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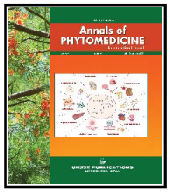

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Phytochemical, antioxidant activity and proximate composition analysis of *Manilkara zapota* L. leaves

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Abstract

Manilkara zapota L., commonly referred to as sapota, is a significant medicinal plant that is used to treat a variety of conditions, including infectious diseases, oxidative stress, and cancer. This study set out to assess the phytochemical analysis, antioxidant, and proximate composition of *M. zapota* leaf. The methanol and ethyl acetate leaf extracts are used to analyse a wide range of phytochemicals, such as total sugars (114.770 ± 0.325 and 151.854 ± 0.358 mg/g), reducing sugars (58.929 ± 0.195 and 39.890 ± 0.318 mg/g), non-reducing sugars (55.117 ± 0.295, and 12.140 ± 0.569 mg/g) total phenolic content (103.950 ± 0.426 and 23.100 ± 0.210 mg GAE/g) and total flavonoid content (25.681 ± 0.366 and 5.064 ± 0.169). The moisture content (4.167%), ash content (6.488%), crude fiber (9.435%), crude fat (5.113%), crude protein (8.407%), total carbohydrate (66.78%), and minerals content in *M. zapota* leaves were evaluated. The FRAP assay (13.007 ± 0.17 mM Fe (II)/ml, 8.49 ± 0.09 mM Fe (II)/ml), total antioxidant capacity (0.64 ± 0.024 mg AAE/ml, 0.552 ± 0.015 mg AAE/ml), DPPH free radical scavenging activity (IC_{50} 34.266 µg/ml, 158.153 µg/ml), and ABTS free radical scavenging activity (IC_{50} 35.02 µg/ml, 167.758 µg/ml) were used to measure the antioxidant activity of the ethyl acetate and methanol extract. The results suggest that *M. zapota* is a decent source of minerals and phytochemicals in both healthy and pharmaceutical settings.

1. Introduction

Natural foods with potentially bioactive ingredients are abundant in medicinal plants, and they have long been utilized as herbal remedies for a widespread range of severe ailments (Moond *et al*., 2023; Devi *et al*., 2023; Poonam *et al*., 2023). Many ailments, such as cancer, obesity and hypertension, oxidative stress, diabetes mellitus, neurological complaints, skincare issues, and inflammations, are treated with medicinal plants. Additionally, they are employed to regulate the expression of various genes (Beniwal *et al*., 2023).

Additionally, plants are a great source of natural antioxidants and antimicrobial agents. Many of the plant materials used in traditional medicines are readily available and relatively less expensive in rural areas than they are in urban areas. In general, plants produce a large number of secondary metabolites, which are a valuable source of antioxidants and microbicides. Numerous natural ingredients with antibacterial and antioxidant qualities have been added to health foods for both medicinal and preservative purposes (Islam *et al*., 2020; Singh *et al*., 2021; Devi *et al*., 2023; Moond *et al*., 2023).

The broad and varied class of phytochemicals known as phenolic compounds is made up of several families of aromatic secondary metabolites found in plants. Because phenolic compounds can chelate metals, scavenge free radicals, and break down radical chain reactions, they have strong antioxidant properties both *in vitro* and *in vivo*. An important class of phytochemicals are flavonoids and other phenolic compounds (Dalal *et al*., 2023). A broad class of naturally finding plant phenolic compounds known as flavonoids, which include flavones, isoflavones, flavonols, chalcones and flavonones, are highly potent antioxidants. With their distinctive C6-C3-C6 structure and free -OH groups linked to aromatic rings, flavonoids scavenge free radicals and prevent lipid oxidation through a variety of other mechanisms, including lipoxygenase inhibition, singlet oxygen quenching, and metal chelation (Aggarwal *et al*., 2022; Kaisoon *et al*., 2011).

Antioxidants are substances that prevent or postpone the oxidation of further molecules by preventing oxidizing chain reactions from starting or continuing. Additionally, antioxidants can defence the body from the harmful effects of reactive oxygen species (ROS) and free radicals. Certain vitamins (A, C, E, and K), carotenoids, flavonoids (isoflavones, flavones, flavonones, catechins, isocatechins, and anthocyanins), polyphenols (ellagic acid, tannins, gallic acid), enzymes, saponins, and minerals (selenium, manganese, copper, zinc, iodine, chromium, *etc*.) are among the numerous antioxidants found in medicinal plants (Kaur *et al*., 2006).

All parts of the plant, including the bark, wood, stems, leaves, pods, flowers, pollen, fruit, roots, and seeds, contain natural antioxidants. Nonetheless, reports have stated that leaves are typically chosen for antioxidant research. *M. zapota* belongs to the Sapotaceae family. It is an 8-15 m tall, glabrous, evergreen tree. Although, it is native to

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Central America and Mexico, it is grown all over India. The seeds have febrifuge, diuretic, and aperient properties. The bark is astringent, febrifuge, and antibiotic. In dentistry, chicle from the bark is utilized. Fruits have a rich, fine flavour and are edible and sweet. The bark is a tonic, and peludism and diarrhea are treated with the infusion. The leaves are used to cure diarrhea, colds, and coughs. There are reports of the leaves having antioxidant and antimicrobial properties as well (Islam *et al*., 2011). The present work aimed to determine phytochemical analysis, proximate composition, and the *in vitro* antioxidant capacity of *M. zapota* leaves extract.

2. Materials and Methods

2.1 Plant material collection

The leaves of *M. zapota* were gathered from CCS Haryana Agricultural University's orchard in Hisar, which is located at 75º.69' E longitude and 29º.15' N latitude. The collected sample was verified by Dr. Anita, Asstt. Scientist, Department of Botany and Plant Physiology, CCS HAU, by using online platform on Atlas of Florida plants and cited as P. Royen, Blumea 7:410, 1953. After being cleaned, plant parts were left to dry at room temperature in the shade. A mechanical grinder was used to grind the dried leaves cutting pieces, which were then placed in an airtight container for storage.

2.2 Proximate composition

In triplicate, the proximate composition (crude fat, moisture, crude protein, ash, total carbohydrate, and crude fibre) of *M. zapota* leaves was ascertained using the standard operating procedures of the Association of Official Analytical Chemists (AOAS). The Maynard method was used to calculate crude fiber (Maynard, 1995). The nitrogen content was ascertained using the Micro-Kjeldahl technique (AOAC, 1990). The percentage of nitrogen content was multiplied by 6.25 to determine the crude protein content. Following microwave-assisted acid digestion, the mineral content of *M. zapota* leaves was examined using inductively coupled plasma mass spectroscopy (ICP-MS) (Jackson, 1973; Ruig, 1986).

2.3 Preparation of *M. zapota* leaf extract in methanol and ethyl acetate for phytochemical analysis

Methanol and ethyl acetate were used in sequential order by the Soxhlet apparatus to extract the dried powder of *M. zapota* leaves. 8 g of *M. zapota* leaf powder was put into a thimble of filter paper of Whatman No. 1. This thimble was kept in a 500 ml circular-bottom flask that houses most of the traditional Borosil Soxhlet apparatus. Up to 1.5 syphons was filled with methanol and ethyl acetate in an amount of at least 200 ml each. Consequently, ethyl acetate and methanol were used as solvent in a Soxhlet utensil to percolate the grounded peel sample. The following characteristics were ascertained using this methanol and ethyl acetate extract: capacity, total sugar, total phenolic content, reducing sugar, total flavonoids, and non-reducing sugar. The extracts were lyophilized again for antioxidant activity after being concentrated to dryness in a rotary evaporator under low pressure.

2.4 Phytochemicals quantitative analysis

2.4.1 Total phenolics

The Folin-Ciocalteu method was used to calculate the total phenolics of the ethyl acetate and methanol *M. zapota* leaves using gallic acid as the standard reference (Singleton and Rossi, 1965). A test tube was packed with 0.5 ml of the Folin-Ciocalteu reagent, 1 ml of Na_2CO_3 (20%, w/v), and 0.5 ml of each extract of ethyl acetate and methanol. After mixing, water was added to attain a final volume of 5 ml. After 20 min of incubation, the mixture was finally centrifuged for 10 min at 6500 rpm. At 735 nm the absorbance of the supernatant solution was determined by using a UV-Vis spectrophotometer. The results were compared with a blank that was prepared in the same way, but with the proper solvent and without the extract. Using a regression equation developed from the gallic acid standard curve, the total phenolic content of the *M. zapota* leaf extract in ethyl acetate and methanol was calculated and expressed as mg GAE/g.

2.4.2 Total flavonoid content

0.5 ml of ethyl acetate and methanol leaves extract, 0.15 ml of 5% $NaNO_2$, and 2 ml of distilled water were added to a test tube. After six min, 0.15 ml of 10% $AlCl_3$ and 1 ml of 1M NaOH were added. The last additions were dilutions, which included 5 ml of distilled water. Catechin was used as a reference to calculate the total amount of flavonoids using an $AlCl_3$ colorimetric test (Marinova *et al*., 2005). The mixture's absorbance at 515 nm was measured using a UV-Vis double beam spectrophotometer in relation to a blank that was prepared using the same procedures but substituted methanol and ethyl acetate for the extract. The total flavonoid content of the leaf extract was calculated using a regression equation derived from the catechin standard curve, and it was expressed as mg CE/g.

2.4.3 Total sugar

Dubois *et al*. (1956) developed a modified version of the method for computing total sugar. 1 ml of phenol solution was added to a test tube containing 0.5 ml of ethyl acetate and methanol, which were used to extract the leaves of the *M. zapota*. 2.5 ml of concentrated H_2SO_4 was then added to the reaction mixture. By using UV-Vis double-beam spectrophotometer, the absorbance of the reaction mixture was determined at 495 nm after the solution was allowed to cool for 30 min. This was done in contrast to a blank, which was created using the same steps but with ethyl acetate and methanol in place of the extract. The regression equation derived from the D-glucose standard curve was used to calculate the total amount of sugar in the leaf extract of *M. zapota*. The results were displayed as mg/g.

2.4.4. Reducing sugar

To determine how much reducing sugar was in the leaf extract, Somogyi (1952) modified the Nelson method (Nelson, 1944). A test tube containing 1 ml of ethyl acetate and methanol extract of *M. zapota's* leaves was filled with 1 ml of alkaline copper reagent. After thoroughly mixing the mixture, it was heated in a hot water bath for thirty minutes. After thoroughly mixing 1 ml of arsenomolybdate reagent at room temperature, it was left to stand for 30 min. The mixture was then diluted upto volume of 10 ml using distilled water. A double beam UV-Vis spectrophotometer was used to measure the reaction mixture's absorbance at 525 nm. To prepare the absorbance of the blank, the same procedure was used, but instead of extract, 1 ml of distilled water was used. The amount of reducing sugars in the extract of ethyl acetate and methanol was determined and stated as mg/g by using the D-glucose standard curve.

2.4.5 Non-reducing sugar

The non-reducing sugars were found by deducting the concentration of reducing sugars from the concentration of total sugars:

Non-reducing sugars = Total sugar – reducing sugar

2.5 Evaluation of antioxidant properties

2.5.1 DPPH free radical scavenging activity

The Hatano *et al*. (1988) methodology was employed to evaluate the DPPH free radical scavenging activity. I ml each of ethyl acetate and methanol extracts (at varying concentrations) and 2 ml of 2, 2'-diphenyl-1-picrylhydrazyl (0.1 mM) radical were combined and vigorously stirred for a duration of 2 min in order to assess the antioxidant activity. After a half an hour dark incubation period at room temperature, the absorbance of the sample and control was measured at 520 nm by :

% DPPH free radical scavenging activity = $[1 - A_1/A_2] \times 100$

where,

A_2 = Absorbance of control; A_1 = Absorbance of sample

2.5.2 ABTS assay

To produce the ABTS radical cation, 5 ml of ABTS (7 mM) was mixed with 5 ml of $(NH_4)_2 SO_4$(2.45 mM) and the mixture was incubated for 18 h. Once the ABTS solution was diluted, the absorbance at 734 nm was nearly 0.75 ± 0.02. The ethyl acetate and methanol extract samples were added to an ABTS radical solution at different concentrations (0.2 ml), and the mixture was left to sit for 4 min at room temperature. A double beam UV-Vis spectrophotometer was used to estimate the sample's absorbance; BHA and a control at 734 nm were used as standards.

The % of scavenged ABTS was calculated using the following formula:

% ABTS free radical scavenging activity = $[1 - A_1/A_2] \times 100$

where,

A_2 = Absorbance of control; A_1 = Absorbance of sample

2.5.3 FRAP assay

The FRAP reagent was prepared by mixing 300 mM of acetate buffer (pH 3.6), 20 mM $FeCl_3.6H_2O$, and 10 mM TPTZ in a 10:1:1 ratio with 40 mM HCl. Following the addition of 0.1 ml of each sample to three millilitres of this FRAP reagent, it was incubated for 5 min at 37°C. A double beam UV-Vis spectrophotometer was used to find the absorbance of the control and sample at 593 nm using $FeSO_4.7H_2O$ as the standard.

2.5.4 Phosphomolybdnum assay

Prieto *et al*. (1999) used the modified phosphomolybdenum method to determine the total antioxidant capacity of leaf extracts of *M. zapota*. Glass vials were filled with 1 ml of each leaf extract and 3 ml of phosphomolybdenum reagent. The mixture was well mixed, sealed, and then incubated for 95 min at 90°C. The vial's contents were then restricted using a UV-Vis double-beam spectrophotometer (Shimadzu) at 695 nm.

2.6 Statistical analysis

In order to get the samples ready for statistical analysis, three were taken. With the use of a beam spectrophotometer and the statistical software SPSS version 23, (Statistical Package for Social Sciences), the data on phytochemicals and proximal composition was presented as mean standard error (SE). A regression analysis was performed on the antioxidant activity IC_{50} values using Origin 2018.

3. Results

3.1 Proximate composition and mineral content

Table 1 presented the approximate composition and mineral content of *M. zapota* leaves. In proximate composition, leaves of *M. zapota* had moisture content (4.167 %), ash content (6.488%), crude fat (5.113%), crude fibre (9.435%), crude protein (8.407%) and total carbohydrate (66.78%). In mineral analysis, leaves of *M. zapota* consist of iron (20.756 ppm), copper (4.78 ppm), zinc (6.656 ppm), manganese (9.43 ppm).

3.2 Phytochemical analysis

M. zapota leaves extract in methanol and ethyl acetate underwent a quantitative analysis to determine the concentrations of various phytochemicals, such as total sugars, non-reducing sugars, reducing sugars, total flavonoids, and total phenolics. The outcomes are shown in Table 2.

Table 1: Proximate composition and minerals analysis of *M. zapota* leaves

Proximate composition	Moisture content (%)	4.167
	Ash content (%)	6.488
	Crude fat (%)	5.113
	Crude fiber (%)	9.435
	Crude protein (%)	8.407
	Total carbohydrate	66.78
Minerals content	Fe (ppm)	20.756
	Cu (ppm)	4.78
	Zn (ppm)	6.656
	Mn (ppm)	9.43

Table 2: Phytochemicals in various solvents extract of the *M. zapota* leaves

S. No.	Phytochemicals	Methanol extract	Ethyl acetate extract
1.	Total phenolics (mg GAE/g)	103.950 ± 0.426	23.100 ± 0.210
2.	Total flavonoid content (mg CE/g)	25.681 ± 0.366	5.064 ± 0.169
3.	Total sugar (mg/g)	114.770 ± 0.325	51.854 ± 0.358
4.	Reducing sugar (mg/g)	58.929 ± 0.195	39.890 ± 0.318
5.	Non-reducing sugar (mg/g)	55.117 ± 0.295	12.140 ± 0.569

3.2.1 Total phenolic content

The Folin-Ciocalteu reagent was used to estimate the extract's total phenol content, which is expressed in gallic acid equivalents (GAE). Using the gallic acid standard curve, the total phenolics of the sample was found to be 103.950 ± 0.426 and 23.100 ± 0.210 mg gallic acid equivalent/g of *M. zapota* leaves methanol and ethyl acetate extract, respectively (Figure 1).

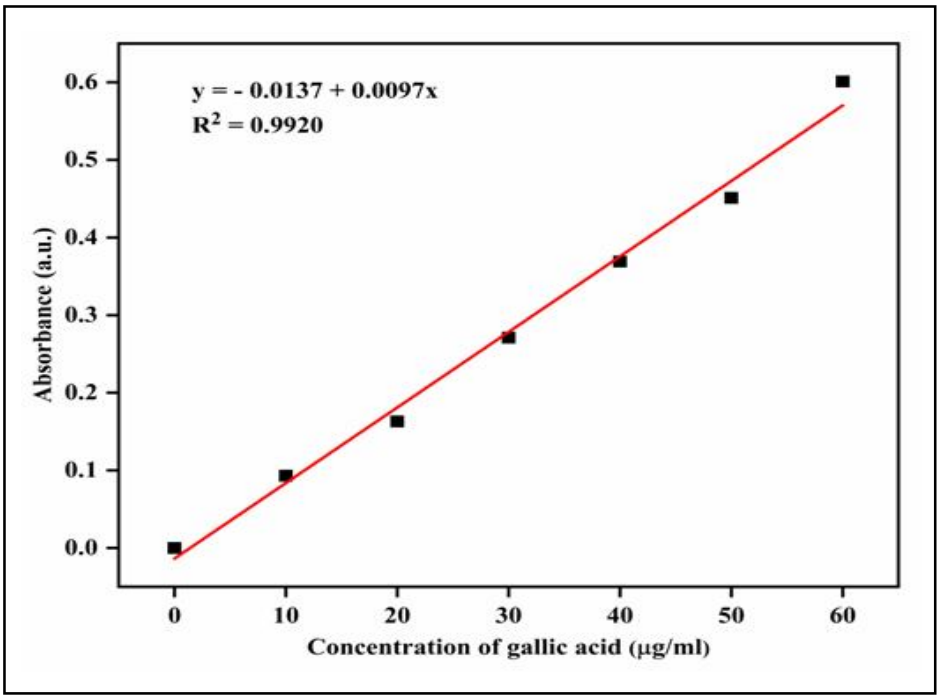

Figure 1: The total phenol standard curve uses gallic acid as the standard.

3.2.2 Total flavonoid content

The amount of total flavonoids found in the ethyl acetate and methanol extract of *M. zapota* leaves was intended using a standard curve with catechin as the reference compound (Figure 2). The regression equation results show that there is a linear relationship between absorbance and catechin concentration. Total flavonoids found were 5.064 ± 0.169 and 25.681 ± 0.366 mg CE/g of the leaves ethyl acetate and methanol extract, respectively.

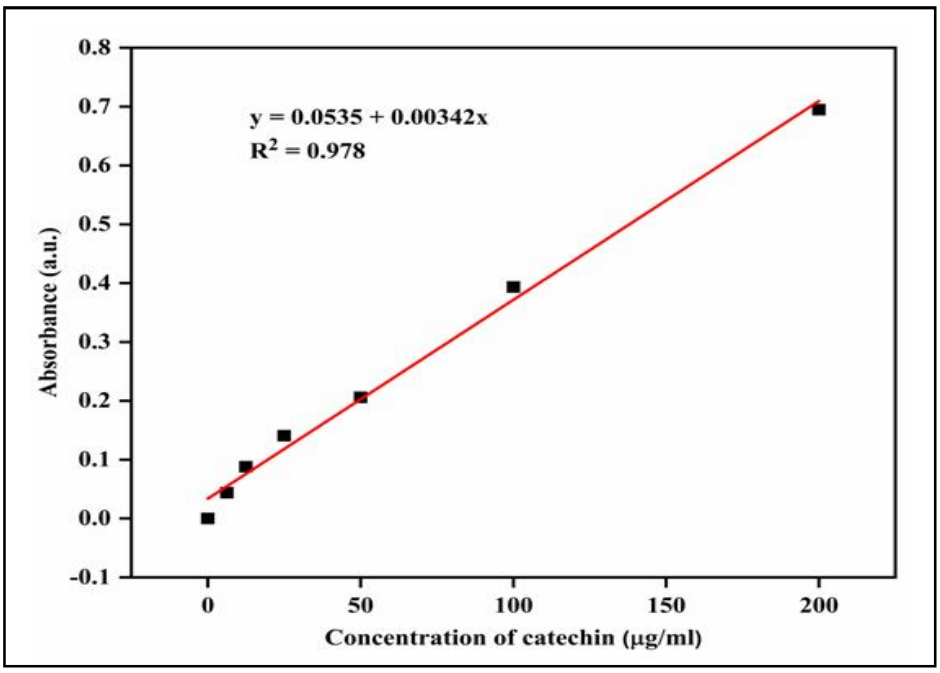

Figure 2: Standard curve for total flavonoids using catechin as the reference.

3.2.3 Total sugar content

D-glucose and a standard curve were used as a point of comparison to estimate the total amount of sugar present in the methanol and ethyl acetate extract of the *M. zapota* leaves (Figure 3). The regression equation demonstrated a linear relationship between D-glucose and absorbance concentration. Regression analysis was used to determine the total sugar content of the ethyl acetate and methanol extract from the leaves ($y = 0.0068 + 0.0011x$, $R^2 = 0.989$). The results indicated that the sugar content was 51.854 ± 0.358 mg/g and 114.770 ± 0.325 mg/g.

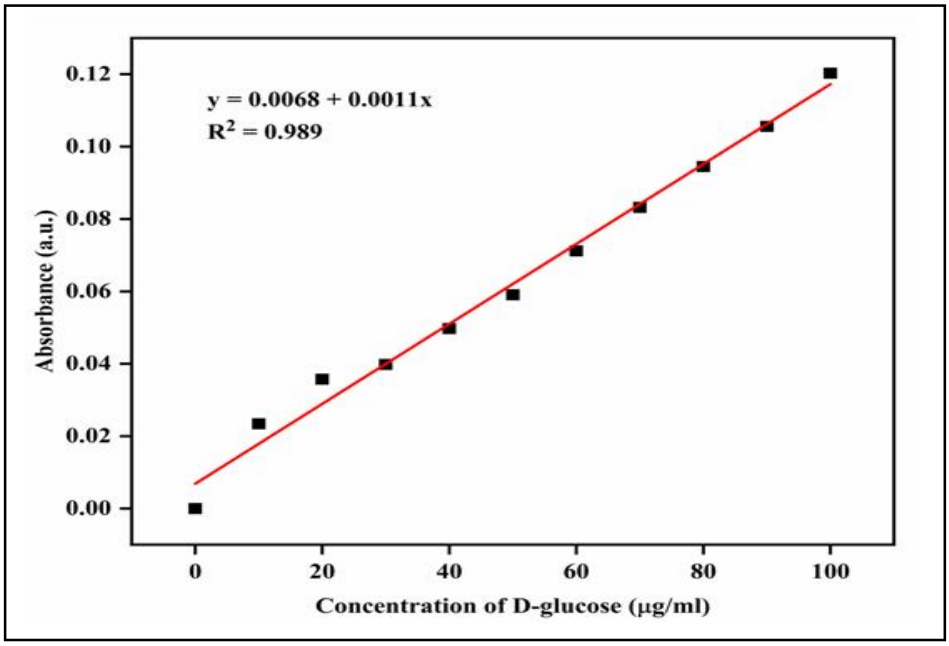

Figure 3: Standard curve for total sugar with reference to glucose.

3.2.4 Reducing sugar

The amount of reducing sugars in the ethyl acetate and methanol extract of the *M. zapota* leaves was determined by creating a standard curve with D-glucose as the reference (Figure 4). Regression analysis results indicated that absorbance and D-glucose concentration were linearly related. The total sugar content of a leaf extract made with methanol and ethyl acetate was calculated using regression analysis ($y = -0.0052 + 0.0018x$, $R^2 = 0.9913$). The findings were 39.890 ± 0.318 mg/g and 58.929 ± 0.195 mg/g, respectively.

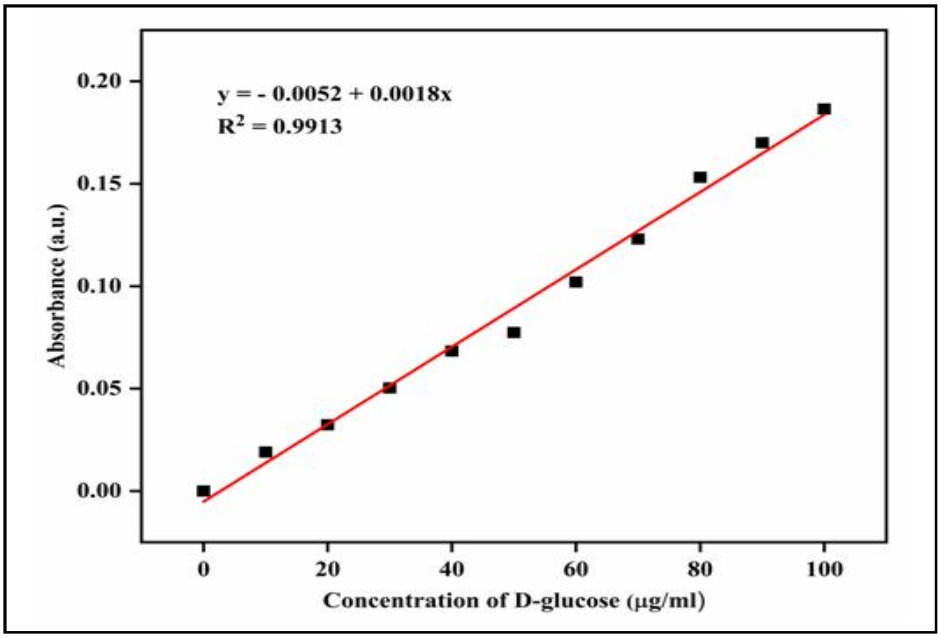

Figure 4: Standard sugar reduction curve using glucose as the point of reference.

3.2.5 Non-reducing sugar

Using the difference between the concentrations of total and reducing sugars, the amount of non-reducing sugars in the *M. zapota* leaves methanol (55.117 ± 0.295 mg/g) and ethyl acetate (50.847 ± 0.178 mg/g) extract was determined.

3.3 Antioxidant activity

3.3.1 DPPH free radical scavenging activity

At 120 µg/ml, ascorbic acid's ability to scavenge DPPH free radical was 86.67%. At 100, 80, 60, 40, and 20 µg/ml, it was 80.32, 71.28, 60.98, 49.01 and 25.38%, respectively. The greatest DPPH free radical scavenging activity was shown by methanol leaves extract at 80 µg/ml, which was followed by concentrations of 83.33, 77.48, 72.514, 66.08, 58.187, 47.07, 35.15 and 16.23%, in that order.

At 350 µg/ml, the ethyl acetate leaf extract exhibited the highest level of activity, scavenging 76.75% of DPPH free radicals. Following this, in that order, were 72.35, 67.66, 60.56, 48.45, 31.53, and 18.37%. Compared to the ethyl acetate extract, which had an IC_{50} of 158.15 µg/ml, the methanol extract had a higher IC_{50} of 34.26 µg/ml. Figure 6 displays the quadratic regression equation for the DPPH free radical scavenging activity's IC_{50} (µg/ml) value.

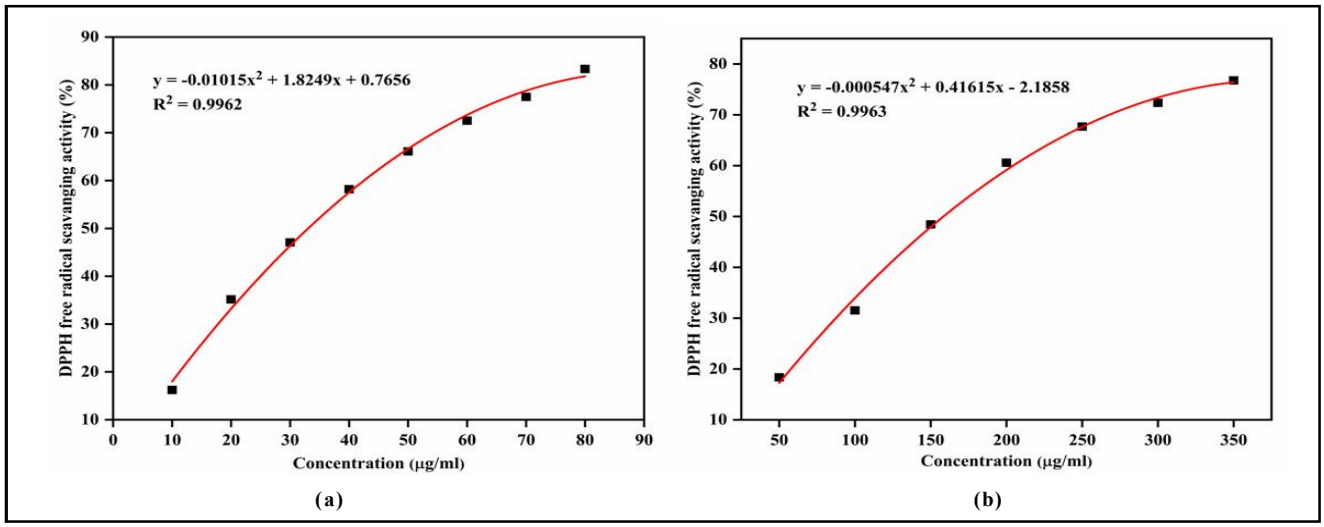

Figure 5: Quadratic regression equation for IC_{50} (µg/ml) of leaves extract DPPH free radical scavenging activity (a) methanol (b) ethyl acetate.

3.3.2 ABTS assay

The percentage of BHA that could scavenge the ABTS free radical was 91.87% at 18 µg/ml, 82.12, 71.73, 58.27, 44.39, and 24.02% at 15, 12, 9, 6, and 3 µg/ml, in that order. Sapota methanol leaves extract had the highest ABTS free radical scavenging activity (87.39%) at 80 µg/ml, followed by 70, 60, 50, 40, 30, 20 and 10 µg/ml. The next highest concentrations were 84.89, 79.76, 71.72, 59.11, 43.56, 22.91, and 6.81%, respectively (Table 3). At 350 µg/ml, the ethyl acetate extract's maximum ABTS free radical scavenging activity was 77.758%, while the lowest values were 73.352, 66.667, 57.564, 45.36, 29.532, and 16.37%, respectively. The ethyl acetate extract's IC_{50} was 167.758 µg/ml, whereas the methanol extract was 34.266 µg/ml. Figure 7 displays the quadratic regression equation for the ABTS free radical scavenging activity's IC_{50} (µg/ml) value.

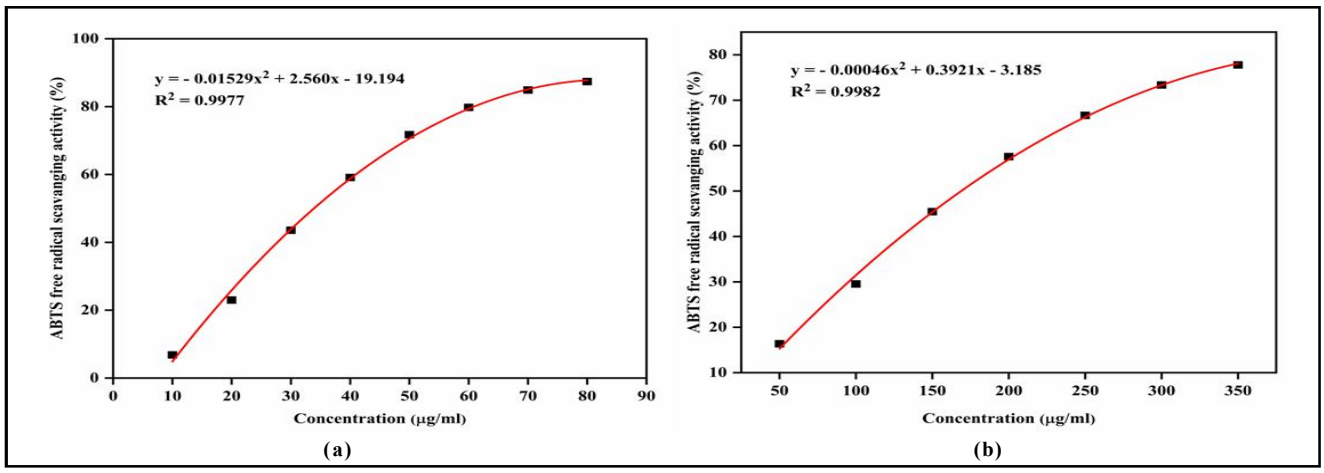

Figure 6: Quadratic regression equation for IC_{50} (µg/ml) of leaves extract ABTS free radical scavenging activity (a) methanol (b) ethyl acetate.

3.3.3 FRAP assay

By reducing a ferric 2,4,6-tripyridyl-s-triazine complex (Fe-TPTZ) to the intense blue colour ferrous form (Fe–TPTZ), the ferrous form of methanol leaves extract (10, 20, 30, 40, 50, 60, 70, and 80 µg/ml) and ethyl acetate leaves extract (50, 100, 150, 200, 250, 300 and 350 µg/ml) were estimated using FRAP tests. The direct correlation between antioxidant concentration and decreased maximal absorption is shown in Figure 8. Methanol and ethyl acetate's ferric reducing capacity rose with concentration, according to the $FeSO_4{\cdot}7H_2O$ standard curve. The results were shown for the appropriate concentration as mM Fe(II)/ml. The ability of methanol leaves extract (13.007 ± 0.17 mM Fe(II)/ml) to reduce ferric iron was found to be higher than that of ethyl acetate leaves extract (8.49 ± 0.09 mM Fe(II)/ml) in Table 3.

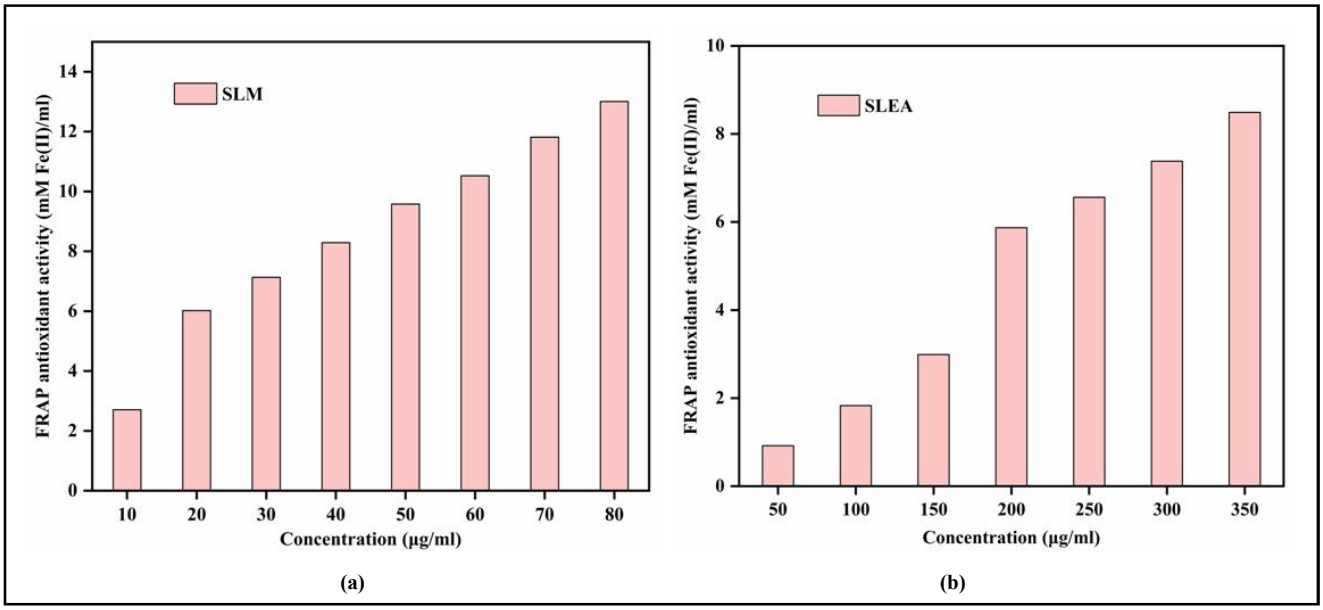

Figure 7: FRAP assay of leaves extract (a) methanol (b) ethyl acetate.

3.3.4 Phosphomolybdnum assay

Mo (VI) was reduced to Mo (V) by antioxidants. The green compound known as phosphomolybdenum complex was produced when Mo (V) reacts with sodium phosphate's phosphate group in an acidic solution. The total antioxidant capacity was estimated with the help of a standard curve using ascorbic acid as a standard and the results were shown in Table 3 as mg AAE/ml of the relevant concentration. It was discovered that the total antioxidant activity of methanol leaves extract (0.64 ± 0.024 mg AAE/ml) was higher than that of ethyl acetate leaves extract (0.552 ± 0.015 mg AAE/ml) and that the two extracts were dose dependent (Figure 9).

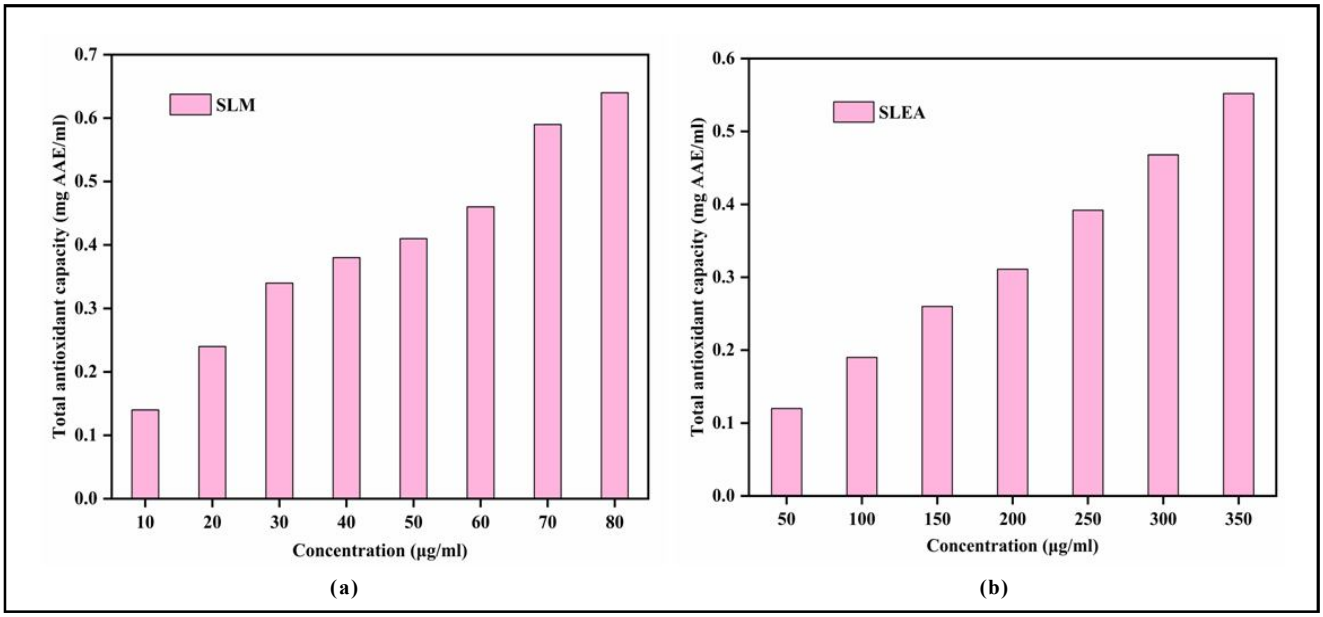

Figure 8: Phosphomolybdnum assay of leaves extract (a) methanol (b) ethyl acetate.

Table 3: Antioxidant properties of *M. zapota* leaf extracts in methanol and ethyl acetate

S.No.	Plant extract	DPPH (IC_{50}) (µg/ml)	ABTS (IC_{50}) (µg/ml)	FRAP (mM Fe(II)/ml)	TAC (mg AAE/ml)
1	SLM	34.266	35.02	13.007 ± 0.17	0.64 ± 0.024
2	SLEA	158.153	167.758	8.49 ± 0.09	0.552 ± 0.015

4. Discussion

This study's primary goal was to determine the phytochemical components of a dried *M. zapota* leaves extract that was used to produce methanol and ethyl acetate. This study found that the methanol extract is a remarkable source of total sugar, non-reducing sugar, total phenolics, total flavonoids, and reducing sugar when it was compared to an ethyl acetate extract from *M. zapota* leaves. A sufficient level of antioxidant capacity is indicated by the presence of flavonoids and phenolic acids in methanol extract, as determined by the phosphomolybdnum assay, ABTS, DPPH free radical scavenging activity, and FRAP.

Depending on how polar the extraction solvents are, the extract's overall phenolic content can change. The results of the investigation show a notable level of activity in the release of secondary metabolites from plant components induced by methanol extract. This might be the outcome of phenolic compounds being widely extracted with polar solvents like methanol, ethanol, and water (Choi *et al*., 2007).

The results of the study are in agreement with the estimate of Islam *et al.* (2011), who evaluated the antioxidant activity, total phenolic content, reducing power capacity, and total flavonoid content of the ethanolic extract of *M. zapota* leaves. According to their report, the total flavonoid content was 984.13 ± 31.39 µg QE/g, and the total phenolic content was 89.67 ± 3.074 mg GAE/g. With IC_{50} values of 68.27 µg/ml, the extract exhibited DPPH radical scavenging activity in comparison to ascorbic acid (IC_{50} 16.17 µg/ml).

Kaneria and Chanda (2012) found that the standard gallic acid (IC_{50}=185 µg/ml) had lower superoxide anion scavenging activity than standard acetone extract (IC_{50}= 78 µg/ml), and that the acetone extract (IC_{50} = 7.6 µg/ml) of Sapota leaves had higher DPPH free radical scavenging activity than standard ascorbic acid (IC_{50}= 11.4 µg/ml). The agar well diffusion method was used to assess the antimicrobial properties. Compared to the other solvent extracts, the acetone extract exhibited a notably greater level of antimicrobial activity.

The antioxidant activity of Sapota leaf extracts was assessed by Chanda and Nagani (2010) using four *in vitro* techniques: DPPH, reducing capacity assessment assay, superoxide, and hydroxyl radical scavenging activity. The acetone extract showed the highest DPPH radical scavenging activity, with an IC_{50} value of 20 µg/ml. Acetone extract (IC_{50} = 140 µg/ml) outperformed standard gallic acid (IC_{50} = 185 µg/ml) in terms of superoxide anion scavenging activity. It showed a strong assessment of capacity reduction.

5. Conclusion

The current study's results suggest that phytochemicals present in the *M. zapota* leaves methanol and ethyl acetate extract may be essential for scavenging organisms that cause oxidative stress. To gain insight into the pharmacological effects of *M. zapota* leaves, quantitative phytochemical research may be helpful. More investigation is required to ascertain the potential applications of *M. zapota's* methanol leaves in the pharmaceutical, medical, and nutritional fields because it has a higher concentration of phytochemicals (TPC 103.950 mg GAE/g and TFC 23.100 mg CE/g) than the ethyl acetate leaves. Additional investigation is necessary to pinpoint the specific components of the antioxidant system and develop applications for the healthcare and food industries.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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