Manilkara sapota and Its Phytochemical Profile

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Article DOI Link: https://zenodo.org/uploads/17542190
DOI: 10.5281/zenodo.17542190

Abstract

Plants remain an indispensable source of food, nutrition, and traditional medicine, with *Manilkara zapota* (Sapotaceae) widely utilized for its therapeutic properties. The present study assessed the phytochemical composition of *M. zapota* fruits from two distinct sites of southern Tamil Nadu, India, through qualitative screening, quantitative estimation, and Gas Chromatography–Mass Spectroscopy (GC–MS) analysis. Among the solvents tested, ethanol yielded the highest diversity of metabolites, including alkaloids, flavonoids, phenols, saponins, amino acids, and proteins, while petroleum ether and chloroform extracts contained fewer constituents. Quantitative assays revealed that Site II fruits possessed significantly higher levels of phenols, flavonoids, and triterpenoids compared to Site I, suggesting environmental factors play a role in metabolite accumulation. GC–MS profiling further confirmed this variation, with 12 compounds identified in Site I and 25 in Site II. Key bioactive constituents such as n-hexadecanoic acid, β-amyrin, urs-12-en-3-ol acetate, camphene, and limonene are known for their antioxidant, antimicrobial, anti-inflammatory, and

cytoprotective activities. The findings emphasize the richness of M. zapota in secondary metabolites and validate its ethnomedicinal applications. Moreover, the influence of both solvent extraction and growth environment on phytochemical yield highlights the importance of standardization in plant-based drug research. Overall, this study provides scientific evidence supporting the pharmacological potential of *M. zapota* fruits and establishes a basis for future therapeutic explorations.

Keywords: *Manilkara zapota*, Sapotaceae, phytochemicals, secondary metabolites, GC–MS, pharmacological activity

Introduction

Plants are vital resources for food and medicine, providing essential nutrients and bioactive compounds that support human health. Approximately 80% of people in developing countries rely on traditional medicine as their primary healthcare system (Tran et al., 2020). Compared to synthetic drugs, plant-derived remedies often show greater therapeutic potential with fewer side effects. They are eco-friendly, biodegradable, renewable, and serve as valuable leads in modern drug discovery (Pandey et al., 2011; Forni et al., 2019).

Medicinal activity in plants is attributed to their diverse phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids (Okwu, 2001). These metabolites contribute to health promotion, economic benefits, and societal development. However, challenges such as insufficient scientific validation and limited funding hinder their wider application (Kamboj, 2000). Historically, many important drugs—including aspirin, digoxin, morphine, and quinine—originated from plants (Gilani & Rahman, 2005).

The Sapotaceae family (order: Ericales) comprises about 800 species of evergreen trees and shrubs distributed across tropical regions. Many members provide edible fruits, latex, and other economically important products. Among them, Manilkara zapota (commonly known as sapodilla, chikoo, or sapota) is widely cultivated in India, Mexico, and Southeast Asia (Fig .1). Traditionally, it has been used for wound healing, inflammation, fever, and gastrointestinal disorders. The fruit is rich in vitamins, fiber, and antioxidants, while its bark, latex, and seeds are employed in various therapeutic and cosmetic applications. Reported pharmacological activities include diuretic, analgesic, antispasmodic, antioxidant, antimicrobial, and anticancer effects. Its seed oil is valued in skincare and hair care formulations, while fruit pulp is beneficial for digestive

and respiratory health.



Fig .1 Manilkara zapota (L.) P. Royen

Plants such as *M. zapota* derive their medicinal value from secondary metabolites produced in response to environmental stresses. Unlike primary metabolites, these compounds are not directly involved in growth or reproduction but play vital roles in defense, pollinator attraction, and ecological adaptation (Korkina et al., 2018). They are classified into major groups such as terpenes, phenolics, alkaloids, and glycosides (Kumar & Pandey, 2013). Polyphenols, especially flavonoids, are abundant and exhibit antioxidant, anti-inflammatory, and antimicrobial activities (Nijveldt et al., 2001; Scalbert et al., 2000). Terpenes, the main constituents of essential oils, contribute to ecological interactions and pharmaceutical applications (Omar et al., 2016).

To identify and characterize these bioactive metabolites, Gas Chromatography–Mass Spectroscopy (GC-MS) is one of the most widely used analytical techniques. GC-MS enables both qualitative and quantitative analysis by separating, detecting, and identifying compounds based on their mass-to-charge ratios (Thomas et al., 2013). It has been applied extensively in phytochemical studies for structural elucidation, isotopic analysis, and compound quantification (Maher et al., 2015). Thus, GC-MS serves as a powerful tool for validating the phytochemical profile and therapeutic potential of *M. zapota*.

In this study, the phytochemical constituents of *M. zapota* fruit extracts collected from two different sites were analyzed using GC-MS, with the objective of identifying and comparing bioactive compounds that may contribute to its medicinal properties.

Methodology

Phytochemical Screening

Collection of Plant Material

Fresh fruits of *Manilkara zapota* L. were collected in February 2025. Two sampling sites were selected:

- Sample I: *M. zapota* plants growing at Murasancode in Kanyakumari District
- Sample II: *M. zapota* plants growing at Kudunulam in Tirunelveli District

Preparation of Plant Extracts (Parekh and Chanda, 2007)

Fresh fruits were collected, washed thoroughly, and air-dried in shade until completely free of moisture. The dried plant material was macerated using a mixer grinder and stored in airtight containers under refrigeration.

Ten grams of powdered fruit were extracted with 250 mL of petroleum ether, ethyl acetate, chloroform, and ethanol separately using a Soxhlet extractor for 8 hours at temperatures below the solvent boiling points. Extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuum at 40 °C using a rotary evaporator. Residues were stored in a freezer until further use.

Qualitative Phytochemical Screening

Each solvent extract was analyzed for different phytochemical constituents using the following methods:

- **Test for Reducing Sugars (Sadhasivam and Manickam, 1996)**

1 mL of filtrate was boiled with 1 mL each of Fehling's solutions A and B. A red precipitate indicated the presence of sugars.

- **Test for Alkaloids (Adegoke et al., 2010)**

0.4 g of extract was mixed with 8 mL of 1% HCl, warmed, and filtered. Filtrates were tested with (a) Mayer's reagent and (b) Dragendorff's reagent. Yellow precipitate (Mayer's) and red precipitate (Dragendorff's) indicated alkaloids.

- **Ninhydrin Test for Amino Acids (Yasuma and Ichikawa, 2000)**

Few drops of Ninhydrin reagent were added to extract and boiled for 2–3 minutes. A bluish-black color indicated amino acids.

- **Ferric Chloride Test for Flavonoids (Raman, 2006)**

Extracts treated with $FeCl_3$ showed blackish-red color, indicating flavonoids.

- **Test for Steroids (Kokate, 1994)**

5 mL extract was dissolved in chloroform and layered with concentrated H_2SO_4. A reddish-brown ring with green fluorescence indicated steroids.

- **Test for Tannins (Trease and Evans, 1989)**

Addition of $FeCl_3$ to extracts produced blue-black, green, or blue-green precipitates, confirming tannins.

- **Test for Terpenoids (Evans, 1997)**

5 mL extract was mixed with chloroform and layered with concentrated H_2SO_4. A

reddish-brown interface indicated terpenoids.

- **Ferric Chloride Test for Phenols (Mace, 1965)**

Extract (50 mg) in distilled water treated with $FeCl_3$ showed dark green color, indicating phenols.

- **Foam Test for Saponins (Kumar et al., 2009)**

Extract shaken with water produced persistent foam for 10 min, confirming saponins.

- **Borntrager's Test for Anthraquinones (Sofowora, 1993; Harborne, 1984)**

Extract treated with chloroform and ammonia produced a bright pink aqueous layer, indicating anthraquinones.

- **Biuret Test for Proteins (Gahan et al., 1984)**

Filtrate treated with $CuSO_4$ and KOH produced a pink color, confirming proteins.

- **Catechin Test (Rinaldo et al., 2010)**

Matchstick dipped in aqueous extract, dried, moistened with HCl, and warmed turned pink/red, indicating catechins.

- **Sugar Test (Sadhasivam and Manickam, 1996)**

Test solution with anthrone and concentrated H_2SO_4 heated to produce green–purple color confirmed sugars.

Quantitative Determination of Non-Enzymatic Antioxidants

- **Total Flavonoids (Naima Saeed et al., 2012)**

Determined using aluminium chloride method with catechin standard. Absorbance measured at 510 nm.

- **Total Phenols (Naima Saeed et al., 2012)**

Measured using Folin–Ciocalteu's reagent and sodium carbonate. Absorbance read at 750 nm.

- **Total Terpenoids (Ghorai et al., 2012)**

Extracts treated with chloroform, H_2SO_4, and methanol. Absorbance measured at 538 nm.

- **GC–MS Analysis (Bagavathi and Ramasamy, 2012)**

The ethanolic extract of M. zapota was analysed using a Perkin-Elmer GC Clarus 500 system equipped with an AOC-201 autosampler and a mass spectrometer. An Elite 5MS fused silica capillary column (30 m × 0.25 mm ID × 0.25 μm df) was employed for the analysis. Helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min, and the injection volume was 2 μL. The injector temperature was maintained at 200 °C, while the ion source temperature was also set at 200 °C. The oven was programmed initially at 110 °C with a 2 min

isothermal hold, followed by a temperature ramp of 10 °C/min up to 200 °C, and then 5 °C/min up to 280 °C, where it was held for 9 min. Mass detection was carried out at 70 eV with a scan range of 45–450 Da and a scan interval of 0.5 s. Data acquisition and processing were performed using Turbo Mass Ver-5.2 software, and the relative abundance of compounds was calculated by peak area normalization.

Results

Phytochemical Screening

Bioactive plant products are gaining increasing attention worldwide, and several pharmacological effects are being investigated for their phytochemical constituents. Manilkara zapota, a member of the Sapotaceae family comprising nearly 65 genera and about 800 species, is recognized for its nutritional and therapeutic properties. Sapota fruit is rich in glucose and calories, serving as a good source of energy. In addition, antioxidants such as ascorbic acid, polyphenols, and flavonoids contribute to skin health by delaying premature ageing, while minerals including calcium, phosphorus, copper, selenium, magnesium, and iron strengthen bones, alleviate joint discomfort, and reduce the risk of osteoporosis.

Qualitative Analysis of Secondary Metabolites

The secondary metabolites of *M. zapota* fruits grown at selected sites demonstrated variation in their availability across different solvent extracts. Petroleum ether and chloroform extracts showed similar profiles, with petroleum ether extracts confirming the presence of steroids and reducing sugars, while chloroform extracts revealed steroids, reducing sugars, and sugars. Ethanolic extracts exhibited a wide range of metabolites, including steroids, triterpenoids, reducing sugars, phenolic groups, proteins, alkaloids, flavonoids, tannins, saponins, amino acids, and sugars.

The aqueous extract of fruits from site I plants contained triterpenoids, reducing sugars, phenolic groups, flavonoids, tannins, and sugars. A similar pattern was observed in fruits from site II. Ethyl acetate extracts from site I and site II revealed the presence of steroids, reducing sugars, tannins, saponins, and sugars.

Conversely, certain metabolites were absent in different extracts. Steroids, proteins, alkaloids, catechins, anthraquinones, and amino acids were not detected in the aqueous extracts from both sites. Petroleum ether extracts lacked triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, tannins, anthraquinones, saponins, amino acids, and sugars. Chloroform extracts showed

the absence of triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, catechins, tannins, anthraquinones, saponins, and amino acids. Ethanolic extracts did not contain catechins or anthraquinones, while ethyl acetate extracts from both sites lacked triterpenoids, phenolic groups, proteins, alkaloids, flavonoids, catechins, anthraquinones, and amino acids.

Quantitative Analysis of Secondary Metabolites

The quantitative analysis of aqueous and ethanolic fruit extracts of *M. zapota* from the selected sites was carried out for total phenols, flavonoids, and triterpenoids. The ethanol extracts consistently showed higher levels of these secondary metabolites compared to aqueous extracts.

In the ethanolic extract of fruits from site II plants exhibited significantly elevated metabolite concentrations compared to fruits from plants of site I. Phenol content was 90.1 ± 0.017 mg/100 g dry wt. in the site II plant fruit extract, while only 37 ± 0.01 mg/100 g dry wt. was observed in the fruits from plants of site I. Similarly, flavonoid levels were 106 ± 0.012 mg/100 g dry wt.in the extract of fruits from site II, nearly double that of extract of fruits from site I plants (45.3 ± 0.021 mg/100 g dry wt.). Triterpenoid content was 61.9 ± 0.03 mg/100 g dry wt. at site II, slightly higher than site I (57.8 ± 0.016 mg/100 g dry wt.).

In aqueous extracts, phenolic content was 22.65 ± 0.043 mg/100 g dry wt. at site I and 27.85 ± 0.051 mg/100 g dry wt. at site II. Flavonoid concentration was 25.7 ± 0.01 mg/100 g dry wt. at site I, increasing to 39.3 ± 0.031 mg/100 g dry wt. at site II. Triterpenoids were 44.7 ± 0.003 mg/100 g dry wt. at site I and 49.9 ± 0.02 mg/100 g dry wt. at site II. Analysis of variance revealed statistical significance with a P value of 0.0201 at the 0.05% level.

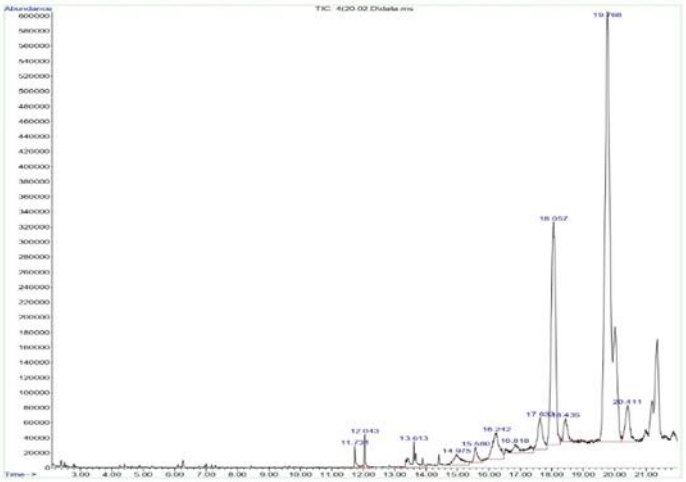
GC–MS Analysis

The ethanolic fruit extract of *M. zapota* was analyzed using gas chromatography–mass spectrometry (GC–MS). The extract from site I revealed the presence of 12 distinct compounds (Fig .2). Structural identification was carried out by comparing mass spectra with reference data in Turbo Mass Ver-5.2 software. The identified compounds included n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, 2(1H)-Naphthalenone octahydro-4a-methyl-7-(1-methylethyl)-(4a.alpha.,7beta.,8a.beta.), Pyrene hexadecahydro-, Tetratetracontane, 1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-, 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine, 2H-3,9a-Methano-1-benzoxepin octahydro-2,2,5a,9-tetramethyl- [3R-

(3alpha.,5a.alpha.,9.alpha.,9a.alpha.)], β-Amyrin, 2-Ethylacridine, Urs-12-en-3-ol acetate (3β) [or α-Amyrin acetate], and Lanosta-8,24-dien-3-ol acetate (3β).

Among these, Urs-12-en-3-ol acetate (3β) or α-Amyrin acetate exhibited the highest peak area (56.45%) with a retention time (Rt) of 19.768 minutes, indicating its dominance in the extract. In contrast, n-Hexadecanoic acid had the lowest peak area (0.59%) at an Rt of 11.73 minutes.

Fig. 2 GC-MS Profile of M. zapota fruits from site I plants



In contrast, the ethanolic fruit extract of plants from site II revealed a more diverse phytochemical profile, with 25 compounds eluted at different retention times (Fig .3). Compound identification was confirmed using the NIST library. The detected compounds included 1-Octanol, 2-butyl; Camphene; Tridecane; Hexadecane; 1,2-Pentadiene; (E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene; trans,cis-2,6-Nonadien-1-ol; 3-Ethyl-3-methylheptane; Bicyclo[4.3.0]nonane, 3-methylene; 2-Propyn-1-amine, N-methyl; 7-Oxabicyclo[4.1.0]heptane, 3-oxiranyl; 1,9-Nonanediol, dimethanesulfonate; Methyl 2,3-di-O-acetyl-4,6-di-O-methyl-α-D-mannopyranoside; n-Hexadecanoic acid; 2H-1-Benzopyran-2-one, 5,7-dimethoxy; 9,17-Octadecadienal,(Z); 7H-Furo[3,2-g][1]benzopyran-7-one, 4,9-dimethoxy-; [1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester; 1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione; Silicic acid, diethyl bis(trimethylsilyl) ester; 2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene; 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine; 1,2-Bis(trimethylsilyl)benzene; Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl; and 2-Ethylacridine.

The compound 2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene (21st

compound) exhibited the highest peak area (27.19%) at an Rt of 16.516 minutes. In contrast, Bicyclo[4.3.0] nonane, 3-methylene (9th compound) displayed the lowest peak area (0.22%) with an Rt of 8.526 minutes.

Fig. 3 GC-MS Profile of M. zapota fruits from site II plants



Discussions

The phytochemical screening of *Manilkara zapota* fruits extracted with different solvents revealed notable variations. Petroleum ether extracts contained only steroids and reducing sugars, while chloroform extracts showed steroids, reducing sugars, and sugars. Ethyl acetate extracts included tannins, and ethanolic extracts exhibited the richest phytochemical profile, containing steroids, triterpenoids, alkaloids, flavonoids, phenols, saponins, amino acids, and proteins. Similar findings of glycosides, phenolics, and terpenoids were reported by Mahajan and Badgujar (2008). The therapeutic activities of these compounds, including antioxidant, anti-inflammatory, antibacterial, and protective roles, are well documented (Okwu, 2001; Waqas-Ahmed et al., 2016; Williams et al., 2004).

Quantitative analysis showed higher concentrations of phenols, flavonoids, and triterpenoids in fruits from Site II compared to Site I, likely due to environmental stress factors (Akula & Ravishankar, 2011). Previous reports also confirm site-dependent variation in phytochemical content (Sebaa & Cherifi, 2021; Singh et al., 2016). Members of the Sapotaceae family are rich in secondary metabolites such as catechins, quercetin derivatives, and phenolic acids, which contribute to diverse pharmacological activities (Baky et al., 2016).

GC–MS analysis confirmed this variability, with Site I extracts yielding 12 compounds, while Site II extracts yielded 25 compounds. Key identified compounds included n-hexadecanoic acid, β-amyrin, urs-12-en-3-ol acetate, camphene, limonene, and tridecane, many of which possess antimicrobial, antioxidant, anti-inflammatory, and cytoprotective properties (Aparna et al., 2012; Pinto et al., 2008; Okoye et al., 2014; Benelli et al., 2018). The greater diversity of compounds at Site II supports the role of abiotic stress in enhancing metabolite production (Muthulakshmi et al., 2012; Kumar et al., 2014).

Overall, the present study confirms that *M. zapota* fruits are a rich source of bioactive phytochemicals, with variations in secondary metabolite content influenced by extraction solvent and environmental conditions. These findings highlight the pharmacological potential of M. zapota and justify further research into the therapeutic applications of its active compounds.

Conclusion

The present investigation highlights the phytochemical richness and pharmacological potential of *Manilkara zapota* fruits. Ethanolic extracts demonstrated the greatest diversity of secondary metabolites, while quantitative analysis revealed significant site-dependent variations, with fruits from Site II containing higher concentrations of phenols, flavonoids, and triterpenoids. GC–MS analysis further confirmed the variability in compound diversity and identified several bioactive metabolites with well-established therapeutic properties.

These results reinforce the role of *M. zapota* as a valuable medicinal plant within the Sapotaceae family and provide a scientific basis for its traditional applications. Moreover, the observed variation in phytochemical composition emphasizes the influence of environmental conditions on metabolite accumulation. Collectively, the findings suggest that *M. zapota* holds strong potential for future pharmacological, nutraceutical, and cosmetic applications, warranting further studies for bioactivity validation and drug development.

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