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Extraction, Phytochemical Investigation and Chromatographic Study of *Manilkara zapota* Seeds

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Abstract: *Manilkara zapota (sapota) seeds are considered an underutilized plant material despite being rich in several bioactive phytochemicals with potential therapeutic importance. The present study was carried out to investigate the phytochemical constituents and chromatographic profile of aqueous extracts obtained from M. zapota seeds. The seeds were collected, shade dried, powdered, and subjected to aqueous extraction by maceration method. Preliminary phytochemical screening was performed using standard qualitative chemical tests to identify the presence of important secondary metabolites. Thin Layer Chromatography (TLC) analysis was carried out using suitable mobile phase systems for separation and characterization of phytoconstituents, while UV–Visible spectroscopic analysis was performed for further confirmation of flavonoid and phenolic compounds.The phytochemical investigation revealed the presence of alkaloids, flavonoids, glycosides, saponins, and terpenoids in the aqueous extract. TLC analysis produced a well-resolved chromatographic spot with an Rf value of 0.69, indicating the possible presence of flavonoid or phenolic compounds. UV–Visible spectroscopic analysis showed a characteristic absorption peak at 285 nm, which further supported the occurrence of phenolic and flavonoid constituents in the extract. The findings suggest that aqueous extraction is an effective method for isolating polar bioactive compounds from M. zapota seeds.Overall, the study highlights the phytochemical richness and potential pharmaceutical significance of Manilkara zapota seeds. The results support their possible application in herbal and nutraceutical formulations; however, advanced analytical and pharmacological studies are required for complete characterization and therapeutic validation of the bioactive constituents.*

Keywords: Manilkara zapota, Sapodilla seeds, Phytochemical screening, Aqueous extraction, Thin Layer Chromatography (TLC), UV–Visible spectroscopy, Flavonoids, Phenolic compounds, Bioactive phytochemicals, Herbal medicine, Chromatographic profiling, Nutraceutical potential, Antioxidant activity, Secondary metabolites, Phytochemical investigation

I. INTRODUCTION

Manilkara zapota (L.) P. Royen, commonly known as sapodilla or Chiku, is an economically important evergreen species of the family Sapotaceae, widely cultivated in tropical regions of Asia, Central America, and India. While the edible fruit is well known for its nutritive and commercial value, the seeds usually discarded as waste represent an under explored reservoir of bioactive phytoconstituents. In recent years, the valorization of agro-industrial waste has gained global attention, prompting scientific interest in the chemical profiling and biological evaluation of M. zapota seeds. [1,2]

Phytochemical investigations have revealed that the seeds contain significant amounts of polar secondary metabolites, including phenolic compounds, flavonoids, tannins, glycosides, saponins, alkaloids, quercitol, fixed oils, and proteins. These compounds are associated with diverse pharmacological properties such as antioxidant, antimicrobial, anthelmintic, anti-inflammatory, and cytoprotective effects. The presence of saponin glycosides (sapotins) and D-quercitol, a cyclitol with medicinal relevance, makes the seeds particularly valuable for therapeutic and nutraceutical research. [1,3,4]

Aqueous extraction is a widely used technique for isolating polar and hydrophilic bioactive constituents from plant matrices. For M. zapota seeds, aqueous extraction offers a safe, eco-friendly, non-toxic, and cost-effective approach to obtain biologically active fractions suitable for phytochemical analysis and in-vitro assays. Water effectively solubilizes glycosides, flavonoid glycosides, phenolic acids, sugars, saponins, and certain proteins, allowing the extract to reflect constituents that may be relevant to traditional or folk medicinal preparations. [1]

Thin Layer Chromatography (TLC) is a simple, rapid, economical, and highly effective chromatographic technique used for the separation, identification, and preliminary characterization of phytochemical constituents present in plant extracts. In TLC analysis, compounds are separated based on their differential adsorption between a stationary phase, usually silica gel, and a mobile solvent phase. The separated phytoconstituents appear as distinct spots with characteristic retention factor (Rf) values. TLC is extensively used in herbal drug standardization, phytochemical fingerprinting, and qualitative analysis of flavonoids and phenolic compounds. Visualization of chromatographic spots is commonly carried out under ultraviolet light at 254 nm and 366 nm or by using specific derivatizing reagents such as ferric chloride and aluminium chloride for phenolics and flavonoids, respectively. [1]

UV-Visible spectroscopy is another important analytical technique widely used for the qualitative and quantitative estimation of flavonoids and phenolic compounds in plant extracts. The technique is based on the absorption of ultraviolet radiation by conjugated π-electron systems present in flavonoid structures. Flavonoids typically exhibit two major absorption bands: Band II in the range of 240–280 nm corresponding to the benzoyl system of ring A, and Band I in the range of 300–380 nm corresponding to the cinnamoyl system of ring B. The position and intensity of these absorption maxima provide valuable information regarding the presence and structural characteristics of flavonoid compounds in plant extracts. UV spectroscopy is therefore considered a rapid, reliable, and cost-effective method for phytochemical screening and flavonoid estimation.

The exploration of *Manilkara zapota* seed extracts aligns with current scientific trends focused on sustainable utilization of plant waste, natural antioxidant discovery, and the development of herbal formulations. As industrial fruit processing generates large quantities of seeds, converting them into promising phytopharmaceutical ingredients supports both environmental sustainability and bioprospecting efforts.

Thus, the extraction and phytochemical investigation of *Manilkara zapota* seeds using aqueous solvents provides critical insight into their chemical composition, guides future pharmacological evaluations, and helps establish the seeds as a potential raw material in herbal and nutraceutical research.

Taxonomy

Sapotaceae family is a diverse and ecologically important family of 800 species and nearly 65 genera. Include shrubs and trees which are widely distributed in tropical regions like America, Asia and South Africa. The members of this family can be easily recognized by the characteristic milky latex exudate and alternate leathery leaves with prominent secondary and tertiary venation, often appearing parallel. The genus Manilkara includes 30-32 species, most of which are economically important and commercially used as a source of fruit, timber and latex. Some of the related species to sapodilla are the sapote [*Pouteria sapota*] which has a large central seed similar to the avocado, the green sapote [*Pouteria viridis*] and the star apple [*Chrysophyllum cainito*]. [1,2,9]

Sr. No.		
1.	Kingdom	Plantae (plants)
2.	Sub kingdom	Tracheobionta (vascular plants)
3.	Super Division	Spermatophyta (seeds plant)
4.	Division	Magnoliophyta (flowering plants)
5.	Class	Magnoliopsida (dicotyledonous)
6.	Sub class	Dilleniida
7.	Order	Ebenales
8.	Family	Sapotaceae

9.	Genus	Manilkara Adans (Manilkara)
10.	Species	M. zapota (L.) P. Royen

Table No. 01: Taxonomic Details of Sapodilla

Synonyms

Sapodilla has been mentioned in literature by a large number of synonyms such as *Achras sapota* L., *Achras zapota* L. var. zapotilla Jacq. *Achras zapotilla* Nutt., *Achras mammosa* L., *Manilkara achras* (Miller) Fosberg, *Manilkara zapotilla* (Jacq.) Gilly, *Sapota zapotilla* (Jacq.) Coville, *Sapota achras* Miller, *Sapota zapotilla* (Coville), etc. The generic names, Manilkara and Achras are the commonly used ones but still the name Achras is controversial and the botanists have no agreement for the proper name. Sapota (zapota) or sapote (zapote) is used for the species name however, this name too is not free of disagreements among authors. The generic name Achras, given by Linnaeus, was based upon a plant and description by the botanist Plumier but unfortunately, the plant that Plumier described is not Sapodilla and thus leading to the misnaming of this genus. Gilly suggested that Manilkara Zapotilla (Jacq.) Gillys's is the only proper name, based on the fact that Manilkara is the earliest recorded name of the Sapodilla Group and zapotilla was used specifically to Sapodilla at the time of its publication. [2,11]

Vernacular Names

Sapodilla is known by a number of vernacular/common names in different countries.

1.	Brazil	Sapoti, sapotilha
2.	Thailand	Lamoot, Lamut, Lamut-farang
3.	English	Sapodilla
4.	Indonesia	Sawu
5.	Cuba	Sapota, sapote
6.	India	Chikoo, chicku, chiku
7.	Mexico	Chicopote, chicozapote
8.	West Indies	Nasebery
9.	Singapore	Ciku
10.	Malaysia	Chikoo

Table No. 02: Vernacular names of Manilkara zapota

Botanical description

Sapodilla is a medium to large sized tree with dense and almost round canopy formed by profuse branching system (sympodial Type). Initially the growth of this tree is slow but after many years, may reach up to 20-30 meters in height. All three parts exude out milky latex known as "chicle".

1.Roots: Sapodilla roots show shallow-root system with a major portion of roots present within the top 75 cm of soil and about 66% of the moisture extracted from the soil is in the first 75 cm.

2.Leaves: The leaves are evergreen and spirally arranged (7-12 × 2-4 cm in size), pinkish brown when young and turns light-dark green at maturity. Secondary veins make a wide angle with the midrib.

3.Flowers: Flowers are small, bisexual, bell-shaped (10mm in diameter), borne singly or in clusters in the axils of leaves near the the ends of branches.

4.Fruit: The fruit of sapodilla is a brown-coloured berry, nearly round and varies from 5-10 cm in width. The unripe fruit is hard and coarse whereas it becomes soft and juicy on maturity.

5.Seeds: Some Sapodilla fruits are seedless but normally they produce 3-12 seeds per fruit. They are hard and brown or black in colour with one white margin. The seeds contain some phytochemicals like sapotin, saponin, achras saponin and the bitter sapotin. [2,9]

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II. LITERATURE SURVEY

Years	Author's	Title	Key Findings
2014	G. Venkateswara Rao et al. [7]	Phytoconstituents from the Leaves and Seeds of *Manilkara zapota* Linn.	The study reported the presence of important phytoconstituents including flavonoids, tannins, alkaloids, and phenolic compounds in seeds and leaves of *M. zapota*, supporting its medicinal significance.
2015	Marie Fomani et al. [16]	Bioactive Phenylethanoids from the Seeds of *Manilkara zapota*	Bioactive phenolic compounds were isolated from seed extracts using chromatographic separation techniques, demonstrating antioxidant and biological potential of seed constituents.
2018	C. Mohanapriya et al.	In-vitro evaluation of secondary metabolites: characterization and antimicrobial activity of *M. zapota* seed extract	TLC and phytochemical screening confirmed the presence of flavonoids, tannins, and phenolic compounds in seed extracts with significant antimicrobial activity
2019	Kalyan Hazra et al. [8]	Phytopharmacognostical Profile of *Manilkara zapota* (L.) P. Royen Seeds	Detailed pharmacognostic, microscopic, and phytochemical evaluation of seeds was performed. Chromatographic and FTIR profiling supported authentication and characterization of seed material.
2020	Yong Yik Shuen and Shukkoor Mohideen Abdul Kade	*M. zapota*: A Phytochemical and Pharmacological Review	The review summarized major phytochemicals including flavonoids, tannins, and polyphenols present in *M. zapota* and highlighted antioxidant, antimicrobial, and anti-inflammatory activities.
2020	Beena Rawat and Amar P. Garg [14]	Characterization of Phytochemicals Isolated from *Cucurbita pepo* Seeds using UV-Vis and FTIR Spectroscopy	The study demonstrated the application of UV-Visible and FTIR spectroscopy in phytochemical characterization of seed extracts, supporting analytical approaches used in phytochemical investigations.
2021	Rupali P. Lade et al. [3]	Phytochemicals and Pharmacological Investigation of Extract of *Manilkara zapota* Linn. Seeds	Ethanolic seed extracts showed the presence of alkaloids, flavonoids, glycosides, tannins, and phenolic compounds with notable pharmacological activity.
2021	Joushan Ara et al.	Phytochemical and Ethnopharmacological Evaluations of Methanolic Extracts of Seed Coat of *Manilkara zapota*	Methanolic seed coat extracts demonstrated antioxidant, anti-inflammatory, and analgesic activities with the presence of major secondary metabolites.
2021	A. Tiwari et al.	Phytochemical Profiling and Anthelmintic Activity of *Manilkara zapota* Leaves and Seeds	Petroleum ether and hydro-ethanolic extracts revealed multiple phytoconstituents and significant anthelmintic activity, emphasizing the influence of extraction solvents on phytochemical recovery.
2022	SP Bangar et al.	A Review of Sapodilla in Human Nutrition and	The review highlighted the occurrence of phenolics, flavonoids, and tannins in sapodilla and emphasized

		Phytochemicals	their antioxidant and therapeutic importance.
2022	Mehedi MA, Arabi II, Islam Z, Das S, Mannan MA.[12]	Extraction of Sapodilla Oil from Sapodilla Seed	Soxhlet extraction using methanol yielded seed oil with potential pharmaceutical applications and emphasized the importance of phytochemical profiling of seed oil fractions.
2023	Maria Fernanda Rivas-Gastelum [16]	Manilkara zapota "chicozapote" as a fruit source of health-beneficial bioactive compounds and its effects on chronic degenerative and infectious diseases	The review discussed flavonoids and phenolic compounds present in *M. zapota* and highlighted chromatographic and spectroscopic techniques for bioactive compound identification.
2023	Md. Anik Mehedi et al. [12]	Extraction, Physio-Chemical Characterization and Antimicrobial Studies of Seed Oil of Sofeda (*Manilkara zapota*)	Seed oil extracts demonstrated antimicrobial activity and physicochemical characterization confirmed the pharmaceutical significance of seed constituents.
2024	Sourajyoti Goswami et al. [11]	Phytochemical Profiling and Anthelmintic Activity of *Manilkara zapota* L. Extract	Integrated phytochemical profiling confirmed secondary metabolites and demonstrated significant biological activity through in-vitro and in-silico approaches.
2024	Ishtiaque Ahmed	Phenolic Composition and Antioxidant Potential of Peel and Pulp of Manilkara zapota	Spectroscopic and chromatographic analyses confirmed phenolic and flavonoid compounds with antioxidant activity, supporting analytical profiling methods in *M. zapota* research.
2025	Manish D. Chandelkar et al. [2]	Phytochemical Characterization and Antimicrobial Efficacy of *Manilkara zapota* Seed Extracts Against Multidrug-Resistant Pathogens	Methanolic and aqueous seed extracts contained flavonoids, tannins, cardiac glycosides, and saponins with significant antimicrobial activity against multidrug-resistant organisms.

III. NEED OF STUDY

Manilkara zapota (sapota) seeds are an under-utilized botanical resource, although several scientific reports indicate that the seeds contain a rich profile of bioactive secondary metabolites such as phenolic, flavonoids, tannins, saponins, glycosides, alkaloids, fatty acids, and lectin-type proteins. Multiple phytochemical reviews and research papers highlight promising antioxidant, antimicrobial, anti-inflammatory, and enzyme-modulating activities, but the seed component remains comparatively less investigated than the fruit pulp or peel. Despite these indications, systematic extraction and chromatographic profiling of sapota seeds are limited, creating significant gaps in understanding their therapeutic potential, chemical diversity, and possible applications in pharmaceutical or nutraceutical formulations. [1,3,4]

1. To Explore Understudied Plant Material

Most published work concentrates on zapota fruit pulp, peel, or leaves. Studies reporting on seeds note the presence of potent phenolic antioxidants and antimicrobial compounds, yet the chemical spectrum is not fully mapped. Therefore,

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extraction and detailed chromatographic studies (TLC) are needed to characterize the seed phytochemicals more comprehensively. [1,8]

2. To Identify Bioactive Phytochemicals of Therapeutic Importance

Research articles show that zapota seed extracts possess measurable DPPH scavenging, antibacterial, and anti-inflammatory properties, suggesting the presence of pharmacologically active molecules. A focused phytochemical investigation can help for further study.

3. To establish chromatographic profiles for quality assessment

Chromatographic tools (TLC, HPTLC, GC–MS) reported in recent literature reveal only partial characterization of sapota seed components. A dedicated chromatography study is essential [6]

4. To Support Valorization of Agricultural Waste

Sapota seeds are typically discarded in fruit processing and household consumption. Studies highlight the presence of bioactive fatty acids, polyphenols, and lectins in these seeds, suggesting they can be converted into: Antioxidants, Antimicrobial agents, cosmetic/nutraceutical raw materials. [1,4]

5. To Contribute to Scientific Understanding and Future Drug Developments The reported activities—antioxidant, antimicrobial, antiproliferative, and enzyme-inhibitory—indicate potential pharmacological relevance. Comprehensive extraction and chromatographic studies can: Therefore, a systematic phytochemical investigation and chromatographic study of Manilkara zapota seeds is essential to:

- Identify and characterize the phytochemical spectrum present in the seeds using suitable extraction procedures.
- Generate chromatographic fingerprints (TLC/HPTLC/HPLC) that can support quality control and standardization of seed-derived products.
- Establish scientific evidence for potential therapeutic applications and guide future pharmacological evaluations.
- Utilize agricultural waste material, promoting cost-effective drug discovery and value addition to the fruit industry.
- Support natural product research by providing data on bioactive molecules that may serve as leads for novel drug development.

IV. AIM AND OBJECTIVE

To systematically extract, phytochemical constituents and chromatographically profile of *Manilkara zapota* (sapota) seeds based on established research findings, in order to identify their bioactive components and evaluate their therapeutic potential for future pharmaceutical or nutraceutical applications.

1. To perform extraction of M. zapota seeds using aqueous solvents.
2. To conduct preliminary phytochemical screening.
3. To optimize an appropriate mobile phase system for achieving effective chromatographic separation and clear resolution of flavonoids, phenolics, tannins, and other polar secondary metabolites.
4. To perform TLC the separation of phytoconstituents present in the aqueous seed extract based on their differential adsorption and migration characteristics.
5. To identify characteristic chromatographic spots and determine their retention factor (Rf) values for preliminary qualitative characterization of phytochemicals
6. To analyze the aqueous extract of Manilkara zapota seed using UV-Vis spectroscopy for the detection and characterization of flavonoid compounds based on their absorption behavior in the ultraviolet region.
7. To correlate chromatographic patterns with phytochemical constituent Chromatographic bands/peaks help confirm compound classes reported in literature (e.g., flavonoids, phenolic acids).

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V. PLAN OF WORK

Sr No.	Work	Duration
01	Literature survey	5 Days
02	Collection and drying	30 Days
03	Extraction	3 to 4 Days
04	Phytochemical investigation	3 Days
05	Thin layer chromatography	5 days
06	Centrifugation	1 day
06	UV Analysis	3 days
07	Result discussion conclusion	5 Days

Table No. 03: Duration of work conducted

Literature Survey
Collection of scientific literature related to *M. zapota* seeds including extraction methods, pharmacognosy, phytochemical studies, traditional uses, toxicity profile, and previous research studies.

↓

Collection and Pre-processing of Seeds
Collection of ripe fruits → Separation of seeds → Washing and drying → Removal of seed coat → Powdering to uniform particle size

↓

Extraction Procedure
Selection of suitable solvent (Water/Ethanol/Methanol/Hydroalcoholic solvent) → Extraction by Maceration/Soxhlet/Shaking method (24–72 hours) → Filtration → Concentration of extract.

↓

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Preliminary Phytochemical Screening
Detection of:
- Alkaloids (Wagner's and Mayer's tests)
- Flavonoids (Shinoda test)
- Tannins (Ferric chloride test)
- Saponins (Foam test)

↓

Thin Layer Chromatography (TLC)

Preparation of mobile phases → Development of TLC plates → Application of spraying reagents (Aluminium chloride, Ferric chloride, Ninhydrin reagent) → Visualization of spots.

↓

UV Spectroscopic Analysis
UV analysis of extracts for characterization of phytoconstituents.

↓

Conclusion and Documentation
Summarization of phytochemical richness → Development of chromatographic fingerprint → Evaluation of medicinal and nutraceutical potential → Documentation of findings with literature support.

VI. MATERIAL & EQUIPMENT

Material

- Plant
- M. zapota seeds powder
- **Chemicals**: Distilled water, Chloroform (few drops, optional – prevents microbial growth), Concentrated HCl, concentrated H_2SO_4, Dragendorff's reagent, Mayer's reagent, Dilute HCL, NaOH, Ferric chloride, Glacial acetic acid, Aluminium chloride, Ninhydrin reagent and silica gel.

Equipment

- Filter paper / muslin cloth.
- Cotton balls
- Mortar & pestle / grinder
- Beaker or conical flasks
- Test tube
- Test tube holder

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- Water bath / hot plate
- Glass slides
- centrifuge
- UV Spectroscope
- Hot air oven
- Funnel
- Self-sealing pouch

Experimental Work

1. COLLECTION:

The new seeds of *Manilkara zapota* were collected from Dive, Saswad 2026. near to Pune on January

Fig.No.01: Collection and drying of *Manilkara zapota* seeds.

2. DRYING OF PLANT MATERIAL :

The seeds were shade dried for one month and coarsely powdered. Extraction of the active ingredient from the seed powder was carried out using the method as pharmacopoeia.

The 30 grams of seeds were clean and collected. It was separated and wipes carefully to eradicate muck and fragments. Seeds were spread and shade dried for 20-25 days. After natural drying of seed weighed, it was found 340g. Dried seeds were coarsely powdered using mortar and pestle or blender.

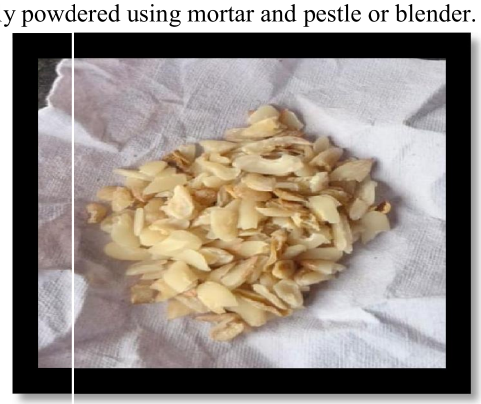

Fig.No. 02: Dried inner content of M. zapota seeds

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3. EXTRACTION:

- A weighed sufficient quantity of the coarse powder of *Manilkara zapota* seeds was transferred into a clean glass beaker. Sufficient distilled water (approximately 50–80 ml) was added to ensure complete immersion of the powder. A few drops of chloroform were incorporated to prevent microbial growth during maceration. The beaker was then covered with a clean cloth, followed by a Petri dish, and kept undisturbed for 3–4 days.
- After the maceration period, the coverings were removed and the mixture was filtered through a muslin cloth to obtain the aqueous extract. The filtrate was subsequently concentrated by gentle heating to reduce its volume and obtain a concentrated extract [5,10]

Fig. No. 03: Maceration of *Manilkara zapota* seeds powder.

4. PHYTOCHEMICALS INVESTIGATION:

Perform standard tests for:
Alkaloids (Wagner's, Mayer's)
Flavonoids (Shinoda test)
Tannins (Ferric chloride) Phenols
Saponins (Foam test)
Steroids, Triterpenoids
Consistent reports: seeds show strong positive tests for tannins, flavonoids, saponins, phenolic.[10]

Phytochemicals screening [6]

	Test	Observation
Alkaloids	1.Hangers Test: Take 1 ml of extract in test tube and add few ml of picric acid solution	Yellow precipitate
	2. Mayer's Test Add few ml of aqueous extract in test tube and add few drops of Mayer's reagent	Creamy color precipitate
	3. Dragendorff's Reagent Test Take 2ml of extract and add few drops of Dragendorff's reagent in test tube	Reddish brown color precipitate

Flavonoids	1.Shinoda Test Take a few drops of aqueous extract and add few small pieces of magnesium turning then add few drops of conc. HCl	Pink, red, orange color precipitate
	2.Alkaline reagent Test Take a 2ml of aq. extract and add few drops of 10% NaOH solution in test tube and add few drops of dil. HCl	Yellow color precipitate
Tannins	Ferric Chloride Test Add few ml of aqueous extract in test tube and add neutral 5% of FeCl3	Blue color precipitate
Glycoside	1.Keller-killiani Test Add a 2ml of aqueous extract in test tube and add few ml of glacial acetic acid then add few drops of FeCl3	Formation of purple ring junction
Saponins	Add 1ml of aq. extract in test tube and add 1ml of water in test tube and shake the test tube for few minutes	Formation of foam
Terpenoid	Add few ml of aqueous extract then add 5ml of con. H2SO4 and add 2ml of chloroform	Reddish brown color precipitate

Table no. 4: Phytochemical screening

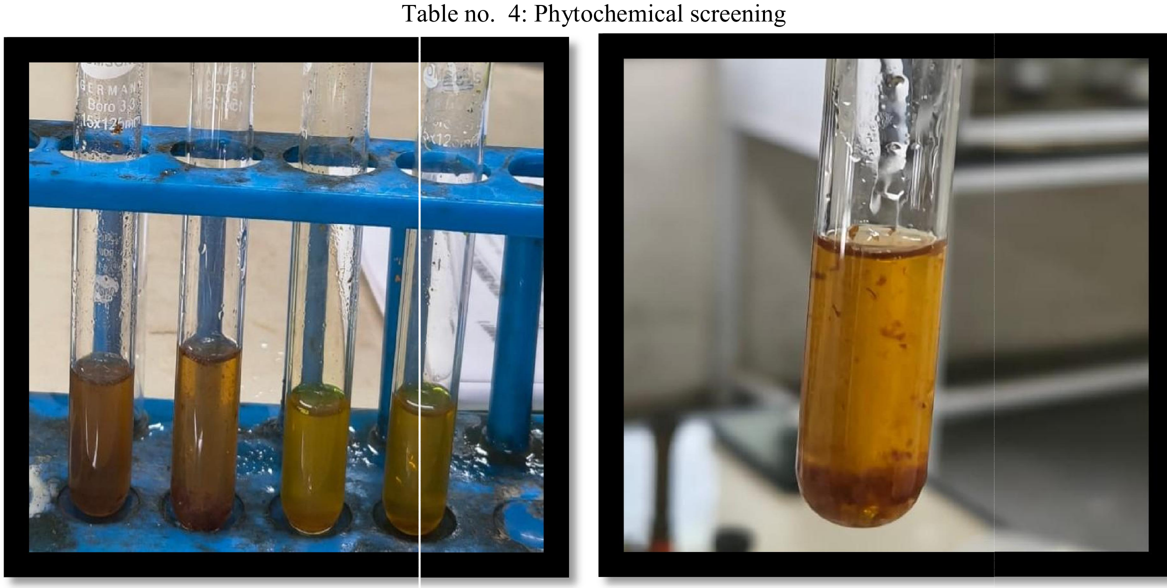

Fig no. 04: Phytochemical test performed.

THIN LAYER CHROMATOGRAPHY:

PRINCIPLE

The principle of TLC plate preparation involves the formation of a thin, uniform, and mechanically stable layer of adsorbent material over a solid support. Silica gel particles are suspended in water or suitable binder solution to form a slurry, which is uniformly spread over the surface of the plate. Upon drying and activation, the adsorbent layer becomes capable of separating compounds based on differential adsorption and partition behavior during chromatographic development.

Composition of slurry

A commonly used ratio for manual TLC plate preparation is:

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Silica gel G = 25 g

Distilled water = 50 mL

This produces a smooth slurry suitable for coating approximately 5–6 plates.

Procedure for manual TLC plate preparation

Step 1: Cleaning of Glass Plates

The glass plates should be thoroughly washed using detergent and water in order to remove dust, grease, and other contaminants that may interfere with uniform coating or chromatographic separation. The cleaned plates should then be rinsed with distilled water followed by acetone or ethanol and dried completely to ensure proper adhesion of the silica layer.

Step 2: Preparation of Silica Slurry

Accurately weighed silica gel G should be transferred into a clean beaker or mortar, after which distilled water should be added gradually while continuously stirring to prevent formation of lumps. The mixture should be stirred uniformly until a smooth, homogeneous slurry of suitable viscosity is obtained. The slurry should neither be too thick nor too watery because improper consistency may produce uneven coating and poor chromatographic performance.

Step 3: Coating of Plates

The prepared slurry should be immediately poured onto the cleaned glass plate because silica suspension tends to settle rapidly. Using a TLC spreader or glass rod, the slurry should be spread uniformly across the entire surface of the plate to obtain a thin layer approximately 0.25 mm thick for analytical TLC.

Care should be taken to maintain:

- Uniform thickness
- Smooth surface
- Absence of cracks or air bubbles
- Uneven coating may lead to irregular solvent migration and distorted chromatographic spots.

Step 4: Air Drying

After coating, the plates should be allowed to dry in air at room temperature for approximately 15–30 minutes in a dust-free environment. This step allows evaporation of excess moisture and improves adhesion of the silica layer to the glass surface.

Step 5: Activation of TLC Plates

The dried plates should be activated by placing them in a hot air oven at approximately:

100–120°C for 30–60 minutes

Activation removes adsorbed moisture from silica gel and increases the adsorption efficiency of the stationary phase. After activation, the plates should be cooled in a desiccator to prevent moisture absorption from the atmosphere.

(a)

(b)

Fig No. 05: TLC Performed.

Procedure of TLC

- A TLC plate coated with silica gel (stationary phase) is taken.
- A small spot of sample extract is applied near the bottom of the plate using a capillary tube.
- The plate is placed in a chamber containing a solvent system (mobile phase).
- The solvent rises upward by capillary action and carries the compounds with it.
- Different compounds move at different speeds depending on their polarity and affinity towards the stationary and mobile phases.
- After the solvent reaches near the top, the plate is removed and dried.
- Spots are visualized under UV light or by spraying reagents.

Mobile Phase Composition (10 mL)

Commonly used ratio:

- Ethyl acetate: Glacial acetic acid: Formic acid: Water [10: 1.1: 1.1: 2.6]
- Ethyl acetate: Formic acid: Water [8: 1: 1]
- Chloroform: Methanol [8: 2]

Solvent	Ethyl acetate	Glacial acetic acid	Formic acid	Water
Volume	6.8 ml	0.75 ml	0.75	1.7ml

- Chloroform: Ethyl acetate: Formic acid [6: 3: 1]

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Calculation for 10 mL

Table no. 05: Composition of Mobile phase for Thin Layer Chromatography (TLC)

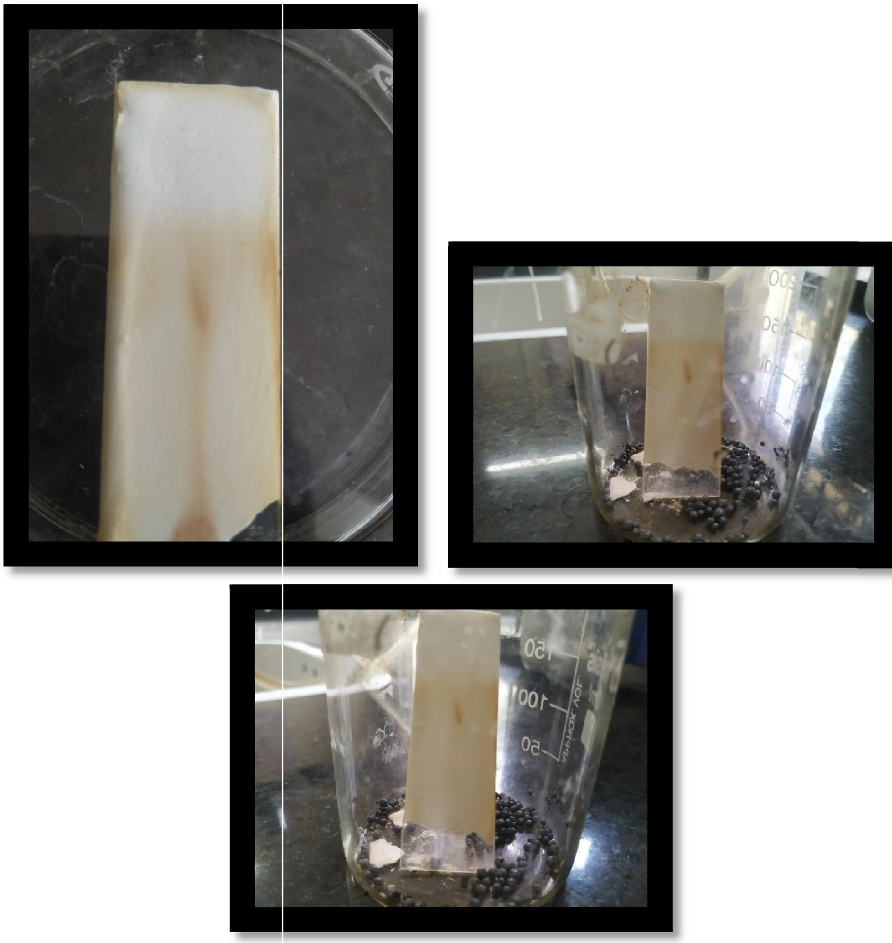

Fig No. 06: Thin Layer Chromatography (TLC)

- Calculation For Retention Factor:

Distance travelled by compound = 3.20

Distance travelled by solvent = 4.60

$$Rf = \frac{\{distance\ travelled\ by\ compound\}}{\{distance\ travelled\ by\ solvent\}} = \frac{3.20}{4.60} = 0.69$$

UV SPECTROSCOPY ANALYSIS:

- Centrifugation of sample:

Centrifugation operates on the principle that when a mixture is rotated at high speed, a centrifugal force is generated, causing denser particles to move outward and settle at the bottom of the centrifuge tube, while lighter soluble components remain in the supernatant. The rate of sedimentation depends on particle size, density difference between particles and solvent, and rotational speed (rpm).

The seed material of Manilkara zapota was first washed, shade-dried, and powdered. The 5g quantity of seed powder was extracted with distilled water under controlled heating conditions to obtain a crude aqueous extract containing both soluble phytochemicals and insoluble plant residues.

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After filtration of the crude extract, the solution was transferred into centrifuge tubes and subjected to centrifugation under the speed of 4000rpm for 20 minutes at room temperature Upon completion of centrifugation, the mixture was separated into two distinct phases:-

Pellet: containing insoluble plant debris, cell wall fragments, and coarse particulate matter and supernatant: containing soluble phytochemicals such as flavonoids, phenolics, tannins, and glycosides. The clear supernatant was carefully collected and used for UV-Visible spectroscopy.

Fig No.07: Process of centrifugation conducted.

- **UV-Visible spectroscopy:**

Ultraviolet–visible spectroscopy is a simple, rapid, an economical analytical technique widely used for the characterization and evaluation of herbal plants and plant-derived products. This method helps in the identification and authentication of phytochemical constituents based on their absorption and transmission of UV and visible light. The spectral pattern obtained from UV–Visible analysis provides important

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information regarding the purity and chemical composition of plant extracts. In addition, UV–Visible spectroscopy plays a significant role in phytochemical research, drug discovery, and toxicity assessment. The spectral data obtained can further assist in designing suitable methods for the isolation and purification of bioactive compounds from plant materials.

The aqueous extract of M. zapota seed was centrifugation at 3000 rpm for 10 minutes to remove insoluble particles and obtain a clear supernatant. The resulting extract was filtered using filter paper and further diluted in a ratio of 1:10 with the same solvent system. The prepared sample was analyzed using a UV-Visible spectrophotometer within the wavelength range of 200-400nm. Characteristic absorption peaks corresponding to flavonoid compounds were observed and recorded for phytochemical characterization of the extract.

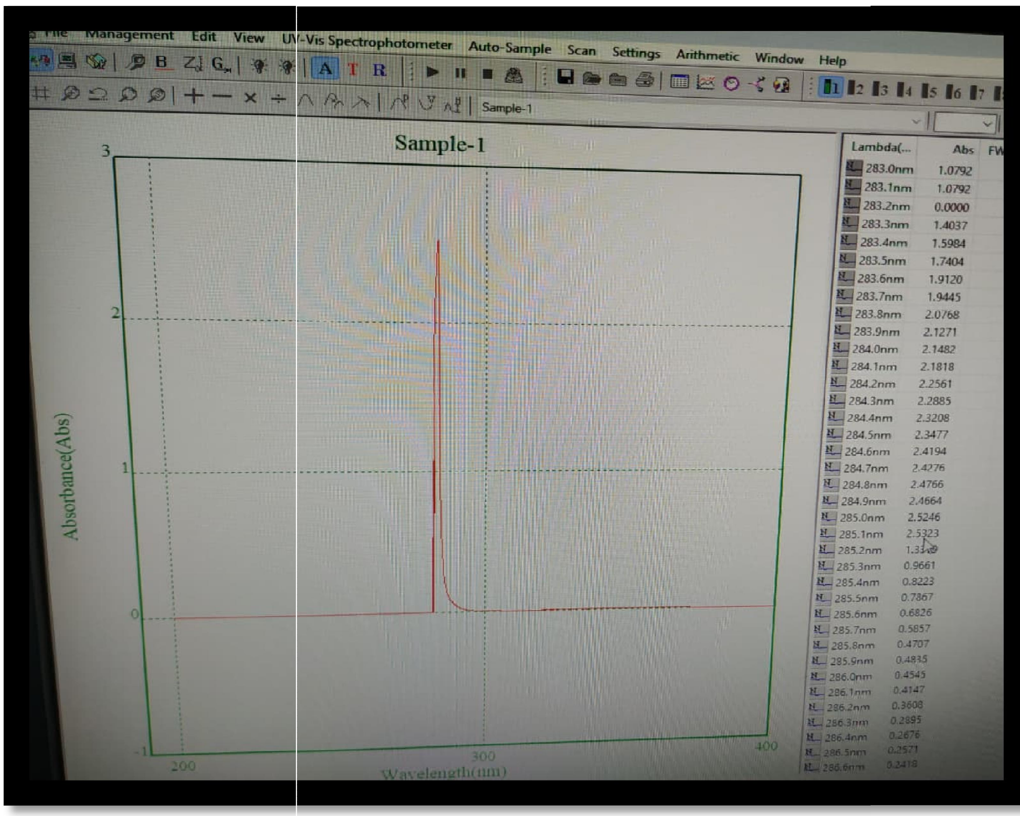

Fig No. 08: Absorbance peak of M. zapota.

Result & Discussion

- **Phytochemical Screening**:

Name of Phytochemical	Result
Hangers test (Alkaloid test)	PRESENT
Mayer’s Test (Alkaloid test)	ABSENT
Dragendorff’s Reagent Test (Alkaloid test)	PRESENT
Shinoda Test (Flavonoids test)	PRESENT
Alkaline reagent test (Flavonoids test)	PRESENT
Ferric Chloride Test (Tannin test)	ABSENT
Keller-killiani Test (Glycoside test)	PRESENT

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Saponins	PRESENT
Terpenoids	PRESENT

Table No. 06: Results of test conducted

The preliminary phytochemical screening of the seed extract showed the presence of alkaloids, flavonoids, glycosides, saponins, and terpenoids. Hager's test and Dragendorff's test confirmed the presence of alkaloids, while Mayer's test showed a negative result. Flavonoids were confirmed by Shinoda and alkaline reagent tests. Glycosides were detected by the Keller–Killiani test. Tannins were absent as indicated by the Ferric chloride test. Overall, the extract contains several important bioactive phytoconstituents which may contribute to its therapeutic potential.

- **Thin Layer Chromatography Analysis :**

The TLC analysis of the aqueous extract of *Manilkara zapota* seed was carried out using a mobile phase consisting of ethyl acetate, glacial acetic acid, formic acid, and water. A distinct and well-resolved spot was observed under UV light with an Rf value of 0.69.

The obtained Rf value was found to be close to the reported standard Rf range of flavonoids in similar solvent systems, which generally lies between 0.60–0.75 depending on the polarity of the mobile phase and stationary phase used. Therefore, the observed Rf value of 0.69 may indicate the presence of flavonoid or certain phenolic constituents in the extract. The selected solvent system provided satisfactory separation of phytochemical constituents on the silica gel stationary phase.

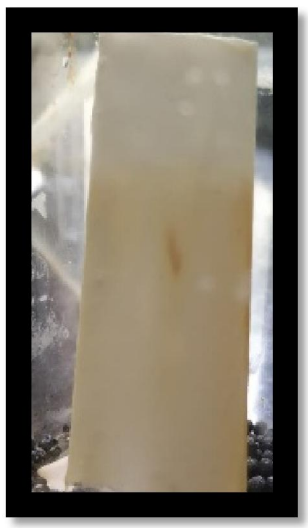

Fig no. 08: TLC plate showing spot with Rf value of 0.69

- **UV-visible spectrophotometric analysis**:

The UV spectrophotometric analysis revealed a major absorption peak at 285 nm. Compounds such as flavonoids, phenolic compounds, and tannins generally exhibit absorption maxima within the range of 270–290 nm due to the presence of aromatic rings and conjugated double-bond systems. Therefore, the observed absorption peak may suggest the presence of flavonoid and phenolic constituents in the aqueous extract of M. zapota seeds.

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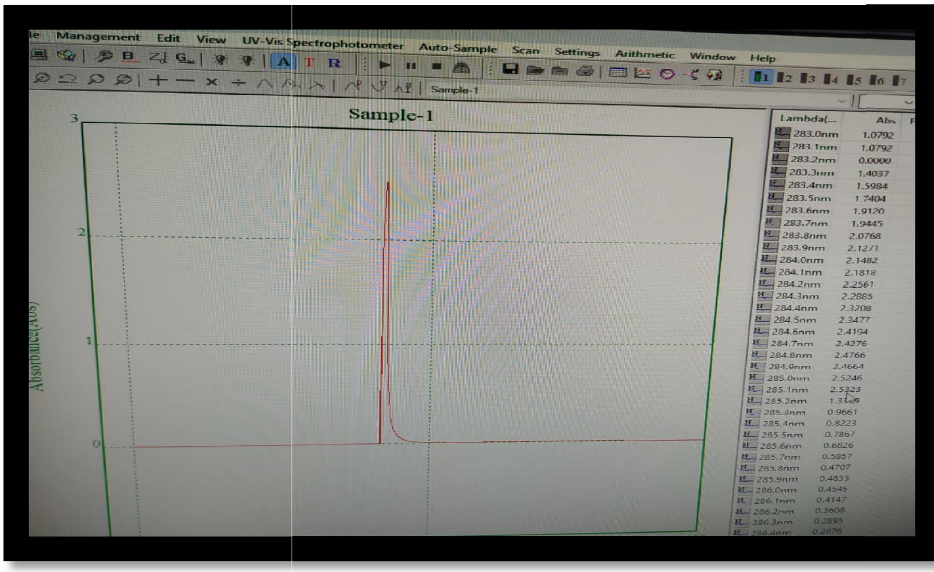

Fig No.08: - Largest absorbance shows at 285nm.

- Preliminary phytochemical screening demonstrated the presence of phenolics, flavonoids, tannins, glycosides, saponins, carbohydrates, proteins, and alkaloids in the aqueous extract. The strong reactions observed for phenolic compounds and tannins are consistent with previous studies reporting the abundance of polyphenolic constituents in sapodilla seeds. These phytochemicals are widely associated with antioxidant, antimicrobial, and anti-inflammatory activities.
- The findings of the present investigation suggest that aqueous extraction is effective for the isolation of polar phytochemicals from M. zapota seeds. Literature reports indicate that polar solvents such as water, methanol, and ethanol generally yield higher amounts of phenolic and flavonoid compounds, whereas non-polar solvents predominantly extract fixed oils and lipophilic constituents. The present results are in agreement with earlier studies demonstrating that M. zapota seeds are a promising source of bioactive phytochemicals with potential pharmaceutical and nutraceutical applications.
- Overall, the present study indicates that *Manilkara zapota* seeds represent a chemically rich and underexplored plant material. However, further investigations involving quantitative phytochemical estimation, advanced chromatographic characterization techniques such as HPLC, HPTLC, GC-MS, and isolation of active constituents are required to establish their complete phytochemical profile and therapeutic potential.

VII. SUMMARY AND CONCLUSION

Manilkara zapota seeds were cleaned, dried, powdered, and subjected to aqueous extraction using distilled water. The aqueous extract yielded polar phytochemicals including tannins, phenolics, flavonoid glycosides, saponins, and water-soluble proteins.

Preliminary phytochemical screening identifies the presence of these major bioactive groups.

Phytochemical screening studies revealed a variety of secondary metabolites: Phenolic acids (gallic acid derivatives, chlorogenic-like compounds), flavonoids (flavan-3-ols such as catechin-type compounds, condensed tannins), tannins (hydrolysable and condensed), saponins (foaming glycosides with mild antimicrobial activity), terpenoids (present in semi-polar fractions), alkaloids (detected in some methanolic extracts), fixed oils (dominant in nonpolar extracts). The abundance of phenolic constituents contributes directly to antioxidant potential, metal-chelating activity, and reducing power.

Aqueous extraction of M. zapota seeds is effective for isolating polar, biologically active phytochemicals. The phytochemical tests confirm that tannins, phenolic, and flavonoid, glycosides are the major constituents in the water extract. The extract demonstrates promising antioxidant and antimicrobial activity, supporting its potential medicinal relevance.

The extraction procedure, supported by phytochemical screening confirmed that water serves as an efficient solvent for isolating hydrophilic phytochemicals while minimizing the degradation of heat-labile constituents. The qualitative chemical tests-especially for phenolics (Ferric chloride test), tannins (lead acetate/gelatin test), and flavonoids (alkaline reagent test) – indicated the polyphenolic richness of the seeds. These findings align with earlier studies demonstrating strong antioxidant and phenolic profiles in M. zapota seed extracts.

Overall, aqueous extraction provides a suitable and environmentally friendly method for preliminary phytochemical investigation and chromatographic profiling of M. zapota seeds. The present investigation demonstrates that M. zapota seeds appears to be promising source of phytochemicals, especially phenolic compounds, flavonoids, tannins, saponins, and glycosidic compounds, which were successfully extracted using aqueous solvent. The aqueous extract exhibited a measurable presence of polar bioactive compounds, consistent with earlier reports that sapodilla seeds contain gallic acid derivatives, catechin-like flavonoids, and condensed tannins.

Based on the findings of the present investigation, it can be concluded that M. zapota seeds represent a promising and underutilized source of bioactive phytochemicals; however, further studies such as quantitative estimation, advanced chromatographic characterization (HPLC/HPTLC/GC–MS), isolation of active compounds, and detailed pharmacological and toxicological evaluations are required to fully establish their therapeutic potential and possible pharmaceutical or nutraceutical applications.

TLC analysis of aqueous extract of M. zapota seed was performed using silica gel TLC plate and a mobile phase containing ethyl acetate, glacial acetic acid, formic acid, and water. After chromatographic development, separated spots were observed under UV light. The calculated Rf value was found to be 0.69, suggesting the presence of phytochemical constituents such as flavonoids or phenolic compounds in the seed extract. The selected mobile phase showed satisfactory separation efficiency for the phytochemicals present in the extract.

Compounds such as flavonoids, phenolic compounds, and tannins generally exhibit absorption maxima in the range of 270–290 nm due to the presence of aromatic rings and conjugated double bond systems. Therefore, the observed peak at 285 nm may indicate the presence of flavonoids and phenolic constituents in the aqueous seed extract of M. zapota.

UV spectrophotometric analysis revealed an absorption peak at 285 nm. Compounds such as flavonoids, phenolic compounds, and tannins commonly exhibit absorption maxima within the range of 270–290 nm due to the presence of aromatic rings and conjugated double-bond systems. Therefore, the observed absorption peak may suggest the presence of flavonoid and phenolic constituents in the aqueous seed extract of M. zapota.

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