Antioxidant, antibacterial, anti-inflammatory, and anticancer activities of the methanol extract from *Manilkara zapota* leaves collected in Vietnam

Nguyen Le Thanh Tuyen[1], Ly Gia Han[1], Le Thi Kim Anh[2], Tran Gia Buu[1*]

[1]Ton Duc Thang University, Ho Chi Minh City, Vietnam
[2]University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, Vietnam
[*]Corresponding author: trangiabuu@tdtu.edu.vn

ARTICLE INFO

DOI:10.46223/HCMCOUJS.tech.en.16.1.4489.2026

Received: June 18th, 2025

Revised: August 06th, 2025

Accepted: August 28th, 2025

Keywords:

antioxidant effect; antibacterial effect; anti-inflammatory potential; anticancer effect; Manilkara zapota

ABSTRACT

Manilkara zapota, a member of the Sapotaceae family, is a well-known and widely distributed plant with various applications in traditional medicine. However, the number of studies about the bioactivities of the leaf extract from this species in Vietnam is limited. This study showed that the methanol leaf extract of *M. zapota* contained relatively high contents of antioxidant compounds, including polyphenols and flavonoids (197.48 ± 2.08mg GAE/g extract and 39.04 ± 0.36mg QE/g extract, respectively). The extract exhibited high antioxidant properties, demonstrated by its potent free radical scavenging activity (IC_{50} of DPPH assay: 2.71 ± 0.11 μg/mL) and its high total antioxidant capacity (218.91 ± 7.84mg AAE/g extract). Additionally, the extract showed a practical anti-bacterial effect against *Bacillus cereus*, *Staphylococcus aureus*, and *Shigella boydii*, with their inhibitory halos ranging from 8.00 - 11.33mm. The extract also exhibited a significant anti-inflammatory potential (IC_{50}: 0.107 ± 0.008 mg/mL). Furthermore, its anti-cancer activities against two cancer cell lines representing soft-tissue sarcoma (RD) and liver (HepG2) cancers were also demonstrated (IC_{50}: 260.27 ± 3.17 and 164.41 ± 1.83 μg/mL, respectively). This is the first study to report the inhibitory effect on protein denaturation and anticancer ability on soft-tissue sarcoma cells of the methanol leaf extract of *M. zapota*. These findings highlight *M. zapota* as a potential candidate for pharmaceutical research and development, particularly for its antioxidant, anti-inflammatory, and anticancer properties.

1. Introduction

The Sapotaceae family, a family of flowering plants belonging to the order Ericales, comprises 53 genera and approximately 1,250 species grown in several regions of the world, most abundant in the tropical and subtropical climates of Asia and South America (Sayma et al., 2025). Among its genera, the *Manilkara* genus is particularly notable for its valuable timber species with numerous practical applications. Currently, about 79 species belonging to the *Manilkara* genus are found in diverse habitats worldwide. Moreover, several Manilkara species, including Manilkara hexandra, Manilkara obovata, Manilkara pellegriniana, Manilkara kauki, Manilkara bidentata, and Manilkara zapota, have garnered scientific attention due to their

promising pharmacological properties. Previous studies have documented a wide range of biological activities of several *Manilkara* species, such as antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, hypolipidemic, hypoglycemic, and anti-cancer activities (Akosung et al., 2021; Hegde & Lakshman, 2023).

M. zapota, also known as "Hong Xiem" in Vietnamese, is a widely cultivated fruit tree in Southeast Asia countries such as Cambodia, Vietnam, Thailand, the Philippines, Malaysia, and Indonesia (Do et al., 2006). Morphologically, it is a large tree that can grow to 10 - 15m in height. The leaves are oblong, green, and alternately arranged. Its flowers are opaque white or green and typically emerge singly from the leaf axils (Yong & Shukkoor, 2020). The fruits are oval-shaped with a yellow-brown color and contain hard, black seeds (Anjali et al., 2018; Bano & Ahmed, 2017). *M. zapota* not only offers nutritional value as a fruit but also holds a long-standing role in traditional medicine. For example, different parts of the plant have historically been utilized to address a wide range of gastrointestinal problems, including constipation, dysentery, and diarrhea, as well as for their diuretic and antipyretic properties in traditional Vietnamese medicine (Do et al., 2006). Beyond its well-established role in traditional medicine, *M. zapota* has emerged as a promising candidate in modern medicine due to its diverse pharmacological activities supported by several extensive studies. For example, the extracts from *M. zapota* exhibited a potent antimicrobial activity against several bacterial strains, including Gram-negative and Gram-positive strains (Kaneria et al., 2009; Nair & Chanda, 2008), as well as against pathogenic nematodes and fungi (Mulvaney et al., 2021). Additionally, the acetone and methanol extracts from *M. zapota* seeds possessed a promising anti-bacterial activity against some pathogens, such as *Shigella flexneri* and *Vibrio cholerae*, with the inhibitory zones ranging from 09 to 17mm (Kothari & Seshadri, 2010). This evidence highlights its potential in managing infectious diseases. In addition, both the ethyl acetate and ethanol extracts from *M. zapota* leaves showed promising free radical scavenging activities with IC_{50} values ranging from 8.28 to 32.16 µg/mL (Ganguly & Rahman, 2014). In the previous study, Kiranmayi and Sureshma (2022) also reported the notable antioxidant properties of the ethanol extract from *M. zapota* leaves via its total antioxidant capacity and scavenging potentials against some free radicals such as OH^-, hydrogen peroxide, and nitric oxide. The anti-inflammatory effect of the methanol bark extract of *M. zapota* via carrageenan and histamine-induced models has been documented (Hossain et al., 2012). According to Tan and Norhaizan (2019), the water extract from *M. zapota* leaves cultivated in Malaysia could induce apoptosis and caspase pathways, which in turn inhibit the colorectal cancer cell line (HT-29). Moreover, the hydro-ethanol fruit extract of *M. zapota* collected in India had a significant cytotoxicity against skin cancer cells (A431) (Rani et al., 2023). However, the number of studies investigating bioactivities of *M. zapota* leaves cultivated in Vietnam, especially in anti-cancer and anti-inflammatory effects, is limited. Recognizing the promising potential of *M. zapota* leaves cultivated in Vietnam in modern pharmacology, this study was performed to expand the existing scientific data about its bioactivities, such as anti-cancer, anti-inflammatory, antioxidant, and anti-bacterial effects, thereby contributing to the advancement of natural product-based pharmaceutical development, particularly in Vietnamese remedies.

2. Materials & methods

2.1. Plant material and extract preparation

In November 2024, fresh leaf samples of *M. zapota* were harvested in Thuan Dien commune, Giong Trom district, Ben Tre province, Viet Nam (10°11'29.8"N 106°24'39.0"E), with an elevation of 5.2m. The samples were identified by the Sanger sequencing method (DNA Sequencing company, Vietnam) with ITS universal primers (ITS-F: 5'-ACGAATTCATGGTCCGGTGAAGTGTTCG-3'; ITS-R: 5'-TAGAATTCCCCGGTTCGC

TCGCCGTTAC-3'). The voucher specimens were stored at the herbarium of the Faculty of Pharmacy, Ton Duc Thang University (voucher number: MZ 16112024-TDT). After harvesting, the fresh leaves were washed, poor quality parts were removed, dried, and ground into medicinal powders (9.7% moisture). A total of 200g of dried leaf powders was soaked with 2L of methanol for 05 days at room temperature (the ratio between powder: solvent was 1:10, w/v). The filtrate was collected via filtering through Whatman filter paper. This process was repeated 03 times, and the filtrates were combined. The excessive solvent was removed under a reduced pressure of 150mbar at 45°C to finally obtain the final extract (55.13g of extract), the methanol extract of *M. zapota* leaves (MMZ), to be used for further qualitative, quantitative, and biological studies (Figure 1). The extraction yield was 27.57%.

Figure 1

Preparation of the M. Zapota Extract

Note. A. The fresh leaves of M. zapota; B. The leaf powder of M. zapota, C. The crude methanol extract of M. zapota leaves. The researcher's data analysis

2.2. Plant identification using DNA sequencing

The genomic DNA was extracted from *M. zapota* leaf samples using the CTAB method (Doyle & Doyle, 1987). The ITS gene region was amplified using the PCR method (Wen & Zimmer, 1996). The PCR reaction was heated as follows: 95°C for 5min, (95°C for 30s, 50°C for 30s, and 72°C for 45s) x 35 cycles, and 72°C for 5min. Then, the amplified DNA product was sequenced using the Sanger method (Sanger et al., 1977). Finally, the obtained DNA sequence was aligned with reference genes from the NCBI database using the Nucleotide BLAST function for species identification. The sample identification process was performed by DNA Sequencing Ltd. (Can Tho City, Vietnam).

2.3. Phytochemical screening

The phytochemicals were screened via standard procedures. Briefly, phenolic compound was determined by $FeCl_3$ 1%, alkaloid was determined by Bouchardat, Dragendorff, and Valse Mayer reagents, flavonoid was determined by cyanidin reaction, tannin was determined by gelatin salt solution, and saponin was determined based on the foaming test (Nguyen & Bui, 2023; Pandey & Tripathi, 2014).

2.4. Determination of total polyphenol content and total flavonoid content

The total polyphenol content of the extract was quantified according to Do et al.'s (2014) approach based on the Folin-Ciocalteu method. The results were calculated based on the

standard curve of gallic acid, and the data from triplicate assays were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The total flavonoid content of the extract was quantified according to Chang et al. (2002) based on the colorimetric method with aluminum chloride. The results from triplicate assays were calculated based on the standard curve of quercetin and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

2.5. Antioxidant activity

The Total Antioxidant Capacity (TAC) of *M. zapota* extract was evaluated by the phosphomolybdenum method according to the procedure of Tran et al. (2025), with some minor modifications. A volume of the reagent mixture (containing 0.6M H_2SO_4, 4mM ammonium molybdate, and 28mM sodium phosphate) was combined with the diluted *M. zapota* extract (ratio 9:1, v/v). The mixture was incubated for 90min at 95°C and then centrifuged at 5,000rpm for 5min. From the results of the absorption spectrophotometric measurement at 695nm, a standard curve was constructed for the positive control, ascorbic acid. The result of the sample was expressed as milligrams of ascorbic acid equivalents per gram of extract (mg AAE/g extract). The DPPH free radical scavenging activity was investigated as described by Nguyen and Tran (2018) with some minor modifications. A mixture of 1.5mL DPPH (0.025mM) and 0.5mL of the sample was thoroughly mixed. It was subsequently incubated in the dark for 30 minutes, and the absorbance was measured at 517nm. The same procedure was performed with the positive control (ascorbic acid) and the negative control (DMSO). The free radical scavenging rate was calculated according to the formula:

$$\% I = \frac{(A_c - A_{t)}}{A_c} \times 100 \quad (1)$$

In which the A_c is the absorbance of the sample without extract, and A_t is the absorbance of the sample containing extract or ascorbic acid.

2.6. Antibacterial activity

A total of 03 Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Shigella boydii)* and 03 Gram-positive bacteria *(Staphylococcus aureus, Enterococcus faecium,* and *Bacillus cereus)* were used for antibacterial tests and stored at the Laboratory of Biology and Pharmacology, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam. All bacterial strains were stored in glycerol stock at -80°C. Bacteria were streaked and grown on TSA overnight; a single colony was then reactivated overnight in TSB.

Bacterial suspension was grown in TSB for 04 - 06 hours (inoculation ratio 1:100, v/v), and the turbidity of bacterial culture was adjusted according to the 0.5 McFarland turbidity standard. The bacterial culture was spread on the surface of TSA agar. The diluted extract (50µL) at concentrations of 100 mg/ml, 50 mg/mL, 25 mg/mL, and the negative control (DMSO) were added to the wells. The positive control was the antibiotic Gentamicin 3 mg/mL added to the well with a volume of 20µL. The agar plates were incubated for 24 hours at 37°C, and the results were read (Pham et al., 2024). The antibacterial ability was assessed through the inhibition zone.

2.7. Anti-inflammatory activity

The anti-inflammatory activity of the extract was determined through the ability to inhibit the denaturation of Bovine Serum Protein (BSA) as described by Tran et al. (2024) with

some modifications. A mixture of 0.9mL of 0.5% BSA with 0.1mL of the extract was mixed and incubated in a heat block at 70°C for 10min. The mixture was stabilized at room temperature, and the absorbance was measured at 660nm. Diclofenac sodium was used as the positive control, and the negative control was established as the sample without extracts or diclofenac.

2.8. Anticancer activity

The cancer cell lines employed in this assay included human soft-tissue sarcoma cells (rhabdomyosarcoma cells-RD) and human hepatoma cells (HepG2) obtained from the collection of UMP Science and Technology Center, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam. The cells were cultured in the appropriate media (DMEM supplemented with 10% fetal bovine serum, 2mM L-glutamine, and 1% streptomycin-penicillin) at a humidified incubator with control temperature (37°C) and CO_2 (5%). The anticancer assay based on the MTT method was described by Lam et al. (2023) with some modifications. The cells were seeded in a 96-well plate format overnight and then incubated with the diluted extracts or reference medicine (doxorubicin) at 37°C, 5% CO_2 for 48h. DMSO was used as a negative control in the assay. After treatment, the media were removed. Next, the MTT reagent and fresh media (without fetal bovine serum) were added to each well and incubated for 4h. Then, the medium was removed, the formazan was dissolved with an appropriate volume of DMSO, and the absorbance was measured at 570nm.

2.9. Statistical analysis

All experiments were conducted in triplicate under identical conditions. The results were presented as mean ± standard deviation (Mean ± SD). GraphPad Prism 10.5.0 and Statgraphics software were used for statistical analysis. Differences were considered statistically significant at a 95% confidence level ($p < 0.05$).

3. Result and discussion

3.1. Plant identification using DNA sequencing

The analyzed product had a size of 815bps with a highly similar sequence to the reference sequence, *Manilkara zapota* voucher J. Clayton 12 (S) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence (Sequence ID: KF686242.1), with 99.71% similarity, 85% query coverage, 1,275 total score and 1,275 max score. This result identified the remedy sample collected in Thuan Dien commune, Giong Trom District, Ben Tre Province, Vietnam as *Manilkara zapota*.

Figure 2

The Top Sequences Producing Significant Alignment with the Sample

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Manilkara zapota voucher J. Clayton 12 (S) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer ...	Manilkara zapota	1275	1275	85%	0.0	99.71%	709	KF686242.1
Manilkara zapota genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, specime...	Manilkara zapota	1275	1275	85%	0.0	99.71%	717	HF542846.1
Manilkara zapota 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA ge...	Manilkara zapota	1214	1214	81%	0.0	99.70%	668	KC952015.1
Manilkara bidentata voucher JSA28 small subunit ribosomal RNA gene, partial sequence; internal transcribed space...	Manilkara bidentata	1205	1205	91%	0.0	95.96%	814	MZ329856.1
Manilkara chicle genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene, specimen ...	Manilkara chicle	1203	1203	90%	0.0	96.07%	758	HF542839.1

Note. The researcher's data analysis using the n-BLAST function

3.2. Phytochemical screening

Preliminary phytochemical investigation of *M. zapota* leaf extract revealed the presence of polyphenols, alkaloids, flavonoids, tannins, and saponins, and the results are shown in Table 1. These findings were closely in line with those reported by Ngongang et al. (2020), who found flavonoids, phenols, and tannins in the methanol extract of *M. zapota* leaves derived from Cameroon. Similarly, Ramos et al. (2022) also confirmed the presence of some bioactive compounds such as phenols, tannins, flavonoids, and alkaloids in the methanol leaf extract of the same species collected in the Philippines. Moreover, four chemical classes, including alkaloids, flavonoids, saponins, and tannins, were detected in the ethanol extract of *M. zapota* leaves, which supported our experimental results (Islam et al., 2013). The presence of these natural compounds in the leaf extract (MMZ) provides the basis for biological activities such as antibacterial, antioxidant, anti-inflammatory, and anticancer effects (Bolat et al., 2024; Liga et al., 2023).

Table 1

Phytochemical Screening of the Extract

	Polyphenol	**Alkaloid**	**Flavonoid**	**Tannin**	**Saponin**
MMZ	+	+	+++	++	+++

Note. (+, ++, +++): positive with increasing levels. The researcher's data analysis

3.3. Total polyphenol content, total flavonoid content, antioxidant activity

The results of quantification of total polyphenols and total flavonoids of the extract were 197.48 ± 2.08mg GAE/g extract and 39.04 ± 0.36mg QE/g extract, respectively (Table 2). This result is quite similar to the previous study of Kaneria et al. (2009), in which total phenol content and flavonoid content of the leaf methanol extract were 194.06 ± 1.21mg GAE/g and 35.55 ± 0.21mg QE/g, respectively. In the previous study, Suhaenah et al. (2024) reported that the ethanol extract from *M. zapota* leaves possessed lower polyphenol and flavonoid contents (150.28mg GAE/g extract and 36.577mg QE/g extract, respectively). Compared with the study of Chanda and Nagani (2010), the aqueous leaf extract had a total polyphenol content of 102.22 ± 3.13mg GAE/g extract and a total flavonoid content of 19.14 ± 0.17mg QE/g extract. According to Miao et al. (2022), flavonoids tend to be dissolved more efficiently in organic solvents, including chloroform, ethanol, and methanol, compared to water. Consequently, the flavonoid content in MMZ extract was greater than that of the aqueous extract reported by Chanda and Nagani (2010). The findings of this experiment further support methanol as a more effective solvent in the recovery of biologically active substances.

The antioxidant capacity of the leaf extract of *M. zapota* showed a significant efficacy based on the ability to neutralize DPPH free radicals, as shown in Table 2, this extract showed an IC_{50} of 2.71 ± 0.11 (μg/mL) compared to the reference substance with an IC_{50} of 1.27 ± 0.13 (μg/mL) which means the antioxidant capacity of the extract was only about 02 times less than ascorbic acid. Furthermore, the total antioxidant capacity of the extract was 218.91 ± 7.84 (mg AAE/g) (Table 2). Previously, the methanol extract of *M. zapota* leaves also showed an excellent ability to neutralize DPPH free radicals when the IC_{50} of the extract was also less than 02 times that of ascorbic acid (Kaneria et al., 2009). Previous reports suggested that the antioxidant activity of plant products was mainly related to the free radical scavenging ability of phenolic compounds (Dzib et al., 2016). Therefore, there is a close relationship between the polyphenol and flavonoid content and the antioxidant capacity of the extract. The leaves of *M. zapota* are a good source of natural antioxidants.

Table 2

Total Polyphenol Content, Total Flavonoid Content, Antioxidant Activity of the Extract

	MMZ	Ascorbic acid
IC_{50} values of DPPH assay (μg/mL)	$2.71 \pm 0.11^{***}$	1.27 ± 0.13
TAC (mg AAE/g)	218.91 ± 7.84	-
Total polyphenol content (mg GAE/g)	197.48 ± 2.08	-
Total flavonoid content (mg QE/g)	39.04 ± 0.36	-

Note. (***): indicates a statistically significant difference between the control group and the test group ($p < 0.001$). The researcher's data analysis

3.4. Antibacterial activity

The antibacterial activity of the extract was assessed against 6 different bacterial strains, and the obtained results are summarized in Table 3. The extract (MMZ) exhibited weak to moderate antibacterial abilities (8.00 - 11.33mm) depending on the concentration tested against 03 bacterial strains (*S. boydii, S. aureus, B. cereus*). The findings indicated that Gram-positive bacteria were more susceptible than Gram-negative bacteria, likely due to the outer membranes present in Gram-negative bacteria, which can act as a barrier to biologically active substances. In comparison to the study of Islam et al. (2013), the ethanol leaf extract showed antibacterial activity against *B. cereus, S. boydii, E. coli, and P. aeruginosa*, but exhibited no effect on *S. aureus.* Variations in antibacterial efficacy may be attributed to differences in extraction solvents or the geographical origin of the plant materials.

Table 3

Results of Antibacterial Activity Survey of the Extract (Mm)

Bacterial strains	MMZ (100 mg/mL)	MMZ (50 mg/mL)	MMZ (25 mg/mL)	Gentamicin (3 mg/mL)
E. coli	-	-	-	19.50 ± 0.50
P. aeruginosa	-	-	-	21.33 ± 0.58
S. boydii	10.17 ± 0.29^{Aa}	-	-	19.67 ± 0.58^{B}
S. aureus	11.17 ± 0.29^{Bb}	8.00 ± 0.50^{Aa}	-	20.83 ± 0.29^{C}
E. faecium	-	-	-	15.83 ± 0.29
B. cereus	11.33 ± 0.29^{Cb}	9.17 ± 0.29^{Bb}	8.33 ± 0.29^{A}	21.33 ± 0.58^{D}

Note. (-): indicates the absence of antimicrobial activity in the extract. In the same column, the superscript lowercase letters "a, b" indicate significant differences among the bacterial strains. In the same row, the superscript capital letters "A, B, C, D" indicate significant differences between the positive control and the diluted extracts ($p < 0.05$). The researcher's data analysis

3.5. Anti-inflammatory activity

The inhibitory effect of the extract on protein denaturation increased from 8.658 ± 0.383% at the concentration of 0.03125 mg/mL to 33.678 ± 0.344% at the concentration of 0.0625 mg/mL. When the concentration of the extract was increased to 0.125 mg/mL, the

inhibitory effect reached 54.408 ± 0.698% (Table 4). There was a direct correlation between the dose of treatment (MMZ) and the inhibitory effect. Furthermore, the MMZ extract exhibited a superior ability to inhibit protein denaturation compared to other plant extracts, such as *Anacardium occidentale, Curcuma zedoaria,* and *Physalis angulate.* For example, the ethanol extract of *A. occidentale* seeds at a concentration of 0.2 mg/mL achieved an inhibitory effect of 45.09 ± 2.55% against protein denaturation (Rajeswaramma & Jayasree, 2018), and *C. zedoaria* ethanol extract at a concentration of 0.2 mg/mL showed an inhibitory effect of 36.78 ± 5.16% (Ullah et al., 2014). In addition, the methanol, ethanol, and water extracts of *P. angulata* leaves at a concentration of 0.125 mg/mL achieved inhibitory effects of 32.6%, 41.6%, and 35.9%, respectively (Shravan et al., 2011). The IC_{50} value of the extract was 0.107 ± 0.008 (mg/mL), which was only about 2.89 times higher than the IC_{50} value of diclofenac sodium (0.037 ± 0.001 mg/mL) (Table 4). This is the first study on the anti-inflammatory effect of *M. zapota* leaf extract by the BSA denaturation inhibition method *in vitro*, showing a potential anti-inflammatory effect. This suggests that *M. zapota* leaves may be a suitable candidate for anti-inflammatory drug development.

Table 4

BSA Denaturation Inhibitory Ability of M. Zapota Extract

Extract		**Diclofenac natri**	
Concentration (mg/mL)	**Inhibition (%)**	**Concentration (mg/mL)**	**Inhibition (%)**
0.125	54.408 ± 0.698	0.1	86.418 ± 0.546
0.0625	33.678 ± 0.344	0.05	61.835 ± 1.396
0.03125	8.658 ± 0.383	0.0125	13.701 ± 1.013
IC_{50} (mg/mL)	0.107 ± 0.008**	IC_{50} (mg/mL)	0.037 ± 0.001

Note. (**): indicates a statistically significant difference between the control group and the test group ($p < 0.01$). The researcher's data analysis

3.6. Anticancer activity

Anticancer activity of the extract against RD and HepG2 cell lines with IC_{50} values of 260.27 ± 3.17 (μg/mL) and 164.41 ± 1.83 (μg/mL), respectively (Table 5). All samples showed an increase in inhibitory activity corresponding to the rise in concentration. The MMZ exhibited a more potent anticancer effect on the HepG2 cell line compared to RD, a trend also observed in the control. In many previous studies, by the MTT method, the anticancer activity of *M. zapota* has been shown on cancer cell lines such as A431, A549, HCT 116, HeLa, HT-29, BT474, Chago-K1, HepG2, KATO-III, SW620, etc. (Chunhakant & Chaicharoenpong, 2019; Rani et al., 2023; Shaniba et al., 2019; Tan & Norhaizan, 2019). To date, this is the first study to demonstrate the anticancer effect of *M. zapota* leaves on human soft-tissue cancer, rhabdomyosarcoma cells (RD). These supplements to the anti-cancer effect of M. zapota suggest further investigation to develop anti-cancer medicine from this material.

Table 5

Anticancer Activity Results of M. Zapota Extract

Cancer cell lines	MMZ		Doxorubicin	
	Concentration (µg/mL)	Inhibition (%)	Concentration (µg/mL)	Inhibition (%)
RD	500	78.32 ± 0.18	5.435	76.24 ± 0.45
	250	49.05 ± 0.33	2.718	64.49 ± 0.24
	125	32.32 ± 0.39	1.359	59.81 ± 0.39
	62.5	22.81 ± 0.18	0.544	18.51 ± 0.10
	31.25	13.19 ± 0.43	0.272	15.81 ± 0.12
			0.136	5.17 ± 0.31
IC_{50} (µg/mL)	260.27 ± 3.17****		1.42 ± 0.19	
HepG2	500	61.34 ± 0.96	2.718	63.41 ± 0.36
	250	57.99 ± 0.72	1.359	52.55 ± 0.67
	125	43.52 ± 0.39	0.544	41.37 ± 0.52
	62.5	28.84 ± 0.41	0.272	30.88 ± 0.81
	31.25	18.50 ± 0.94	0.136	17.97 ± 0.06
			0.054	12.96 ± 1.25
IC_{50} (µg/mL)	164.41 ± 1.83****		1.09 ± 0.07	

Note. (****): indicates a statistically significant difference between the control group and the test group ($p < 0.0001$). The researcher's data analysis

4. Conclusions

This study provided scientific information on Manilkara zapota leaves harvested in Ben Tre province, Viet Nam, encompassing qualitative, quantitative, and biological activities. Some natural compounds found in the methanol extract of the leaves were polyphenols, alkaloids, tannins, and saponins. This extract also showed relatively high total polyphenol and total flavonoid contents (197.48 ± 2.08mg GAE/g extract and 39.04 ± 0.36mg QE/g extract, respectively). The results showed that the extract had significant DPPH free radical scavenging activity (IC_{50} = 2.71 ± 0.11 µg/mL), compared with ascorbic acid standard (IC_{50} = 1.27 ± 0.13 µg/mL) and a relatively high total antioxidant capacity (218.91 ± 7.84mg AAE/g extract). The extract could inhibit the growth of 3 bacterial strains: *B. cereus, S. aureus,* and *S. boydii* with antibacterial zone diameters of 8.00 - 11.33mm. For the first time, the methanol extract from *M. zapota* leaves has shown a potent inhibitory effect on albumin denaturation (IC_{50} = 0.107 ± 0.008 mg/mL), compared with the positive control diclofenac sodium (IC_{50} = 0.037 ± 0.001 mg/mL). Although the cytotoxicity of the extract on RD and HepG2 cancer cells was weak (IC_{50} = 260.27 ± 3.17 µg/mL and IC_{50} = 164.41 ± 1.83 µg/mL, respectively), this study is the first publication to document the anticancer ability of the methanol extract from *M. zapota* leaves on the RD cell line. The results of the study show that *M. zapota* leaves are a potential medicinal source with many notable biological activities, such as antioxidant, anti-inflammatory, and anticancer activities. The results of the study serve as the premise for developing drugs and functional foods from M. zapota leaves to combat diseases related to cancer, inflammation, and oxidative stress, while also contributing to environmental protection through the utilization of this agricultural by-product.

SCIENTIFIC CONTRIBUTION

The manuscript clearly identifies a research gap; the manuscript extends/refines existing theories; the manuscript proposes a new theoretical and analytical model; the manuscript introduces/improves research methods; the manuscript provides new datasets and empiricalevidence; the manuscript presents statistically and practically significant findings; themanuscript offers policy/managerial/technological implications; the manuscript opens newdirections for further research.

AUTHOR CONTRIBUTIONS

CRediT: **Nguyen Le Thanh Tuyen**: Conceptualization, Methodology, Investigation, Writing - Original Draft; **Tran Gia Buu**: Conceptualization, Methodology, Investigation, Software, Data Curation, Formal Analysis; Supervision, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing; **Ly Gia Han**: Resources, Investigation, Data Curation, Formal Analysis, Writing - Original Draft; **Le Thi Kim Anh**: Investigation, Formal Analysis, Writing - Original Draft.

FUNDING

This research received no external funding.

NO CONFLICT OF INTEREST STATEMENT

The author declares that they have no conflict of interest.

References

Akosung, E., Kenmogne, S. B., Lobe Songue, J., Longue Ekon, J. P., Lateef, M., Happi, E. N., Langat, M. K., Tabekoueng, G. B., Toze, F. A. A., Stammler, H. G., Lenta, B. N., Wansi, J. D., Sewald, N., Ali, M. S., & Waffo, A. F. K. (2021). Bioactive constituents from *Manilkara obovata* (Sabine & G. Don) J. H. Hemsl. *Natural Product Research, 35*(22), 4347-4356. https://doi.org/10.1080/14786419.2020.1713123

Anjali, Garg, V., Dhiman, A., Dutt, R., & Ranga, S. (2018). The genus *Manilkara*: An update. *The Pharma Innovation Journal, 7*(1), 316-318.

Bano, M., & Ahmed, B. (2017). *Manilkara zapota* (L.) P. Royen (Sapodilla): A review. *International Journal of Advance Research, Ideas and Innovations in Technology, 3*(6), 1364-1371.

Bolat, E., Sarıtaş, S., Duman, H., Eker, F., Akdaşçi, E., Karav, S., & Witkowska, A. M. (2024). Polyphenols: Secondary metabolites with a biological impression. *Nutrients, 16*(15), Article 2550. https://doi.org/10.3390/nu16152550.

Chanda, S. V., & Nagani, K. V. (2010). Antioxidant capacity of *Manilkara zapota* L. leaves extracts evaluated by four *in vitro* methods. *Nature and Science, 8*(10), 260-266.

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis, 10*(3), 178-182. https://doi.org/10.38212/2224-6614.2748

Chunhakant, S., & Chaicharoenpong, C. (2019). Antityrosinase, antioxidant, and cytotoxic activities of phytochemical constituents from *Manilkara zapota* L. bark. *Molecules, 24*(15), Article 2798. https://doi.org/10.3390/molecules24152798

Do, B. H., Dang, C. Q., Bui, C. X., Nguyen, D. T., Do, D. T., Pham, H. V., Vu, L. N., Pham, M. D., Pham, M. K., Doan, N. T., Nguyen, T., & Tran, T. (2006). *Medicinal plants and medicinal animals in Vietnam*. Science and Technics Publishing House.

Do, Q. D., Angkawijaya, A. E., Tran, P. N. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis, 22*(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin, 19*(1), 11-15.

Dzib-Guerra, W. D., Escalante-Erosa, F., García-Sosa, K., Derbré, S., Blanchard, P., Richomme, P., & Peña-Rodríguez, L. M. (2016). Anti-advanced glycation end-product and free radical scavenging activity of plants from the Yucatecan flora. *Pharmacognosy Research, 8*(4), 276-280. https://doi.org/10.4103/0974-8490.188883

Ganguly, A., & Rahman, S. A. (2014). Evaluation of the cytotoxic, antimicrobial, antioxidant, anthelmintic and CNS depressant activities of *Manilkara zapota* leaf (Sapotaceae). *World Journal of Pharmaceutical Research, 4*(1), 272-283.

Hegde, M. M., & Lakshman, K. (2023). Phyto-pharmacological review of genus manilkara. *International Journal of Herbal Medicine, 11*(5), 1-13. https://doi.org/10.22271/flora.2023.v11.i5a.882

Hossain, M. H., Jahan, F., Howlader, M. S. I., Dey, S. K., Hira, A., Ahmed, A., & Sarkar, R. (2012). Evaluation of anti-inflammatory activity and total flavonoids content of *Manilkara zapota* (Linn.) bark. *International Journal of Pharmaceutical and Phytopharmacological Research, 2*(1), 35-39.

Islam, M. R., Parvin, M. S., Banu, M. R., Jahan, N., Das, N., & Islam, M. E. (2013). Antibacterial and phytochemical screening of ethanol extracts of *Manilkara zapota* leaves and bark. *International Journal of Pharma Sciences, 3*(6), 394-397.

Kaneria, M., Baravalia, Y., Vaghasiya, Y., & Chanda, S. (2009). Determination of antibacterial and antioxidant potential of some medicinal plants from Saurashtra Region, India. *Indian Journal of Pharmaceutical Sciences, 71*(4), 406-412. https://doi.org/10.4103/0250-474X.57289

Kiranmayi, G. V. N., & Sureshma, M. S. (2022). Antioxidant and antidepressant activities of ethanolic leaf extract of *Manilkara zapota*. *International Journal of Pharmceutical Sciences and Research, 13*(4), 1581-1590. https://doi.org/10.13040/IJPSR.0975-8232

Kothari, V., & Seshadri, S. (2010). In vitro antibacterial activity in seed extracts of *Manilkara zapota*, *Anona squamosa*, and *Tamarindus indica*. *Biological Research, 43*(2), 165-168. https://doi.org/10.4067/S0716-97602010000200003

Lam, K. K., Dinh, H. M., Ngo, H. D., Tran, B. G., & Dam, M. S. (2023). Biological activities of thymine extracted from ethyl acetate of *Cordyceps neovolkiana* DL0004 isolated in Vietnam. *Research Journal of Biotechnology, 18*(8), 97-105.

Liga, S., Paul, C., & Péter, F. (2023). Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques. *Plants, 12*(14), Article 2732. https://doi.org/10.3390/plants12142732

Miao, L., Zhang, H., Yang, L., Chen, L., Xie, Y., & Xiao, J. (2022). Flavonoids. In S. M. Nabavi & A. S. Silva (Eds.), *Antioxidants effects in health* (pp. 353-374). Elsevier. https://doi.org/10.1016/B978-0-12-819096-8.00048-3

Mulvaney, L. C. M., Xavier-Júnior, F. H., Rodrigues, A. M. S., Stien, D., Allegretti, S. M., & Garcia, V. L. (2021). Antimicrobial and anthelmintic activities of the ethanolic extract, fractions and isolated compounds from *Manilkara zapota* L. P. Royen (Sapotaceae). *The Journal of Pharmacy and Pharmacology, 73*(3), 377-387. https://doi.org/10.1093/jpp/rgaa030

Nair, R., & Chanda, S. (2008). Antimicrobial activity of *Terminalia catappa, Manilkara zapota* and *Piper betel* leaf extract. *Indian Journal of Pharmaceutical Sciences, 70*(3), 390-393. https://doi.org/10.4103/0250-474X.43012

Ngongang, F. C., Fankam, A. G., Mbaveng, A. T., Wamba, B. E., Nayim, P., Beng, V. P., & Kuete, V. (2020). Methanol extracts from *Manilkara zapota* with moderate antibacterial activity displayed strong antibiotic-modulating effects against multidrug-resistant phenotypes. *Investigational Medicinal Chemistry and Pharmacology, 3*(1), Article 37. https://dx.doi.org/10.31183/imcp.2020.00037

Nguyen, T. T. L., & Bui, C. C. (2023). Investigation of the extraction process and preliminary phytochemical analysis from Henna leaves (*Lawsonia inermis* L.). *Can Tho Journal of Medicine and Pharmacy, 59*, 80-85. https://doi.org/10.58490/ctump.2023i59.487

Nguyen, Y. T. H., & Tran, B. G. (2018). The effect of humidity and temperature on total polyphenol content and antioxidant activity of black garlic produced from peeled cloves of garlics. *Journal of Science and Technology - IUH, 31*(1), 88-96. https://doi.org/10.46242/jst-iuh.v31i01.363

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry, 2*(5), 115-119.

Pham, T. V., Hoang, T. X., Nguyen, N. H., Do, H. B., Vo, H. N. H., & Tran, B. G. (2024). Chemical composition, anti-bacterial activity and molecular docking studies of essential oil isolated from Sa Sam Nam (*Launaea sarmentosa*). *Journal of Oleo Science, 73*(5), 787-799. https://doi.org/10.5650/jos.ess23254

Rajeswaramma, G., & Jayasree, D. (2018). In vitro anti-inflammatory activity of *Anacardium occidentale* seed extract. *IOSR Journal of Dental and Medical Sciences, 17*(1), 18-22. https://doi.org/10.9790/0853-1701081822

Ramos, A., Alvarez, M. R., Delica, K., Moreno, P. G., Abogado, R., Grijaldo, S. J., de Juan, F., Deniega, F. M., Basingan, M., Radivas, C. M., Heralde Iii, F., Completo, G. C., Padolina, I., & Nacario, R. (2022). Antioxidant and anticancer activities of *Manilkara zapota* and *Lansium domesticum* Leaves coupled with metabolomics analysis using molecular networking. *Vietnam Journal of Chemistry*, *60*(5), 578-588. https://doi.org/https://doi.org/ 10.1002/vjch.202100110

Rani, A., Gupta, T., Kangra, K., Garg, V., & Dutt, R. (2023). Evaluation of antioxidant, antibacterial and anticancer activity of fruit and leaf extracts of *Manilkara zapota* against A431 skin cancer cell lines. *South African Journal of Botany, 153*, 219-226. https://doi.org/10.1016/j.sajb.2022.12.023

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, 74*(12), 5463-5467. https://doi.org/10.1073/pnas.74.12.5463

Sayma, S., Revad, T., Pandya, H., & Solanki, H. (2025). Sapotaceae family as a source of natural therapeutics: A review on bioactive compounds and medicinal potential. *International Journal of Scientific Research and Technology, 2*(4), 365-371. https://doi.org/10.5281/ zenodo.15236653

Shaniba, V. S., Aziz, A. A., Jayasree, P. R., & Kumar, P. M. (2019). *Manilkara zapota* (L.) P. Royen leaf extract derived silver nanoparticles induce apoptosis in human colorectal carcinoma cells without affecting human lymphocytes or erythrocytes. *Biological Trace Element Research, 192*(2), 160-174. https://doi.org/10.1007/s12011-019-1653-6

Shravan, K. N., Kishore, G., Siva, K. G., & Sindhu, P. E. S. (2011). In vitro anti-inflammatory and anti-arthritic activity of leaves of *Physalis angulata* L. *International Journal Pharmacy and Industrial Research, 1*(3), 211-213.

Suhaenah, A., Hartina, S., & Aminah (2024). Determination of phenolic and flavonoid content of ethanol extract of Manila Sawo leaf (*Manilkara zapota* L.) from Wajo District by UV-VIS spectrophotometry. *Pharmaceutical Reports, 3*(2), 50-55. https://doi.org/10.33096/pharmrep.v3i2.311

Tan, B. L., & Norhaizan, M. E. (2019). *Manilkara zapota* (L.) P. Royen leaf water extract triggered apoptosis and activated caspase-dependent pathway in HT-29 human colorectal cancer cell line. *Biomedicine & Pharmacotherapy, 110*, 748-757. https://doi.org/10.1016/j.biopha.2018.12.027

Tran, B. G., Do, H. B., Tran, T. T. X., Pham, T. V., & Do, T. D. (2024). A comparative analysis of antioxidant, anti-inflammatory, and anticancer activities of the crude and fractionated extracts from *Distichochlamys benenica*. *The EuroBiotech Journal, 8*(4), 184-194. https://doi.org/10.2478/ebtj-2024-0018

Tran, T. T. X., Pham, T. V., Lam, L. T., Hua, B. T., Nguyen, N. B., Do, H. B., & Tran, B. G. (2025). Anti-oxidant and anti-bacterial effects of Diospyros kaki leaf extract in Vietnam. *Ho Chi Minh City Open University Journal of Science - Engineering and Technology, 15*(1), 15-27. https://doi.org/10.46223/HCMCOUJS.tech.en.15.1.3502.2025

Ullah, H. A., Zaman, S., Juhara, F., Akter, L., Tareq, S. M., Masum, E. H., & Bhattacharjee, R. (2014). Evaluation of antinociceptive, *in-vivo* & *in-vitro* anti-inflammatory activity of ethanolic extract of *Curcuma zedoaria* rhizome. *BMC Complementary and Alternative Medicine, 14*, Article 346. https://doi.org/10.1186/1472-6882-14-346

Wen, J., & Zimmer, E. A. (1996). Phylogeny and biogeography of *Panax* L. (the Ginseng Genus, Araliaceae): Inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution, 6*(2), 167-177. https://doi.org/10.1006/mpev.1996.0069

Yahia, E. M., & Gutierrez-Orozco, F. (2011). Sapodilla (*Manilkara achras* (Mill) Fosb., syn *Achras sapota* L.). In *Postharvest biology and technology of tropical and subtropical fruits* (pp. 351-363e). Woodhead Publishing.

Yong, K. Y., & Shukkoor, M. S. A. (2020). *Manilkara zapota*: A phytochemical and pharmacological review. *Materials Today: Proceedings, 29*(1), 30-33. https://doi.org/10.1016/j.matpr.2020.05.688