Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/fr915b52

PHENOLIC COMPOSITION AND ANTIOXIDANT POTENTIAL OF PEEL AND PULP OF MANILKARA ZAPOTA

Ishtiaque Ahmed[1*], Fatima Hafeez[2], Ayesha Hameed[3], Rabia Ameer[4]

[1*]Institute of Chemistry, University of Sargodha, Punjab, Pakistan.
[2]Lecturer, University of the Punjab Lahore, Punjab, Pakistan.
[3]Senior Lecturer, M. Islam Medical & Dental College, Gujranwala, Punjab, Pakistan.
[4]Student, Pakistan Institute of Quality Control, Lahore, Punjab, Pakistan.

***Correspondence Author:** Ishtiaque Ahmed,
*Institute of Chemistry, University of Sargodha, Punjab, Pakistan.
Email: ahmedishtiaque1989@gmail.com

ABSTRACT

Introduction: Natural antioxidants derived from plant sources have gained substantial attention owing to their potential therapeutic applications and role in combating oxidative stress-related disorders. *Manilkara zapota* (sapodilla), a tropical fruit of nutritional and medicinal significance, represents a promising yet underexplored source of phenolic and flavonoid compounds with potent antioxidant activities. However, comprehensive profiling of its bioactive constituents, particularly in its peel a typically discarded agro-industrial byproduct remains limited in the scientific literature.

Aims and Objectives: The present study was conducted to evaluate the phenolic composition and antioxidant potential of *M. zapota* peel and pulp extracts. Specific objectives included: (i) determination of total phenolic content (TPC) and total flavonoid content (TFC); (ii) qualitative and quantitative profiling of individual phenolic acids using HPLC; and (iii) assessment of antioxidant activity via the DPPH free radical scavenging assay.

Methodology: Peel and pulp samples of *M. zapota* were subjected to extraction using solvents of varying polarity (100% methanol, 80% methanol, 100% ethanol, 80% ethanol) combined with two extraction techniques: magnetic stirring and orbital shaking. TPC was determined using the Folin–Ciocalteu assay, TFC by aluminum chloride colorimetric method, individual phenolic acids were quantified through HPLC analysis, and antioxidant activity was assessed via the DPPH assay, with IC_{50} values calculated for comparative analysis. Statistical significance was established at $p < 0.05$.

Results and Findings: Extraction yields varied from 19.82% to 47.34%, with the highest yields recorded for 80% methanol extracts using magnetic stirring. The TPC ranged from 17.50 to 53.21 mg GAE/g dry weight, while TFC varied between 4.81 and 24.56 mg CE/g dry weight. Thirteen phenolic acids were identified, with gallic acid, quercetin, and chromatotrophic acid consistently present across extracts. The strongest antioxidant activity (lowest IC_{50} = 1.05 mg/mL) was observed in peel extracts obtained with 80% methanol using orbital shaking. The antioxidant potential followed the trend: 80% methanol > 80% ethanol > 100% methanol > 100% ethanol.

Conclusion: The findings confirm that *M. zapota* peel and pulp are rich in phenolic acids and flavonoids with significant antioxidant potential. The superior extraction efficiency achieved with 80% methanol highlights its applicability for recovering bioactive phytochemicals. These results strongly advocate for the valorization of *M. zapota*, particularly its peel, as a sustainable source of natural antioxidants for application in food, nutraceutical, and pharmaceutical industries.

Keywords: *Manilkara zapota*, antioxidant activity, phenolic acids, flavonoids, DPPH assay, HPLC, nutraceuticals, functional foods

INTRODUCTION

The progressive degradation of food matrices through oxidative mechanisms represents a critical challenge in food science and human nutrition, significantly compromising the nutritional quality, safety, and shelf life of consumables. Lipid peroxidation and oxidative rancidity, predominantly triggered by reactive oxygen species (ROS) and free radical chain reactions, are principal contributors to the deterioration of lipids and related biomolecules in foodstuffs during prolonged storage and thermal processing. These oxidative processes not only result in substantial economic losses within the food industry but also pose public health concerns due to the formation of toxic secondary metabolites and diminished nutrient bioavailability [1,2]. Oxidative stress defined as an imbalance between pro-oxidants and antioxidants in biological systems has been mechanistically implicated in the pathogenesis of a broad spectrum of chronic and degenerative disorders, including diabetes mellitus, cardiovascular diseases, oncogenesis, and neurodegenerative conditions such as Alzheimer's disease [3]. Consequently, the mitigation of oxidative damage through effective antioxidant interventions remains a focal point of contemporary biomedical and nutritional research. Antioxidants, defined as molecules capable of inhibiting or delaying oxidation at low concentrations relative to the oxidizable substrates, are pivotal in arresting free radical propagation via diverse mechanisms including radical scavenging, metal chelation, and enzymatic inhibition [4,5].

Antioxidants are broadly categorized into primary (chain-breaking) antioxidants, which directly quench lipid radicals to form stable, non-propagating species, and secondary (preventive) antioxidants, which impede oxidation indirectly by chelating pro-oxidant metal ions, scavenging oxygen, or decomposing hydroperoxides [6,7]. The increasing awareness of the potential toxicological risks associated with synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tertiary butylhydroquinone (TBHQ) has intensified research into natural alternatives derived from plant-based sources, which exhibit multifaceted bioactive properties alongside their antioxidative capabilities [8,9]. Plant-derived phenolic compounds constitute the predominant class of natural antioxidants, encompassing a heterogeneous group including phenolic acids, flavonoids, tocopherols, carotenoids, and vitamin C. Their antioxidant efficacy is primarily attributed to their redox properties, enabling them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal ion chelators [10]. The structural diversity within phenolics such as hydroxylation patterns and conjugated double bond systems critically determines their radical scavenging capacity and overall bioactivity [11]. Among these, flavonoids such as quercetin and catechin, tocopherols with their phenolic chromanol rings, and carotenoids with conjugated polyene chains have been extensively characterized for their potent antioxidative functions [12–14].

Despite extensive research on phenolic profiles and antioxidant properties in commonly consumed fruits and vegetables, tropical fruits remain underexplored despite their vast phytochemical diversity and nutritional potential [15]. Manilkara zapota (commonly known as sapodilla or chiku), a member of the Sapotaceae family, is a tropical evergreen tree native to the New World tropics and widely cultivated in South Asia, including Pakistan. The fruit is valued not only for its organoleptic qualities characterized by a caramel-like sweetness and grainy texture but also for its rich nutrient profile comprising carbohydrates, dietary fiber, essential vitamins (notably vitamin C and B-complex vitamins), and trace minerals [16,17]. Given the growing interest in functional foods and nutraceuticals, elucidating the phenolic composition and antioxidant potential of Manilkara zapota, particularly differentiating between peel and pulp fractions, is imperative. The peel, often considered an agro-industrial waste, is hypothesized to harbor a higher concentration of bioactive phenolics and antioxidant compounds relative to the pulp, thus representing a valuable resource for natural antioxidant extraction and utilization [18]. Comprehensive phytochemical profiling, coupled with robust in vitro antioxidant assays, will advance our understanding of the fruit's contribution to oxidative stress modulation and its potential applications in food preservation and human health.

Aims & Objectives of the Study

This study aims to systematically quantify and characterize the phenolic constituents in both the peel and pulp of Manilkara zapota and to evaluate their antioxidant capacities using standardized analytical methodologies. The findings will contribute to the expanding database on tropical fruit bioactives, fostering the development of novel natural antioxidant formulations and supporting dietary recommendations for disease prevention through enhanced intake of phenolic-rich foods.

METHODOLOGY

The present experimental work was conducted at the laboratories of the Department of Chemistry (Ibn-e-Sina Block) and the Department of Pharmacy, University of Sargodha, Sargodha, Pakistan. The research design was systematically structured to investigate the phenolic composition and antioxidant potential of both the peel and pulp of *Manilkara zapota* (commonly known as sapodilla or chiku). The methodological framework comprised the extraction of bioactive compounds, qualitative and quantitative evaluation of phenolic constituents, and the assessment of antioxidant potential using standardized in vitro assays.

Sample Collection and Preparation: Fresh fruit samples of *Manilkara zapota* were procured from local markets in Sargodha, with random selection to minimize sampling bias. Upon procurement, the fruits were manually peeled, and both peel and pulp were separated and chopped into small pieces. These were subjected to air drying on aluminum foil sheets under ambient environmental conditions to prevent thermal degradation of thermolabile phytochemicals. The dried material was then finely ground using a laboratory grinder, and the resulting powdered peel and pulp samples were stored in airtight polythene bags at refrigerated conditions (4°C) to preserve their biochemical integrity until further analysis.

Reagents, Chemicals, and Analytical Instruments: All solvents and chemicals employed in this study were of analytical reagent (AR) grade, procured from Merck (Germany). Major chemicals included Folin-Ciocalteu reagent, gallic acid, catechin, quercetin, aluminum chloride, sodium carbonate, sodium nitrite, sodium hydroxide, ascorbic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, sinapic acid, vanillic acid, trichloroacetic acid (TCA), butylated hydroxyanisole (BHA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Analytical procedures were supported by advanced laboratory equipment, including an analytical balance (Shimadzu AW 220), magnetic stirrers, orbital shaker (Optima OS-752), electric water bath, rotary vacuum evaporator, UV-visible spectrophotometer (Cecil CE-7200), and high-performance liquid chromatography (HPLC) system (Shimadzu LC-10A, Japan) equipped with a C18 reversed-phase column.

Preparation of Plant Extracts: Extracts of *M. zapota* peel and pulp were prepared using both magnetic stirring and orbital shaking methods with four solvent systems: 100% ethanol, 100% methanol, 80% ethanol (80:20 v/v), and 80% methanol (80:20 v/v). The solid-to-solvent ratio was maintained at 1:10 (w/v). Extraction was performed for 10 hours using the orbital shaker and 8 hours using the magnetic stirrer at room temperature. Filtration of the extracts was conducted using Whatman filter paper, followed by successive re-extractions of the residues to ensure exhaustive extraction. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at controlled temperatures to prevent degradation of phenolic constituents. The semi-solid crude extracts were stored at 3–5°C in airtight containers for further phytochemical and antioxidant analyses. The percentage yield of each extract was calculated gravimetrically.

Determination of Total Phenolic Content (TPC): The total phenolic content (TPC) of each extract was quantified using the Folin–Ciocalteu colorimetric assay, following the protocol described by Kalpana et al. Briefly, 0.5 mL of extract (2 mg/mL) was mixed with 0.1 mL of 0.5N Folin–Ciocalteu reagent, followed by incubation for 15 minutes. Subsequently, 2.5 mL of saturated sodium carbonate solution (7% w/v) was added, and the mixture was incubated for an additional 30 minutes at ambient temperature. Absorbance was measured at 700 nm using a UV-visible spectrophotometer (CECIL CE-7200). The phenolic content was determined using a gallic acid calibration curve (10–200 ppm, $R^2 = 0.998$), and results were expressed as mg gallic acid equivalents (GAE) per gram of dried sample (mg GAE/g dry weight). Each assay was conducted in triplicate, and mean values were reported.

Determination of Total Flavonoid Content (TFC): Total flavonoid content (TFC) was evaluated according to the method of Zhishen et al., utilizing a colorimetric assay. For each analysis, 1 mL of extract (10 mg/mL) was introduced into a 10 mL volumetric flask, and the volume was brought to 5 mL with distilled water. Subsequently, 0.3 mL of 5% sodium nitrite solution was added, and the mixture was allowed to stand for 5 minutes. Then, 0.3 mL of 10% aluminum chloride was added, followed by a further 6-minute incubation at room temperature. Finally, 2 mL of 1M sodium hydroxide was incorporated, and the total volume was adjusted to 10 mL with distilled water. After thorough mixing, absorbance was recorded at 510 nm. Catechin was used as the reference standard for constructing a calibration curve (10–500 ppm, $R^2 = 0.9911$), and results were expressed as mg catechin equivalents (CE) per gram of dried sample. All measurements were performed in triplicate.

Phenolic Profile Analysis by HPLC: The qualitative and quantitative profiling of individual phenolic acids in *M. zapota* peel and pulp was carried out using high-performance liquid chromatography (HPLC). Analysis was performed on a Varian HPLC system equipped with a reversed-phase C18 column (250 mm × 4.6 mm). An isocratic elution was conducted using an acidified acetonitrile-water mobile phase at a constant flow rate of 1.0 mL/min. Injection volume was maintained at 20 µL. Detection of phenolic acids was achieved at 280 nm, and identification was based on retention time comparison with known reference standards. Quantification of individual phenolic acids was determined using external calibration curves generated from pure standards.

Antioxidant Activity Assessment by DPPH Assay: The antioxidant activity of each extract was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, following the methodology described by Zhuang et al. Briefly, 1 mL of extract (concentrations ranging from 0.25 to 2 mg/mL) was mixed with 2 mL of 0.1 mM DPPH methanolic solution. The volume was adjusted to 4 mL with methanol, and the mixtures were incubated in the dark for 30 minutes at room temperature. The decrease in absorbance was measured at 515 nm. Butylated hydroxyanisole (BHA) was used as the positive control. The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging Activity} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

where $A_{control}$ represents the absorbance of the DPPH solution without the sample, and A_{sample} represents the absorbance of the sample. The concentration of extract required to achieve 50% inhibition of DPPH radicals (IC_{50}) was determined graphically.

Statistical Analysis: All experimental measurements were performed in triplicate, and data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to assess the statistical significance of differences among sample groups, with significance considered at $P < 0.05$. Post hoc analysis was conducted where applicable. Statistical analyses were performed using IBM SPSS Statistics (version 25.0, IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

This study comprehensively investigated the phenolic composition and antioxidant potential of Manilkara zapota (sapodilla) peel and pulp extracts. A strategic combination of extraction techniques orbital shaking and magnetic stirring along with solvents of varying polarity (absolute and aqueous ethanol and methanol), was employed to systematically evaluate extract yields, total phenolic content (TPC), total flavonoid content (TFC), individual phenolic acid profiles, and antioxidant activity using the DPPH radical scavenging assay. The experimental framework was designed to maximize the retrieval of bioactive constituents and to establish scientifically robust correlations between phytochemical composition and antioxidant efficacy.

Extract Yields: The yield of bioactive compounds from M. zapota peel and pulp was significantly influenced by the polarity of the solvent and the extraction technique employed. Consistent with phytochemical extraction principles, methanol particularly in its aqueous form (80%) demonstrated superior extractive capacity relative to ethanol. The extract yields ranged from 19.82% to 47.34%,

with the highest yield (47.34%) observed for pulp extracts using 80% methanol with magnetic stirring, and the lowest (19.82%) for peel extracts using 100% ethanol with orbital shaking.

Table 3.1: Extract Yields (g/100g) from Peel and Pulp of Sapodilla in Different Solvents

Sample Nature	**100% Methanol**	**80% Methanol**	**100% Ethanol**	**80% Ethanol**
Peel shaker	40.25 ± 1.02	44.18 ± 1.68	39.81 ± 0.93	21.52 ± 1.03
Pulp shaker	37.34 ± 0.98	47.34 ± 1.22	17.86 ± 1.53	29.56 ± 0.47
Peel stirrer	43.25 ± 0.42	37.35 ± 2.01	38.54 ± 0.68	19.51 ± 0.26
Pulp stirrer	39.15 ± 2.12	35.89 ± 0.54	32.25 ± 0.35	27.24 ± 0.11

Mean values ± SD (n = 3)

The extraction efficiency followed the trend: 80% methanol > 100% methanol > 80% ethanol > 100% ethanol. Magnetic stirring consistently provided higher extraction efficiency than orbital shaking. These findings align with previous studies by Vijay and Kothari, confirming that extraction dynamics, climatic variations, and solvent polarity are critical determinants in maximizing phytochemical recovery.

Total Phenolic Content (TPC): Phenolic compounds are widely recognized for their significant contribution to the antioxidant capacity of plant-based materials due to their ability to neutralize free radicals through redox mechanisms. The TPC of M. zapota extracts ranged from 17.50 to 53.21 mg GAE/g dry weight. The highest TPC was recorded in the pulp extract derived from 100% methanol using magnetic stirring, while the lowest TPC was associated with the peel extract in 100% ethanol using orbital shaking.

Table 3.2: Total Phenolic Content (GAE mg/g DW) from Peel and Pulp of Sapodilla in Different Solvents

Sample Nature	***100% Methanol***	***80% Methanol***	***100% Ethanol***	***80% Ethanol***
Peel stirrer	***33.21 ± 0.15***	***30.89 ± 0.07***	***24.87 ± 0.21***	***39.32 ± 0.14***
Pulp stirrer	***53.21 ± 0.19***	***45.29 ± 0.24***	***35.17 ± 1.05***	***35.79 ± 0.09***
Peel shaker	***21.85 ± 0.25***	***20.86 ± 0.13***	***17.50 ± 0.09***	***27.88 ± 0.50***
Pulp shaker	***41.23 ± 0.11***	***37.53 ± 0.12***	***31.58 ± 0.18***	***22.97 ± 0.29***

Mean values ± SD (n = 3)

These findings corroborate with reports by Sultana et al. and Hegazy and Ebraheem, who demonstrated enhanced phenolic recovery with aqueous methanol systems. The superior phenolic content in pulp extracts highlights M. zapota as a promising nutritional source of antioxidant phytochemicals with applications in functional foods.

Phenolic Acid Profile: High-performance liquid chromatography (HPLC) analysis provided detailed characterization of individual phenolic acids in M. zapota peel and pulp extracts. Thirteen phenolic acids were assessed, including gallic acid, syringic acid, ferulic acid, vanillic acid, chlorogenic acid, caffeic acid, sinapic acid, and quercetin. The concentration of individual phenolic acids varied across different solvent systems, with total amounts ranging from 10.62 to 50.18 mg/kg.

Table 1: Individual Profile of Phenolic Acids (mg/kg) for Peel and Pulp Extracts of Sapodilla (Excerpt)

Sample Nature	**Total Phenolic Acids (mg/kg)**
Peel, 80% MeOH	35.97
Pulp, 80% MeOH	23.20
Peel, 100% EtOH	42.45
Pulp, 100% MeOH	38.69

The predominance of gallic acid, quercetin, and chromatotrophic acid in these extracts is of significant biological relevance. Their well-documented anticancer, neuroprotective, and anti-inflammatory activities highlight the functional potential of M. zapota extracts.

Total Flavonoid Content (TFC): Flavonoids represent another essential class of plant secondary metabolites contributing to the antioxidant potential and therapeutic versatility of M. zapota. The TFC of the extracts ranged from 4.81 to 24.56 mg CE/g dry weight. The maximum flavonoid content was recorded in pulp extracts obtained with 80% methanol via magnetic stirring, while the lowest was found in peel extracts extracted with 100% ethanol using the same technique.

Table 3.4: Total Flavonoid Content (CE mg/g DW) from Peel and Pulp of Sapodilla in Different Solvents

Sample Nature	**100% Methanol**	**80% Methanol**	**100% Ethanol**	**80% Ethanol**
Peel stirrer	9.2 ± 0.09	13.27 ± 0.11	4.81 ± 0.17	10.07 ± 0.02
Pulp stirrer	11.41 ± 0.12	16.57 ± 0.03	7.89 ± 0.06	12.29 ± 0.32
Peel shaker	17.22 ± 0.23	21.10 ± 0.01	15.59 ± 0.13	18.71 ± 0.16
Pulp shaker	22.16 ± 0.04	24.56 ± 0.07	16.17 ± 0.08	20.78 ± 0.22

Mean values ± SD (n = 3)

These results corroborate earlier findings and affirm that aqueous methanol is particularly effective for flavonoid recovery from plant matrices. The substantial flavonoid content in both peel and pulp extracts substantiates the traditional medicinal use of M. zapota.

Antioxidant Activity (DPPH Radical Scavenging Assay): The antioxidant potential of M. zapota peel and pulp extracts was assessed using the DPPH radical scavenging assay. The IC50 values for the extracts ranged from 1.05 to 5.68 mg/mL. The lowest IC50, indicative of the strongest antioxidant activity, was observed in the peel extract obtained with 80% methanol using the orbital shaker, while the highest IC50 was recorded for the pulp extract with 100% ethanol using magnetic stirring.

Table 3.5: Free Radical Scavenging Activity by DPPH (IC50 mg/mL) from Peel and Pulp of Sapodilla in Different Solvents

Sample Nature	**100% Methanol**	**80% Methanol**	**100% Ethanol**	**80% Ethanol**
Peel stirrer	1.58 ± 0.04	1.32 ± 0.09	1.68 ± 0.21	2.54 ± 0.11
Pulp stirrer	2.88 ± 0.06	3.94 ± 0.05	5.68 ± 0.07	3.21 ± 0.04
Peel shaker	1.95 ± 0.01	1.05 ± 0.03	4.26 ± 0.30	3.48 ± 0.16
Pulp shaker	3.74 ± 0.24	2.69 ± 0.01	2.59 ± 0.02	1.48 ± 0.08

Mean values ± SD (n = 3)

The antioxidant activity trend of 80% methanol > 80% ethanol > 100% methanol > 100% ethanol further supports the hypothesis that phenolic-rich extracts exhibit superior radical scavenging efficiency. These findings substantiate the functional relevance of M. zapota peel and pulp extracts in mitigating oxidative stress-related pathologies and promoting human health.

Extracts	Sample Nature	Phenolic Acids (mg/kg)													Total phenolic acids amount (mg/kg)
		Gallic Acid	Caffic Acid	Chlorogenic Acid	Quercitin	Sinapic Acid	Syringic Acid	Chromatographic	Ferulic Acid	Cinnamic	Vanillic Acid	4-hydroxy – 3 –	p- coumeric Acid	m- coumeric Acid	
100 % methanol	Peel shaker	7.5	5.5	-	0.47	3.6	-	7.02	-	-	8.06	-	2.5	1.24	35.89
	Pulp shaker	7.5	-	-	0.58	-	-	9.08	6.08	3.65	-	2.3	0.94	-	30.13
	Peel stirrer	5.04	-	6.3	1.74	-	-	4.66	-	-	-	2.6	-	1.12	21.46
	Pulp stirrer	6.4	-	-	1.85	-	-	14.4	-	-	2.9	13.14	-	-	38.69
80% methanol	Peel shaker	3.25	-	-	0.54	-	-	11.9	8.2	8.39	-	15.64	1.18	1.08	50.18
	Pulp shaker	6.3	5.42	-	0.5	2.37	-	6.68	4.75	-	-	-	1.15	-	27.17
	Peel stirrer	2.7	-	6.24	1.3	-	1.65	6.19	10.3	7.59	-	-	-	-	35.97
	Pulp stirrer	6.02	-	-	2.22	1.43	0.95	9.08	-	-	3.5	-	-	-	23.20
100 % ethanol	Peel shaker	4.06	-	4.9	3.6	-	1.3	26.8	-	-	-	-	1.19	0.6	42.45
	Pulp shaker	2.15	-	-	1.53	1.15	2.15	10.3	-	2.4	-	8.7	3.17	-	31.55
	Peel stirrer	4.23	3.4	-	0.35	0.75	-	5.2	3.38	-	8.9	-	0.48	-	26.69
	Pulp stirrer	5.6	-	-	0.3	5.5	-	0.12	18.5	-	-	-	0.76	-	30.78

80% ethanol	Peel shaker	0.64	4.9	-	0.04	-	-	1.2	9.2	2.48	-	7.4	-	-	25.86
	Pulp shaker	0.86	1.19	-	0.56	1.01	1.15	3.7	0.28	1.14	-	-	-	0.73	10.62
	Peel stirrer	4.8	-	-	0.35	-	0.84	6.19	-	4.02	-	-	-	-	16.21
	Pulp stirrer	4.17	-	-	2.12	0.89	-	21.8	-	-	-	7.7	0.55	-	37.23

Table 1: Individual Profile of Phenolic Acids (mg/kg) for peel and pulp extracts of sapodilla.

DISCUSSION

The efficiency of phytochemical extraction is contingent upon the polarity of the solvent, the nature of the plant matrix, and the operational extraction conditions. Consistent with established phytochemical extraction principles, methanol owing to its high polarity demonstrated superior extraction capacity relative to ethanol, particularly when used in aqueous form (80%) [19]. The extraction yield for *M. zapota* peel and pulp varied from 19.82% to 47.34% across different solvent systems and techniques. Notably, the highest yield (47.34%) was achieved with 80% methanol using the magnetic stirring technique, whereas the lowest yield (19.82%) was obtained with 100% ethanol using the orbital shaker. Comparative analysis revealed the solvent efficacy trend as 80% methanol > 100% methanol > 80% ethanol > 100% ethanol. Additionally, the extraction technique significantly influenced yield outcomes, with magnetic stirring outperforming orbital shaking. The enhanced extraction observed with aqueous methanol is attributed to the optimal balance of solvent polarity, facilitating superior solubilization of polar phenolic constituents. Statistical analysis confirmed significant differences ($p < 0.05$) in extraction yields among solvent systems and techniques. These findings are consistent with reports by Vijay [20] and extend upon the observations by Kothari [58], further corroborating the influence of solvent polarity, extraction dynamics, and fruit matrix characteristics on phytochemical recovery [21,22]. Phenolic compounds are recognized as principal contributors to the antioxidant potential of plant matrices due to their inherent radical scavenging capabilities and redox behavior [23]. Using the Folin-Ciocalteu colorimetric assay, the total phenolic content of *M. zapota* extracts ranged from 17.50 to 53.21 mg GAE/g dry weight. The lowest TPC value was observed in the peel extract derived using 100% ethanol with orbital shaking, whereas the maximum TPC was recorded in the pulp extract obtained with 100% methanol using magnetic stirring. A comparative evaluation revealed that aqueous solvents (80% methanol and ethanol) consistently facilitated higher phenolic recovery than their absolute counterparts, with methanol demonstrating superior performance. This aligns with previous investigations by Sultana et al. [24] and Hegazy and Ebraheem [25], who reported enhanced phenolic recovery with aqueous ethanol and methanol systems. The variability in TPC across extraction systems can be attributed to solvent polarity, molecular affinity for phenolics, and the differential solubility of phenolic subclasses present in the fruit matrix. The higher phenolic content in pulp extracts, particularly those obtained with methanol, underscores the nutritional potential of *M. zapota* pulp as a source of antioxidant compounds. These findings provide further evidence supporting the inclusion of sapodilla-derived products in functional food formulations aimed at mitigating oxidative stress-related pathologies [26]. High-performance liquid chromatography (HPLC) analysis provided detailed insights into the individual phenolic acid composition of *M. zapota* peel and pulp extracts (Table 1). A total of thirteen phenolic acids were assessed, including gallic acid, syringic acid, ferulic acid, vanillic acid, chlorogenic acid, caffeic acid, sinapic acid, and quercetin. The concentration of individual phenolic acids varied substantially across solvents and extraction techniques, with total amounts ranging from 10.62 mg/kg to 50.18 mg/kg. Chromatotrophic acid, gallic acid, and quercetin were consistently

detected in all extracts. The peel extracts exhibited higher concentrations of chromatotrophic acid, particularly in the 100% ethanol extract from the orbital shaker (26.8 mg/kg). Quercetin was most abundant in pulp extracts derived from magnetic stirring using 100% methanol, while gallic acid displayed a broad distribution across both peel and pulp extracts. The predominance of phenolic acids such as gallic acid and quercetin in the extracts is of significant biological relevance. Gallic acid is widely recognized for its anticancer, anti-inflammatory, and neuroprotective properties, particularly in modulating pathways associated with neurodegenerative diseases such as Alzheimer's and Parkinson's diseases [27,28,29]. Additionally, its role in inhibiting amyloid aggregation through modulation of α-synuclein confirms its therapeutic promise. Similarly, quercetin exhibits potent antiviral activity, notably against hepatitis C virus (HCV), and possesses bronchodilatory effects that mitigate inflammatory responses [30,31]. The presence of p-coumaric acid, reported to inhibit carcinogenic nitrosamine formation and confer gastroprotective effects [83], further reinforces the health-promoting attributes of *M. zapota* extracts.

Total Flavonoid Content (TFC): Flavonoids are integral constituents of plant antioxidant defense systems and contribute substantially to their pharmacological activities, including antimicrobial, anti-inflammatory, and anti-carcinogenic effects [32]. The total flavonoid content (TFC) of *M. zapota* extracts, expressed as mg catechin equivalents (CE) per gram of dry weight, ranged from 4.81 to 24.56 mg CE/g DW. The maximum TFC was recorded in pulp extracts extracted with 80% methanol using magnetic stirring, while the lowest TFC was found in peel extracts obtained with 100% ethanol using magnetic stirring. The order of flavonoid extraction efficiency across solvents was 80% methanol > 100% methanol > 80% ethanol > 100% ethanol, corroborating trends observed for TPC. The preferential extraction of flavonoids using aqueous methanol reflects their moderate polarity and improved solubility in mixed solvent systems. Statistical analysis indicated significant differences in TFC values among extraction methods ($p < 0.05$). These results are congruent with findings from Hegazy and Ebraheem [33] and Ghasemi et al. [Ghasemi et al., 2009], further validating the optimized extraction of flavonoids using methanolic systems. The substantial flavonoid content identified in *M. zapota* extracts affirms its traditional use in managing inflammation, gastrointestinal disorders, and oxidative stress-mediated cellular damage.

The DPPH free radical scavenging assay is widely employed for evaluating the antioxidant potential of plant extracts, owing to its simplicity, reproducibility, and relevance in free radical neutralization assessments [34]. In this study, the IC_{50} values of *M. zapota* extracts, representing the concentration required to scavenge 50% of DPPH radicals, ranged from 1.05 to 5.68 mg/mL. The lowest IC_{50} (indicative of the highest antioxidant activity) was observed in the peel extract using 80% methanol with the orbital shaker, while the highest IC_{50} was recorded for the pulp extract in 100% ethanol with magnetic stirring. The antioxidant potential followed the trend 80% methanol > 80% ethanol > 100% methanol > 100% ethanol. This pattern supports the hypothesis that phenolic-rich extracts, particularly those obtained with aqueous methanol, exhibit superior radical scavenging efficiency. These findings are in agreement with prior reports by Sultana et al. [35] and Kalpana et al. [80], reinforcing the established correlation between phenolic content and antioxidant activity [36,37]. The strong antioxidant potential demonstrated by *M. zapota* peel and pulp extracts substantiates their application in functional foods and nutraceutical formulations designed to counteract oxidative stress-induced pathologies. The comprehensive analysis of *M. zapota* demonstrated that the peel and pulp are rich sources of phenolic and flavonoid compounds with considerable antioxidant potential. The study underscores the superior efficacy of 80% methanol as an extraction solvent, particularly when combined with magnetic stirring, for maximal recovery of bioactive phytochemicals. The findings strongly advocate for the valorization of *M. zapota* particularly its peel, often discarded as agro-industrial waste as a valuable source of natural antioxidants for applications in food, pharmaceutical, and cosmetic industries.

CONCLUSION

The present study comprehensively elucidated the phenolic composition and antioxidant potential of *Manilkara zapota* (sapodilla) peel and pulp, employing optimized extraction methodologies and a

multifaceted analytical approach. The findings demonstrated that both the peel and pulp of *M. zapota* are significant reservoirs of phenolic acids and flavonoids, contributing substantially to their overall antioxidant potential. Among the various solvent systems utilized, 80% methanol in conjunction with magnetic stirring consistently yielded the highest extractable bioactive compounds, as evidenced by superior total phenolic content (TPC), total flavonoid content (TFC), and potent free radical scavenging activity indicated by lower IC_{50} values in the DPPH assay. Notably, the phenolic acid profiling by HPLC revealed the consistent presence of therapeutically relevant compounds such as gallic acid, quercetin, and p-coumaric acid, further reinforcing the medicinal value of this tropical fruit. The superior antioxidant activity observed, particularly in the pulp extracts, positions *M. zapota* as a promising candidate for incorporation into nutraceutical, pharmaceutical, and functional food products. Furthermore, the data advocate for the valorization of sapodilla peel, traditionally considered agro-industrial waste, as an untapped source of potent natural antioxidants with potential applications in oxidative stress mitigation and chronic disease prevention.

Future Recommendations

While the current study has successfully characterized the antioxidant potential and phenolic profile of *M. zapota* extracts, several avenues remain open for further exploration. Future investigations should be directed toward *in vivo* assessments of the bioavailability, metabolic pathways, and pharmacokinetics of the identified phenolic constituents to corroborate their physiological relevance in biological systems. Moreover, advanced analytical techniques such as LC-MS/MS and NMR spectroscopy should be employed for a more comprehensive characterization of minor and structurally complex phenolic compounds. The exploration of synergistic interactions between different phytochemical classes present in *M. zapota*, particularly between flavonoids and phenolic acids, would provide deeper insights into their cumulative therapeutic potential. Additionally, the potential application of encapsulation or nano-formulation techniques could be examined to enhance the stability and targeted delivery of *M. zapota* bioactives in functional food and pharmaceutical formulations. Industrial-scale extraction feasibility studies, combined with cost-benefit analyses, would further facilitate the translation of these laboratory findings into commercially viable antioxidant formulations. Ultimately, leveraging the full potential of *M. zapota* requires a multidisciplinary approach integrating food chemistry, pharmacology, biotechnology, and product development sciences.

Conflict of Interest

The authors declare no conflict of interest related to this study

REFERENCES

1. Gordon M. H., Pokorný J., Yanishlieva N., Measuring antioxidant activity. Antioxidants in foods,a practical application, 2001, 73-84.
2. Lachance P.A., Nakat Z., Jeong W.S., Antioxidants: an integrative approach. 2001, 17, 835-838
3. Yanishlieva N., Pokorný J., Inhibiting oxidation. Antioxidants in food,practical application. 2001, 22-70.
4. Huang D., Ou B., Prior R.L., The chemistry behind antioxidant capacity assays. Journal of Agricultural & Food Chemistry, 2005, 53, 1841-1856.
5. Pokorný J, Korczak J., Preparation of natural antioxidants Antioxidants in food, practical application. 2001, 311-341.
6. Decker E.A., Warner K., Richards M.P., Shahidi F., Measuring antioxidant effectiveness in food. Journal of Agricultural & Food Chemistry, 2005, 53:4303-10.
7. Gordon .M.H, Pokorný .J, Yanishlieva N., Measuring antioxidant activity .Antioxidants in food: practical application. 2001, 73-84.
8. Schwarz K, Bertelsen G, Nissen LR, Gardner PT, Heinonen MI, Tijburg L., Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant

assays based on radical scavenging, lipid oxidation and analysis of the principal antioxidant compounds. European Food Research Technology, 2001, 212:319-328.
9. Gordon M.H., Measuring antioxidant activity. Antioxidants in food: practical application. 2001, 73-84.
10. Decker EA, Warner K, Richards MP, Shahidi F. Measuring antioxidant effectiveness in food. Journal of Agricultural & Food Chemistry 2005, 53, 4303-4310.
11. Pokorný J., Korcza. J., Preparation of natural antioxidants. Antioxidants in foods, practical application. 2001, 311-341.
12. Barlow S.M., Toxicological aspects of antioxidants used as food additives. . Food Antioxidants. 1990, 253-307.
13. Shi H, Noguchi N, Niki E. Introducing natural antioxidants. Antioxidants in food, practical application: 2001, 147-158.
14. Barlow S.M., Toxicological aspects of antioxidants used as food additives. Food antioxidants. 1990, 251-309.
15. Wanasundara U.N., Shahidi F., Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chemistry 1998, 63, 335-342.
16. Pratt D, Hudson BJF., Natural antioxidants not exploited commercially. Food antioxidants, 1990, 171-191.
17. Block G. Vitamin-C, cancer and aging. 1990, 16, 55-58.
18. Delgado-V. F.,. Jimenez A. R , and Paredes-Lopez. O., Natural pigments: carotenoids, anthocyanins, and betalains characteristics, biosynthesis, processing, and stability. Critical reviews in food science and nutrition. . 2000, 40 (3), 173-289.
19. Zhishen, J; Mengchemg, T; Jianming, W ; The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chemistry, 1999, 64, 555-559.
20. Kalpana, R; Mital, K; Sumitra, C; Vegetable and fruit peels as a novel source of antioxidants, Journal of Medicinal plants Research, 2011, 5(1), 63-71.
21. Sajid, Z. I; Anwar, F; Rasul, G; Alkharfy, K. M; Gilani, A. H; Antioxidant, antimicrobial properties and phenolics of different solvent extracts from barks, leaves and seeds of Pongamia pinnata (L.) pierre, Molecules,2012, 17, 3917-3932.
22. Zhuang, Y; Chen, L; Sun, L; Cao, J; Bioactive characteristics and antioxidant activities of nine peppers, Journal of Functional Foods, 2012, 4, 331-338.
23. Sajid, Z. I; Anwar F; Rasul G; Alkharfy K. M; Gilani A. H; Antioxidant, antimicrobial properties and phenolics of different solvent extracts from barks, leaves and seeds of Pongamia pinnata (L.) Molecules, 2012, 17, 3917-3932.
24. Kothari. V; Seshadri . S; In vitro antibacterial activity in seed extracts of Manilkara zapota, Anona squamosa, and Tamarindus indica. Biological Research, 2010, (43)2, 165-168.
25. Wetzstein, H.Y. Z; Zangh, N. R; Wetzstein, M.E; Characterization of Attributes Related to fruit size in Pomegranate. Horticultural Science, 2011, 46(6), 908-912.
26. Karadeniz, F; Burdurlu, H. S; Koca, N; Soyer, Y; Antioxidant potential of fruits and Vegetables grown in Turkey, Turkish Journal of Agriculture, 2005, 29, 297-303.
27. Akram, N; Anwar, F; Raza, S. A; Effects of Sunlight exposure on the quality and oxidative stability of sunflower and soybean oils, Asian Journal of Chemistry, 2009, 21(4), 2789-2798.
28. Liu, Y; Pukala, T. L.; Musgrave, I. F.; Williams, D. M.; Dehle, F. C.; Carver, J. A.; Gallic acid is the major component of grape seed extract that inhibits amyloid fibril formation. Bio-organic & Medicinal Chemistry, 23 (23): 6336–6340.
29. Wang, Y. J.; Thomas, P; Zhong, J. H.; Bi, F. F.; Kosaraju, S; Pollard, A; Fenech, M; Zhou, X. F; Consumption of grape seed extract prevents amyloid-beta deposition and attenuates inflammation in brain of an Alzheimer's disease mouse. Neurotoxicity Research, 2013, 15 (1), 03–14.
30. Liu, Y; Carver, J. A.; Calabrese, A. N; Pukala, T. L. (2014). Gallic acid interacts with α-synuclein to prevent the structural collapse necessary for its aggregation. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2014, 1844 (9), 1481–1485

31. Pisonero V, S; García-M. M,V; Jorquera F; Majano P, L; Benet M; Jover R; González .G, J; Sánchez C, S; Modulation of PI3K-LXRα-dependent lipogenesis mediated by oxidative/nitro sative stress contributes to inhibition of HCV replication by quercetin. Laboratory Investigation, 2014, 94 (3), 262–274.
32. Asthma. University of Maryland Medical Center, 2013.
33. Li B. B; Smith B; Hossain H; Separation and purification on technologies, 2013, 48(2) , 182-188.
34. Mokbel M, S; Hashinaga F; Evaluation of the antioxidant potential of extracts from buntan (Citrus grandis osbeck) fruit tissues. Food Chemistry, 2006, 94, 529-534.
35. Siddhuraja P. Becker K; Antioxidant properties of various solvents of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam) leaves. Journal of Agriculture and Food Chemistry, 2003, 51, 2144-2155.
36. Sultana B ;Anwar F; Przybylski R; Antioxidant potential of corncob extracts for stabilization of corn oil subjected to microwave heating, Food Chemistry, 2007,104, 997-1005.
37. Hegazy A , E ; Ibraheem M , I ; Antioxidant activities of orange peel extracts, World of Applies Sciences Journal, 2012, 18(5), 684-688.