LRF-783
[1-7]

RESEARCH ARTICLE Legume Research- An International Journal,

Polyphenol Content, Antioxidant Properties and Trypsin Inhibition Activity of Methanol Seed Extract and Fractions of White Velvet Bean from Vietnam

D.T. Ha[1], P.T.T. Ha[2,3], P.T. Tuyen[4], R.J. Henry[3], T.T. Loi[1], T.N. Chi[5]

10.18805/LRF-783

ABSTRACT

Background: White Velvet bean (*Mucuna pruriens)* is a very valuable plant due to its medical potential. Different parts of the plant have been studied by various researchers. However, the seeds, which contain a lot of bioactive compounds, have been considered of substantial medicinal importance.

Methods: The total phenolic content, total flavonoid content, antioxidant and trypsin inhibition properties in the methanol extract of white Velvet bean (WVB) seeds and its fractions (methanol, n-hexane, ethyl acetate, n-butanol and aqueous) were studied.

Result: The highest total phenolic content was recorded in the methanol extract (206.3 mg GAE g^{-1}). The highest total flavonoid content was in the n-hexane fraction (7.75 mg rutin g^{-1}). The methanol extract showed the highest total antioxidant potential (261.5 mg AAE g^{-1}). The methanol extract also had the strongest DPPH radical scavenging activity and the highest level of trypsin inhibitory activity compared to the other fractions with an IC_{50} value (35.3 µg mL^{-1}).

Key words: Antioxidant, Flavonoids, Phenolics, Trypsin inhibition, White Velvet bean.

INTRODUCTION

Legumes contain high levels of phytochemicals such as phenolic acids, flavanols, flavones, flavanols, flavanones, isoflavones, anthocyanins, tannins and other phenolics (Dias *et al.,* 2020). The Velvet bean *(Mucuna pruriens)* is a plant of the Fabaceae family (Rajeshwar *et al.,* 2005a) subfamily Papilionaceae, including species commonly known as Cowhage, Cowitch, Bengal bean, Buffalo bean, Mucuna, Itchy bean, among others that include approximately 150 species of annual and perennial legumes (Kavitha and Thangamani, 2014). Velvet bean is a medicinal plant, that originated in Asia and grows in India, Pakistan and Bangladesh, among many other countries. It is now cultivated in Australia and is also found in some provinces in Vietnam (Khare, 2016). It is a good food source as it is a viable dietary source of protein (Pugalenthi *et al.,* 2005) due to its high protein concentration (23–35%) along with its digestibility, comparable to that of other legumes such as soybeans, rice beans and lima beans (Gurumoorthi *et al.,* 2003). In oriental medicine, Traditional medicine suggests that the seeds of *Mucuna pruriens* have the potential to protect against the consequences of snake bites and scorpions or stings by insects (Siddhuraju *et al.,* 1996; Aguiyi *et al.,* 1999; Chikagwa-Malunga *et al.,* 2009). This medicinal herb is also used to remove worms and roundworms from the body. Nowadays, *Mucuna* is used for the treatment of Parkinson's disease (PD) (Cilia *et al.,* 2017).

According to Sathiyanarayanan and Arulmozhi (2007), the Mucuna plant has medicinal potential in all its components. Velvet bean seeds contain approximately 5% levodopa, which is their primary phenolic compound (Chikagwa-Malunga *et al.*, 2009). because levodopa is a substance that is commonly prescribed for the initial management of Parkinson's disease (PD).

Multiple studies show that velvet bean has many benefits over synthetic levodopa when provided to patients with Parkinson's disease, given that long-term use of synthetic levodopa is associated with several adverse effects (Tharakan *et al.,* 2007).

Food-additive proteinase inhibitors are often used to protect myofibrillar proteins from endogenous proteinases.

[1]Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam.
[2]High Agricultural Technology Research Institute for Mekong Delta (HATRI), Vietnam.
[3]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia.
[4]Faculty of Forest Resources and Environmental Management, Vietnam National University of Forestry, Hanoi, Vietnam.
[5]Faculty of Agriculture and Food Technology, Tien Giang University, Vietnam.

Corresponding Author: P.T.T. Ha and P.T. Tuyen; High Agricultural Technology Research Institute for Mekong Delta (HATRI), Vietnam; Faculty of Forest Resources and Environmental Management, Vietnam National University of Forestry, Hanoi, Vietnam.
Email: phamthithuhabt@gmail.com; tuyenpt@vnuf.edu.vn

How to cite this article: Ha, D.T., Ha, P.T.T., Tuyen, P.T., Henry, R.J., Loi, T.T. and Chi, T.N. (2024). Polyphenol Content, Antioxidant Properties and Trypsin Inhibition Activity of Methanol Seed Extract and Fractions of White Velvet Bean from Vietnam. Legume Research. DOI: 10.18805/LRF-783

Submitted: 24-11-2023 **Accepted:** 16-01-2024 **Online**:14-02-2024

Such inhibitors derived from legumes, which normally inhibit trypsin, are safe, effective, thermally stable and affordable. Trypsin inhibitors from legume seeds prevent heat-activated proteinases in fish muscle or surimi from softening mince or surimi (Yi-Shen *et al.,* 2018). This study evaluated the trypsin-inhibitory ability of white velvet beans.

Studies showed velvet bean seed extract compounds offer several health benefits (Siddhuraju and Manian, 2007). Kumar *et al.* (2010) found that the phenolic-rich ethyl acetate and methanolic extracts of the complete *M. pruriens* plant (MEMP) scavenge free radicals and are strong antioxidants. Due to their high phenolic content, Mucuna seeds have strong antioxidant capacity and could be used in medicine to prevent brain-related diseases, cancer, inflammation, neurodegeneration, menstruation, diabetes, arthritis and cardiovascular disease (Jayasri *et al.,* 2009). There are various varieties of cultivated velvet beans and all are botanically *Mucuna pruriens* var. utilis. Traditionally, two varieties of seeds (black and white) are available and are used interchangeably in the name velvet bean. To better understand velvet bean seed compounds, this study measured the total phenolic and flavonoid content of the methanol extract and its fractions for White velvet bean (WVB) from Vietnam and evaluated their antioxidant and trypsin inhibitor activities. The research findings are carried out as parameters of quality that establish a foundation for the execution of remedial actions and ongoing enhancement of the standard of WVB or its related products.

MATERIALS AND METHODS

Plant material

The seeds of WVB were collected from Ben Tre province, Vietnam in September 2022 and were used for antioxidant assays. The experiment was carried out at the laboratory of the Faculty of Applied Sciences, Ton Duc Thang University, Vietnam from September 2022 to April 2023. The main steps and methods to experiment are described below (Fig 1 and 2).

Plant sample collection and handling

One hundred grams of dried and milled WVB seeds into powder were soaked in a glass bottle containing a total of 1000 mL of methanol (MeOH) for three days at room temperature. The methanol extract was filtered using a vacuum filter and the solvent was subsequently removed using a rotary evaporator (HEID_31039, Germany). The resulting residue was then weighed. The dried crude extract was then resolved in 95% methanol and fractionated successively in n-hexane, ethyl acetate, n-butanol and H_2O to separate polar and non-polar compounds in the crude extract (Ha *et al.,* 2021). Fig 3 shows the specific steps involved in the fraction's procedure. The solvent was separated from the mixture using a rotary evaporator and the weight of each residue was measured.

Total phenolic content determination (TPC)

The methodology outlined by Ganesan *et al.* (2007) was utilized to determine the total phenolic contents and fractions of the WVB extract. Absorbance was determined at 720 nm using a spectrophotometer (Jenway 6705, USA). The gallic acid equivalents per gram (GE g^{-1}) were utilized as a measure of the total phenolic contents. Triplicate analyses of the samples were performed.

Total flavonoid content determination (TFC)

The total flavonoid contents of the WVB extract and its fractions were identified using the method that was laid out by Júnior *et al.* (2015). Total flavonoid content was measured with the aluminium chloride colourimetric assay. Absorbance was determined at 510 nm with rutin as a standard. The amount of total flavonoids was measured in rutin equivalents per gram of material. Triplicate analyses were performed on the samples.

Total antioxidant capacity (TAC)

The determination of the total antioxidant capacity of the WVB extracts and fractions was conducted utilizing the phosphomolybdenum method, as described in the studies of Kaushik *et al.* (2009) and Prieto *et al.* (1999). The absorbance values were recorded at 695 nm against a blank (water without plant extract) and ascorbic acid as the standard. The samples were analyzed in triplicate.

Free radical scavenging capacity (DPPH assay)

The scavenging effect of phenolics in the methanol extract and its fractions was determined by Amarowicz *et al.* (2007). Absorbance was determined at 517 nm. The capacity to scavenge free radicals (A%) was calculated as follows:

$$A\ (\%) = \frac{\text{Absorbance control - Absorbance sample}}{\text{Absorbance control}} \times 100$$

In this case, the absorbance sample denoted the absorbance of the extracted sample when compared to absorbance control, which represented the absorbance of the control reaction.

Trypsin inhibition activity (TIA)

The reaction mixtures (2.0 mL) contained 0.06 mg trypsin in 1.0 mL. 25 mM Tris-HCl buffer (pH 7.4) and 1.0 mL of different fractions of seeds (10, 100 and 1000 µg mL^{-1} of final volume for extracts and 10, 25 and 50 µg mL^{-1} for compounds). After incubating the solutions for 5 min at 37°C, 1.0 mL of 0.8% (w/v) casein was added. After that, the mixes were incubated for another 20 min. Afterwards, 2.0 mL of 70% (v/v) perchloric acid was added to stop the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was measured at 280 nm against the buffer as a blank (Patel and Zaveri, 2014). The samples were examined in triplicate.

The following formula was used to determine the percentage of inhibition:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance (Control) - Absorbance (Test)}}{\text{Absorbance (Control)}} \times 100$$

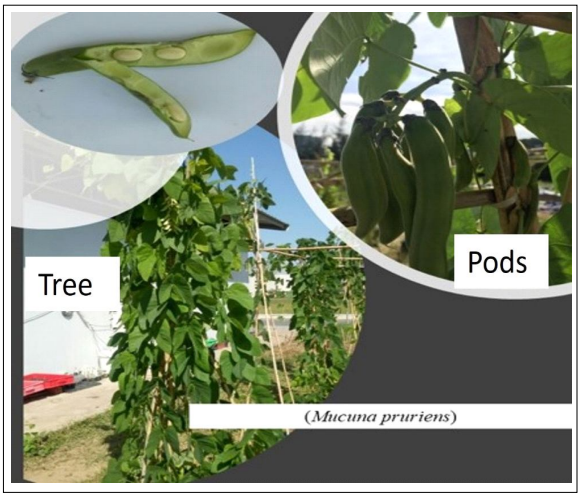

Fig 1: White Velvet bean tree; pods and pods in cross-section.

Statistical analysis

Statistical analysis by the method of analysis of variance (ANOVA) was performed on Minitab 19 software and expressed as ± SD value. The smallest difference was significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Estimation of TPC and TFC

Phenolic compounds are a prevalent and widely dispersed category of secondary metabolites found in plants. They can take the form of basic molecules such as phenolic acids, phenylpropanoids and flavonoids, or they can be highly polymeric compounds (Lin *et al.,* 2016). A large number of different kinds of phenolics have been reported to be in the seed coat of legume grains (Troszynska *et al.*, 2002). Previous research demonstrated the total phenolic content

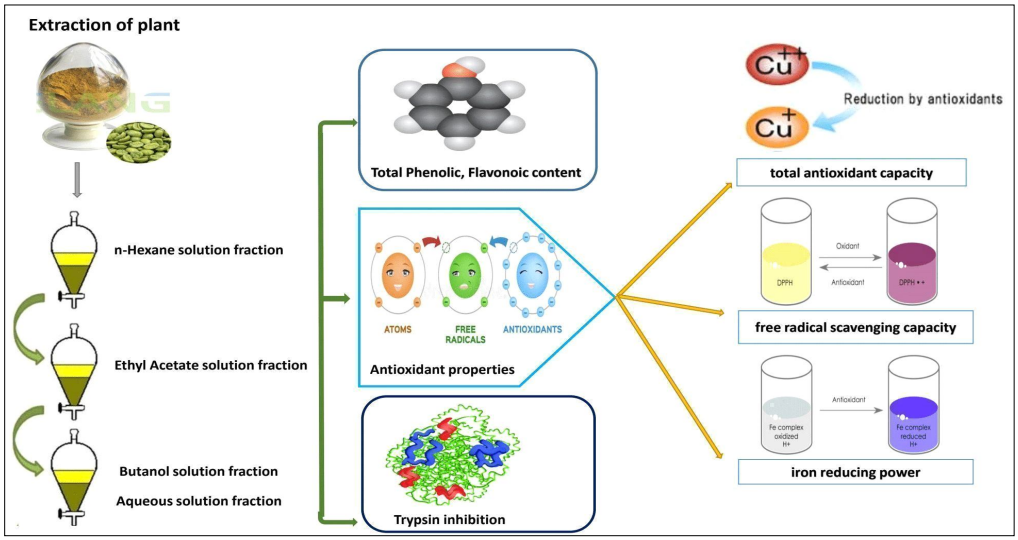

Fig 2: General experimental procedure summary.

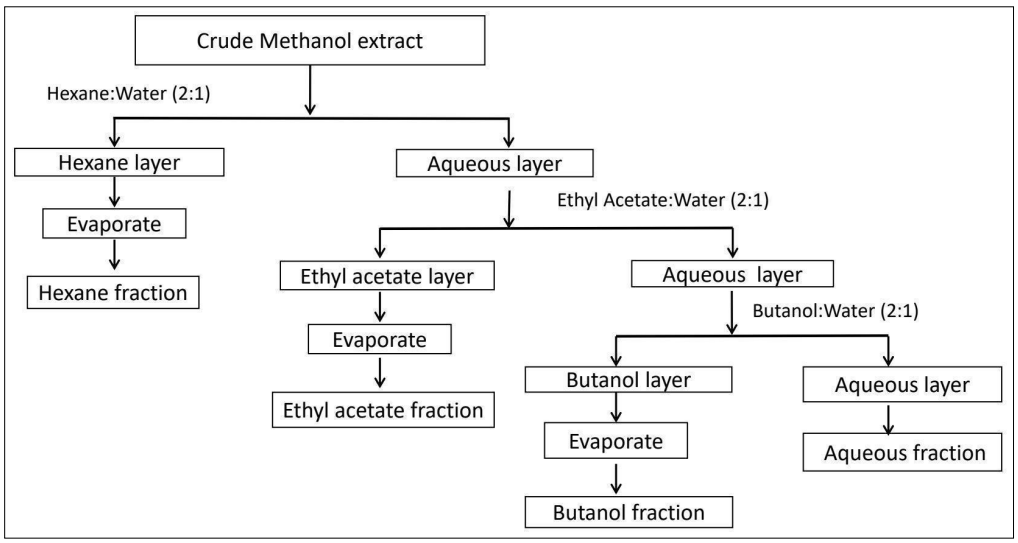

Fig 3: Fractionation of the crude methanol extract.

of velvet beans was 33.0 mg g^{-1} gallic acid (Rajeshwar *et al.* 2005a) and 3730±15.5 mg, equivalent to gallic acid (GAE) g^{-1} (Jimoh *et al.* (2020). In this study, TPC was calculated from the standard curve (y = 0.002x - 0.017, R^2 = 0.992). Table 1 shows that TPC in methanol extract of WVB had the highest result (206.3 mg GAE g^{-1}) followed by n-butanol (65.0 mg GAE g^{-1}), aqueous (52.7 mg GAE g^{-1}), ethyl acetate (49.3 mg GAE g^{-1}) and n-hexane (10.7 mg GAE/g) ($p<0.05$). These results are also different from the levels of phenolic compounds found by Longhi *et al.* (2011) in mucuna seed at 24 ± 0.2 g 100 g^{-1} of extract and by Adebowale *et al.* (2005) (7.75 ± 0.02 g per 100 g of dry seeds) in the same species.

The TFC was measured by the aluminium chloride colourimetric assay. The n-hexane fraction had the highest TFC (7.75 mg rutin g^{-1}), followed by methanol (5.49 mg rutin g^{-1}), ethyl acetate (2.92 mg rutin g^{-1}), aqueous (0.91 mg rutin g^{-1}) and n-butanol (0.34 mg rutin g^{-1}). An earlier study found that velvet bean seeds have substantial quantities of flavonoids in their ethyl acetate, butanol and water fractions (Widowati *et al.,* 2010). Theansungnoen *et al.* (2022) the polarity of the extracting solvent affects the amount of phenolic and total flavonoids in each extract. According to Aryal *et al.* (2019), the phenolic and flavonoid content levels may vary slightly due to the attendance of varying amounts of sugars, carotenoids, or ascorbic acid, as well as the environmental variance, length of extraction, or extraction methods.

Antioxidant activity (TAC and DPPH)

Antioxidant activity refers to the capacity of redox molecules to effectively eliminate free radicals that are observed in biological systems and food (Bunea *et al.,* 2011). The antioxidant activity of the methanol extract and its fraction of the WVB seeds were determined by TAC and DPPH as mentioned in Table 2 and 3, respectively.

Table 2 presents the TAC obtained through the phosphomolybdenum assay and was calculated from the standard curve (y = 0.0038x + 0.0383, R^2 = 0.9900). Results were expressed as ascorbic acid equivalent per g in the methanol extract and its fractions. At 261.5 mg AAE g^{-1}, the methanol extract was the most powerful antioxidant, followed by the aqueous extract at 84.4 mg AAE g^{-1}, n-hexane at 74.6 mg AAE g^{-1}, ethyl acetate at 28.85 mg AAE g^{-1} and n-butanol at 22.6 mg AAE g^{-1}. The total antioxidant capacity results showed antioxidant potential in the following order: methanol > aqueous > n-hexane > ethyl acetate > n-butanol. In this study, methanol extract had the highest total phenolic content (206.33 mg GAE g^{-1}) and the highest total antioxidant capacity (261.48 mg AAE g^{-1}). The results indicated that the TAC and the concentration of phenolic compounds in the methanol extract and its fractions were positively correlated. Nur *et al.* (2019) also reported that the phenolic and flavonoid contents of the extract and fractions played a role in increasing antioxidant activity, both in the DPPH scavenging assay and iron reduction power activity.

The DPPH radical scavenging activity results are shown in Table 3 as a comparison with the known antioxidant ascorbic acid (Vitamin C). Among the fractions, the methanol fraction had the highest activity. At a concentration of 60 µg mL^{-1}, the scavenging activity of methanol, n-hexane, n-butanol, ethyl acetate and aqueous fractions was 89.2 %, 41.3%, 57.2%, 48.0% and 61.7%, also at the same concentration, the activity of ascorbic acid was 92.1%. In Table 3, data represents the IC_{50} of the methanol extract and fractions with standard ascorbic acid, the free radical scavenging activity of different extracts and ascorbic acid was in the following order: ascorbic acid > methanol > aqueous > ethyl acetate > n-butanol > n-hexane. The DPPH radical scavenging activity assay showed that at 60 µg mL^{-1}, the methanol extract had the highest antioxidant activity, lowest IC_{50} (30.1%) and maximal reduction activity. A study conducted by Rajeshwar *et al.* (2005b) found that *Mucuna pruriens* methanol extract had strong antioxidant properties. At 100 µg mL^{-1}, *Mucuna pruriens* methanol extract and BHT showed 90.2 and 94.0% inhibition, respectively, with IC_{50} values of 38.5 µg and 15 µg mL^{-1}, as determined by DPPH radical. In this study, although the n-hexane fraction had the highest total flavonoid concentration (7.75 mg rutin g^{-1}), the methanol extract exhibited the strongest antioxidant properties based on total antioxidant capacity, radical scavenging activity and reducing power assay results. That could be because, in the seeds of WVB, the polarity of the solvent had a

Table 1: Measurement of TPC and TFC in the methanol extract and its fractions from seeds of WVB.

Fractions	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg rutin/g extract)
Methanol	206.33±1.26^{a}	5.49±0.01^{b}
n-Hexane	10.67±0.29^{e}	7.75±0.01^{a}
Ethyl acetate	49.33±0.76^{d}	2.92±0.01^{c}
n-Butanol	65.00±0.50^{b}	0.34±0.03^{e}
Aqueous	52.17±0.76^{c}	0.91±0.02^{d}

Values in the column followed by a different letter superscript (a-e) are significantly different ($p<0.05$) based on one-way ANOVA. Results are expressed as mean ± standard deviation of three replications. GAE: Gallic acid equivalent.

Table 2: Total antioxidant capacity in the methanol extract and its fractions from seeds of WVB.

Fractions	Total antioxidant capacity (mg AAE/g extract)
Methanol	261.48±0.40^{a}
n-Hexane	74.64±0.61^{c}
Ethyl acetate	28.85±0.15^{d}
n-Butanol	22.62±0.40^{e}
Aqueous	84.38±0.40^{b}

Values in the column followed by a different letter superscript (a-e) are significantly different ($p<0.05$) based on one-way ANOVA. Results are expressed as mean ± standard deviation of three replications. AAE: Ascorbic acid equivalent.

Table 3: The IC_{50} values of DPPH scavenging effect in methanol extract and its fractions from seeds of WVB.

	Fractions and ascorbic acid					
Concentration (µg/ml)	Ascorbic acid	Methanol	n-Hexane	Ethyl acetate	n-Butanol	Aqueous
10	41.53±0.03^{e}	17.47±0.08^{e}	3.76±0.08^{e}	13.39±0.10^{e}	9.05±0.09^{e}	15.84±0.13^{e}
25	58.94±0.03^{d}	44.54±0.18^{d}	14.58±0.14^{d}	24.61±0.13^{d}	18.73±0.09^{d}	28.18±0.08^{d}
30	61.69±0.62^{c}	52.72±0.08^{c}	17.55±0.10^{c}	31.17±0.06^{c}	25.52±0.12^{c}	31.13±0.19^{c}
35	64.24±0.09^{b}	59.39±0.18^{b}	23.52±0.08^{b}	38.64±1.48^{b}	31.04±0.04^{b}	35.64±0.16^{b}
60	92.09±0.07^{a}	89.18±0.11^{a}	3.76±0.08^{e}	57.24±0.10^{a}	48.01±0.12^{a}	61.71±0.13^{a}
IC_{50}	18.22	30.11	71.50	51.00	61.63	48.80

Values in the column followed by a different letter superscript (a-e) are significantly different ($p<0.05$) based on one way ANOVA. Results are expressed as mean ± standard deviation of three replications.

Table 4: The IC_{50} values of trypsin inhibition activity in methanol extract and its fractions from seeds of WVB.

	Fractions and indomethacin					
Concentration (µg/ml)	Indomethacin	Methanol	n-Hexane	Ethyl acetate	n-Butanol	Aqueous
10	19.09±0.01^{e}	18.16±3.74^{d}	3.82±0.20^{e}	5.47±0.20^{e}	9.43±0.49^{e}	4.68±0.72^{e}
25	48.83±0.04^{d}	33.96±2.04^{c}	7.33±0.15^{d}	9.92±0.13^{d}	16.78±0.02^{d}	11.92±0.79^{d}
30	55.50±0.02^{c}	46.10±3.21^{b}	8.58±0.13^{c}	11.55±0.08^{c}	19.48±0.27^{c}	16.17±0.69^{c}
35	64.24±0.07^{b}	52.21±2.48^{b}	9.12±0.13^{b}	12.48±0.26^{b}	22.06±0.47^{b}	20.42±1.15^{b}
60	99.19±0.06^{a}	80.66±1.72^{a}	15.62±0.11^{a}	22.98±0.08^{a}	39.09±0.43^{a}	33.18±2.34^{a}
IC_{50}	27.99	35.26	195.94	134.63	78.08	89.75

Values in the column followed by a different letter superscript (a-e) are significantly different ($p<0.05$) based on one-way ANOVA. Results are expressed as mean ± standard deviation of three replications.

significant effect on the chemical profile of the secondary metabolite of the fraction (Verawati *et al.*, 2016).

Trypsin inhibition activity

Inhibitors of nutritional trypsin function as an enzyme that diminishes the activity of chymotrypsin and trypsin in the pancreas, thereby impeding the digestion and assimilation of proteins and preventing the development of pancreatic hyperplasia (Kårlund *et al.*, 2021; Dhaliwal *et al.*, 2022). The processing of grain legumes and other crops containing trypsin inhibitors is thus essential (Nkhata *et al.,* 2018). The data in Table 4 shows the results of the trypsin inhibitory testing for the methanol extract of WVB and fraction as being equivalent to the standard medicine indomethacin. The methanol extract showed the most activity compared to the other components. The IC_{50} values for the conventional pharmaceutical indomethacin were compared to those of the methanol extract and its fractions in Table 4. The sequence in which the various fractions and indomethacin demonstrated their capacity to inhibit activity was as follows indomethacin (28.0 µg mL^{-1}) > methanol (35.3 µg mL^{-1}) > n-butanol (78.1 µg mL^{-1}) > aqueous (89.8 µg mL^{-1}) > ethyl acetate (134.6 µg mL^{-1}) > n-hexane (195.9 µg mL^{-1}). *M. pruriens* seeds from Brazil have been reported to contain trypsin inhibitors (Adive and Celia, 1998). Vadivel and Janardhanan (2000) also found trypsin inhibitor activity in velvet bean seed collected from three different locations in Western Ghats, South India.

CONCLUSION

The current research found that the methanol extract had modest amounts of total flavonoids and the highest levels of total phenolics. Methanol extract of WVB seeds also shows strong antioxidant activities by TAC and DPPH as well as the highest amount of trypsin inhibitory activity compared to the other fractions, with an IC_{50} value of 35.3 g mL^{-1}. The findings of this study will provide critical information for further research into this underutilized legume for nutritional purposes, as well as for the increase of new, cheaper and safer food products utilized for human food or animal feed.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Adebowale, Y.A., Adeyemi, A., Oshodi. A.A. (2005). Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. Food Chemistry. 89(1): 37-48.

Adive B.I.U., Celia, R.C. (1998). Brazilian *Mucuna pruriens* seeds (Velvet Bean) lack hemagglutinating activity. Journal of Agricultural and Food Chemistry. 46(4): 1450-1452.

Aguiyi, J.C., Igweh, A.C., Egesie, U.G. and Leocini, R. (1999). Studies on possible protection against snake venom using *Mucuna pruriens* protein immunization. Fitoterapia. 70: 21-26.

Amarowicz, R., Zegarska, Z., Pegg, R.B., Karamac, M. and Kosinska (2007). Antioxidant and radical scavenging activities of a barley crude extract and its fraction. Czech Journal of Food Sciences. 25: 73-80.

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel). 8(4): 96.

Bunea, A., Rugina, O.D., Pintea, A.M., Sconta, Z., Bunea, C.I., Socaciu, C. (2011). Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from romania. Notulae Botanicae Horti. Agrobotanici Cluj-Napoca. 39: 70-76.

Chikagwa-Malunga, S.K., Adesogan, A.T., Sollenberger, L.E., Badinga, L.K., Szabo, N.J., Litell, 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.

Cilia, R., Laguna, J., Cassani, E., Cereda, E., Pozzi, N. G., Isaias, I.U., Contin, M., Barichella, M. and Pezzoli, G. (2017). *Mucuna pruriens* in Parkinson disease: A double-blind, randomized, controlled, crossover study. Neurology. 89: 432-438.

Dhaliwal, S.K., Dhillon, S.K., Gill, B.S., Kaur, G., Sirari, A. and Sharma, S. (2022). Effect of genetic elimination of Kunitz Trypsin inhibitor on agronomic and quality traits in soybean [*Glycine max* (L.) Merril]. Legume Research. 45(1): 32-38. doi: 10.18805/LR-4205.

Dias, R., Oliveira, H., Fernandes, I., Simal-Gandara, J., Perez-Gregorio, R. (2020). Recent advances in extracting phenolic compounds from food and their use in disease prevention and as cosmetics. Critical Reviews in Food Science and Nutrition. 1-22.

Ganesan, P., Kumar, C. S., Bhaskar, N. (2007). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresource Technology. 99. 2717-2723.

Gurumoorthi, P., Pugalenthi, M., Janardhanan, K. (2003). Nutritional potential of five accessions of a south Indian tribal pulse *Mucuna pruriens var. utilis*; II Investigation on total free phenolics, tannins, trypsin and chymotrypsin inhibitors, phytohemagglutinin and *in vitro* protein digestibility. Tropical and Subtropical Agroecosystems. 1: 153-158.

Ha, P.T.T., Linh, D.T.N., Dat, M.T.T. and Trang, P.T.T. (2021). Inhibitory *in vitro* effects of Basil (*Ocimum basilicum*) leaf extracts on cholesterol esterase activity and the growth of *Escherichia coli.* Journal of Food Processing and Preservation. 45(12): e16105.

Jayasri, M.A., Mathew, L., Radha, A. (2009). A report on the antioxidant activities of leaves and rhizomes of *Costus pictus* D. Don. International Journal of Integrative Biology. 5(1): 20-26.

Jimoh, M.A., Idris, O. A., Jimoh, M.O. (2020). Cytotoxicity, phytochemical, antiparasitic screening and antioxidant activities of *Mcuna pruriens* (Fabaceae). Plants. 9(9): 1-13.

Júnior, S.Q., Carneiro, V.H.Q., Fontenelle, T.P.C., Chaves, L.D. S., Mesquita, G.X., Brito, T. V., Prudêncio, R.S., *et al.* (2015). Antioxidant and anti-inflammatory activities of methanol extract and its fractions from the brown seaweed *Spatoglossum schroederi.* Journal of Applied Phycology. 27: 2367-2376.

Kårlund, A., Paukkonen, I., Gómez-Gallego, C., Kolehmainen, M. (2021). Intestinal exposure to food-derived protease inhibitors: Digestion physiology- and gut health-related effects. Healthcare (Basel). 9(8): 1002.

Kaushik, U., Lachake, P., Shreedhara, C.S., Ram, H.N.A. (2009). *In vitro* antioxidant activity of extracts of *Avipattikar churna*. Pharmacologyonline. 3: 581-589.

Kavitha, C., Thangamani, C. (2014). Amazing bean "*Mucuna pruriens*": A comprehensive review. Journal of Medicinal Plants Research. 8(2): 138-143.

Khare, C.P. (2016). Ayurvedic Pharmacopeial Plant Drugs: Expanded Therapeutics. CRC Press. 373-374.

Kumar, D.S, Muthu, K.A, Smith, A.A., Manavalan, R. (2010). *In vitro* antioxidant activity of various extracts of whole plant of *Mucuna pruriens* (Linn). International Journal of PharmTech Research. 2: 2063-2070.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H., Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes. Molecules. 21(10): 1374.

Longhi, J.G., Perez, E., de Lima, J.I., Cândido, L.M.B. (2011). *In vitro* evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity. Brazilian Journal of Pharmaceutical Sciences. 47(3): 535-544.

Nkhata, S.G., Ayua, E., Kamau, E.H., Shingiro, J.B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Science and Nutrition. 6(8): 2446-2458.

Nur, S., Mubarak, F., Jannah, C., Winarni, D.A., Rahman, D.A., Hamdayani, L.A., Sam, F.J. (2019). Total phenolic and flavonoid compounds, antioxidant and toxicity profile of extract and frations of paku atai tuber (*Angiopteris ferox Copel*). Food Research. 3(6): 734-740.

Patel, S.S., Zaveri, M.N. (2014). Trypsin and protein denaturation inhibitory activity of different fractionation and isolated compound of leaf and root of *Justicia Gendarussa*. International Journal of Pharmaceutical Sciences and Research. 5(12): 5564-5571.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry. 269: 337-341.

Pugalenthi, M., Vadivel, V., Siddhuraju, P. (2005). Alternative food/feed perspectives of an under-utilized legume *Mucuna pruriens* utilis- A review. Plant Foods for Human Nutrition. 60: 201-218.

Rajeshwar, Y., Gupta, M., Mazumder, U.K. (2005b). *In vitro* lipid peroxidation and antimicrobial activity of Mucuna pruriens seeds. Iranian Journal of Pharmacology and Therapeutics. 4: 32-35.

Rajeshwar, Y., Kumar, S.G.P., Gupta, M., Mazumder, K.U. (2005a). Studies on *in vitro* antioxidant activities of methanolic extract of *Mucuna pruriens* (Fabaceae) seeds. European Bull of Drug Research. 13: 31-39.

Sathiyanarayanan, L., Arulmozhi, S. (2007). *Mucuna pruriens*: A comprehensive review. Pharmacognosy Review. 1: 157-162.

Siddhuraju, P., Manian, S. (2007). The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] seeds. Food Chemistry. 105: 950-958.

Siddhuraju, P., Vijayakumari, K., Janardhanan, K. (1996). Chemical composition and protein quality of the little-known legume, velvet bean [*Mucuna pruriens* (L.) DC]. Journal of Agricultural and Food Chemistry. 44(9): 2636-2641.

Tharakan, B., Dhanasekaran, M., Mize-Berge, J., Manyam, B.V. (2007). Anti-Parkinson botanical *Mucuna pruriens* preventes levodopa induced plasmid and genomic DNA damage. Phytotherapy Research. 21(12): 1124-1126.

Theansungnoen, T., Nitthikan, N., Wilai, M., Chaiwu, P., Kiattisin, K., Intharuksa, A. (2022). Phytochemical analysis and antioxidant, antimicrobial and antiaging activities of ethanolic seed extracts of four *Mucuna* species. Cosmetics. 9: 14.

Troszynska, A., Estrella, I., Lopez-Amores, M. L., Hernandez, T. (2002). Antioxidant activity of pea (*Pisum sativum* L.) seed coat acetone extract. LWT-Food Science and Technology. 35: 158-164.

Vadivel, V., Janardhanan, K. (2000). Nutritional and antinutritional composition of velvet bean: An under-utilized food legume in South India. International Journal of Food Sciences and Nutrition. 51: 279-287.

Verawati, Aria, M., Arel, A., Ryanto, E. (2016). Antioxidant activity and total flavonoid content of fractions of piladang [*Solenostemon scutellarioides* (L.) Codd] leaf extract. Scholars Research Library. 8(18): 67-71.

Widowati, W., Ratnawati, H., Rusdi, U.D., Winarno, W., Immanuel, V. (2010). Phytochemical assay and antiplatelet activity of fractions of Velvet bean seeds (*Mucuna pruriens* L.). HAYATI Journal of Biosciences. 17(2): 85-90.

Yi-Shen, Z., Shuai, S., FitzGerald, R. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. Food and Nutrition Research. 62: 1290.