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Original Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* DPPH ASSAY OF ETHANOLIC EXTRACT OF MUCUNA PRURIENS SEED

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ABSTRACT

Objective: To detect the bioactive phytoconstituents of Mucuna pruriens seed extract and to estimate invitro antioxidant activity.

Methods: Preliminary phytochemical analysis were done to determine the active phytochemical constituents and DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay method was used for the estimation of *In vitro* antioxidant activity.

Results: Preliminary phytochemical analysis of Mucuna pruriens seed extract showed the presence of chief bioactive components like phenols, flavonoids, tannins, alkaloids, triterpenes and phytosterols. In antioxidant assay, Mucuna pruriens seed extract exhibited a maximum DPPH radical scavenging activity of 72.95% against the standard control with a maximum activity of 93.24% for the same concentration (0.2 mg/ml). This is a clear indication of strong antioxidant property of the drug.

Conclusion: Mucuna Pruriens seeds are abundant source of natural antioxidants, and important bioactive phytoconstituents.

Keywords: Mucuna pruriens, Reactive oxygen species, *In vitro* antioxidant activity, DPPH assay, Natural antioxidants, Flavonoids

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INTRODUCTION

Since prehistoric times, plants have been a plentiful source of drugs. Nowadays medicinal plants continue to serve as an important source of novel bioactive compounds which revolutionized the treatment of wide spectrum of diseases. The continued search for plant secondary metabolites as natural antioxidants has gained momentum in recent years.

In living systems, aerobic metabolism and environmental stresses generate free radicals, mainly reactive oxygen species (ROS) [1]. Amplified ROS levels can damage structure of biomolecules and in turn alters their functional characteristics, lead to cellular dysfunction and even cell death [2]. The cumulative effect of increased ROS induces oxidative stress in organ systems and play a crucial role in the pathogenesis of cancer, cardiovascular diseases and various age related and neurodegenerative disorders like Parkinson's disease [3-5]. These Cellular ROS are regulated by interaction of complex antioxidant machineries in living systems [6]. Nature itself has bestowed living systems with numerous antioxidant molecules, that enhances antioxidant capacity of the plasma and minimise the opposing effects of free radicals in living system thus aiding as an important therapeutic agent against degenerative diseases [7].

Mucuna Pruriens Linn. belonging to Fabaceae family is one of the prevalent and broadly used drug in the alternate system of medicine. The seeds of M. pruriens were found to be therapeutically effective in various conditions like impotence and neurological disorders [8]. Previous research studies on the M. pruriens seeds reported about the antidiabetic, anti-inflammatory, antipyretic, antiparkinsonian and aphrodisiac properties [9, 10]. The efficacy of its seed in degenerative disorders irradiates a wide area of research by exploring the multifaceted pharmacotherapeutics. Based on these ethnomedicinal leads, the present study was undertaken to determine the phytoconstituents and to evaluate the antioxidant activity of Mucuna pruriens seed extract.

MATERIALS AND METHODS

Collection and authentication of plant material

The seeds of Mucuna pruriens were obtained from Department of Dravyaguna Vijnana, VPSV Ayurveda College, Kottakkal. The drug was identified and authenticated by Dr. Sreekala. K, Senior Scientist, Centre for Medicinal Plant Research, Aryavaidyasala, Kottakkal. The seeds were washed, shade dried and powdered.

Preparation of extract

Powdered drug was subjected to cold alcoholic extraction with ethanol in 1:3 ratio. 2 kg of the coarse powder of seeds of *Mucuna Pruriens* was taken in an aspirator bottle, 6 litres of ethanol added and this mixture shaken occasionally for 72 h. Then the extract was filtered by using Whatman No.1filter paper on a Buchner funnel for three times before decanting and pooling. The solvent removed by vacuum distillation in a rotary evaporator at 40 °C.

Preliminary phytochemical analysis

The ethanolic extract of test drug was subjected to preliminary phytochemical analysis for the detection of bioactive constituents using standard procedures described in 'Trease and Evans' Pharmacognosy, 16th edition [11].

Test for steroids

First the alcoholic extract of test drug was mixed with minimum quantity of choloroform. Then 3–4 drops of acetic anhydride and one drop of concentrated H2SO4 was added. Formation of purple colour that changed in to blue or green indicated the presence of steroids.

Test for triterpenoids

Noller's Test: The substance was warmed with tin and thionyl chloride. Pink coloration indicated the presence of triterpenoids.

Test for reducing sugars

The test sample was mixed with Fehling's solutions II and I. Formation of red color precipitate indicated the presence of reducing sugars.

Test for glycosides

The alcoholic extract of Mucuna pruriens seed was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added, made into a paste and warmed gently over water bath. Dark green colour was appeared which indicated the presence of glycosides.

Test for alkaloids

To the test substance few drops of acetic acid was added, followed by Dragendorff's reagent and shaken well. Formation of orange-red color precipitate indicated the presence of alkaloid.

Test for phenolic compounds

The extract was dissolved in alcohol and then added a drop of neutral ferric chloride (5%). Development of intense blue colour indicated the presence of phenolic compounds.

Test for catechin

To the test substance in alcohol, Ehrlich's reagent was added followed by few drops of concentrated HCl. Formation of pink colour indicated the presence of catechins.

Test for saponins

The substance was shaken with water. Copious lather formation indicated the presence of saponins.

Test for flavonoids

Shinado's Test: To the substance in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes. Red colour showed the presence of flavonoids.

Test for tannins

The alcoholic extract of test sample mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

Test for anthroquinones

To the test substance, magnesium acetate solution was added. The presence of anthroquinones indicated by the development of pink colour.

***In vitro* antioxidant assay**

In vitro DPPH assay was done to estimate the antioxidant activity of ethanolic extract of Mucuna Pruriens seed using the standard method. [12] A solution of 0.1 mm DPPH was prepared and 1 ml of this solution was added to 1 ml of four different concentrations (0.025 mg/ml, 0.05 mg/ml, 0.1 mg/ml and 0.2 mg/ml) of the ethanolic extract of the test drug and 1 ml of methanol. The reaction mixture was then incubated at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was noted and the absorbance of the mixture was measured at 517 nm using ultra violet spectrophotometer. Ascorbic acid was used as reference and DPPH solution in methanol was taken as the control. Estimation of the ability of test drug to scavenge DPPH free radical was calculated using the following formula

The percentage of DPPH radical scavenging activity = [(Abs control)−(Abs sample)]/(Abs control) x 100;

Where, Abs control is the absorbance of the control and Abs sample is the absorbance of samples.

Principle of DPPH assay

Scavenging of DPPH free radical is the basis of this antioxidant assay. DPPH (1, 1diphenyl-2-picryl hydrazyl) represents the stable free radical with Purple color. The Antioxidant scavenger molecule scavenges free radical from DPPH thereby DPPH accepting a hydrogen (H) atom from antioxidant molecule resulting in the Reduction of DPPH to DPPH2. This produces change in color from purple to yellow with the associated decrease in absorbance at 515 nm. This color change is monitored spectrophotometrically and the estimation of antioxidant property was done based on the formulae [DPPH+[H-A] →DPPH-H+(A)].

RESULTS

Preliminary phytochemical analysis

The results of the preliminary phytochemical analysis of Mucuna pruriens is given in table 1. Phytochemical analysis of mucuna pruriens seeds revealed the presence of phenolic compounds, flavonoids, tannins, alkaloids, glycosides, phytosterols, triterpenes, saponins and reducing sugars.

Table 1: Preliminary phytochemical screening of ethanolic extract of *Mucuna Pruriens* seeds

S. No.	Phytochemical analysis	Test	Result
1.	Test for Steroids	Salkowski test	Present
2.	Test for Triterpenoids	Noller's Test:	Present
3.	Test for Reducing sugars	Fehling's test	Present
4.	Test for Glycosides	Liebermann's Test	Present
5.	Test for Alkaloids	Dragendorff's test	Present
6.	Test for Phenolic compounds	Ferric chloride test	Present
7.	Test for Catechin	Ehrlich's test	Absent
8.	Test for Saponins	Foam test	Present
9.	Test for Flavanoids	Shinoda test	Present
10.	Test for Tannins	Gelatin test	Present
11.	Test for Anthraquinones	Borntrager's test	Absent

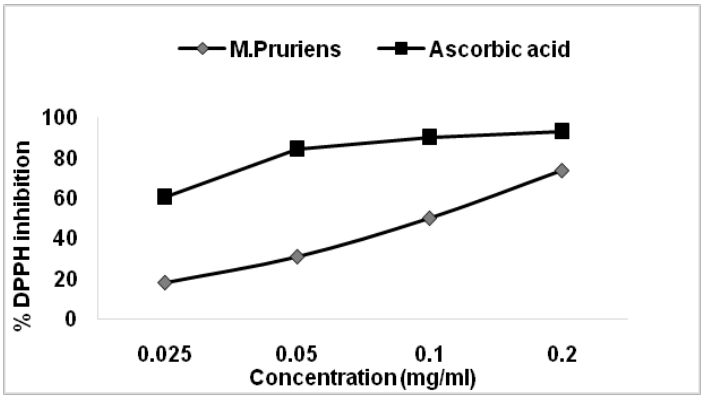

Fig. 1: DPPH radical scavenging activities of mucuna pruriens seed extract and ascorbic acid

***In vitro* DPPH antioxidant assay**

Mucuna pruriens seed extract showed DPPH radical scavenging activity of 18.24 %, 31.13%, 50.27% and of 72.95% at different concentrations of 0.025 mg/ml, 0.05 mg/ml, 0.1 mg/ml and 0.2 mg/ml respectively. The corresponding percentage of DPPH radical scavenging activity of reference control ascorbic acid were 60.79%,84.65%, 90.41% and 93.24%. Showed a maximum activity of 93.24% at the same concentration (table 2). A significant percentage inhibition of DPPH by mucuna Pruriens seed extract (72.95) was observed at the highest concentration of 0.2 mg/ml as represented in the fig. 1.

Table 2: Percentage DPPH radical inhibition of mucuna pruriens seed extract and Ascorbic acid at different concentrations

Concentrations tested (mg/ml)	% DPPH radical inhibition	
	M. Pruriens seed extract	Ascorbic acid
0.025	18.24	60.79
0.05	31.13	84.65
0.1	50.27	90.41
0.2	73.95	93.24

DISCUSSION

Phytochemical analysis

In the present study, preliminary phytochemical analysis showed that the Mucuna Pruriens seeds possess bioactive ingredients like alkaloids, flavonoids, saponins, phenolic components, triterpenoids, steroids, reducing sugars, tannins and cardiac glycosides. Apart from this, it also contains high fibre content. This study supports the findings of Swamy *et al.* reported that ethanolic extract of mucuna seeds contain alkaloids, carbohydrates, flavonoids, steroids, amino acids, triterpenoids, saponin, tannin, protein and phenol [13]. While phytochemical evaluation of the methanolic extract of Mucuna Pruriens seeds by Hadimani *et al.* noted the absence of saponins and triterpenoids [14]. This variation in the phytochemical constitution may be attributed to the fact that genomic composition and ambient environmental conditions, harvesting techniques, postharvest processing, storage conditions, widespread use of pesticides, frequent adulteration and microbial contamination may result in notable variation in chemical profile of medicinal plant material [15].

In vitro DPPH antioxidant assay

Increased oxidative stress and Reactive oxygen species (ROS) formation damage the structure of biomolecules and modify their function. Natural antioxidants minimize the adverse effects of free radicals by scavenging action. It has beneficial effects in maintenance of health, management of age related diseases, ameliorating the harmful effects of toxic agents both chemical and physical [16].

Scavenging of DPPH free radical by the antioxidants in the test sample constitutes the basis of this antioxidant assay [17]. DPPH (1, 1diphenyl-2-picryl hydrazyl) is a stable free radical with red colour which turns yellow when scavenged. DPPH assay uses this character to estimate free radical scavenging activity. Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability. In the present study, the test drug showed a significant antioxidant activity while comparing with the standards used.

The therapeutic efficacy of herbal drugs largely depends on the different phytochemical compounds present in it [18]. The powerful antioxidant activity of Mucuna pruriens seed extract can be attributed to the diverse phytoconstituents present in the drug. Previous studies have already reported the antioxidant potential of many of these compounds. Tannins are important phytochemical compound that possess antioxidant properties [19]. Flavonoids possess membrane stabilizing properties and inhibit lipid peroxidation in liver, brain, pancreas etc [20]. Phenols have antioxidant properties which carry out their protective activity on cells either by checking the free radical generation or by scavenging the free radicals [21]. Various studies have reported the antioxidant and anti-inflammatory potentials of saponins [22, 23]. Bioactive components like alkaloids and phytosterols are also considered effective in conditions like stress and depression [24]. Tri terpenoids, and reducing sugars are known antioxidants with antiproliferative properties, used in cancer research [25, 26].

Various studies on Mucuna pruriens seeds as an active prophylactic and therapeutic agent for radiation counter measures, combating cancers and aged related diseases [27]. Can be ascribed to the potential antioxidant properties of the drug, as evident in the present study.

CONCLUSION

Present study justifies the therapeutic potentials of Mucuna pruriens seed extract as a strong antioxidant agent due to the presence of diverse bioactive components like phenols, flavonoids, tannins, alkaloids, triterpenes and phytosterols. The outcome of this study is relevant for further researches on Mucuna pruriens seed extract as a viable source of natural antioxidants with wide area of application in different fields of sciences.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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