EVALUATION OF *INVITRO* FREE RADICAL SCAVENGING POTENTIAL OF VARIOUS EXTRACTS OF WHOLE PLANT OF *Mucuna pruriens* (Linn)

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Summary

The antioxidant activity of various extracts of whole plant of *Mucuna pruriens* (Linn) was investigated in various *in-vitro* methods. The antioxidant activity was evaluated by Total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard Ascorbate and total flavonoids content respectively. The methanolic extract of *Mucuna pruriens* was found to more effective in the total antioxidant activity. The IC_{50} values of the methanolic extract of *Mucuna pruriens* and ascorbate were found to be 100µg/ml and 410µg/ml respectively. The methanolic extract of *Mucuna pruriens* was found more effective in FRAP assay than that of petroleum ether and ethyl acetate extracts. But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Mucuna pruriens* showed the better result. The methanolic extract of *Mucuna pruriens* contains high amount of flavonoids than that of other two extracts. Moreover, the results were observed in a concentration dependent manner. All the above in vitro studies clearly indicate that the methanolic extract of Mucuna pruriens has a better antioxidant activity. These in vitro assays indicate that this plant extracts is a better 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, Total antioxidant activity, FRAP assay, Total flavonoids.

Introduction

Oxygen free radicals are formed in tissue cells by many endogenous and exogenous causes such as metabolism, chemicals, and ionizing radiation[1]. Oxygen free radicals may attack lipids and DNA giving rise to a large number of damaged products[2]. Iron is known to be involved in the generation of reactive oxygen species (ROS) and in the formation of highly toxic hydroxyl radicals from other active oxygen species such as hydrogen peroxide[3, 4]. The enhanced generation of ROS *in vivo* could be quite deleterious, since they are involved in mutagenesis, apoptosis, ageing, and carcinogenesis[4].

Antioxidants are important in the prevention of human diseases. Naturally occurring antioxidants in leaf vegetables and seeds, such as ascorbic acid, vitamin E and phenolic compounds, possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing[5, 6, 7]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[8]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties.

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[9]. Traditionally, in India, the seeds of *Mucuna pruriens* are used as a tonic and aphrodisiac for male virility. It has been reported to be antidiabetic[10]. 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[11]. The anti-epileptic and anti-neoplastic activity of methanol extract of *Mucuna pruriens* has been reported[12]. It had been reported analgesic and anti-inflammatory activities[13]. It also used as a fertility agent in men[14].

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.

Materials 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 Botanical 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[15] for 24 hrs. Then 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:

Total antioxidant activity (Phosphomolybdic acid method) [16]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)[16]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^{0}C for 90 min.

After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay[17]

A modified method of Benzie and Strain (1996)[17] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM $Fecl_3.\ 6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total flavonoids[18]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

Results and Discussion

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation[19]. Phenolic compounds and flavonoids are major constituents of most of the plants reported to possess antioxidant and free radical scavenging activity[20]. Therefore, the importance of search for natural antioxidants has increased in the recent years so many researchers focused the same[21].

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Mucuna pruriens* presented in Table 1. The petroleum ether extract of *Mucuna pruriens* exhibited a maximum total antioxidant activity of 80.01 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Mucuna pruriens* and ascorbate were found to be 240µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Mucuna Pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	39.72 ± 0.045	26.87 ± 0.076
2	250	50.68 ± 0.024	30.30 ± 0.054
3	500	58.08 ± 0.091	60.64 ± 0.022
4	1000	80.01 ± 0.021	55.23 ± 0.014
		IC_{50} = 240 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of ethyl acetate extract of *Mucuna pruriens* presented in Table 2. The ethyl acetate extract of *Mucuna pruriens* exhibited a maximum total antioxidant activity of 85.75 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Mucuna pruriens* and ascorbate were found to be 160µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Mucuna Pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	41.91 ± 0.021	26.87 ± 0.076
2	250	79.17 ± 0.047	30.30 ± 0.054
3	500	85.47 ± 0.072	60.64 ± 0.022
4	1000	85.75 ± 0.039	55.23 ± 0.014
		IC_{50} = 160 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of methanolic extract of *Mucuna pruriens* presented in Table 3. The methanolic extract of *Mucuna pruriens* exhibited a maximum total antioxidant activity of 87.12 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Mucuna pruriens* and ascorbate were found to be 100µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Mucuna Pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	51.50 ± 0.012	26.87 ± 0.076
2	250	76.98 ± 0.049	30.30 ± 0.054
3	500	86.02 ± 0.036	60.64 ± 0.022
4	1000	87.12 ± 0.024	55.23 ± 0.014
		IC_{50} = 100 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the result clearly indicated the methanolic extract of *Mucuna pruriens* was found to more effective than petroleum ether and ethyl acetate extract. But when compare all the extracts with standard the methanolic extract of *Mucuna pruriens* was found strong antioxidant activity. The IC_{50} of the methanolic extract of *Mucuna pruriens* and Ascorbate were found to be 100µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Mucuna pruriens* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the petroleum ether extract of *Mucuna pruriens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/ml for petroleum ether extract and ascorbate were found to be 38.31% and 98.07% respectively. The IC_{50} values of petroleum ether extract and ascorbate were recorded as 1200µg/ml and 50µg/ml respectively.

Table 4: FRAP assay of Pet. ether extract of *Mucuna pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	21.91 ± 0.020	72.04 ± 0.014
2	250	31.81 ± 0.037	82.05 ± 0.034
3	500	36.79 ± 0.029	86.04 ± 0.026
4	1000	38.31 ± 0.022	98.07 ± 0.041
		IC_{50} = 1200 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the ethyl acetate extract of *Mucuna pruriens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/ml for ethyl acetate extract and ascorbate were found to be 59.80% and 98.07% respectively. The IC_{50} values of ethyl acetate extract and ascorbate were recorded as 780µg/ml and 50µg/ml respectively.

Table 5: FRAP assay of Ethyl acetate extract of *Mucuna pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Ascorbate)
1	125	19.77 ± 0.016	72.04 ± 0.014
2	250	23.46 ± 0.011	82.05 ± 0.034
3	500	37.14 ± 0.029	86.04 ± 0.026
4	1000	59.80 ± 0.021	98.07 ± 0.041
		IC_{50} = 780 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the methanolic extract of *Mucuna pruriens* and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/ml for methanolic extract and ascorbate were found to be 70.16% and 98.07% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 280µg/ml and 50µg/ml respectively.

Table 6: FRAP assay of Methanolic extract of *Mucuna Pruriens*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	37.03 ± 0.044	72.04 ± 0.014
2	250	47.98 ± 0.029	82.05 ± 0.034
3	500	57.99 ± 0.036	86.04 ± 0.026
4	1000	70.16 ± 0.013	98.07 ± 0.041
		IC_{50} = 280 µg/ml	**IC_{50} = 50 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. . But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Mucuna pruriens* showed the moderate result.

Total flavonoids

Flavonoids present in food of plant origin are also potential antioxidants[22], [23]. Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities [24]. The total amount of flavonoids content of various extract of whole plant of *Mucuna pruriens* was presented in Table 7.

Table 7: The total flavonoids content of various extracts of whole plant of *Mucuna pruriens*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Mucuna pruriens*	0.017 ± 0.007
2	Ethyl acetate extract of *Mucuna pruriens*	0.305 ± 0.002
3	Methanolic extract of *Mucuna pruriens*	2.378 ± 0.023

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

Based on the result the methanolic extract of *Mucuna pruriens* was found higher content of flavonoids than that of petroleum ether and ethyl acetate extract of *Mucuna pruriens.*

Conclusion

The present study was clearly indicated the methanolic extract of *Mucuna pruriens* showed strong antioxidant activity by total antioxidant activity and FRAP assay when compared with standard Ascorbate. But the ethyl acetate extract showed moderate activity when compared with standard Ascorbate. In addition, the methanolic extract of *Mucuna pruriens* was found to contain a noticeable amount of flavonoids, which play a major role in controlling antioxidants. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

Acknowledgments

The authors would like to thank University Grants Commission (UGC), New Delhi, India, for providing financial support for this investigation.

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