Phytochemical Evaluation and Antioxidant Potential of *Manilkara zapota* Leaf Extract for Oral Health Applications

Swati gokhe, Ekta Mishra, Niharika Thakur, Chandni Rai

Pinnacle Biomedical Research Institute

ABSTRACT

Oral health is an essential component of overall well-being and is closely linked to general health. The rise of chronic oral diseases such as dental caries and periodontitis necessitates the exploration of natural, cost-effective alternatives for prevention and treatment. *Manilkara zapota* (sapodilla), a plant known in traditional medicine, was investigated for its potential antioxidant and therapeutic properties in oral health applications. Methanolic extracts of *Manilkara zapota* leaves were obtained using Soxhlet extraction and yielded 9.679%, while petroleum ether extracts yielded 2.916%. Preliminary phytochemical screening of the methanolic extract revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, and proteins, while the petroleum ether extract showed no significant constituents. Quantitative analysis showed a high total phenolic content (85.48 mg GAE/g) and flavonoid content (84.92 mg RE/g). Antioxidant activity was evaluated using DPPH radical scavenging and reducing power assays. The methanolic extract demonstrated 67.97% DPPH inhibition with an IC_{50} of 39.084 µg/ml, compared to 81.606% inhibition and an IC_{50} of 21.391 µg/ml for ascorbic acid. The extract also showed moderate reducing power, suggesting electron-donating capability. Additionally, a herbal oral rinse was formulated using the extract, indicating potential for development into a natural oral care product. These findings support the use of *Manilkara zapota* as a natural source of antioxidants with promising applications in preventive dentistry and oral health management.

Keywords : Oral Health, *Manilkara zapota*, Methanolic extract, DPPH, Oral rinse

1. INTRODUCTION

Oral health is a fundamental component of overall well-being, encompassing not just the absence of disease but also the ability to speak, smile, taste, and eat comfortably and without pain or discomfort. Maintaining good oral health is essential for an individual's quality of life, as it affects their physical, mental, and social well-being. Poor oral health can lead to significant pain, discomfort, and even disability, impacting an individual's ability to perform daily activities, work, and engage in social interactions. The consequences of oral diseases extend beyond the mouth, as emerging research suggests links between oral health and systemic conditions, such as cardiovascular diseases, diabetes, and respiratory infections. This interconnection underscores the importance of oral health as a critical aspect of general health and highlights the need for effective preventive and treatment strategies **(Sheiham et al., 2005).**

Oral diseases, including dental caries (tooth decay) and periodontitis (gum disease), are among the most common chronic diseases affecting humanity. According to the World Health Organization (WHO), dental caries affects 60-90% of school-going children in developing countries, and untreated dental caries is the most prevalent health condition globally. Periodontitis, characterized by the loss of teeth and supporting bone, is also widespread and can lead to significant discomfort, aesthetic issues, and functional impairments **(Petersen et al., 2003).** The economic burden of treating these diseases is immense, with dental care often being one of the most expensive health services. In some countries, the treatment of oral diseases ranks fourth in terms of healthcare expenditure, highlighting the need for cost-effective preventive measures, especially for economically disadvantaged populations.

Plants have long been a source of medicinal compounds, and many traditional remedies incorporate plant extracts to maintain oral health. One such plant is Manilkara zapota, commonly known as sapodilla, which has been used in traditional medicine for various ailments. Recent scientific investigations have revealed that Manilkara zapota possesses significant antioxidant activity, attributed to its rich phytochemical composition, including flavonoids, phenols, and saponins. These compounds have been shown to scavenge free radicals, reduce oxidative stress, and exhibit anti-inflammatory effects, all of which are beneficial for maintaining oral health and preventing disease.

This exploration aims to delve into the potential of Manilkara zapota as a natural source of antioxidants for promoting oral health. By examining the extraction efficiency, phytochemical content, and antioxidant activity of Manilkara zapota, we can gain insights into its potential applications in preventive dentistry, pharmaceuticals, and nutraceuticals. Furthermore, understanding the mechanisms by which this plant extract exerts its beneficial effects can pave the way for developing innovative and effective strategies to combat oral diseases and improve overall health.

2. MATERIAL AND METHODS

2.1 Extraction

Selection and Collection of plant:

Plant and plant parts was selected on the basis of ethno-botanical survey. Fresh leaves of *Manilkara zapota,* free from disease were collected from local area of Bhopal.

2.1.1 Soxhlet Extraction:

Dried and ground powder of *Manilkara zapota* leaves were placed in a Soxhlet apparatus and extracted with solvent in a round bottom flask containing glass beads for 24 h. After extraction, the solvent was removed from the extract in a vacuum rotary evaporator. Methanol was used as solvent. Extracts were collected in air tight container **(Şahin et al., 2011).** Extraction yield of all extracts were calculated using the following equation below:

$$\text{Formula of Percentage yield} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100$$

2.2 Qualitative phytochemical analysis of plant extract

Manilkara zapota extracts obtained was subjected to the preliminary phytochemical analysis. The extracts were screened to spot the presence or absence of many active constituents like carbohydrates, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, fats or fixed oils, protein, amino acid and tannins.

The extract of Manilkara zapota was subjected to a series of qualitative phytochemical tests to identify the presence of various bioactive constituents. Carbohydrates were confirmed using Molisch's, Fehling's, and Benedict's tests, indicating the presence of both general and reducing sugars. Alkaloids were detected through Mayer's, Hager's, and Wagner's tests, with characteristic precipitate formation. Triterpenoids and steroids were identified using Salkowski's and Libermann-Burchard's tests, while flavonoids were confirmed by the lead acetate and alkaline reagent tests. Phenolic compounds and tannins showed positive results with ferric chloride, gelatin, and lead acetate tests. The froth test confirmed the presence of saponins, and solubility tests in chloroform and ethanol indicated the presence of fats and oils. Proteins and amino acids were identified through Biuret's and Ninhydrin tests. Lastly, the presence of glycosides, including cardiac and anthraquinone types, was confirmed using Borntrager's, Legal's, and Keller-Killiani tests. These findings highlight the diverse phytochemical profile of Manilkara zapota, supporting its potential for pharmacological and therapeutic applications (**Kokate et al., 1986**).

2.3 Quantitative Tests

2.3.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. *Manilkara zapota* extracts (0.2 ml) was mixed with 2.5 mL of Folin-Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 2 mL of sodium bicarbonate (7.5%, w/v) was added to the mixture. After standing for 2 hrs at room temperature, absorbance was measured at 760 nm. Aqueous solutions of known gallic acid concentrations in the range of 20–100 µg/ml were used for calibration. Results were expressed as mg gallic acid equivalents (GAE)/g sample. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically **(Al-Rimawi et al., 2016).**

2.3.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The determination of total flavonoids was performed by Aluminium chloride method **(Al-Rimawi et al., 2016).** Distilled water (1.5 mL) was added to 0.5 mL of *Manilkara zapota* extract in a test tube. Then, 0.150 mL of 5% sodium nitrite solution was added, followed by 0.150 mL of 10% aluminum chloride solution. Test tubes were incubated at ambient temperature for 6 minutes, and then 2 mL of 4% sodium hydroxide was added to the mixture. Immediately, the volume of reaction mixture was made to 5 mL with distilled water. The mixture was thoroughly mixed using test tube shaker and the absorbance of the yellow to orange color developed was determined at 510 nm. Aqueous solutions of known rutin concentrations in the range of 20–100 µg/ml were used for calibration and the results were expressed as mg rutin equivalents (RE)/g sample.

2.4 In vitro anti oxidant activity

2.4.1 DPPH assay

Free radical scavenging activity of extracts of *Manilkara zapota*, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Ali et al **(Ali et al., 2013).** Different volume of extracts/standard (20 – 100µg/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation: % scavenging activity=[(A0-A1)/A0]×100. Where A0 is the absorbance of the control and A1 is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC50 value was calculated using linear regression analysis.

2.4.2 Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of extracts

Stock solutions of extracts of *Manilkara zapota* were prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extracts of *Manilkara zapota* (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri-chloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Shimadzu-1700). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard **(Choi et al., 2002).**

2.3 Preparation of Oral rinse

The oral rinse was prepared using 0.3 g of sucrose as a sweetener, 0.001 g of sodium benzoate as a preservative, and 0.01 g of sodium lauryl sulfate as a foaming agent. These were dissolved in 9.5 mL of distilled water. Four dilution of formulation were prepared by adding 100, 200, 300 and 400µg (F1, F2, F3 and F4) per 10 ml of *Manilkara zapota* extract to the oral rinse, and the mixture was placed in an orbital shaker. Finally, a total volume of 10 mL of *Manilkara zapota* extract based oral rinse was used for further studies **(Kumar et al., 2022).**

3. RESULTS

3.1. Percentage Yield

In phytochemical extraction, the percentage yield is very crucial in order to determine the standard efficiency of extraction for a specific plant, various sections of the same plant or different solvents used.

Table 1: Percentage Yield of crude extracts of *Manilkara zapota* extract

S. No.	Solvent	Colour of extract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleum ether	Dark Green	92.22	2.69	2.916%
2.	Mehanol	Dark Green	89.57	8.67	9.679

3.2 Preliminary Phytochemical study

Table 2 Phytochemical testing of *Manilkara zapota* extract

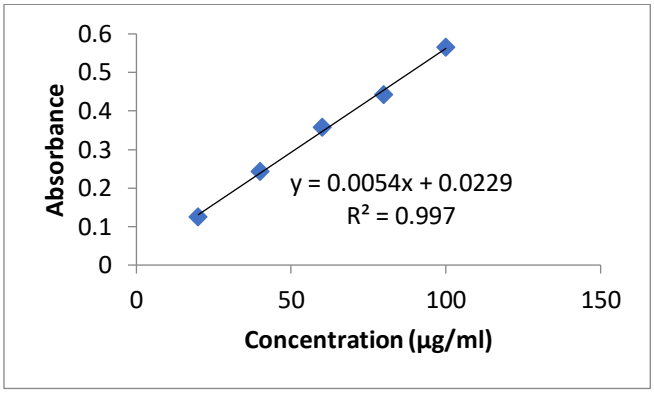
Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Methanolic extract
Alkaloids		
Dragendroff's test	Absent	Present
Mayer's reagent test	Absent	Present
Wagner's reagent test	Absent	Present
Hager's reagent test	Absent	Present
Glycoside		
Borntrager test	Absent	Present
Legal's test	Absent	Present
Killer-Killiani test	Absent	Present
Carbohydrates		
Molish's test	Absent	Present
Fehling's test	Absent	Present
Benedict's test	Absent	Present
Barfoed's test	Absent	Present
Proteins and Amino Acids		
Biuret test	Absent	Present
Flavonoids		
Alkaline reagent test	Absent	Present
Lead Acetate test	Absent	Present
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	Present
Saponin		
Foam test	Absent	Present
Test for Triterpenoids and Steroids		
Salkowski's test	Absent	Absent
Libbermann-Burchard's test	Absent	Absent

3.3 Quantitative Analysis

3.3.1 Total Phenolic Content

Table 3: Standard Table of Gallic Acid

S. No.	Concentration(µg/ml)	Absorbance (OD at 760 nm)
1	20	0.125
2	40	0.243
3	60	0.358
4	80	0.442
5	100	0.565



Graph 1: Graph represents the standard curve of Gallic acid

3.3.2 Total Phenolic Content in extract

Table 4: Total Phenolic Content in *Manilkara zapota* extract

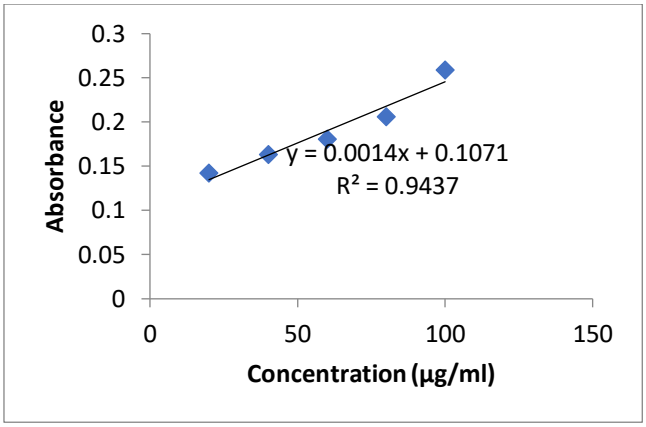
S. No	Absorbance(OD at 760 nm)	TPC in mg/gm equivalent of Gallic Acid
1	0.393	74.2 mg/gm

The total Phenolic content of the *Manilkara zapota* extract with respect to Gallic acid was evaluated 85.48 mg/gm.

3.3.3 Total Flavonoid Content

Table 5: Standard Reading of Rutin

S.No	Concentration(µg/ml)	Absorbance (OD at 510 nm)
1	20	0.142
2	40	0.163
3	60	0.181
4	80	0.206
5	100	0.259



Graph 2: Graph represent standard curve of Rutin

3.3.4 Total Flavonoid Content in the extract

Table 6: Total Flavonoid Content in *Manilkara zapota* extract

S. No	Absorbance(OD at 510 nm)	TFC in (mg/gm) equivalent of Rutin
1	0.168	61mg/gm

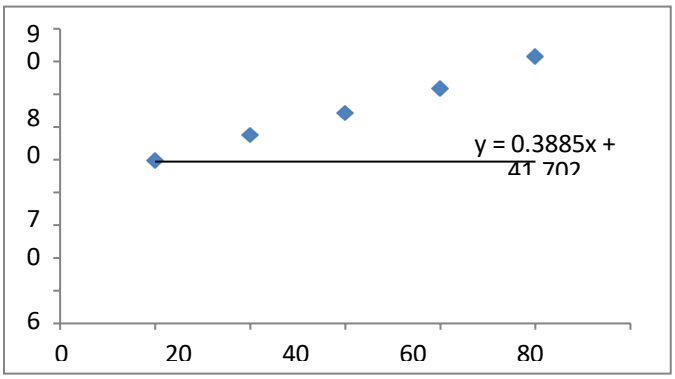
Total Flavonoid content of *Manilkara zapota* extract was calculated using Rutin as standard was found to be 84.92mg/gm.

3.4 Determination of antioxidant Activity of fruit juice

3.4.1 *In Vitro* Anti-Oxidant Activity by DPPH Radical Scavenging Activity

Table 7: DPPH scavenging capacity of the Standard Ascorbic acid

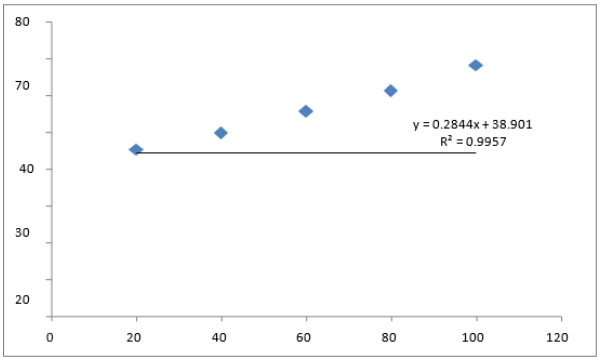
Ascorbic acid		
Concentration	**Absorbance**	**% inhibition**
20	0.474	49.894
40	0.402	57.505
60	0.338	64.270
80	0.267	71.775
100	0.174	81.606
Control	**0.946**	
IC 50	**21. 391**	



Graph 3: Graph represent Standard curve of Ascorbic acid

Table 8: DPPH scavenging capacities of the *Manilkara zapota*

Paper boat		
Concentration	**Absorbance**	**% Inhibition**
20	0.518	45.24
40	0.475	49.78
60	0.42	55.60
80	0.367	61.20
100	0.303	67.97
Control	**0.946**	
IC50	**39.084**	

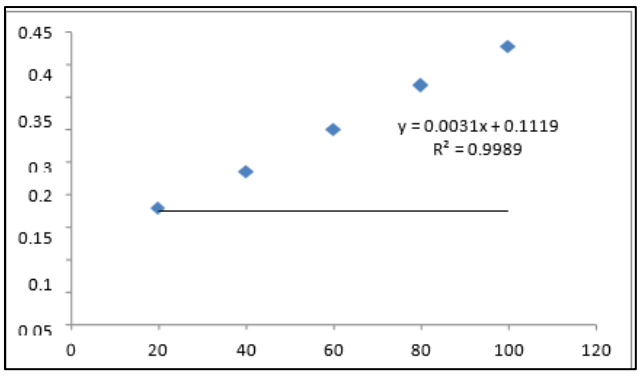


Graph 4: Graph of DPPH Radical Scavenging Activity of *Manilkara zapota*

5.7.2 Reducing power assay

Table 9: Standard Table of Ascorbic acid

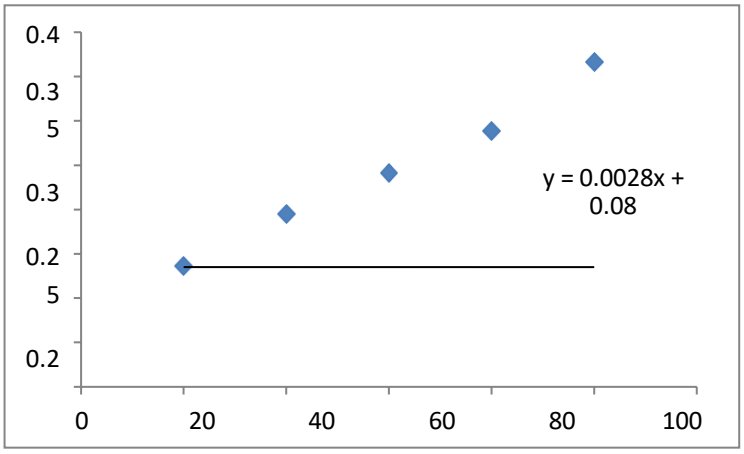
Concentration (µg/ml)	**Absorbance**
20	0.178
40	0.234
60	0.298
80	0.367
100	0.426



Graph 5: Graph represent standard curve of Ascorbic Acid

Table 10: Reducing power activity of *Manilkara zapota*

Concentration (Paper boat)	Absorbance
20	0.137
40	0.195
60	0.241
80	0.289
100	0.366



Graph 6: Graph represents the standard curve of *Manilkara zapota*

4. DISCUSSION

The extraction efficiency of Manilkara zapota was influenced by solvent polarity, with the methanolic extract yielding 9.679% and petroleum ether extract yielding 2.916% from 100 g of plant powder. Qualitative phytochemical screening revealed that the methanolic extract contained alkaloids, flavonoids, phenols, and saponins, while the petroleum ether extract lacked all tested components. Quantitative analysis showed that the total phenolic content was 74.2 mg GAE/g, and total flavonoid content was 61.00 mg RE/g, highlighting significant antioxidant potential. The methanolic extract demonstrated notable in vitro antioxidant activity, with 67.97% DPPH radical scavenging and an IC_{50} of 39.084 µg/ml, compared to 81.606% and 21.391 µg/ml for ascorbic acid. Additionally, the extract exhibited moderate reducing power, confirming its ability to act as an electron donor and indicating potential therapeutic benefits in combating oxidative stress-related diseases.

5. CONCLUSION

Oral health is a critical component of overall well-being, affecting speech, diet, quality of life, and general health. Oral diseases, including dental caries and periodontitis, are prevalent and costly, posing a significant public health challenge. These diseases not only cause pain and discomfort but are also linked to systemic health issues such as cardiovascular diseases, diabetes, and respiratory problems. The economic burden of treating oral diseases is substantial, especially in low-income countries, where preventive strategies are urgently needed. The methanolic extract of Manilkara zapota shows promising properties that could be harnessed for oral health benefits. Methanolic extraction is more effective than petroleum ether, yielding a richer profile of bioactive compounds, including alkaloids, flavonoids, phenols, and saponins. The extract demonstrates significant in vitro antioxidant activity, with notable DPPH radical scavenging ability and an IC_{50} value indicating its potential as a natural antioxidant. The presence of substantial total phenolic and flavonoid contents further supports the extract's antioxidant potential, suggesting it could mitigate oxidative stress-related diseases. The extract's ability to act as an electron donor and its moderate reducing power indicate potential therapeutic benefits in combating oxidative stress, which is relevant to oral health and systemic diseases. Given these findings, Manilkara zapota extract could be explored for various applications in oral health, such as developing socially acceptable, easily available, and cost-effective preventive measures for oral diseases, incorporating the extract into dental products, mouthwashes, or functional foods to enhance oral health and overall well-being, and justifying additional investigations into the extract's specific mechanisms of action, safety, and efficacy in clinical settings. In summary, promoting oral health through innovative and cost-effective strategies is essential, and Manilkara zapota extract shows promise as a natural antioxidant source with potential applications in preventing and managing oral diseases.

6. REFERENCES

- Sheiham A. Oral health, genral health and quality of life. *Bull World Health Organ.* 2005;83:644–5.
- Petersen PE. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century- -the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol.* 2003;31(Suppl 1):3–23.
- Şahin, S., Bilgin, M., & Dramur, M. U. (2011). Investigation of oleuropein content in olive leaf extract obtained by supercritical fluid extraction and soxhlet methods. *Separation Science and Technology, 46*(11), 1829-1837.
- Kokate, C. K. (1986). Preliminary phytochemical analysis. Practical Pharmacognosy. 1st ed. New Delhi: Vallabh Prakashan, 111.
- Al-Rimawi, F., Rishmawi, S., Ariqat, S. H., Khalid, M. F., Warad, I., & Salah, Z. (2016). Anticancer activity, antioxidant activity, and phenolic and flavonoids content of wild Tragopogon porrifolius plant extracts. *Evidence-Based Complementary and Alternative Medicine, 2016.*
- Ali, M. S., Amin, M. R., Kamal, C. M. I., & Hossain, M. A. (2013). In vitro antioxidant, cytotoxic, thrombolytic activities and phytochemical evaluation of methanol extract of the A. philippense L. leaves. Asian Pacific Journal of Tropical Biomedicine**.** 3(6), 464-469.
- Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay –guided comparison. *Plant Sci.*;163:1161–8.