

Pharmacognostic Evaluation of *Momordica dioica* Fruit

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ABSTRACT: The purpose of this study was to determine the pharmacognostic profile of *Momordica dioica* (MD) fruit in accordance with World Health Organization recommendations to ensure the purity, safety, and efficacy of this medicinal plant. The macroscopic and microscopic properties of the focus plant were examined as standardization parameters. Preliminary phytochemical screening was performed on petroleum ether and methanol extracts. The extracts were also utilized to analyze total phenol and flavonoid levels as well as a Thin Layer Chromatography analysis. The fruit of plants displayed helpful diagnostic traits in terms of shape, size, color, odor, surface properties, and microscopic pictures. The results showed that the amounts of total ash, acid-insoluble ash, and water-soluble ash were $7.4 \pm 0.1\%$ w/w, $2.3 \pm 0.1\%$ w/w, and $5.2 \pm 0.1\%$ w/w, respectively. The extractive values of ethanol, water, and ether were determined to be $17.8 \pm 0.3\%$ w/w, $20.5 \pm 0.5\%$ w/w, and $4.1 \pm 0.2\%$ w/w, respectively. The loss on drying was $10.23 \pm 0.72\%$ w/w, and the foreign matter was $1.0 \pm 0.8\%$ w/w. Glycosides, carbohydrates, phenolic substances, flavonoids, alkaloids, terpenoids, proteins, saponins, lipids, steroids, and tannins were all found during the phytochemical screening. The total flavonoid concentration in the methanol extract of MD was discovered to be 125 mg/g of quercetin equivalent, while the total phenol content was determined to be 64 mg/g of gallic acid equivalent. Charantin was detected in methanol extract at R_f 0.45, and a violet spot emerged when compared to the marker. The information obtained from this study will be useful for the authentication of MD fruit and quality control. It would be beneficial to establish pharmacopoeial standards using qualitative and quantitative microscopic features.

Keywords: *Momordica dioica*, Macroscopy, Microscopy, Preliminary physiochemical screening, Physicochemical parameters.

INTRODUCTION

Traditional medicinal herbs are still used in various cultures all over the world for their basic healthcare requirements, despite recent advancements in modern medicine. Due to their efficiency, low cost, and ease of availability, medicinal plants have traditionally been used as a form of therapy in many traditional medical systems. Unfortunately, the lack of standards for drug authenticity makes crude medications of natural origin vulnerable to adulteration and substitution. In consequence, this will have an impact on the pharmaceuticals' strength, excellence, and purity. A Pharmacognostic investigation must be carried out to ensure the validity of herbal medicine (Dash *et al.*, 2021). Herbal medicines have been utilized for many years to treat a variety of illnesses. Many human illnesses have been treated with herbs, which are widely available, and have a lower risk of side effects (Swapna Patel *et al.*,

et al., 2020; Sharma *et al.*, 2021). In many nations, where 35% of medicines contain natural components, the use of therapeutic plants is growing (Rakh and Chaudhari 2010). The World Health Organization, better known as the WHO, has established specific standards for the assurance of safety, and strong quality control profiles, and also outlines the requirements for standardizing herbs, herbal products, and other forms of healthcare (Deb *et al.*, 2014).

Momordica dioica is a perennial, dioecious cucurbitaceous climbing creeper. Kankoda, kakrol, spiny gourd, teasle gourd, akakara, bodakakara, kakor, kantola, golbandra, parora, kheksa, dharkarela, and batkarila are some of its other names (Jha *et al.*, 2019; Ameen *et al.*, 2022). It is found on the Deccan Plateau and in central India, where it is indigenous to Asia. In addition to Bangladesh, Nepal, Myanmar, China, and Pakistan, it is also dispersed outside of India

(Mukherjee *et al.*, 2022; Nabi *et al.*, 2002). It is used to treat and prevent a wide range of illnesses. The plant's fruit is used for its numerous therapeutic benefits. The plant's leaves are anti-helminthic and aphrodisiac, and its fruits have hepatoprotective, diuretic, alexiteric, laxative, stomachic, and anti-hyperglycemic effects (Thiruvengadam *et al.*, 2013; Devendra *et al.*, 2009; Ilango *et al.*, 2008). The root juice has astringent, energizing, and antibacterial properties. Additionally, it was said to have anti-inflammatory, anti-microbial, hepatoprotective, renoprotective, analgesic, and antioxidant activities (Hassan *et al.*, 2022). It is frequently consumed as a healthy vegetable in addition to being used as a disease-curing agent. Among all the cucurbits, the fruits of this plant are notable for having a high concentration of carotene (162 mg for every 100 g of edible portions). Thiamine, Vitamin C, niacin, and riboflavin, are some of the vitamins and minerals that are abundant in fruits, along with fiber, proteins, fatty acids (linoleic and oleic acid), and minerals including calcium, phosphorus, magnesium, iodine, and iron (Choudhary *et al.*, 2017; Talukdar and Hossain *et al.*, 2014; Swathi *et al.*, 2020). Different plant parts from *M. dioica* supply a variety of phytoconstituents, including alkaloids, glycosides, steroids, flavonoids, triterpenoids, and ursolic acid (Weerasinghe and Dahanayake 2021; Kumar and Bhowmik 2010; Jha *et al.*, 2019).

Based on these facts and in order to establish the quality control standards for this important ayurvedic medicine, this research was created to carry out the pharmacognostic parameters, including microscopic characteristics, powder microscopy, physicochemical analysis, and phytochemical analysis of *M. dioica* fruits, supported by thin layer chromatography fingerprint of its various extracts. Additionally, a standardization effort for the total phenol and flavonoid content of extracts was made.

MATERIAL AND METHODS

Collection and Authentication of Plant material. The fruits of *Momordica dioica* Roxb. were collected from the local market of Anand. Authenticated by Dr. R. R. Acharya. Head & Research Scientist (Veg.), Main Vegetable Research Station, Anand agricultural university, Anand - 388 110, Gujarat (India) against a voucher specimen AAU/ MVRs/EST/53/2021 on Dated 10/05/2021. The fruits used for the studies were sun-dried.

Reagents and chemicals. The whole inventory of chemicals and reagents used in the current investigation was purchased from Merk Life Science Pvt. Ltd. in Vikhori, Mumbai. Analytical grading was done on all other reagents.

Preparation of crude drug powder The *Momordica dioica* fruit was cleaned, rinsed, and chopped into little pieces before being dried in the sun for 7 to 8 days. After being pulverized at a monitored temperature in a plant sample grinder, the dried fruit (Fig. 1) was then placed in a plastic container for storage (Krishna *et al.*, 2014).



Fig. 1 (A) Dried fruit (B) Crude drug powder of *Momordica dioica*.

Macroscopic examinations. Systematically studying the macroscopic characteristics of *M. dioica* fruits. The analysis of morphological characteristics assists in the separation of the medicine from related species and adulterants. Sensory organs were used to examine the organoleptic characteristics of the crude medication. The morphological characteristics, such as color, size, form, odor, and taste, were observed.

Microscopic evaluation. Using a blade, a transverse section from the fruit pericarp was cut off, stained according to accepted procedures, and then studied under a microscope. Zeiss Axio Lab. A1 microscope fitted with Zeiss Axio CamERc5s was used to take photomicrographs of the microscopical sections (Kumar *et al.*, 2016; Sudhakaran, 2016).

Powder microscopy study. Phloroglucinol and strong hydrochloric acid were used to stain the sample in a 1:1 ratio, mount it on a glass slide, and view it under a microscope. Applying distilled water to the sample allows researchers to determine the presence of biological components.

Determination of foreign content. A thin coating was created using around 100 g of *M. dioica* fruit powder. The foreign object was separated and weighed after being observed with the unaided eye. It was calculated how much of the content was from abroad (Krishna *et al.*, 2014).

Loss on drying. If medicinal plants are not properly dried and kept, moisture can cause toxigenic fungus and insect damage, which can lead to quality degradation. When 2 g of a sample is reduced to a constant weight under controlled circumstances at 105°C, the sample loss expressed as a percentage equals the quantity lost (Kim *et al.*, 2013).

Determination of the size of starch grains and Calcium oxalate crystals. Using a compound microscope, determine the size and form of calcium oxalate crystals and starch grains. The stage micrometer should be used to calibrate the eyepiece micrometer, and the factor should be determined. *Momordica dioica* powder is placed in a watch glass along with a diluted iodine solution. Add glycerin to a slide and mount the dyed starch grains on it. While putting powder in a watch glass, adding water to it, and mounting it on the slide will allow you to measure the crystals of calcium oxalate. By focusing them on the line of the eyepiece micrometer, you can measure the diameter. Take note of how many divisions the starch granules cover. Measure at least 50 measurements, multiply the divisions the factor covers, and calculate the range and average in micrometers (µm) (Khandelwal, 2008).

Total ash value. Total ash content displays the physiological mineral content of the medicinal plants as well as the amount of foreign components that have been blended in during processing. In a previously fired tared crucible of silica, the air-dried aerial sections of MD were precisely weighed (2 g). The dried substance was heated in a muffle furnace to 600°C till it becomes white, then cooled in a desiccator, and the weight was recorded. Calculations were made to determine the ash percentage in relation to the medication that had been air-dried (Sharma *et al.*, 2021)

Water-soluble ash and acid-insoluble ash. The crucible containing the complete ash was filled with water and hydrochloric acid (25 mL), and it was then heated for five minutes. The insoluble material was collected on ash-free filter paper, washed with hot water, and ignited at a temperature no higher than 450°C. In relation to the air-dried medication, the proportion of water-soluble and acid-insoluble ash was determined (Khandelwal, 2008; Mukherjee, 2008).

Alcohol and water soluble extractive value. 5 g of the coarsely powdered crude drug were weighed, macerated for 24 hours in 100 mL of an iodine flask containing 70% V/V alcohol and water with regular shaking for 6 hours, and then left to stand for 18 hours. The solution was quickly filtered, and the filtered solution was dried at 105°C in an evaporating dish. With reference to the medicine that was shade-dried, the percentage of the extract that is soluble in alcohol and water was calculated (Singh *et al.*, 2010).

Extraction. Petroleum ether and methanol were used as solvents, and the soxhlet extraction method was chosen as the standard method. 100 g of powdered, ground-up *Momordica dioica* Roxb. fruit was placed in filter paper and placed into the Soxhlet apparatus for each extraction. The system was heated until the solvent was boiling after the solvent (900 mL) was added. Reflux remained for 8 hours. Use Whatman filter paper to filter the extracts after extraction. At 60 °C and 450 mmHg, the main solvent was evaporated in a rotating vacuum evaporator. The content was then dried for two hours at 65 °C in an oven with moving air. The flask was weighed once it had cooled down in a desiccator for an hour. The ratio of the mass of the extracted material to the mass of the raw material utilized was used to determine the extraction yield for all solvent systems. The outcomes are displayed using the average ± standard deviation. Prior to analysis, the extracts were shielded from light and kept at 4-5 °C. A triplet of the Soxhlet extractions was made (Pereira *et al.*, 2017).

Preliminary phytochemical screening. To identify the various classes of phytoconstituents including glycosides, carbohydrates, phenolic compounds, flavonoids, alkaloids, terpenoids, proteins, saponins, lipids, steroids, and tannins, preliminary phytochemical analyses of petroleum ether and methanol extracts of *M. dioica* fruits were carried out in accordance with the reported methods (Shaikh and Patil 2020).

Determination of Total Phenolic Contents by UV Spectrophotometer. By using a UV spectrophotometer, the total phenolic content of the

methanolic extract was determined using the Folin-Ciocalteu technique. Gallic acid was used as a standard phenolic component to create an external calibration curve. Test tubes were filled with samples (2 mL, in triplicates), 1.0 mL of Folin-Ciocalteu's reagent, and 0.8 mL of sodium carbonate (7.5%). After mixing, the tubes were let to stand for 30 minutes. Using a UV-visible spectrophotometer, absorption at 765 nm was determined. The amount of total phenolics was calculated as milligrams of gallic acid equivalents (GAE) per gram of dry material (Samshuddin *et al.*, 2015; Kupina *et al.*, 2019).

Determination of Total Flavonoid Contents by Colorimetric Method. The flavonoid content of the methanolic extract of *M. dioica* fruits was examined using Chang *et al.*'s aluminum chloride colorimetric technique. A standard calibration curve was created using quercetin, a standard flavonoid molecule. The fruit extract sample's flavonoid concentration is listed as Quercetin equivalent (mg/g of dry mass) (Shraim *et al.*, 2021).

Development of TLC. *M. dioica* fruit samples were extracted with methanol, and the supernatant from the centrifugation process was employed as a test solution for TLC analysis. TLC was used with the test solution and standard charantin. As the mobile phase, benzene: methanol (8:2) was employed. After being sprayed with 10% sulfuric acid in ethanol and dried at 100°C in a hot air oven, the produced TLC plate's R_f value was recorded (Shanmugapriya and Poornima 2014).

RESULTS AND DISCUSSION

Macroscopic examinations. The morphology of the fruit component that can be seen with the naked eye or magnifying glass is evaluated using a technique called macroscopic analysis. In order to discern the traits and ascertain the precise identification of crude pharmaceuticals, macroscopic evaluation is crucial. The fruit has a short beak, obtuse, and is heavily covered in soft spines. Fruit that is immature is green and turns yellow with age (Fig. 2). The red pulp that surrounds the seeds is spherical, broadly ellipsoid, somewhat compressed, and irregularly corrugated. The fruit is 1-4 inches long and almost ovoid or ellipsoid in shape. The seed is many, ovoid, smooth, and dark brown. Fruits taste bitter and have an unpleasant smell.



Fig. 2. Macroscopy of *Momordica dioica* fruit.

Microscopic evaluation. The fruit's transversely cut surface has a spherical shape and many exterior longitudinal rugose folds (Fig. 3A). The pericarp is divided into an outer, middle, inner, and endocarp. The

glandular and multicellular trichomes are present on the epidermal cells, which are isodiametrically tangentially elongated (Fig. 3B, F, G). With huge, isodiametric, or radially elongated cells that appear to be empty of the cell sap and a network of tiny intercellular gaps, outer mesocarp tissue is made up of eight to ten layers (8–10). Middle mesocarp tissue has thick-walled cells with lignin and is polygonal in form with four to five layers (Fig. 3C). The interior mesocarp is made up of

numerous layers of thin-walled cells that exhibit an enormous amount of starch relative to the outer layers (Fig. 3 E). The central mesocarp is covered in tiny, floppy bundles of bicollateral vascular tissue that are organized in a ring (Fig. 3 D). Endocarp tissue is thin and transparent and is made up of tiny, thin-walled, and tangentially elongated cells. The endosperm is made up of polygonal parenchyma surrounded by cotyledon tissue, oil globules, and aleurone grains (Fig. 3H).

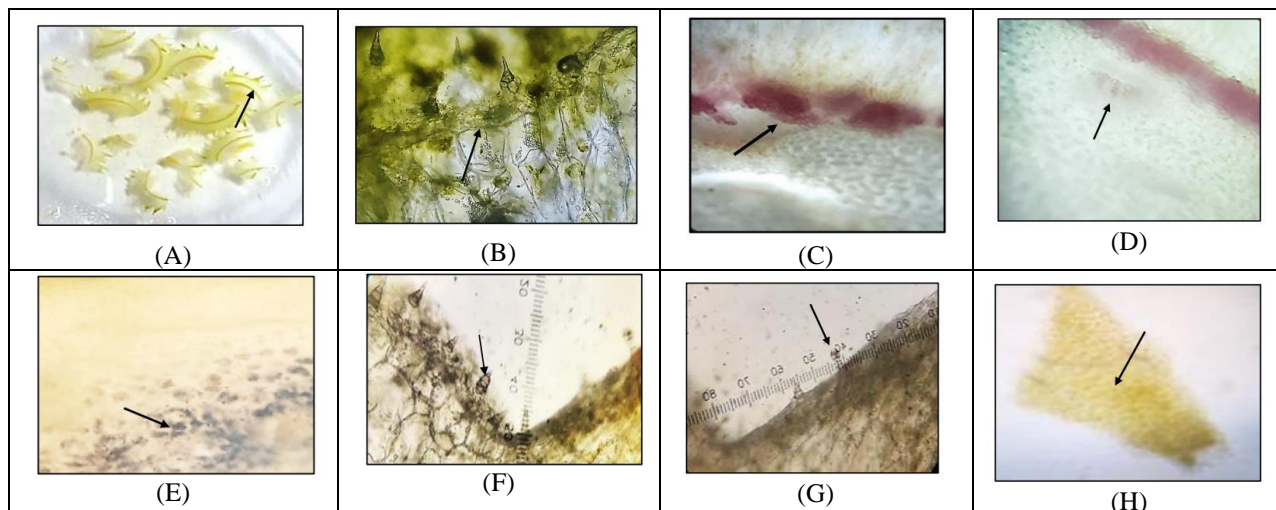


Fig. 3. Cross sections of the immature fruit of *Momordica dioica* (A) An External longitudinal rugose folds (B) Epidermis (C) Middle mesocarp with thick-walled lignin cells (Stained with Phloroglucinol and concentrated HCL) (D) Inner Mesocarp with scattered Vascular Bundle (Stained with Phloroglucinol and concentrated HCL) (E) Inner mesocarp with thin-walled cells containing a lot of starch grains (F) Multicellular covering trichomes (G) Glandular trichomes (H) Endosperm (Stained with alcoholic picric acid).

Powder Microscopy. *Momordica dioica* dried fruits were examined for powder properties. The powder had a characteristic bitter taste and light brown color. When examined under a microscope, fruit powder reveals the presence of the Middle Mesocarp, fibers, epidermis,

starch grain, Glandular trichomes, Multicellular trichomes, Prism-like Calcium Oxalate Crystals, Spiral Vessels, Endosperm with Polygonal Cells, Secretory Cells.

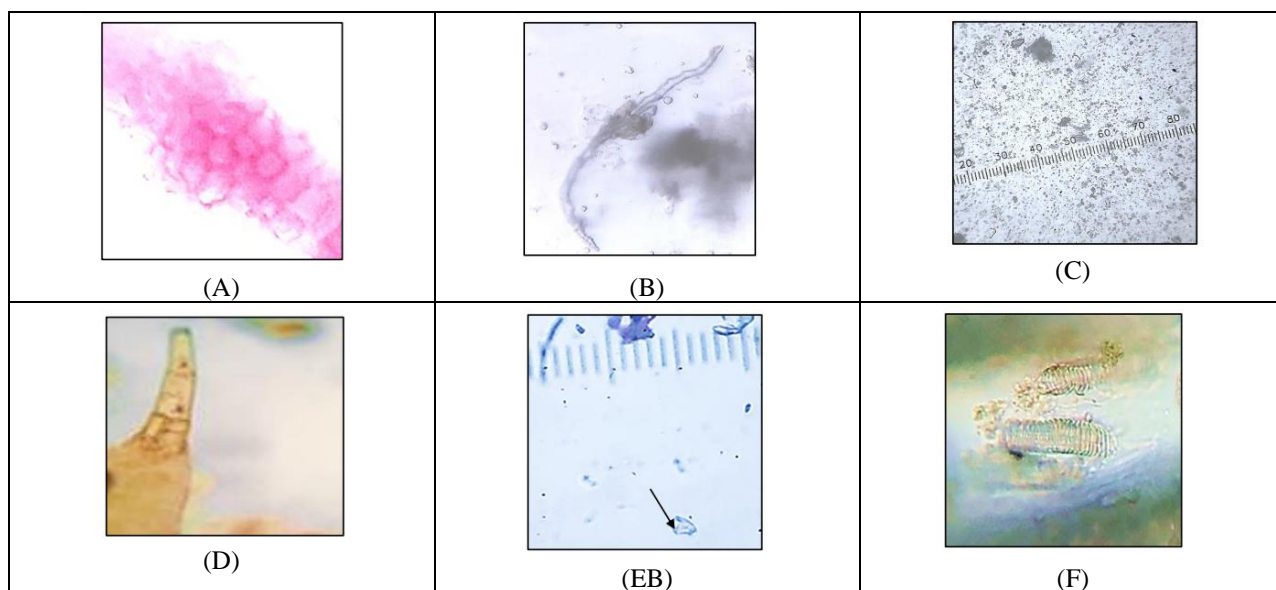


Fig. 4. Powder microscopy of *Momordica dioica* fruit (A) Middle mesocarp (B) Fibers (C) Starch grains (D) Multicellular trichome (E) Prism type crystal (F) Spiral Vessels.

Determination of foreign content and Loss on drying. One of the key elements in evaluating the stability and deterioration of medications and formulations is loss on drying. Moisture content was calculated taking into account these details. *Momordica dioica* (MD) powder had a foreign matter level of $1.0 \pm 0.8\%$, and the dried aerial parts had a moisture content of $10.23 \pm 0.72\%$ w/w.

Determination of the size of starch grains and Calcium oxalate crystals. Starch grains of MD were simple and compound, oval to round in shape, and the diameter of small grains, large grains, and average grains was $2\ \mu$ to $9\ \mu$, $10\ \mu$ to $23\ \mu$, and $10\ \mu$ respectively. No striations are visible and only very occasionally a hilum can be distinguished as a small dot. The presence of prism shape calcium oxalate crystals was large, single, and well-developed. The size of crystals was $13\ \mu$ to $20\ \mu$ and $23\ \mu$ average size.

Ash values & extractive value. Ash values are significant as a qualitative benchmark because they can be used to assess the quality and purity of crude drugs. The mineral content of a crude drug, like carbonate, oxalate, and silicate, can be determined based on the ash value. In general, water-soluble ash is used to calculate the amount of inorganic compounds present in crude drugs and is a crucial indicator of whether or not the crude drug is exhausted by water, while acid-insoluble ash, which is primarily composed of silica, may indicate contamination with earthy material. The water, alcohol, and ether extractive values play a vital role in evaluating crude drugs and give an idea about the nature of the chemical constituents present in them. The alcohol-soluble extractive value revealed an important parameter in the evaluation of crude drugs. Based on the less extractive value it can be caused by certain factors such as exhaustion of crude drugs, adulteration, and due to the incorrect method during drying and storage. The water-soluble extractive value also revealed the same purpose as the alcohol-soluble extractive. The percentage of total ash, acid-insoluble ash, water-soluble ash, ethanol-soluble extractive, water, and ether-soluble extractive are presented in Table 1. The total ash, acid-insoluble, and water-soluble ash values were observed to be $(7.4 \pm 0.1)\%$ w/w, $(2.3 \pm 0.1)\%$ w/w, and $(5.2 \pm 0.1)\%$ w/w respectively where the extractive value of ethanol, water, and ether was found to be $(17.8 \pm 0.3)\%$ w/w, $(20.5 \pm 0.5)\%$ w/w and $(4.1 \pm 0.2)\%$ w/w respectively (Singh *et al.*, 2010; Dash *et al.*, 2021).

Table 1: Physico-chemical analysis of *Momordica dioica*.

Standardization parameters	Mean \pm SEM (% w/w)
Total ash	7.4 ± 0.1
Acid insoluble ash	2.3 ± 0.1
Water soluble ash	5.2 ± 0.1
Ethanol soluble extractive values	17.8 ± 0.3
Water soluble extractive values	20.5 ± 0.5
Ether soluble extractive values	4.1 ± 0.2

SEM: Standard error of the mean. Extraction. Using a Soxhlet device, petroleum ether and methanol were used to extract the fruit powder of *Momordica dioica* Roxb. The yield of the extracts from the *M. dioica* fruit in petroleum ether and methanol was 5.03% and 15.63%, respectively. The petroleum ether extract was an oily-viscous yellowish brown that solidified at 28°C and the methanol extract was Semi-solid Dark Brown (Budrat and Shotipruk 2008).

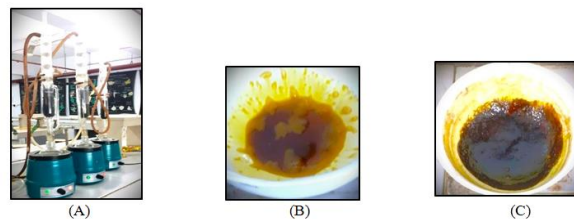


Fig. 5 (A) Soxhlet extraction (B) Petroleum ether extract (C) Methanol extract of *Momordica dioica* fruit.

Preliminary phytochemical screening. The preliminary phytochemical analysis can be used as a standardization tool to recognize, confirm, and detect falsification in order to guarantee quality control of the market's supply of crude drugs. The phytochemical screening of the petroleum ether and methanol extracts revealed the presence of proteins, alkaloids, carbohydrates, glycosides, steroids, terpenoids, tannins, saponins, phenolic compounds, and flavonoids in the methanol extract. Terpenoids and steroids are found in petroleum ether extract. Table 2 presents the findings (Bhandary *et al.*, 2012).

Table 2: Results of preliminary phytochemical investigation of extracts.

Sr. No.	Chemical components	Petroleum ether extract	Methanol extract
1.	Alkaloids	-	+
2.	Carbohydrates	-	+
3.	Antraquinones	-	-
4.	Glycosides	-	+
5.	Cardiac glycosides	-	+
6.	Steroids	+	+
7.	Terpenoids	+	+
8.	Tannins	-	+
9.	Saponins	-	+
10.	Flavonoids	-	+
11.	Phenol	-	+
12.	Proteins	-	+

+ = Present - = Absent

Total phenolic content and total flavonoid content. The total flavonoid concentration in the MD methanol extract was found to be 125 mg/g of quercetin equivalent, while the total phenol content was determined to be 64 mg/g of gallic acid equivalent. Figs. 6A and 6B show the standard calibration curve visually. Findings regarding the overall flavonoid and phenol content in the study plant point to significant antioxidant elements that are responsible for neutralizing free radicals and preventing the development of various chronic illnesses, including cancer and cardiovascular disease (Swapna *et al.*, 2020).

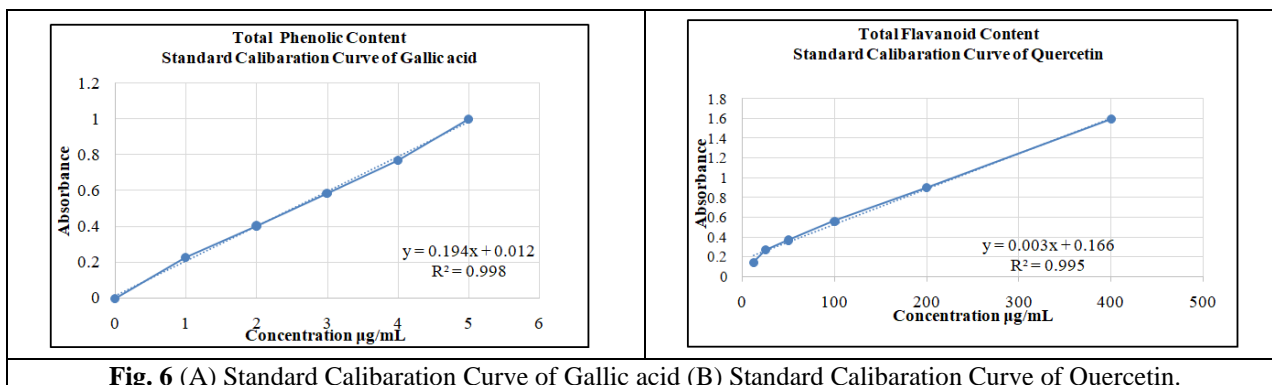


Fig. 6 (A) Standard Calibration Curve of Gallic acid (B) Standard Calibration Curve of Quercetin.

Development of Chromatographic TLC Fingerprint.

The bioactive chemicals are often better identified using thin-layer chromatography. The TLC profiling of all the plant extracts in the current investigation once again demonstrated the presence of several metabolites, including alkaloids, flavonoids, phenols, steroids, saponins, and tannins. Fig. 7 displays the TLC profiling findings. Charantin was detected in a methanol extract at R_f 0.45. When 10% sulfuric acid in alcohol was sprayed onto the TLC plate and heated at 100°C for 2-3 minutes, a violet spot became visible (Shanmugapriya and Poornima 2014).



Fig. 7. TLC of (A) Methanol extract of MD Fruit (B) Marker.

CONCLUSIONS

The quality and safety of herbal medications have given rise to various issues, and the Indian herbal business is expanding rapidly. Macroscopy, microscopy, and proximate analysis, among other standardization parameters of *Momordica dioica* Roxb. fruit examined in the current study, may be used as a quick and precise tool in herbal research for the identification and adulteration of the study herb as well as to set quality standards and specifications for therapeutic safety, efficacy, and shelf-life of herbal drugs.

FUTURE SCOPE

The study's findings suggest that pharmacognostic standardization and a preliminary phytochemical analysis of *Momordica dioica* Roxb. fruit are crucial for supplying useful details about standardization for specific plants. Additionally, this standardization is helpful for identifying and creating pertinent monographs for accurate identification purposes to avoid issues caused by irresponsible parties who want

to increase their earnings in spite of the lack of standardization for specific species. The above study is useful in identifying the difference between contaminated products and making it simpler to distinguish herbal drugs from the same or different species.

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Conflict of Interest. None.

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