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PHYTOCHEMICAL ANALYSIS AND INVESTIGATION OF ANTIHEMOLYTIC AND ANTIOXIDANT PROPERTIES OF MUCUNA PRURIENS SEED EXTRACT

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ABSTRACT

The phytochemical profiling, antihemolytic activity and antioxidant capacity of seed extract of *Mucuna pruriens* was demonstrated. Extraction was performed using four solvents (Ethanol, Methanol, Hexane and n-Butanol) by Soxhlet extraction and Magnetic Stirrer methods. Extraction yields were determined but the best results were obtained using Ethanol solvent. Antihemolytic assay demonstrated that the seed extract does not cause lysis of RBCs. EC_{50} values of the *M. pruriens* seed extract after performing DPPH radical scavenging assay and Iron chelating assay were found to be 71.48 µg/mL and 70.19 µg/mL respectively.

KEYWORDS: Antihemolytic, antioxidant, Iron chelating, *Mucuna pruriens*, Phtyochemical, Radical scavenging.

INTRODUCTION

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic drugs and the new drug is necessary to search for new antioxidant from alternative sources. Phytochemicals from medicinal plants showing antioxidant activities have the potential of filling this need. In this growing interest, many of the phytochemical bioactive compounds from medicinal plants have shown many pharmacological activities. Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles against various free radical mediated diseases.[1]

The plant *M. pruriens*, widely known as "velvet bean," has long been used in traditional Ayurveda Indian medicine, for diseases including Parkinsonism (Sathiyanarayanan *et al*., 2007). The seeds of *Mucuna pruriens* have been used for treating many dysfunctions in Tibb-e-Unani (Unani Medicine). The plant and its extracts have been long used in tribal communities as a toxin antagonist for various snake bites.[2]

A methanol extract of *M. pruriens* seeds has demonstrated significant in vitro anti-oxidant activity (Rajeshwar, 2005).

Free radicals that have one or more unpaired electrons are produced during normal and pathological cell metabolism. Reactive oxygen species (ROS) react readily with free radicals to become radicals themselves. Anti-oxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breakage.[3] Many flavonoids are well known for their significant antioxidant activity (Pietta, 2000; Dai and Mumper, 2010), Nakayama, 1994; Ozen et al., 2011). Generally, phenolics have been considered powerful antioxidants in vitro and proved to be more potent than vitamin C and E and carotenoids (Rice-Evans et al., 1995, 1996).

MATERIALS AND METHODS

Plant material

The seeds of *M. pruriens* were chosen to evaluate antihemolytic and antioxidant activities. The seeds were collected from the local market of Bangalore, Karnataka.

Preparation of herbal extracts

Dried and powdered seeds of *M. pruriens* (60 g) was extracted using 4 solvents. Soxhlet extraction was carried out using 350 mL ethanol and methanol for 72 hrs. Magnetic stirrer was used to carry out extraction using Hexane and n-butanol.

Phytochemical analysis

i Screening for Alkaloids (Dragendroff's test)

100µL of Dragendroff's reagent was added to 200µL of extract and mixed well.

ii Screening for Tannins

10% and 1% $FeCl_3$ was added to 200µL of methanol and the other three extract respectively.

iii Screening for Terpenoids (Salkowski's test)

200µL chloroform + 3-4 drops of conc. Sulphuric acid were added to 200µL extract.

iv Screening for glycoside (Libermann test)

200µL of chloroform + 200µL of glacial acetic acid + drops of conc. Sulphuric acid were added to the extract.

v Screening for steroids

200µL chloroform + 3-4 drops of conc. Sulphuric acid were added to 200µL extract.

vi Screening for Saponins (foam test)

200µL of distilled water was added to 200µL of plant extract. Shook well and warmed.

vii Screening for flavonoids

Few drops of NaOH solution was added to plant extract followed by the addition of dil. HCl.

viii Screening for mucilages

500µL of absolute alcohol was added to 200µL of plant extract and was allowed to dry.

ix Screening for volatile oils

Few drops of dil. NaOH and dil. HCl were added to 200µL of plant extract and shake.

x Screening for phenolic compounds

500µL of distilled water and few drops of 5% $FeCl_3$ were added to 500µL of extract.

xi Screening for carbohydrates (Benedict's test)

Few drops of Benedict's reagent were added to plant extract.

xii Screening for proteins (Xanthoproteic test)

Few drops of conc.$HNO_3$was added to 200µL of extract.

Blood sampling

Blood sample was collected from a healthy volunteer in heparinized polypropylene tube. After immediate centrifugation (1000 rpm, 10 min), plasma and buffy coat layers were removed and erythrocytes were washed with Phosphate Buffer Saline (PBS).

Antihemolytic Assay

Diluted RBCs were added to different concentrations of ethanol plant extract. The cells were incubated at 37°C for 1 hour after which the reaction was centrifuged at 300 rpm for 5 minutes. Supernatant of each reaction was transferred to a micro titre plate and absorbance was measured at 590nm. Percentage haemolysis was calculated by the formula.

[% Haemolysis = (Control - sample)*100/Control].

Antioxidant Assays

Sample preparation

Dried seed powder extract was solubilized in ethanol-water (1:1 ratio) to a final concentration of 1mg/mL. Sample stock solution (1mg/mL) was diluted to final concentrations of 400, 200, 100, 50 and 25µg/mL for various antioxidant assays. Reference standard chemical (Ascorbic acid) was used for comparison in all assays.

DPPH radical scavenging assay

100µL of 0.5mM DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) was added to 200µL of plant samples of different concentrations. The experiments were conducted in duplicates. The reaction mixture was placed in dark at room temperature. After 30 minutes, absorbance was measured at 518nm using ELx800 ELISA plate reader and converted into percentage antioxidant activity (AA) by the formula.

$\% A.A = [(Ab_{control} - Ab_{sample}) * 100] / Ab_{control}$

As a standard reference compound, ascorbic acid was employed. The degree of discoloration indicated the scavenging potential of the antioxidant compound.

Iron chelating assay

50µL of O-phenanthroline and 125µL of $FeCl_3$ were added to 25µL of different concentrations of plant extract. The reaction mixtures were prepared in duplicates and were incubated for 10 minutes. After which the intensity of the colour was measured at 517nm using ELx800 ELISA plate reader and converted into percentage antioxidant activity (AA) by the formula.

$\% A.A = [(Ab_{control} - Ab_{sample}) * 100] / Ab_{control}$

RESULTS AND DISCUSSION

Extraction yield

Mucuna pruriens seed ethanol extract yielded 23.5 g of crude sample while Methanol, n-Butanol and Hexane yielded 20 g, 8.08 g and 7.54 g respectively. Ethanol appears to be the best solvent used giving the maximum yield [Table 1]. Hence ethanol extract was used for further analysis.

Table. 1: Yield of crude extract of *M. pruriens* seeds in different solvents.

SL. No.	Solvent	*M. pruriens seeds*
1.	Ethanol	60g in 350mL Yield = 23.5g
2.	Methanol	60g in 350mL Yield = 20.0g
3.	n-Butanol	60g in 350mL Yield = 8.08g
4.	Hexane	60g in 350mL Yield = 7.54g

Phytochemical analysis

Table. 2: Qualitative Analysis of phyto-compounds in various extracts of *M. pruriens.*

Secondary metabolite	Methanol	Ethanol	Hexane	n-Butanol
Alkaloids	++	++	+	+
Tannins	+++	++	-	+
Terpenoids	-	-	-	-
Steroids	+	+	+	+
Glycosides	-	-	-	-
Saponins	-	-	-	-
Flavonoids	+	+	-	-
Mucilage	+	-	-	-
Volatile Oils	+	+	-	-
Phenolic Compounds	+	+	-	-
Carbohydate	++	++	-	+
Proteins	+	+	-	-

+++: Very high presence; ++: High presence; +: Low presence; -: No presence

Compared to all the other solvent extracts, methanolic seed extract has higher number of secondary metabolites with greater degree of precipitation. But ethanol seed extract was used for further analysis due to greater yield.

Antihemolytic assay assessment

Table. 3: Absorbance values of haemolysis caused by *Mucuna pruriens* seed extract on RBCs.

Sample	**Treat**	**Absorbance**	**% Haemolysis**
Negative Control	PBS	0.790	0.000
Positive Control	1% SDS	0.160	80.260
Sample	**Conc.(µg/mL)**	**Absorbance**	**% Haemolysis**
Control	0	0.790	0.000
Ethanolic Extract of *M. pruriens*	25	0.930	0.000
	50	0.810	0.002
	100	0.680	0.005
	200	0.640	0.006
	400	0.560	0.010

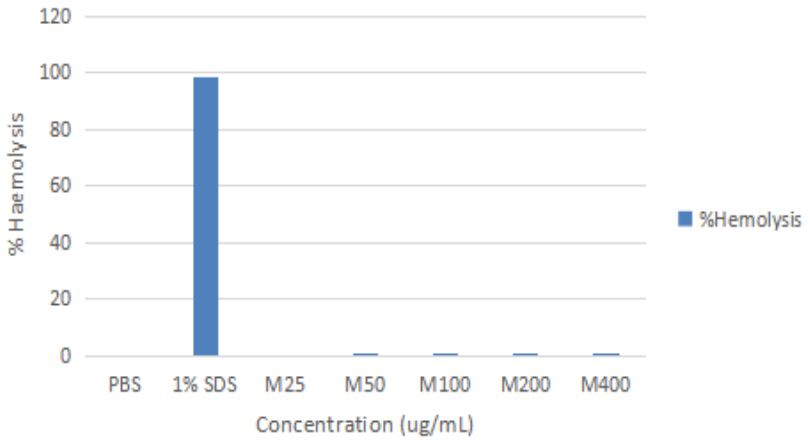

Fig. 1: % Hemolysis of *M. pruriens* (M25-M400- concentration of *M. pruriens*).

The selected plant extracts tested for their hemolytic abilities showed no hemolysis of red blood cells. This suggests that the samples can be used for therapeutics.

Antioxidant activity assessment

DPPH radical scavenging activity

Table. 4: % Antioxidant Activity of plant extract compared with standard (Ascorbic Acid).

Concentration(µg/mL)	**% A.A [Standard]**	**% A.A [Plant extract]**
25	22	18.22
50	40.5	42
100	55.3	49.74
200	67	51.73
400	88	63.76

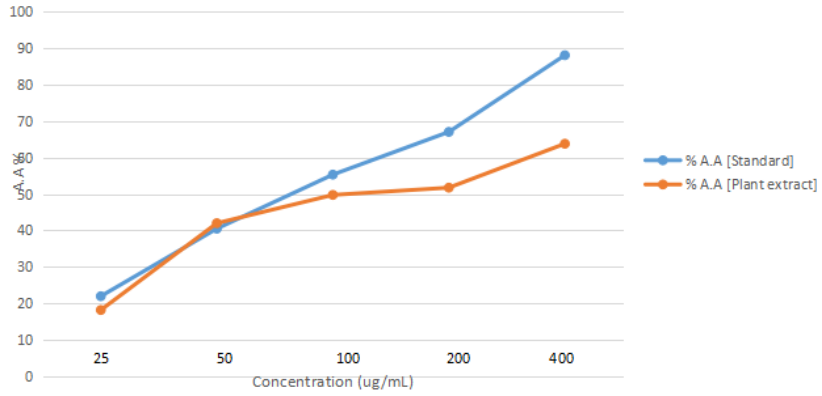

Fig. 2: % A.A at different concentrations (Comparison of Plant extract with Standard)

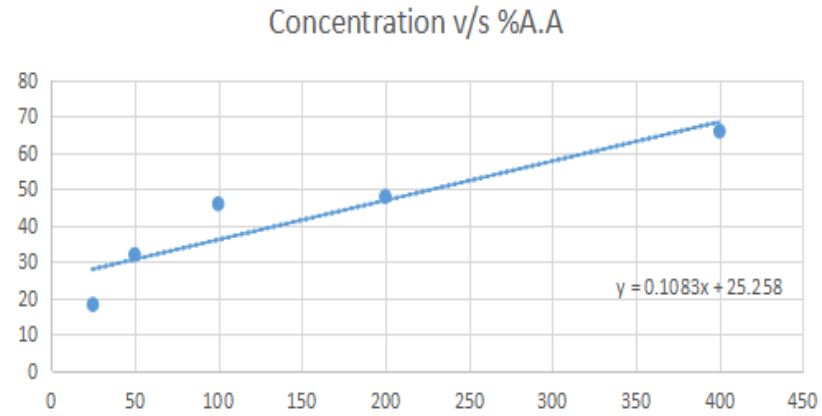

Fig. 3: EC_{50} by linear regression (EC_{50}= 71.48 µg/mL).

DPPH radical scavenging assay of *M. pruriens* ethanol extract showed good correlation with the standard Ascorbic acid. Results of the present investigation imply that *M. pruriens* contain phyto-constituents that are proficient of donating hydrogen to a free radical in order to rescue the potential impairment.

Iron chelating activity

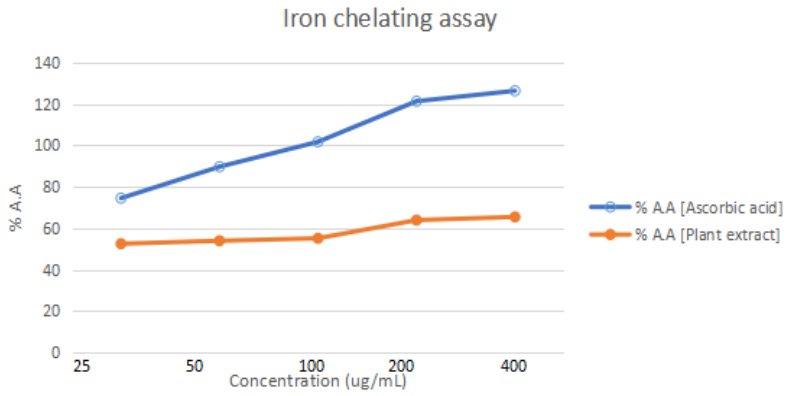

Fig. 4: % A.A at different concentrations (Comparison of Plant sample with Standard).

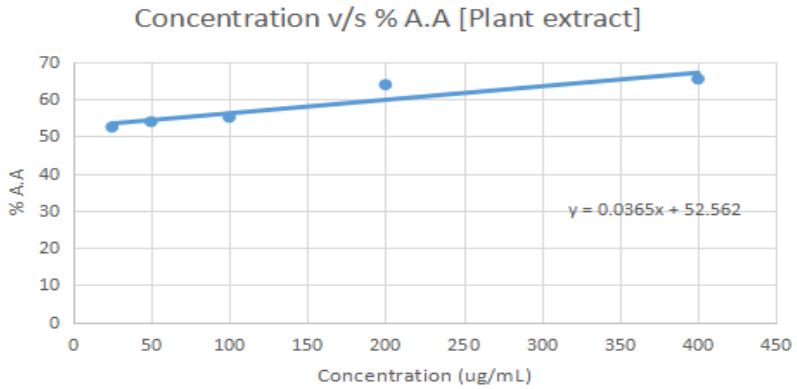

Fig. 5: EC_{50} by linear regression (EC_{50}= 70.19µg/mL).

A dose dependent mode was observed for iron chelating activity. Antioxidant activity of *M. pruriens* was significantly different from standard ascorbic acid.

CONCLUSION

The present study demonstrates the phytochemical profiling, antihemolytic and antioxidant activity of seed extract of *Mucuna pruriens*. It can be concluded that extracts obtained from higher polarity solvents (Ethanol and Methanol) had higher yield compared less polar and non-polar solvents. The result of phytochemical analysis showed that the plant is rich in many secondary metabolites- alkaloids, tannins, flavonoids, steroids and phenolic compounds. Safety profile of ethanol seed extract was assessed by antihemolytic assay. Antioxidant assay results provide important evidence that the seed extract of *M. pruriens* is a potent source of protective agent against the oxidative process. This antioxidant property could be attributed to the presence of flavonoids and phenolic compounds as indicated in the literature. In conclusion, *Mucuna pruriens* maybe a potent source of protective chemicals against free radical oxygen species. These findings make the title plant interesting for the search of new therapeutic agents. Further investigation is necessary to determine the medicinal properties of the plant *in vivo* and *in vitro*.

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REFERENCES

1. Jamuna Senguttuvan, Subramaniam Paulsamy, Krishnamoorthy Karthika. (Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities). Asian Pac J Trop Biomed, 2014; 4(1): 359-S367.
2. Lucia Raffaella Lampariello, Alessio Cortelazzo, Roberto Guerranti, Claudia Sticozzi, Giuseppe Valacchi. (The Magic Velvet Bean of Mucuna pruriens), Journal of Traditional and Complementary Medicine, 2010; 2(4): 331-339.
3. Mhamed Ramchoun, Khalid Sellam, Hicham Harnafi, Chakib Alem *et al.* (Investigation of antioxidant and antihemolytic properties of *Thymus satureioides* collected from Tafilalet Region, south-east of Morocco). Asian Pacific Journal of Tropical Biomedicine, 2015; 5(2): 93-100.
4. Sridhar KR, Rajeev Bhat. (Agro botanical, nutritional and bioactive potential of unconventional legume-Mucuna). Livestock Research for Rural Development, 2012; 19(9): 1-34.
5. Tayyaba Afsar, Suhail Razak, Muhammad Rashid Khan, Saadia Mawash *et al.* (Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of *Acacia hydaspica* R. Parker aerial parts). BMC Complement Altern Med., 2016; 16(2): 258.
6. Suna Sabuncuoğlu, Didem Şöhretoğlu. (Evaluation of antihemolytic and antioxidant activities of Geranium tuberosum subsp. tuberosum with in vitro models). Pharmaceutical Biology, 2012; 50(11): 1374–1379.
7. Maria Shabbir, Muhammad Rashid Khan, Naima Saeed. (Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of *Maytenus royleanus* leaves). BMC Complementary and Alternative Medicine, 2013; 13(8): 143.