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Antioxidant and Phytochemical Analysis of Leaf Extract of Velvet Bean (*Mucuna pruriens* (L): An Experimental Analysis in Wistar Albino Rats

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ABSTRACT: Several disorders have pathophysiologies that have been linked to oxidative stress and a deficient antioxidant system. Seeds of *Mucuna pruriens* are used to treat and manage a range of disorders, were examined for antioxidants and phytochemicals, but there are very little or no information on the presence of phytochemicals and antioxidants in the leaf extracts of *Mucuna pruriens*. Based on their power to block the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, the extracts' potential as free radical scavengers were evaluated. The extract's phytochemical content was tested using ethanol and distilled water. To test the antioxidant activity of the extracts, malondialdehyde (MDA). Superoxide dismutase (SOD). and catalase (CAT) levels in the liver of albino rats treated with carbon tetrachloride (CCl_4) were evaluated. A single injection of CCl_4 (3 mL/kg body weight) was given to the animals after they had received the extract for six consecutive days at a dose of 400 mg/kg body weight each. The most extensively utilized antioxidant was vitamin C. The extract contained saponins, tannins, anthraquinones, terpenoids, flavonoids, and alkaloids according to a phytochemical analysis of *Mucuna pruriens*. Extract reduced the DPPH radical in a concentration-dependent manner and it is statistically equal to vitamin C (P >0.05) in terms of inhibition. In comparison to the positive control, the extracts markedly decreased (P 0.05) the levels of liver MDA while markedly boosting (P 0.05) the levels of SOD and CAT. These findings call attention to the extract's antioxidant capabilities and may shed light on the therapeutic usage of this plant.

Keywords: Phytochemicals, DPPH, MDA, SOD, Traditional Medicine, *Mucuna pruriens*, Antioxidants, CNS Activities, Adverse drug reactions, Vitamin-C, Albino rats.

INTRODUCTION

Since the dawn of time, people have used plants to prevent and treat diseases. Recent studies have revealed that plants have healing properties (Salmerón *et al.,* 2020). Different parts of the plant are used traditionally in many varieties of diseases in India (Mbuni *et al.,* 2020). *Mucuna pruriens* (Velvet Bean) belongs to Fabaceae, whose seeds are believed to treat a variety of diseases such as parkinsonism, aphrodisiacs, snake bites, diabetes mellitus cancer, epilepsy, helminthiasis, diarrhoea, scorpion stings, malaria, ulcers, infertility, and elephantiasis by consuming the seed extract of Velvet Bean (DeFilipps and Krupnick 2018).

But based on the literature there are very few conditions treated with *Mucuna pruriens* leaves. Phytochemicals are bioactive substances found in plants that protect against disease via macronutrients. Non-nutritive substances known as secondary metabolites affect its natural colour and taste. There are many varieties of phytochemicals with free radical scavenging action; they help to control the risk of multiple varieties of fatal conditions. Phytochemicals, including carotenoids, flavonoids, and phytosterols, are present in plants (Maharaj *et al.,* 2022). Reactive oxygen-free radicals have been connected to several diseases and ageing. The body produces free radicals, which cause inflammation, and lipid peroxidation contributes to oxidative stress and tissue damage. Malondialdehyde (MDA) and 4-hydroxyalkanals are formed during the dissolution of polyunsaturated fatty acid peroxides (Forman and Zhang 2021). Superoxide anion is quickly converted to oxygen and hydrogen peroxide with the aid of the enzyme superoxide dismutase (SOD) (Pizzino *et al.,* 2017). Many physiological and pathophysiological processes rely on the superoxide radical, Catalase (CAT) are the enzymes responsible for converting hydrogen peroxide to water where oxygen speeds up, as a result, H_2O_2 accumulates in the body and oxidises proteins, lipids, and DNA, resulting in mutagenesis and cell death. This enzyme is a crucial part of the body's defence against oxidative damage, as

it is involved in the conversion of toxic superoxide radicals to non-toxic ones (Pizzino *et al.,* 2017).

MATERIAL AND METHODS

Extract collection, extraction & authentication: In April 2019, fresh leaves were collected from a village in Tamil Nadu, India, called Pollachi. They were identified by Mr. Rambabu, Professor and HOD of Botany, Vikas Degree College, Vissanapeta, Andhra Pradesh. The leaves were washed with fresh water and immediately used. The extraction was done separately with distilled water and ethanol. The extract was obtained by removing solvents from the leaves using rotary evaporators.

Phytochemical Evaluation: The following phytochemicals were identified in *Mucuna pruriens* leaf extract: alkaloids, anthraquinones, tannins, saponins, terpenoids, flavonoids, and cardiac glycosides. These phytochemicals have been found to possess various medicinal properties, such as antioxidant, anti-inflammatory, and neuroprotective effects. Further research is needed to explore their potential therapeutic applications.

Animal care & division: Twenty-four adult Wistar albino rats weighing between 150 and 250 grams were brought from the Animal House Facility, Department of Pharmacology, Karuna Medical College, Palakkad, India. They were divided into four groups of six rats each and kept in the animal house facility for seven days to acclimatise. Rats were given free access to food and water before and during the experiment.

Distilled water was used to prepare the extraction solution

Group-1: Distilled water (control) (q.s.)
Group-2: MP extract (400 mg/kg) p.o. + CCl_4
Group-3: Vitamin C (10mg/ml) p.o. + CCl_4
Group-4: CCl_4 (3.0mL/kg) i.p
Group-5: Distilled water with olive oil. (q.s.)

A. Antioxidant Property Assessment: The antioxidant activity of the extracts was investigated by two methods:

(i) Substitute vitamin C as a reference antioxidant.
(ii) Measuring MDA, SOD, and CAT levels by inducing CCl_4

1. Substituting vitamin C as a reference antioxidant: Vitamin C has antioxidant activity. The extracts free radical scavenging properties against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals were measured at 517 nm. The extracts and concentrations of vitamin C used were 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 mg/ml. The % of free radical scavenging activity was calculated using the following formula:

Inhibition of DPPH FRS activity in %
= (A1-A2) / A1 × 100
A1: Absorbance of the Control
A2 : Absorbance of the MP Extract

2. Measuring MDA, SOD, and CAT levels by inducing CCl_4 (Ohkawa *et al.,* 1979). The endangering the liver groups 2 to 5 received a single intraperitoneal dose of 3.0 mL/kg body weight of CCl_4 and olive oil on the seventh day (1:1). Blood samples were collected from the animals via cardiac puncture under mild anaesthesia with diethyl ether after an overnight fast. Anticoagulant-free specimen bottles were used for the samples. Furthermore, the liver was quickly removed, perfused with cold normal saline, and homogenised in 0.25 M sucrose in phosphate buffer (0.2 M, pH 7.4). Further, the samples were tested to determine MDA, SOD (Superoxide) and CAT (Catalase) levels in the liver to determine oxidative damage and *Mucuna pruriens*' antioxidant properties.

Statistical analysis: Statistical analysis was carried out using analysis of variance (ANOVA). Dunnett's multiple comparisons test (P 0.05) was used to compare the means.

RESULTS AND DISCUSSION

Table 1 presents the phytochemical analysis of leaf extracts. Flavonoids, steroids, alkaloids, PC-phenolic compounds, quinine, glycosides, and tannins were found in the extract. Table 2 & Fig. 1 displays the amount of DPPH inhibition. It appears that all of the extracts possess antioxidant properties based on their ability to treat the above conditions when subjected to the inhibition of DPPH. (Kedare and Singh 2011) describe the method as quick, easy, affordable, and popular for determining a compound's capacity to act as a hydrogen donor or free radical scavenger.

Table 1: Results of Phytochemical Analysis of *Mucuna pruriens* with Different Solvents.

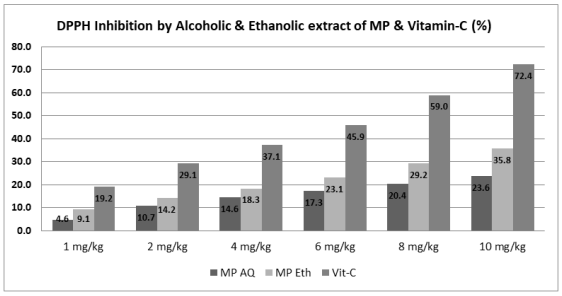
Phytochemical analysis of *Mucuna pruriens*									
Sr. No.	**Solvents**	**Phytochemicals**							
		F	St	A	S	PC	Q	G	T
1.	Ethyl Acetate	✓	×	✓	×	✓	×	×	✓
2.	Ethanol	✓	×	✓	✓	✓	×	✓	✓
3.	Acetone	✓	×	✓	×	✓	×	×	✓
4.	Hexane	✓	×	✓	×	✓	×	×	✓
5.	Petroleum ether	✓	×	✓	×	✓	×	✓	✓
6.	Chloroform	✓	×	✓	×	✓	×	✓	✓
7.	n-butanol	✓	×	✓	×	✓	×	✓	✓
8.	Methanol	✓	×	✓	×	✓	✓	✓	✓
9.	Water	✓	×	✓	✓	✓	×	✓	✓
10.	n-propyl alcohol	✓	×	✓	×	✓	×	✓	✓
11.	Diethyl ether	✓	×	✓	×	✓	✓	✓	✓
F-Flavonoids, St-Steroids, Alkaloids, PC-Phenolic Compounds, Saponins, Quinine, G-Glycosides, and Tannins									

Table 2: DPPH Inhibition by Alcoholic and Ethanolic Extracts of MP and Vitamin-C (%).

Dose in mg/ml	Mucuna (water extract)	Mucuna (Ethanolic Extract)	MP+Vitamin -C
0.1	4.667 ± 0.92	9.143 ± 0.97	19.24± 1.8
0.2	10.72 ± 1.4	14.21 ± 0.94	29.14 ± 1.05
0.4	14.56 ± 1.85	18.32 ±1.99	37.13 ± 2.55
0.6	17.34 ± 1.65	23.12 ± 1.49	45.85 ± 9.77
0.8	20.37 ± 6.45	29.24 ± 4.96	58.96 ± 3.49
10	23.61 ± 3.92	35.83 ± 9.9	72.37 ± 7.7
Values mentioned in the mean SD, n = 3,			

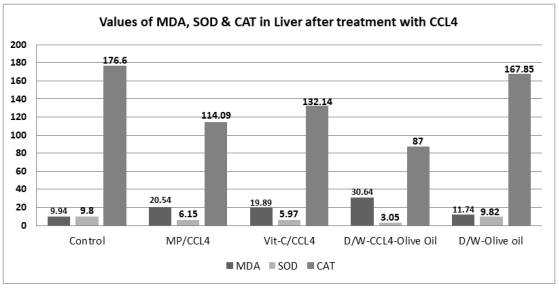
Table 3: Post-treatment values of MDA, SOD, and CAT in the liver.

Group	MDA n mols/mg Protein	SOD Units/ mg Protein	CAT Units/ mg Protein
Control	9.94 ± 0.85	9.8 ± 1.24	176.6 ± 15.08
MP/ CCl_4	20.54 ± 2.73	6.15 ± 0.98	114.09 ± 4.64
Vit-C/ CCl_4	19.89 ± 2.65	5.97 ± 0.48	132.14 ± 2.05
D/W-CCl_4-Olive Oil	30.64 ± 2.81	3.05 ± 0.42	87 ± 9.830
D/W-Olive oil	11.74 ± 0.96	9.82 ± 1.24	167.85 ± 15.52
n = 6, values given in (mean SD).			



(X-Axis indicates the dose of extract in mg/Kg & Y-Axis indicates the percentage of inhibition)

Fig. 1. Inhibition of DPPH by *Mucuna pruriens* (Aqueous & Ethanolic extract).



(X-Axis: The substances used for the experiment; Y-Axis: The values of MDA, SOD & CAT)

Fig. 2. The values of MDA, SOD & CAT in Liver after the treating the animals with CCl_4.

Antioxidants in intricate biological structures can also be quantified using this method using both liquid and solid samples (Munteanu and Apetrei 2021). Percentage of DPPH inhibition by *Mucuna pruriens* was significantly reduced ($P < 0.05$) compared with vitamin C. *Mucuna pruriens* and vitamin C inhibited the enzyme in a dose-dependent manner. An analysis of a compounds reactivity using the DPPH test is done by using a stable free radicle called DPPH. Its visible absorption band is at 517 nm and can be measured using method of (Baliyan *et al.,* 2022). The odd electron pairs off in the presence of a free radical scavenger, which causes the absorbance to decrease and changes the colour of the DPPH solution from deep violet to light yellow. The degree of the absorbance decrease reveals the antioxidant (radical scavenger) activity of the substance (Shekhar and Anju 2014) (Table 3 and Fig. 2). How the extracts impacted the levels of CAT, SOD, and MDA in the liver is shown in Table 3 and Fig. 2. The MP and Vit-C groups showed significantly greater MDA levels and lower levels of SOD and CAT activity when compared to the untreated control group, respectively (P 0.05). This is consistent with the finding of CCl_4 hepatotoxicity (Singh *et al.*, 2017). The outcomes were altered and reversed by pre-treating with vitamin C or leaf extracts. Compared to the control groups, the pre-treated groups had considerably reduced MDA concentrations. Yet, compared to the positive control, the pre-treated groups' SOD and CAT activities were noticeably higher. The antioxidant properties of the extracts are supported by the significant increase in the levels of SOD and CAT activities, indicating their potential use as natural sources of antioxidants. These findings support the antioxidant effects of the extracts. (Singh *et al*., 2017) reported the finding of CCl_4 hepatotoxicity. The results were altered and reversed by pre-treating with leaf extracts or vitamin C. MDA concentrations were substantially lower in the pre-treated groups than in the control groups. Yet, compared to the positive control, the pre-treated groups' SOD and CAT activities were noticeably higher. These findings back up the extracts' antioxidant properties.

Oxidative stress and free radical injury are the main causes of liver tissue damage. Antioxidant enzymes, which halt degeneration, serve as the first line of defence against this form of damage (Cichoż-Lach and Michalak 2014). Studies have shown that diets rich in antioxidants can help reduce oxidative stress and protect liver function. Additionally, lifestyle changes such as reducing alcohol consumption and increasing physical activity can also help prevent liver damage. Monitoring oxidative damage and lipid peroxidation can be done by looking at MDA levels in the liver. Lipid peroxidation is one of the fundamental processes through which free radicals harm tissue (Atiba *et al.*, 2016). One important family of compounds that act as major antioxidants or free radical scavengers are plant phenolics, which include flavonoids and tannins (Tungmunnithum *et al*., 2018). Similar to how vitamins

work, terpenoids also regulate metabolism and act as antioxidants (Grassmann, 2005). The antioxidant capabilities of velvet bean (*Mucuna pruriens*) leaf extract may have a substantial impact on how it is used in disease prevention and therapy. Antioxidants protect cells and vital biomolecules from the harmful effects of free radical oxidative stress. By eliminating free radical intermediates, they prevent subsequent oxidation processes and stop free radical-caused chain reactions (Lobo *et al.*, 2010). The extract's pattern of DPPH inhibition is supported by these findings. The existence of the identified phytochemicals may be the cause of the extract's antioxidant action.

CONCLUSIONS

This research is important as it could lead to the development of new drugs or supplements that can help prevent or treat diseases associated with oxidative stress. Further studies are needed to fully understand the potential health benefits of these leaves and their antioxidant properties.

FUTURE SCOPE

The presence of phytochemicals and antioxidants has a key role in tissue protection and is highly helpful in treating neurobehavioral disorders like anxiety, depression, and dementia with the leaves of *Mucuna pruriens* without any adverse drug reactions as these are natural substances with no side effects. Further research on the effectiveness of *Mucuna pruriens* in treating these disorders can lead to the development of new natural remedies and drugs that can provide relief to patients without the harmful side effects of synthetic drugs. Additionally, exploring the potential of other plants with similar properties can expand the range of natural treatments available for neurobehavioral disorders.

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Conflict of Interest. None.

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