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In-Vitro evaluation of antidiabetic, antioxidant, and anti-inflammatory activities in *Mucuna pruriens* seed extract

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Abstract

Background *Mucuna pruriens var. utilis* (Wall. ex Wight) belonging to the family Fabaceae. Renowned for its diverse array of phytochemicals, this plant has been historically employed in the treatment of various ailments. The objectives of this study are to evaluate the anti-diabetic, anti-inflammatory, and antioxidant properties of the optimized *M. pruriens var. utilis* seed extract.

Methods The in-vitro anti-inflammatory activity of *M. pruriens var. utilis* ethanolic extracts was scrutinized using the Human Red Blood Cell (HRBC) method. To evaluate antioxidant activity, ABTS and DPPH assays were employed. Furthermore, the antidiabetic activity was assessed through α-amylase and α-glucosidase inhibition assays.

Results In the ethanolic extract of *M. pruriens var. utilis* numerous phytoconstituents were found by doing a phytochemical analysis (alkaloids, flavonoids, phenols, saponins, steroids, glycosides, tannins). The total phenolic and flavonoid content were determined to be 112.07 ± 1.21 mg of gallic acid equivalents GAE/g and 101.41 ± 1.08 mg of quercetin equivalents QE/g respectively. In this investigation ethanolic extract of *M. pruriens var. utilis* exhibited a high anti-inflammatory, antioxidant and antidiabetic activities in a dose-dependent manner. The *M. pruriens var. utilis* extract shows that anti-inflammatory activity 32.26 ± 3.23%, potent antioxidant effect by ABTS radical scavenging assay IC_{50} 67.46 ± 1.45 µg/mL and DPPH radical scavenging assay IC_{50} 63.34 ± 2.27 µg/mL and in addition, showed promising antidiabetic potential by inhibiting α-amylase IC_{50} 33.42 ± 1.35 µg/mL and α-glucosidase IC_{50} 28.34 ± 1.41 µg/mL.

Conclusion These findings provide additional support for the traditional medicinal use of *M. pruriens var. utilis* in treating inflammation, oxidative stress, and diabetes mellitus.

Keywords α--amylase, α-glucosidase, Antioxidant, *M. pruriens var. utilis*, Anti-inflammatory

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Introduction

Nature offers an extensive array of plants renowned for their healing properties in addressing various human ailments. Due to their minimal toxicity and cost-effectiveness, medicinal plants have been utilized by numerous cultures throughout history to treat a diverse range of maladies [1]. This longstanding tradition has piqued considerable interest in the development of herbal medications, often associated with the presence of phytoconstituents. According to data from the world health organization (WHO), an estimated 80% of the global population primarily depends on medicinal plants for fundamental healthcare needs [2]. Over the past few years, there has been a growing focus on studying the therapeutic potential of natural plant-based antioxidants, whether in the form of raw extracts or integrated into functional foods. These investigations aim to understand their effectiveness in health management, particularly in addressing conditions such as diabetes and inflammatory processes [3, 4]. The bioactive elements present in extracts exhibit synergistic interactions, believed to be advantageous in addressing chronic, multifaceted disorders that involve various pathways [5, 6]. Recent studies have established connections between inflammation, oxidative stress, and diabetes. However, understanding the underlying mechanism is challenging due to the dual role of oxidative stress as both a signaling agent and a destructive force, influencing transcriptional control and cell cycle regulation [7, 8].

Diabetes stands as a debilitating chronic condition, marked by a series of events characterized by the reduction or obstruction of insulin release from the pancreas. Inadequate insulin production or utilization results in hyperglycemia, leading to elevated blood glucose levels, and over time, contributing to damage in organs and tissues [9, 10]. The growing prevalence of diabetes underscores the imperative for utilizing medicinal herbs in both treatment and prevention [11, 12]. A rising number of individuals seek ways to integrate natural bioactive substances into alternative medicine, drawn to their advantages of low toxicity, affordability, minimal side effects, and easy accessibility [13]. According to previous investigation it was found notable α-glucosidase inhibitory activity was observed in the methanol and water extracts of *P. malayana* leaf. The IC_{50} values for these extracts were found to be 2.71 and 6.75 µg/mL, respectively. Active anti-diabetic metabolites identified in the extracts included palmitic acid, 1,3,5-benzenetriol, β-tocopherol, α-tocopherol, cholesta-7,9(11)-diene-3-ol, 24-epicampesterol, stigmast-5-ene, and monopalmitin [14].

Inflammation, a natural defense mechanism of the body, arises in response to damage or infection, presenting with edema, redness, and pain in the affected area [15]. This process triggers the release of diverse hydrolytic enzymes, the extravasation of fluids, the restoration and healing of the injured site, vasodilation, heightened blood vessel permeability, and an elevation in blood pressure [16, 17]. These inflammatory reactions additionally play a role in activating and generating free radicals, including reactive oxygen and nitric oxide, from immune system cells. This process leads to localized tissue damage and lipid peroxidation [18].

Oxidative stress induces the liberation of reactive free radicals, particularly OH (hydroxyl radicals), which disrupt the normal metabolic pathways in the body, affecting all biomolecules [19]. This disturbance contributes to the onset of various chronic diseases, including cancer, autoimmunity, diabetes, cardiovascular issues, neurodegenerative diseases, and premature aging in humans [20]. Antioxidants play a crucial role in mitigating oxidative stress by preserving an equilibrium between the production of free radicals and the occurrence of oxidative stress [21, 22]. Phytochemicals, such as phenolic acids and flavonoids, are believed to be strongly associated with the antioxidant properties of natural products widely promoted for their potential health benefits [23, 24]. These compounds constitute a significant portion of the non-sugar components in honey, influencing not only its color and sensory characteristics but also playing a role in its bioactivity, particularly in terms of antioxidant capabilities [25].

Traditional Chinese Medicine Velvet bean, scientifically known as *M. pruriens var. utilis*, and belonging to the Fabaceae family, is a widely distributed plant found in southern China and eastern India. The isolation of levodopa L-dihydroxyphenylalanine or (L-DOPA) from *M. pruriens var. utilis* seeds in 1937 marked a significant milestone, as levodopa is now effectively employed to manage the motor symptoms of Parkinson's disease [26, 27]. *M. pruriens var. utilis*, seeds encompass a diverse array of phytochemical substances, including carbohydrates and amino acids, alongside anti-nutrient components like polyphenols, phytates, tannins, and saponins [28, 29]. Numerous pharmacological studies have underscored the multifaceted therapeutic potential of *M. pruriens var. utilis*, demonstrating its anti-Parkinson [30], antidiabetic [31], antioxidant [32, 33], anti-inflammatory [34], and antibacterial activities [35], as well as its effects on male fertility and aphrodisiac properties [36, 37]. Despite the historical use of *M. pruriens var. utilis* in traditional medicine, the specific phenolic and flavonoid compounds responsible for its various therapeutic activities have remained elusive. This study, however, aims to delve into the in-vitro evaluation of the antidiabetic, antioxidant, and anti-inflammatory activities of *M. pruriens var. utilis* plant extracts.

Materials and methods

Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), ethanol (ENL), ferric chloride (FC), lead acetate (LA), sodium hydroxide (SOH), dilute HCl (dil HCL), gallic acid (GA), Alluminium trichloride ($AlCl_3$), Iodine solution (IOS), trichloro acetic acid (TCA) quercetin (QRN), ferric chloride ($FeCl_3$), were purchased from Sigma Chemical Co. (St. Louis, MO, USA); potassium chloride, potassium acetate, phosphate buffer, 2-deoxy-D-ribose, thiobarbituric acid (TBA), HCl, H_2SO_4, H_2O_2 were purchased from Sigma-Aldrich, folin-ciocalteus's phenol reagent and sodium carbonate were obtained from Merck (Dam-stadt, Germany).

Collection and identification of plant samples

The seeds of *M. pruriens var. utilis*, was collected (January 2023) from Kanpur, Uttar Pradesh India. It was authenticated by Dr. Sunita Garg taxonomist, and former chief scientist at RHMD, *CSIR* National Institute of Science Communication and Information Resources *(NISCAIR)*, identified the specimen using the available literature. The plant specimen was photographed, and a voucher specimen (TR No- NIScPR/RHMD/Consult/2023/4347-48-2) on dated 07/02/2023 was placed and preserved and deposited properly in the herbarium of the Department of *(NISCAIR)* Raw Materials Herbarium and Museum, Delhi, India.

Extraction preparation

A 1 kg dried seed sample of *Mucuna pruriens var. utilis* was crushed into a very fine powder using an electric grinder, yielding 830 g of powder. This powder was then stored in a refrigerator at 4 °C until further use. The entire 830 g of powder was soaked in 8300 ml of commercial-grade ethanol (maintaining a ratio of 1:10, powder to ethanol) for 7 days. The mixture was first filtered through muslin cloth and then re-filtered using Whatman filter paper (#01). The filtrate was collected for use in the extraction process [9, 15]. The remaining residue was immersed in 90% ethanol for 10 days and then filtered again. All the filtrates were combined and concentrated using a rotary evaporator under reduced pressure. The concentrated solution was dried in an oven at 40 °C, resulting in a semi-solid mass of 14.39 g. This semi-solid mass was further evaporated in a fume hood until fully dry, then stored in airtight containers for future use [10, 15].

Physicochemical analysis

Various physicochemical parameters (foaming index, loss on drying, total ash, acid insoluble ash, water-soluble ash, swelling index, ethanol-soluble extractive and water-soluble extractive) were analyzed according to the official Pharmacopoeias and other literatures [38–40].

Preliminary phytochemical screening

The seeds extract was examined for the presence of various phytoconstituents (carbohydrates, alkaloids, flavonoids, saponins, glycosides, tannins and phenolic according to the well-established methods [41–43].

Total flavonoids content determination (TFC)

The TFC of the extracts was determined by dissolving 1 mg of extract with 1.5 ml methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. This mixture was incubated at room temperature for 30 min. The absorbance was measured at 415 nm using a UV-Vis Double Beam Spectrophotometer (Systronic). The similar treatment was performed for variable concentrations of quercetin 10–50 µg/ml. The TFC was calculated using quercetin as standard, and values were expressed in terms of mg of quercetin equivalents QE/g [44].

Total phenolic content determination (TPC)

The extract 0.1 ml (1 mg/ml) was added to 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) was added, followed by 2.0 ml of 7.5% sodium carbonate solution. The reaction mixture was incubated for 1.5 h at room temperature in dark, and the absorbance was measured at 765 nm using a UV-Vis Double Beam Spectrophotometer (Systronic). The similar treatment was performed for variable concentrations of gallic acid (10–50 µg/ml). The total phenol content was subsequently calculated using gallic acid standard curve. The results were expressed as mg of gallic acid equivalents GAE/g [44].

HPLC analysis

The *M. pruriens var. utilis* extract was analyzed using chromatography on the Shimadzu LC2050 series, which was equipped with a UV detection system. A Phenomenex Luna C18 column with dimensions of 4.6×250 mm and a particle size of 5 µm was employed at a temperature of 25 °C. The mobile phase comprised a mixture of water and methanol at a volumetric ratio of 60:40, with a flow rate of 1 mL/min. The samples were observed and measured at a specific wavelength of 282 nm. A sample, which had been filtered using a 0.45 µm PTFE syringe filter, was injected into the rheodyne injector using a volume of 20 µL. Data analysis and recording of chromatogram was accomplished using Lab Solutions software [45].

Calibration curve

A calibration curve was generated using seven different levodopa concentrations ranging from 5 to 35 µg/ml. The

levodopa concentrations were diluted with an appropriate mobile phase. A calibration curve was constructed by plotting the concentration of levodopa against the corresponding peak area. Regression equations were then derived from the data [45].

In-vitro antioxidant potential of *M. pruriens var. Utilis*

Antioxidant Activity The antioxidant activity of selected extract was assessed by DPPH free radical scavenging assay and ABTS radical cation decolorization assay. The experiments were accomplished in triplicates and the antioxidant activity was calculated using following written formula:

$$\text{Percentage of scavenging} = \frac{A_0 - A_t}{A_0} \times 100 \quad (1)$$

.Where, as $A_{0\ =}$ absorbance of control, $A_{t\ =}$ absorbance of sample, However, IC_{50} values were determined on the basis of Probit analysis.

DPPH Radical scavenging activity

DPPH assay was used to determine free radical scavenging capacity. 1.0 mL ethanolic solution of DPPH 0.1 mM was mixed with 1.0 mL of different concentrations of *M. pruriens var. utilis* extract and positive control ascorbic acid. Further, the reaction mixture tubes were incubated for 20 min and the absorbance was observed at 517 nm. Reduction in absorbance was indicated as stronger DPPH radical-scavenging activity [46–48]. Percentage scavenging was calculated according to the formula (1).

ABTS Radical cation decolorization activity

The $ABTS^+$ scavenging assay was screened according to the previously described literatures [49–51]. Briefly, 1 mL of $ABTS^+$ solution 7.0 mM was added in 1 mL of potassium persulfate 2.45 mM solution and placed in the dark for 16 h for the generation of $ABTS^+$ radical cation. Further, ethanol was used to dilute $ABTS^+$ solution l until the absorbance displayed as 0.70±0.05 at 734 nm. Then, 50 µL of the samples (different concentrations) and ascorbic acid (as reference standard) were mixed with prepared diluted 1.9 mL of $ABTS^+$ solution. The final test mixtures were allowing to stand for 6 min at room temperature and absorbance was measured at 734 nm [51, 52]. Percentage scavenging was calculated by the formula (1).

In-vitro antidiabetic potential of M. pruriens var. Utilis

Assay of α-Amylase inhibition

α-Amylase inhibitory activity was assayed according to the procedure described by Yadav et al. [52], with a slight modification. α-Amylase activity was determined using soluble starch 1% as a substrate in 0.02 mol/l sodium phosphate buffer pH 6.9. *M. pruriens var. utilis* extracts with concentration 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL were mixed with substrate solution made up to a total volume of 150 µL; 10 µL of α-amylase solution 1 unit/mL/min was added. After incubation at 25 °C for 30 min, 30 µL of dinitrosalicylic acid reagent was added and incubated at 90 °C for 5 min. The absorbance was measured at 540 nm. Acarbose α-amylase inhibitor was used as a positive control. The assay was performed in triplicate. The percentage of inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = \left[\left(A_{\text{control (without extract)}} - A_{\text{sample}}\right) / A_{\text{control(withoutextract)}}\right] \times 100$$

Where A is the absorbance reading measured at 540 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the α-Amylase activity under the assay conditions.

Assay of α-Glucosidase inhibition

The inhibition of α-glucosidase assay is a modification of the method previously described by Yadav et al. [52]. *M. pruriens var. utilis* extracts with concentration 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL were mixed with 0.1 mol/l potassium phosphate buffer pH 6.9 made up to total volume of 150 µL; 10 µL of α-glucosidase solution 1 unit/mL/min was added. The mixer was incubated at 37 °C for 15 min. Then, 10 mL of 3 mM pnitrophenyl-α-D-glucopyranoside (PNP-G) was added, and the mixture was re-incubated at 37 °C for 10 min. The reaction was terminated by the addition of 30 mL of 0.1 M sodium carbonate. The amount of released product p-nitrophenol was measured at 405 nm using a UV-spectrophotometer to estimate the enzymatic activity. Acarbose α-glucosidase inhibitor was used as a positive control. The assay was performed in triplicate. The percentage of inhibition was calculated using the following formula:

$$\text{Percentage of inhibition} = \left[\left(A_{\text{control (without extract)}} - A_{\text{sample}}\right) / A_{\text{control(withoutextract)}}\right] \times 100$$

Where A is the absorbance reading measured at 405 nm.

The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the α-glucosidase activity under the assay conditions.

Anti-inflammatory potential of *M. pruriens var. Utilis*

HRBC membrane stabilization method

Fresh whole blood was ethically obtained from a healthy human donor, collected into EDTA tubes to prevent clotting. The blood samples were centrifuged at 2000× g for 10 min at 4 °C after being diluted with normal saline solution (0.9%, in a 1:4 ratio). This centrifugation process was repeated thrice until the supernatant appeared clear. The resulting packed cells were quantified and reconstituted to form a 10% v/v HRBC (Human Red Blood Cell) suspension using normal saline solution for use in the experiment [53].

Hypotonicity-induced hemolysis

Hypotonicity-induced hemolysis was assessed using an isotonic solution composed of 154 mM NaCl in a 10 mM sodium phosphate solution with a pH buffer of 7.4. To investigate the effects, a stock RBC suspension (50 µl) was mixed with 5 ml of a hypotonic solution containing *M. pruriens var. utilis*, ethanolic extract at concentrations of 100, 200, 300, 400, and 500 µg/ml. A control sample was prepared using a drug-free solution. After a 10-minute incubation at room temperature, the mixture was centrifuged at 5000 rpm for 5 min. The absorbance of the supernatant was measured at 560 nm using a UV-spectrophotometer. Aspirin (ASP) at a concentration of 200 µg/ml served as the standard. The percentage inhibition of hemolysis was calculated using the formula:

$$\%\,\text{Membrane stability} = 100 \times 1 - \left(\frac{\text{OD2} - \text{OD1}}{\text{OD3} - \text{OD1}}\right)$$

Where, OD_1 = Test sample unheated; OD_2 = Test sample heated and OD_3 = Control sample heated.

Egg albumin protein denaturation

Inflammatory and arthritic diseases may arise from the denaturation of proteins, leading to the generation of autoantigens. Therefore, compounds capable of inducing protein denaturation hold promise for the development of anti-inflammatory drugs. The experimental setup involved preparing a reaction mixture consisting of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline (PBS, pH 6.4), and varying concentrations 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml of *M. pruriens var. utilis* extract, totaling 5 ml. A control solution containing the same volume of distilled water was used for comparison. The mixture was then incubated for 15 min at 37.2 °C, followed by heating for 5 min at 70 °C. Absorbance was measured at 560 nm. As a standard, Aspirin (ASP) was used at final concentrations of 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml, with absorbance measurements taken similarly.

The inhibition of protein denaturation was calculated using the following formula:

$$\%\,\text{inhibition egg albumin protein denaturation} = 100 \times 1 - \left(\frac{\text{OD2} - \text{OD1}}{\text{OD3} - \text{OD1}}\right)$$

Where, OD_1 = Test sample unheated; OD_2 = Test sample heated and OD_3 = Control sample heated.

Statistical analysis

GraphPad Prism 8.2 software GraphPad Prism Software Inc., San Diego, CA, USA was employed for statistical analysis. The data are presented as Mean ± SEM. Statistical comparisons were conducted through a one-way analysis of variance test, followed by a Dunnet's t-test. Statistical significance was denoted by $^*p < 0.001$.

Results

Physicochemical analysis

Physicochemical analysis is a crucial aspect of the study of medicinal plants and natural products, encompassing the identification, authentication, and evaluation of the physical, chemical, and biological characteristics of plant materials. This multidisciplinary field combines elements of botany, chemistry, and pharmacology to ensure the quality, safety, and efficacy of herbal medicines. Moisture, extractive value, ash value, swelling, and foaming index are displayed in Table 1. The values were found within the mentioned limits of standard pharmacopoeias [38, 39].

Table 1 The physicochemical analysis results *M. pruriens var. Utilis*

Parameters	Value %
Loss on drying	4.8
Water soluble extractive value	13.9
Ethanol soluble extractive value	16.4
Acid insoluble ash value	6.1
water-soluble ash	1.5
Total Ash value	5.8
Swelling index	1.6
Foaming index	4.3

Screening for phytochemical constituents

Phytochemical examination of *M. pruriens var. utilis* seeds extract confirmed the presence of alkaloids, flavonoids, phenolic, saponins, steroids, carbohydrates, cardiac glycosides and tannins (Table 2). The phytoconstituents such as: tannins, coumarins, and flavonoids consist antioxidant, anti-inflammatory, antibacterial properties [54, 55], and also enhance wound healing by improving the survivability of collagen fibers, strengthening the strength of collagen fibers either by enhancing the circulation [56]. Therefore, the major constituents such

Table 2 The outcomes of qualitative phytochemical screening of *M. pruriens var. Utilis*

Test performed	Ethanolic extract
Alkaloids	
Mayer's reagent	++
Dragendorff's reagent	++
Wagner's reagent	++
Iodine solution	++
Flavonoids	
Shinoda test	++
Zinc turning	+
H_2SO_4 solution	++
Phenolic compounds	
Lead acetate test	++
$Fecl_3$ test	++
Tannins	
Lead acetate solution	++
Ferric chloride solution	+
Saponins	
Foam test	++
Borntrager's test	++
Steroids	
Salkowasld test	++
Liebermann's reagent	++
Carbohydrates	
Molisch's test	++
Fehling's test	++
Cardiac glycosides	
Legal test	+
Keller killiani Test	-

+ present, ++ strong present, - absent

flavonoids, phenols and alkaloids present in *M. pruriens var. utilis* might play a major role in the process of anti-diabetic and anti-inflammatory activity.

Evaluation of total phenolic and flavonoid contents

The total phenolic and flavonoid content of *M. pruriens var. utilis* ethanolic extract was determined using a colorimetric technique. The total phenolic content of the ethanolic extract was found to be 112.07 ± 1.21 mg Gallic acid equivalents GAE/g of dry extract. However, the flavonoid content was found to be 101.41 ± 1.08 mg of quercetin equivalents QE/g of dry extract.

HPLC analysis

The retention time of levodopa was 4.4 min Fig. 1. The peak eluted in the HPLC chromatogram of *M. pruriens var. utilis* extract is depicted in Fig. 2. The peak eluted at 4.4 min was identified as levodopa. By comparing the standard HPLC chromatograms, the peaks eluted at other retention times were found to be levodopa. The regression equation was found y = 125.7x − 100.6 and correlation coefficient (r^2) values were found to be 0.995. The levodopa concentration from *M. pruriens var. utilis* extract was quantified using regression equation. The levodopa concentration was found 105 mg per one gram of *M. pruriens var. utilis* extract.

Effect of M. pruriens var. utilis on antioxidant activity

Reactive oxygen species (ROS) and nitrogen species have been recognized for their therapeutic potential in various lifestyle diseases, including diabetes and liver conditions. Studies have indicated that during the initial phase of an inflammatory response, free radicals directly engage with DNA, promoting cell growth and division [57–59]. Consequently, medications or formulations with free radical-scavenging abilities could be valuable adjuvants in the treatment of diseases such as diabetes and malignancies [57–61]. In this context, the antioxidant properties of *M. pruriens var. utilis*, were explored (Table 3). The seed extract of *M. pruriens var. utilis*, along with the standard drug L-ascorbic acid, demonstrated a 50%

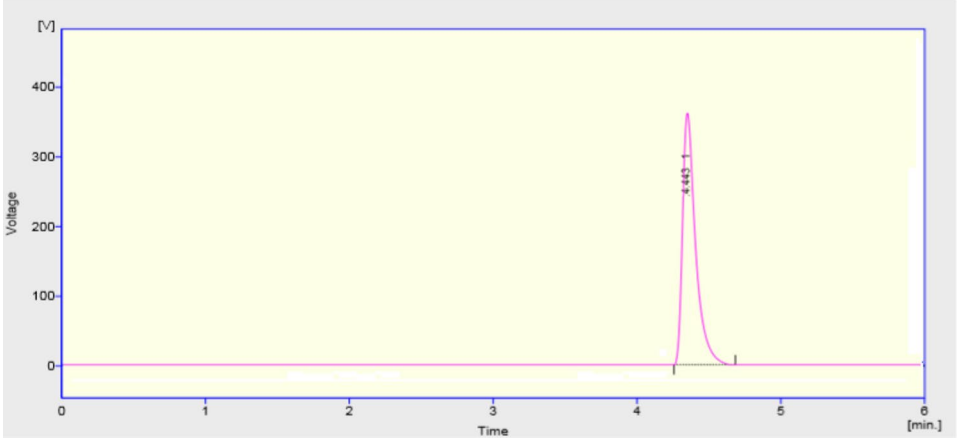

Fig. 1 HPLC chromatograms of levodopa. Concentration of levodopa is 23.71 µg/mL

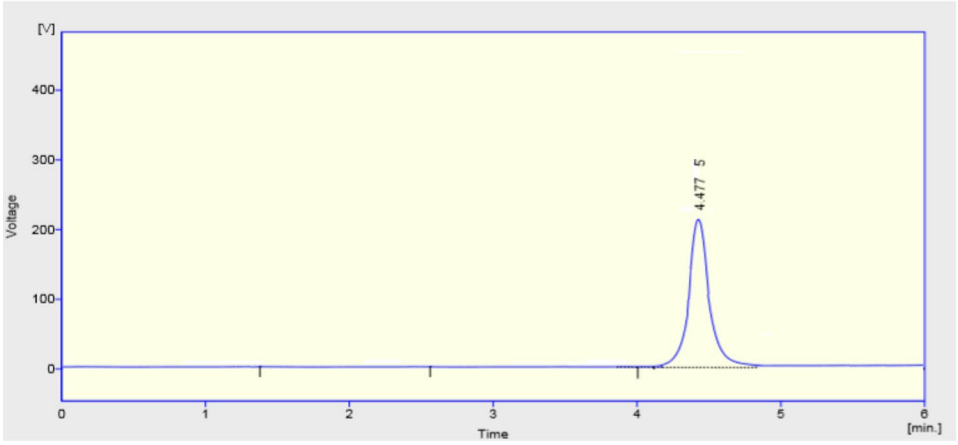

Fig. 2 HPLC chromatogram of *Mucuna pruriens* extract. The peak indicates the presence of levodopa. The concentration of levodopa is 20.37 µg/mL

Table 3 *M. pruriens var. Utilis* has antioxidant properties

Drugs	DPPH IC_{50} value µg/mL	ABTS IC_{50} value µg/mL
M. pruriens var. utilis	63.34 ± 2.27*	67.46 ± 1.45*
Ascorbic acid	9.07 ± 1.23	4.31 ± 1.18

Each statistics represents the Mean±SEM (n=3) * Significant difference (P<0.001) when compared to standards (Dunnet's t-test)

Table 4 *In-vitro α-glucosidase* and *α-amylase* inhibitory activity of *M. pruriens var. Utilis* extracts

Compound	α-glucosidase IC_{50} µg/mL	α-amylase IC_{50} µg/mL
M. pruriens var. utilis extract	28.34 ± 1.41	33.42 ± 1.35*
Acarbose	28.81 ± 1.23	29.66 ± 2.51

A statistically significant difference in means in comparison to standard is marked by a symbol: *p<0.001

DPPH inhibitory scavenging effect at 63.34±2.27 µg/mL and 9.07±1.23 µg/mL, respectively. *M. pruriens var. utilis*, exhibited an 8-fold lower DPPH scavenging potential compared to ascorbic acid, suggesting its potential as an adjuvant in antidiabetic action. A recent study [48], on *Oroxylum indicum* reported a DPPH scavenging IC_{50} of 149.97±1.10 µg/mL, emphasizing *M. pruriens var. utilis*, stronger DPPH scavenging potency among traditionally recognized antioxidant plants.

Furthermore, *M. pruriens var. utilis*, and ascorbic acid displayed $ABTS^+$ scavenging activity with IC_{50} values of 67.46±1.45 µg/mL and 4.31±1.18 µg/mL, respectively. In comparison, *O. indicum* bark extract showed 50% inhibitory potential at 80.10±0.90 µg/mL against $ABTS^+$ scavenging assay [48]. The comparative analysis highlighted *M. pruriens var. utilis* significant $ABTS^+$ scavenging capacity compared to *O. indicum* seed extract, indicating its notable role in reducing oxidative stress. The antioxidant results affirm that *M. pruriens var. utilis* can protect deoxyribose in the presence of hydroxyl radicals and exhibit a higher potential for scavenging DPPH and $ABTS^+$ free radicals [9].

Effects of M. pruriens var. utilis, on α-glucosidase and α-amylase antidiabetic activity

In this study, in-vitro investigations into the antidiabetic activity of *M. pruriens var. utilis*, often focus on their effects on α-glucosidase and α-amylase enzymes. These enzymes play critical roles in carbohydrate digestion, and their inhibition can be a promising strategy for managing diabetes [62]. Researchers explore the potential of natural and synthetic substances to modulate α-glucosidase and α-amylase activity, aiming to retard the breakdown of complex carbohydrates and reduce postprandial glucose levels [4]. Substances exhibiting inhibitory effects on these enzymes may hold therapeutic promise in controlling blood glucose levels, providing a foundation for the development of antidiabetic agents. The identification of compounds with significant in-vitro antidiabetic activity contributes to ongoing efforts to discover effective treatments for diabetes through a better understanding of the molecular mechanisms involved in carbohydrate metabolism. The results are shown in Table 4.

In the α-glucosidase and α-amylase assays, *M. pruriens var. utilis* had IC_{50} of 28.34±1.41 µg/mL and 33.42±1.35 µg/mL, while the acarbose standard, showed IC_{50} of 28.81±1.23 µg/mL and 29.66±2.51 µg/mL, respectively. *M. pruriens var. utilis*, is a more potent α-glucosidase and α-amylase inhibitor. Therefore, based on the presented results and a comparison with other similar studies, *M. pruriens var. utilis* has an excellent potential to inhibit α-glucosidase, and α-amylase enzymes.

Effect of M. pruriens var. utilis and ASP on egg albumin protein denaturation

The in-vitro anti-inflammatory activity was studied using egg albumin protein denaturation method. Table 5 shows the percent inhibition of egg albumin protein

Table 5 Effect of ASP and *M. pruriens var. utilis* on egg albumin protein denaturation

Sample	Conc. (µg/ml)	Absorbance at 560 nm			% inhibition of egg albumin protein denaturation			% Inhibition (Mean ± SD)
		A	b	c	a	b	c	
ASP	100	0.53	0.51	0.53	14.52	17.74	14.52	15.59 ± 1.86
	200	0.49	0.46	0.45	20.97	25.81	27.42	24.73 ± 3.36
	300	0.44	0.42	0.41	29.03	32.26	33.87	31.72 ± 2.46
	400	0.37	0.36	0.37	40.32	41.94	40.32	40.86 ± 0.93
	500	0.16	0.15	0.13	74.19	75.81	79.03	76.34 ± 2.46
M. pruriens var. utilis	100	0.53	0.53	0.51	14.52	14.52	17.74	15.59 ± 1.86
	200	0.45	0.43	0.45	27.42	30.65	27.42	28.49 ± 1.86
	300	0.44	0.42	0.44	29.03	32.26	35.48	32.26 ± 3.23
	400	0.50	0.52	0.53	35.12	37.13	38.61	36.95 ± 1.43
	500	0.52	0.54	0.53	44.61	42.21	43.37	43.39 ± 0.97

Table 6 Effect of ASP and *M. pruriens var. Utilis* on hypotonicity induced hemolysis of RBC membrane

Sample	Conc. (µg/ml)	Absorbance at 560 nm			% of inhibition of RBC hemolysis			% Inhibition (Mean ± SD)
		a	b	c	a	b	c	
ASP	100	0.81	0.84	0.83	12.17	14.36	13.71	13.41 ± 0.91
	200	0.72	0.73	0.72	20.14	22.24	20.67	21.01 ± 0.89
	300	0.58	0.60	0.61	34.81	35.61	33.37	34.59 ± 0.92
	400	0.55	0.52	0.54	42.72	43.47	42.44	42.87 ± 0.43
	500	0.48	0.46	0.45	58.42	55.37	56.28	56.69 ± 1.27
M. pruriens var. utilis	100	0.80	0.78	0.79	17.75	16.32	18.37	17.48 ± 0.85
	200	0.68	0.65	0.66	24.16	22.33	25.03	23.84 ± 1.12
	300	0.65	0.63	0.63	29.42	30.22	28.11	29.25 ± 0.86
	400	0.58	0.57	0.55	34.42	35.61	34.22	34.75 ± 0.61
	500	0.50	0.48	0.53	41.81	40.44	39.72	40.65 ± 0.86

denaturation for different concentrations of *M. pruriens var. utilis* and aspirin. The *M. pruriens var. utilis* exhibited good anti-inflammatory properties that are comparable with standard aspirin. Protein denaturation is a process by which proteins lose their tertiary and secondary structures by application of external stress compounds such as strong acid, base, concentrated inorganic-organic salt. Protein denaturation is a well-documented causative of inflammation. During this process, the proteins lose their quaternary structure, thereby inducing aggregation, which activates deleterious inflammatory signals [62, 63].

It is well established that protein denaturation inhibitors' agents function by sup pressing different types of inflammatory mediators involved in the inflammation process. Denaturation of proteins is a well-documented cause of inflammation; drugs such as Phenylbutazone, salicylic acid, sodium diclofenac, and flufenamic acid show dose-dependent ability against protein denaturation. It has also been reported that various plant extracts and their isolated compounds showed good anti-inflammatory activity comparable to that of synthetic anti-inflammatory drugs [64].

Effect of M. pruriens var. utilis and ASP on hypotonicity induced hemolysis of HRBC

Hypotonic solutions can cause hemolysis, where red blood cells accumulate excessive fluids, leading to the rupture of their membranes. This damage makes the cells more vulnerable to harm from free radicals, which can induce lipid peroxidation [53]. This, in turn, increases membrane permeability, facilitated by inflammatory mediators. Stabilizing the membrane can prevent the release of serum proteins and fluids into the surrounding tissue [65]. Table 6 displays the percentage inhibition of hypotonicity induced hemolysis in human red blood cells treated with ASP and *M. pruriens var. utilis.*

The extracts displayed a concentration-dependent inhibition of hemolysis. The ethanolic seed extract of *M. pruriens var. utilis* may stabilize the membrane of red blood cells by preventing the release of lytic enzymes and other active inflammatory mediators.

Discussion

This study expands on our previous research, which highlighted the potential of the ethanol extract derived from *M. pruriens var. utilis* seeds to demonstrate robust in-vitro anti-diabetic, anti-inflammatory, and antioxidant properties. The evaluation of *M. pruriens var. utilis* involved assessing these attributes. Numerous studies

have focused on the simultaneous utilization of probiotic bacterial families, including *Lactobacillaceae* and *Bifidobacteriaceae*, along with phenolic compounds. Several polyphenols and their metabolites have been shown to enhance the growth of probiotics within the human intestinal tract [66]. α-glucosidase inhibitors can effectively impede the release of D-glucose from oligosaccharides and disaccharides present in complex carbohydrates within our diet. This retardation in glucose absorption results in reduced blood glucose levels following a meal, contributing to the mitigation of postprandial hyperglycemia [67]. Lipoxygenase (LOX) facilitates the reaction involving unsaturated fatty acids, leading to the generation of active lipid metabolites. These metabolites have been associated with the development of various disease conditions, including diabetes, in the context of pathogenesis [68].

M. pruriens var. utilis exhibited remarkable activity as α-glucosidase inhibitors, efficiently inhibiting the absorption of disaccharides. This dual functionality could play a role in augmenting the extract's effectiveness in preventing adipogenesis and combating obesity, ultimately resulting in substantial weight loss. In future studies, it is crucial to investigate the potential effects of the optimized extract on glucose and lipid metabolism in relevant tissues, such as skeletal muscle and the liver [69].

The results from the ABTS and DPPH assays indicate that *M. pruriens var. utilis* possesses antioxidant properties. Specifically, the DPPH inhibitory activity of *M. pruriens var. utilis* was observed to be consistent IC_{50} 63.34±2.27 µg/mL with the findings reported in a previous study IC_{50} 10.85 µg/Ml [14]. *M. pruriens var. utilis*, as assessed by the ABTS assay, displayed the characteristics of an effective antioxidant inhibitor. Comprehensive investigations were undertaken to encompass a diverse range of compounds. Unlike the DPPH assay, which exclusively quantifies hydrophobic antioxidants, the ABTS assay is specifically designed to measure the antioxidant activity of hydrophilic compounds [70]. A hypothesis posits an increase in oxidative stress among individuals with diabetes. The action of oxygen free radicals may trigger lipid peroxidation, setting in motion the glycation of proteins. Consequently, this process can deactivate enzymes, modify collagen structure and function, and disrupt basement membrane homeostasis. This sequence of events may lead to a range of complications, both immediate and prolonged, ultimately culminating in the onset of diabetes [71].

Inflammation represents an intricate bodily response aimed at addressing damages in cells, tissues, and organs resulting from stimuli like mechanical injuries, allergens, burns, microbial infections, and other toxic substances. This process activates macrophages, leukocytes, mast cells, and complement factors [72]. Inflammation is linked to diabetes, and the current approach to treating DM p/lkrimarily relies on parenteral insulin and oral anti-diabetic drugs. Given the significant side effects associated with oral hypoglycemic agents, there is a necessity to explore novel anti-diabetic compounds with minimal side effects and heightened therapeutic efficacy [73, 74].

The impact of *M. pruriens var. utilis* on HRBC membrane stabilization was assessed by quantifying hemoglobin levels in samples following the inhibition of egg albumin protein denaturation at elevated temperatures. *M. pruriens var. utilis* demonstrated an anti-inflammatory effect by dose-dependently decreasing inhibition of egg albumin protein denaturation at high temperatures. As a benchmark, ASP was employed as the standard drug for comparison [75]. *M. pruriens var. utilis* achieved the highest level of percentage inhibition at a concentration of 500 µg/ml=43.39±0.97%.

Conclusion

The investigation yielded noteworthy findings, indicating that the ethanolic extracts derived from *M. pruriens var. utilis* seeds effectively inhibited HRBC membrane stabilization, antidiabetic and displayed robust antioxidant properties by scavenging both DPPH and ABTS radicals. Additionally, the extracts exhibited a dose-dependent inhibition of carbohydrate-metabolizing enzymes. These collective results strongly suggest the substantial medicinal potential of *M. pruriens var. utilis* seed ethanolic extract as a versatile agent with anti-inflammatory, antioxidant, and antidiabetic properties. However, to gain a comprehensive understanding of the precise mechanisms underlying these activities, further efforts are warranted for the isolation and characterization of bioactive compounds from the *M. pruriens var. utilis* seed extracts. Such endeavors are crucial for unraveling the intricate therapeutic pathways and laying the foundation for the development of novel and effective therapeutic agents.

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Author contributions

Conceptualization and supervision: Amita Verma; Data collection: Jagat Pal Yadav, Abhishek Singh, Seema Yadav; Study design: Amita Verma and Jagat Pal Yadav Writing the manuscript: Jagat Pal Yadav; Statistical analysis: Jagat Pal Yadav and Prateek Pathak; Review and final editing of the manuscript: Jagat Pal Ydav, Narahari N. Palei, Amita Verma, and Prateek Pathak.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
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