



Original Research Paper

In Vitro Antioxidant Activity of root extract of *Mucuna Pruriens*

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ABSTRACT

There are an increasing number of studies on pharmaceutical properties of medicinal plants growing wild in various climates of India. However, there is little information available concerning antioxidant activity of medicinal plants growing wild in northeastern India. *Mucuna pruriens* is a medicinal plant used for the management of different human and livestock diseases in India. The antioxidant potential of the roots from this herb was investigated using its hydro alcoholic extract. All the extracts were rich in phenols, proanthocyanidins and flavonoids. The root extract exhibited low DPPH scavenging activity. Again all the extracts showed high ABTS scavenging activity with a correlation between total phenolic content. Therefore, it would seem likely that both solvents were able to extract those compounds which are responsible for the antioxidant activity of *Mucuna pruriens* root extract.

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1. INTRODUCTION

Plants have been one of the important sources of medicines since the beginning of the human civilization. plants still continue to be the major source of drugs in modern as well as traditional medicine throughout the world¹.

The Recent studies indicate that antioxidants used at appropriate doses improve the tumor response towards chemotherapy and radiation therapy. It was also shown that antioxidants selectively suppress the growth of cancer cells without affecting normal cells². This is a widely accepted hypothesis by the physicians who are nutrition minded. The most commonly used antioxidants in cancer treatment include vitamins, minerals, enzymes and other nutrients. These constituents are considered as a part of antioxidant regimen along with chemotherapy³. Different antioxidants work at different levels in the cell. The selection of the antioxidants/nutrients is based on the type of cancer, chemotherapy and the condition of the patient. Various antioxidants used in cancer therapy are Beta carotene, Vitamin-C, Vitamin- E, Lipoic acid, Poly MVA, Bioflavonoid and Selenium⁴. The main aim of the present study is to find out antioxidant activity in *Mucuna pruriens* root extract by using *in vitro* methods

2. MATERIALS AND METHOD

Plant extracts

A novel way to deal with plant choice is a mechanized choice strategy or writing data choice procedure (LIST) that corresponds organic action, natural actualities and chemotaxonomical data utilizing NAPRALERT database. The HPLC grade root extract of plant part was purchased from Bioextract, bangalore, India⁹⁻¹¹.

All the reagents used for this study were of Analytical grade and purchased from authentic chemical supplier.

HYDROGEN PEROXIDE SCAVENGING (H₂O₂) ASSAY

Among reactive oxygen species (ROS), hydrogen peroxide (H₂O₂) is a relatively stable, non-radical oxidant, which can diffuse across biological membranes. It is produced by 2-electron reduction of molecular oxygen or by dismutation of the superoxide anion radical. A large number of assays developed for the determination of HPS activity depend on the oxidation of a spectrophotometric, fluorogenic or chemiluminogenic probe (detector molecule) by H₂O₂ as the oxidation catalyst.

Procedure

The ability of plant extract to scavenge hydrogen peroxide can be

estimated by making a solution of hydrogen peroxide (40mm) in phosphate buffer (50mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230nm using a spectro photometer. Extract (20-60mueg/ml) in distilled water is added to hydrogen peroxide and absorbance at 230nm is determined after 10min against blank solution containing phosphate buffer with hydrogen peroxide.

H₂O₂ is known to be a strong oxidant; it may act as a reductant under certain conditions. The molar absorptivity for H₂O₂ was: $\epsilon = 1.42 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, and the linear concentration range: 2.11×10^{-6} to $9.91 \times 10^{-5} \text{ M}$ (linear correlation coefficient: $r = 0.9992$). The relative standard deviation (RSD) within this concentration range was approximately 1.9%. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the equations; $\text{LOD} = 3s_{bl}/m$ and $\text{LOQ} = 10s_{bl}/m$, respectively, where s_{bl} is the standard deviation of a blank and m is the slope of the calibration line. The LOD and LOQ for H₂O₂ were found to be $6.3 \times 10^{-4} \text{ mM}$ and $2.1 \times 10^{-3} \text{ mM}$, respectively⁶⁻⁸.

DNA damage in the presence of Cu (II) + H₂O₂ are more likely copper-peroxide complexes, with similar reactivity to singlet oxygen and/or hydroxyl radical rather than hydroxyl free radical. The percentage of hydrogen peroxide scavenging is calculated as follows

Formula

$$\% \text{Scavenged (H}_2\text{O}_2) = [(A_i - A_t)/A_i] \times 100$$

Where A_i is the absorbance of control and A_t is the Absorbance of test.

REDUCING POWER METHOD (RP)

The total antioxidant activity of given plant root extract was evaluated by FRAP method, the results being expressed as Ascorbic Acid Equivalent Antioxidant Capacity, AEAC).

Procedure

This method is based on principles of increase in the absorbance of reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride, which is measured in 700nm. Increase in the absorbance of the reaction mixture indicates the reducing power of the samples¹⁶. In the method 2.5ml of 0.2M phosphate buffer (pH6.6) and 2.5ml of K₃Fe(CN)₆ (1% w/v) are added to 1.0ml of sample dissolved in distilled water. The resulting mixture is incubated at 50 degree Celsius for 20 min, followed by the addition of 2.5 ml of trichloro acetic acid (10% w/v). The mixture is centrifuge at 3000rpm for 10 min to collect the upper layer of the solution (2.5ml), mixed with distilled water (2.5ml) and 0.5ml of FeCl₃ (0.1%w/v). The absorbance is then measured at 700nm against blank sample¹⁷⁻²¹.

Statistical analysis

Descriptive statistical analyses were performed using Excel software for calculating the means and the standard error of the mean. Results were expressed as the mean standard deviation (SD). Using SPSS software for Windows (version 13), the data were evaluated by two-way Analysis Of Variance (ANOVA)

3.RESULT AND DISCUSSION

Table no.1 Phytochemical screening of *Mucuna pruriens* root extract

Chemical Constituents	Root extract
Carbohydrates	-
Glycosides	+
Alkaloids	+
Flavonoids	+
Phenols	+
Fixed oils	-
Steroids	+
Saponins	+
Gums & mucilage	-
Proteins & free amino acids	-

(+) → Present (-) → Absent

Table no. 2 Data showing Scavenging activity assessment by H₂O₂ assay

S.No	Concentration	Wavelength	Absorbance
1	Blank	230nm	0.226±0.2
2	Plant extract (0.5mg/ml)	230nm	0.238±0.16
3	Plant extract (1mg/ml)	230nm	0.326±0.11

Values are expressed as the mean ± S.D; Statistical significance value are calculated by one way ANOVA followed by Dunnett's test.

Table no. 3 Data showing Scavenging activity assessment by Reducing Power Method assay

S.No	Concentration	Wavelength	Absorbance
1	Blank	700nm	0.48±0.009
2	Plant extract (0.5mg/ml)	700nm	0.69±0.013
3	Plant extract (1mg/ml)	700nm	0.9±0.210

Values are expressed as the mean ± S.D; Statistical significance value are calculated by one way ANOVA followed by Dunnett's test.

The idea in the present study is to measure the HPS activity of polyphenols (i.e. flavonoids, simple phenolic acids, and hydroxycinnamic acids) and other antioxidant compounds (i.e. ascorbic acid) with a simple, low-cost and versatile colorimetric procedure¹³⁻¹⁵. In the most common UV-method for determination of HPS activity, scavenging ability depends on the change of the absorbance value at 230 nm when H₂O₂ concentration is decreased by scavenger compounds. Since the UV-method suffers from both the interference of some phenolics in real

samples having strong absorption in the UV-region and from inefficient degradation of H₂O₂ with polyphenols in the absence of Cu (II), HPS activity of polyphenols was alternatively determined without interference by directly measuring the concentration of undegraded H₂O₂. The results indicated that the plant root extract exhibited good antioxidant capacity²².

4. CONCLUSION

In conclusion, we defined that given root extracts possess certain antioxidant effects, which implied that other products could be considered for exploitation in addition to polyunsaturated fatty acids²³. The correlation analysis between the antioxidant capacity and the total polyphenol and flavonoid contents indicates that there are likely to be different types of antioxidants. Therefore, we need to conduct further work to isolate and identify the other antioxidant components present in root extract of this plant²⁴.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. REFERENCES

- 1) Chevion S, Roberts MA, Chevion M (2000) The use of cyclic voltammetry for the evaluation of antioxidant capacity. *Free Radic Biol Med* 28: 860-870.
- 2) Halliwell B, Gutteridge JMC (1989) *Free radicals in biology and medicine*. Clarendon Press, Oxford.
- 3) Chance PA, Sies H and Boveris A (1979) A hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59: 527-605.
- 4) Ames BM, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid produces an antioxidant defense in humans against oxidant and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* 73: 6858-6862.
- 5) Stocker R, Yamamoto Y, McDonagh A, Glazer AN and Ames BN (1987) Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043-1045.
- 6) Frei B, England L and Ames BN (1989) Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 86: 6377-6381.
- 7) Raoof JB, Ojani R and Beitollahi H (2007) Electrocatalytic determination of ascorbic acid at chemically modified carbon paste electrode with 2, 7-bis (ferrocenyl ethynyl) fluoren-9-one. *Int J Electrochem Sci* 2: 534-548.
- 8) Tomita IN, Manzoli A, Fertonani FL and Yamanaka H (2005) Amperometric biosensor for ascorbic acid. *Electrochim Acta* 50: 37-43.
- 9) Rajeshwar Y, Kumar GPS, Gupta M and Mazumder UK. Studies on in vitro antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. *Eur Bull Drug Res*. 2005; 13: 31-39.
- 10) Kumar KVA, Srinivasan KK, Shanbhag T and Rao SG. Aphrodisiac activity of the seeds of *Mucuna pruriens*. *Ind Drug*. 1994; 31: 321-327.
- Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. *Urology*. 1996; 48(6): 835-850.

- 11) Champatisingh C, Lejeune H, Gomez E, Bosmans E, Ballet F, Saez J and Jegou B. Human Leydig cells and Sertoli cells are producers of interleukins-1 and-6. *J Clinical Endocrinol Metabol*. 1997; 82: 1426-1433.
- 12) Tripathi YB and Upadhyay AK. Antioxidant property of *Mucuna pruriens* Linn. *Current Sci*. 2001; 80(11): 1378
- 13) Ahmad MK, Mahdi AA, Shukla KK, Islam N, Jaiswar SP and Ahmad S. Effect of *Mucuna pruriens* on semen profile and biochemical parameters in seminal plasma of infertile men. *Fertil Steril*. 2008; 90(3): 627-635.
- 14) Shukla KK, Mahdi AA, Ahmad MK, Jaiswar SP, Shankwar SN and Tiwari SC. *Mucuna pruriens* Reduces Stress and Improves the Quality of Semen in Infertile Men. *Evid Based Complement Alternat Med*. 2010; 7(1): 137-44.
- 15) Suresh S, Prithiviraj E, Lakshmi NV, Ganesh MK, Ganesh L and Prakash S. Effect of *Mucuna pruriens* (Linn.) on mitochondrial dysfunction and DNA damage in epididymal sperm of streptozotocin induced diabetic rat. *J Ethnopharmacol*. 2012; 9, 145(1): 32-41.
- 16) Ray AD, Keela CA, Maikhana M. Observation Of Chemical Excitants Of Cutaneous Pain In Man. *J Physiol*. 1953;12:376-51.
- 17) Lorenzetti E, Macissac S, Anasson JT, Avang DVC, Buckles D. The Phytochemistry, Toxicology And Food Potential Of Velvet Bean (*Mucuna Adans Ssp. Fabaceae*). In Buckles D, Osiname O, Galliba MM, Galiano G. *Cover Crop Of West African Contributing To Sustainable Agriculture* IDRC, Ottawa, Canada And IITA, Ibadan, Nigeria, P.57.
- 18) Bektas, Apak, R., 2008. Hydroxyl radical detection with a salicylate probe using modified CUPRAC spectrophotometry and HPLC. *Talanta* 77, 90-97.
- 19) Bozin, B., Mimica-Dukic, N., Samojlik, I., Goran, A., Igic, R., 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chemistry* 111, 925-929.
- 20) Campos, C., Casado, A., 2009. Evaluation of the copper(II) reduction assay using bathocuproinedisulfonic acid disodium salt for the total antioxidant capacity assessment: the CUPRAC-BCS assay. *Analytical Biochemistry* 392, 37-44.
- 21) Cheeseman, J.M., 2006. Hydrogen peroxide concentrations in leaves under natural conditions. *Journal of Experimental Botany* 57, 2435-2444.
- 22) Chen, J.H., Ho, C.-T., 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acids compounds. *Journal of Agricultural and Food Chemistry* 45, 2374-2378.
- 23) Coudray, C., Talla, M., Martin, S., Fatome, M., Favier, A., 1995. High-performance liquid chromatography-electrochemical determination of salicylate hydroxyl-ation products as an in vivo marker of oxidative stress. *Analytical Biochemistry* 227, 101-111.
- 24) Demirci C, ekic, S., So'zgen Bas, kan, K., Tu'tem, E., Apak, R., 2009. Modified cupric reducing antioxidant capacity (CUPRAC) assay for measuring the antioxidant capacities of thiol-containing proteins in admixture with polyphenols. *Talanta* 79, 344-351.