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ANTIOXIDANT, ANTIMICROBIAL ACTIVITY WITH MINERAL COMPOSITION AND LCMS BASED PHYTOCHEMICAL EVALUATION OF SOME *MUCUNA* SPECIES FROM INDIA

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ABSTRACT

The study was designed to demonstrate the phytochemical composition and to evaluate minerals, antioxidant and antibacterial activities of different Mucuna species. Liquid Column Mass Spectrometry (LC-MS) analysis was performed to identifies the phytoconstituents like phenolic, flavonoids, alkaloids, vitamins, amino acids, anti-cancer agent, antiretroviral agents, antidiabetic agents, anti-inflammatory and anti-Parkinson's drug. Minerals essential for healthy functioning of the human body were resoluted from these species using atomic absorption spectroscopy. The antioxidant activity was evaluated by using the DPPH and ABTS assay. The antimicrobial activities of various Mucuna extracts were performed against some micro-organisms (Gram positive and Gram-negative micro-organism) using agar well diffusion method. The results of the present study revealed variation between some different Mucuna species. The results of LCMS phytochemical profiling indicate flavonoids and related polyphenols responsible for various pharmacological activities were the major compounds in the Mucuna seeds. Estimated mineral content in various Mucuna species shows the importance of these new wild varieties (Mucuna bracteata and Mucuna imbricata). Thus, serve as a great platform for the food industry due to their macro and micronutrients contents in significant amount. The strong antioxidant and antimicrobial activity present in the wild Mucuna imbricata seeds may be good alternative to the communally used of Mucuna species. They can also be used as a potential source of food and anti-Parkinson's drug in the pharmaceutical industry. Thus, the present article puts focus on the possible use of two wild Mucuna species as a substitute for Mucuna pruriens var utilis and herbal remedy against neurodegenerative disorders.

KEY WORDS

Antioxidants, Anti-microbial, LC-Q-TOF/MS, Minerals, Mucuna, Parkinson's disease.

INTRODUCTION

People are dealing with various neurodegenerative diseases principally Parkinson disease, Alzheimer's disease, schizophrenia, mental retardation, etc. Parkinsonism is an age-related commonest neurodegenerative disorder, with pathological feature characterized by progressive deterioration of dopaminergic neurons, initiating a chain of symptoms. Its clinical manifestation comprises of bradykinesia, hypokinesia inflexibility, rest tremor, non-motor features comprising depression, psychosis autonomic dysfunction and ultimately induces programmed cell death. The prevalence and occurrence of Parkinson disease is at its peak in European nations shadowed by few Asian countries. Thus, future aging society may have to face a severe socioeconomic burden created by the medical expenses done on this foremost neurologic disease[1–3]. The diverse adverse side effects induced

after long-term handlings of available conventional pharmacotherapeutic agents against Parkinson, has forced to opt for the herbal remedies. In this context, tropics of Africa and Asia were found to be traditionally using compounds extracted from the *Mucuna (M.) pruriens* (velvet bean or cowitch) as dietary supplements. Regular supplementation of this legume reduces Parkinson's by increasing dopamine content in the brain and regulating mood swings[4]. The Cognition enhancing activity in *Zandopa* (*Mucuna pruriens*) medicines suggests its use for anti-Parkinson's activity. Parkinson disease is the most common neurodegenerative disease being treated by using *Mucuna* species, having antioxidant [5], anti-inflammatory [6], analgesic[5], antimicrobial [7] and antidiabetic values[8]. *Mucuna* belonging to family Fabaceae includes approximately 150 species of annual and perennial legumes. The seeds of this plant are rich source of levodopa: dopamine precursor that helps to recover the symptoms of this disease. Diet rich in leguminous plants fulfil the requirement of protein that is the reason for common use of *Mucuna* species in feed and food by peoples in tropical locations[9]. In addition *Mucuna* species acts as green manure[10] and mainly used in production of pharmaceutical formulation [11, 12] to treat several disorders like Diabetes [8], nervous disorders [13], sexual disorder [14] etc. *Mucuna* is a common and rich source of antioxidant compounds which helps to protect oxidative damage. Earlier reports indicates that the optimization of solvents and extraction techniques have great impact on phytochemical composition of *Mucuna* species [15]. Nutritional, anti-nutritional content, proximate analysis and anti-inflammatory effects of *M. pruriens* var *utilis* is well known and have wide range of health-promoting and pharmaceutical properties [16]. Employing *Mucuna* for the treatment of Parkinson's disease is common these days, which may create overburden on the well-studied *M. pruriens* var *utilis.* Due to wide spread use, range of health-promoting and pharmaceutical properties *M. pruriens* var *utilis* overexploited from natural habitats results scarcity. To overcome this problem or to release the pressure on *M. pruriens* var *utilis* researchers have focused their interests on various biotechnological approaches and engaged to find superior substitute.

Considering this, the present study was designed to evaluate optimization of different solvents for the extraction of antioxidant and antimicrobial activity with mineral analysis. Similarly, phytochemical compositions of the different *Mucuna* species were studied by using LCMS-MS technique.

2. METHODS AND MATERIAL

2.1 Chemical

High-grade chemicals mandatory for LCMS analysis were taken from Himedia and SD Fine chemicals, Mumbai, India. All other chemicals required for the experiments were of analytical grades and purchased from Sigma (St Louis MO, USA).

2.2 Collection and sample preparation

Mucuna (*Mucuna bracteata, Mucuna imbricata* and *Mucuna pruriens* var *utilis)* samples were collected from various parts of Indian subcontinent identified, authenticated and deposited in herbarium (Voucher no-SVG008, SVG003, SVG009 respectively). Seeds obtained from full grown pods were powdered and air-dried using mortar pestle. The obtained powder was stored in the airtight zip bag to retain its dryness. 10 mg powder of different *Mucuna* species were extracted by using10 ml of four different solvents (Water, Acetone, Methanol and Ethanol). The samples were incubated at -20^{0}C for overnight and sonicated for 10 min. The extracts were then centrifuged at 10000 rpm for 15 min and the supernatant was harvested and used for further analysis.

2.3.1 Free Radical Scavenging Assay (DPPH)

Free radical scavenging potential of *Mucuna* samples extracted in various solvents was determined using DPPH radical scavenging assay described by Brand-Williams et al [17] with slight modifications [18]. Absorbance was measured at 517 nm using spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The inhibition percentage was calculated using the following formula which represents free radical scavenging activity.

$$\text{DPPH RSA}(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A\ \text{control}} \times 100$$

Where; A control is the absorbance of DPPH reagent; 'A sample' is the absorbance of test sample.

2.3.2 ABTS Radical activity

ABTS or (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation decolourization assay was used to measure free radical scavenging activity. The measurement was performed according to the method described by Re et al [19]. Working ABTS reagent was prepared by using stock solution of ABTS and potassium persulfate. 0.5 ml of *Mucuna* sample was allowed to

react with 1.5 ml ABTS reagent and absorbance was measured at 734 nm. All the measurements were estimated in triplicate. The results were expressed as percentage inhibition of ABTS radical by given equation

$$\text{ABTS radical scavenging effect (\%)} = \frac{\text{A control-A sample}}{\text{Acontrol}} \text{X100}$$

Where, A control is absorbance of ABTS radical + methanol.

A sample is absorbance of ABTS radical + *Mucuna* extract.

2.4 LCMS-MS Analysis Chemical Profiling

Extracts of 1 mg/ml *Mucuna* species samples were prepared using 0.01M formic acid: methanol (2:8 v/v). The samples were kept in the solvent for overnight and sonicated for 10 min. The extracts were then centrifuged at 10,000 rpm for 10 min and supernatant was taken out and store in freeze for further LCMS analysis. UHPLC–QTOFMS (Ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry) instrument (Agilent Series 1290) equipped with a degasser, binary gradient pump, column thermostat, autosampler and UV detector was used for analysis.

Agilent jet stream source was used for Dual AJS electrospray 205 ionization, a negative mode was used (Agilent 1290Technologies, CA, USA). ZORBAX RRHD Eclipse column used for the analysis was plus reversed phase C18 analytical column (100 mm ×2.1 mm, 1.8 µm particle sizes) kept at a temperature of 40 °C. Sample injection volume was 4 µl. The mobile phases were containing of 0.1% formic acid in water (eluent A) and 0.1% formic acid in methanol (eluent B). The following gradient elution was carried out: eluent B 5–45% from 0 to 8 min; eluent B 45–90% from 8 to 10 min, B 90% from 10 to 15 min; eluent B 3% from 15 to 20min (for equilibration). A flow rate of 0.4 mL/min was maintained. Data produced was selected on the basis of chromatographic and mass spectrometric performance. The data was processed for profiling using Mass-Hunter Qualitative software (Agilent, version B.06.00). To identify the metabolites, the mass accuracy was calculated with the procedure "Find Compounds by Molecular Feature", which contains the algorithm molecular feature extraction (MFE). All the compounds were detected by MS detection technique. The detection limits were calculated and least concentration producing a reproductive peak with a signal-to-noise ratio greater than three was recorded[20].The reference masses were 121.05m/z and 922.00 m/z.

2.5 Mineral analysis.

The mineral content of dried powdered materials of *Mucuna* species were quantified by using atomic absorption spectrophotometer. Two-gram fine seed powder of different *Mucuna* species were converted into ash by using muffle furnace at 550^{0}C in clean porcelain crucible for 20 min. The produced white residues of ash were dissolved in 5ml HNO_3/HCL (1:1 v/v). Solutions were heated on hot plate till brown fumes of HCL disappeared. Later 5 ml of deionised water was added, and mixture was heated till the development of colorless solution. The resulting solutions were filtered through a Whatman No. 42 filter paper and transferred into 100 ml volumetric flasks. Solutions were diluted to the desired volume with deionised water. The metals form the extracts were determined using a Perkin–Elmer 8650 AAS method described by Nahapetian and Bassiri [21].

2.6 Anti-microbial study

A agar well diffusion method, which is widely used to learn the antimicrobial activity of various plant extracts [21, 22]. Previously prepared *Mucuna* species seed extracts in different solvents were used for this analysis. Microorganisms namely *Escherichia coli* (NCLM2832), *Bacillus cereus* (NCLM2703), *Proteus vulgaris* (NCLM2813), *Pseudomonas aeruginosa* (NCLM5032) and *Salmonella typhimurium* (NCLM2501) procured from National Chemical Laboratory Pune were used for this study. The stock cultures were maintained on nutrient agar slants at 37^{0}C. The fresh working cultures were prepared by inoculating a loopful of microbial culture in 3 ml of saline solution. 0.5 ml of this active suspension was spread aseptically on the nutrient agar plate. By using sterile cork borer four wells were bored in the plates. Extracts of different *Mucuna* species were introduced aseptically inside each labelled well, plates were kept in the freezer for 20 min for extract diffusions and incubated at 37^{0}C for 48 hrs. The respective solvents were used as a control.

All analyses were carried out in triplicates. The results of antioxidant assay, mineral analysis were performed from the averages of samples reading (mean ± standard deviation) using Excel 2007.

3. RESULTS AND DISCUSSIONS

The main purpose of this article is to investigate the pharmacological constituents of some *Mucuna* species, which could be useful in preparation of formulations for

clinical use against Parkinson's disease. The use of vitamins, minerals and other supplements in the Parkinson's disease treatment helps in reducing its symptoms and progression.

3.1.1 Antioxidant study using DPPH

The DPPH was a convenient, fast and widely used technique to determine free radical scavenging activity of various biological samples. Hydroxyl radical was an extremely reactive free radical formed in a biological system and has concerned as a highly damaging species in the free radical pathology, capable of damaging almost every molecule found in the cell. The discoloration of mixture indicates the free radical scavenging power of the antioxidant. The scavenging effect of different *Mucuna* samples in different extraction solvents is given in Figure 1. This represented that *M. imbricata* have highest content of radical scavenging activity followed by M. bracteata than *M. pruriens* var *utilis.* DPPH radical scavenging activities of plant extracts varied from 29.67 ± 1.73 to 52.47 ± 1.28 %, which represents variation in the activity in different solvents. Results revealed highest antioxidant capacity in the ethanolic extract of *M. imbricata* (52.47 ± 1.28 %) and lowest in water extract of *M. pruriens* var *utilis* (29.67 ± 0.74%). Results confirm that ethanol is a best solvent for extraction of antioxidant compounds. Similar type of scavenging activity results were observed in M. *pruriens* in ethanolic extract by Kavith[24]. Whereas Aware et al [6]reported that water is best solvent for antioxidant activity in *M. macrocarpa*.

3.1.2 Antioxidant study using ABTS activity

ABTS radicals reacts rapidly with antioxidants under wide range of pH and also checks the effect of pH on antioxidant [25].The relative antioxidant ability to scavenge the radical ABTS has been studied using various solvent for *Mucuna* species. *M. imbricata* followed by *M. bracteata* has good ABTS activity in various extracts. The extent of inhibition of the absorbance of ABTS radicals is plotted in Figure 2. Results of ABTS activity revels that ethanolic extract of all *Mucuna samples* is having significantly stronger ABTS scavenging potency. Similar type of result were shown by Jivad et al.[26] in medicinal plant like *Coronopus Didymus.*

3.2 Metabolic profiling using LC-QTOF-MS

This was first report of metabolic profiling of different *Mucuna* species. LC-QTOF-MS is one of the newest, fast and simple technology which was used to make metabolic profile of sample, synthesis[27], degradation pathway[28] and detection of primary and secondary metabolites[28]. Data obtained from LCMS analyses were depicted in supplementary file. Table 1 gives an account of the major compounds found in seed samples of different *Mucuna* species. The possible compounds were selected on the basis of vital use and quality score among the number of phytochemical compounds and metabolites identified. Many compounds detected in LCMS analyses in different *Mucuna* species have wide range of biological activities like anti-Parkinson's, anti-cancer, anti-oxidants, anti-inflammatory, anti-microbial, anti-retroviral activity. Well-studied metabolites are present in the *M.*species like phenolics, flavonoids, L-DOPA[29], different derivatives of Gallic acid, catechin, different amino acids, alkaloids, quercetin, glycosides, tannic acid, and Tubastatin.

There are numerous constituents like flavonoids, alkaloids, saponins, tannins which are responsible for anti-inflamentory activity in *Mucuna* species is described Javedet al [30]. L-DOPA is principal compound commonly present in all *Mucuna* species responsible for anti-Parkinson's activity[15,28].There are some anticancer compounds present in the *Mucuna, mainly Spergualin,* Sanggenon G, Isopentenyl adenosine[32], Spisulosine etc[33]. Whereas some compounds like Terconazole[34], Validamycin A[33], clavulanate[35] present in the *Mucuna* species have the antimicrobial activity. Apart from that there are few anti-retroviral compound present in the *Mucuna* species they are Zidovudine[36], Tilorone[37].Cumulative effect of some compounds in the drug formulation help to heal Parkinson[30] Preceding report and method validation for levodopa from the rat plasma was performed on LCMS by Yang et al [38]. Similar type of study for determination of phytochemical compounds in *Ardisia elliptica was carried out by using LCMS [39].*

3.3 Mineral analysis

Ash is the inorganic residue remaining after heating and in presence of oxidizing agents by removing the water and organic matter, which make available a measure of minerals within a sample. The mineral analysis results represented in table 1. revealed highest quantities of K, Ca, Zn, Fe, Co concentration in *M.imbricata*; whereas *M.bracteata* showed the highest amount of N, Na, and S. However *M.pruriens var utilis has lowest* concentration of minerals than the other *Mucuna* species under study. The previous report by Gowrishankar[40] and Vermani and Chauhan[41] suggests that Ca, Fe, K, P, Ca and Mg are mineral

element essentially required in the formation of bone and teeth. There are some minerals like Zinc, Ferrous, Copper, Manganese, Molybdenum which are present in very lower (ppm) concentrations (Trace element) but performs a vital role in haemoglobin and collagen formation in brain cells[42]. *M. bracteata* is species used as food in some part which is richer in mineral content than *M. pruriens.* Zinc is present in high amount in *M. imbricata* which is significant component of metallo enzyme and stimulator for immune system. Zinc deficiency leads to malnutrition and growth failure[43] similarly high zinc content was reported in *M. pruriens* by Mercy et al [44]. Calcium content is maximum in *M. imbricata* which plays main role in signal transport in a neuron which is significant in the restoration of neurodegenerative disorder like Parkinson's disease. This Calcium content is similar to *M. pruriens* reported by Fathima et al [45]. As per results showed by Patil et al [29] in *M. sanjappae,* the Potassium content was maximum incomparison to other minerals. The plant containing minerals play vital role [41] whereas deficiency of mineral have perilous effect on health [46].

3.4 Antimicrobial study

The results obtained by antibacterial activity experiments of various extracts are given in figer.3 which shows all extracts of *Mucuna* species under study have superior antimicrobial activity than control. Ethanol, acetone and methonalic extracts of *Mucuna* species showed maximum growth inhibitory activity than water extracts. The broadest antimicrobial spectrum of zone of inhibition was found in *M. imbricata* followed by *M. bracteata* and *M. pruriens* var *utilis*. *M. imbricata* ethanolic extract showed highest activity against *Bacillus cereus* (Fig.3 B) followed by *Pseudomonas aeruginosa* (Fig.3 D) and other microorganisms. On the other hands, *M. pruriens* var *utilis* showed a narrow spectrum of zone of inhibition against all micro-organisms under study (Fig.3A, C, D). The water extracts of *Mucuna* species does not have any zone of inhibition in bacterial growth in *Mucuna* species. The overall observation specifies that *M. bracteata* and *M. imbricata* has more impact on different hazardous micro-organisms. along with some selected bacteria are checked for their antimicrobial activity in *M. pruriens* using methanolic extract by Salauet al[47] Their observation states that all the bacteria under study have good activity at 160 mg/ml. Whereas results by Kusuma et al [48] and Mastan et al [49] concludes that *M. pruriens* have good spectrum of zone of inhibition in methanolic extract comparison to chloroform and hexane.

Table 1- Mineral analysis of different four *M.* samples.

Sr.no	Parameter	*M. imbricata*	*M. Pruriens Var utilis*	*M. bracteata*	*M. Zendopa*
1	Nirogen %	0.22±0.001	0.61±0.01	0.72±0.031	0.67±0.14
2	Phosphrous %	0.17±0.002	0.29±0.09	0.15±0.005	0.19±0.074
3	Potassium %	26.25±0.03	17.22±0.01	20.52±0.07	15.36±0.045
4	Calcium %	4.46±0.5	3.31±0.47	2.09±0.83	3.12±0.04
5	Magnesium %	3.04±0.008	2.75±0.074	2.5±0.003	3.5±0.008
6	Sulphur %	2.09±0.32	1.37±0.09	5.94±0.65	0.82±0.06
7	Sodium %	2.05±0.06	2.89±0.09	2.92±0.012	1.69±0.04
8	Zinc ppm	119.6±0.14	104.28±0.09	88.35±0.35	89.3±0.54
9	Ferrous ppm	127.5±0.09	115.3±0.35	94.86±0.045	95.88±0.09
10	Copper ppm	134.42±0.64	85.1±0.19	132.9±0.58	67.5±0.1
11	Mangenese ppm	78.52±0.07	21.42±0.65	39.9±0.08	40.42±0.45
12	Molybdenum ppm	28.24±0.07	8.26±0.95	19.44±0.951	45.59±0.557

Values are expressed as mean± SD (n=3)

Fig 1. Antioxidant activities of *Mucuna* extracts tested by DPPH radical scavenging activity. Error bars represent standard deviations (n = 3).

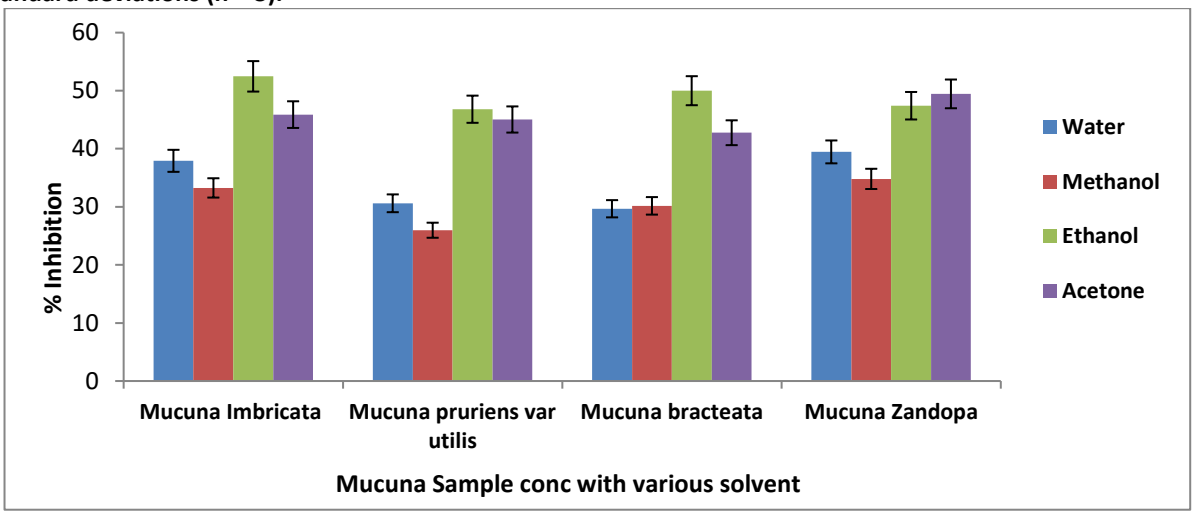


Fig 2. Antioxidant activities of *Mucuna* extracts tested by ABTS radical scavenging activity. Error bars represent standard deviations (n = 3).

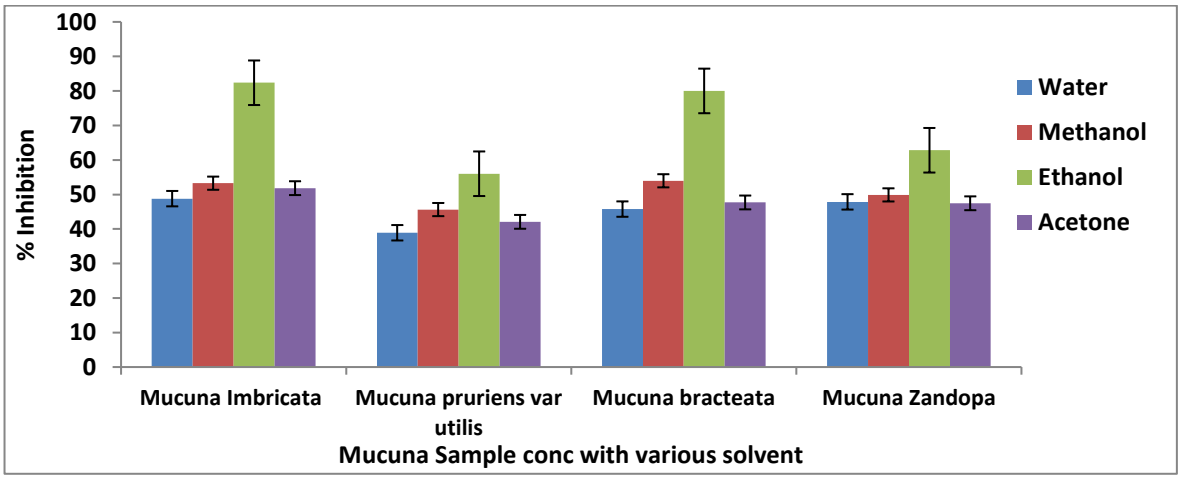
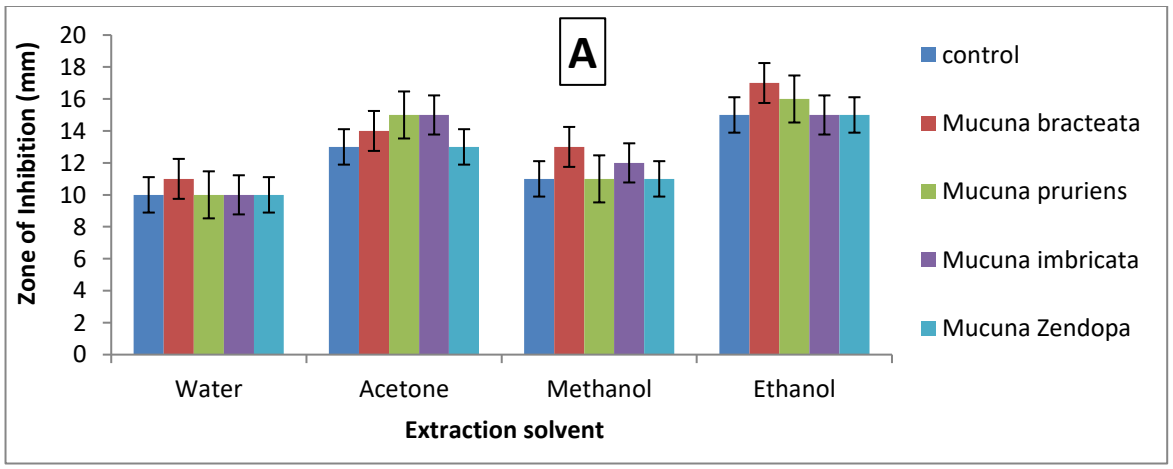
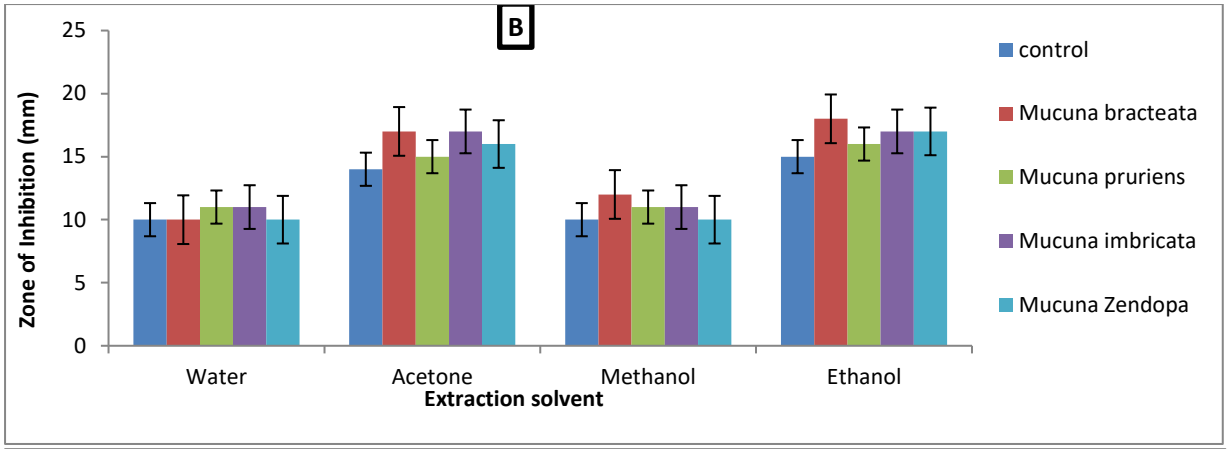


Fig 3. Zone of inhibition for Antimicrobial screening test of various solvents seed extract of different *Mucuna* samples against some bacterial strains A) *Escherichia coli* B) *Bacillus cereus* C) *Proteus vulgaris* D) *Pseudomonas aeruginosa* E) *Salmonella typhimurium*.

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Extraction solvent
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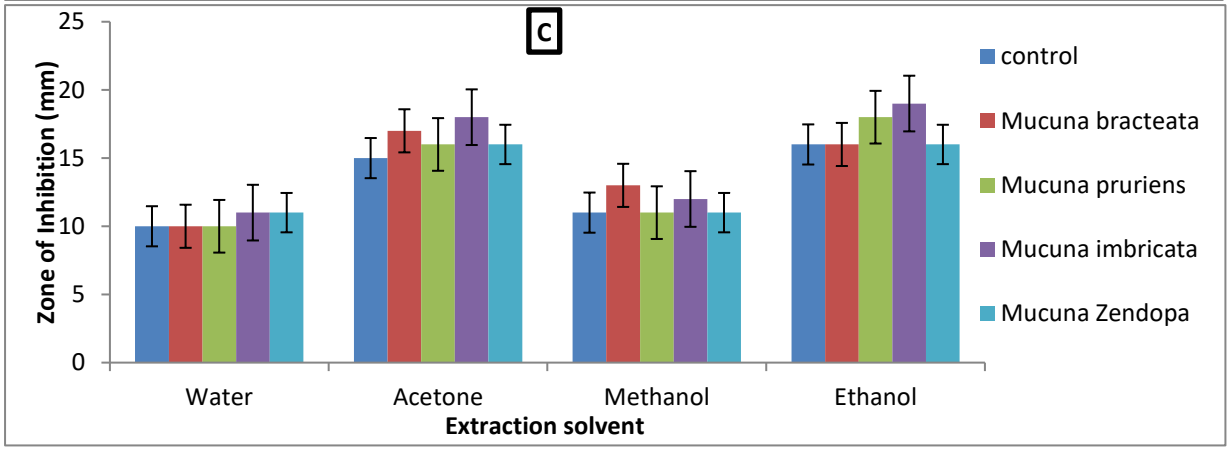
Mucuna pruriens
Mucuna imbricata
Mucuna Zendopa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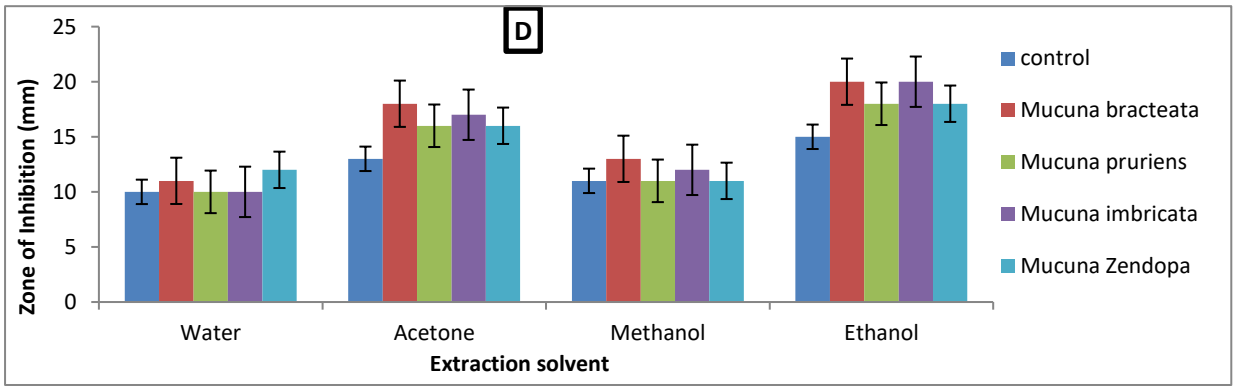
Mucuna imbricata
Mucuna Zendopa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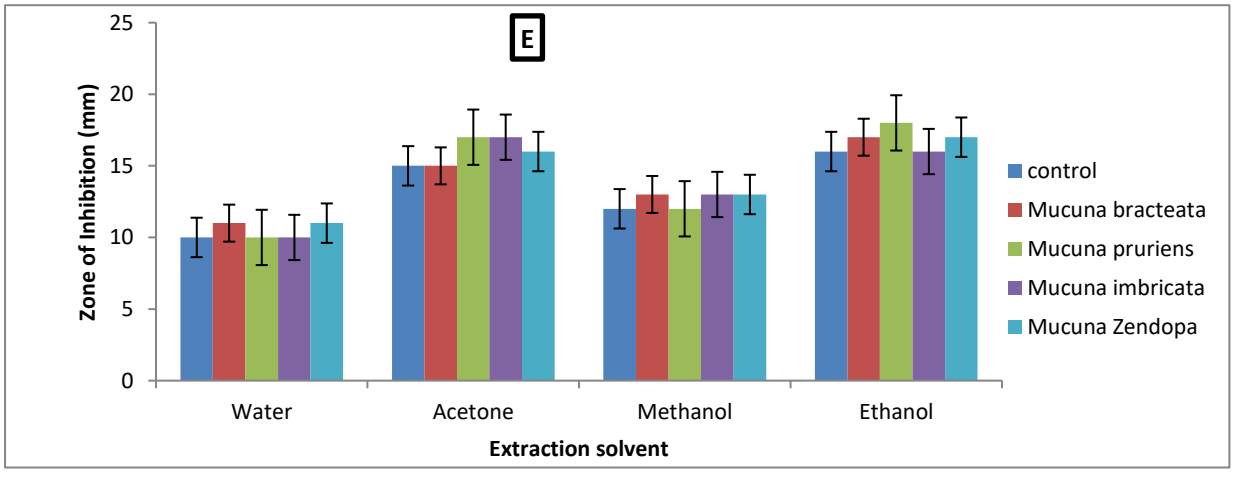
Mucuna imbricata
Mucuna Zendopa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Mucuna bracteata
Mucuna pruriens
Mucuna imbricata
Mucuna Zendopa

CONCLUSIONS

All the findings in the present results demonstrates that newly discovered and unnoticed wild species like *Mucuna imbricata* and *Mucuna bracteata* also act as good candidate sources of antioxidant, Minerals, phytochemicals and antimicrobial compounds than that of regularly used *Mucuna pruriens* var *utilis*. The results revealed that ethanolic extracts of *Mucuna imbricata* have a potential source of antioxidant and antimicrobial activity. Metabolic profiling of different *Mucuna* species concludes that various pharmacological and pharmaceutical compounds present in the *Mucuna imbricate and Mucuna bracteata crucially contribute in dealing with neurological disorders*. Even the mineral content in these neglected *Mucuna* species is high which may be beneficial to overcome various disorders caused by mineral deficiency. In the present study, some interesting expectation about the antimicrobial activities in Gram positive and Gram-negative micro-organisms obtained will help in developing effective drugs to treat several diseases. From this result it was concluded that *Mucuna* species *(Mucuna imbricata* and *Mucuna bracteata*) have reliable source of antimicrobial activity, antioxidants and different vital phtyto compounds over *Mucuna pruriens* var *utilis*. Phytochemical analysis in present study will definitely help to reduce pressure on *Mucuna pruriens var utilis. Thus, critical investigation of phytochemical with its biological activities,Mucuna imbricata and Mucuna bracteata may be used as superior substitute.*.

Conflict of Interest-

There is no any conflicts of interest.

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REFERENCE

[1] Y. Winter, S. von Campenhausen, H. Brozova, J. Skoupa, J.P. Reese, K. Bötzel, K. Eggert, W.H. Oertel, R. Dodel, E. Ruzicka, Costs of Parkinson's disease in Eastern Europe: A Czech cohort study, Park. Relat. Disord. 16: 51–56, (2010).

[2] J. Lökk, Caregiver strain in Parkinson's disease and the impact of disease duration, Eur J Phys Rehabil Med. 44: 39–45, (2008).

[3] C. Dowding, C. Shenton, S. Salek, A Review of the Health-Related Quality of Life and Economic Impact of Parkinson's Disease, Drugs Ageing. 23:693–721, (2006).

[4] P. Remy, M. Doder, A. Lees, N. Turjanski, D. Brooks, Depression in Parkinson's disease: Loss of dopamine and noradrenaline innervation in the limbic system, Brain. 128:1314–1322, (2005).

[5] S.S. Nipate, In vitro antioxidant activity of ethanolic extract of Mucuna pruriens (L.) DC. Seeds Journal of Pharmacy Research, 5: 2769–2772, (2012).

[6] C. Aware, R. Patil, S. Gaikwad, S. Yadav, V. Bapat, J. Jadhav, Evaluation of L-dopa, proximate composition with in vitro anti-inflammatory and antioxidant activity of Mucuna macrocarpa beans: A future drug for Parkinson treatment, Asian Pac. J. Trop. Biomed. 7: 1097–1106, (2017).

[7] A.A. Mostafa, A.A. Al-Askar, K.S. Almaary, T.M. Dawoud, E.N. Sholkamy, M.M. Bakri, Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, Saudi J. Biol. Sci. 25: 361–366, (2017).

[8] D. Donati, L.R. Lampariello, R. Pagani, R. Guerranti, G. Cinci, E. Marinello, Antidiabetic oligocyclitols in seeds of Mucuna pruriens, Phyther. Res. 19: 1057–1060(2005).

[9] S. As, A.F. Ingredient, F.O.R. Small, E. Of, E. On, N. Value, Ruminants and Effect of Ensiling On Its Agroecosystems, Crop Sci. 1: 97–105, (2003).

[10] M.A. Florentín, M. Peñalva, A. Calegari, R.D. Translated, M.J. Mcdonald, Green Manure/Cover Crops and Crop Rotation in Conservation Agriculture on Small Farms, 2010.

[11] J.T.M. Ngatchic, S.D. Sokeng, N.Y. Njintang, T. Maoundombaye, J. Oben, C.M.F. Mbofung, Evaluation of some selected blood parameters and histopathology of liver and kidney of rats fed protein-substituted mucuna flour and derived protein rich product, Food Chem. Toxicol. 57: 46–53, (2013).

[12] R.R. Patil, M.R. Rane, V.A. Bapat, J.P. Jadhav, Phytochemical Analysis and Antioxidant Activity of Mucuna sanjappae : A Possible Implementation in the Parkinson ' s Disease Treatment, Journal of Pharmaceutical and Medicinal Research,2: 48–51,(2016).

[13] R.R. Patil, a. R. Gholave, J.P. Jadhav, S.R. Yadav, V. a. Bapat, Mucuna sanjappae Aitawade et Yadav: a new

species of Mucuna with promising yield of anti-Parkinson's drug L-DOPA, Genet. Resour. Crop Evol. 62: 155–162, (2014).

[14] Y.I. Ding, J. Kinjo, C. Yang, T. Nohara, Triterpenes from *mucuna* bird woodiana, Phytochemistry., 30: 3703–3707, (1991).

[15] L. Lampariello, A. Cortelazzo, R. Guerranti, C. Sticozzi, G. Valacchi, The magic velvet bean of mucuna pruriens, J. Tradit. Complement. Med. 2: 331–339(2012).

[16] K. Yadav, Mukesh, P. Upadhyay, S. Purohit, B.. Pandey, H. Shah, Phytochemistry and pharmacological activity of Mucuna pruriens: a review, Int. J. Green Pharm. 11: 69–723,(2017).

[17] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. 28: 25–30,(1995).

[18] K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, D. Hawkins Byrne, Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts, J. Food Compos. Anal. 19: 669–675,(2006).

[19] R. Re, N. Pellegrini, a. Proteggente, a. Pannala, M. Yang, C. Rice-Evans, Antioxidant Activity Applying an Improved Abts Radical Cation Decolorization Assay, Free Radic. Biol. Med. 26: 1231–1237,(1999).

[20] A. Das, P. Rushton, J. Rohila, Metabolomic Profiling of Soybeans (Glycine max L.) Reveals the Importance of Sugar and Nitrogen Metabolism under Drought and Heat Stress, Plants. 6: 21, (2017).

[21] Perkin Elmer Coorporation, Analytical Methods for Atomic Absorption Spectroscopy, Anal. Methods. 216, (1996).

[22] S. Magaldi, S. Mata-Essayag, C. Hartung De Capriles, C. Perez, M.T. Colella, C. Olaizola, Y. Ontiveros, Well diffusion for antifungal susceptibility testing, Int. J. Infect. Dis. 8: 39–45,(2004).

[23] C. Valgas, S. Souza, Screening methods to determine antibacterial activity of natural products, Brazilian Journal: 369–380,(2007).

[24] Kavitha K, evaluation of total phenols , total flavonoids , antioxidant , and anticancer activity of mucuna pruriens seed extract *Asian Journal of Pharmaceutical and Clinical Research*, 11: 1–5,(2018).

[25] K. Lemańska, H. Szymusiak, B. Tyrakowska, R. Zieliński, A.E.M.F. Soffers, I.M.C.M. Rietjens, The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones, Free Radic. Biol. Med. 31: 869–881,(2001).

[26] N. Jivad, Z. Rabiei, Asian Paci fi c Journal of Tropical Biomedicine, Apjtb. 5: 789–795,(2015).

[27] J. Wang, L. Zhou, H. Lei, F. Hao, X. Liu, Y. Wang, H. Tang, Simultaneous Quantification of Amino Metabolites in Multiple Metabolic Pathways Using Ultra-High Performance Liquid Chromatography with Tandem-mass Spectrometry, Sci. Rep. 7: 1–16,(2017).

[28] B. Jaegle, M.K. Uroic, X. Holtkotte, C. Lucas, A.O. Termath, H.G. Schmalz, M. Bucher, U. Hoecker, M. Hülskamp, A. Schrader, A fast and simple LC-MS-based characterization of the flavonoid biosynthesis pathway for few seed(ling)s, BMC Plant Biol. 16: 1–15,(2016).

[29] Contin. M, Lopane. G, Passini. A, Poli. F, Iannello. C, Guarino. M, Mucuna pruriens in Parkinson Disease. Clin. Neuropharmacol. 38, 201–203.(2015)

[30] A.A. Yavuz, B. Glas, M. Ihle, H. Hacioglu, K. Wehefritz, (12) Patent Application Publication (10) Pub. No.: US 2014/0270163 A1, 1, (2014).

[31] S.N. Rai, H. Birla, S.S. Singh, W. Zahra, R.R. Patil, J.P. Jadhav, M.R. Gedda, S.P. Singh, Mucuna pruriens protects against MPTP intoxicated neuroinflammation in Parkinson's disease through NF-$κ$B/pAKT signaling pathways, Front. Aging Neurosci. 9: 1–14,(2017).

[32] F. Di, M.E. Chirurgia, E. Gorincioi, N 6 -Isopentenyl Adenosine, a Promising Anticancer Agent : Synthesis of Novel Analogues , Evaluation of Antiproliferative Activity and Insight Into, Evaluation, (2010).

[33] P. Singh Sisodiya, Plant Derived Anticancer Agents: a Review, Int. J. Res. Dev. Pharm. Life Sci. 2: 293–308, (2013).

[34] E.L. Tolman, D.M. Isaacson, M.E. Rosenthale, J.L. McGuire, J. Van Cutsem, M. Borgers, H. Van den Bossche, Anticandidal activities of terconazole, a broad-spectrum antimycotic, Antimicrob. Agents Chemother. 29: 986–991,(1986).

[35] P. Maple, W. Brumfitt, J.M.T. Hamilton-Miller, A review of the antimicrobial activity of the fluoroquinolones, J. Chemother. 2: 280–294,(1990).

[36] G. Turk, G. Moroni, S. Pampuro, M.C. Brin, H. Salomo, Antiretro v iral activity and cytotoxicity of novelzido v udine (AZT) derivatives and the relation to their chemical structure, Int J Antimicrob Agents,20: 282–288,(2002).

[37] M. Markowitz, J.O. Morales-ramirez, B. Nguyen, C.M. Kovacs, R.T. Steigbigel, D.A. Cooper, R. Liporace, R. Schwartz, R. Isaacs, L.R. Gilde, L. Wenning, As Monotherapy for 10 Days in Treatment-Naive HIV-1 – Infected Individuals, 43: 509–515(2006).

[38] G. Yang, F. Zhang, L. Deng, C. Chen, Z. Cheng, J. Huang, J. Liu, H. Jiang, Development and validation of an LC-MS/MS method for simultaneous quantification of levodopa and MD01 in rat plasma and its application to a pharmacokinetic study of mucuna pruriens extract, Biomed. Chromatogr. 30: 1506–1514,(2016).

[39] J. Iqbal, B.A. Abbasi, T. Mahmood, S. Kanwal, B. Ali, S.A. Shah, Asian Paci fi c Journal of Tropical Biomedicine, Asian Pac. J. Trop. Biomed. 7: 1129–1150,(2017).

[40] R. Gowrishankar, M. Kumar, V. Menon, S.M. Divi, M. Saravanan, P. Magudapathy, B.K. Panigrahi, K.G.M. Nair,

K. Venkataramaniah, Trace element studies on tinospora cordifolia (Menispermaceae), Ocimum sanctum (Lamiaceae), moringa oleifera (Moringaceae), and phyllanthus niruri (Euphorbiaceae) using PIXE, Biol. Trace Elem. Res. 133: 357–363, (2010).

[41] A. Vermani, A. Chauhan, Physico-Chemical Analysis of Ash of Some Medicinal Plants Growing in Uttarakhand, India, Appl. Sci. 8: 88–91, (2010).

[42] R. Bhat, K. Kiran, A.B. Arun, A.A. Karim, Determination of mineral composition and heavy metal content of some nutraceutically valued plant products, Food Anal. Methods. 3: 181–187, (2010).

[43] R. Wapnir, Zinc deficiency, malnutrition and the gastrointestinal tract., J. Nutr. 130: 1388S–92S, (2000).

[44] E. Mercy, O. Sunday, Proximate Composition and Mineral Analysis of Mucuna utilis (Velvet Bean), IOSR J. Appl. Chem. 8: 42–45, (2015).

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[45] K.R. Fathima, P.T. Soris, V.R. Mohan, Nutritional and Antinutritional Assessment of Mucuna pruriens (L.) DC var. pruriens an Underutilized Tribal Pulse, Advances in bioresearch, 1: 79–89, (2010).

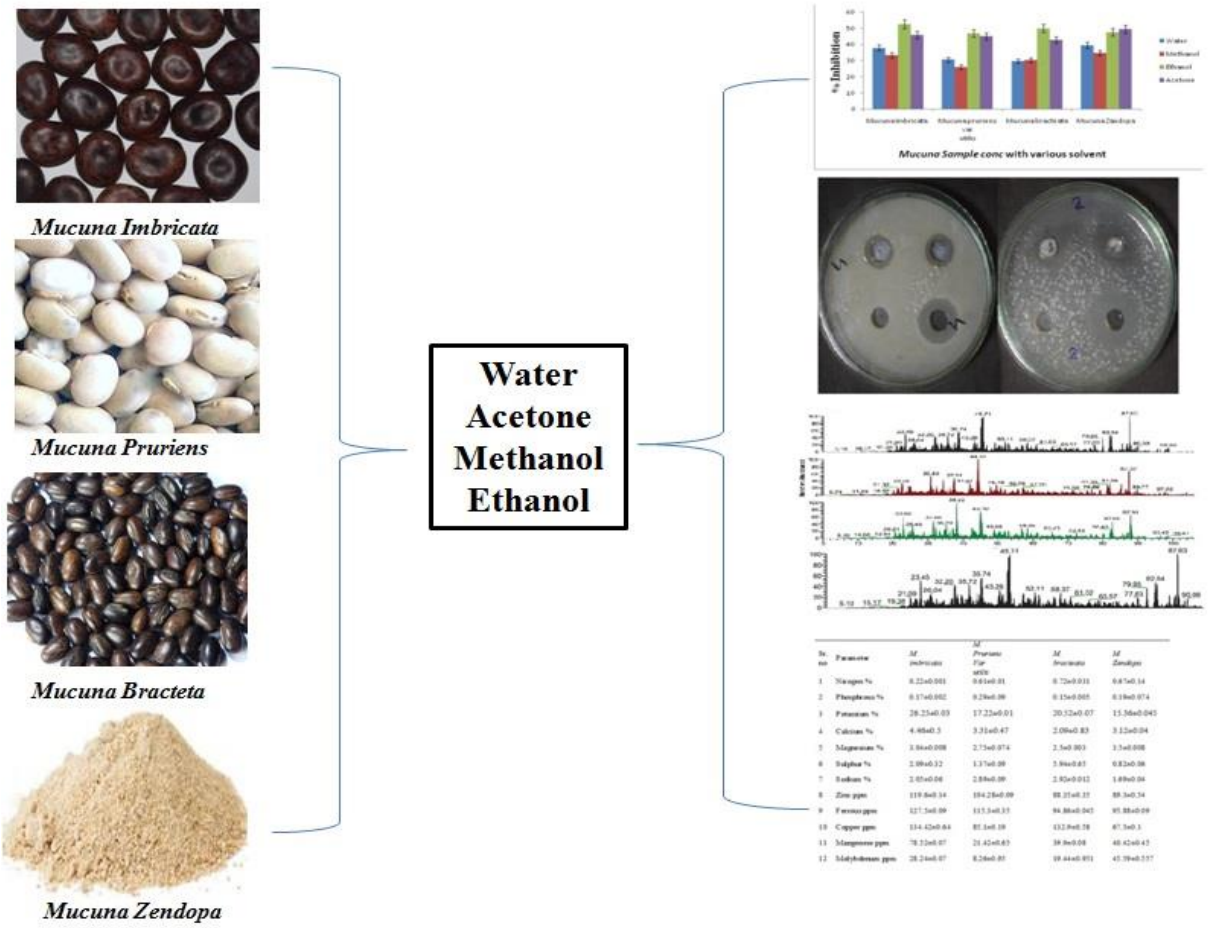
[46] J. Radwinska, K. Zarczynska, Effects of mineral deficiency on the health of young ruminants, J. Elem. 19: 915–928, (2014).

[47] A.O. Salau, O.M. Odeleye, Antimicrobial activity of Mucuna pruriens on selected bacteria, African J. Biotechnol. 6: 2091–2092, (2007).

[48] S. Kusuma, O.A. Kumar, K.L. Narayana, S. Pola, K.V. Reddy, In vitro Physicochemical, Phytochemical, Antimicrobial and Antidiabetic studies on Mucuna pruriens (Linn.) DC seeds, Int. J. Bioassays. 4650–4657, (2016).

[49] S.A. Mastan, P.J. Ramayya, L.M. Naidu, K. Mallikarjuna, Antimicrobial activity of various extracts of Mucuna pruriens leaves, Biomed.Pharmacol.J, 2: 55–60, (2009).

Graphical Abstract:



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