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Comprehensive Phytochemical Profiling and Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of *Mucuna pruriens* Leaves

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ABSTRACT

Mucuna pruriens also known as Velvet Beans is an annual climbing shrub that has been claimed traditionally to possess anti anemic potentials. *Mucuna pruriens* is one of the most promising wild underutilized medicinal legume belonging to family Fabaceae. It is used in ayurvedic as well as various traditional systems of medicine. This plant was widely utilized in treatment of various disorders. Also, it is a rich source of nutrients as well as used as a flavoring agent in the bakery industry. The traditional use of plants has declined due to scarcity of plant species, because of human activities and also by over grazing by animals. Therefore, it has become the need of hour to conserve these plant species. The present study was aimed to investigate leaves phytochemicals by preliminary phytochemical screening and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis in five different solvents. Preliminary phytochemical screening revealed presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids and terpenoids. The chemical profiling revealed the presence of high-value phytochemicals like terpenes, alkaloids and phenolic compounds in these extracts. GC-MS analysis revealed twenty-four and thirty bioactive compounds from the leaves and seeds respectively and it was solvent specific. GC–MS analysis led to the identification of several fatty acids, including linoleic acid, palmitic acid, oleic acid, and stearic acid, which are known for their therapeutic and nutritional benefits. These findings support the potential of *M. pruriens* leaves as a source of bioactive compounds and essential fatty acids, highlighting their relevance in nutraceutical and pharmaceutical applications. However, reported bioactive compounds highlight its nutritional importance and validate the use of the plant to cure various disorders by traditional practitioners.

1. Introduction

Herbal remedies were used to treat various diseases from ancient times. The phytoconstituents derived from natural sources create an attention for researchers due to their potential use in therapeutic treatment (Bhusare *et al.,* 2021a). The natural compounds synthesized by plants as integral components in their defense mechanism, also work for the welfare of humanity. These natural compounds have various medicinal properties, majorly Antimicrobial and Antioxidants (Singh *et al*. 2020a). In recent days huge amounts of synthetic medicines are explored but traditional remedies are gaining popularity day by day. Also, structural complexity makes chemical synthesis of important metabolites an unviable option, and makes plants the only source (Bhusare *et al.,* 2021 b). The plant derived phytoconstituents classified into two categories like primary and secondary metabolites depending upon their role and properties. The primary metabolite includes amino acids, proteins, lipids and carbohydrates etc. which play an important role in growth and development of plants. Whereas, the secondary metabolites viz alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides playing crucial role in plant defence mechanism against different stresses like biotic and abiotic stress (Simsek & Whitney, 2021).

A B

Fig 1. A) Flowering twig and B) seeds of Mucuna pruriens(L). DC

The *Mucuna pruriens* shown in fig 1, is one of the most promising medicinal plants used in the ayurvedic system, and in various traditional medicinal systems of different countries (Simmons, 2018). This plant possesses vital applications such as sedative, to relieve urinary tract infections, treatment of chest complaints, to treat snake bite intoxication and used in flavoring agents in cakes, sweet breads and candy. Also, it is a rich source of nutrients (Devhade *et al.,* 2015). The medicinal plants are widely used by all sections of the populations in India whether directly as folk remedies or through the medicine men because of their less expensive, easy availability and without any side effects. In rural areas of remote villages, where economic conditions are very low, people used the medicinal plants directly or by local methods of preparation. Their folk remedies are not widely common, non-documented properly and it is only transmitted orally (Ahmad, 2016). This leguminous crop is grown in tropical and sub-tropical areas of the world. In Asian and African nations including India, the Philippines, Nigeria, Ghana, Brazil, and Malawi, it is consumed as food. In India, the plant is usually used by traditional medicine practitioners for the treatment of asthma, bronchial infection, liver diseases, diabetes, gonorrhea, inducing labor and treatment of edema, feverish pain, sore throat, female sterility, oliguria, and vaginitis (Singh *et al.* 2020b). The immature pods and leaves are utilized as vegetables and the roasted seeds are eaten in some parts of Asia (Bindu *et al.,* 2023). However, the crop is still underexplored with regard to its detailed chemo profiling and the contribution of these phytochemicals to the nutraceutical and pharmacological potentials of this plant. So, many of its high-value constituents, preferably secondary metabolites, are still unidentified and further pharmacological studies are needed to assess their biological effects (Sruthi *et al.,* 2023). The various metabolites from plants are mainly associated with antioxidant activity and have medicinal significance. Therefore, the present investigation aims to reveal metabolites from wild underutilized medicinally important legume *Mucuna pruriens* leaves and seeds and their bioactive potential for medicinal significance.

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Studies analyzing the composition of MP grown in India have shown high percentages of protein (28.23%) and carbohydrate (60.03%) and a low content of lipids (2.69%), and a mineral composition of good nutritional quality (Siddhuraju and Becker, 2003). Studies on the nutritional composition of MP have characterized this leguminous plant grown mainly in India; however, it is known that the nutritional composition of foods is influenced by climatic variations, soil composition, temperature, surrounding vegetation, and rainfall volume (Luizão, 2007).

2. Material and Methods

2.1 Collection of plant material

The plant materials of *Mucuna pruriens* were collected from the local market of Lucknow in India. The collected leaf samples were rinsed with clean tap water and dehydrated under shadow conditions until suitable for the pulverizing process.

The well-dried plant leaf samples were pulverized individually, and grounded samples were filtered through standard four filters and refrigerated for further extraction (Narayanan *et al.,* 2021).

2.2 Identification of plant

Identification of plant material was done and authenticated with the help of standard flora; The Flora of British India (Hooker, 1890).

2.3 Sample preparation

The dry pods and fresh leaves were collected from *Mucuna pruriens*. Further seeds were removed from dry pods. The immature and infected or having diseased condition leaves were sorted out. The fresh and clean seeds were cut into small pieces by using a knife and small pieces of seeds were ground by electric mixture grinder. Further grind seeds and fresh leaves were crushed in liquid nitrogen. Prepared leaves were stored in an airtight plastic container and preserved in the refrigerator for further experimentation.

2.4 Extract preparation

The standard hot plate extraction method (Narayanan *et al.,* 2021) was applied to extract the phytochemical ingredients present in the powdered leaf samples of *Mucuna pruriens*. The 15-gram powder was filled in the thimble (made up of filter paper) and extracted successively with petroleum ether, chloroform, acetone, ethanol, and methanol solvent in 150 ml for 24 hours using Soxhlet extraction assembly. The temperature of the apparatus was maintained at the boiling point for each solvent. The obtained extracts were filtered through Whatman filter paper for free and clear extract. These extracts were evaporated and concentrated up to 10 ml. The resultant 10 ml extract was again filtered and stored in small sterile airtight bottles at 4 °C temperatures in the refrigerator. In brief, about 15 g of powdered leaf samples of *Mucuna pruriens* were dissolved in 150 mL of various solvents such as benzene, ethanol, and methanol in 250-mL conical flasks. These individual reaction mixers were placed over the water bath for 20 min at 55 °C, the extracts were filtered then, and using Whatman filter paper and filtrates were stored at 4 °C for study.

2.5 GC-MS Analysis

Sample was injected in an GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Splitless. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature 45 °C for 2 min then 140 °C at 5°C/ min and finally increased to 280 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out at 70 eV. The running time of the GC was from 9.10 min – 52.0 min. NIST14.L library (2020) was then searched to compare the structures of the compounds with that of the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST20.L).

3. Result and Discussion

3.1 Determination of Extraction Yield

The dried sample powder (1.0 gm) was weighed, placed in a cheese cloth and kept in thimble; Methanol solvent used for extraction. Solvent was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser. The crushed spice was loaded into the thimble, which was then placed inside the Soxhlet extractor. The solvent was heated using the heating mantle as the solvent boils, vapour starts to rise to the extraction chamber, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reached the siphon it was poured back into the flask and the cycle began again. The process ran for a total of 6 hours. The collected extract was concentrated using a rotary evaporator to dryness and then weighed.

Determination of Extraction Yield

extraction yield (%) = (weight of extract after evaporation solvent and freeze drying/ dry weight of the sample) × 100

Table 1: Yield obtained from samples after extraction

S. No	Sample Code	Yield content(%)
1	ML	8.21

3.4 GC-MS Analysis Result

The GC–MS chromatogram of the *Morus alba* leaf extract (Figure 3.2) revealed the presence of multiple phytochemical constituents eluted at various retention times, indicating the complex chemical nature of the extract. The total ion chromatogram (TIC) showed a series of sharp peaks between 8 and 50 minutes, corresponding to different volatile and semi-volatile compounds. Notable peaks were observed at retention times 11.05, 21.60, 28.65, 36.73, 42.93, and 44.60 minutes, among others, suggesting the presence of compounds such as phenolic derivatives, terpenoids, fatty acids, and esters. The most intense peak appeared at a retention time of 42.93 minutes, indicating the compound with the highest abundance in the extract, possibly a dominant bioactive phytochemical. Peaks around 36–45 minutes correspond to medium- to long-chain compounds, which are typically associated with antioxidant and antimicrobial activities.

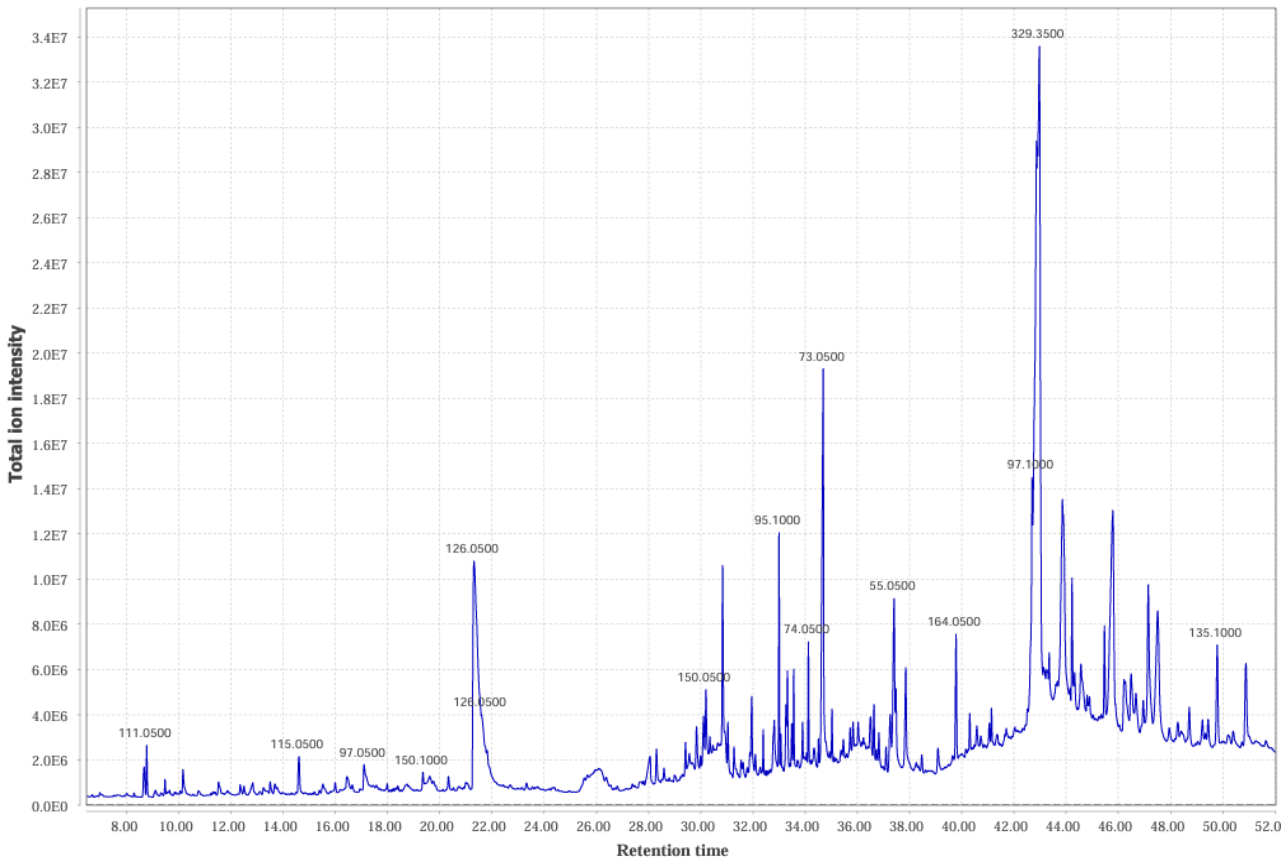
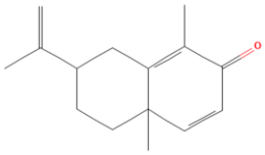


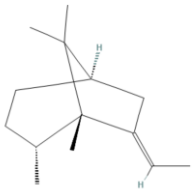
Fig. 2 GC-MS Spectrum of *Mucuna pruriens extract depicting peaks for various bioactive components*

Table 2: GC-MS profile of major bioactive compounds observed in extract of Mucuna pruriens leaves

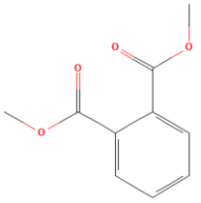
R. Time	Area	Area %	Height	Height%	Compound Names
29.854	26098756	1.59	6745838	1.70	1,2-Dehydro-.alpha.-cyperone
16.504	54841351	3.33	7490608	1.89	Catechol
33.565	14832646	0.90	7698494	1.94	8-Hydroxyageraphorone
31.408	17850312	1.08	8115863	2.05	(3aR)-3a,7-Dimethyl-2,3,3a,4,5,6-hexahydro-8
16.918	40194632	2.44	9161768	2.31	4-Vinylphenol
34.688	33583754	2.04	9160105	2.31	n-Hexadecanoic acid
9.046	23789354	1.45	9303425	2.35	2-Furancarboxaldehyde, 5-methyl-
34.525	19972520	1.21	10119937	2.56	Dibutyl phthalate
27.718	84411830	5.13	13259881	3.35	p-Cymene-2,5-diol
35.095	34586138	2.10	14966792	3.78	Xysmalogenin
23.380	84122301	5.11	15721007	3.97	Dimethyl phthalate
33.498	35145213	2.13	16051255	4.05	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hex
28.613	59588762	3.62	18766959	4.74	Benzene, (1,1,4,6,6-pentamethylheptyl)-
32.489	72641995	4.41	27125481	6.85	(1R,2R,5R,E)-7-Ethylidene-1,2,8,8-tetramethy
32.767	113414725	6.89	34499359	8.71	(1R,2R,5R,E)-7-Ethylidene-1,2,8,8-tetramethy



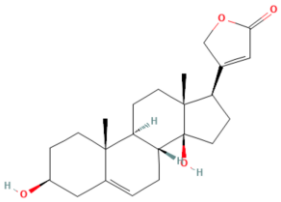
1,2-Dehydro-.alpha.-cyperone



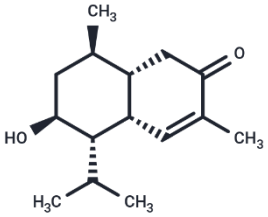
(1R,2R,5R,E)-7-Ethylidene-1,2,8,8-tetramethyl



Dimethyl phthalate



Xysmalogenin



8-Hydroxyageraphorone

Fig 3: Major compounds observed in GC-MS scanning of *Mucuna pruriens* extract

3.5 Discussion

The GC–MS profiling of the *Mucuna pruriens* leaf extract revealed a diverse range of phytochemical compounds, demonstrating the plant's complex chemical composition and therapeutic potential. The detection of twenty-four bioactive constituents, including catechol, 4-vinylphenol, p-cymene-2,5-diol, dimethyl phthalate, n-hexadecanoic acid, and xysmalogenin, indicates that the extract is rich in compounds with significant pharmacological and biological properties. The presence of these compounds aligns with previous studies that reported the abundance of phenolic derivatives, fatty acids, terpenoids, and phthalates in medicinal plants (Shelke *et al.,* 2022; Sruthi *et al.,* 2023). Among the detected compounds, **n**-hexadecanoic acid (palmitic acid) and dimethyl phthalate are noteworthy due to their well-documented antioxidant, anti-inflammatory, and antimicrobial activities (Martin *et al.,* 2006). Similarly**,** p-cymene-2,5-diol and catechol are phenolic compounds known for their strong radical-scavenging properties, which contribute to the antioxidant potential of *M. pruriens* extracts. These findings support earlier reports highlighting the correlation between phenolic content and antioxidant efficacy in leguminous and medicinal plants (Tavares *et al.,* 2015; Simsek & Whitney, 2024). The major peak observed at 42.93 minutes in the chromatogram represents the compound with the highest abundance, suggesting it may be a dominant bioactive component contributing to the overall therapeutic effect. The identification of fatty acids such as palmitic acid and oleic acid further underscores the nutritional and medicinal importance of *M. pruriens*, as these essential fatty acids play key roles in maintaining cellular integrity and preventing oxidative stress (Frota *et al.,* 2008). The solvent extraction using methanol appeared to be particularly effective in recovering these bioactive compounds, supporting the suitability of polar solvents for extracting phenolics, flavonoids, and fatty acids (Tiwari *et al.,* 2011). The Soxhlet extraction method enhanced compound recovery by maintaining constant solvent contact and heat, allowing for efficient extraction of medium- to long-chain molecules observed in the GC–MS profile.

4. Conclusion

The present study successfully demonstrated the phytochemical richness and chemical diversity of *Mucuna pruriens* leaves through preliminary screening and GC–MS analysis. The findings confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, phenols, tannins, terpenoids, and saponins, which contribute to the plant's therapeutic potential. The GC–MS profiling revealed twenty-four bioactive compounds, including catechol, 4-vinylphenol, *p*-cymene-2,5-diol, *n*-hexadecanoic acid (palmitic acid), and xysmalogenin, known for their antioxidant, anti-inflammatory, antimicrobial, and nutraceutical properties. The identification of essential fatty acids such as linoleic, palmitic, and oleic acids highlights the nutritional significance of *M. pruriens* as a source of health-promoting compounds. The study also underscores the effectiveness of methanolic extraction for isolating polar bioactives and fatty acids from plant tissues. Overall, these results validate the traditional medicinal use of *M. pruriens* and support its potential application in nutraceutical, pharmaceutical, and functional food formulations. Further in vivo and clinical investigations are recommended to explore the pharmacological efficacy and safety of the identified compounds, paving the way for its utilization in developing natural therapeutic agents.

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