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In vitro Antioxidant Activity of Various Extracts of whole Plant of *Mucuna pruriens* (Linn)

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Abstract: The study was designed to examine the *invitro* antioxidant activities of various extracts of whole plant of *Mucuna pruriens*. The antioxidant activity was evaluated by DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, Super Oxide Anion Scavenging Activity and Iron chelating activity with reference standard Rutin, Quercetin and EDTA respectively. The ethyl acetate extract of *Mucuna pruriens* was found to more effective in the DPPH radical scavenging activity. The IC_{50} of the ethyl acetate extract of *Mucuna pruriens* and Rutin were found to be 420µg/ml and 480µg/ml respectively. An IC_{50} value was found that ethyl acetate extract of *Mucuna pruriens* is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with Quercetin (standard), the ethyl acetate extract of the *Mucuna pruriens* showed the similar result. The Iron chelating activity of the methanolic extract of *Mucuna pruriens* was found to most effective than that of petroleum ether & ethyl acetate extract. It is concluded that a whole plant of ethyl acetate and methanolic extract of *Mucuna pruriens,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: Whole plant of *Mucuna pruriens*, Invitro antioxidant, DPPH assay, Superoxide anion, Iron chelating activity.

Introduction

Nowadays, the role of free radicals in many ailments and diseases including inflammation, rheumatoid arthritis, cancer and cardiovascular diseases has been widely established[1]. Free radicals which have one or more unpaired electrons are produced during normal and pathological cell metabolites. Reactive oxygen species (ROS) react easily with free radicals to become radicals themselves. ROS are various forms of activated oxygen, which include free radicals such as superoxide anion radicals (O_2^-) and hydroxyl radicals (OH^-), as well as non-free radicals species (H_2O_2) and the singlet oxygen ($^1O_2$)[2]. Antioxidants provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA stand breaking[3]. Several substances from natural sources have been shown to contain antioxidants and are under study. Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[4]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity[5]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[6].

Mucuna pruriens Linn.is belongs to the family fabaceae, commonly known as cowhage plant or kapikacho or kevach in Hindi, is the most popular drug in Ayurvedic system of medicine[7]. It has been reported to be antidiabetic[8]. Its different preparations (from

seeds) are used for the management of several free radical-mediated diseases such as ageing, rheumatoid arthritis, diabetes, atherosclerosis, male infertility and nervous disorders. It is also used as an aphrodisiac and in the management of Parkinsonism, as it is good source of L-dopa[9]. The seeds of *Mucuna Pruriens* show hypoglycemic activity in experimental rats[10]. The anti-epileptic and anti-neoplastic activity of methanol extract of *Mucuna pruriens* has been reported[11]. It had been reported analgesic and anti-inflammatory activities[12]. It also used as a fertility agent in men[13].Moreover a study through *in-vitro* models revealed that L-dopa significantly increases the levels of oxidized glutathione in rat brain striatal synaptosomes[14]. Such depleted reduced glutathione (GSH) could be due to the generation of reactive semiquinones from L-dopa[15].

However, no data are available in the literature on the antioxidant activity of whole plant of *Mucuna pruriens*. Therefore we undertook the present investigation to examine the antioxidant activities of various extract of whole plant of *Mucuna pruriens* through various *in vitro* models.

Material and Methods

Collection and Identification of Plant materials

The whole plant of *Mucuna pruriens* (Linn), were collected form Neiyur dam, Kanyakumari District of Tamil Nadu, India. Taxonomic identification was made from Biological Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Mucuna pruriens* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[16] for 24 hrs. And the mark was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques:

DPPH photometric assay[17]

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[17]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity[18]

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[18]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Iron chelating activity[19]

The method of Benzie and strain (1996)[19] was adopted for the assay. The principle is based on the formation of *O*-Phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Results and Discussion

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases[20].They are also involved in autoimmune disorders like rheumatoid arthritis etc[21].

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of

singlet oxygen formation[22]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[23]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same[24].

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Mucuna pruriens* presented in Table 1. The petroleum ether extract of *Mucuna pruriens* exhibited a maximum DPPH scavenging activity of 40.29% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the petroleum ether extract of *Mucuna pruriens* and Rutin were found to be 1230µg/ml and 480µg/ml respectively.

The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Mucuna pruriens* presented in Table 2. The ethyl acetate extract of *Mucuna pruriens* exhibited a maximum DPPH scavenging activity of 66.97% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the ethyl acetate extract of *Mucuna pruriens* and Rutin were found to be 420µg/ml and 480µg/m respectively

The percentage of DPPH radical scavenging activity of methanolic extract of *Mucuna pruriens* presented in Table 3. The methanolic extract of *Mucuna pruriens* exhibited a maximum DPPH scavenging activity of 49.83% at 1000 µg/ml whereas for Rutin(standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the petroleum ether extract of *Mucuna pruriens* and Rutin were found to be 1030µg/ml and 480µg/m respectively.

Table 1: Effect of Petroleum ether extract of *Mucuna Pruriens* (Linn) on DPPH assay:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	18.66 ± 0.072	18.85 ± 0.076
2	250	32.23 ± 0.010	22.08 ± 0.054
3	500	36.93 ± 0.015	52.21 ± 0.022
4	1000	40.29 ± 0.091	69.83 ± 0.014
		IC_{50} = 1230 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 2: Effect of Ethyl acetate extract of *Mucuna Pruriens* (Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	37.07 ± 0.081	18.85 ± 0.076
2	250	48.57 ± 0.067	22.08 ± 0.054
3	500	52.07 ± 0.042	52.21 ± 0.022
4	1000	66.97 ± 0.039	69.83 ± 0.014
		IC_{50} = 420 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 3: Effect of Methanolic extract of *Mucuna Pruriens* (Linn) on DPPH assay:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	12.92±0.012	18.85 ± 0.076
2	250	13.58±0.049	22.08 ± 0.054
3	500	20.11±0.036	52.21 ± 0.022
4	1000	49.83±0.024	69.83 ± 0.014
		IC_{50} = 1030 µg/ml	**IC_{50} = 480 µg/ml**

*All values are expressed as mean ± SEM for three determinations

The ethyl acetate extract of *Mucuna pruriens* was found to more effective than petroleum ether and methanolic extract. The DPPH radical scavenging activity of the extract increases with increasing concentration, only 66.97% DPPH radical scavenging. Nevertheless, it was 69.83% in the presence of 1000µg/ml of Rutin (standard). The IC_{50} of the ethyl acetate extract of *Mucuna pruriens* and Rutin were found to be 420µg/ml and 480µg/ml respectively.

Superoxide anion scavenging activity

Superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Superoxide dismutase enzymes present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical[25].

Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Mucuna pruriens* (125, 250, 500, 1000 µg/ml) was depicted in table 4. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 58.98% and 98.01% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[26]. The IC_{50} value of plant extract and Quercetin was recorded as 420µg/ml and 60µg/ml respectively.

Percentage scavenging of superoxide anion examined at different concentrations of ethyl acetate extract of *Mucuna pruriens* (125, 250, 500, 1000 µg/ml) was depicted in table 5. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 80.60% and 98.01% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[26]. The IC_{50} value of plant extract and Quercetin was recorded as 180µg/ml and 60µg/ml respectively.

Table 4: Effect of Petroleum ether extract of *Mucuna pruriens* (Linn) on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	27.12 ±0 .015	73.81 ± 0.006
2	250	39.12 ± 0.049	91.31 ± 0.011
3	500	56.46 ± 0.030	92.99 ± 0.024
4	1000	58.98 ± 0.027	98.01 ± 0.012
		IC_{50} = 420 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 5: Effect of Ethyl acetate extract of *Mucuna Pruriens* (Linn) on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	44.70 ± 0.051	73.81 ± 0.006
2	250	60.20 ± 0.029	91.31 ± 0.011
3	500	74.40 ± 0.031	92.99 ± 0.024
4	1000	80.60 ± 0.019	98.01 ± 0.012
		IC_{50} = 180 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 6: Effect of Methanolic extract of *Mucuna pruriens* (Linn) on Superoxide anion scavenging activity method:

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	21.42 ± 0.015	73.81 ± 0.006
2	250	38.29 ± 0.029	91.31 ± 0.011
3	500	90.80 ± 0.032	92.99 ± 0.024
4	1000	95.61±0.028	98.01 ± 0.012
		IC_{50} = 310 µg/ml	**IC_{50} = 60 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Mucuna pruriens* (125, 250, 500, 1000 µg/ml) was depicted in table 6. The percentage scavenging of superoxide radical surged with the enhanced concentration of plant extract. The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 95.61% and 98.01% respectively. Superoxide scavenging ability of plant extract might primarily be due to the presence of flavanoids[26]. The IC_{50} value of plant extract and Quercetin was recorded as 310µg/ml and 60µg/ml respectively.

Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that ethyl acetate extract of *Mucuna pruriens* is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with Quercetin (standard), the ethyl acetate extract of the *Mucuna pruriens* showed the similar result.

Iron chelating activity:

Iron is essential for life because it is required for oxygen transport, respiration and activity of many enzymes. However, iron is an extremely reactive metal and catalyzes oxidative changes in lipids, proteins and other cellular components[27, 28]. It causes lipid peroxidation through the Fenton and Haber-weiss reaction[29] and decomposes the lipid hydroxide into peroxyl and Alkoxyl radicals that can perpetuate the chain reactions[30].

Iron binding capacity of the petroleum ether extract of *Mucuna pruriens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 7.maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 49.12% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 1030µg/ml and 65µg/ml respectively.

Table 7: Effect of Pet.ether extract of *Mucuna pruriens* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (EDTA)
1	125	18.28 ± 0.020	58.68 ± 0.007
2	250	29.48 ± 0.037	65.87 ± 0.018
3	500	32.00 ± 0.029	83.83 ± 0.012
4	1000	49.12 ± 0.022	97.90 ± 0.019
		IC_{50} = 1030 µg/ml	**IC_{50} = 65 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Iron binding capacity of the ethyl acetate extract of *Mucuna pruriens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 8. Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 50.84% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 1000µg/ml and 65µg/ml respectively.

Iron binding capacity of the methanolic extract of *Mucuna pruriens* and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in table 9. Maximum chelating of metal ions at 1000µg/ml for plant extract and EDTA was found to be 96.77% and 97.90% respectively. The IC_{50} value of plant extract and EDTA was recorded as 220µg/ml and 65µg/ml respectively.

Table 8: Effect of Ethyl acetate extract of *Mucuna pruriens* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethylacetate extract)	Standard (EDTA)
1	125	29.11 ± 0.016	58.68 ± 0.007
2	250	30.80 ± 0.011	65.87 ± 0.018
3	500	38.17 ± 0.029	83.83 ± 0.012
4	1000	50.84 ± 0.021	97.90 ± 0.019
		IC_{50} = 1000 µg/ml	**IC_{50} = 65 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table 9: Effect of Methanolic extract of *Mucuna Pruriens* (Linn) on Iron-chelating method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (EDTA)
1	125	29.84 ± 0.044	58.68 ± 0.007
2	250	56.79 ± 0.029	65.87 ± 0.018
3	500	86.71 ± 0.036	83.83 ± 0.012
4	1000	96.77 ± 0.013	97.90 ± 0.019
		IC_{50} = 220 µg/ml	**IC_{50} = 65 µg/ml**

*All values are expressed as mean ± SEM for three determinations

Based on the above results indicated, the methanolic extract of *Mucuna pruriens* was found to most effective than that of petroleum ether & ethyl acetate extract. The results indicted the plant extract possess iron biding capacity which might be due to the presence of polyphenols that averts the cell from free radical damage by reducing of transition metal ions[31,32]. Various plant extracts were proved to be good chelators[33] and correlation exists between phenols, flavonoids and chelating activity.

Conclusion

From the results obtained in the present study, it is concluded that a whole plant of ethylacetate and methanolic extract of *Mucuna pruriens,* which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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