Int J Ayurvedic Med,Vol 17 (1), 2026; 209-213

Ayurveda Sahiti Prabha's

International Journal of Ayurvedic Medicine

ISSN No: 0976-5921

Research Article

Proximate evaluation and Assessment of Antioxidant activity of *Mucuna pruriens* leaf extract

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Received: 26-06-2025 Accepted: 07-02-2026 Published: 31-03-2026

Abstract

Abstract: *Mucuna pruriens* also known as a velvet bean belonging to the Fabaceae family which is a miraculous plant. Plants from Fabaceae or Leguminosae family are known to have multiple nutritional factors and different constituents showing therapeutic importance. Researchers have investigated few of the aspects with the seeds of the said plant. In the current research, overall profiling of leaves has been focused. Leaves of the plant are easily available for research as well as contain many storage components in the form of primary and secondary metabolites of the plant. Proximate analysis gives the general profile of the plant revealing valuable and important information about quality of the plant part used. It provides information on organic and inorganic matter with respect to moisture content, ash content, volatile matter content, ash, fixed carbon, content of some biomolecules etc. which can be responsible for pharmacological effect. Qualitative estimation of phytoconstituents showed the predominant presence of alkaloids, tannins, cardiac glycosides and terpenoids. Elemental analysis of plant leaves also revealed the information on presence of some essential metals like Manganese, Copper, Cobalt and Zinc. Significant anti-oxidant activity shown by the plant leaf extracts was assessed by DPPH and FRAP assay. This study can further be used for testing further therapeutic properties of the leaves against prominent groups of diseases.

Keywords: Elemental analysis, Ash Content, Phytoconstituents, Radical scavenging activity, Therapeutic, Extractive value.

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v17i1.6292

Introduction

Knowledge on Indian Traditional medicines or Herbal medicines is being proven as a boon to cure to many diseases. Herbal medicines can interact with other pharmaceutical formulations to give best therapeutic results. Herbal medicine emphasizes on human health which requires personalized and a holistic approach. Traditional medicine has its origin in current cultures which involves medicinal use of plants to prevent and treat multiple infections.Herbal medicine plays vital role in maintaining the health and wealth of mankind. Majority of world population use herbal medicines. The World Health Organization (WHO) reports that approximately 21,000 plants have been used for medicinal purposes(1). Herbs have stood the test of time for their safety, efficacy, cultural acceptability and minimal side effects (2). Therapeutic power of some plants is mainly due to the presence of some secondary metabolites, which collectively are referred to as phytochemicals(3). These phytochemicals have potential to be developed as herbal medicines or could serve as precursors for modern medicine (4).

Proximate evaluation of a plant reveals the information about ash content and extractable matter. Ash value indicates the quality and purity of the drug which is in the powder form. Ash of any organic material is composed of their non-volatile inorganic components(5). Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts). A high ash value is an indication of contamination, substitution, adulteration in the crude drug. The ash remaining followed by ignition of herbal drugs is determined for various parameters like total ash, acid-insoluble ash and water-soluble ash(6). Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. Water soluble ash is the difference in weight between the total ash and the residues after treatment of the total ash with water. This is used as a means of evaluation of the crude drugs for the presence of active constituents in the herbal material. An excess of water in herbal crude drug encourages microbial growth, fungal growth, and deterioration. Moisture is an evitable component of crude drugs, which needs to be eliminated to aid

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their preservation(7).The test for loss on drying of crude drug determines the presence of both water and volatile matter for the materials that absorb moisture easily or deteriorate in presence of water(8). Elemental analysis of plant is performed by Atomic Absorption Spectroscopy (AAS) for four essential metals(9).Qualitative screening of phytoconstituents are simple tube tests which reveal the presence of some important secondary metabolites. These reactions are specific to presence of alkaloids, tannins, terpenoids, saponins, cardiac glycosides, etc. On completing the preliminary estimation, the plant profile and its nutritive value can be confirmed(10,11). Natural defense mechanism of the body shows power to prevent any kind of disease development by using antioxidants. In case of failures of these mechanisms, external anti-oxidants are supplemented. Natural antioxidants are preferred over synthetic anti-oxidants due to their side effects. Many of the general diseases are a result of oxidative stress(12). Oxidative stress is damage to the cells and tissues because of accumulation of reactive oxygen species (ROS) and free radicals. These radicals are found to be accumulated at different sites in the analysis of majority of disorders. These entities are results of some of the natural pathways of body like metabolism and some external parameters like smoking. Free radicals and ROS react and damage the biomolecules like proteins, lipids and carbohydrates of the body leading to different diseased conditions(13). Antioxidants are the molecules which fight with these free radicals. They neutralize these free radicals and ROS(14).Many of the plants belonging to Leguminosae family are having high antioxidant potential which can further be proven significant in showing action against prominent diseases.

Main aim of the study is to complete proximate evaluation along with assessment of antioxidant potential. Prominent objectives of the study are to use plant leaves for estimating organic and inorganic content of the dried ash as well as crude powder. Further the research emphasizes on determining radical scavenging activity of leaf extract as well as crude plant leaves by weight.

Materials and Methods

Mucuna pruriens which is commonly known as velvet beans belonging to Fabaceae or Leguminosae family. The used plant material has been under the research for pharmacological activities of the seed as a plant part. Whereas in the current research work we are using leaves to evaluate their antioxidant potential. Leaves are the plant parts available in abundance in all the seasons. The plant is collected from Charkop, Kandivali (Mumbai) area in spring season of 2021. Leaves were cleaned, dried and ground in a fine powdered form for further use.

Materials

Proximate evaluation was performed as per Ayurvedic Pharmacopoeia of India. The protocols for determination of total ash content, acid insoluble ash content, water soluble ash content, moisture content and establish the extractive matter have been followed as per the Appendix 2 of Ayurvedic Pharmacopoeia of India(15).

Elemental Analysis by Atomic Absorption Spectroscopy

Acid digestion of the sample was carried out for the ash of dried plant leaf powder. Nitric acid, perchloric acid and sulphuric acids were used individually as well as in mixture 3:1:1v/v/v. Sample effective concentration aspirated was prepared 1000ppm.

Respective standards were also prepared in acids and pure solvents in the concentration of working stocks as 1000ppm for Manganese, 5ppm for Zinc, 50ppm for Cobalt and 10ppm for Copper. Metal lamps and their analyzing wavelength are given in Table 1.

Instrument details: Atomic Absorption Spectrophotometer Model number- AA-7000F Manufactured by Shimadzu

Table 1: Name of the metal with its lamp used and wavelength of detection

Metal	Detector lamp	Absorption maxima
Manganese	P624 Lumina hollow cathode lamp (HCL)	279.5nm
Cobalt	P813 HCL	240.7nm
Copper	P624 HCL	324.8nm
Zinc	P867 HCL	213.9nm

Phytochemical Screening of Plant Extract

Plant leaf extracts were prepared in five different solvents as mentioned in the table for qualitative estimation of some important phytoconstituents or secondary metabolites. The details of the tests performed are given in Table 2.

Table 2: Name of the phytoconstituents and tests

Phytoconstituents	Test	Expected observation
Alkaloids	Dragendorff's test	Orange coloured precipitate
Alkaloids	Mayer's test	White coloured precipitate
Saponins	Foam tube test	Foam formation/ frothing observed
Cardiac glycosides	Brown ring test	Brown ring at interphase indicating the presence of deoxy sugar which is characteristic of cardiac glycosides. Violet or greenish ring appears. Below the ring, coloration is observed.
Flavonoids	Yellow coloration test	Yellow coloration
Tannins	Ferric chloride test	Bluish black coloration
Terpenoids	Brown ring test	Brown ring or coloration at the base of test tube

Anti-oxidant Activity

Plant extracts were subjected to assessment of anti-oxidant potential by two methods as stated below. These are the two most efficient, simple, rapid and easy methods to evaluate free radical scavenging antioxidant potential of the plant which may in turn reveal a therapeutic profile of the plant.

DPPH assay

This is simple colorimetric evaluation based for the assessment of scavenging activity which uses 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a stable free radical. Antioxidant which when comes in contact with it, decreases the absorbance as a result of neutralization of free radical. Plant extracts and DPPH reagent is mixed and absorbance is measured after some time at 517nm(16). The plant sample is diluted 1:4.

FRAP assay

Reduction of ferric ion to ferrous ion by antioxidant in an acidic environment is measured using simple colorimetric assay. Antioxidant activity of plant extract is measured by increase in the

absorbance at 590nm. Under the acidic condition of pH 3.2, intensely blue coloured solution will form as a result of reduction reaction(17).

Results and Discussion

Proximate evaluation reveals the profile of plant for its organic and inorganic content. As per the polarity gradient of the used solvents, content of extractable matters is observed in the current research. Extraction yield, Proximate organic, inorganic content, elemental analysis, the total phenolic contents and antioxidant activities of the tested plant materials certainly help to ascertain the potency of the tested medicinal plant materials as potential source of natural antioxidants to be used for nutraceutical and functional food applications(18).

Figure 1: Proximate evaluation

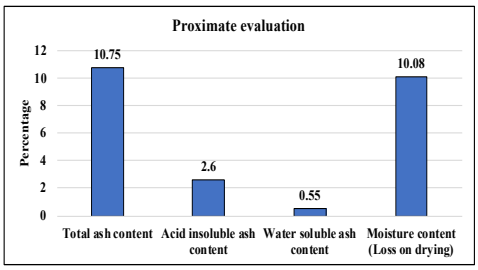


Figure 2: Extractive value in percentage

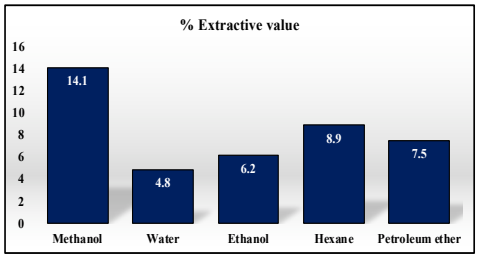


Figure 3: % content of elemental analysis by AAS

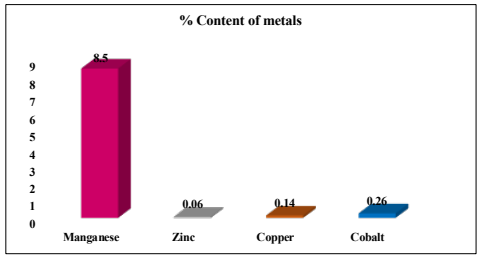


Table 3: Plant Antioxidant activity by two simultaneous methods

Name of the sample and	Antioxidant activity
DPPH method 100ppm	39%
DPPH method 500ppm	50%
Sample by FRAP assay	56nmol/g dried weigh

Essential metals like Manganese, Zinc, Cobalt and Copper play a significant role in important metabolic functions of human body like biological processes, for example as a cofactor for a variety of enzymes, including photosynthesis, the transfer of electrons within biological molecules, iron metabolism, the scavenging of free radicals, and various neurological functions(19).

Plant leaves are showing presence of multiple phytoconstituents known for their significant therapeutic properties as shown in Table 4.

Table 4: Results of Qualitative tests for phytoconstituents

Phyto-constituents	Methanol extract	Ethanol extract	Water extract	Hexane extract	Petroleum ether extract
Alkaloids	+	+	-	-	+
Saponins	-	-	-	-	-
Cardiac glycosides	+	-	+	-	-
Flavonoids	-	-	-	-	-
Tannins	+	+	+	-	-
Terpenoids	+	-	-	+	+

Conclusion

As depictable in the summarized result of Figure 1 of proximate ash content evaluation, considerable variation was observed. This is due to the physiological and biochemical composition of the plant. This will reflect upon the biological or pharmacological perspective of the plant. Nutritional value of plant also depends upon the proximate evaluation. Moisture content gives an overview and helps deciding the shelf life of the plant. High ash value of plant concludes that plant is high mineral content. Moisture content is the amount of loss on drying of water and volatile substances. Moisture is sometime used for estimation of the qualitative of food. However, the amount of moisture content is one of the main factors in storage, due to the proliferation of microorganisms, such as fungi and mold. Higher extractive values in polar solvents like methanol for both the plant extracts show presence of high number of polar components in leaves. Extractive value of crude drugs gives us prima facia information about chemical nature of the components present as shown in Figure 2. It further helps in standardization of solvent for quantitative analysis. Ash content and relative solubility reveals the organic and inorganic composition of the plant. Extractable matter shows some specific polar and nonpolar components being soluble in respective solvents having relative similar polarities. Elemental analysis shows highest content of Mn followed by Cobalt followed by copper followed by Zinc. Figure 4-7 show the calibration curve by Atomic Absorption Spectroscopy for all 4 metals. Figure 3 gives overall comparative composition of all 4 metals.

Tests for secondary metabolites show the presence of some therapeutically important phytoconstituents like alkaloids, tannins, cardiac glycosides and terpenoids as depicted in Table 3. Further quantification can be done in order to identify specific constituents from the above classes. These phytoconstituents show significant medicinal properties which can be further used for formulation studies. Methanolic extract shows presence of majority of phytoconstituents. For further studying the specific secondary metabolites qualitatively or quantitatively respective solvents can be chosen. Previously published work on standardization of solvent for extraction of polyphenols also reveals that methanol is the best solvent that can be used for extraction. Previous research work has shown the presence of tannins (Gallic acid equivalent), polyphenols and their antibacterial potential. Quantification assay has shown significant amount of tannins being present in the extract of plant leaves. (20).

Table 4 shows results for DPPH and FRAP assay of the plant showing significant antioxidant potential. Components exhibiting antioxidant potential should be isolated and characterized. Antioxidant activity of the plant reveals many new therapeutic avenues of the plant like anticancer property, antidiabetic activity, inflammation, neurobiology, cardio vascular disorders. Plant

leaves may show multiple such activities and hence can be proven medicinally very important.

Figure 4: Cobalt Calibration curve (240.7nm)

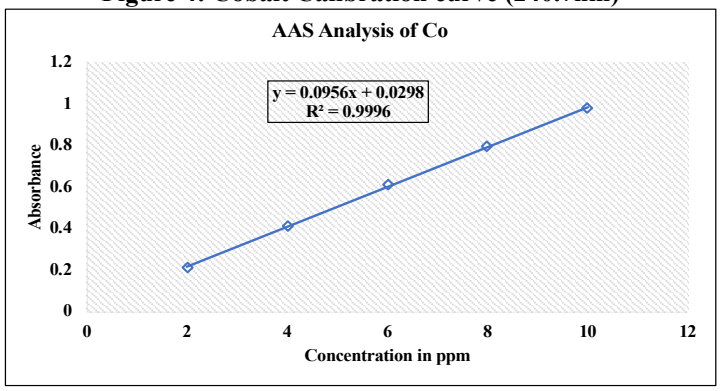


Figure 5: Copper Calibration curve (324.8nm)

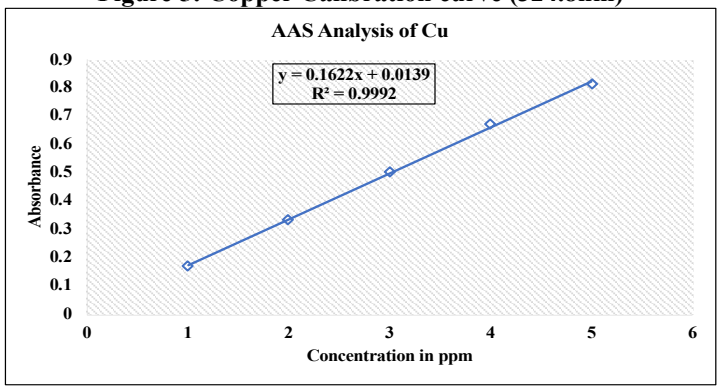


Figure 6: Zinc Calibration curve (213.9nm)

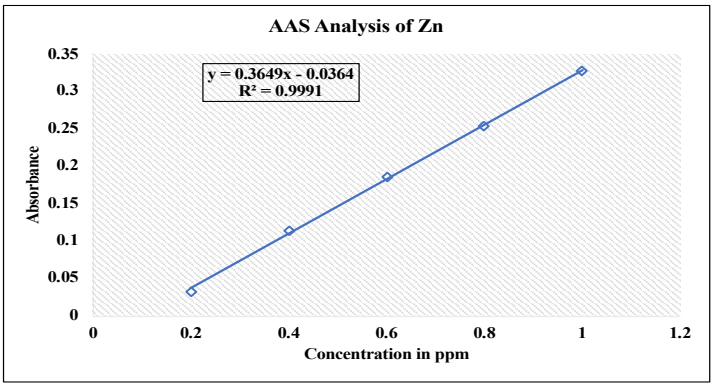
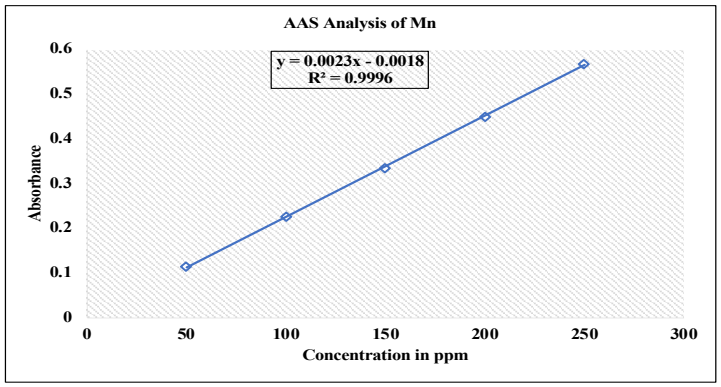


Figure 7: Manganese Calibration curve (279.5nm)



Acknowledgements

I want to thank Department of Bioanalytical Sciences, Ramnarain Ruia Autonomous College, Mumbai and Department of Chemistry, St. Xavier's College (Autonomous), Mumbai and for their unconditional support throughout the progression of the work.

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