Comparative Studies on Antioxidant and Anticancer Activities of Leaves and Fruit's *Manilkara zapota* (L.)

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Abstract

*Manilkara zapota (*L.*)* (sapota), a member of the Sapotaceae family, is highly regarded for its traditional and medicinal value, being used across various cultures to treat a wide range of ailments. This study aims to comprehensively investigate the qualitative and quantitative properties, antioxidant activities, and potential anticancer effects of *M. zapota* extracts. *Manilkara zapota (*L.*)* (sapota) leaves and fruit were collected from local market, then carefully dried, ground into powder, and subjected to aqueous extraction. Qualitative analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, anthraquinones, and polyphenols. Notably, tannins were absent in *M. zapota* leaves extract but present in fruit extract, while glycosides were detected only in the leaves extract. Quantitative analysis showed significantly higher levels of total flavonoids and phenolics in *M. zapota* fruit extracts compared to leaves extract. FT-IR analysis identified the functional groups present in the extracts. Further, free radical scavenging assays, including the DPPH and reducing power assay, demonstrated superior antioxidant activity in *Manilkara zapota (*L.*)* fruit extract compared to leaves extract. Additionally, the anticancer potential against breast cancer cell lines was assessed, revealing significant antiproliferative effects in the *Manilkara zapota (*L.*)* (sapota) fruit extract. Overall, the findings highlight the strong antioxidant and anticancer properties of *Manilkara zapota (*L.*)* fruit extract in comparison to its leaves extract.

Keywords: *Manilkara zapota (*L.*)* - sapota - flavonoids - phenolics - antioxidant activity - anticancer properties.

دراسات مقارنة على الأنشطة المضادة للأكسدة والمضادة للسرطان لأوراق وثمار فاكهة السابوتا Manilkara zapota (L)

الملخص العربي

فاكهه السابوتا Manilkara zapota **(L)** والتى تعتبر من عائلة Sapotaceae، تحظى بتقدير كبير نظرا لقيمتها التقليدية والطبية، حيث يتم استخدامها في مختلف الثقافات لعلاج مجموعة واسعة من الأمراض. تهدف هذه الدراسة إلى إجراء تحقيق في الخصائص النوعية والكمية، وأنشطة مضادات الأكسدة، والتأثيرات المحتملة المضادة للسرطان لمستخلصات السابوتا M. zapota حيث تم جمع الأوراق وفاكهة السابوتا من السوق المحلي ، ثم تم تجفيفها بعناية، وطحنها إلى مسحوق، وإخضاعها

للاستخلاص المائي. وقد كشف اجراء التحليل النوعي عن وجود القلويدات ، الفلافونويدات، الصابونين، التانين، التيربينويدات ،الأنثراكينونات والبوليفينول. ومن الجدير بالذكر أن مادة التانين كانت غائبة في مستخلص الأوراق لفاكهة السابوتا ولكنها موجودة في مستخلص الفاكهة، في حين تم الكشف عن الجليكوسيدات في مستخلص الأوراق فقط. كما أظهر التحليل الكمي مستويات أعلى بكثير من إجمالي مركبات الفلافونويد والفينولات في مستخلصات فاكهة السابوتا مقارنة بمستخلص الأوراق. كما تم استخدام تحليل FT-IR لتحديد المجموعات الوظيفية الموجودة في المستخلصات. وقد أظهرت فحوصات مسح الجذور الحرة، بما في ذلك DPPH نشاطًا مضادًا للأكسدة متفوقًا في مستخلص فاكهة السابوتا مقارنة بمستخلص الأوراق. بالإضافة إلى ذلك، تم تقييم القدرة المضادة للسرطان ضد خطوط خلايا سرطان الثدي، حيث كشف عن تأثيرات كبيرة مضادة لتكاثر الخلايا السرطانية لمستخلص الفاكهة. بشكل عام، تسلط النتائج الضوء على الخصائص القوية المضادة للأكسدة والمضادة للسرطان لمستخلص فاكهة السابوتا Manilkara zapota **(L)** مقارنة بمستخلص أوراقها.

الكلمات المفتاحية: Manilkara zapota **(L)**– السابوتا– فلافونيدات– فينولية– النشاط المضاد للأكسدة– خصائص مضادة للسرطان.

Introduction

The global demand for studying plants has witnessed a steady increase over the past few decades, owing to their abundance in diverse and potent chemical compounds with medicinal properties. The potential of plants in the discovery of novel remedies against emerging and existing diseases. Their significant pharmacological efficacy, minimal toxicity profile, and economic viability have prompted extensive exploration into their medicinal attributes (Rai et al., 2008). In addition to their nutritional value, plants play a crucial role in mitigating oxidative damage by impeding lipid peroxidation through various mechanisms, including scavenging free radicals and eliciting antioxidant enzyme activity (Nirmala and Ramanathan, 2011).

Free radicals are implicated in the root cause of numerous fatal ailments such as cancer, diabetes, heart failure, and stroke (Pham-Huy et al., 2008). Furthermore, the indiscriminate use of commercial antioxidant and anticancer agents has led to the emergence of multiple drug resistance and various adverse effects, including hypersensitivity reactions, immune suppression, abdominal discomfort, and anorexia. This predicament necessitates the exploration of novel and efficacious antioxidant and anticancer agents as substitutes for existing therapies (Dharini et al., 2010).

Despite their myriad side effects, chemotherapeutic drugs remain the cornerstone of cancer treatment. However, given the gravity of the situation, researchers are actively investigating natural medicinal resources as adjuncts or alternatives to conventional chemotherapeutic protocols (Kooti et al., 2017). Moreover, phytochemicals possessing antioxidant properties have exhibited potential in inhibiting carcinogenesis (Zhang et al., 2015).

*Manilkara zapota (*L.*)* , belonging to the family Sapotaceae, is a versatile tree species known for its edible fruits and medicinal properties. It is commonly cultivated in tropical and subtropical regions, thriving in diverse habitats ranging from coastal plains to foothills and river valleys. Despite its widespread occurrence, the medicinal potential of *Manilkara zapota (*L.*)*, remains underexplored, presenting opportunities for further research and development of plant-based therapeutics (Bhattacharya & Kaur, 2016). *Manilkara zapota (*L.*)* is known by various names such as sapota, Chiko, naseberry and sapodilla in American, Asian, and European countries Chandrasekaran. It is often cultivated in home gardens, orchards, and agroforestry systems, where it serves as a valuable source of nutritious fruits and timber. The tree prefers well-drained, sandy loam soils and thrives in regions with adequate rainfall and sunlight (Gomathy et al., 2013).

The various parts of *Manilkara zapota (*L.*)* , including the fruit, leaves, bark, and seeds, possess pharmacologically active compounds with diverse medicinal properties. The fruit, characterized by its sweet and flavorful pulp, is rich in vitamins, minerals, and dietary fibers, making it a nutritious addition to the diet (Bashir et al., 2019). These nutritional components contribute to various health benefits, such as supporting immune function, promoting healthy skin and vision, and aiding digestion. The high fiber content also helps in regulating bowel movements and preventing constipation (Katsirma et al., 2021). Additionally, it exhibits antioxidant, anti-inflammatory, and antimicrobial activities, which may confer health benefits such as improved immune function and protection against infectious diseases (Chunhakant and Chaicharoenpong, 2019). The leaves of *Manilkara zapota (*L.*)* have been traditionally used for treating ailments such as diarrhea, dysentery, and respiratory infections. They contain bioactive compounds with antidiabetic, antihypertensive, and analgesic properties, which hold promise for the development of novel therapeutics. Furthermore, the bark and seeds of *Manilkara zapota (*L.*)* , contain secondary metabolites with antimalarial, antifungal, and anticancer activities, suggesting their potential in combating various diseases (Bashir et al., 2019).

To our knowledge from the literatures, there was no information about the comparative study on the phytochemical contents and biological activities of *Manilkara zapota (*L.*)* and its relationship to breast cells cytotoxicity. In this work, phytochemical contents, biological activities and cytotoxicity of *Manilkara zapota (*L.*)* from leaves and fruit were assessed, evaluated and compared.

Materials and Methods:

Materials.

All the chemical reagents used in this experiment were of analytical grade purchased from Loba Chemicals, India.

Fresh *Manilkara zapota (*L.*)* (sapota) leaves and fruits were collected in May 2023 from a local market in Saudi Arabia.

Methods.

Preparation of Aqueous Extract .

The leaves and fruit were thoroughly washed with distilled water to remove any external particulate matter and seeds. Subsequently, the plant materials were shade-dried until they reached a clean and dry state and then ground separately into a fine powder using an electric grinder. Each powder was meticulously preserved in a sealed glass container, protected from light exposure, to uphold its quality for future utilization. Around 10 grams of the powdered substance were placed into 100 ml of distilled water. The mixture was vigorously stirred using a magnetic stirrer at a constant temperature for 20 minutes (Mendonca et al., 2001). After this period, the mixture was filtered using Whatman filter paper to separate the solid remnants from the liquid filtrate. The collected filtrate was then promptly refrigerated at 4°C to preserve its chemical composition for further experimental procedures (Saranyaadevi et al., 2014).

Phytochemical Screening.

Investigation for alkaloids, glycosides, flavonoids, phenols, saponins, tannins and terpenoids, as well as total phenolic contents were conducted for plant extracts following established protocols as documented in previous studies. Harborne (1973), Trease and Evans (1989) and Sofowora (1993).

Gas Chromatography Mass Spectrometry (GC-MS) Analysis.

The methodology outlined by Dawra et al. (2021) was followed to identify the volatile compounds in the aqueous extracts of both leaves and fruit**.**

FT-IR analysis.

The FT-IR spectrophotometer was utilized to obtain the FT-IR spectrum of the sample at room temperature, covering the spectral range from 400 to 4000 cm-1.

DPPH Free Radical Scavenging Potential.

The methodology mentioned by Koleva et al., (2001) was employed to assess the antioxidant potential of plant extracts, focusing on their ability to scavenge or transfer hydrogen/electrons against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). A volume of 0.1ml of extract (5-100 µg/ml) was mixed with 3ml of freshly prepared 0.001M DPPH in methanol. After a 30-minute incubation time, the sample absorbance was done at 517 nm with the help of a UV-visible spectrophotometer (Shimadzu, UV-2450). The radical scavenging activity was determined as % inhibition using the following formula. The % Inhibition was calculated using the formula [(Ao - Ae)/Ao] × 100, where Ao represents the absorbance without the extract and Ae represents the absorbance with the extract or standard. Gallic acid served as the standard antioxidant chemical in this study.

Determination of Ferric Reducing Ability Power (FRAP).

The evaluation of the reducing capacity of the plant extracts followed the methodology proposed by Oyaizu, (1986). A mixture comprising plant extract (5-100 µg) in 1ml of distilled water, 2.5 ml of 0.2 molar phosphate buffer (pH

6.6), and 2.5 ml of 1% potassium ferricyanide was prepared. This mixture was placed at 50°C for 20 minutes. After the period of incubation, the next step involved the addition of 2.5 ml of a solution containing 10% trichloro acetic acid and the resultant mixture was centrifuged at 1800 rpm for 10 minutes. Subsequently, supernatant with the volume of 2.5ml was mixed with 2.5 ml of distilled water and 0.5 ml of a solution containing 0.1% FeCl3. The absorbance at 700 nm was then measured using a UV-visible spectrophotometer (Shimadzu, UV-2450).

The reducing activity was determined as % inhibition using the equation:

% Inhibition = [(Ao - Ae)/Ao] × 100

Where Ao represents the absorbance without the extract and Ae represents the absorbance with the extract or standard. Ascorbic acid served as the standard antioxidant compound in this analysis.

Cytotoxicity test for plant extracts (MTT Assay).

The evaluation of cell cytotoxicity for *Manilkara zapota (*L.*)* fruit extracts on breast cancer cell lines (MDA MB 231) was conducted using a colorimetric assay described by Mosmann (1983), which measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase enzyme. The level of formazan is directly proportional to the number of living cells and % inhibition. The intensity of color was assessed through a straightforward colorimetric assay, with results analyzed using a multi-well scanning spectrophotometer (ELISA reader).

Statistical analysis:

The measurements were carried out three times, and then the collected data underwent a multi-way analysis of variance. Mean comparisons were performed using Tukey's multiple range test with SPSS version 20.0. The significance of differences between means was determined at a p-value < 0.05.

Results and Discussion:

Phytochemical Screening.

Table 1 displayed the presence of phytochemical compounds in the aqueous extracts of *M. zapota* leaves and fruit. Analysis revealed that plant extracts contained alkaloids, flavonoids, glycosides, saponins, tannins, terpenoids, anthraquinone, and polyphenols. These complex mixtures of bioactive compounds are known for their various therapeutic effects, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, which may contribute to the health benefits of *Manilkara zapota (*L.*)* . Flavonoids and polyphenols, in particular, are strongly linked to their ability to scavenge free radicals and inhibit cancer cell growth. The difference was tannin and glycosides content. Tannin was absent in leaves extract that was present in the fruit extract. Glycosides weren't detected in fruit extracts were present in leaves extract. The presence of tannins in fruit but not in leaves of *M. zapota* can be attributed to a combination of protective, developmental, physiological, environmental, and genetic factors (Santos et al., 2019). These factors influence

the synthesis, accumulation, and distribution of tannins in different plant parts, reflecting the complex interplay between plant biology and environmental adaptation. The presence or absence of tannins in specific plant parts can also be regulated by genetic factors (Wang et al., 2020). Differential gene expression and regulation may lead to the synthesis of tannins in fruit but not in leaves, reflecting tissue-specific metabolic pathways and responses to environmental stimuli.

Total phenolic content and flavonoid content by (GC-MS) Analysis.

This study provides information on the total phenolic and flavonoid content in the leaves and fruit extract of *Manilkara zapota (*L.*)*, as shown in Table 2. The highest levels of phenolic and flavonoid content were detected in the fruit extract, reaching (348.64 ± 22.43) mg GAE/g and (42.56 ± 4.81) mg quercetin equivalents/g, respectively. The results indicated that *M. Zapota* leaves and fruit extracts have significant phenolic and flavonoid levels, which are important phytochemicals with antioxidant properties. The phenolic and flavonoid levels of the extracts may be influenced by several factors, such as the maturity stage, the extraction method, and the environmental conditions (Deng et al., 2014). The phenolic and flavonoid compounds in *M. Zapota* may contribute to its medicinal benefits, such as anti-inflammatory, anti-diabetic, and anti-cancer effects (Tamsir et al., 2020).

FT-IR spectrum of the extract.

FT-IR analysis of the plant extracts revealed the presence of several functional groups, highlighting the complex chemical composition and potential biological functions of the extracts. Specifically, the spectrum displayed distinct absorption peaks that corresponded to various functional groups (Figure 1A – 1B), further underscoring the diversity of bioactive components present in the plant. The presence of CH_2 groups in alkanes, responsible for the hydrophobic properties of the extract, was confirmed by the detection of characteristic peaks within the range of 1446 - 2917 cm^{-1} (Abdel- Hakim et al., 2012). These hydrophobic components play crucial roles in various biological actions and are often associated with the bioavailability and efficacy of natural extracts. Furthermore, the identification of C=O bonds in carboxylic acids, esters, alcohols and spanning the spectral region of 1242 – 1731 cm^{-1}, suggests the involvement of these functional groups in hydrogen bonding and the polarity of the extract (Ahlam and Mustafa, 2022). The existence of such functional groups is often linked with the solubility and interactions of bioactive compounds, influencing their potential biological activities (Devi and Battu, 2019). Moreover, the presence of N-H groups in amine and amide compounds, observed at 3289 cm^{-1}, is indicative of nitrogen fixation and may contribute to the biological activity of the extract. These functional groups are frequently found in compounds with diverse pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities (Tobias et al., 2009). Additionally, the detection of N-O groups in nitro compound at 1535 cm^{-1} suggests reveals a nitro asymmetric stretch (Maria et al., 2018). This peak

suggest the stretching vibrations of nitrogen-oxygen bonds within the nitro group (Mohammed et al., 2024). Nitro groups are used as directing groups and reactive intermediates in organic synthesis and they are found in molecules with pharmacological applications (Aubucho et al., 1999). Antioxidants play a crucial role in scavenging free radicals and mitigating oxidative stress-related damage, thus contributing to the extract's potential therapeutic benefits (Gawade et al., 2020). The presence of constituents with potential antioxidant properties within the extract (Gomathi and Maneemegalai., 2023). Overall, the FT-IR analysis highlights the diverse chemical composition of the extract, incorporating a variety of functional groups associated with different biological activities. These findings confirmed the potential of the extract as a source of bioactive compounds with multifaceted pharmacological properties, warranting further exploration and investigation.

DPPH free radical scavenging activity.

The DPPH neutralization percentage of *Manilkara zapota (*L.*)* leaves and fruit extracts exhibited dependence on concentration. This indicates that with increasing concentration of the extracts, their ability to scavenge DPPH radicals also increased. DPPH, a stable free radical, serves as a common indicator for assessing the antioxidant activity of plant extracts (Porto et al., 2000). The aqueous fraction of the fruit demonstrated the highest activity in scavenging free radicals when compared to the leaves extract. This suggests that the fruit extract contains more water-soluble antioxidants than the leaves. The concentration of DPPH radicals significantly decreased due to the scavenging activity exhibited by both *M. Zapota* leaves and fruit extracts, as well as the standard compound, gallic acid (Figure 2). Specifically, at a concentration of 100 µg/ml, the leaves extract demonstrated a 60.0% scavenging effect on the DPPH radical, while the fruit extract exhibited a stronger scavenging effect of 76.92%. In comparison, the standard gallic acid showed a scavenging effect of 84.62%. These results indicate that both leaves and fruit extracts possess substantial antioxidant activity, with the fruit extract displaying greater potency. Remarkably, the fruit extract exhibited antioxidant activity comparable to that of gallic acid, a pure compound, despite the fruit extract being a complex mixture of phytochemicals. Previous studies have also reported that the antioxidant potential of *M. zapota* fruit peel extract, as assessed by the DPPH assay, demonstrated a higher percentage of DPPH neutralization than ascorbic acid (Kumar et al., 2024). Further, our results were in line with Gomathy et al., (2013), who compared the antioxidant potential of *M. Zapota* fruit pulp extract with other fruits like apple, banana, guava and mango using DPPH assay and found that it had the highest percentage of DPPH neutralization among them.

Determination of Ferric Reducing Ability Power (FRAP).

The outcomes of the reducing power assay revealed that the extracts from *Manilkara zapota (*L.*)* leaves and fruit possessed the capability to reduce Fe3+ to Fe2+ in a manner dependent on concentration. As depicted in Figure 3, the aqueous fruit extract demonstrated the most substantial reducing power of the

other plant sample tested, even surpassing the standard antioxidant, ascorbic acid. Conversely, the aqueous leaves extract exhibited the least reducing power compared to fruit. These results imply that *M. Zapota* fruit contains phytochemicals capable of donating electrons and scavenging free radicals, thus potentially mitigating oxidative harm. Furthermore, a significant positive correlation was noted between the phenolic content and the reducing power of the extracts, suggesting that phenolic compounds predominantly contribute to the antioxidant capabilities of *M. Zapota* extracts. This aligns with the findings which have shown that phenolic compounds extracted from Zapota fruit peel exhibit notable antioxidant and anti-inflammatory properties (Tavanappanavar et al., 2024). Likewise, Prabhu et al., (2018) found that phenolic compounds extracted from Sapota leaves possess potent antioxidant and anticancer properties.

Cytotoxic activity.

The evaluation of cytotoxicity on MDA-MB-231 breast cell lines induced by the aqueous extract from *Manilkara zapota (*L.*)* fruit (as a potent extract compared to leaves) compared with doxorubicin as a standard chemotherapeutic reference drug compound was conducted using the micro-culture tetrazolium assay (MTT). Various concentrations (10-100 µg/ml extract) of the extract from dose-response curve were tested. Figure 4 illustrates the results of the cytotoxicity assessment, indicating the influence of *M. Zapota* fruit extract on MDA-MB-231 cells. The *M. Zapota* aqueous extract displayed notable cytotoxic effects on MDA-MB-231 cell lines, with its toxicity escalating proportionally with dosage. These results are consistent with the research conducted by Tan et al. (2018), who examined the cytotoxic effects of *M. Zapota* leaf methanol extract and found significant toxicity against HeLa human cervical cancer cells. This suggests a broad spectrum of anticancer activity associated with different parts of the *M. Zapota* plant. The cytotoxic effects of *M. Zapota* fruit extract are probably attributed to its rich array of bioactive compounds, including polyphenols, carotenoids, and tannins. These substances are well-known for their antioxidant characteristics, which are strongly linked to their ability to combat cancer. Antioxidants play a critical role in maintaining human health by neutralizing free radicals and binding to harmful substances, thereby reducing oxidative stress and potentially hindering the progression of cancer (Zhang et al., 2015).The relationship between antioxidant and anticancer activities underscores the importance of natural sources like *M. Zapota* in cancer prevention and treatment. The significant cytotoxicity exhibited by the extract against MDA-MB-231 cell lines highlights its potential as a therapeutic agent in combating breast cancer.

Conclusion:

The primary objective of this investigation was to delve into the chemical makeup, antioxidative, and anticarcinogenic attributes of aqueous extracts derived from both the leaves and fruit of *Manilkara zapota (*L.*)* . Our findings reveal that the aqueous extracts from both *Manilkara zapota (*L.*)* leaves and

fruit are teeming with phytochemical compounds, displaying auspicious antioxidative and anticarcinogenic properties. The chemical analysis divulged a wide assortment of bioactive constituents within the aqueous extracts of *M. Zapota* leaves and fruit. These bioactive compounds likely contribute to the observed biological effects of the extracts. The presence of an array of secondary metabolites, including polyphenols, flavonoids, terpenoids, and alkaloids, suggests the potential therapeutic significance of *M. Zapota* in traditional healing practices and the exploration of natural product-derived medications. Nevertheless, further research endeavors are imperative to comprehensively comprehend the mechanisms of action and clinical applicability of *M. Zapota* extracts in both disease prevention and treatment.

Tables and Figures:

Table 1: Preliminary Phytochemical Constituents of *Manilkara zapota (*L.*)* Leaves and Fruit Extracts

S.NO	Phytochemicals	leaves Extract	Fruit Extract
1	Alkaloids	+	+
2	Flavanoids	+	+
3	Glycosides	+	–
4	Saponins	+	+
5	Tannins	–	+
6	Terpenoids	+	+
7	Anthraquinone	+	+
8	Polyphenol	+	+

+ Indicate Present – Indicate Absent

Table 2. Quantitative Analysis of Phytochemical Constituents of *Manilkara zapota (*L.*)* Leaves and Fruit Extracts.

Extract	Total phenolic Content (mg GAE/g of plant extract)	Total Flavonoid content (mg QE/g of plant extract)
Leaves	226.11 ± 16.24	25.89 ± 4.81
Fruit	348.64 ± 22.43	42.56± 4.81

All values are expressed as Mean ± SD for three determinations; GAE- Gallic acid equivalent; QE -Quercetin equivalent

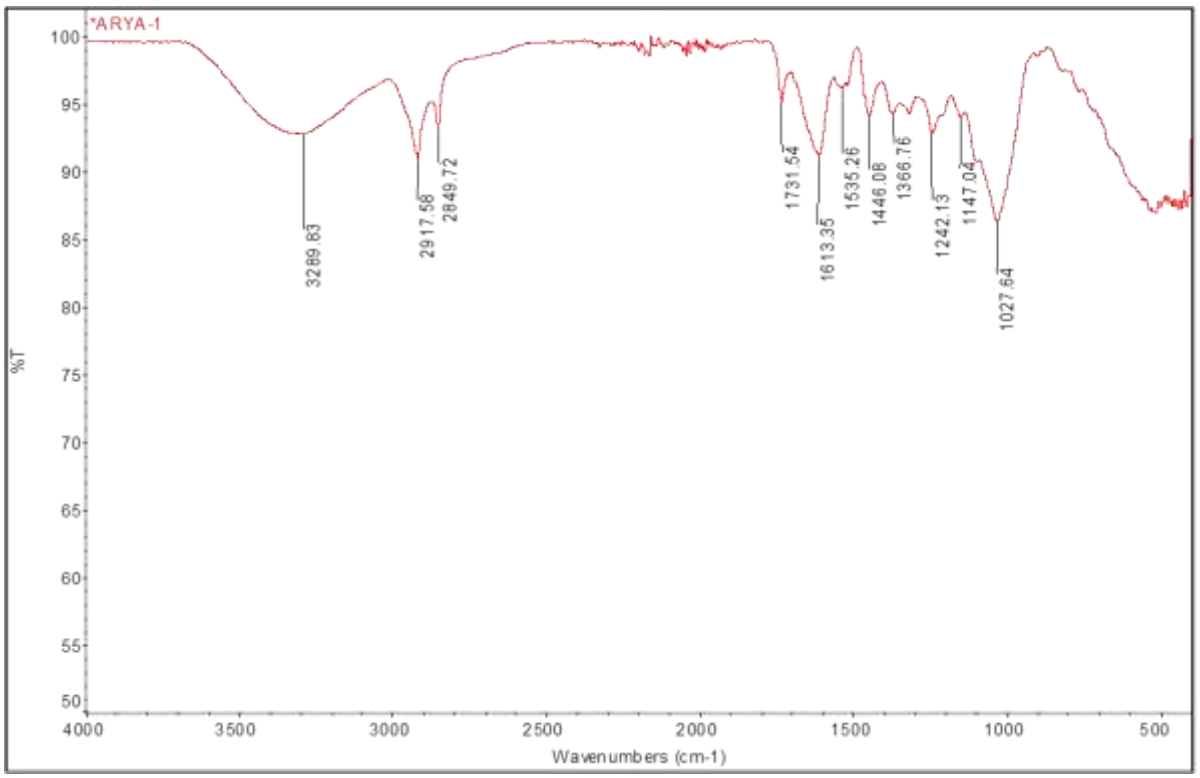

Figure 1A: FT-IR Spectrum of Aqueous Extract of *Manilkara zapota* (L.) Leaves.

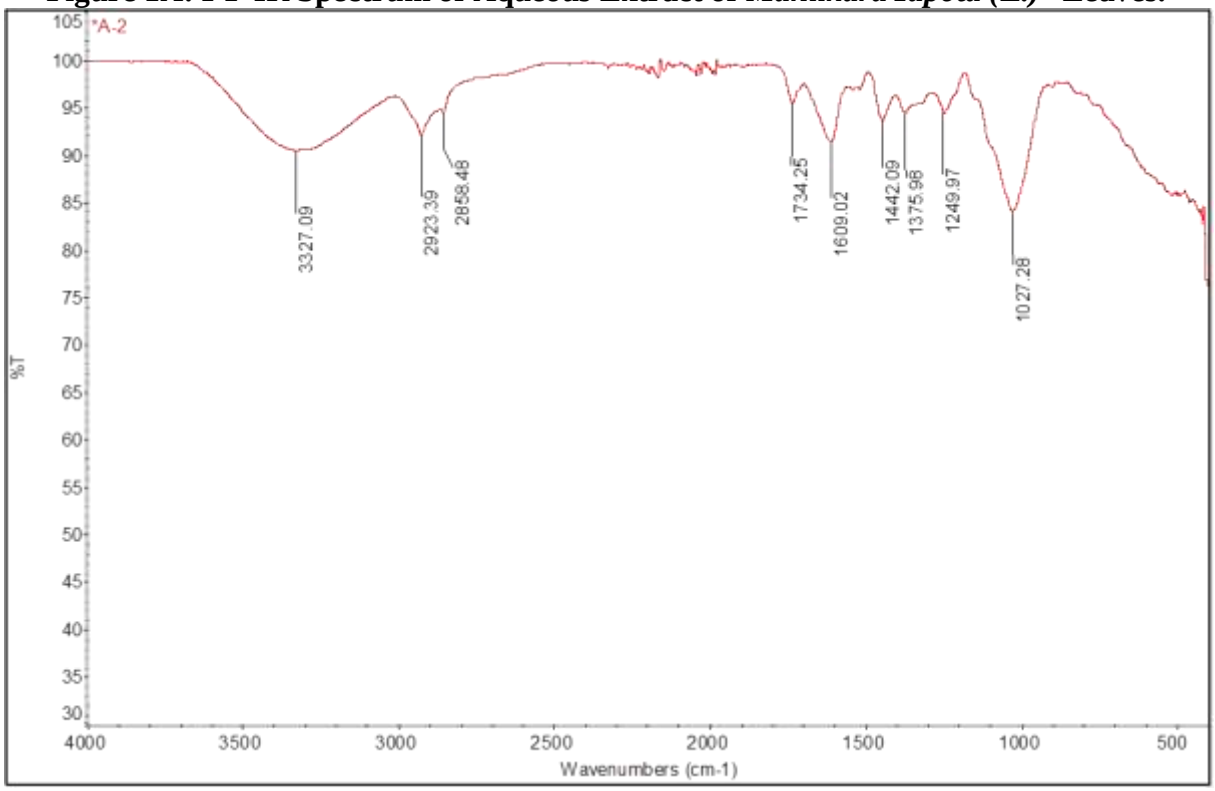

Figure 1B: FT-IR Spectrum of Aqueous Extract of *Manilkara zapota* (L.) Fruit.

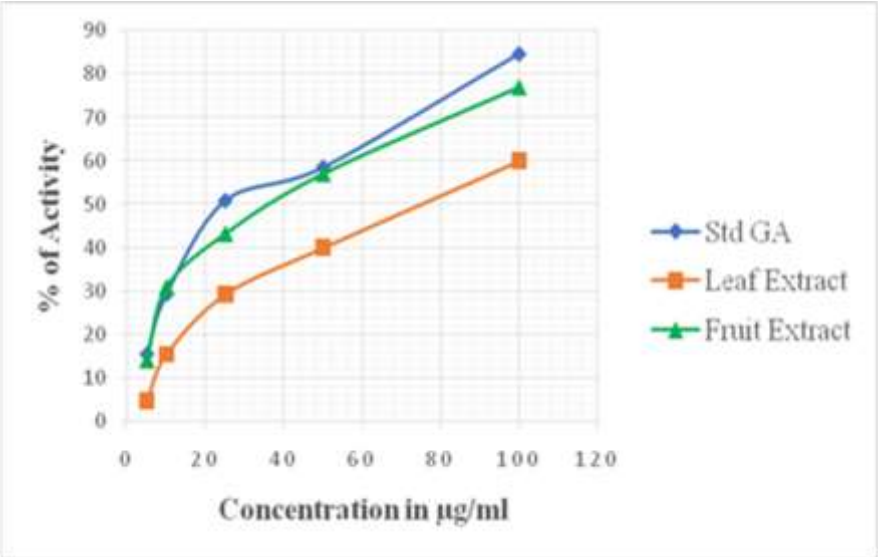

Figure. 2: Free Radical-Scavenging Activity of *Manilkara zapota* (L.) Leaves and Fruit Extracts.

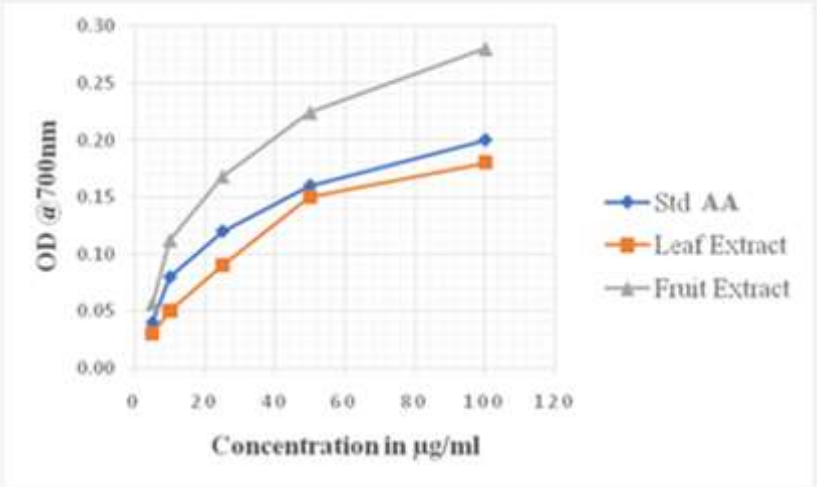

Figure.3: Reducing Power of *Manilkara zapota* (L.) Leaves and Fruit Extracts.

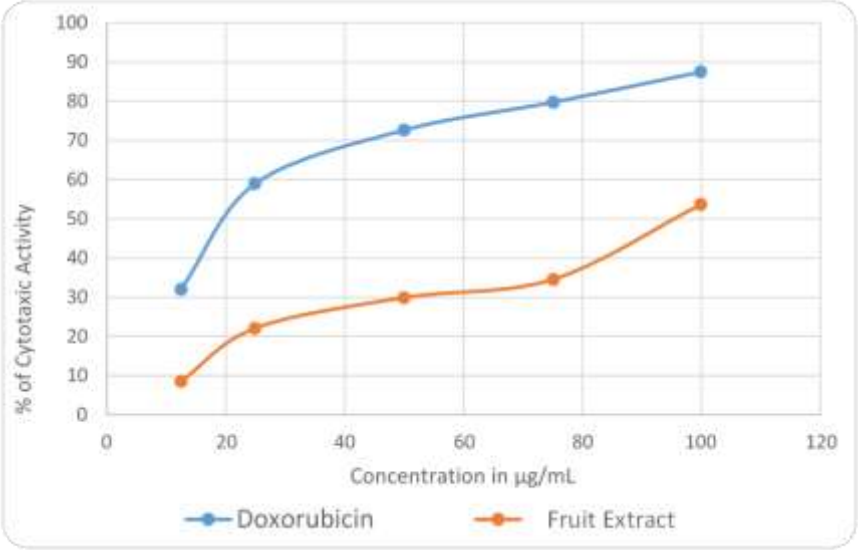

Figure 4: Cytotoxic Activity of *Manilkara zapota* (L.) Aqueous Fruit Extract

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