

Phytochemical characterization and antidiabetic potential of standardized total methanolic extract and phytosomes of *Momordica dioica roxb. ex Willd.* fruit

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Abstract

Objective: *Momordica dioica* climbing creeper plant fruits and leaves are traditionally used as a medicinal agent in asthma, leprosy, bronchitis, fever, aphrodisiac, stomachic, and constipation. This study aims in standardization of the fruit of *M. dioica Roxb. ex Willd.* by determining the pharmacognostical parameters, toxic residue analysis and antidiabetic effect of MeOH extract and phytosomes. **Methods:** Pharmacognostical parameters, total phenolic and flavonoid contents, toxic residue analysis were studied as per World Health Organization guidelines. High performance thin layer chromatography (HPTLC) system was used for fingerprinting of methanolic extract of *M. dioica* fruits. Antidiabetic activity of standardized total effect of a MeOH extract and phytosomes was studied using streptozotocin-nicotinamide model in rats. **Statistical Analysis Used:** Statistical analysis was performed using analysis of variance followed by Dunnett's test and by Tukey's test. **Results:** The macroscopic and powdered microscopy revealed useful diagnostic features of *M. dioica*. Physicochemical parameters and phytochemical analysis are proved as useful tools to differentiate the powdered drug material. Total phenolic and flavonoid contents in the methanolic extract were found to be 208 ± 0.1 mg/g and 0.0245 ± 1.01 µg/g, respectively. HPTLC analysis showed the presence of phytoconstituent gallic acid. The levels of all heavy metals were found to be within limits. Aflatoxins and pesticides residues were found to be absent in fruit. The antidiabetic activity of total effect of a MeOH extract in high dose and phytosomes formulation in low dose was found to be significant. **Conclusions:** The outcome of this study might prove beneficial in formulation development in novel drug delivery system and herbal industries for identification, characterization, and standardization of *M. dioica* fruits.

Key words: Antidaibetic, chromatographic technique, *Momordica dioica*, pharmacognostical parameters, phytochemicals, toxic residue

INTRODUCTION

Momordica Dioica

M. dioica Roxb. (Family: Cucurbitaceae) commonly known as spine gourd or teasels gourd is an annual or perennial climber. It is native of tropical regions of Asia with extensive distribution in China, Japan, parts of South East Asia, and India and is cultivated for its fruits which are used as a vegetable.^[1] It is known as Kakora in Hindi.

As per an Indian system of traditional medicine, roots are used in head trouble, for treating urinary calculi. The plant fruits and leaves are traditionally used as a medicinal agent in asthma, leprosy, bronchitis, fever, tridosha,

aphrodisiac, and anthelmintic properties. The fruit is used as stomachic; treating constipation.^[2] *M. dioica* fruits proved to be effective in controlling drug-induced nephrotoxicity and curing renal damages.^[3] The fruit is cooked in a small amount of oil and consumed for treating diabetes.^[4] The leaves have reported for having strong antioxidant, hepatoprotective action.^[5]

The extract of the dried roots of this plant was successfully evaluated for its abortifacient and estrogenic activity.^[6] The

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plant is claimed to have anti-inflammatory, hepatoprotective,^[7] antiallergic and analgesic,^[8] and antibacterial activity,^[9] Phytochemical investigation revealed the presence of the traces of alkaloids, lectins, sitosterol, saponin glycosides, triterpenoids, saponins, long chain aliphatic hydrocarbons, tannins and fixed oil.^[10,11]

Based on these facts and to establish the quality control parameters of this valuable ayurvedic drug, the standardization of fruits of *M. dioica* was performed according to standard methods of the World Health Organization (WHO) and guidelines of different pharmacopeia for herbal drugs. Therefore, this research work was designed to carry out the pharmacognostical parameters such as macroscopic characters, powder microscopy, physicochemical, and phytochemical analysis of *M. dioica* fruits supported by high performance thin layer chromatography (HPTLC) fingerprint of its different extracts. Toxic residue analysis was also performed to determine the safety parameters of the fruit of *M. dioica*. Antidiabetic activity was evaluated of total methanolic extract and phytosomes formulation of fruit of *M. dioica* using streptozotocin-nicotinamide (STZ-NAD) model.

MATERIALS AND METHODS

Materials

The phospholipids including Lipoid® S45 from Lipoid Co, Ludwigshafen, Germany, and Metformin from IndSwift Labs, Mohali, Quercetin and Gallic acid from Dabur Labs, New Delhi, were obtained as gift samples. NAD was procured from Sigma-Aldrich. STZ was procured from Santa Cruz Biotechnology®, Inc. All other chemicals and reagents used in this study were AR grade.

Collection and Authentication of Drug

The fruits of *M. dioica* were collected from local area of Loharu, District Bhiwani (Haryana) and Authenticated by Dr. K. Pradeep Senior Scientist at National Herbarium of Cultivated Plant, National Bureau of Plant Genetic Resources, New Delhi, India, against a voucher specimen NHCP/NBPGR/2013-23. The fruits of *M. dioica* were thoroughly washed with water to remove the impurities and dried under shade. The dried fruits were powdered and stored in air tight container for further.

Morphological Characters

The study of morphological characters helps to distinguish the plant from the similar species and adulterants. The organoleptic features of the crude drug were observed with sensory organs and were analyzed for color, odor, taste, shape and size.^[12]

Powder Microscopy

The dried fruit powder of *M. dioica* was examined for its microscopic characters. The powder of the fruit was passed through sieve No. 60 and observed under a microscope for the microscopical characters. The specimen was treated with chemical reagents. The coloring matters were removed by boiling with chloral hydrate and viewed under microscope after mounting it on a glass slide using glycerin and covered with a cover slip. Then, the powder was stained with phloroglucinol in the presence of hydrochloric acid for the lignified structures, and again it was viewed under microscope. Further iodine solution was used to locate the starch.^[13]

Preparation of Extracts

The dried powdered plant material of *M. dioica* fruit (500 g) was extracted in a Soxhlet apparatus on heating mantle, first with petroleum ether to remove lipids and then with methanol. The methanolic extract was concentrated on Rotavapor under reduced pressure and stored in a closed container for further use.

Physicochemical Parameters

The physicochemical parameters of *M. dioica* fruit were carried out as per WHO guidelines and different procedures in pharmacopeia. The standardization studies of different physicochemical parameters include extractive values, different ash values (total ash value, water soluble ash value, acid insoluble ash value, and sulfated ash values), moisture content, loss on drying, and foreign organic matter (WHO, 2002).^[14]

Preliminary Phytochemical Testing

The preliminary phytochemical investigations of alcoholic and aqueous solvent extracts of *M. dioica* fruits were performed as per the reported methods, to detect the various classes of phytoconstituents such as carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, lipids, steroids, and tannins (WHO, 1998).^[15] Total phenols and flavonoids contents in the fruit extracts were also analyzed by ultraviolet (UV) spectroscopy as per standard procedures.^[16,17]

Determination of Total Phenolic Contents by UV Spectrophotometer

Folin-Ciocalteu method was used to determine the total phenolic contents of methanolic extract by UV spectrophotometer. An external calibration curve of gallic acid as standard phenolic compound was plotted. The total phenolic contents of the methanolic extract of fruits were

calculated with the help of a standard calibration curve^[16] and are reported as gallic acid equivalent (mg/g of dry mass).

Determination of Total Flavonoid Contents by Colorimetric Method

The aluminum chloride colorimetric method of Chang *et al.* was used to analyze the flavonoid content of methanolic extract of *M. dioica* fruits.^[17] Quercetin (standard flavonoid compound) was used to make a standard calibration curve. The flavonoid contents in the sample of fruits extract are reported as gallic acid equivalent ($\mu\text{g/g}$ of dry mass).

Development of Chromatographic HPTLC Fingerprint

Profile of methanolic extract of *M. dioica* was used to obtain HPTLC fingerprinting. HPTLC fingerprint profile of the methanolic extract of *M. dioica* was developed to confirm the occurrence of different phytopharmaceuticals. Different combinations of solvent systems were tried to obtain an excellent separation for analysis. The satisfactory resolution for separation of compounds presented in methanolic extract of *M. dioica* was obtained in the toluene:ethyl acetate:formic acid (5:4:1) solvent system.

The investigations were performed in an air-conditioned room maintained at a temperature of 22°C and 55% humidity. Precoated silica gel HPTLC aluminum plates 60F-254 (20 cm \times 10 cm, 0.2 mm thicknesses, 5-6 μm particle size, E-Merck, Germany) were used for chromatographic separation. The extract (5 μL) was spotted as bands of 6 mm width with the help of the auto sampler fitted with a 100 μL Hamilton syringe. The plates were prepared using a solvent system of toluene:ethyl acetate:formic acid (5:4:1) as mobile phase gave the best resolution of gallic acid. The solvent system was transferred to CAMAG twin trough plate development chamber lined with filter paper and pre-saturated with mobile phase (30 mL). The resulted plates were air dried and scanned. A spectrodensitometer (scanner 3, CAMAG) equipped with win CATS planar chromatography manager (version 1.3.0) software was employed for the densitometry measurements, spectra recording, and data processing. Absorption/remission was then measured at a scan speed of 20 mm/s. Chromatograms were recorded at the wavelength of 254 and 366 nm. The retention factor (Rf) value of each compound separated on plate and data of peak area of each band were recorded.

Toxic Residues Analysis

Determination of heavy metal residues

Heavy metals residues, viz., Cu, Zn, Cd, Mn, Fe, Hg, As, and Pb in the *M. dioica* extracts were analyzed as per the WHO guidelines.^[15]

Determination of pesticide residues

Gas chromatography-mass spectrometer was used to determine the presence of pesticide residues, including organochlorines (alderin, β -hexachlorocyclohexane, and endosulfan) and organophosphates (malathion) in the *M. dioica* fruit extract by the WHO guidelines.^[15]

Aflatoxin analysis

M. dioica fruits extracts were subjected to aflatoxins analysis by HPLC method as per the WHO guidelines.^[18]

Experimental Animals and Research Protocol

Swiss albino rats (200-250 gm) of either sex were obtained from Chandigarh College of Pharmacy, Landran, India. Animals were maintained at a temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of 45-55%. The food pellets and water was available *ad libitum* to animals. The experiment was conducted after obtaining ethical committee permission from the Institutional Animal Ethics Committee of Chandigarh College of Pharmacy, Landran, and (Mohali) Punjab, India. No: IAEC/FEB16/019 in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals, India.

Induction of diabetes and determination of serum glucose level

The selected animals, weighing between 200 and 250 g fasted overnight were administered with STZ 60 mg/kg i.p route after 15 min of administration of NAD 120 mg/kg body weight.^[19] STZ was dissolved in citrate buffer having pH 4.5, and NAD was dissolved in normal saline solution. This model has been used earlier to induce diabetes by Shirwaikar *et al.*^[20] The diabetes initiation was established by measuring fasting blood sugar level after 1 week. Animals having blood glucose level more than 250 mg/dl were selected for the evaluation of antidiabetic activity of the extract.

The diabetic rats after confirmation of stable hyperglycemia were divided into five different groups of 6 rats each. That day was considered as the 0 day.

The rats were divided into following groups of six animals each:

- Group I: Normal control group (distilled water orally 2 ml/kg body weight).
- Group II: Diabetic controlled (STZ 60 mg/kg, and NAD 120 mg/kg, and distilled water orally 2 ml/kg body weight).
- Group III: Standard antidiabetic drug (metformin dose 50 mg/kg body weight and STZ 60 mg/kg and NAD 120 mg/kg).
- Group IV: Methanolic extract of plant *M. dioica* (dose 250 mg/kg body weight and STZ 60 mg/kg and NAD 120 mg/kg).

- Group V: Phytosomes formulation (dose 100mg/kg body weight and STZ 60 mg/kg and NAD 120 mg/kg).

The drugs dissolved in 1 ml of distilled water, were administered orally via a standard or gastric cannula. Antihyperglycemic activity in diabetic rats was assessed by reduction in fasting blood glucose level. Blood samples were collected from overnight fasted rats at 0 day, 7th day, and 15th day by retro-orbital venipuncture technique using microcapillary to determine blood glucose levels. The blood was then centrifuged at 2500 rpm for 10 min to get clear serum. Blood glucose in plasma was determined by glucose oxidase method earlier used by Shirwaikar *et al.*, (2006).^[19] 10 µL of serum and 1ml of working reagent were mixed and incubated for 15 min at 37°C. The absorbance of the sample (As) and standard (Astd) (CDH New Delhi, India) solutions was measured against blank at 505 nm using UV-Visible spectrophotometer (Shimadzu 1700 IPC).

Glucose level was estimated using the formula:

$$\text{Glucose (mg/dl)} = \text{As/Astd} \times 100;$$

Whereas As = Sample reading; Astd = Standard reading.

Statistical analysis

Statistical analysis was performed using repeated measure analysis of variance (ANOVA) followed by Dunnett's test and two-way ANOVA followed by Tukey's test at 5%. Results were expressed as mean \pm standard error mean. The difference showing a $P = 0.05$ or lower was considered to be statistically significant.

RESULTS

Morphological Description

Color

Fruit is shortly beaked, obtuse with inner red kernel, densely covered with soft spines. Immature fruit is green and yellow at maturity. Seeds are rounded broadly ellipsoid, slightly compressed, and irregularly corrugated enclosed in red pulp.

Odor and taste

Unpleasant and bitter.

Shape and size

Fruit is nearly ovoid or ellipsoid in shape, and 1-4 inches long. Seeds are numerous, ovoid, compressed, smooth, dark brown to light yellowish-orange, borne on parietal placenta.

Powder Microscopy

Microscopically, the powder of fruits shows the presence of epidermis, prismatic calcium oxalate crystals, non-glandular uniseriate unicellular/multicellular trichomes, starch grain, and endosperm with polygonal cells, secretory cells, anomocytic stomata, spiral vessels, and fibers [Figure 1].

Physicochemical Standardization

The purity and quality of the drug was evaluated by determining different physicochemical parameters included alcohol soluble and water soluble extractive values, total ash value, acid insoluble ash value, water soluble ash value, moisture content, and foreign organic matter. The results of physicochemical parameters are summarized in Table 1. The moisture content in the fruits of *M. dioica* was found to be 8.3 %w/w which indicating that the drug was properly dried and well-stored [Table 1].

Preliminary Phytochemicals Analysis

The results of preliminary qualitative phytochemical screening showed the presence of carbohydrates, glycosides, phenolic compounds, flavonoids, alkaloid, proteins, saponins, lipids, tannins, and steroids. All these chemicals constituents except anthraquinone glycoside were found to present in the extracts. The results of phytochemicals analysis are given in Table 2.

Total Phenolic Contents

The total phenolic contents of methanolic extract of *M. dioica* were determined by UV spectrophotometric method. The total content of phenolic compounds was found to be (208 \pm 0.1) mg/g of gallic acid equivalent in methanolic

Table 1: Results of physicochemical parameters of *M. dioica* fruit

Physicochemical parameters	Average values (%w/w)
Moisture content	8.3 \pm 1.2
Ash values	
Total ash value	12 \pm 0.11
Acid insoluble ash value	2.5 \pm 0.21
Water soluble ash value	7 \pm 0.22
Foreign organic acid	5.0 \pm 0.31
Extractive value	
Alcohol soluble extractive values	6.8 \pm 0.13
Water soluble extractive values	21.0 \pm 0.02

M. dioica: Momordica dioica

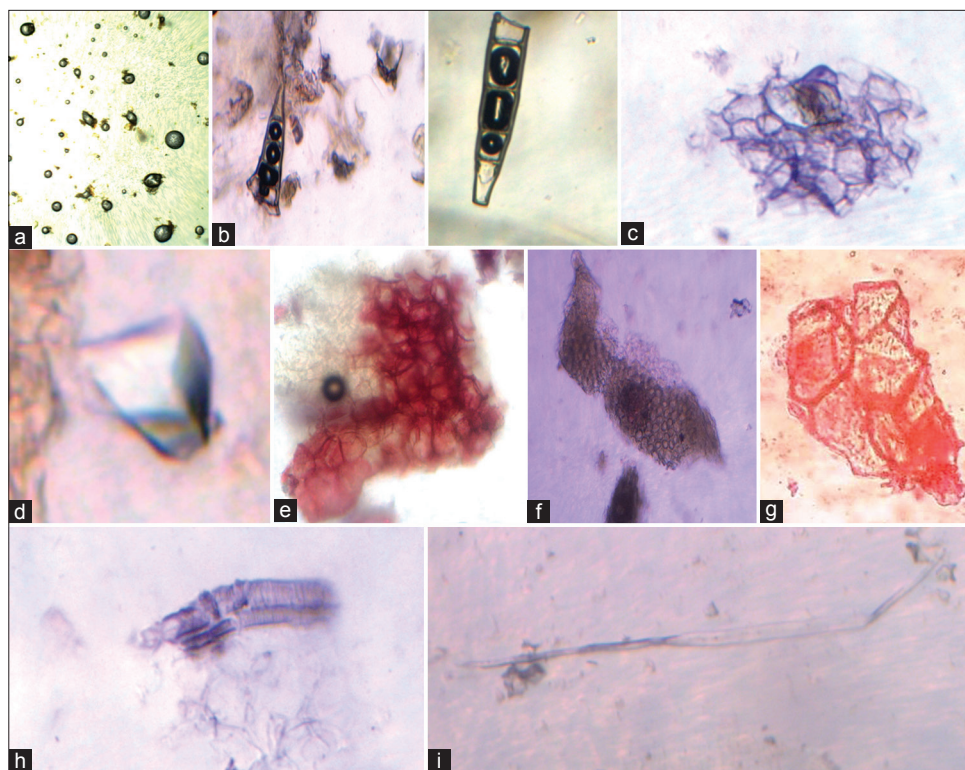


Figure 1: Powder microscopic images of different cells: (a) Starch grain, (b) non-glandular trichome, (c) epidermis, (d) prismatic calcium oxalate crystals, (e) mesocarp, (f) secretory cells, (g) endosperm polygonal cells, (h) anomocytic stomata and spiral vessels and (i) fibers

Table 2: Results of phytochemical screening of *M. dioica* fruit

Type of phytoconstituents	Alcoholic extract	Aqueous extract
Carbohydrates	+	+
Cardiac glycosides	+	+
Anthraquinone glycoside	-	-
Tannin	+	+
Flavonoids	+	+
Steroid	+	+
Terpenoid	+	+
Saponin	+	+
Alkaloid	+	-
Proteins	+	+

M. dioica: *Momordica dioica*

extract of *M. dioica* fruits. The given values are mean \pm SD of three different determinations.

Total Flavonoids Content

The flavonoid content of methanolic extract of *M. dioica* was determined by UV spectrophotometric method. The total content of flavonoids was found to be $(0.0245 \pm 1.01) \mu\text{g/g}$ quercetin equivalent in methanolic extract of *M. dioica*

fruits. The given values are expressed as mean \pm SD of three different determinations.

HPTLC Fingerprint Profile of Methanolic Extract of *M. Dioica*

HPTLC fingerprinting of methanolic extract of *M. dioica* fruits was carried out using toluene:ethyl acetate:formic acid (5:4:1) solvent system to confirm the presence of phenolic compound gallic acid in the extract [Figure 2]. HPTLC chromatogram when observed under at wavelength 254 nm in UV light and showed four peak having R_f value 0.81, 0.76, 0.58, and 0.23 which was compared with a standard sample of gallic acid having R_f value 0.57.

Determination of Heavy Metal Content

The heavy metals (Cu, Zn, Cd, Mn, Fe, Hg, As, and Pb) were analyzed using atomic absorption spectrophotometer in the fruit extracts of *M. dioica*. All essential safety precautions were implemented to avoid potential contamination in the sample according to the WHO guidelines. Lead and Hg were found to be below detection level (bdl). The level of As and Cd concentration was found to be 0.6 ± 2.11 and 0.01 ± 0.21 mg/kg. The level of Zn concentration was found to be (6.5 ± 0.11) mg/kg. Cu was found to be 4 ± 2.11 mg/kg. Mn and Fe were found to be 0.9 ± 1.21 and 10.04 ± 0.11 mg/kg, respectively, in the sample of *M. dioica* fruits extract.

The level of heavy metals follows of hierarchy, i.e., ($\text{Fe} > \text{Zn} > \text{Cu} > \text{Mn} > \text{As} > \text{Cd} > \text{Hg} > \text{Pb}$) and the level of all heavy metals were found to be within limits as compared to the standard of the WHO and shown in Table 3 and Figure 3.

Determination of Pesticide Residues

The presence of four pesticide residues like organochlorines and organophosphates were investigated in the extracts using gas chromatography-mass spectrometer as per guidelines of the WHO.^[21] All four pesticides were found to be absent in the samples of *M. dioica* fruits extract.

Aflatoxin Analysis

Various aflatoxins, e.g., B1, B2, G1, and G2 were investigated but were found to be absent in the samples of *M. dioica* fruits extract.

Antidiabetic Activity

Treatment with the methanolic extract in the dose 250 mg/kg body weight exhibited remarkable glycemic control in diabetic

rats as evident by significant decrease ($P < 0.001$) in the levels of fasting blood glucose when compared with treatment with metformin [Table 4 and Figure 4]. Further treatment with phytosomes formulation in the low dose (100 mg/kg body weight) showed more significant blood glucose lowering effect as compared to conventional total methanolic extract group.

A significant increase ($P < 0.001$) in body weight was also observed in diabetic rats [Table 5 and Figure 5]. The weights of diabetic rats increase significantly with increase in time and in formulation group.

DISCUSSION

The quality control of crude drugs and herbal formulation is of paramount importance in justifying their acceptability in the modern system of medicine. However, one of the major problems faced by the herbal drug industry is non-availability of rigid quality control profile for herbal material and their formulations.^[22] Standardization is an essential measurement

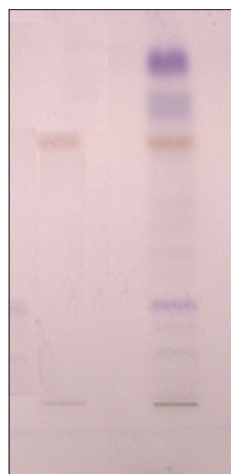


Figure 2: Thin layer chromatography profile for test sample of methanolic extract of *Momordica dioica* and standard sample of gallic acid

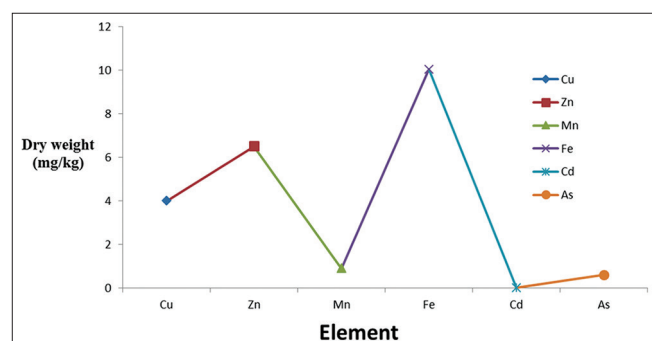


Figure 3: Heavy metal content in *Momordica dioica* fruits compared with maximum permissible limits

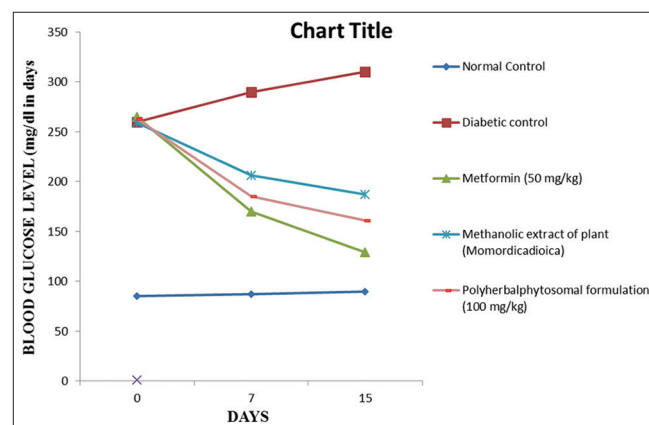


Figure 4: Effect of methanolic extract and phytosomes formulation plants *Momordica dioica* on serum glucose level in streptozotocin-nicotinamide induced diabetic rats

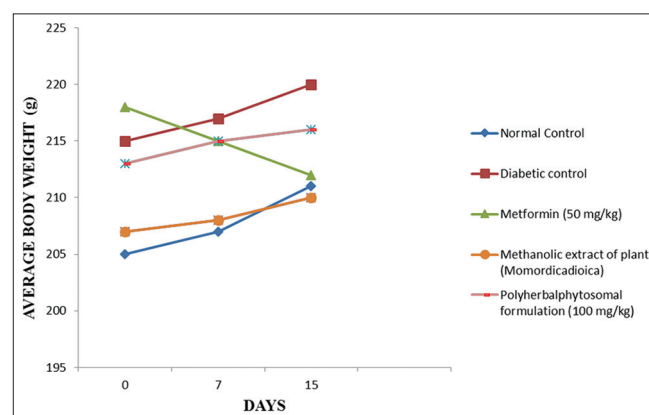


Figure 5: Effect of methanolic extract and phytosomes formulation plants *Momordica dioica* on body weight in streptozotocin-nicotinamide induced diabetic rats

Table 3: Results of heavy metal content of *M. dioica* fruit

Limits of heavy metals	Element dry weight (mg/kg)							
	Cu	Zn	Mn	Fe	Cd	Hg	As	Pb
Practical yield	4±2.11	6.5±0.11	0.9±1.21	10.04±0.11	0.01±0.21	bdl	0.6±2.11	bdl
Limits as per WHO	20	50	1	100	0.3	1	3	10

M. dioica: *Momordica dioica*, WHO: World Health Organization, bdl: Below detection level

Table 4: The effect of 2-week treatment with methanolic extract of fruit of *M. dioica* on blood glucose levels (mg/dl) after STZ-NAD induced diabetic rats

Groups	Treatment	Blood glucose level (mg/dl in days)		
		0 day	7 th day	15 th day
Group I	Normal control	85±1.8	87±1.45	90±1.33
Group II	Diabetic control	260±1.4	290±2.12	310±3.12
Group III	Metformin (50 mg/kg)	265±2.71	170±2.55	129±2.01
Group IV	Methanolic extract of plant (<i>M. dioica</i>)	259±2.24	206±1.52	187±2.02
Group V	Phytosome formulation (100 mg/kg)	263±1.12	185±1.12	161±2.02***

M. dioica: *Momordica dioica*, STZ-NAD: Streptozotocin-nicotinamide

Table 5: The effect of 2-week treatment with methanolic extract of fruit of *M. dioica* on body weight (g) after STZ-NAD induced diabetic rats

Groups	Treatment	Average body weight (g)		
		0 day	7 th day	15 th day
Group I	Normal control	205±1.8	207±2.16	211±2.13
Group II	Diabetic control	215±2.2	217±2.16	220±2.3
Group III	Metformin (50 mg/kg)	218±1.2	215±2.3	212±2.21
Group IV	Methanolic extract of plant (<i>M. dioica</i>)	207±2.01	208±1.22	210±2.22
Group V	phytosome formulation (100 mg/kg)	213±2.22	215±2.12	216±1.02

M. dioica: *Momordica dioica*, STZ-NAD: Streptozotocin-nicotinamide

for ensuring the quality control of the herbal drugs and also encompasses the entire field of study from birth of a plant to its clinical application.

M. dioica Koen. (Cucurbitaceae) has many medicinal and therapeutic actions that have been scientifically validated and documented. The present investigation deals with all the physicochemical and pharmacognostical perspectives of its fruit. It was concluded from physicochemical parameters that total ash value was found to be the highest (12% w/w) while acid insoluble ash was lowest (2.5% w/w). Hot extractive values revealed that the percentage yield of aqueous extract was highest 21% w/w and alcohol soluble extractive values were found to be lowest (6.8% w/w). The phytochemical investigation indicates the presence of the potent phytoconstituents such as alkaloids, carbohydrates, flavonoids, proteins, amino acids, phenols, tannins, and glycosides. The findings of this research will help in the evaluation, identification, and authentication of the plant.

Heavy metals are inorganic materials which are highly toxic to human beings even at very low concentrations. Moreover,

the dietary intake of contaminated plants with heavy metals could also lead to dangerous consequences for the health of humans and animals.^[23]

However, the trace elements are very necessary for humans to survive but their high concentrations toxic to human body and can lead to various ailments and toxicities.

A total number of four pesticides were tested in the samples, but none of the pesticides was found to be present in the samples of *M. dioica* fruits extract.

Secondary metabolites produced by fungi are known as mycotoxins and aflatoxins.^[24] The *Aspergillus* species produce aflatoxins B1, B2, G1, and G2 which are aflatoxins are known to cause liver cancer in human beings.^[25] No aflatoxin was detected in the samples of *M. dioica* fruits extract. This study using biochemical assays pertaining to blood glucose levels of different animal models reveals that the methanolic extract (in high dose 250 mg/kg body weight) of fruit of *M. dioica* was found to have the significant antidiabetic activity but the phytosomes formulation was

found to possess significant antidiabetic activity even in the low dose.

CONCLUSION

This study was an attempt to establish the diagnostic characteristics of *M. dioica* fruit. The results obtained could be employed as suitable quality control measures to ensure the quality, safety, and efficacy of this herbal drug material. The various parameters and phytochemical studies indicate that it is a useful plant to investigate for phytochemical and biological assays. HPTLC fingerprinting may assist as handy data for the standardization of the drug. Toxic residue analysis such as heavy metal, pesticide's, and aflatoxin analysis serve as useful tool for preparation of safety parameters of herbal drug. To our knowledge, this is the first study of its kind on this plant (*M. dioica*) species, therefore, it is of much importance in future research on this plant.

The total methanolic extract from fruit of *M. dioica* in the dose 250 mg/kg body weight showed a significant hypoglycemic effect in STZ-NAD model when compared with the standard (metformin) group. This study using biochemical assays pertaining to blood glucose levels of different animal models reveals that the methanolic extract (in high dose 250 mg/kg body weight) of fruit of *M. dioica* was found to have the significant antidiabetic activity.

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