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Research Article

Pharmacognostic and Physico-chemical standardization of *Macrotyloma uniflorum* (Lam.) verdc. edible seed

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ABSTRACT: **Introduction:** *Macrotyloma uniflorum* (Lam.) Verdc. (Family Fabaceae) commonly known as horse gram is largely cultivated in Australia, Burma, India and Sri Lanka. In the present study an attempt has been made to standardize this ethnopharmacologically useful seed on the basis of pharmacognostical, physico-chemical and phytochemical characteristics. **Methods:** The external morphology of seed was observed by using simple microscope. The percentage of foreign matter, loss on drying, crude fiber, swelling and foaming index of dried seed powder were determined. Total ash, water soluble ash, acid insoluble ash, sulphated ash and fluorescence property were also determined. Extraction, qualitative and quantitative analysis of seed samples was measured for total phenols and tannins and total flavonoids, along with TLC and HPTLC. **Results:** The seeds were odorless, slightly acrid and nonaromatic. The loss on drying was 1.45%, the total ash was 7.95% and the water soluble ash was 2.90% whereas acid insoluble and sulfated ash showed low content (3.10 and 1.81% respectively). Acetone (18.42%) and methanol (15.58%) soluble extracts showed the presence of alkaloid, glycoside, steroid, triterpenoid, carbohydrate, tannin, phenolics, saponin, flavonoid, amino acid and mucilage (except starch) phytoconstituents. The methanol extract was rich in flavonoid (8.59 mg/gm) whilst the acetone extract showed a high content of total polyphenols and tannins (8.91 and 5.23 mg/gm respectively). TLC showed the presence of chlorogenic acid and ferulic acid, whereas HPTLC confirmed the presence of quercetin and chlorogenic acid. **Conclusion:** The present study established pharmacognostic, physico-chemical, phytochemical, microscopic and fluorescence characterizations of *M. uniflorum* seed along with identification of bioactive flavonoid compounds.

KEYWORDS: Flavonoid, HPTLC, *M. uniflorum*, Seed microscopy, TLC, Quantitative estimation

INTRODUCTION

Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector, WHO recommends and encourages the use of traditional herbs/remedies because of easy availability and affordability.[1] A lack of proper documentation, stringent quality control and standardization has hindered the acceptance of the alternative medicines in the developed countries. There is a growing concern for documentation of research work carried out on traditional medicines needed for regulatory control.[2] The maintenance of herbal medicinal quality depends on both scientists and the manufacturers and consumers.

Herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants, seasonal, ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotics.[3] The assurance of standardization of medicinal plant in term of safety, quality and efficacy has become an important issue. It becomes extremely important to make an effort towards standardization of the plant material used for therapeutic purposes. The process of standardization can be achieved by stepwise pharmacognostic studies and minimizing the inherent variation of natural product composition through quality assurance practices applied to cultivation and manufacturing processes.[4] In the present study an attempt has been

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made to standardize the ethnopharmacologically useful seeds of *Macrotyloma uniflorum* (Lam.) Verdc. (Family Fabaceae) on the basis of pharmacognostic, physico-chemical and phytochemical characteristics.

M. Uniflorum (synonym *Leichhardt biflorus, Dolico cavallino, D. biflorus*) commonly known as horse gram is cultivated in dry areas of Australia, Burma, India and Sri Lanka. It is an annual or perennial plant growing in the summer. It matures in 40 days for forage and 120–180 days for seed. The plant is used as a vegetable in India and the seed is known as the poor man's pulse in southern India. Consumption of horse gram seeds, after soaking/dry heating followed by cooking, along with cooked rice or pearl millet is a common practice among the rural people in India.[5] Seeds are black or brown in color, odorless, slightly acrid in taste and ovular in shape. The chemical composition is comparable with commonly cultivated legumes.[6] According to Ayurveda seed is bitter, acrid, hot, dry and used as astringent, anthelmintic, antipyretic and uterine stones, tumours, asthma, bronchitis, hiccup, urinary discharges, heart-troubles, disease of the brain and eyes, intestinal colic, piles, leucoderma, inflammation, liver troubles etc. Its decoction was used traditionally in leucorrhoea and menstrual dysfunctions.[6] Horse gram is an excellent source of iron and molybdenum.

MATERIAL AND METHODS

Plant materials

The *M. uniflorum* seeds were collected in the month of Nov-Dec 2010 from Godavari District, Andhra Pradesh. The plant was authenticated by Dr. Zea Ul Hasan, Astt. Professor, Head, Dept. of Botany, Safia Science College and a specimen voucher no. 192/BOT/Safia/10 was preserved for future reference. Dust and debris were removed from the seeds and dried in an oven at 40°C. Dried seeds were powdered with a domestic grinder passed through sieve 40/60 and stored in air tight container.

Macroscopic and microscopic analysis

The external seed morphology (nature, color, odour and taste) were noted. Other structural peculiarities including size, shape and texture were observed by using simple microscopy.[7] The seeds were soaked overnight in water and cut into 2–5 mm pieces without compression and immediately transferred into FAA solution (95% ethyl alcohol: glacial acetic acid: formalin: water in 50:5:10:35) for one day to fix the tissues. The pieces were embedded with paraffin wax. The paraffin embedded specimens were sectioned with the help of rotary microtome giving a thickness of 10–12 µm. Dewaxing of the sections was performed by customary procedure.[8] The sections were stained with hemalum and safranin. A drop of HCl and phloroglucinol were used to detect lignified cell in the cut sections.[9] The microphotographs were captured using trinocular microscope (Jyoti Scientific, Gwalior) with digital Olympus Camera.

Physico-chemical studies

The percentage of foreign matter, loss on drying, crude fiber, swelling and foaming index of dried seed powder were determined.[10] Total ash, water soluble ash, acid insoluble ash and sulphated ash were also determined according to the method described in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plants materials.[10] The dried seed powders were subjected to fluorescence color reaction analysis as is and also after treating separately with water, 10% NaOH, 10% KOH, acetic acid, 1N HCl, 1N HNO_3, 1N H_2SO_4, ammonia, 5% $FeCl_3$, 5% iodine and methanol against normal and ultra-violet light at 254 nm.[11,12]

Preparation of extracts

Coarse seed powders (50 gm) were defatted with a sufficient quantity of petroleum ether (40–60°C) with the aid of Soxhlet apparatus for 24 h. The defatted seed cakes (5 gm each) were then extracted separately with 100 ml each of ethyl acetate, chloroform, methanol, ethanol, acetone and water for 48 h by maceration and then filtered to obtain respective extracts. The petroleum ether fraction obtained after defatting was recovered as a petroleum ether extract after filtration. The extracts in different solvent were collected separately and the volume reduced under low pressure. Each extract (25 ml) was used to determine the percentage extractive values of seeds in different solvents.[10]

Extraction of seed for qualitative and quantitative analysis

Powdered seed samples were defatted by using petroleum ether in the ratio of sample: solvent (1:10 w/v) with occasional shaking at room temperature for 24 h. The extract was filtered through Whatman filter paper and air-dried. The residue was extracted again with methanol for 24 h, filtered and re-extracted with an additional quantity of methanol for three hours. The combined methanol extracts (ME) were stored for further analysis.

The residue left out after air-drying were again extracted by occasional shaking with 70% acetone at room temperature for 24 h, filtered and re-extracted for 3 h. The respective combined acetone extracts (AE) was evaporated under reduced pressure using a rotary vacuum evaporator. The ME and AE thus obtained were used for

qualitative phytochemical analysis and estimation of total phenolic and tannin content.

Preliminary phytochemical screening

Preliminary phytochemical screening of the seed extracts in different solvents has been performed by standard methods to detect the alkaloid, glycoside, steroid triterpenoid, carbohydrate, tannin, phenolic, saponin, flavonoid, amino acid, inulin, mucilage and starch phytoconstituents.[13,14]

Quantitative estimation of phytoconstituents

Determination of total phenols and tannin contents

The total phenolic content of the freeze-dried ME and AE were determined according to previously described methods.[15] Aliquots (0.5 ml, 0.1 mg/ml) of the ME and AE extracts were made up to a volume of 1 ml with distilled water. Then 0.5 ml of Folin Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20% w/v) were added sequentially in each tube. After vortexing the reaction mixture the tubes were incubated in the dark for 40 min at room temperature. The absorbance was recorded at 725 nm against the reagent blank with a double beam UV/Visible spectrophotometer (EI model no.1372, Japan). The amount of total phenolics was calculated as pyrogallol equivalents (even though pyrogallol was used as a standard in this work, it is not a natural constituent of horse gram or legumes in general) from the calibration curve by linear regression.

The tannin content was estimated in freeze-dried ME and AE extracts after treatment with polyvinyl polypyrrolidone (PVPP) following standard methods.[15] PVPP content (100 mg/ml in distilled water) was taken in a test tube and to this 1.0 ml of the ME and AE were added. The mixtures were vortexed and kept at 4°C for 15 min. the mixtures were subsequently centrifuged (3000 gm for 10 min at room temperature) and the supernatants were collected. These supernatants would have only simple phenolics other than tannins as PVPP is known to precipitate the tannins as a residue. The phenolic content of the supernatant was measured as mentioned above and expressed as the content of non-tannin phenolics on a dry weight basis. From the above results, the tannin content of the sample was calculated in percentage as follows:

$$\text{Tannin content} = \text{total phenolics} - \text{non-tannin phenolics}$$

Estimation of total flavonoid

The aluminium chloride colorimetric technique was used for estimation of flavonoids.[16] ME and AE (0.5 ml each) were taken (100 mg/ml of methanol and acetone respectively) in test tube and mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. The reaction preceded at room temperature for 30 min and the absorbance was subsequently measured at 415 nm. The calibration curve was plotted by preparing quercetin standard solution across a range of 10–70 ppm in methanol. The amount of flavonoid was calculated from the standard quercetin graph.[17]

Thin layer chromatography

Freshly coated plates with silica gel G_{254} was allowed to air dry in room temperature and transferred to an oven for activation maintained at 110ºC for 30 min. AE was dissolved in methanol and filtered through Whatman filter paper. Detection of maximum possible flavonoid present in AE was done with solvent system toluene: ethyl acetate: formic acid 5:3:2, reported to give good resolution for the presence of flavonoid.[18] The plates were placed into the developing chamber and allowed to run until reaching a height of approximately 10 cm from the point of application. The spraying agent used for detection of spots was vanillin sulphuric acid reagent. After development, the plates were kept in oven maintained at 110ºC for optimal color development. Standard sample of the flavonoids gallic acid was gift sample from Phytomed Pharma (Rajkot), whilst chlorogenic acid and ferulic acid was purchased from Himedia (Mumbai) and used for co-TLC to compare the Rf values.

High performance thin layer chromatography

HPTLC was performed on a CAMAG system which includes a pre-coated plate (silica gel $60F_{254}$, Merck), high pressure sample injector of 100 µl capacity, an HPTLC development chamber and scanning UV- cabinet. Samples for HPTLC were prepared in methanol, filtered with the Whatman filter paper. Toluene, ethyl acetate and formic acid in the ratio of 5:3:2 was used as the mobile phase. Spotting was done by the auto-injector on a 10×10 cm pre-coated silica plate. A 2.0 µl aliquot of sample was used for banding. Plates were put in the development chamber and allowed to run up to an optimum height of 9 cm. Plates were dried with a mechanical blower and they were observed under UV light. HPTLC of standard quercetin (gift sample from Phytomed Pharma, Rajkot), gallic acid, chlorogenic acid and ferulic acid was also performed following the same conditions. The scanning of the plate

was done at 254 nm wavelength. Data analysis was done with the help of CAMAG- Linomat 5 software system.

Antioxidant assay

Extract solutions of ME and AE were prepared in methanol and acetone respectively, filtered and diluted to desired concentration. Different concentrations of the ME and AE (from 100 to 1000 mg/ml) were assayed for in vitro antioxidant ability. Liver homogenate 10% w/v was prepared in 0.15 M potassium chloride and centrifuged at 6000 rpm (4°C) for 20 min and the supernatant was used for the study. All these estimations were done on Shimadzu (UV-1800 pharmaspec, Japan) spectrophotometer.

Estimation of glutathione

Glutathione was estimated by following the method of Tietze.[19] Glutathione reduces H_2O_2 directly to water or react directly with the free radicals such as O_2, OH* and O* by a radical process, which yields thiol radicals. This thiol radical or the sulfhydryl group present in glutathione forms a colored complex with DTNB which was measured calorimetrically at 412 nm.

Estimation of superoxide dismutase (SOD)

The effect on the superoxide radical production was evaluated using the Cytochrome C reduction method of McCord and Fridovic.[20] SOD is a powerful enzyme catalysing the conversion of O_2* to H_2O_2 and O_2. SDO was estimated as the inhibition of rate of reduction of cytocrome C by the superoxide radical observed at 560 nm.

Estimation of lipid peroxidase (LPO)

Based on the principles of Pulla Reddy and Lokesh, lipid peroxidation was initiated in the rat liver microsomes by ferric ions which leads to the formation of small amounts of malondialdehyde (MDA major product of lipid peroxidation).[21] Thiobarbituric acid reacts with MDA forming a pink chromogen which was detected spectrophotometrically at 532 nm.

RESULTS

Macroscopic Characters of seed

The seeds of *M. Uniflorum* used for the present study was of a dark black colored variety. The seeds were odorless, tasted slightly acrid and were nonaromatic. The size of the seeds was of 0.5−0.6 × 0.4−0.5 cm, ovular in shape, surrounded by the integuments and fully developed endosperm [Figure 1].

Microscopic evaluation of seed

The transverse section of seed showed dicotyledon consisting of plumule, redical, testa, epidermis, cotyledon, endosperm, parenchymatous cells and tigellum. Plumule (shoot apical meristem) and testa (root apical meristem) were made up of parenchymatous cells, which form the stem and root are developed on germination of seed. Testa was thick, 1–2 layered and appeared yellowish brown whereas, epidermis layer was attached to inner side of testa and appeared as single layer. Endosperm was composed of thick walled oval/circular cells attached closely with each other. Parenchymatous cells are fully packed simple, oval and rounded in

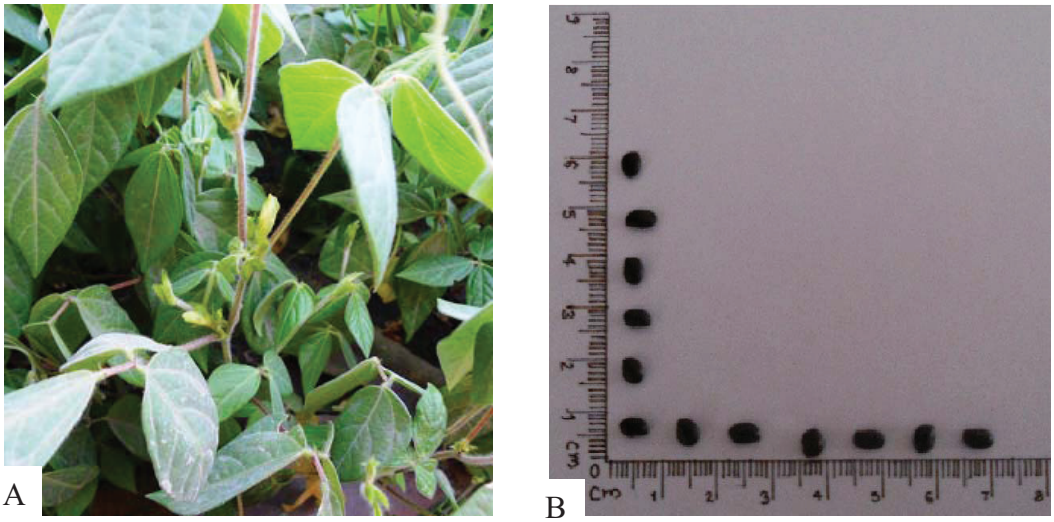

Figure 1. *Macrotyloma uniflorum* lam. (A) whole plant and (B) seeds.

shape. Parenchymatous cells were imbedded inside the endosperm and contain thick cotyledon to an axis called tigellum. The tigellum showed a protruding plumule and radical hidden between the cotyledons [Figure 2].

Physico–chemical evaluation

The results for physico-chemical parameters showed that only 0.82% of foreign matter were present in *M. Uniflorum* seed. The recorded loss on drying was 1.45%. The total ash content was found to be 7.95% whereas the water soluble ash was 2.90%. The acid insoluble and sulfated ash showed low content i.e. 3.10 and 1.81% [Table 1]. The result of fluorescence studies of seed powder using different reagents and solvents are given in Table 2. Seed powder did not showed fluorescence in acidic media like acetic acid, HCl, HNO_3 and H_2SO_4 but in other solvent it showed yellow and green fluorescence.

Extraction in different solvent

The results suggest that the seed has high acetone (18.42%) and methanol (15.58%) soluble extractive value in comparison to the chloroform (1.97%), petroleum ether (2.04%), ethyl acetate (4.26%), water (8.52%) and ethanol (7.20%) soluble extractive values [Table 3].

Preliminary phytochemical test for ME and AE

The extractive value for methanol and acetone extract was 21.05 and 30.26% respectively. Methanol extract showed presence of phytoconstituents like alkaloid, glycoside, steroid and triterpenoid, carbohydrate, tannin and phenolics, saponin, flavonoid, amino acid, inulin and mucilage except starch. The acetone extract showed the presence of all constituents except inulin and starch [Table 4].

Quantitative estimation of Phytoconstituents

The content of *M. uniflorum* methanol extract was higher with rich presence of flavonoid (8.59 mg/gm). The acetone extract showed a high content of total polyphenols and tannins (8.91 and 5.23 mg/gm respectively).

TLC and HPTLC analysis

AE showed the presence of three compounds. Matching Rf indicated presence of two constituents as chlorogenic acid and ferulic acid with one unidentified compound [Figure 3]. HPTLC of AE indicated presence of two constituents as quercetin and chlorogenic acid with two unidentified compounds [Figure 4].

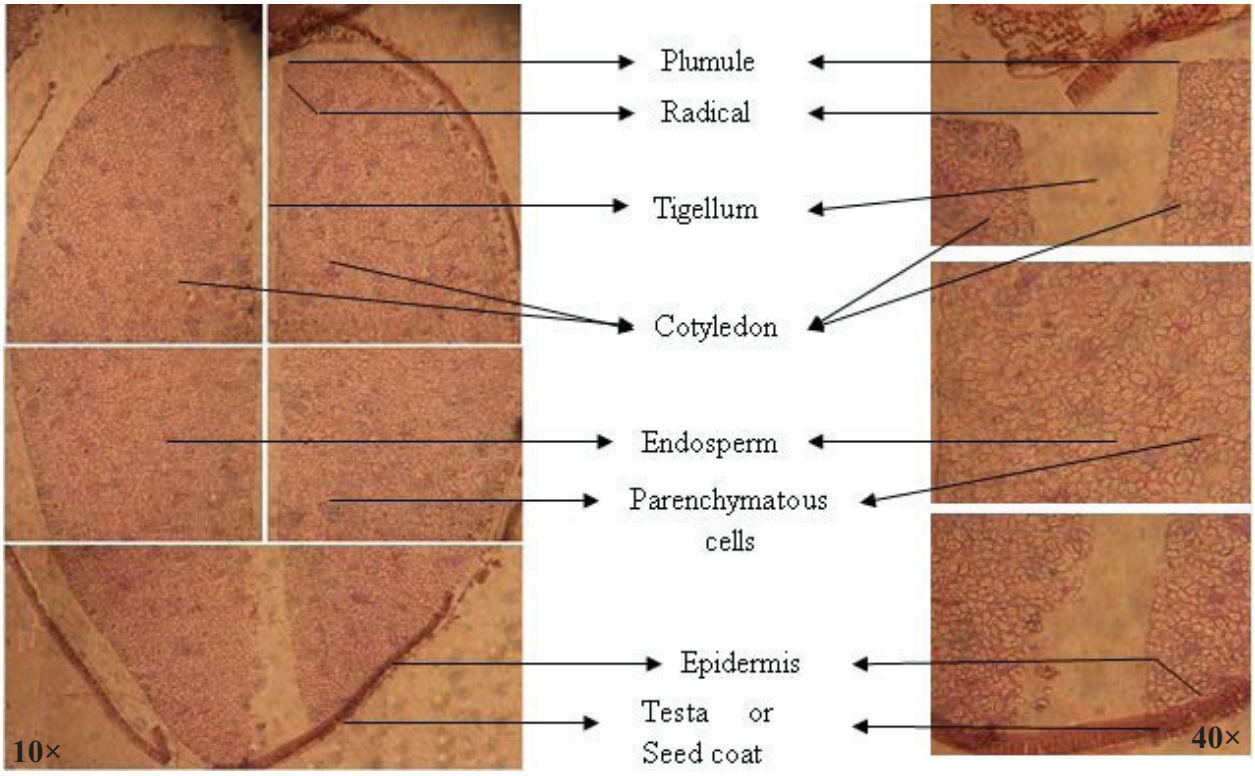

Figure 2. The transverse section of seed showing dicotyledon consisting of plumule, redical, testa, epidermis, cotyledon, endosperm, parenchymatous cells and tigellum. The parenchymatous cells are imbedded inside the endosperm and contain thick cotyledon to an axis called tigellum. The tigellum showed a protruding plumule and radical hidden between the cotyledons.

Table 1: Physico-chemical evaluation of *M. uniflorum* seed

Parameter	Value
% Foreign matter	0.82
% Loss on drying	1.45
% Crude fiber content	12.90
Swelling index	3.21
Foaming index	11.24
% Total ash	7.95
% Water soluble ash	2.90
% Acid insoluble ash	3.10
% Sulfated ash	1.81

Table 2: Fluorescence analysis of *M. uniflorum* seeds powder under visible and UV light

Treatment	Visible Light	UV Light
Dry powder	Blackish brown	Reddish brown
Powder + Water	Golden	Greenish yellow
Powder + 10% NaOH	Orange red	Yellowish green
Powder + 10% KOH	Orange red	Fluorescence green
Powder + Acetic acid	Creamy white	No fluorescence
Powder + 1N HCl	Light brown	No fluorescence
Powder + 1N HNO_3	Light yellow	No fluorescence
Powder + 1N H_2SO_4	Reddish brown	No fluorescence
Powder + Ammonia	Light yellow	Yellowish green
Powder + 5% $FeCl_3$	Yellow	Fluorescence green
Powder + 5% Iodine	Orange red	Yellow
Powder + Methanol	Orange red	Yellow

Table 3: Extractive values of *M. uniflorum* seed in different solvent

Solvent	Extractive value in % w/w	Color
Petroleum ether	2.04	Off white
Ethyl Acetate	4.26	Yellowish white
Chloroform	1.97	Off white
Methanol	15.58	golden brown
Ethanol	7.20	Orange Red
Acetone	18.42	light yellow
Water	8.52	Dark reddish brown

Antioxidant activity

M. uniflorum seed methanol extract showed potent increase in SOD and glutathione level with weak LPO scavenging ability. Acetone extract showed good lipid peroxide inhibition and enhancement of glutathione where as SOD has not increased significantly [Table 5].

Table 4: Quantitative and qualitative analysis of *M. uniflorum* seed extracts for presence of different phytoconstituents

	Phytoconstituents	Methanol extract	Acetone extract
Quantitative	Total polyphenols	4.26	8.91
	Total Flavonoid	8.59	7.34
	Tannins	3.21	5.23
Qualitative	**Alkaloids**		
	Mayer's reagent	+	+
	Dragendroff's reagent	+	+
	Wagner's reagent	+	+
	Hager's reagent	+	–
	Glycoside: General test	+	+
	Steroid and Triterpenoid		
	Liebermann test	+	+
	Salkowski test	+	+
	Carbohydrate		
	Molisch test	+	+
	Barfoed test	+	+
	Tannins and Phenolic		
	Ferric chloride test	+	+
	Gelatin test	+	+
	Lead acetate test	+	–
	Saponin: Froth formation test	+	+
	Flavonoids		
	Alkaline reagent test	+	+
	Zinc hydrochloride test	+	+
	Amino acid	+	+
	Inulin	+	–
	Mucilage	+	+
	Starch	–	–

"+" = Presence of constituent "–" = Absence of constituents

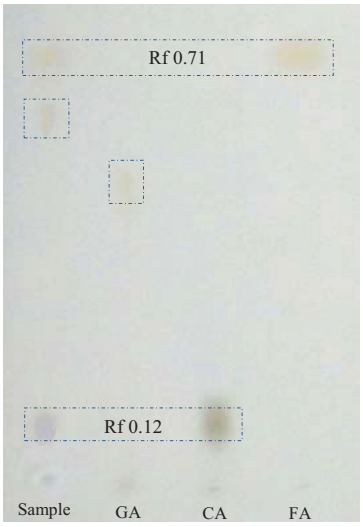

Figure 3. TLC analysis of acetone extract of *M. uniflorum* seed showing flavonoid profile. Sample: Acetone extract of *M. uniflorum* seed. Sample showed three spots with respective Rf of 0.11, 0.56 and 0.71, GA: Gallic acid (Rf 0.40), CA: Chlorogenic acid (Rf 0.12), FA: Ferulic acid (Rf 0.71). Solvent system Toluene: Ethyl acetate: Formic acid 5:3:2.

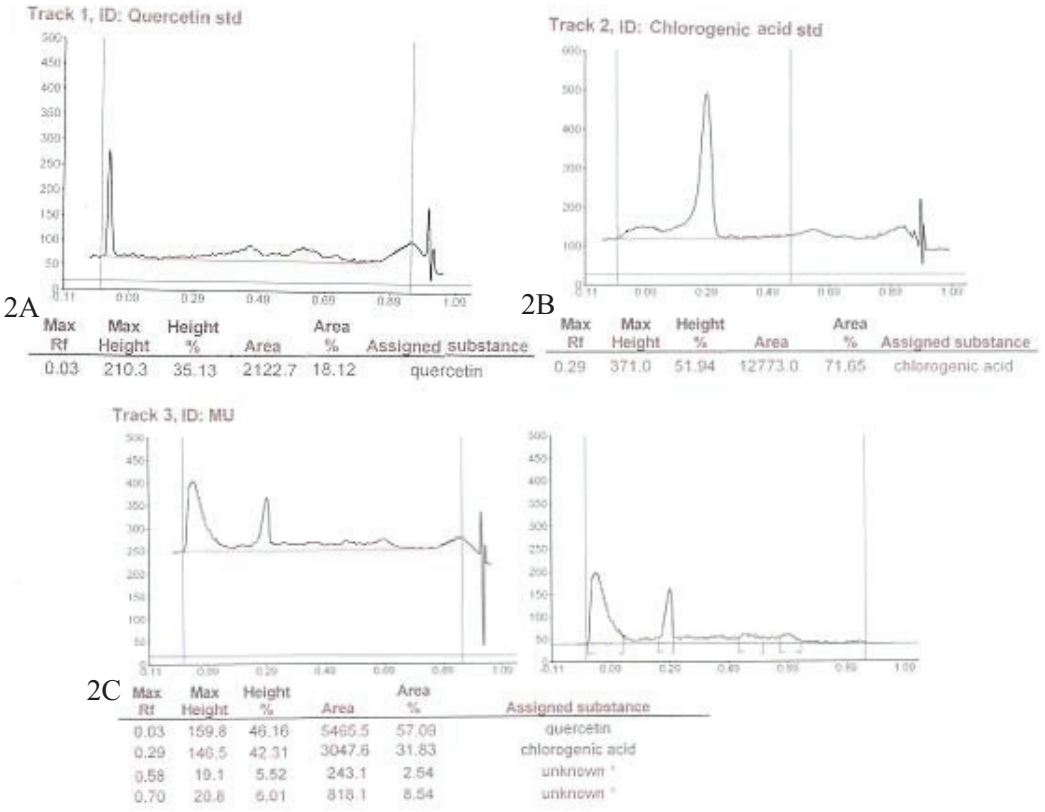

Figure 4. HPTLC analysis of Acetone extract of *M. uniflorum* seed scanned at 254 nm. 2A: Peak of standard quercetin showing Rf at 0.03; 2B: Peak of standard chlorogenic acid showing Rf at 0.29; 2C: HPTLC profile of acetone extract of *M. uniflorum* seed showing peak at Rf 0.03 (quercetin), Rf 0.29 (chlorogenic acid), Rf 0.58 (unknown) and Rf 0.70 (unknown).

Table 5: Antioxidant activity profile of *M. uniflorum* seed methanol (ME) and acetone (AE) extracts

Concentration (µg/ml)	Glutathione µg/gm of liver (M ± SEM)		Lipid peroxidase nmol/gm of protein (M ± SEM)		Superoxide dismutase unit/mg of protein (M ± SEM)	
	ME	AE	ME	AE	ME	AE
100	38.44 ± 1.87	32.28 ± 1.87	56.00 ± 2.58	273.00 ± 6.58	23.22 ± 1.88	15.48 ± 1.88
250	50.20 ± 2.71	56.00 ± 2.71	41.00 ± 2.69	136.00 ± 4.69	46.45 ± 2.63	23.22 ± 1.63
500	60.52 ± 2.34	100.8 ± 4.34	15.00 ± 1.64	108.00 ± 4.64	154.83 ± 4.83	54.19 ± 2.94
750	64.56 ± 3.74	102.24 ± 3.74	7.70 ± 0.84	92.00 ± 2.84	270.96 ± 7.94	61.93 ± 2.83
1000	146.32 ± 5.13	209.96 ± 4.13	4.40 ± 0.67	53.00 ± 1.67	286.45 ± 8.96	69.67 ± 3.96

DISCUSSION

Identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameters is still more reliable, accurate and inexpensive despite the availability of analytical techniques. Macroscopic and microscopic determination of a plant is the first step towards establishing its identity and purity and should be carried out before any other tests are undertaken.[22] To ensure batch to batch consistency, homogeneity and reproducible quality of herbal products, proper control of starting material is essential. The present study was focused on the macroscopic, microscopic, physico-chemical, phytochemical properties of grinded seeds including identification phytoconstituents by chromatographic methods.

Microscopic evaluation allows more detailed examination of crude plant parts and staining with various reagents enable to identify the organized cellular structure like embryo, testa, tegmen, alleurone layer, cotyledons and

endosperm present in the seed. The results of microscopy showed characteristics which will be useful for identifying and distinguishing *M. uniflorum* seed from other similar pulses often used as an adulterant.[23] The physico-chemical parameters ensure the purity and quality of the drug. The foreign matters were present in negligible amount signifying genuine quality of seeds and first hand collection from nonpolluted area.[24] Loss on drying was also only 1.45%, showed very less moisture content and fully dried well preserved condition of the seeds. Moisture content of the crude drug should be minimized in order to prevent decomposition either due to chemical change or microbial contamination.

Evaluation of total ash is helpful in determining authenticity, purity and inorganic materials such as oxides of Ca, Mg, K, Na, Si, P and Fe as well as minute quantities of other elements in the crude drugs.[25] Total ash content of seed was found to be relatively lower which may be due to low content inorganic materials. The total ash value is not a very reliable indicator of impurity present in a crude drug, as there is always possibility of presence of physiological and non-physiological substances like calcium oxide, phosphates, silicates, silica, sand and soil. Authentication of crude drugs by acid insoluble ash analysis should also be performed which particularly indicates the contamination with silicious material such as earth and soil.[26,27] The results showed the low content of silicious material in the seed. Fluorescence analysis of seed powder in different organic and inorganic solvents showed their characteristic fluorescent color. The fluorescence character of powdered drugs plays a vital role in the determination of quality and purity of the drug material.

The results indicate that the seeds have high methanol and acetone soluble extractive value in comparison to the petroleum ether, ethyl acetate, chloroform, ethanol and water. The results of preliminary phytochemical test revealed the presence of various phytochemical compounds in the seeds which are known to have multiple therapeutic importances in traditional medicine. The higher methanol and acetone soluble extractive value indicates the presence of phytocompounds such as polyphenols, tannins and flavonoids in the seed. The methanolic extract of *D. biflorus* whole plants possesses antihyperlipedimic activity.[28] Administration of *D. biflorus* extract to rabbits with high-fat diet induced oxidative stress showed improvement in antioxidant enzymes such as superoxide dismutase, catalase and increased glutathione concentration.[29] Chaitanya et al (2010) reported the antiurolithiatic activity of *M. uniflorum* seed extract on ethylene glycol induced urolithiasis in albino rats.[30] *M. uniflorum* seed showed significant hepatoprotective properties in D-galctosamine and paracetamol induced hepatotoxicity on rats.[31] Ravishankar and Vishnu Priya (2012) reported in vitro antioxidant activity of ethanolic seed extracts of *M. uniflorum*.[32]

All these studies reported potential medicinal potential of *M. uniflorum* seed extract in different solvents but phytopharmacological correlation for identification of bioactive compounds are lacking. Perumal and Sellamuthu (2007) reported presence of extractable total phenolics and tannins in raw and dry heated seed sample of *M. uniflorum* extracted with methanol and acetone.[33] Antioxidant and free radical scavenging activities were also reported where the acetone extract was more potent in vitro. The five different phenolic acid namely 3,4-dihydroxy benzoic acid, p-hydroxy benzoic acid, p-coumaric acid, ferulic and synaptic acid are found in *M. uniflorum*. It also contains the flavonoids and glycosides like kampferol-3-O-β D glycoside, β-sitosterol, stigmasterol and isoflavone diglycoside.[34,35]

Successive methanol and acetone extraction following petroleum ether defatting was done looking in to the higher extractive value in these two solvents expecting to extract out maximum number and quantity of phytoconstituents. Perumal and Sellamuthu (2007) have reported potent in vitro antioxidant and free radical scavenging activities of acetone extract.[33] TLC and HPTLC analysis of acetone extract having highest yield and polyphenol with flavonoid content was processed for identification of individual compounds. Chromatographic studies showed presence of quercetin, chlorogenic acid and ferulic acid like phenolic acid. These secondary metabolites have been reported to possess promising therapeutic activities, like antioxidant, antihyperlipedimic, hepatoprotective, anticancer, immune deficiency and antiaging activities which can be utilized to develop potential drugs for therapeutic purposes.[63,37,38] In antioxidant activity profile it was found that *M. uniflorum* seed methanol and acetone extract inhibit oxygen derived free radicals, like superoxide radical *in vitro* with a relatively high anti-lipid peroxidation and very potent increase in glutathione concentration. In this basis it could be concluded that the bioactive components of *M. uniflorum* seed extracts can act as primary and secondary antioxidants by scavenging free radicals and enhancing level of radical scavenging enzyme gluthione. They may have beneficial effect on prevention of diseases where reactive oxygen species are involved.[39,40] The extract can also be given to patients as supplements receiving radiation therapy as they can protect against the damage of free radicals generated during radiation therapy.

The present study established pharmacognostic, physico-chemical, phytochemical, microscopic and fluorescence characterizations of *M. uniflorum* seed along with identification of bioactive flavonoid compounds. These characteristics will be helpful for establishing quantitative and qualitative standardization of herbal preparations containing *M. uniflorum* seed. Further studies are in progress in order to explore of detailed pharmacological profile of rich flavonoid and polyphenol content having antioxidant property.

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