



Research Article

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Qualitative and quantitative phytochemical analysis of methanolic extract of *Manilkara zapota* leaves

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ABSTRACT

Background: Medicinal plants are valuable sources of structurally diverse bioactive compounds that contribute significantly to drug discovery and development. *Manilkara zapota* has been traditionally used for various therapeutic purposes; however, comprehensive chemical characterization of its leaf extract remains limited. **Objective:** To preliminary phytochemical screening to determine the main classes of secondary metabolites in the extract, along with the determination of predominant functional groups by FTIR spectroscopy and the identification of bioactive compounds using GC-MS to explore the chemical composition of extract. **Materials and Methods:** Shade-dried leaves of *M. zapota* were extracted with methanol using a Soxhlet apparatus. Co-extracted latex constituents were removed by acid-induced coagulation. Preliminary phytochemical screening was performed on both latex-removed and untreated extracts. FTIR spectroscopy was employed to identify major functional groups, while GC-MS analysis was carried out to characterise bioactive constituents. **Results:** Qualitative screening revealed the presence of steroids, terpenoids, alkaloids, tannins, phenolic compounds, flavonoids and saponins. FTIR analysis confirmed the presence of functional groups corresponding to alcohols, alkanes, conjugated alkenes and esters. GC-MS analysis identified several bioactive compounds, including glycopeptide derivatives, phenolic compounds, fatty acid esters, monoglycerides and terpenoid constituents. **Conclusion:** The findings demonstrate that *M. zapota* leaves are rich in diverse phytochemicals, supporting their potential therapeutic applications.

Keywords: *Manilkara zapota*, Phytochemical Screening, Methanolic Extract, FTIR Spectroscopy, GC-MS Analysis, Bioactive Compounds.

INTRODUCTION

Plants produce a wide array of bioactive compounds commonly referred to as phytochemicals, for their survival during adverse conditions [1]. Most bioactive compounds reside in plant secondary metabolites and have played a crucial role in the development of modern medicines [2]. Based on the pathways through which they are biosynthesized, secondary metabolites are broadly classified into three major groups such as phenolic compounds, terpenes and steroids and alkaloids [3]. These bioactive substances exhibit a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, anticancer, neuroprotective, immunomodulatory and cardioprotective effects [4]. Identification of such phytochemical constituents present in plant materials helps in understanding their potential therapeutic effects and provides direction for further pharmacological studies [5].

M. zapota (L.) P. Royen, commonly known as sapedilla (chikoo or sapota) and formerly as *Achras sapota* L., belongs to the family Sapotaceae [6]. Leaf extracts of *M. zapota* have been extensively investigated and reported to exhibit a broad spectrum of pharmacological activities, including antioxidant and hepatoprotective effects [7], antimicrobial activity [8], antitumour potential [9], antidiabetic and antilipidaemic properties [10], antidiarrhoeal activity [11], as well as anti-inflammatory and anti-arthritis effects [12]. Nevertheless, detailed chemical characterisation of the leaf constituents using advanced analytical tools remains insufficient.

The present study was therefore designed to systematically profile the phytochemical constituents of methanolic leaf extract of *M. zapota* using qualitative assays in conjunction with FTIR and GC-MS techniques.

MATERIAL AND METHODS

Plant material collection and authentication

Fresh leaves of *M. zapota* were collected in April 2025 from Mannuthy, Thrissur, Kerala, India (10.5306° N, 76.2589° E). Taxonomical authentication was done by the Research and Postgraduate Department of Botany, St. Thomas College (Autonomous), Thrissur, Kerala and a voucher specimen was deposited in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary and Animal Sciences (CVAS), Mannuthy for reference.

Preparation of methanolic extract

The collected leaves were shade-dried, pulverised and extracted with 99% methanol using a Soxhlet apparatus at 67 °C. The co-extracted polymeric latex constituents in the methanolic leaf extract were removed by acid-induced coagulation with 0.5 N HCl, followed by filtration. The filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator, neutralized with sodium bicarbonate and the resulting salts removed by decantation. Residual solvent was evaporated at room temperature.

Preliminary phytochemical screening

The methanolic extract of *M. zapota* leaves (acid treated and untreated extract) was subjected to phytochemical screening to qualitatively assess the presence of major classes of bioactive constituents like steroids, triterpenes, diterpenes, alkaloids, glycosides, tannins, phenolic compounds, flavonoids and saponins by following standard protocols [13-15].

FTIR spectroscopy

FTIR Analysis was performed to characterize the functional groups in the plant extract using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy with a diamond crystal (Spectrum Two™, Perkin-Elmer Ltd., Singapore). A small quantity of dried plant extract was placed on the ATR crystal and pressed uniformly using the built-in pressure arm to ensure optimal contact of sample. Background spectra were recorded prior to analysis and the sample spectra were obtained in absorbance mode over the range of 4000-400 cm⁻¹. The functional groups were assigned by comparing the position (wavenumber, cm⁻¹), shape and intensity of absorbance peaks with reference spectra from the FTIR Functional Group Database Table with Search (InstaNANO) [16].

GC-MS analysis

The leaf extract was analyzed using a Gas chromatography (Trace 1300) coupled to a tandem mass spectrometer (Thermo Scientific TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS) at the Central Instruments Laboratory (CIL), CVAS, Mannuthy, Kerala. The extract was dissolved in methanol and filtered through a 0.22 µm syringe filter, injected (1 µL) into a TSQ-2MS capillary column (30 m × 0.25 mm, 0.25 µm film), with helium as the carrier gas at 1 mL/min. The oven was initially set at 80 °C for 2 min, then increased to 150 °C at 15 °C/min and held for 1 min and finally increased to 250 °C at 10 °C/min and held for 5 min, with a total run time of 35.45 min. Mass spectrometry was performed in electron impact mode at 70 eV, scanning m/z 50-500 and compounds were identified by comparing obtained mass spectra using the NIST MS Search 2.0 library.

RESULTS

Qualitative phytochemical analysis

Qualitative phytochemical screening (Table 1) of the latex-removed methanolic extract and untreated methanolic extract of *M. zapota* leaves confirmed the presence steroids, triterpenoids, diterpenoids,

alkaloids, glycosides, tannins, phenolic compounds, flavonoids and saponins.

FTIR analysis

ATR-FTIR analysis of the latex-removed methanolic extract confirmed the presence of key functional groups (Table 2, Figure 1). Hydrogen-bonded O-H stretching indicated alcohols or phenols, while C-H stretching and bending confirmed alkane groups. C=C stretching suggested the presence of conjugated alkenes or aromatic rings. Strong C-O stretching vibrations indicated the presence of esters, ethers and primary alcohols.

GC-MS analysis

GC-MS analysis of extract revealed several phytochemicals (Table 3, Figure 2) of which the major compounds were methyl N-(N-benzyloxycarbonyl-β-L-aspartyl)-β-D-glucosaminide (3.06%), 2,6-bis(1,1-dimethylethyl) phenol (2.85%), 9-octadecenoic acid derivatives (2.11%), 1-monolinoleoylglycerol derivatives (1.88%), isolongifolene, 7,8-dehydro-8a-hydroxy- (1.15%), longifolenaldehyde (1.15%).

DISCUSSION

A previous study reported the presence of alkaloids, flavonoids, tannins, saponins, and phytosterols in the methanolic extract of *M. zapota* leaves, while these metabolites were absent in the chloroform extract [17]. Similarly, another study demonstrated that methanolic leaf extracts contained tannins, alkaloids, steroids and glycosides in comparison to acetone extracts [18]. These findings closely aligned with the phytochemical profile observed in the present study.

FTIR analysis in the present study revealed a broad absorption band in the range of 3200-3550 cm⁻¹, indicative of O-H stretching vibrations corresponding to alcohol functional groups. A similar broad absorption band has been previously reported in the methanolic leaf extract of *Manilkara zapota*. The characteristic peaks corresponding to O-H stretching (alcohols), C-O stretching (primary alcohols), C-H stretching (alkanes) and C=C stretching (conjugated alkenes) observed in the present study were in agreement with earlier reports on the functional group profile of methanolic and ethanolic extracts of *M. zapota* leaf [17,19]. Among the phytochemicals identified by GCMS analysis in the present study, Phenol, 2,6-bis(1,1-dimethylethyl) has been previously reported in the acetone extract of *M. zapota* seeds [20]. 9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, 2,6-bis(1,1-dimethylethyl) a flavonoid, has been reported in the methanol:water fraction of *Manilkara hexandra* stem bark [21]. Additionally, 9-Octadecenoic acid (Z)-, methyl ester and 9,12-Octadecadienoic acid (Z, Z)-, methyl ester was earlier identified in the methanolic extract of *M. zapota* seeds [22]. 4-Hydroxy-β-ionone and 1,2-Benzenedicarboxylic acid has been reported previously in GCMS analysis of ethanolic leaf extracts of *M. zapota* [19].

Phytochemicals identified in the present study have been previously reported to exhibit a wide range of pharmacological activities. N-(N-benzyloxycarbonyl-β-L-aspartyl)-β-D-glucosaminide, the compound found at the highest concentration in the present study has been reported with significant antioxidant and neuroprotective effects and has been suggested as a potential depressant candidate for the management of Alzheimer's disease [23]. Phenol, 2,6-bis(1,1-dimethylethyl) was reported with anti-inflammatory, antioxidant, antimicrobial and antidiabetic activities [24-26]. 1-Monolinoleoylglycerol trimethylsilyl ether has been identified as a promising bioactive compound exhibiting potent antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthmatic, and diuretic properties [27,28]. 1,2-Benzenedicarboxylic acid was reported to have antioxidant and antimicrobial activities [29]. Collectively, the presence of these bioactive compounds supports the pharmacological relevance of the phytochemicals identified in the present study.

Table 1: Results of qualitative screening of secondary metabolites in the methanolic leaf extract of *Manilkara zapota*

Phytochemical class	Test	Result	
		Pretreated methanolic extract	Untreated methanolic extract
Steroids	Salkowski's test	+	+
	Liebermann's test	+	+
Triterpenoids	Salkowski's test	+	+
	Liebermann's test	+	+
Diterpenoids	Copper acetate test	+	+
Alkaloids	Dragendorff's test	+	+
	Mayer's test	-	-
	Wagner's test	+	+
	Hager's test	+	+
Glycosides	Keller-Kiliani test	+	+
	Concentrated H ₂ SO ₄ test	+	+
Tannic and phenolic compounds	Ferric chloride test	+	+
Flavonoids	Lead acetate test	+	+
Saponins	Foam test	+	+ *
+ indicates presence; - indicates absence; * formation of less foam compared to untreated methanolic extract			

Table 2: Functional groups identified in *M. zapota* leaves extract by FTIR

Absorption (cm ⁻¹)	Appearance	Absorption peak range (cm ⁻¹)	Group	Compound Class
3302	Strong, broad	3200-3550	O-H stretching (hydrogen-bonded)	Alcohol/ Phenols
2924.38	Medium	2840-3000	C-H stretching	Alkanes
1607.00	Medium	1600-1650	C=C stretching	Conjugated alkene/ Aromatic ring
1445.64	Medium	1450	C-H bending	Alkane
1204.88	Strong	1163-1210	C-O stretching	Esters/Ethers
1040.01	Strong	1050-1020	C-O stretching	Primary alcohol
510.88, 463.19	Strong, broad	400-600	M-O stretching	Metal-oxygen (inorganic) probably arising from metal-coordinated biomolecules such as chlorophyll.

Table 3: List of Phytochemical Constituents Identified in *M. zapota* Leaf Extract Using GC-MS

S. No.	RT (min)	Compound identified	Peak area (%)	Molecular formula	Molecular weight
1	5.08	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans-	2.11	C ₂₈ H ₄₄ O ₄	444
2	5.08	9-Octadecenoic acid (Z)-, phenylmethyl ester	2.11	C ₂₅ H ₄₀ O ₂	372
3	5.52	Methyl N-(N-benzyloxycarbonyl-beta-l-aspartyl)-beta-d-glucosaminide	3.06	C ₁₉ H ₂₆ N ₂ O ₁₀	442
4	5.82	Columbin	0.15	C ₂₀ H ₂₂ O ₆	358
5	5.94	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.33	C ₁₄ H ₂₂ O	206
6	6.27	(-)-Myrtenol, tert-butyldimethylsilyl ether	0.16	C ₁₆ H ₃₀ OSi	266
7	6.76	5,7-Dodecadiyn-1,12-diol	0.22	C ₁₂ H ₁₈ O ₂	194
8	6.91	4-Hydroxy-β-ionone	0.11	C ₁₃ H ₂₀ O ₂	208
9	7.21	Cubedol	0.19	C ₁₅ H ₂₆ O	222

10	8.31	Epoxyastaphiaton	0.15	C ₁₅ H ₁₈ O ₄	262
11	8.65	4,6,10,10-Tetramethyl-5-oxatricyclo [4.4.0.0(1,4)] dec-2-en-7-ol	0.40	C ₁₃ H ₂₀ O ₂	208
12	8.78	1-Buten-3-one, 1-(2-carboxy-4,4-dimethylcyclobutenyl)-	0.41	C ₁₁ H ₁₄ O ₃	194
13	8.78	Cyclopenta[1,3] cyclopropa [1,2] cyclohepten-3(3aH)-one	0.41	C ₁₃ H ₁₈ O	190
14	8.86	Methyl 2,4-tridecadienoate	0.25	C ₁₄ H ₂₀ O ₂	220
15	9.96	Dasycarpidan-1-methanol, acetate (ester)	0.13	C ₂₀ H ₂₆ N ₂ O ₂	326
16	22.35	Phenol, 2,6-bis(1,1-dimethylethyl)-	2.85	C ₁₄ H ₂₂ O	206
17	23.83	Diethyl phthalate	1.13	C ₁₂ H ₁₄ O ₄	222
18	26.29	2-(2,6,6-Trimethylcyclohex-1-enyl) cyclopropanecarboxylic acid, methyl ester	0.43	C ₁₄ H ₂₂ O ₂	222
19	27.39	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	1.15	C ₁₇ H ₂₄ O ₃	276
20	27.39	7-Tetracyclo [6.2.1.0(3,8)0(3,9)] undecanol, 4,4,11,11-tetramethyl-	1.15	C ₁₅ H ₂₄ O	220
21	27.39	Isolongifolene, 7,8-dehydro-8a-hydroxy-	1.15	C ₁₅ H ₂₄ O	220
22	27.39	Longifolinaldehyde	1.15	C ₁₅ H ₂₄ O	220
23	27.76	1,2-Benzenedicarboxylic acid	0.71	C ₆ H ₄ (COOH) ₂	166
24	27.76	Tricyclo [5.4.3.0(1,8)] tetradecan-6-one, 4-ethenyl-3-hydroxy-2,4,7,14-tetramethyl	0.71	C ₂₀ H ₃₂ O ₂	304
25	27.76	2-Methyl-4-tert-octylphenol	0.71	C ₁₅ H ₂₄ O	220
26	28.76	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl] cyclohexan-1-perhydrol	0.74	C ₁₄ H ₂₄ O ₄	256
27	29.33	N-[5-(3-Hydroxy-2-methylpropenyl)-1,3,4,5-tetrahydrobenzo[cd]indol-3-yl]-N-methylacetamide	0.72	C ₁₈ H ₂₂ N ₂ O ₂	298
28	29.33	1-Propyl-3,6-diazahomoadamantan-9-ol	0.72	C ₁₂ H ₂₂ N ₂ O	210
29	29.92	α-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate	0.57	C ₁₇ H ₇ BO ₄ Si ₂	404
30	30.77	2-Monolinolenin, 2TMS derivative	1.12	C ₂₇ H ₅₂ O ₄ Si ₂	496
31	31.05	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	1.10	C ₁₄ H ₄₂ O ₆ Si ₇	503
32	33.28	1-Monolinoleoylglycerol trimethylsilyl ether	1.88	C ₂₇ H ₅₄ O ₄ Si ₂	498

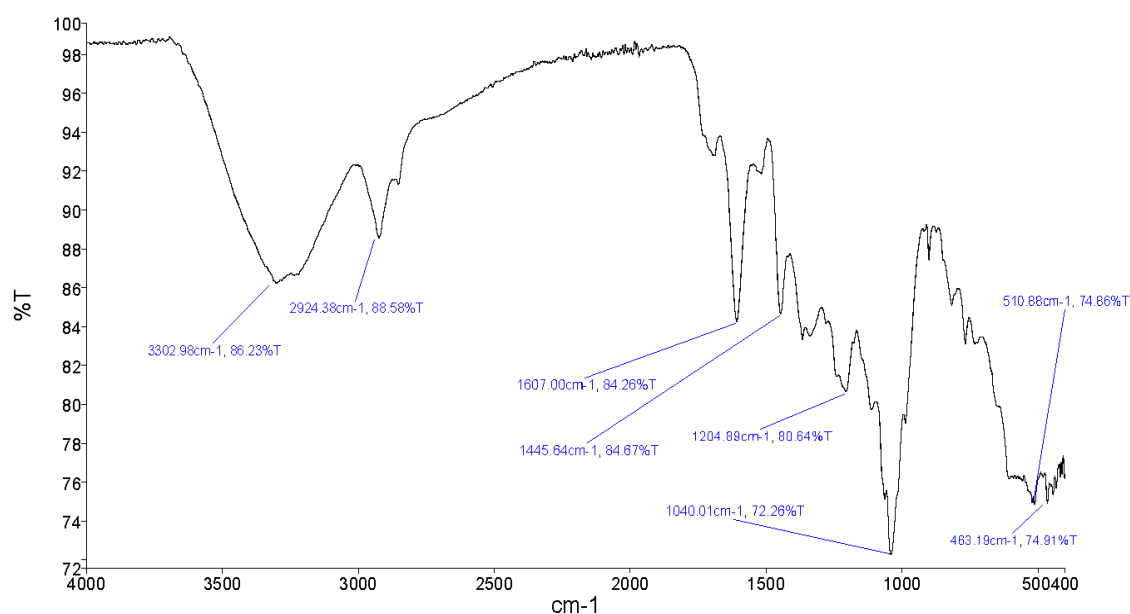


Figure 1: FTIR spectra of methanolic extract of *M. zapota* leaves

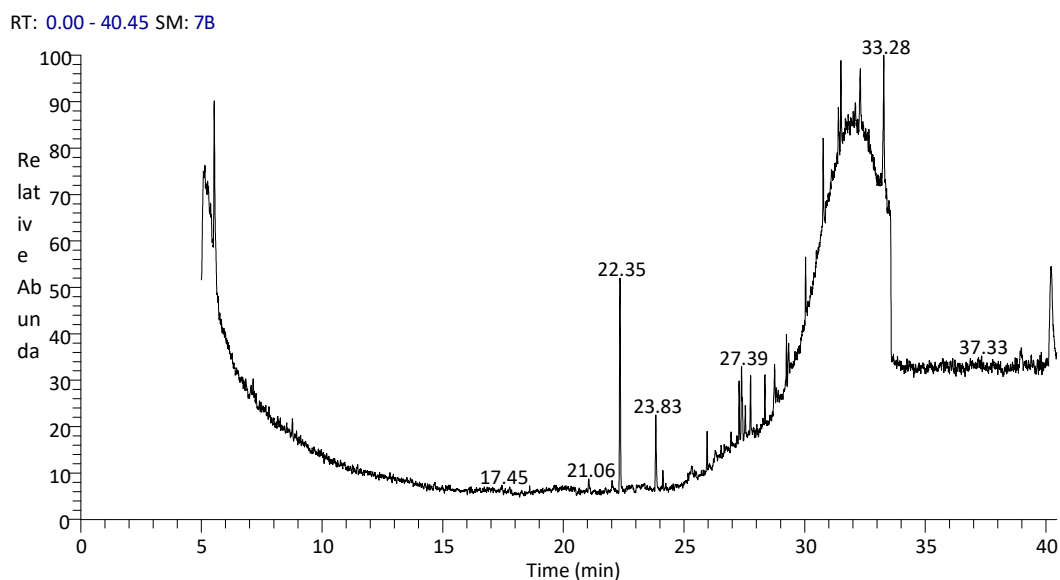


Figure 2: Gas chromatography-tandem mass spectrometry chromatogram of methanolic extract of *M. zapota* leaves

CONCLUSION

The present study provides a comprehensive phytochemical profile of *M. zapota* leaf extract through preliminary screening, FTIR, and GC-MS analyses. The results confirmed the presence of multiple classes of secondary metabolites, along with diverse functional groups indicative of alcohols, alkanes, alkenes, and esters. GC-MS analysis further identified key bioactive constituents such as glycopeptide derivatives, phenolic compounds, monoglycerides, fatty acid esters and terpenoids. Overall, the findings support the chemical richness of *M. zapota* leaves and provide a scientific basis for their reported pharmacological activities, highlighting their potential for further bioactivity-guided and mechanistic studies.

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Conflict of interest

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