Barragán 1421, Guadalajara 44430, México.

[6] Centro Universitario del Norte, Universidad de Guadalajara. México 23 Km 191, 46200 Colotlán, México.

[7] Instituto de Investigación en Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara 44340, México.

* Corresponding author: gabriela.camargo@academicos.udg.mx (G. Camargo-Hernández).

Received: 19 November 2025. Accepted: 13 April 2026. Published: 27 April 2026.

Abstract

Traditional methods for extracting bioactive compounds from *Mucuna pruriens* involve high solvent use, long extraction times, and low yields. As a result, finding more efficient extraction techniques remains an important research focus. In this context, ultrasound-assisted extraction has become a promising alternative for extracting flavonoids from *M. pruriens*. These compounds play a crucial role in various applications, including nutraceuticals, pharmaceuticals, medicine, and cosmetics. Therefore, this study aimed to optimize the extraction yield of flavonoid content from *Mucuna pruriens* seeds by employing a Box-Behnken Design, focusing on the effects of ultrasound power, pulse cycle, and extraction time as factors, and using an acidified ethanol-water solution (80:20 v/v plus 2% HCl 2N) as the extraction solvent. Under optimal conditions, the ultrasound-assisted extraction method yielded a higher flavonoid content (77.30 µg/mL) than the magnetic stirring technique (60.92 µg/mL). High-performance liquid chromatography (HPLC) analysis identified and quantified significant amounts of gallocatechin, epicatechin, and epigallocatechin. Fourier-transform infrared (FTIR) spectroscopy suggested the presence of additional compounds, including amino acids, proteins, glycerides, aliphatic compounds, and alkaloids. Antioxidant assessments indicated that the optimal ultrasound conditions enhanced antioxidant activity in the DPPH and FRAP assays (323.51 and 259.07 mmol TE/g, respectively) compared to the conventional extraction method (294.13 and 164.19 mmol TE/g, respectively). Toxicity evaluations were conducted by exposing *C. elegans* to varying concentrations of extracts from both methods for 24 h, with LC_{50} values calculated at 143.65 µg/mL for the ultrasound extract under optimal conditions and 132.18 µg/mL for the magnetic stirring method. These findings provide support for the functional applications of *Mucuna pruriens* flavonoids as natural antioxidants.

Keywords: Velvet bean; ultrasound extraction; Box–Behnken design; antioxidant compounds; *Caenorhabditis elegans*.

DOI: https://doi.org/10.17268/sci.agropecu.2026.036

Cite this article:
Ramírez-Contreras, L. A., Anaya-Esparza, L. M., Montalvo-González, E., Sánchez-Enríquez, S., Silva-Jara, J. M., Rubio-Castillo, Á. E., Hernández-Hernández, L., & Camargo-Hernández, G. (2026). Extraction of flavonoids from *Mucuna pruriens* seeds by ultrasound: Evaluation of their antioxidant properties and toxicity in *Caenorhabditis elegans*. *Scientia Agropecuaria*, *17*(2), 513-527.

1. Introduction

Mucuna pruriens (L.) DC., commonly known as cowage or velvet bean, is a tropical legume in the family Fabaceae. It is native to Southeast Asia and is cultivated worldwide (Chandrasiri-Waliwita, 2021). It features long, thin stems, non-consecutive, lance-shaped leaves, and white blooms with a blue-purple hue. The pods are hairy, thick, and leathery, averaging 4 inches in length, and contain 4 to 6 deep brown seeds (Kedam, 2017). According to the literature, all parts of the *M. pruriens* plant exhibit therapeutic and nutritional properties (Sowdhanya et al., 2024). Conversely, in Mexico, *M. pruriens* is considered an invasive plague (CONABIO, 2009). *M. pruriens* seeds

are widely used in the Ayurvedic system to treat central nervous diseases and other medical conditions; moreover, in several countries, they have been investigated for their anti-Parkinsonian, antioxidant, anti-inflammatory, antimicrobial, antihypertensive, and anti-diabetic properties (Parvatikar et al., 2023; Sowdhanya et al., 2024). Therefore, *M. pruriens* seeds have attracted considerable attention in academic research due to their nutritional compounds (proteins, lipids, minerals, carbohydrates, fiber) and phytochemicals (including non-protein amino acid-derived L-DOPA, polyphenols, tannins, saponins, and flavonoids), which demonstrate therapeutic properties (Pathania et al., 2020; Yadav et al., 2017).

The extraction of bioactive molecules from plant tissues is an active research area with potential applications in both cosmetics and pharmaceuticals (Jitpimai et al., 2023). The most common methods for extracting bioactive compounds from *M. pruriens* seeds include maceration (Chaudhary, De, Bhadra, & Mukherjee, 2015; Jitpimai et al., 2023), soaking (Hajibeglou et al., 2023), boiling (Han, Bae, & An, 2022), Soxhlet extraction (Rachsee et al., 2021), heat reflux (Nwaoguikpe, Braide, & Ujowundu, 2011), and decoction (Avalos et al., 2023). However, these extraction methods used high amounts of solvents (petroleum ether, methanol, ethanol, and water), long extraction times (24 – 168 h), and exhibited low-extraction yields; moreover, due to these harsh extraction conditions, bioactive compounds may degrade or oxidize, affecting their potential biological properties and applications (Bitwell et al., 2023). On the other hand, some non-traditional extraction methods, such as supercritical fluid extraction (Costa et al., 2018), microwave-assisted and ultrasound-assisted extractions (Dhanani et al., 2015) have been investigated for extracting bioactive compounds from *M. pruriens* seeds, because they are environmentally friendly and sustainable techniques for extracting bioactive molecules from plant tissues (Sang et al., 2025). In recent years, ultrasonic extraction (20 - 100 kHz) has been studied as a green method for extracting bioactive compounds from plant materials (Sayem et al., 2024; Sang et al., 2025). It is based on the propagation of sound waves in a liquid medium, in which the implosion of cavitational bubbles disrupts plant cell walls, facilitating solvent diffusion and releasing phytochemicals (Dhanani et al., 2015). This method offers several advantages, including a short extraction time, reduced solvent demand, reduced degradation of bioactive compounds, improved preservation of biological activity, higher yields, and lower energy consumption compared to conventional extraction techniques (Hernández-Estrada et al., 2024; Tesoro et al., 2022).

In this context, it has been reported that ultrasonic extraction (using a 35 kHz ultrasound bath at 480 Watts for 15 min) is sufficient to obtain higher amounts of phenols and L-DOPA from *M. pruriens* seeds than the reflux method, in a shorter processing time. Nonetheless, the authors suggested that further research is needed to develop a mathematical model to optimize the extraction process for higher yields of bioactive compounds from *M. pruriens* (Dhanani et al., 2015). Additionally, Aware et al. (2019) optimized the ultrasonic extraction process of flavonoids from *Mucuna macrocarpa* using response surface methodology, employing an ultrasound bath and considering the extraction time (5 – 15 min) and ultrasound power (10 – 30 W) as variables, with water as the solvent. They reported that ultrasound is more efficient at extracting bioactive flavonoids from *M. macrocarpa* than static or shaking extraction. Additionally, it is essential to mention that no studies have been performed on the extraction of flavonoids from *M. pruriens* seed powder by ultrasound coupled with a sonicator probe, where several studies have reported that ultrasound-assisted extraction using a sonicator probe is more effective than ultrasound bath to extract bioactive molecules from plant tissues (Anaya-Esparza et al., 2023; Chemat et al., 2019).

Nonetheless, it is crucial to recognize that certain plant-based compounds can be toxic, a multifaceted issue shaped by factors such as their structural properties, dosage, and timing of exposure (Bhardwaj et al., 2024). In this context, over the last twenty years, *Caenorhabditis elegans* has been extensively used as a model organism in drug discovery and to investigate the *in vivo* mechanisms of bioactive compounds. This invertebrate model is particularly advantageous for studying the toxicity of phytochemicals from *M. pruriens* seeds due to its short life cycle, predictable development, easy cultivation, and small size (Rangsinth et al., 2019; Zarroug et al., 2023). Previous investigations have examined the potential of *M. pruriens* seed extracts to inhibit the aggregation of the alpha-synuclein protein and their neuroprotective effects in transgenic *C. elegans* strains (Anjaneyulu et al., 2020). However, the toxicity of *M. pruriens* seed extracts obtained by various extraction methods remains unexplored in this model. Furthermore, the existing literature on the ultrasound-assisted extraction of flavonoids from *M. pruriens* seeds is limited. Therefore, this work aimed to evaluated the effect of ultrasound power (X_{UP}: 80% – 100%), pulse cycle (X_{PC}: 1:1 – 3:1 s on/off), and extraction time (X_{ET}: 2 – 6 min) on flavonoids extraction from *M. pruriens* seed powder by ultrasound and compare their effectiveness against magnetic stirring, evaluating their antioxidant properties and toxicity against *C. elegans*.

2. Methodology

2.1 Details of plant material and reagents

The *M. pruriens* seeds were purchased from an online marketplace (Figure 1a). Seeds of uniform size were carefully chosen, excluding damaged specimens, and pulverized using an industrial-grade impact mill (Retsch SR300, Germany). The resulting *M. pruriens* seed powder was sifted through a steel mesh screen (Humboldt, AASHTO M92, IL, USA) to yield a fine powder of 500 µm (Figure 1b). The seed powder was stored in polystyrene containers at room temperature pending analysis. The reagents and solvents used in this investigation were of analytical or HPLC grade.

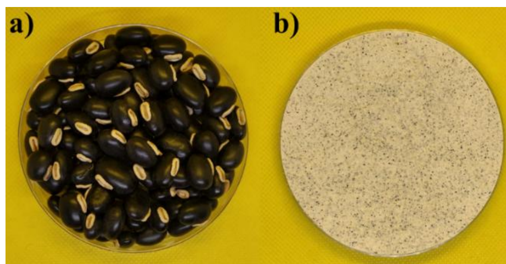


Figure 1. *Mucuna pruriens* seeds (a) and powder seed (b).

Additionally, a structural analysis of the *M. pruriens* seed powder was performed using FTIR, with a spectrometer coupled to attenuated total reflectance (Agilent Cary 630 FTIR, Australia) at room temperature and wavelengths ranging from 4000 to 450 cm^{-1}, with 24 scans and a resolution of 2 cm^{-1}. The FTIR spectra of *M. pruriens* seed powder is shown in Figure 2, which is consistent with previous reports for *M. pruriens* seeds (see supplementary material, Table S1).

2.2 First phase: Optimization of the ultrasound-assisted extraction method

2.2.1 Experimental design

The experimental design was formulated using Statistica version 10 software (StatSoft, Tulsa, OK, USA). A Box-Behnken design for three coded factors (X_{UP}, X_{PC}, and X_{ET}), three levels (–1, 0, +1), and three central points (15 experimental runs) was implemented to identify the optimal conditions for ultrasound-assisted extraction of total flavonoids (Y_{FLA}, mg CE/g). The three independent variables examined were ultrasound power (X_{UP}, 80%, 90%, and 100%), pulse cycle (X_{PC}, 1:1, 2:1, and 3:1 s on/off intervals), and extraction time (X_{ET}, 2, 4, and 6 min). Extraction runs were performed in a random sequence to minimize systematic errors.

2.2.2 Ultrasound-assisted extraction

Flavonoids were extracted using an ultrasonic probe (6 mm in diameter) coupled to a high-intensity ultrasonic processor (XMSJ, PZ-550LI, Zhengzhou City, China) with an output power of 550 watts and a frequency of 20 kHz. The process involved mixing 500 mg of *M. pruriens* seed powder with 25 mL of acidified ethanol-water (80:20 v/v, 2% v/v HCl at 2 M) solution (Pérez-Jiménez et al., 2008). An ice bath maintained a constant extraction temperature (25 ± 2 °C). After sonication under specific experimental conditions, cold centrifugation was performed at 4 °C (Hermle Z32HK; Wehingen, Germany) for 10 min (8000 × g). Then, the supernatants were gathered and stored at -20 °C for subsequent analysis.

2.2.3 Determination of total flavonoids

To determine the total flavonoid content, 430 µL of a 5% w/v sodium nitrate solution (Sigma-Aldrich, USA) was added to a conical tube, mixed with 100 µL of *M. pruriens* extract, and incubated for 5 minutes. Subsequently, 300 µL of 10% aluminum chloride (Golden-Bell, Mexico) was added, and the mixture was incubated for an additional minute. Then, 400 µL of sodium hydroxide (NaOH 1 M) was added and homogenized using a vortex mixer, and 200 µL of the mixture was placed in a 96-well plate and read at a wavelength of 490 nm (Khorasani Esmaeili, Mat Taha, Mohajer, & Banisalam, 2015) in a microplate reader (ACCURIS Instruments, SmartReader MR-9600, Nankín, China). A calibration curve (R^2 = 0.999) was constructed with catechin (Sigma-Aldrich, USA), and the results were expressed as mg equivalents of catechin per gram of dry extract (mg QE/g).

It must be noted that this study was conducted in three experimental phases, as outlined in Figure 3.

Following the ultrasound extractions, response surface methodology was employed to identify optimal ultrasound conditions for extracting total flavonoids from *M. pruriens* seed powder. Consequently, a 2nd-order polynomial model incorporating all terms (linear, quadratic, and interaction) was employed to predict the response (Equation 1).

$$Y = b_0 + \sum_{i=1}^{n} (b_i x_i) + \sum_{i=1}^{n} (b_i x_{ii}^2) + \sum_{j=1 \neq i}^{n} (b_{ij} x_i x_j) + \varepsilon \quad (1)$$

Y: is the projected response (total flavonoids), b_0 is model constant, b_i are model coefficients in its linear form, b_{ii} are model coefficients in its quadratic form, b_{ij} is the model interaction coefficient, *xi* and *xj* stands for the coded levels of the independent variable (ultrasound power, pulse cycle, and extraction time), and ε is the experimental error.

The F-ratio was utilized to evaluate model suitability. A lack-of-fit test was employed to assess the adequacy of the fitted model. Concurrently, the R-square and R-adjusted values were examined at a 95% confidence interval to evaluate model performance.

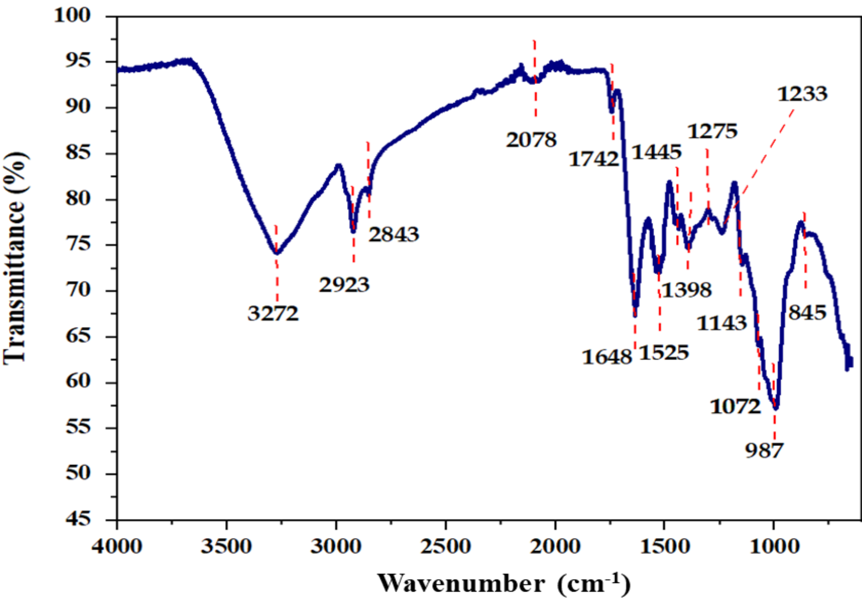


Figure 2. FTIR spectra of *Mucuna pruriens* seed powder.

Figure 3. Workflow for experimental process.

2.2.4 Modeling and optimization of extraction process parameters

2.2.5 Experimental validation of optimal ultrasound-assisted extraction

The optimal ultrasound-assisted extraction conditions (85% ultrasound power, 3:1 s on/off pulse cycle, and 5 min extraction time), obtained through response surface methodology analysis for total flavonoids, were experimentally validated to assess the model's accuracy by comparing predicted and experimental values.

2.2.6 Statistical analysis

Response surface methodology and analysis of variance (ANOVA; $p < 0.05$) were used to analyze data obtained in the first phase. Tukey's test ($\alpha = 0.05$) was employed to compare the extraction runs from the Box-Behnken design ($p < 0.05$). The results were analyzed using STATISTICA software version 10 (Statsoft, Tulsa, OK, USA). Data were presented as means ± standard deviation. All experiments (extractions and measurements) were performed in triplicate (n=3).

2.3 Second phase: Magnetic stirring extraction

Magnetic stirring extraction was conducted by mixing 500 mg of *M. pruriens* seed powder with 25 mL of acidified ethanol-water (80:20 v/v with 2% v/v HCl at 2M) solution (Pérez-Jiménez et al., 2008) in an amber glass bottle and agitated at room temperature (25 °C) at 400 rpm for 60 min, using a magnetic stirrer (Thermo Fisher Scientific, Cimarec+, SP88857100, MA, USA), followed by cold centrifugation at 4 °C (8000×g) for 10 min. The supernatants were gathered and stored at -20 °C for further analysis.

2.4 Third phase: Comparison of extraction methods *in vitro* and *in vivo*

2.4.1 Determination of total flavonoids

The extracts obtained from previous sections (2.2.5 and 2.3) were used to quantify the total flavonoid content, following the method described in Section 2.2.3. The efficacy of ultrasound-assisted extraction was evaluated using Equation (2), with total flavonoid content as the response variable (Aguilar-Hernández et al., 2019).

$$\text{Effectiveness (n-times)} = \frac{\text{Total flavonoids by UAE (g)}}{\text{Total flavonoids by conventional extraction (g)}} \quad (2)$$

2.4.2 Antioxidant capacity by ABTS, DPPH, and FRAP

The extracts obtained from the previous sections (2.2.5 and 2.3) were used to assess the antioxidant capacity of *M. pruriens* seed extracts (ABTS, DPPH, and FRAP). For assessing DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, a 96-well plate was used, where 260 µL of DPPH solution (Sigma Aldrich, USA) at 190 µM was mixed with 40 µL of the *M. pruriens* seed extract. This mixture was then incubated with agitation (200 rpm) for 30 min in the dark. Subsequently, the absorbance was measured at 517 nm using a plate reader. A calibration curve ($R^2 = 0.992$) was generated using the Trolox standard (Sigma-Aldrich, USA), and the results were reported as millimoles of Trolox equivalents per gram (mmol TE/g) (Prior et al., 2005). The DPPH reagent was mixed with methanol and prepared 15 min before use.

To evaluate the ABTS•+ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging capacity, 35 µL of the extract was combined with 265 µL of ABTS solution (Sigma Aldrich, USA) in a 96-well plate. The mixture was then shaken at 200 rpm in the dark for 10 min. The absorbance was recorded at 734 nm using a plate reader. A calibration curve ($R^2 = 0.999$) was constructed with the Trolox standard (Sigma-Aldrich, USA), and the results were reported as millimole Trolox equivalent per gram (mmol TE/g) (Re et al., 1999). The $ABTS^{\bullet+}$ solution was made by mixing ABTS reagent (7 mM) and potassium persulfate buffer (2.45 mM) overnight (16 h) and diluted with phosphate buffer (pH 7.4) to an absorbance of 0.7±0.02 at 734 in a UV-Vis spectrophotometer (UV-5100PC, Shangai Metash Instruments CO, LTD, China).

To conduct the FRAP (Ferric Reducing Antioxidant Power) assay, 264 µL of FRAP solution, 36 µL of extract, and 9 µL of distilled water were mixed in a test tube. This mixture was shaken (200 rpm) in the dark (30 min). Then the absorbance (595 nm) was measured in a plate reader, using 200 µL of the mixture added to a 96-well plate (Benzie & Strain, 1996). A calibration curve ($R^2 = 0.997$) was constructed using a Trolox standard (Sigma-Aldrich, USA), and results were expressed as millimole Trolox equivalent per gram (mmol TE/g). The FRAP solution consisted of acetate buffer (0.3 M, pH 3.6), $FeCl_3$ (20 mM), and TPTZ (2,4,6-tris(2-pyridyl) triazine; 10 mM) at a 10:1:1 volume ratio.

2.4.3 High-Performance Liquid Chromatography (HPLC) analysis

To identify phenolic compounds in *M. pruriens* seed extracts obtained under optimal ultrasound-assisted extraction conditions and magnetic stirring extraction, HPLC analysis was performed using the method of Aguilar-Hernández et al. (2019). The ethanolic extracts were evaporated to dryness at room temperature and redissolved into 1 mL of acidified

water containing 2% acetic acid (v/v). Then, it was filtered through 0.22 µm membrane filters and injected (30 µL) into an HPLC system (Agilent Technologies 1260 Infinity, Waldbronn, Germany-equipp) equipped with a photodiode array detector and a C18 reverse-phase column (250 mm long, 4.6 mm in diameter, 5 µm particle size; Thermo Scientific, Sunnyvale, CA, USA). The mobile phase consisted of acidified water (2% acetic acid), designated as eluent A, and a mixture of acidified water (0.5% acetic acid) and methanol, designated as eluent B. The samples and standards underwent analysis through a gradient program starting 0% B (0 - 35 min), following to 35% B (35 - 55 min), and increasing to 75% B (55 - 60 min), and 100% B (60 - 70 min), and returning to 0% B, all at a flow rate of 0.4 mL/min. The peak areas were detected at 280 and 320 nm. Quantification of flavonoid acids was performed using a calibration curve of standards ranging from 0.5 to 300 µg/mL, expressed in µg/100 g.

2.4.4 Toxicity against *C. elegans*

The toxicity of *M. pruriens* seed extract was assessed using *C. elegans* N2 wild-type nematodes obtained from the Caenorhabditis Genetics Center (CGC, Minnesota, USA). Experiments were performed on 1-day adult age-synchronized worms, following standard methods. The nematodes were grown at 20 °C on Petri dishes containing nematode growth medium and *Escherichia coli* OP50 bacteria as their food supply (Gallegos-Saucedo et al., 2020). To assess toxicity, 10 age-synchronized N2 nematodes were transferred into 96-well microplates containing M9 culture medium and varying concentrations of *M. pruriens* seed extract (50 - 1000 µg/mL), prepared by ultrasound-assisted extraction with magnetic stirring. Subsequently, the nematodes were incubated at 20 °C for 24 h. For the negative control, nematodes were incubated in M9 culture medium. Following the incubation period, nematode viability was evaluated by assessing their sensitivity to tactile stimuli using a platinum wire. The results of three independent assays, each conducted in triplicate, were averaged (Leite et al., 2020).

2.4.4 Statistical analysis

Total flavonoids, DPPH, ABTS, FRAP, and HPLC analysis data were examined using a Student T-test ($\alpha = 0.05$), using STATISTICA software version 10 (Statsoft, Tulsa, OK, USA). On the other hand, for the toxicity test, data were analyzed using a one-way ANOVA ($p < 0.05$; $p < 0.01$; $p < 0.001$; $p < 0.0001$) with Dunnett's multiple comparison test performed using GraphPad Prism software v.8.0.1 (GraphPad Software, Inc., San Diego, CA, USA). Data were presented as means ± standard deviation. All experiments (extractions and measurements) were performed in triplicate (n = 3).

3. Results and discussion

3.1 Ultrasonic extraction of flavonoids from *Mucuna pruriens* seed powder

Some studies have reported that *M. pruriens* seeds contain phenolic acids and flavonoids (Aware et al., 2019; Nwaoguikpe et al., 2011). Table 1 presents the experimental runs, including the predicted and experimental values of total flavonoids, along with their relative error. The results showed statistically significant differences in ultrasound-assisted extraction of flavonoids across different experimental conditions ($p < 0.05$).

Table 1
Box-Behnken design, observed and estimated values of total flavonoids, and the error rate following ultrasonic extraction from *Mucuna pruriens* seed powder

Run	Predictors[1]			Response variables		Relative error
	X_{UP} (%)	X_{PC} (s on/off)	X_{ET} (min)	Experimental FLA[2]	Predicted FLA[3]	(%)
1	90	3:1	2	76.42 ± 1.28ab	76.00	0.55
2	90	3:1	6	75.19 ± 0.21b	75.34	– 0.19
3	90	2:1	4	70.65 ± 0.53de	70.33	0.45
4	100	2:1	6	77.03 ± 0.21a	76.88	0.19
5	90	1:1	6	72.79 ± 0.09c	72.94	– 0.20
6	90	2:1	4	70.12 ± 0.12e	70.28	– 0.22
7	90	2:1	2	68.07 ± 0.21f	69.07	–1.44
8	80	3:1	4	76.48 ± 0.42ab	76.63	–0.19
9	80	1:1	4	65.55 ± 0.12h	65.70	– 0.22
10	80	2:1	6	71.88 ± 0.53cd	71.73	0.20
11	100	1:1	4	72.47 ± 0.53c	72.59	– 0.16
12	80	2:1	2	66.61 ± 0.42gh	66.46	0.22
13	100	3:1	4	67.41 ± 0.32fg	67.53	– 0.17
14	90	2:1	4	70.71 ± 0.40de	70.33	0.54
15	90	1:1	2	66.67 ± 0.12fgh	66.25	0.63

All values are means ± standard deviation (n = 3). Different letters in the column indicate statistically significant differences between treatments, as determined by the Tukey test ($\alpha = 0.05$). X_{ET}: Extraction time; X_{PC}: Pulse cycle; X_{UP}: Ultrasound power; FLA: Total flavonoids (mg CE/g dry basis). [1]Catechin equivalent; [2]Values were predicted using a 2nd-order polynomic equation, $R^2 = 0.9889$.

The highest total flavonoid content was achieved at 100% ultrasound power, a 2:1 s on/off pulse cycle, and 6 min of extraction time (77.03 mg CE/g) or at 80% ultrasound power, 3:1 s on/off pulse cycle, and 4 min of extraction time (76.48 mg CE/g). Ultrasound power and pulse cycle influence total flavonoid outcomes, but they are also affected by the pulse cycle. These values are higher than those reported for recovering flavonoids from *M. pruriens* seed powder soaked during 24 h (42 mg/g) or boiled to 98 °C for 60 min (35 mg/g) (Nwaoguikpe et al., 2011).

The effectiveness of ultrasound-assisted extraction is due to the cavitation phenomenon (microbubble implosion and microjets), which enhances flavonoid extraction and recovery by breaking down plant cell walls and allowing better solvent penetration into plant material (Vinatoru et al., 2017). Moreover, the pulsed mode of ultrasound may reduce the formation of cavitation bubbles but enhance bubble collapse strength (Kumar & Srinivasa Rao, 2020). In contrast, extraction time is a critical parameter in UAE, as prolonged extraction times may degrade bioactive compounds (Albarri & Sahin, 2023; Hosseini et al., 2019). Therefore, achieving an optimal balance between extraction time and pulse cycle is crucial. It has been observed that shorter extraction times necessitate higher duty cycles to quickly break down cell walls; however, these effects are influenced by the matrix composition (Kobus et al., 2022). Some studies have demonstrated that ultrasound-assisted extraction is a viable alternative to efficiently extract L-DOPA from *M. pruriens* seeds (Tesoro et al., 2022) and flavonoids from seeds of other *Mucuna* species, such as *M. macrocarpa* (Aware et al., 2019).

3.2 Response surface methodology analysis for flavonoids from *Mucuna pruriens* seed powder

Response surface method was used to establish the optimal ultrasound-assisted extraction conditions for the recovery of flavonoids from *M. pruriens* seed powder. The statistically significant effects of the independent variables (X_{UP}, X_{PC}, and X_{ET}) on the UAE of FLAs were evaluated by analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$) (Table S2). The statistical analysis reveals that all independent variables were significant ($p < 0.05$), except for X_{PC}^2 ($p > 0.05$). Furthermore, the lack of significant values ($p > 0.05$) in the ANOVA suggested that the proposed model is not significant for total flavonoid extraction, indicating good predictive capabilities (Rheem, 2023). Furthermore, the high determination coefficient (R^2 = 0.9889) indicated strong agreement between experimental and predicted observations, with errors below 2% (Table 1). Aware et al. (2019) reported that during the ultrasound-assisted extraction of flavonoids from *Mucuna macrocarpa* beans, the extraction time and ultrasonic power were significant ($p < 0.05$) in the ANOVA model; however, the interactive effect of extraction time and ultrasonic power was non-significant ($p > 0.05$). Moreover, the authors reported that ANOVA models for flavonoids exhibited a non-significant lack-of-fit (*p*-value: 0.0764) and a determination coefficient of 0.9856. Several studies have shown that in the optimization of ultrasound-assisted processes for extracting bioactive compounds from plant sources, specific components of the ANOVA model may be statistically insignificant without compromising the model's predictive capabilities (Fernández-Barbero et al., 2019; Hernández-Estrada et al., 2024; Rashad et al., 2023).

The mathematical model (Equation 3) for total flavonoids was constructed using the estimated regression coefficients, with statistically nonsignificant ($p > 0.05$) coefficients omitted to improve the model's predictive power (Table S3), as suggested by Jabbar et al. (2015). The polynomial model exhibited a determination coefficient greater than 0.97, indicating strong agreement between the predicted and experimental data. This aligns with findings from diverse studies that applied ultrasound-assisted extraction to recover bioactive compounds from plant materials, reporting determination coefficient values ranging from 0.85 to 0.99 (Anaya-Esparza et al., 2018; Hernández-Estrada et al., 2024; Jabbar et al., 2015).

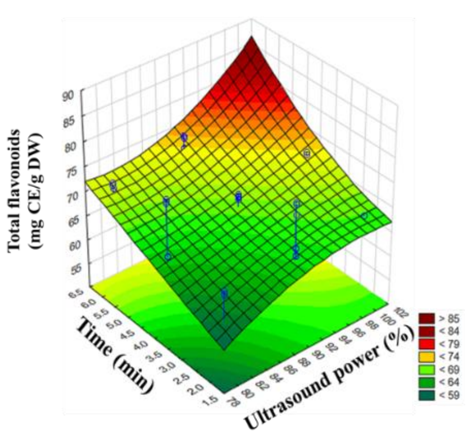
$$\text{Total flavonoids (mg CE/g; } R^2 = 0.9759) = -193.03 + 5.20X_{UP} - 0.02X_{UP}^2 - 84.58X_{PC}^2 + 1.81X_{ET}^2 + 0.15X_{UP}*X_{PC} - 0.10X_{UP}^2*X_{PC}^2 - 0.002X_{UP}^2*X_{PC}^2 - 2.73X_{UP}*X_{ET} + 0.011X_{UP}^2*X_{ET} - 0.92X_{PC}*X_{ET} \quad (3)$$

The interactive effects of independent variables (X_{UP}, X_{PC}, and X_{ET}) on the total flavonoid content in *M. pruriens* seed powder are illustrated by elliptical response surface plots (Figure 4a, b, and c). These plots help visualize the optimal region for maximizing flavonoid recovery. The findings indicate that ultrasound-assisted extraction of flavonoids from *M. pruriens* seed powder occurred under all experimental conditions, regardless of ultrasound power, pulse cycle, or extraction time. For a 1:1 s on/off pulse cycle, extracting high flavonoid content requires 100% ultrasound power for at least 6 min (Figure 4a). With a 2:1 s on/off pulse cycle, the maximum flavonoid content can be obtained by using 100% ultrasound power for 6 min

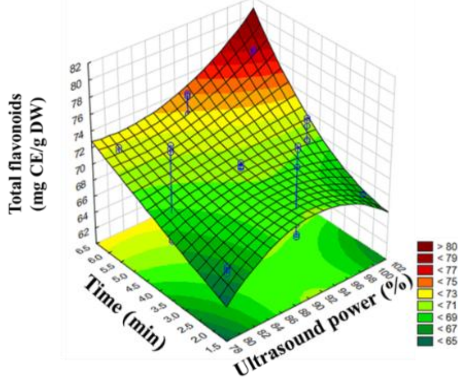
(Figure 4b). In contrast, a 3:1 s on/off pulse cycle demands 80% – 85% of ultrasound power for 6 min to achieve a high flavonoid recovery content (Figure 4c). According to Albarri & Sahin (2024), the extraction time and ultrasonic power significantly influenced the recovery yield of phenolic compounds extracted from *Moringa oleifera* leaves by ultrasound. Furthermore, the effect of extraction time and ultrasound power on the ultrasound-assisted extraction process is dependent on the pulse cycle, which reduces the number of cavitation bubbles but enhances the intensity of bubble collapse (Kumar & Srinivasa Rao, 2020). Moreover, it has been indicated that the pulse cycle significantly influences bubble formation (growth and implosion) during cavitation, enhancing or affecting the extraction process (Anaya-Esparza, Ramos-Aguirre, Zamora-Gasga, Yahia, & Montalvo-González, 2018).

Additionally, the Pareto Chart at a 95% confidence level shows the effects of the linear, quadratic, and interaction terms of the independent variables on the extraction of flavonoids by ultrasound (Figure 4f). The most critical parameters for extracting flavonoids from *M. pruriens* seed powder by ultrasound were the interactive effect of *XUP*XPC* (negative effect), followed by X_{ET} and X_{PC} (positive effects). It has been reported that adequate extraction time and ultrasound pulse cycle settings can promote bubble formation during cavitation, thereby enhancing cellular disruption and compound extraction. Furthermore, pulsed ultrasound mode applied during the extraction process allows for better control of temperature (reduced thermal gradients) and facilitates the release of phenolic compounds (Kumar & Srinivasa Rao, 2020).

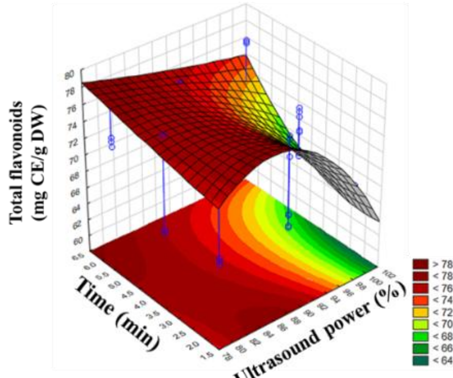
a)At 1:1 s on/off pulse cycle



b) At 2:1 s on/off pulse cycle



c) At 3:1 s on/off pulse cycle



d) Pareto Chart

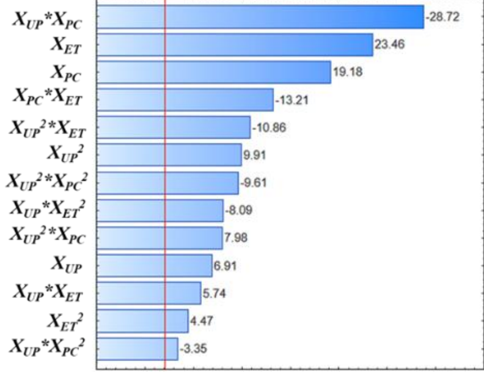


Figure 4. Response surface plots (a,b,c) and Pareto (d) Chart indicating the effect of ultrasonic extraction on the total flavonoids content from *Mucuna pruriens* seed powder at 1:1, 2:1, and 3:1 second on/off pulse cycle, respectively. X_{ET}: Extraction time; X_{PC}: Pulse cycle; X_{UP}: Ultrasonic power.

3.3 Validation of the ultrasonic-assisted extraction conditions for flavonoids from *Mucuna pruriens* seed powder and comparison with magnetic stirring extraction

The optimal ultrasound conditions for extracting total flavonoids from *M. pruriens* seed powder were 85% ultrasound power and a 3:1 s-on/s-off pulse cycle for 5 min, yielding a predicted flavonoid content of 71.41-81.19 mg CE/g. These conditions were experimentally validated (Table 2). The experimental result (77.30 mg CE/g) showed strong concordance with the predicted result (76.25 mg CE/g), confirming the adequacy and robustness of the fitted model for response. Additionally, the effectiveness of this optimal ultrasound-assisted extraction was compared with the conventional magnetic stirring extraction method. The total flavonoid content when ultrasound-assisted extraction was applied (77.30 mg CE/g) was 1.27 times higher than magnetic stirring extraction (60.92 mg CE/g, $p < 0.05$). However, the ultrasound process exhibited a 90% reduction in time compared to magnetic stirring extraction.

3.4 Antioxidant activity

Regarding antioxidant activity, significant differences were observed among extraction methods for DPPH, ABTS, and FRAP ($p < 0.05$) (Table 2). Significant differences in DPPH antioxidant activity were observed between ultrasound-assisted extraction (323.51 mmol TE/g) and magnetic stirring (294.13 mmol TE/g). For ABTS, the UAE (262.95 mmol TE/g) exhibited the lowest value compared to magnetic stirring (266.44 mmol TE/g) ($p < 0.05$). In contrast, the ultrasound-assisted extraction showed the highest FRAP activity (259.07 mmol TE/g) compared to magnetic stirring extraction (164.12 mmol TE/g) ($p < 0.05$). It has been reported that the antioxidant activity measured by DPPH and ABTS assays from *Justicia spicigera* extracts obtained by ultrasound-assisted extraction did not exhibit significant differences ($p > 0.05$) compared to magnetic stirring but differed significantly from thermal decoction extraction ($p < 0.05$) (Anaya-Esparza et al., 2018).

A 2020 study conducted by Jimoh et al. (2020) observed that the extract derived from *M. pruriens* seeds, at a concentration of 0.01 mg/mL, has the capacity to inhibit approximately 80% of the DPPH radical, with a value higher than that of rutin (approximately 60%). In another study, values ranging from 29.28 to 123.43 mmol TE/g were reported for this radical-linked activity in extracts from the pods of *Mucuna pruriens* (including the seeds) (Avalos et al., 2023). Meanwhile, for the ABTS radical, it has been demonstrated that a concentration of 0.04 mg/mL of ethanolic extract from *M. pruriens* seeds can inhibit nearly 100% of the radical (Jimoh et al., 2020). Additionally, it has also been shown that the *M. pruriens* seeds extract possesses good activity in reducing the activity of ferric ions (0.66 mg $FeSO_4$/mg extract) compared to other standard antioxidant compounds such as gallic acid and Trolox (mean values of 0.36 and 0.55 mg $FeSO_4$/mg, respectively) (Chookiat et al., 2024). Therefore, by contrasting the findings of this study with those previously conducted by other authors, it is suggested, as proposed by Iftikar et al. (2020) that the bioactive compounds present in *M. pruriens* seeds exhibit antagonistic, neutral, or synergistic effects, which influenced the response in radical activity, as well as in the antioxidant mechanism (via electron transference, electron donation, or chelating metals).

3.5 HPLC analysis of *M. pruriens* seed powder extracts

Regarding HPLC analysis, gallocatechin, epicatechin, and epigallocatechin were identified as the flavonoids in *M. pruriens* seed extracts obtained using an optimal ultrasound-assisted extraction and magnetic stirring method (Table 3). Under ultrasound-assisted extraction, gallocatechin was the most abundant flavonoid extracted (36,664 µg/100 g) followed by epigallocatechin (7869 µg/100 g), and epicatechin (1206 µg/100 g), whereas under magnetic stirring, similar pattern was observed, but in lower contents ($p < 0.05$) for all identified flavonoids (gallocatechin: 3257 µg/100 g; epigallocatechin: 1749 µg/100 g, and epicatechin: 318 µg/100 g).

Table 2
Comparison of experimentally optimal ultrasound-assisted extraction conditions with the magnetic stirring extraction method

Parameter	[1]UAE	[2]MS
Total flavonoids (mg CE/g)	77.30 ± 0.65a	60.92 ± 0.44b
DPPH (mmol TE/g)	323.51 ± 0.35a	294.13 ± 0.74b
ABTS (mmol TE/g)	262.95 ± 0.17b	266.44 ± 0.29a
FRAP (mmol TE/g)	259.07 ± 0.47a	164.19 ± 0.31b
Effectiveness (n-times [1]UAE *vs.* MS)	1.27	

All values are means ± standard deviation (n = 3). Different letters on each line indicate statistically significant differences in extraction methods by Student's T-test (α = 0.05). [1]UAE: Ultrasound-assisted extraction conditions at 85% ultrasound power, 3:1 s on/off pulse cycle, and 5 min extraction time. [2]MS: Magnetic stirring at 400 rpm for 60 min. *Effectiveness was calculated considering the total flavonoid content for ultrasound extraction.

Table 3
Comparison of the flavonoid compound profile of *M. pruriens* seeds using conventional and ultrasound extraction

Identified flavonoids (µg/ 100 g)	UAE	Conventional extraction
Gallocatechin	36664.88 ± 750.92[a]	3257.91 ± 170.92[b]
Epicatechin	1206.03 ± 20.97[a]	318.05 ± 15.65[b]
Epigallocatechin	7869.03 ± 1046.58[a]	1749.39 ± 58.79[b]

Data are expressed as mean ± SD (n = 3). Different lowercase letters indicate significant statistical differences between treatments by Student T-test (α = 0.05) ($p < 0.05$). UAE: Ultrasound-assisted extraction using optimal extraction conditions.

Notably, the yield of gallocatechin was approximately 11 times higher with ultrasound-assisted extraction than with magnetic stirring extraction. Moreover, the extraction yields of epigallocatechin and epicatechin were 4.5 and 3.7 times higher, respectively, with ultrasound-assisted extraction than with magnetic stirring. Several studies have demonstrated that cavitation bubbles generated by the high-frequency ultrasound can disrupt the cell walls of various plant matrices, thereby enhancing the release of these compounds (Xiang et al., 2024). In a previous study, different flavonoids were identified in *Mucuna pruriens* seeds from methanol acidified with formic acid 1%, such as catechin (37000 µg/100g), epicatechin (31000 µg/100g), rutin (362000 µg/100g), quercetin (587000 µg/100g), kaempferol (73000 µg/100g), and luteolin (295000 µg/100g) (Adefegha et al., 2017). Additionally, it has been reported that the presence of catechin (57800 µg/100g), quercetin 3-glucoside (13000 µg/100g), and kaempferol 3-glucoside (19300 µg/100g) in *M. pruriens* seeds, using methanol as solvent (Tavares et al., 2020). Therefore, although previous studies have identified compounds related to gallocatechin, such as catechin and epicatechin, no study to date has quantified this flavonoid and epigallocatechin in the *M. pruriens* seeds. Gallocatechin comprises two components, a flavan-3-ol structure and a galloyl group. The biosynthesis of flavan-3-ols and other flavonoid compounds, such as epicatechin and epigallocatechin, occurs via the primary shikimic acid-phenylpropanoid-flavonoid pathway and involves four main derivatization reactions, including hydroxylation, glycosylation, acylation, and polyphenol condensation reactions (Jiang et al., 2025). Catechins are a class of flavonoids characterized by their diverse biological activities, including antimicrobial, antifungal, anticarcinogenic, and neuroprotective effects, which are attributed to their unique chemical structure (Bae et al., 2020; Mita et al., 2024; Molina-Hernández et al., 2022). Furthermore, due to this property, these compounds have been utilized in the cosmetics industry, as they provide protection against UV-induced effects and exhibit anti-inflammatory activity (Mita et al., 2024). Additionally, during HPLC analysis, we detected a signal not identified by any of our standards, with a retention time exceeding 5 min (Figure 5). Intharuksa et al. (2023) identified L-DOPA by HPLC in *M. pruriens* seeds and in other *Mucuna* species, with a retention time of approximately 5 min. Thus, based on the literature, we hypothesize that this signal may be attributable to L-DOPA (Intharuksa et al., 2023; Rima et al., 2023; Vilairat et al., 2023).

The seeds of *M. pruriens* are known for their high L-DOPA content. L-DOPA plays a crucial role as a precursor to the neurotransmitter dopamine. Unlike externally administered dopamine, which cannot cross the blood-brain barrier, L-DOPA can. This characteristic makes L-DOPA an important therapeutic option for neurodegenerative disorders such as Parkinson's disease, in which dopamine levels within neurons are reduced (Ndayiragije et al., 2024). Moreover, L-DOPA exhibits antioxidant properties, which are advantageous in treating conditions such as cancer, aging, cardiovascular, neurodegenerative, and inflammatory diseases (Yadav et al., 2023). Furthermore, in this study, we identified several phenolic acids, including protocatechuic acid, caffeic acid, 3,4-dihydroxyphenylacetic acid, and neochlorogenic acid, in extracts obtained by both methods (data not shown). Some of these phenolic compounds are consistent with those previously reported in other studies; specifically, chlorogenic acid, caffeic acid, and gallic acid have been identified in *M. pruriens* seed extracts (Adefegha et al., 2017; Tavares et al., 2020).

3.6 Effect of *Mucuna pruriens* seed extracts on *C. elegans* worm mortality

Plant-derived products play a crucial role in enhancing human health and preventing diseases. However, high-dose, prolonged consumption of nutraceuticals may lead to adverse effects (Taroncher et al., 2021). The invertebrate model *C. elegans* is commonly employed to investigate the toxicological and pharmacological effects of various substances, including *M. pruriens*. In this study, a dose-dependent reduction in survival was noted following 24 h of exposure to *M. pruriens* seed extract (Figure 6). For the extract obtained by ultrasound, the groups of worms exposed to doses

ranging from 100 to 1000 µg/mL showed statistically significant differences compared with the control group maintained in M9 medium (Figure 6a). Similarly, for the extract obtained via magnetic stirring, the groups of worms exposed to doses ranging from 100 to 1000 µg/mL also showed statistically significant differences compared with the control group in M9 (Figure 6b). In both cases, lower worm survival was observed at higher concentrations of *M. pruriens* extracts, regardless of the extraction method.

The LC_{50} values for both extracts were 143.65 µg/mL for the *M. pruriens* extract obtained by the ultrasound method and 132.18 µg/mL for the extract obtained under magnetic stirring (Table S4). Other studies have documented the anthelmintic properties of plant extracts, compared with approved pharmacological treatments for infections, such as levamisole, when administered to this nematode (Katiki et al., 2013; Widaad et al., 2022). In this context, LC_{50} values of 73 mg/mL, 0.65 mg/mL, 1.03 mg/mL, and 2.14 mg/mL have been established following the administration of extracts from *Leucaena leucocephala*, *Rhus typhina*, *Acer rubrum*, and *Rosa multiflora*, respectively. In contrast, a concentration of 8 mg/mL of levamisole is entirely lethal for this organism. These observations suggest that the extract of *M. pruriens* seeds may be a promising candidate for anthelmintic activity compared with other plant extracts. However, the harmful effects that might result from ingesting this plant could be attributed to the consumption of high doses of antinutritional compounds, such as L-DOPA; moreover, the intake of substantial quantities of *M. pruriens* seeds, can result in adverse effects, including digestive and neurological disorders in humans (Kulkarni et al., 2025; Maillot, Schmitt, & Marteau, 2022). Our findings suggest a promising safety margin for future pharmacological and toxicological evaluations of *M. pruriens* seed powder in other animal models, including vertebrates such as mice, given the strong correlation between the LC_{50} value in *C. elegans* and the LD_{50} in murine models (Gao et al., 2018). Moreover, further studies that conduct a more thorough toxicity assessment, including stress response evaluation, differentiation potential assessment, and gene expression analysis, would enhance our confidence in its safety profile and provide a more detailed understanding of the seed effects.

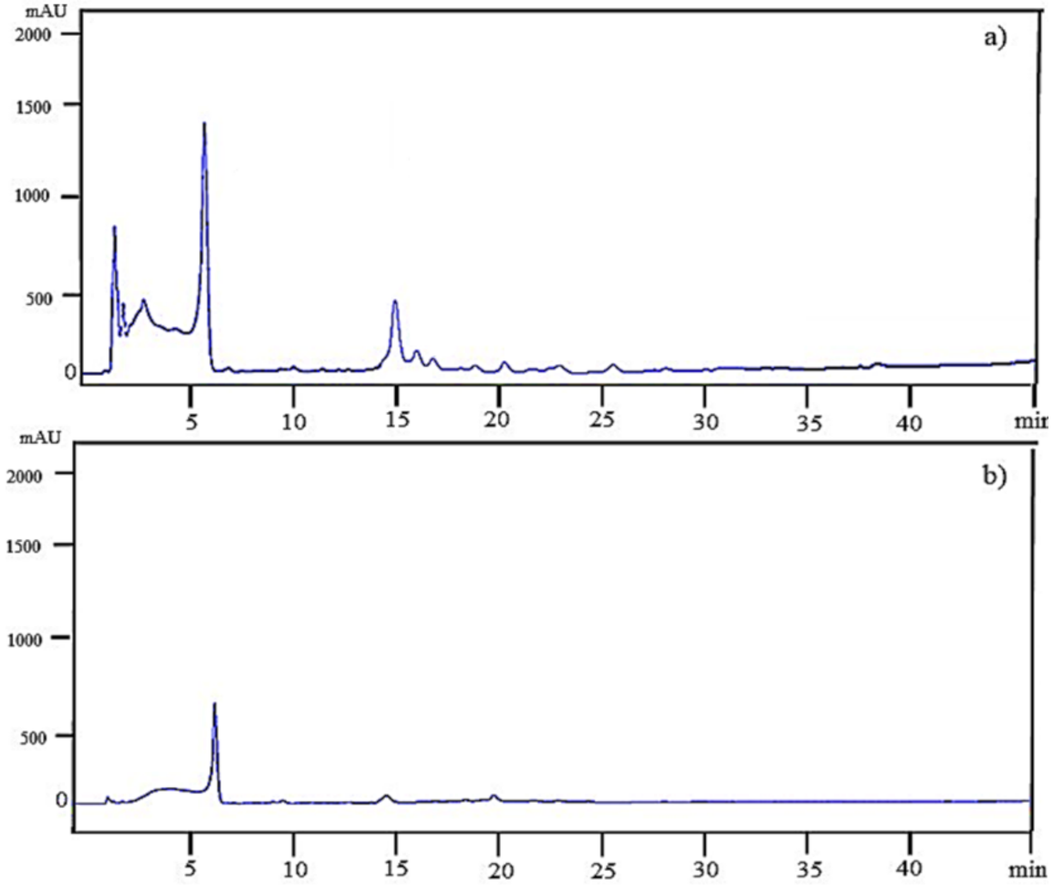


Figure 5. Chromatograms of ultrasound-assisted extraction (a) and magnetic stirring extraction (b) of *Mucuna pruriens* seeds.

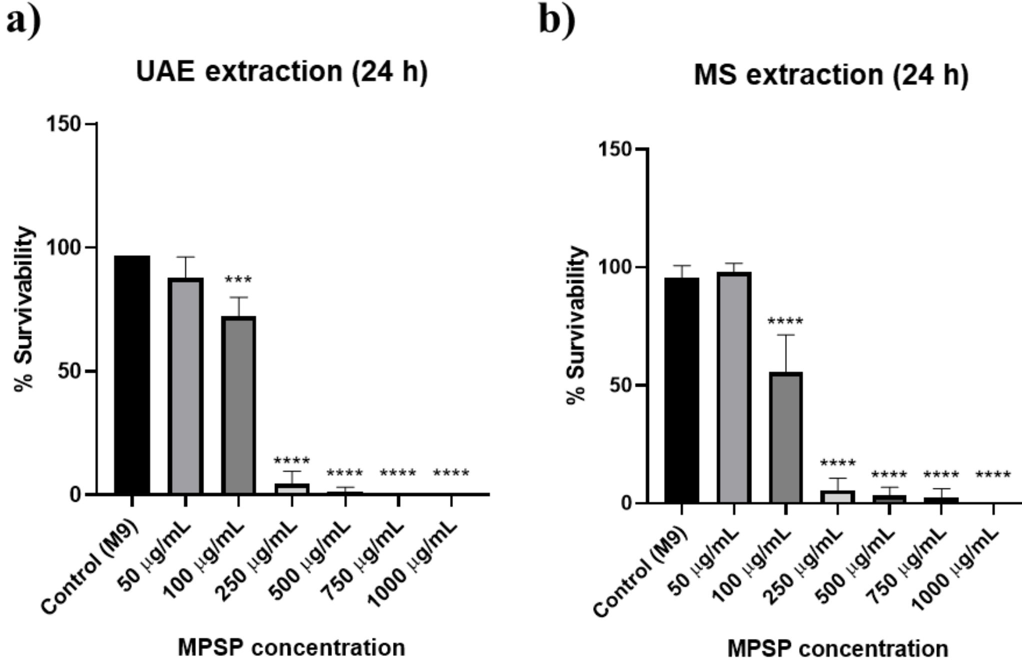


Figure 6. Toxicity of *Mucuna pruriens* seed powder from UAE and MS extraction. (a) The percentage of *C. elegans* mortality in MSPS from UAE extraction. (b) The percentage of *C. elegans* mortality from MS extraction. Data is presented as means ± SD and is compared to the M9 buffer as the negative control. Statistical significance was performed using a one-way ANOVA with Dunnett's multiple comparison test and is denoted by asterisks. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. n = *30 worms*.

4. Conclusions

Mucuna pruriens seeds are extensively studied and utilized for their medicinal properties. Consequently, developing effective extraction techniques to enhance the yield of bioactive compounds from *M. pruriens* seed powder is a current research focus. This study assessed various ultrasound-assisted extraction parameters (ultrasound power, pulse cycle, and extraction duration) to maximize flavonoid recovery from *M. pruriens* seeds and successfully optimized them using response surface methodology. The predictive model was fitted to a 2nd-order polynomial equation, with the interaction effect of ultrasound power and pulse cycle being the most influential. The optimal ultrasound-assisted extraction conditions (85% ultrasound power, 3:1 s on/off pulse cycle, and 5 min extraction time) yielded a 1.27-fold higher total flavonoid content with antioxidant properties compared to the magnetic stirring method, in 90% less time. Additionally, we examined both extraction methods using the nematode *C. elegans* to assess the toxicological impact of *M. pruriens* seeds. In both scenarios, concentrations between 100 and 1000 µg/mL significantly affected the survivability of *C. elegans*.

This research underscores the application of ultrasound as a favorable alternative for extracting flavonoids from *M. pruriens* seeds. These compounds show considerable potential for therapeutic, pharmaceutical, and cosmetic applications. Additional research is necessary to evaluate the potential biological properties of *M. pruriens* seed extracts and assess their toxicological effects on other *in vivo* models.

Acknowledgments

Luis Antonio Ramírez-Contreras (CVU: 1319432) gratefully acknowledges the financial support for the scholarship from SECIHTI-México for Doctoral studies in the Biociencias program from the Centro Universitario de Los Altos (CUALTOS) of the University of Guadalajara. Thanks to Andrea Quetzalli Uribe Torres and Gerardo Alcala Aceves for technical support as part of their activities of the "Early incorporation into Research Program" from CUALTOS, and to the Instituto de Investigación en Ciencias Médicas from CUALTOS for the infrastructure support.

Author Contributions

L. A. Ramírez-Contreras: investigation, writing—original draft preparation. L. M. Anaya.-Esparza: project administration, conceptualization, formal analysis, writing—original draft preparation, review and editing, supervision. E. Montavo-González: investigation, writing—original draft preparation, review, and editing. S. Sánchez-Enriquez: resources, methodological supervision, writing—original draft preparation. J. M. Silva-Jara: investigation, data analysis, writing—original draft preparation. A. E. Rubio-Castillo: formal analysis, writing—original draft preparation. L. Hernández-Hernández: methodological supervision, discussion of results, writing—original draft preparation, supervision. G. Camargo-Hernández: project administration, conceptualization, methodological supervision, review and editing, writing—original draft preparation, review and editing.

Conflicts of Interest

The authors declare no conflicts of interest.

ORCID

L. A. Ramírez-Contreras https://orcid.org/0009-0002-6063-8834
L. M. Anaya-Esparza https://orcid.org/0000-0001-9194-1719
E. Montalvo-González https://orcid.org/0000-0002-3760-0806
S. Sánchez Enríquez https://orcid.org/0000-0002-6153-3703
J. M. Silva-Jara https://orcid.org/0000-0001-8742-6247
Á. E. Rubio-Castillo https://orcid.org/0000-0002-8869-8559
L. Hernández-Hernández https://orcid.org/0000-0002-8570-5848
G. Camargo-Hernández https://orcid.org/0000-0003-0853-9396

References

Adefegha, S. A., Oboh, G., Oyeleye, S. I., Dada, F. A., Ejakpovi, I., & Boligon, A. A. (2017). Cognitive enhancing and antioxidative potentials of velvet beans (*Mucuna pruriens*) and horseradish (*Moringa oleifera*) seeds extracts: A comparative study. *Journal of Food Biochemistry*, *41*(1), e12292. https://doi.org/10.1111/jfbc.12292

Aguilar-Hernández, G., García-Magaña, M. d. L., Vivar-Vera, M. d. l. Á., Sáyago-Ayerdi, S. G., Sánchez-Burgos, J. A., Morales-Castro, J., Anaya-Esparza, L. M., & Montalvo González, E. (2019). Optimization of ultrasound-assisted extraction of phenolic compounds from *Annona muricata* by-products and pulp. *Molecules*, *24*(5), 904.

Anjaneyulu, J., Vidyashankar, R., & Godbole, A. (2020). Differential effect of Ayurvedic nootropics on C. elegans models of Parkinson's disease. *Journal of Ayurveda and integrative medicine*, *11*(4), 440-447. https://doi.org/10.1016/j.jaim.2020.07.006

Albarri, R., & Şahin, S. (2024). Monitoring the recovery of bioactive molecules from *Moringa oleifera* leaves: microwave treatment vs ultrasound treatment. *Biomass Conversion and Biorefinery*, *14*(1), 1059-1071.

Anaya-Esparza, L. M., Aurora-Vigo, E. F., Villagrán, Z., Rodríguez-Lafitte, E., Ruvalcaba-Gómez, J. M., Solano-Cornejo, M. Á., Zamora-Gasga, V. M., Montalvo-González, E., Gómez-Rodríguez, H., Aceves-Aldrete, C. E., & González-Silva, N. (2023). Design of experiments for optimizing ultrasound-assisted extraction of bioactive compounds from plant-based sources. *Molecules*, *28*(23), 7752. https://doi.org/10.3390/molecules28237752

Anaya-Esparza, L. M., Ramos-Aguirre, D., Zamora-Gasga, V. M., Yahia, E., & Montalvo-González, E. (2018). Optimization of ultrasonic-assisted extraction of phenolic compounds from *Justicia spicigera* leaves. *Food Sci Biotechnol*, *27*(4), 1093-1102. https://doi.org/10.1007/s10068-018-0350-0

Avalos, B. I., Ojeda, G. A., Spinnenhirn, E. D., Acevedo, B. A., & Vallejos, M. M. (2023). Evaluation of phenolic compounds and antioxidant capacity in *Mucuna pruriens* and *Cajanus cajan* pods extracts. *Food Chemistry Advances*, *3*, 100503. https://doi.org/10.1016/j.focha.2023.100503

Aware, C., Patil, R., Vyavahare, G., Gurav, R., Bapat, V., & Jadhav, J. (2019). Processing Effect on L-DOPA, *In vitro* protein and starch digestibility, proximate composition, and biological activities of promising legume: *Mucuna macrocarpa*. *J Am Coll Nutr*, *38*(5), 447-456. https://doi.org/10.1080/07315724.2018.1547230

Bae, J., Kim, N., Shin, Y., Kim, S.-Y., & Kim, Y.-J. (2020). Activity of catechins and their applications. *Biomedical Dermatology*, *4*(1), 8. https://doi.org/10.1186/s41702-020-0057-8

Benzie, I. F. F., & Strain, J. J. (1996). The rerric 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

Bhardwaj, P., Aiman, A., Iram, F., Saifi, I., Basir, S. F., Hassan, I., Islam, A., & Manzoor, N. (2024). Natural compound toxicity: An egregiously overlooked topic. In N. Manzoor (Ed.), *Advances in Antifungal Drug Development: Natural Products with Antifungal Potential* (pp. 653-673). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-5165-5_25

Bitwell, C., Indra, S. S., Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, *19*, e01585. https://doi.org/10.1016/j.sciaf.2023.e01585

Chandrasiri Waliwita, W. A. L. (2021). Chapter 27 - Sri Lankan medicinal herbs used for the management of neurodegenerative diseases of the brain. In D. Ghosh (Ed.), *Nutraceuticals in Brain Health and Beyond* (pp. 379-389). Academic Press. https://doi.org/10.1016/B978-0-12-820593-8.00027-6

Chaudhary, S. K., De, A., Bhadra, S., & Mukherjee, P. K. (2015). Angiotensin-converting enzyme (ACE) inhibitory potential of standardized Mucuna pruriens seed extract. *Pharm Biol*, *53*(11), 1614-1620. https://doi.org/10.3109/13880209.2014.996820

Chemat, F., Abert-Vian, M., Fabiano-Tixier, A. S., Strube, J., Uhlenbrock, L., Gunjevic, V., & Cravotto, G. (2019). Green extraction of natural products. Origins, current status, and future challenges. *TrAC Trends in Analytical Chemistry*, *118*, 248-263. https://doi.org/10.1016/j.trac.2019.05.037

Chookiat, S., Theansungnoen, T., Kiattisin, K., & Intharuksa, A. (2024). Nanoemulsions containing *Mucuna pruriens* (L.) DC. seed extract for cosmetic applications. *Cosmetics*, *11*(1), 29.

CONABIO. (2009). *Stizolobium pruriens* - ficha informativa. https://www.gob.mx/cms/uploads/attachment/file/221056/Mucuna_pruriens_utilis_final.pdf

Costa, A. C. C., Oliveira, L., Dorea, M. A., Leite, G., Amorim, R., Neto, E. D. S. M., Júnior, L. N., Silva, R. C., Silveira, E. L., & Fontelles, M. J. (2018). PO-029 Supercritical carbon dioxide extract of *Mucuna pruriens* inhibits brain cancer tumour growth in rats. *ESMO Open*, *3*, A239. https://doi.org/10.1136/esmoopen-2018-EACR25.564

Dhanani, T., Singh, R., Shah, S., Kumari, P., & Kumar, S. (2015). Comparison of green extraction methods with conventional extraction method for extract yield, L-DOPA concentration and antioxidant activity of *Mucuna pruriens* seed. *Green Chemistry Letters and Reviews*, 8(2), 43-48. https://doi.org/10.1080/17518253.2015.1075070

Fernández-Barbero, G., Pinedo, C., Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Palma, M., & García-Barroso, C. (2019). Optimization of ultrasound-assisted extraction of bioactive compounds from jabuticaba (*Myrciaria cauliflora*) fruit through a Box-Behnken experimental design. *Food Science and Technology*, *39*(4), 1018-1029. https://doi.org/10.1590/fst.16918

Gallegos-Saucedo, M. d. J., Camargo-Hernández, G., Castillo-Romero, A., Ramírez-Herrera, M. A., Bañuelos-Pineda, J., Pereira-Suárez, A. L., Hernández-Chávez, A., & Hernández-Hernández, L. (2020). GABAergic system's injuries induced by sodium sulfite in *Caenorhabditis elegans* were prevented by the anti-oxidative properties of dehydroepiandrosterone sulfate. *Neurotoxicity Research*, *38*(2), 447-460. https://doi.org/10.1007/s12640-020-00207-y

Gao, S., Chen, W., Zeng, Y., Jing, H., Zhang, N., Flavel, M., Jois, M., Han, J.-D. J., Xian, B., & Li, G. (2018). Classification and prediction of toxicity of chemicals using an automated phenotypic profiling of *Caenorhabditis elegans*. *BMC Pharmacology and Toxicology*, *19*(1), 18. https://doi.org/10.1186/s40360-018-0208-3

Hajibeglou, A., Sudagar, M., Azizinezhad-Jolandan, F., & Alaei, K. (2023). The effects of *Mucuna pruriens* and *Basella alba* extracts on sex reversal in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Research*, *2023*, 1-6. https://doi.org/10.1155/2023/8252139

Han, D.-G., Bae, M.-J., & An, B.-J. (2022). Anti-inflammatory activity of velvet bean (*Mucuna pruriens*) substances in LPS–stimulated RAW 264.7 macrophages. *Molecules*, *27*(24), 8797.

Hernández-Estrada, S., Anaya-Esparza, L. M., González-Torres, S., Hernández-Villaseñor, L. A., Gómez-Rodríguez, V. M., Ramírez-Vega, H., Villagrán, Z., Ruvalcaba-Gómez, J. M., Rodríguez-Barajas, N., & Montalvo-González, E. (2024). Extraction of soluble phenols and flavonoids from native Mexican pigmented corn kernel powder by ultrasound: Optimization process using response surface methodology. *Applied Sciences*, *14*(17), 7869. https://doi.org/10.3390/app14177869

Hosseini, S. S., Khodaiyan, F., Kazemi, M., & Najari, Z. (2019). Optimization and characterization of pectin extracted from sour orange peel by ultrasound assisted method. *International Journal of Biological Macromolecules*, *125*, 621-629. https://doi.org/10.1016/j.ijbiomac.2018.12.096

Iftikhar, M., Zhang, H., Iftikhar, A., Raza, A., Begum, N., Tahamina, A., Syed, H., Khan, M., & Wang, J. (2020). Study on optimization of ultrasonic assisted extraction of phenolic compounds from rye bran. *LWT*, *134*, 110243. https://doi.org/10.1016/j.lwt.2020.110243

Intharuksa, A., Denduangboripant, J., Chansakaow, S., Thongkhao, K., & Sukrong, S. (2023). HPLC and DNA barcoding profiles for identification of the selected twelve Mucuna species and its application for detecting prohibited aphrodisiac Mucuna products. *Heliyon*, *9*(3), e14130. https://doi.org/10.1016/j.heliyon.2023.e14130

Jabbar, S., Abid, M., Wu, T., Hashim, M. M., Saeeduddin, M., Hu, B., Lei, S., & Zeng, X. (2015). Ultrasound-assisted extraction of bioactive compounds and antioxidants from carrot pomace: A response surface approach. *Journal of Food Processing and Preservation*, *39*(6), 1878-1888. https://doi.org/10.1111/jfpp.12425

Jiang, X., Liu, N., Liu, Y., Gao, L., & Xia, T. (2025). Exploration of the biosynthesis of galloylated catechins in tea plants. *Beverage Plant Research*, *5*(1).

Jitpimai, K., Ngiwsara, L., Lang, W., Panichpat, T., Mingma, R., Svasti, J., & Wongchawalit, J. (2023). Evaluation of alcoholic extracts of *Mucuna pruriens* (L.) DC. var. utilis for antibacterial, antioxidant and cytotoxic activities toward human cancer cell lines. *Chiang Mai Journal of Science*, *50*(5), 1-15.

Jimoh, M. A., Idris, O. A., & Jimoh, M. O. (2020). Cytotoxicity, phytochemical, antiparasitic screening, and antioxidant activities of *Mucuna pruriens* (Fabaceae). *Plants (Basel)*, *9*(9). https://doi.org/10.3390/plants9091249

Katiki, L. M., Ferreira, J. F. S., Gonzalez, J. M., Zajac, A. M., Lindsay, D. S., Chagas, A. C. S., & Amarante, A. F. T. (2013). Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. *Veterinary Parasitology*, *192*(1), 218-227. https://doi.org/10.1016/j.vetpar.2012.09.030

Kedam, T. (2017). The traditional uses and pharmacological activities of *Mucuna pruriens* (L)DC: A comprehensive review. *Indo American Journal of Pharmaceutical Research*, *7*, 7516.

Khorasani Esmaeili, A., Mat Taha, R., Mohajer, S., & Banisalam, B. (2015). Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover). *Biomed Res Int*, *2015*, 643285. https://doi.org/10.1155/2015/643285

Kobus, Z., Krzywicka, M., Starek-Wójcicka, A., & Sagan, A. (2022). Effect of the duty cycle of the ultrasonic processor on the efficiency of extraction of phenolic compounds from *Sorbus intermedia*. *Scientific reports*, 12(1), 8311. https://doi.org/10.1038/s41598-022-12244-y

Kulkarni, S. R., Thokchom, B., Abbigeri, M. B., Bhavi, S. M., Singh, S. R., Metri, N., & Yarajarla, R. B. (2025). The role of L-DOPA in neurological and neurodegenerative complications: a review. *Molecular and Cellular Biochemistry*, 1-22.

Kumar, A., & Srinivasa Rao, P. (2020). Optimization of pulsed-mode ultrasound assisted extraction of bioactive compounds from pomegranate peel using response surface methodology. *Journal of Food Measurement and Characterization*, *14*(6), 3493-3507. https://doi.org/10.1007/s11694-020-00597-9

Leite, N. R., de Araújo, L. C. A., Dos Santos da Rocha, P., Agarrayua, D. A., Ávila, D. S., Carollo, C. A., Silva, D. B., Estevinho, L. M., de Picoli Souza, K., & Dos Santos, E. L. (2020). Baru Pulp (*Dipteryx alata* Vogel): Fruit from the brazilian savanna protects against oxidative stress and increases the life expectancy of *Caenorhabditis elegans* via SOD-3 and DAF-16. *Biomolecules*, *10*(8). https://doi.org/10.3390/biom10081106

Maillot, A., Schmitt, C., & Marteau, A. (2022). Poisoning after ingestion of *Mucuna pruriens* Seeds on Reunion Island. *Wilderness Environ Med*, *33*(1), 122-124. doi:10.1016/j.wem.2021.10.004

Mita, S. R., Husni, P., Putriana, N. A., Maharani, R., Hendrawan, R. P., & Dewi, D. A. (2024). A recent update on the potential use of catechins in cosmeceuticals. *Cosmetics*, *11*(1), 23.

Molina-Hernández, J. B., Scroccarello, A., Della Pelle, F., De Flaviis, R., Compagnone, D., Del Carlo, M., Paparella, A., & Chaves López, C. (2022). Synergistic antifungal activity of catechin and silver nanoparticles on *Aspergillus niger* isolated from coffee seeds. *LWT*, *169*, 113990. https://doi.org/10.1016/j.lwt.2022.113990

Ndayiragije, E., Caumul, P., Joondan, N., Bhowon, M. G., & Laulloo, S. J. (2024). l-Tyrosine and l-DOPA: Promising scaffolds for the synthesis of biologically active compounds. Comptes Rendus. *Chimie*, *27*(G1), 299-317.

Nwaoguikpe, R., Braide, W., & Ujowundu, C. (2011). The effects of processing on the proximate and phytochemical compositions of *Mucuna pruriens* seeds (velvet beans). *Pakistan Journal of Nutrition*, *10*(10), 947-951.

Parvatikar, P. P., Patil, S. M., Patil, B. S., Reddy, R. C., Bagoji, I., Kotennavar, M. S., Patil, S., Patil, A. V., Das, K. K., Das, S. N., & Bagali, S. (2023). Effect of *Mucuna pruriens* on brain NMDA receptor and tau protein gene expression in cerebral ischemic rats. *Frontiers in Physiology*, *14*. https://doi.org/10.3389/fphys.2023.1092032

Pathania, R., Chawla, P., Khan, H., Kaushik, R., & Khan, M. A. (2020). An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. *3 Biotech*, *10*(6). doi:10.1007/s13205-020-02253-x

Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz- Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, *41*(3), 274-285. https://doi.org/10.1016/j.foodres.2007.12.004

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, *53*(10), 4290-4302. https://doi.org/10.1021/jf0502698

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

Rheem, S. (2023). Optimizing food processing through a new approach to response surface methodology. *Food science of animal resources*, 43(2), 374-381. https://doi.org/10.5851/kosfa.2023.e7

Rangsinth, P., Prasansuklab, A., Duangjan, C., Gu, X., Meemon, K., Wink, M., & Tencomnao, T. (2019). Leaf extract of *Caesalpinia mimosoides* enhances oxidative stress resistance and prolongs lifespan in *Caenorhabditis elegans*. *BMC Complementary and Alternative Medicine*, *19*(1). https://doi.org/10.1186/s12906-019-2578-5

Rashad, S., El-Chaghaby, G., Lima, E. C., & Simoes Dos Reis, G. (2023). Optimizing the ultrasonic-assisted extraction of antioxidants from *Ulva lactuca* algal biomass using factorial

design. *Biomass Conversion and Biorefinery*, *13*(7), 5681-5690. https://doi.org/10.1007/s13399-021-01516-8

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rima, Ishmayana, S., Made Malini, D., & Soedjanaatmadja, U. M. S. (2023). Nutritional content and the activities of l-Dopa (L-3,4-dihydoxyphenyalanine) from *Mucuna pruriens* L. DC seeds of Central Java accession. *Arabian Journal of Chemistry*, *16*(1), 104390. https://doi.org/10.1016/j.arabjc.2022.104390

Sang, X., Zhen, F., Lv, P., Zhang, Z., Qu, B., & Wang, Y. (2025). Green and chemical-free pretreatment of flavonoids in tea plant seed husk using ultrasound-cold isostatic pressure synergistic extraction. *Food Chemistry*, *478*, 143725. https://doi.org/10.1016/j.foodchem.2025.143725

Sayem, A. S. M., Ahmed, T., Mithun, M. U. K., Rashid, M., & Rana, M. R. (2024). Optimising ultrasound-assisted extraction conditions for maximising phenolic, flavonoid content and antioxidant activity in hog plum peel and seed: A response surface methodology approach. *Journal of Agriculture and Food Research*, *18*, 101312. https://doi.org/10.1016/j.jafr.2024.101312

Sowdhanya, D., Singh, J., Rasane, P., Kaur, S., Kaur, J., Ercisli, S., & Verma, H. (2024). Nutritional significance of velvet bean (*Mucuna pruriens*) and opportunities for its processing into value-added products. *Journal of Agriculture and Food Research, 15*, 100921. https://doi.org/10.1016/j.jafr.2023.100921

Taroncher, M., Vila-Donat, P., Tolosa, J., Ruiz, M. J., & Rodríguez-Carrasco, Y. (2021). Biological activity and toxicity of plant nutraceuticals: an overview. *Current Opinion in Food Science, 42*, 113-118. doi:https://doi.org/10.1016/j.cofs.2021.05.008

Tavares, R. l., Vasconcelos, M. H. A. d., Dutra, M. L. d. V., D'Oliveira, A. B., Lima, M. d. S., Salvadori, M. G. d. S. S., Pereira, R. d. A., Alves, A. F., Nascimento, Y. M. d., Tavares, J. F., Guzman-Quevedo, O., & Aquino, J. d. S. (2020). *Mucuna pruriens* administration minimizes neuroinflammation and shows anxiolytic, antidepressant and slimming effects in obese rats. *Molecules*, *25*(23), 5559.

Tesoro, C., Lelario, F., Ciriello, R., Bianco, G., Di Capua, A., & Acquavia, M. A. (2022). An overview of methods for L-dopa extraction and analytical determination in plant matrices. *Separations*, *9*(8), 224. https://doi.org/10.3390/separations9080224

Vilairat, C., Kobtrakul, K., & Vimolmangkang, S. (2023). Enhanced physicochemical stability of the L-DOPA extract of *Mucuna pruriens* seeds by adding *Phyllanthus emblica*. *Molecules, 28*(4), 1573.

Vinatoru, M., Mason, T. J., & Calinescu, I. (2017). Ultrasonically assisted extraction (UAE) and microwave assisted extraction (MAE) of functional compounds from plant materials. *TrAC Trends in Analytical Chemistry*, *97*, 159-178. doi:https://doi.org/10.1016/j.trac.2017.09.002

Widaad, A., Zulkipli, I. N., & Petalcorin, M. I. R. (2022). Anthelmintic effect of *Leucaena leucocephala* extract and its active compound, mimosine, on vital behavioral activities in *Caenorhabditis elegans*. *Molecules*, *27*(6), 1875. Retrieved from https://www.mdpi.com/1420-3049/27/6/1875

Xiang, Y., Liu, Z., Liu, Y., Dong, B., Yang, C., & Li, H. (2024). Ultrasound-assisted extraction, optimization, and purification of total flavonoids from Daphnegenkwa and analysis of their antioxidant, anti-inflammatory, and analgesic activities. *Ultrasonics Sonochemistry, 111*, 107079. https://doi.org/10.1016/j.ultsonch.2024.107079

Yadav, M., Upadhyay, P., Purohit, P., Pandey, B., & Shah, H. (2017). Phytochemistry and pharmacological activity of *Mucuna pruriens*: A review. *International Journal of Green Pharmacy, 11*, 69-73. https://doi.org/10.22377/ijgp.v11i02.916

Yadav, R. K., Yadav, R. A., & Kostova, I. (2023). Structural, conformational and spectroscopic investigations of a biologically active compound: L-Dopa. *Applied Sciences*, *13*(24), 13336.

Zarroug, S. H. O., Bajaman, J. S., Hamza, F. N., Saleem, R. A., & Abdalla, H. K. (2023). *Caenorhabditis elegans* as an *in vivo* model for the discovery and development of natural plant-based antimicrobial compounds. *Pharmaceuticals*, *16*(8), 1070. https://doi.org/10.3390/ph16081070