

A Preliminary Investigation for Standardization of Hydroalcoholic Extract of *Mucuna pruriens* (Kapikaccu): An Important Drug Used to Treat Parkinson's Disease in Ayurveda

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Abstract

Keywords

- ▶ hydroalcoholic extract of *Mucuna pruriens*
- ▶ phytochemical analysis
- ▶ HPTLC

Establishment of standardization for the Ayurvedic formulations is most important for its chemical compounds, biological action, and its quality reassurance in production and manufacturing of traditional herbal medicines. As most of the drugs are standardized, drug companies are using substitute drugs instead of true drugs. So, to make finest superiority drugs it is necessary to validate raw drugs. Observing the existing trend in mind, hydroalcoholic extract of *Mucuna pruriens* seeds (HAMP) was subjected to standardize the procedures for phytochemical tests. The separation of bioactive substances from HAMP was performed using both manual methods and high-profile thin layer chromatography. From the current study, it is revealed that the seed contains alkaloids, steroids, carbohydrates, tannins, flavonoids, and coumarins, which gave the medicine numerous therapeutic properties.

Introduction

As per the World Health Organization (WHO), approximately 80 to 85% of the world's population use herbal medicines for their health care.^{1,2} As the usage of herbal medicines has increased, issues regarding their quality, safety, and efficacy have risen up.³ WHO has even evolved guidelines for the validation of plant-based drugs.⁴ As standardization is a burning topic in Ayurvedic drug formulations, it is most important for the establishment of its biological activity, chemical profile, and quality assurance in production and manufacturing of herbal drugs. As Ayurvedic medicines are supervised by the Drugs and Cosmetics Act, there is increased awareness for quality assurance to the public and the necessity for developing the standards for the purpose of quality control by the manufacturers as well as by the drug control authorities.⁵

Mucuna pruriens (MP) commonly called velvet bean is a tropical legume indigenous tree in Africa and tropical Asia.

As it is one of the best sources of protein content in many African countries, it is used as food for humans and also as animal feed.⁶ It is used as a forage, fallow, green manure crop enriched with proteins, amino acids, glutathione, lecithin, gallic acid, β -sitosterol, and L-3,4-dihydroxyphenylalanine (L-DOPA), vitamins such as niacin, ascorbic acid, synthesizing dopamine linked with mood and sex.⁷ The fatty acids present are palmitic, oleic, stearic, behenic, linoleic, and linolenic acid. It possesses antioxidant, hypoglycemic, lipid-lowering, and neuroprotective activities.⁸ MP seedpods are covered with hair which are rich in 5-hydroxytryptamine and if they come in touch with the skin it causes severe itching.^{9,10} In Ayurveda, to treat Parkinson's disease (PD) (also called *Kampavata*), MP has been used for a long time. Many kinds of research have found the presence of L-DOPA in the seeds of MP. Various clinical trials have been performed on PD patients for its safety, bioavailability, and action.^{11,12} It is reported that MP has antiurolithiatic,¹³ antidiabetic,^{14–16} anticancer, and antioxidant properties.¹⁷ As the plant has

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numerous therapeutic properties and uses in the field of medicine, one should be aware of its photochemistry.

So to prepare the best quality MP drug, it is necessary to authenticate raw drugs. Keeping the current trend in mind, it was subjected to standardize the procedures. For the current study, genuinity indicating parameters for MP were derived.

Materials and Methods

Plant Material

The seeds of MP were collected from the Sri Dharmastala Ayurveda Medical College and Research Center, Udupi, Karnataka, India. The plant material was stored in ambient conditions for further study.

Preparation of Extracts

The MP seeds were dried in the shade and powdered in our research laboratory with the help of pulverizer. The hydroalcoholic extract of *Mucuna pruriens* (HAMP) was prepared by soaking 500 g of powdered seeds of the MP in 2 L of 50% ethanol and 50% cold distilled water for 24 hours, filtered, and concentrated by evaporating on water bath till free from water. The extract is stored in an airtight container under normal temperature.¹⁸

Phytochemical tests, such as tests for alkaloids, steroids, saponins, tannins, flavonoids, phenol, coumarins, triterpenoids, carboxylic acid, resin, quinine, and high-profile thin layer chromatography (HPTLC), were performed as per the WHO guidelines,¹⁹ Ayurvedic Pharmacopoeia,²⁰ and Indian Pharmacopoeia.²¹

Preliminary Phytochemical Tests

Tests for Alkaloids

- Dragendorff's test*: A few milligrams of HAMP extract were dissolved in alcohol and few drops of acetic acid and Dragendorff's reagent were added, and then shaken well. An orange-red precipitate formed indicates the presence of alkaloids.²²
- Wagners's test*: A few milligrams of extract were dissolved in acetic acid and a few drops of Wagner's reagent were added. A reddish-brown precipitate formed indicates the presence of alkaloids.²³
- Mayer's test*: A few milligrams of HAMP extract were dissolved in acetic acid and few drops of Mayer's reagent were added. A dull white precipitate will be formed if the alkaloids are present.²⁴
- Hager's test*: A few milligrams of extract were dissolved in acetic acid and 3 mL of Hager's reagent was added. Formation of a yellow precipitate indicates the presence of alkaloids.²⁵

Tests for Carbohydrates

- Molisch test*: To the HAMP, along the sides of the test tube, 1 mL of α -naphthol solution and concentrated sulfuric acids were added. If carbohydrates are present then a violet color will be formed at the junction of the two liquids.²⁶

- Fehling's test*: Few milligrams of HAMP were mixed with equal quantities of Fehling's solution A and B. The mixture was warmed in a water bath. If carbohydrates are present then the formation of a brick-red precipitate is seen.²⁷
- Benedict's test*: To 5 mL of Benedict's reagent, a few milligrams of the extract were added, and boiled for 2 minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.²⁸

Test for Steroids

- Liebermann-Burchard test*: To the extract dissolved in chloroform, 1 mL of acetic acid and 1 mL of acetic anhydride were added, then heated on a water bath and cooled. Few drops of concentrated sulfuric acid were added along the sides of the test tube. The appearance of a bluish green color indicates the presence of steroids.²⁹
- Salkowski test*: The HAMP was dissolved in chloroform and equal volume of concentrated sulfuric acid was added. Formation of bluish red to a cherry red color in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.³⁰

Test for Saponins

To a few milligrams of HAMP, sodium bicarbonate (NaHCO_3) was added and shaken. Stable froth formation indicates the presence of saponin.³¹

Test for Tannins

To the HAMP, a few drops of dilute solution of ferric chloride (FeCl_3) were added. Formation of dark blue color shows the presence of tannins.³²

Test for Flavonoids

Shinoda's test: To HAMP in alcohol, a few magnesium turnings and few drops of concentrated hydrochloric acid were added and heated on a water bath. Formation of red to pink color indicates the presence of flavonoids.³³

Test for Phenol

To HAMP in alcohol, add two drops of alcoholic ferric chloride. Formation of blue to blue-black indicates the presence of phenol.³⁴

Test for Coumarins

To the HAMP in alcohol, a few drops of 2 N sodium hydroxide (NaOH) solutions were added. Dark yellow color formation indicates the presence of coumarins.³²

Test for Triterpenoids

The HAMP was warmed with tiny bits and a few drops of vinyl chloride. Formation of pink color indicates the presence of triterpenoids.³⁵

Test for Carboxylic Acid

HAMP dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.³⁶

Test for Resin

Few milligrams of the sample were mixed with water and acetone. Turbidity indicates the presence of resin.³⁷

Test for Quinine

Few milligrams of HAMP were treated with 0.5% of NaOH. If quinine is present it gives deep coloration like pink, purple, or red.³⁵

High-Profile Thin Layer Chromatography

One gram of powdered sample was dissolved in 10 mL ethanol and kept for cold percolation for 24 hours and filtered. 4, 8, and 12 μ L of the above sample were applied to a pre-coated silica gel F254 on aluminum plates to a bandwidth of 7 mm using Linomat 5 TLC applicator. The plate was developed in n-butanol:acetic acid:water (4:1:1). The developed plates were visualized in short ultraviolet (UV) and long UV, and then derivatized with vanillin sulfuric acid reagent and scanned under 254 and 366 nm and white light at 620 nm. Retention factor (R_f), the color of the spots, and densitometric scan were recorded.³⁸

Results and Discussion

Qualitative and quantitative analysis of phytochemical compounds present in HAMP by colorimetry (\rightarrow Tables 1 and 2), photo documentation (\rightarrow Fig. 1), the unique R_f values (\rightarrow Table 3), densitometric scan, and densitogram (\rightarrow Figs. 2–4) were obtained at different wavelengths from HPTLC.

Concentration of different phytochemicals of HAMP detected by HPTLC at wavelength 366 nm are alkaloids (17.13%), carbohydrates (17.19%), steroids (19.59%), tannins (25.86%), flavonoids (10.81%), and Coumarins (9.42%).

The phytochemical tests were performed to serve as a preliminary test for the standardization of the HAMP formulation. Tests for alkaloids, carbohydrates, steroids, tannins, flavonoids, and coumarins are positive for HAMP. Photo

Table 1 Results of preliminary phytochemical tests

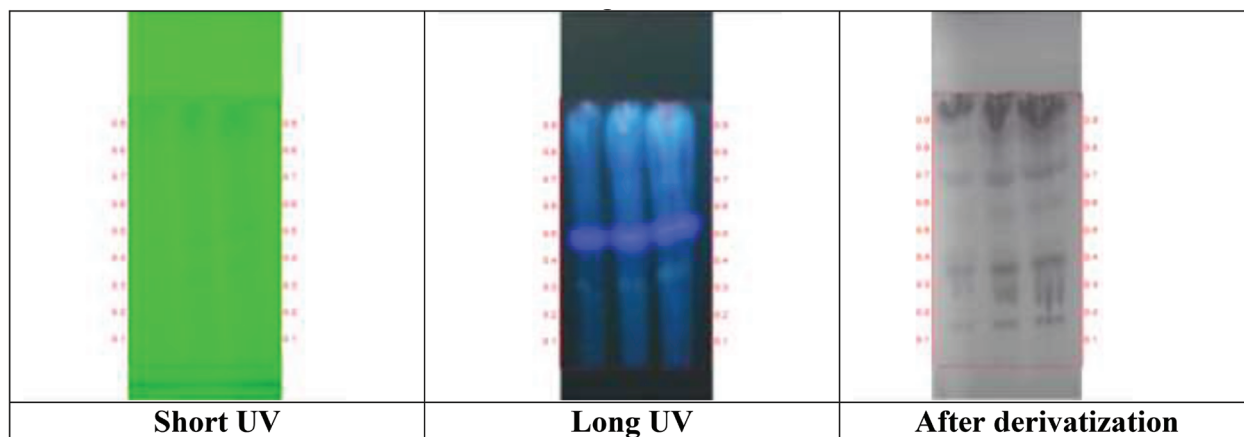
Test	HAMP
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+
Flavonoids	+
Saponins	-
Terpenoid	-
Coumarins	+
Phenol	-
Carboxylic acid	-
Resins	-
Quinine	-
Amino acids	-

Abbreviation: HAMP, hydroalcoholic extract of *Mucuna pruriens* seeds.

Table 2 Results of preliminary phytochemical tests

S. no.	Tests	Color if positive	HAMP
1	Alkaloids		
	Dragendroff's test	Orange precipitate	Red solution
	Wagner's test	Red precipitate	Red solution
	Mayer's test	Dull white precipitate	Dull white precipitate
	Hager's test	Yellow precipitate	
2	Steroids		
	Liebermann-Burchard test	Bluish green	Green color is appeared in the chloroform and colorless in the acid layer
	Salkowski test	Bluish red to cherry red	Red solution at the upper layer and clear at the base layer
3	Carbohydrate		
	Molisch test	Violet ring	Violet ring
	Fehlings test	Brick red precipitate	Ink blue solution
	Benedicts test	Red precipitate	Bluish green solution
4	Tannin		
	With FeCl_3	Dark blue or green or brown	Dark brown
5	Flavonoids		
	Shinoda's test	Red to pink	Light pink
6	Saponins		
	With NaHCO_3	Stable froth	No froth
7	Triterpenoids		
	Tin and thionyl chloride test	Red	Gray color
8	Coumarins		
	With 2 N NaOH	Yellow	Yellow color
9	Phenols		
	With alcoholic ferric chloride	Blue to blue black, brown	Dark brown
10	Carboxylic acid		
	With water and NaHCO_3	Brisk effervescence	No effervescence
11	Resin		
	With aqueous acetone	Turbidity	No turbidity
12	Quinine		
	5% NaOH	Pink/purple/red	Green colored solution
13	Amino acids		
	Ninhydrine reagent	Purple color	Green solution

Abbreviation: HAMP, hydroalcoholic extract of *Mucuna pruriens* seeds.



Track 1: HAMP – 4 μ L
 Track 2: HAMP – 8 μ L
 Track 3: HAMP – 12 μ L

Solvent system: n-Butanol:acetic acid:water (4:1:1)

Fig. 1 High-profile thin layer chromatography (HPTLC) photo documentation of ethanolic extract of hydroalcoholic extract of *Mucuna pruriens* seeds (HAMP).

Table 3 R_f values of samples

Short UV	Long UV	After derivatization
–	–	0.17 (D purple)
0.36 (D green)	0.36 (F Aq. blue)	–
–	–	0.39 (D purple)
–	–	0.47 (L purple)
0.52 (D green)	0.52 (FD blue)	–
–	–	0.59 (D purple)
0.61 (D green)	–	–
–	–	0.72 (D purple)
–	–	0.84 (D purple)

Abbreviations: D, dark; F, fluorescent; L, light; UV, ultraviolet.

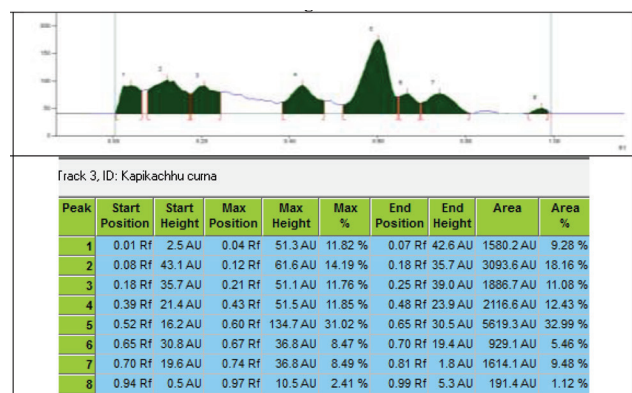


Fig. 2 Densitometric scan at 254 nm.

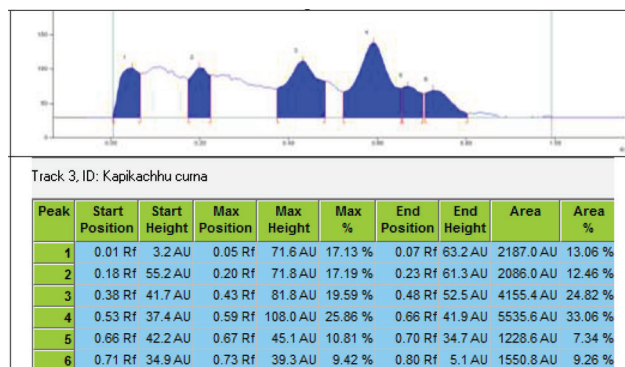


Fig. 3 Densitometric scan at 366 nm.

documentation, the unique R_f values, densitometric scan, and densitogram obtained at different wavelengths from HPTLC can be used as a fingerprint to identify HAMP powder. Similar findings about the presence of the compounds in the MP has been reported by several authors.^{39,40} Several reports on MP about anti-venom,⁴¹ hypoglycemic,⁴² anti-Parkinson's,¹¹ antimicrobial,⁴³ antioxidant,⁴⁴ and aphrodisiac activity⁴⁵ has been documented. Our present phytochemical analysis of HAMP also confirms similar types of compounds found in other studies. Thus, it can be used for further various clinical trials.

Conclusion

By preserving the fundamental aspect of the Ayurvedic drug, standardization requires a rational approach. The main

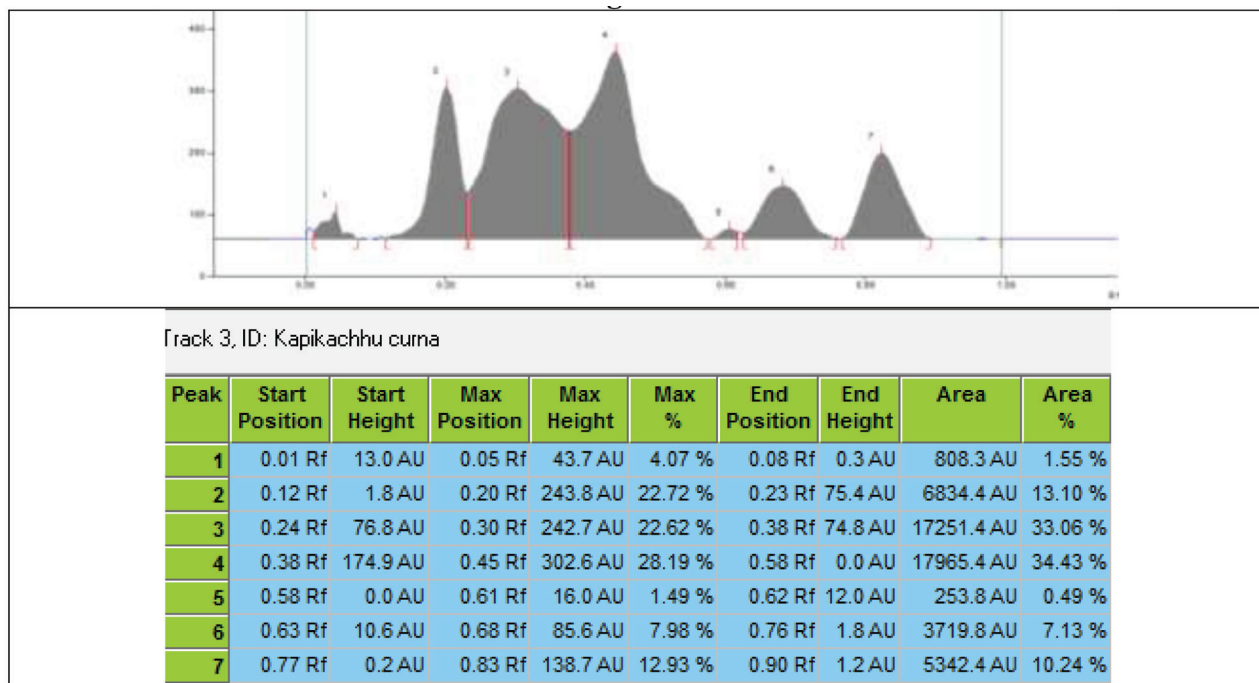


Fig. 4 Densitometric scan after derivatization at 620 nm.

obstacle in the standardization of the Ayurvedic drug is the identification of its biological source. Drugs from different geographical source may vary with its active constituent and it may not be feasible to standardize drug chemically. The parameters used in this work ensure the quality control of HAMP. The results found through this study were rapid, reproducible, and could be used for routine monitoring of HAMP. HAMP is endowed with various biological properties and hence efforts have been made here to provide scientific data on the same.

Conflict of Interest

None declared.

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