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PHYTOCHEMICAL EXTRACTION, SCREENING AND ANTIMICROBIAL AND IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF PLANT *MUCUNA PRURIENS*

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Abstract: Mucuna pruriens, also known as velvet bean or cowhage, is a leguminous plant native to tropical Africa and Asia, esteemed for its medicinal properties in Ayurvedic medicine. Rich in bioactive compounds like L-DOPA, it shows promise in treating neurological disorders such as Parkinson's disease. Its vine-like growth, distinctive lavender to purple flowers, and irritating pod hairs define its morphology. While it offers antioxidant, anti-inflammatory, and immunomodulatory benefits, caution is advised due to potential side effects and drug interactions. Cultivated widely in Indian states like Kerala and Karnataka, it serves cultural and therapeutic roles, including nerve tonic and aphrodisiac. Its contributions to soil fertility and sustainable agriculture are also recognized. Ongoing research aims to further understand its potential in healthcare and agriculture.

Phytochemical extraction, screening, and different in-vitro free radical scavenging activities and antimicrobial activity of this plant show that we can use this pant as a potential bioactive molecule because this plant possesses good free radical scavenging activity and antimicrobial activity.

Keywords: *Mucuna pruriens*, phytochemical screening, Invitro free radical, scavenging activity, DPPH assay, H2o2 Assay.

Introduction:

Mucuna pruriens, also known as velvet bean or cowhage, is a leguminous plant native to tropical regions of Africa and Asia [1]. It is renowned for its medicinal properties and has been used for centuries in traditional Ayurvedic and herbal medicine systems.

The plant is characterized by its vine-like growth habit, habit with long, twining stems and clusters of lavender to purple flowers. Its most distinctive feature is the presence of small hairs on its pods, which can cause intense itching upon contact, hence the name “pruriens” is derived from the Latin word for itching [2]. Mucuna pruriens is valued for it has a high concentration of bioactive compounds, particularly L-DOPA (levodopa), a precursor to the neurotransmitter dopamine. This is a result of the role of dopamine in motor control and mood regulation, it is a significant herb in the treatment of various neurological disorders, including Parkinson's disease, due to dopamine's role in motor control and mood regulation [3]. Mucuna pruriens contains a variety of other bioactive compounds, including alkaloids, flavonoids, and sterols, which contribute to its antioxidant, anti-inflammatory, and immunomodulatory effects [4]. These properties make it potentially beneficial for supporting possible to support cardiovascular health, enhancing fertility, and strengthening the immune system. However, it's important to note It should be noted, however, that Mucuna pruriens may also have side effects and interactions with certain medications, particularly medicines, especially when consumed in high doses or for extended long periods.

In India, states like Kerala, Karnataka, Tamil Nadu, Andhra Pradesh, Telangana, Maharashtra, Madhya Pradesh, Odisha, West Bengal, and Assam are among the most popular locations for Mucuna pruriens. It thrives in warm, humid settings and frequently occurs as a weed in agricultural fields, along roadsides, and in waste areas [5].

For thousands of years in India, Mucuna pruriens has been farmed and used in traditional Ayurvedic medical practices due to its cultural value and therapeutic benefits. The plant is revered in Ayurveda for its numerous health advantages, including usage as a nerve tonic, aphrodisiac, and therapy for illnesses such as Parkinson's disease, male infertility, and neurological problems. It is also known that Mucuna pruriens assists in soil development and sustainable agriculture [6].

This herb is commonly used in Ayurveda, an ancient Indian medical practice. This study covers the morphology, traditional phytochemical usage, pharmacological properties, and analytical methodologies of this plant, taking into consideration recent significant results.

BOTANICAL DESCRIPTION:

Mucuna pruriens, recognized as an annual climbing shrub, exhibits remarkable characteristics throughout its growth cycle. Initially, as a young plant, it is densely covered with fuzzy hairs [7], gradually losing them as it matures, revealing a smoother surface. The plant's vines can extend to impressive lengths, surpassing 15 meters, providing ample support for its climbing habit. [8]. The leaves, typically tripinnate, vary in shape from oval to reverse ovate, rhombus-shaped, or broadly ovate, featuring sharp tips and deeply serrated edges. During its immature stage, both surfaces of the leaves are adorned with fine hairs, contributing to its distinct appearance [9].

In addition to its foliage, Mucuna pruriens produces striking axial panicles of flowers, adding to its ornamental allure. Ranging from 15 to 32 cm in length, these plants bear numerous blooms, accompanied by leaves measuring approximately 12.5 mm long [10] The flowers themselves, bell-shaped and smooth, exhibit a vibrant array of hues from purple to white, enhancing the plant's aesthetic appeal. Notably, the sepals are equal to or longer than the petals, further defining its floral structure. The diminutive flag petal, measuring merely 1.5 mm, contrasts with the impressive wings extending between 2.5 to 3.8 cm [11].

As Mucuna pruriens progresses through its lifecycle, it transitions to the fruiting stage, developing leguminous pods devoid of wings. These pods, ranging from 4 to 13 cm in length and 1 to 2 cm in width, are covered in a hairy husk, encapsulating up to seven seeds [12]. This culmination of reproductive structures signifies the completion of the plant's lifecycle, embodying its botanical significance and contributing to its ecological role.

MATERIALS AND METHODS

Collection of Seeds:

Mucuna pruriens (M. pruriens), routinely known as Mucuna or velvet bean, is a popular medicinal plant belonging to the legume family and common in tropical and subtropical areas worldwide. In India, it is widely found in the eastern part of the country. The seeds of *M. pruriens* were collected from the Local market of Vijayapura, Karnataka, India. The seeds of the plant were given for authentication by the Department of Botany KCP Science College, Vijayapura, Karnataka. The seeds were dried under shade and made into a powder [13].

Preparation of Plant Extract

A 20-gm sample of powdered material was sieved through a number 40 sieve. The sieved powder underwent hot percolation in a Soxhlet apparatus 200 ml of using 99.9% ethanol as a solvent for 24 hours at a temperature range of 50-60°C, the solvent was removed by rotary evaporator [14].

Phytochemical Analysis:
Phytochemical analysis to screen the plants for the presence of alkaloids, flavonoids, glycosides, reducing sugars, saponins, steroids, triterpenoids, tannins, carbohydrates and proteins were performed according to the method described by Sofowora (1993) and Evans (1998) [15,16]

Test for Alkaloids:
Four test tubes were used for each of the different drug samples. Few drops of the following reagents manager's reagent, Drangendorff's reagent. Wanger's reagent, Hanger's reagents were added. The presence of precipitate in at least 3 or all of the above reagentsindicate the presence of alkaloids (Evans 1989).

Test for Carbohydrates:
A few drops of Molisch's reagent were added to 2ml of each of the extracts in two tubes. A small quantity of concentrated sulphuric acid was then added and allowed to form a lower layer. A purple ring at the interface of the liquids indicates the presence of carbohydrates. Each mixture was then shaken and allowed to stand for 2 minutes and

diluted with 5ml of water. A purple precipitate also showed the presence of carbohydrates (Evans, 1989).

Test for proteins:
To the different extracts, 40% NaoH+dilute copper sulphate solution was added blue color indicates the presence of proteins.

Test for Saponins:
A small quantity of the different extracts was boiled. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of the distilled water in a test tube. The test tube was corked and shaken vigorously for about 30 seconds, then it was allowed to stand for half an hour. A honeycomb fourth was an indicator of the presence of saponins (Sofowora, 1993.

Test for Tannins (Ferric chloride test):
A portion of the different extracts was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green color indicates the presence of tannins (Evans,1989).

Test for steroids (Salkwoski's test):
5 drops of concentrated H2SO4 was added to 1ml of each extract and observed for red coloration (Waterman 1993).

Test for Flavonoids (Shinoda test):
To the different extracts were added four pieces of magnesium fillings followed by a few drops of concentrated hydrochloric acid. A pink or red color indicates the presence of flavonoids.

Test for Glycosides:
A portion of the aqueous extract was added fehlings reagent and boiled for 2 minutes.
A brick-red coloration indicates the presence of glycosides.
Test for Reducing sugars:
To the 1ml of each extract 2ml of Fehling's solution A & B is added mixed and observe for formation of red cuprous oxide precipitate is an indication of reducing sugars.

Antibacterial activity of Plant Extract

The efficacy of ethanol extracts from Mucuna pruriens seeds against pathogenic bacteria like E-coli and Streptococcus sp, Klebsiella planticola, and Klebsiella pneumoniae was assessed using the agar well diffusion method [17]. The bacterial cultures were spread onto Muller Hinton agar plates, and three wells were created in each plate. Different volumes of Mucuna pruriens seed extract (2.5, 5, and 10 µL) were added to the wells, while commercial antibiotic discs served as controls. The plates were then incubated at 37°C for 24 hours. Following incubation, the presence or absence of inhibition zones around the wells was observed and recorded. This procedure was repeated thrice for accuracy [18].

Invitro Free Radical Scavenging Assay:

DPPH Assay

A mixture of 0.5ml of varying concentrations (20,40,60,80,100ug\ml) of Mucuna pruriens seed extract and the standard solution was added to 2ml of working solution (0.2mM) of DPPH. The mixture was then left to incubate for 30 minutes at room temperature in a dark environment [19]. The control utilized in this instance had the reagent but no extract or medication. The Labman UV Visible Spectrophotometer (LMSP-UV1200PC) was used to detect the absorbance at 517 nm. The following formula was used to determine the percentage of antioxidant or radical scavenging activity:

% Antioxidant activity = [Ac-As\Ac] x100

Where Ac and as are the absorbance of the control and sample, respectively. Ascorbic acid is been used as the reference standard. The results were analyzed in replica [20].

HYDROGEN PEROXIDE ESTIMATION:

A 40 mM hydrogen peroxide solution was made in phosphate buffer (pH 7.4). The final volume was adjusted to 1 milliliter using distilled water after Mucuna pruriens seed extract (25, 50, 100, 200, and 400 ug/ml) in distilled water was added to 0.6 milliliter of hydrogen peroxide solution (40 mM). The drug extract was absent from the control, which nevertheless included the reagent. A blank solution including phosphate buffer without hydrogen peroxide was used to measure the absorbance of hydrogen peroxide at 230 nm using a Labman UV visible spectrophotometer (LMSP-UV1200pc) ten minutes later [21]. Using the following formula, the proportion of hydrogen peroxide scavenging of both standard compounds and ethanol extract was determined:

%Scavenged [H2O2] = [(Ac-As) \Ac]x100.

Where Ac is the absorbance of control and as is the absorbance of the sample of extract or standards. Ascorbic acid was used as a reference standard. The results were analyzed in triplicate.

Result and Discussion:

- **Preparation of Plant Extract**

  The principle behind Soxhlet extraction is based on the repeated cyclical extraction and distillation of a solvent through a solid sample.

20 gm of Mucuna pruriens seeds powder extract yield 1.35gm of extract which is used further for different tests.

❖ **Preliminary phytochemical analysis Result**

Table 1 shows the different preliminary phytochemical analysis resul

Sr. No.	Chemical test	Ethanol extract
1.	Test for steroids	+ +
2.	Test for alkaloids	+ +
3.	Test for flavonoids	++
4.	Test for glycosides	+
6.	Test for carbohydrates	+ +
7.	Test for proteins	+ +
9.	Test for tannins	+ +
10.	Test for triterpenoids	+

Table 1: preliminary phytochemical analysis results

Antibirobial activity

Antibacterial assay was carried out by agar well diffusion method after 18 to 24 hrs of incubation. Table 2 shows the result of the Zone of inhibition of different concentrations of ethanol extract of Mucuna pruriens by the different methods

S.No	Bacterial species	Zone of Inhibition		
		125	250	500
01	E-coli	0.89	0.99	1.1
02	Streptococcus sp, Klebsiella planticola	0.79	0.85	0.9
03	Klebsiella pneumoniae	0.99	1.25	1.55

Table 2: Antimicrobial activity

Invitro Free Radical Scavenging Assay

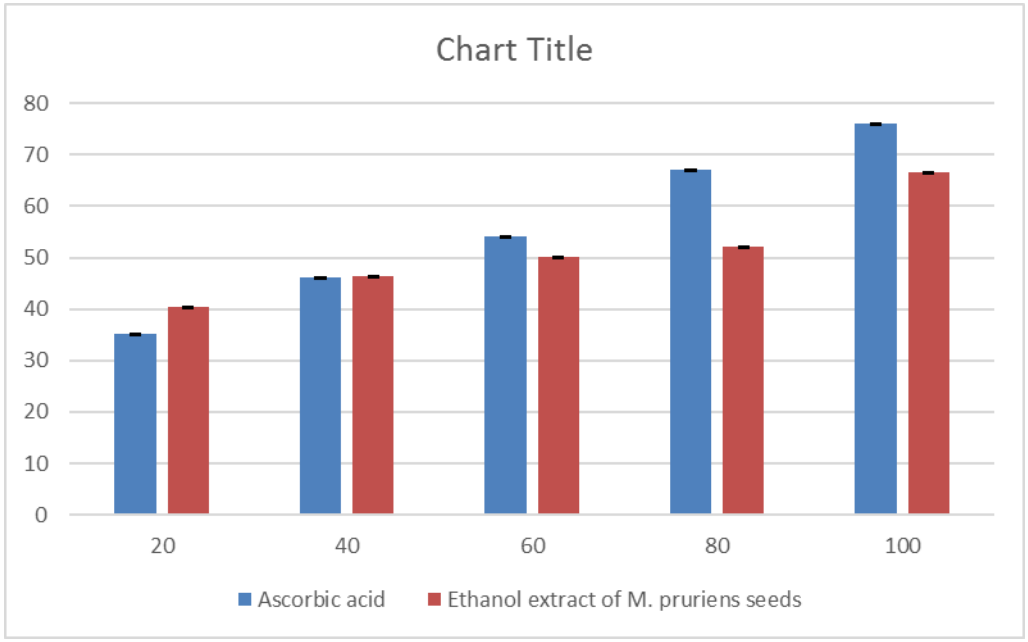
Reactive oxygen species (ROS) raise the risk of degenerative illnesses by causing oxidative stress, which can harm cells and genes. Even though the body may not produce enough antioxidants naturally, taking antioxidant supplements is advised. Natural antioxidants found in fruits, vegetables, and whole grains are preferred over synthetic antioxidants due to their lack of toxicity. Mucuna pruriens has strong antioxidant qualities due to its effective

extraction methods. It is high in phenolic compounds, sterols, alkaloids, and betanin. As construction increases the scavenging activity also increases and this activity is compared with the standard which is ascorbic acid as shown in table 3 and graph 1.

Table 3: 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging ability (DPPH) assay

Concentration (µg/ml)	**% Scavenging activity**	
	Ascorbic acid	***Ethanol extract of M. pruriens* seeds**
20	35.06 ± 0.01	40.5± 0.01**
40	46.03 ± 0.01	46.4± 0.01
60	54.05 ± 0.01	50.1± 0.01**
80	67.12 ± 0.02	52.03 ± 0.01**
100	76.03 ± 0.03	66.54 ± 0.01**

Data is presented as the mean standard error of the mean (n = 3). The values were significantly different when compared to the standard at the same concentration (standard-ascorbic acid) ** $p < 0.01$.



Graph 1 DDPH Assay of seed extract of *Mucuna pruriens*

H2O2 Assay calculation

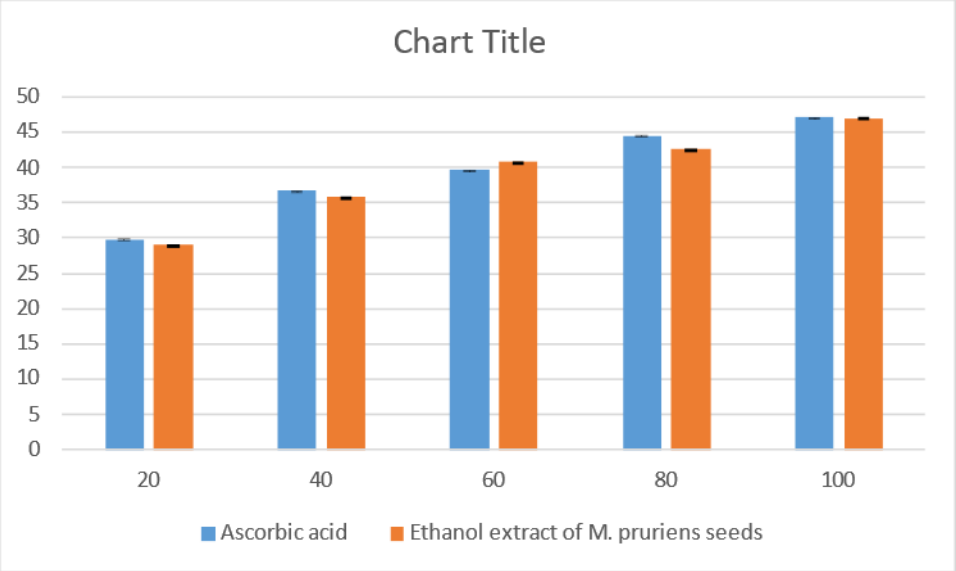
H2O2 is highly important because of its ability to penetrate biological membranes. H2O2 itself is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in the cells. Thus, removing H2O2 is very important for the protection of food systems. The hydrogen peroxide (H2O2) scavenging activity of Mucuna pruriens extract was carried out along with reference standard ascorbic acid. The hydrogen peroxide (H2O2) scavenging activity of Mucuna pruriens along with standard reference ascorbic acid is depicted in Table 4, graph 2.

Table 4: Hydrogen Peroxide Scavenging Activity

Concentration (**µg/ml**)	% Scavenging activity	
	Ascorbic acid	***Ethanol extract of M. pruriens* seeds**
20	29.82 ± 0.01	28.96± 0.01
40	36.7 ± 0.01	35.8± 0.01
60	39.6 ± 0.01	40.7± 0.01
80	44.5 ± 0.02	42.60± 0.01
100	47.03 ± 0.03	47.00 ± 0.01

Data is presented as mean ± standard error of the mean (n = 3). Values not significantly different when compared to the standard (ascorbic acid)

Table 4: H2O2 assay



Graph 2 : H2O2 Assay of seed extract of Keywords: *Mucuna pruriens.*

Conclusion:

This study on Mucuna pruriens seed extract sounds promising and multifaceted. By conducting a phytochemical investigation, researchers are delving into the constituents of the plant, which could have implications for both traditional and modern medicine. The presence of various compounds like alkaloids, carbohydrates, flavonoids, and others indicates a rich chemical profile with potential health benefits.

The antibacterial activity against different strains of bacteria suggests the extract could be explored further as a natural antimicrobial agent. Additionally, the antioxidant properties observed through assays like DPPH and H2O2 indicate its potential for combating oxidative stress, which is implicated in various health issues.

Integrating seed extract as a bio-supplement alongside conventional medicine is an intriguing approach that could enhance treatment outcomes. This blend of traditional and modern approaches not only adds scientific depth but also holds promise for more holistic healthcare solutions.

Further research could focus on isolating and characterizing specific bioactive compounds within the extract to better understand their mechanisms of action and potential therapeutic applications. Additionally, clinical studies could investigate the efficacy and safety of incorporating Mucuna pruriens seed extract into treatment regimens for various health conditions.

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