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Original Article

Comprehensive Analysis of Secondary Metabolites in *Manilkara Zapota L.*: Qualitative and Quantitative approaches

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ABSTRACT

The research presents an in-depth examination of the secondary metabolites found in *Manilkara zapota* L. (sapodilla) utilizing both qualitative and quantitative methodologies. Fresh leaves and seeds were gathered, processed, and extracted with methanol and acetone. Phytochemical analysis identified significant bioactive compounds, including tannins, alkaloids, steroids and glycosides, primarily in the methanol extracts of the leaves. The antioxidant capacity was assessed using the DDPH assay, revealing that acetone extracts, especially from the seeds, exhibited superior antioxidant activity compared to those extracted with methanol.

Keywords: Secondary Metabolites, *Manilkara Zapota L.*, Qualitative, Quantitative approaches

INTRODUCTION

Plants contain a wide variety of phytochemicals, such as phenolics, flavonoids, terpenoids, saponins, tannins, glycosides, and alkaloids. The antioxidant effects observed in plants are primarily linked to the presence of phenolic compounds. These compounds are classified as secondary metabolites and are characterized by their aromatic rings that include hydroxyl groups. They combat free radicals by either donating an electron or a hydrogen atom. in addition to chelating an metal ions. Among the various phytochemicals, phenolic acids and flavonoids represent the largest and most significant groups (Tamsir *et al*., 2020). One of nature's wonders, *Manilkara zapota* (L.) P. Royen, often referred to as sapodilla, chickoo, or sapota, is a member of the Sapotaceae family, which has roughly 65 genera and 800 species. The sapodilla, also known as *Manilkara zapota* (L.) P. Royen, it's a fruit-bearing tree that has primarily been grown in tropical regions such as South East Asia and Mexico (Bano at el., 2017). *M. Zapota* may be useful in preventing dyslipidemia, diabetes, obesity, and its consequences (Barbalho *et al*., 2015). Flavonoids and bioactive phenolic compounds are among the phytochemicals found in it. The fruit has antibacterial and antioxidant properties in addition to being a strong source of minerals. Lupeol acetate, oleanolic acid, apigenin-7-O-a-L-rhamnoside, myricetin-3-O-a-L-rhamnoside, and caffeic acid are the main components that were extracted from *M. Zapota* leaves (Shazly *et al*., 2012). It is widely grown for commercial use, particularly in the Philippines, Indonesia, Vietnam, Malaysia, India and Thailand. Additionally, it is significantly cultivated in Bangladesh, Cambodia, sir Lanka and Pakistan (Rivas-Gastelum *et al*., 2023). This study examines the reproductive phenology, breeding system and pollination of this species across two distinct environments: medium-height subdeciduous forests and homegardens ("solares") within a Mayan community in Yucatan. Notable differences were observed between the two environments regarding the timing of flower and mature fruit production, as well as the quantity of mature fruits. Trees in homegardens exhibited higher flower production levels remained consistent across both settings. Additionally, mature fruits from homegardens were of superior quality, characterized by greater fresh weight (Salianas *et al*., 2007).

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Sr no.	Species names
1.	*Manilkara celebica H.*
2.	*Manilkara dardanoi D.*
3.	*Manilkara elata M.*
4.	*Manilkara fasciculate (Warb)*
5.	*Manilkara hexandra (Roxb.)*
6.	*Manilkara jaimiqui (C. Wright ex Griseb)*
7.	*Manilkara bidentate (A.DC)*
8.	*Manilkara kauki L.*
9.	*Manilkara longifolia (A.DC.)*
10.	*Manilkara spectabilis P.*

Taxonomy

This group comprises shrubs and trees that are commonly found in tropical areas such as America, Asia and Africa. Members of this family are easily identifiable due to their distinctive milky latex and the presence of alternate leathery leaves featuring parallel secondary and tertiary veins (Bano *et al*., 2017).

Classification

Kingdom	Plantae
Class	Dicotyledons
Subclass	Gamopetalae
Series	Inferae
Order	Ebenales
Family	Sapotaceae
Genus	*Manilkara*
Species	*M. zapota*

The classification according to Benthum and Hooker.

Vernacular names

Country	Common names
India	Chikoo
Thailand	Lamoot
English	Sapodilla
Indonesia	Sawu
Cuba	Sapota
Brazil	sapoti
Mexico	Chicopote
West Indies	Naseberry
Singapore	Ciku
Malaysia	Chikoo

Procedure

Collection of plant material

Fresh leaves and seeds of *Manilkara zapota* L. were collected in the month of January, 2025, from Shahibaug, Ahmedabad, Gujarat, India. The leaves and seeds were separated, washed thoroughly with tap water, shade dried, crushed to fine powder and stored in air tight bottles.

Sample preparation

The leaves, seeds, of *M. zapota* were manually separated and thoroughly washed with running tap water. Subsequently, they were dried in a ventilated dryer at a temperature of 35 °C for 72 hours before being ground into a powdered form.

Leaves and seeds extract

About 10g leaves and seeds powder were dissolve in 100ml acetone and methanol and then put it in shaker for 24 hours. The suspension was processed using a vacuum pump in conjunction with filter paper Lastly, the filtrates from acetone and methanol extracts were dried at room temperature or on hot plate at the temp. 60 c.

Yield of extract:

The yield is calculated in percentage (%) from the following formula.

$$\text{Percentage yield} = \frac{\text{Weight of dry extract} \times 100}{\text{Weight of plant powder}}$$

Qualitative Tests for Phytochemical Screening:

The phytochemical screening was carried out for all samples as per the standard method.

Test of Steroids: (Khandelwal, K. 2008, Melad, F. M., *et al*., 2023)
Salkowaski Reaction:
Few milligrams of gum was taken in the taste tube. 2 ml of chloroform and 2 ml of conc. sulphuric acid was added from the side of the taste tube. The taste tube was shaken for a few minutes. The development of red colour in the chloroform layer indicated the presence of sterols.

Tests for Flavonoids:

Shinoda Test:

Few milligrams of gum was taken in the taste tube and dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta colour is developed within a minute or two if Flavonoids are present.

Alkaline Reagent Test:

Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

Lead Acetate test:

Extracts were treated with a few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Tests for Amino Acids:

Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for a few minutes. Formation of a blue colour indicates the presence of amino acid.

Xanthoproteic Test:

The extracts were treated with a few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.

Test for sugars:

Fehling's Solution Test:

Fehling solution A and Fehling solution B Both were mixed in equal volumes immediately before use. A little of the test residue was dissolved in water, and a few ml of the Fehling's solution was added to it. This mixture was then warmed. If a red precipitate of cuprous oxide is obtained, reducing sugars are present.

Test for Glycosides:

Legal's Test:

Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Tests for Alkaloids:

Wagner's Test:

Few ml extract are added in the test tube and treated with a 4-5 drops of Wagner's reagent (along the sides of test tube), Formation of brown/reddish colour Precipitate indicates the presence of alkaloids (Wagner, H. 1993; Banu, K. S., *et al*., 2015).

Mayer's Test:

Few ml extract are added in the test tube and treated with a two drops of Mayer's reagent (along the sides of taste tube), formation of creamy white colour precipitate indicates the presence of alkaloids (Evans, W. C. 2009).

Dragendroff's Test:

Few ml extract are added in the taste tube and treated with few drops of Dragendroff's reagent (along the sides of taste tube), formation of reddish/brown precipitate indicates the presence of alkaloids (De Silva, G. O., *et al*., 2017; Kumar, M. K., *et al*., 2011).

Tests for Tannins:

Ferric Chloride Reagent Test:

1 ml extract are added in the test tube and treated with 2 ml 5% ferric chloride reagent, Formation of dark green/bluish black colour indicates the presence of tannins (Audu, S. A. *et al*., 2007).

Lead Acetate Test:

1 ml Extract are added in the test tube and treated with 3 ml 10% lead acetate solution, Formation of White precipitate indicates the presence of tannins (Shaikh, J. R., *et al*., 2020).

Quantitative estimation of total Tannin content

The total tannin content was assessed using the Folin-Ciocalteu method. A volume of 0.1 ml of the sample extract was introduced into a 10 ml volumetric flask, which contained 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, and 1ml of a 35% sodium carbonate solution. The flask was then filled to the 10ml mark with distilled water. The resulting mixture was thoroughly shaken and allowed to stand at room temperature for 30 minutes. Reference standard solutions of tannic acid were prepared in concentrations of 20,40,60,80 and 100 ug/ml using at 700 nm with an UV/Visible spectrophotometer. The tannin content was determined in triplicate and expressed as milligrams of tannic acid equivalents per gram of dried sample (Naima saeed *et al.*, 2012).

Antioxidant Activity

A paper-based assay utilizing 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) has been developed for efficient, cost-effective, and low-consumption analysis of antioxidant activity. This device was created through a lamination technique, resulting in a circular test zone with a diameter of 5mm, which contains the DPPH reagent. The analysis is performed in a single step by applying an antioxidant, the DPPH radicals are reduced to stable DPPH molecules, leading to a colour transition from deep violet to pale yellow (Biois, M. S., 1958).

METHOD:

The DPPH (2,2-diphenyl-1-picrylhydrazly) reagent in the DPPH technique, which was developed by Marsden Blois in 1958 to measure a substance's capacity to scavenger free radicles. Firstly, take plant extract having different concentration followed by adding 3ml DPPH solution. After that make final volume up to 1ml with solvent i.e. Methanol & Acetone. Incubate it for 30 min under dark condition. Using UV spectrophotometer. The absorbance at 517nm was determined. For Ascorbic acid similar procedure was followed for the standard series with concentration 1mg/1ml.

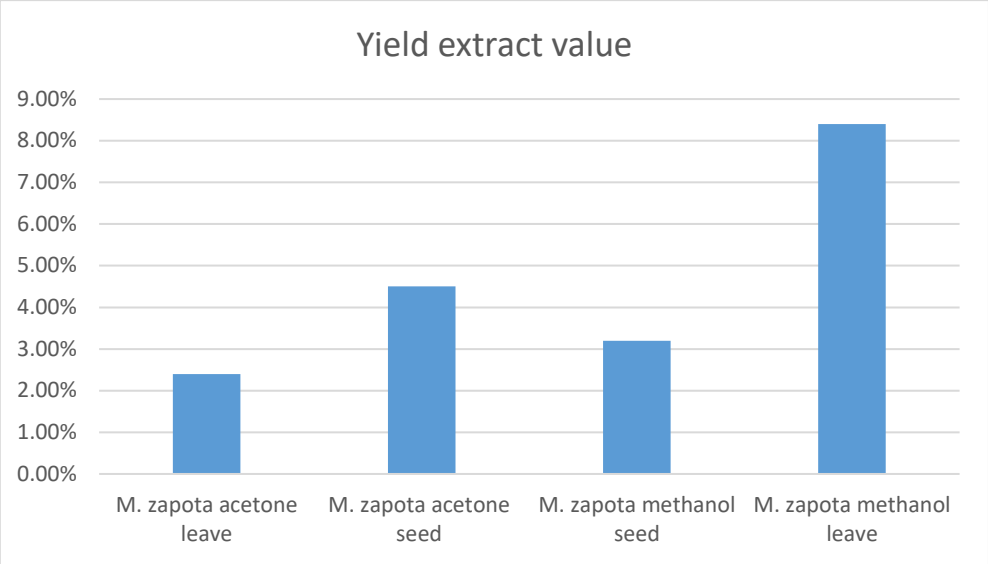
RESULT:

Calculations of % yield of the extract:

% yield= (extract obtained/total amount of crude drug) ×100

Plant	Acetone leaves	Acetone seed	Methanol seed	Methanol leave
Manilkara zapota. L	2.4%	4.5%	3.2%	8.4%

Graph



DISCUSSION

The extraction yield of manilkara zapota L. was influenced by both the solvent and the specific part of the plant utilized. The methanol extract from the leaves produced the highest yield at 8.4% demonstrating methanol's effectiveness in extracting bioactive compounds from leaf material. In contract, the acetone extract from seeds yielded a moderate 4.5%, while the acetone extract from leaves resulted in the lowest yield of 2.4%, indicating that acetone is more suitable for extracting from seeds. The methanol extract from seeds yielded 3.2%, suggesting that extracting compounds from seeds than from leaves. These differences underscore the significance of choosing the appropriate solvent to enhance extraction efficiency, with methanol being particularly advantageous for leaf extract.

Phytochemical screening

All the tests performed for the presence of absence of phytochemicals are tabulated below. The "+" sign indicates presence while the "-" sign indicates absence of the phytochemical in the plant extract.

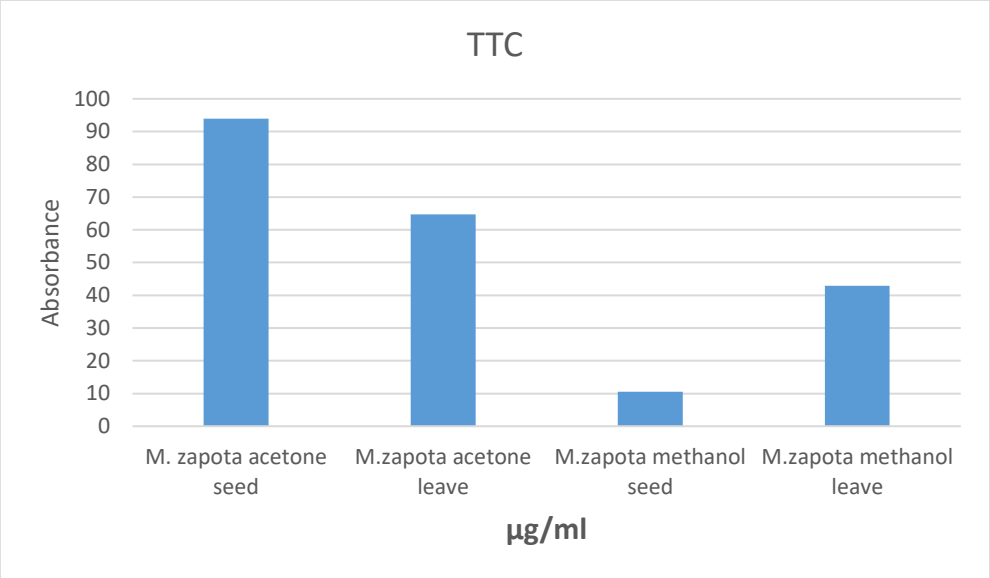
Detections	Tests	Methanol seeds	Methanol leaves	Acetone seeds	Acetone leaves
Detection of Reducing Sugars	Fehling"s test	-	-	-	-
Detection of glycosides	Legal's test	-	+	-	+
Detection of Amino acids	Ninhydrin test	-	-	-	-
	Xanthoproteic test	+	+	-	-
Detection of flavonoids	Shinoda's test	-	-	-	-
	Alkaline reagent test	-	+	-	+
	Lead acetate test	-	+	-	+
Detection of tannin	Ferric Chloride reagent	+	+	+	-
	Lead sub acetate test	-	-	-	-
Detection of alkaloid	Dragendroff's test	-	-	+	+
	Mayer's test	-	+	-	+
	Wagner"s test	+	+	-	-
Detection of steroids	Salkowski's test	-	+	-	-
	Libermann-Burchard's test	-	+	+	-

DISCUSSION

The phytochemical analysis of plant extract utilizing various solvents (methanol and acetone) demonstrated differences in the occurrence of bioactive compounds. Notably, methanol extracts, especially from leaves, exhibited the greatest variety of phytochemicals, including tannins, alkaloids, steroids and glycosides, indicating that methanol is a more efficient solvent for extraction. The detection of flavonoids was minimal, with only the alkaline reagent test yielding positive results for methanol leaves. While reducing sugars and amino acids were largely absent, tannins and alkaloids were notably present, particularly in methanol extract. Additionally, steroids were identified in some extracts, suggesting potential medicinal benefits. These results imply that the plant contains significant bioactive compounds that warrant further investigation for therapeutic uses.

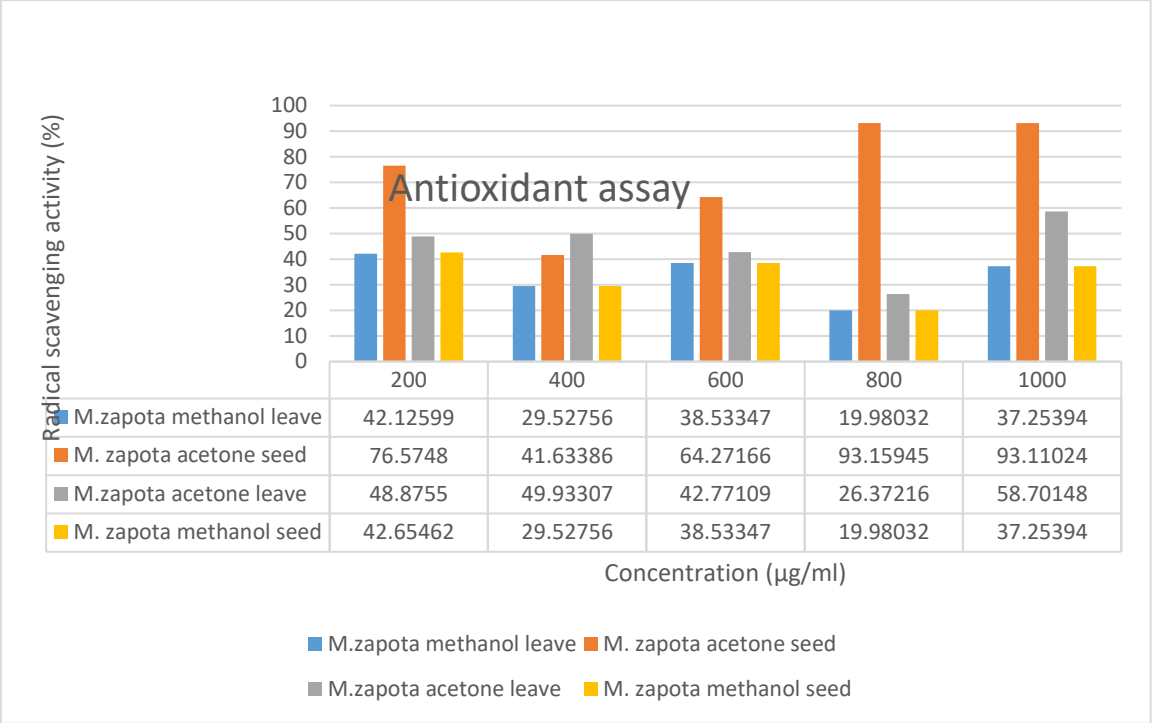
Total Tannin content (TTC)

Sample	Concentration µg/ml
M. zapota Acetone seed	93.94±109.60
M.zapota Acetone leave	64.75±1.38
M.zapota methanol seed	10.55±1.01
M.zapota methanol leave	42.87±8.83



DISCUSSION

The graph illustrates the absorbance values of extracts from *Manilkara zapota* in a TTC assay, differentiated by solvent type (acetone and methanol) and plant part (seed and leaf). The extract from seeds using acetone demonstrates the highest absorbance, signifying the greatest bioactivity, followed closely by the acetone extract from leaves. In contrast, the methanol seed extract displaying the least activity. This indicates that acetone is a more efficient solvent for the extraction of bioactive compounds, especially from seeds. The results underscore the differences metabolite content influenced by the type of solvent and the specific plant part, highlighting the potential of acetone extract from M.zapota for uses that require high antioxidant or metabolic activity.



DISCUSSION

In contrast, the methanol leaf and seed extracts show significantly lower antioxidant activity, with a noticeable drop at higher conc. This suggests that acetone extracts are more effective in extracting bioactive compounds responsible for antioxidant properties. The overall trend shows that antioxidant activity increases with concentration, particularly for acetone extracts, while methanol extracts display variability. These findings highlight the potential of *M. zapota* acetone seed and leaf extracts as strong natural antioxidants, warranting further phytochemical investigations.

CONCLUSION

Acetone has proven to be a more efficient solvent for the extraction of bioactive metabolites, with seed extracts exhibiting the highest levels of absorbance and bioactivity. Phytochemical analysis indicated that methanol extracts, particularly those derived from leaves, possessed a broader range of bioactive compounds, such as tannins, alkaloids, steroids and glycosides. These finding underscore the potential of *M. zapota* as a natural source of antioxidants and bioactive substances, reinforcing its applicability in medicinal and pharmaceutical fields. Additional research is necessary to investigate its therapeutic potential and to refine extraction techniques for optimal effectiveness

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