

# Extraction, Characterization and Applications of Latex of *Manilkara zapota*

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## Abstract

In this study, bioactivity and application of plant latex of *Manilkara zapota* in agriculture fields had been studied. Latex was collected, extracted and characterized with TLC, UV-Vis and GCMS analyses. Latex was also subjected for seed germination study, pot study and insecticidal activity. This latex was found to increase water holding capacity and soil porosity and soil structure was improved by latex. It was also inducing the root formation and enhances crop yields. It also shown insecticidal activity against mealy bugs.

**Keywords:** *Manilkara zapota*; latex; bioactivity; insecticidal activity.

## INTRODUCTION

Laticifers are highly specialized elongated secretory plant cell distributed over the whole plant. It is in charge of secreting and storing the plant latex<sup>1</sup>. Plant latex is a secondary metabolite of plant which normally in milky white, yellow or orange. Roots, stem, leaves, unripe fruits and barks are typically the part with high amount of latex<sup>2</sup>. In order response to turgor pressure in laticifers, latex is secreted only when the plant is suffering from mechanical injury such as insect bites or an incision on it. Various bioactive compounds can be detected in plant latex such as alkaloids, terpenoids, tannins, proteins, sugar, saponins, starches, resins, and gums. This is because it consists of content from vacuoles of laticifers and emulsion are formed from it. Hence, the plant latex acts as defence system of the plant. Latex normally will coagulate in a short time and cover the damaged tissue to protect the plant from attack by insect again during its recovery period<sup>3</sup>.

*Manilkara zapota* (sapodilla) is a latex bearing plant from the genus *Manilkara* which belongs to Sapotaceae family. It is also known as sapodilla, naseberry, chicku and chikoo. *M. zapota* is a tropical tree which mostly is found in India, Malaysia and South America<sup>4</sup>. Its latex, fruits and timber are commercially used. The latex produced by *M. zapota* is milky white colour. Traditionally, latex of it is used to manufacture the chewing gum which is chicle-based and acts as filling for tooth cavities. Latex of *M. zapota* mainly contain tannins, flavonoids, alkaloids, saponins, polysaccharide and polyphenolic compounds. These components contributed to different bioactivities.

Fertilizers are one of the factors which largely influenced the growth of plants. It increased the crop yields by enhancing and sustaining the soil quality. Agriculture fields employ insecticides to prevent their plantations from being destroyed by insects as well as microorganisms<sup>5</sup>. Massive usage of inorganic fertilizer and synthetic chemical insecticides resulting air pollution, water pollution, soil pollution<sup>6,7</sup>. Chemical insecticides also impact via food commodities. Apart from that, the health of workers in manufacturing chemical insecticides as well as agricultural farm workers are significantly affected since they long-terms exposed to toxic chemicals. Hence, latex from *M. zapota* had been used as fertilizer to determine whether it promotes the growth of the plant and natural insecticides to protect the attack from insects<sup>8,9</sup>. This study was done to determine the bioactivity of latex of sapodilla and its role in agriculture such as its ability to enhance the growth of plants and its potential as insecticides.

## Materials and Methods

### Collection of Latex

Latex was collected from the unripe sapodilla fruits by making an incision on it (Figure 1). Latex was collected, dried and used for further study and characterization.



**Figure 1. Latex from *M. zapota***

### Extraction, Characterization and bioactivity of Plant Latex,

25 ml of double distilled water was added to 2g of dried. The mixture was centrifuged at 6000 rpm for 15 minutes. Supernatants were lyophilized and used for the study. The latex extracts were characterized by Thin Layer Chromatography, UV-Visible spectroscopy and GC-MS analysis (SHIMADZU, QP2010 PLUS). Antibacterial activity against *E. coli* and *S.aureus* was performed and antioxidant activity was performed using DPPH assay <sup>10</sup>.

### Seed Germination Study

Petri dishes were autoclaved to prevent any contamination occurred during seed germination study. The petri dishes were fully covered with cotton and 5 seeds were placed / petri dish. In control set, the seeds were added with 10 drops of water / petri dish / day <sup>11</sup>, where latex dispersed in water and added as 10 drops as above mentioned. Humidity was maintained. On 12<sup>th</sup> day and 20<sup>th</sup> day, the length of shoots and roots were measured and recorded. Germination rate were calculated with the following equation <sup>12</sup>.

$$\text{Germination Rate} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100\%$$

### Soil properties

Pots were prepared and added with 1000 g non fertile soil collected from construction site (which acted as control), where in test 1000 g of non-fertile soil was added with 10 g latex / 10 ml water and mixed thoroughly. Both latex-treated and non-treated soils were tested for water holding capacity and soil porosity as follows. Water holding capacity was measured by percolation method. A filter funnel with filter paper was placed onto the measuring cylinder. 100 g of soil samples were filled on to the filter funnel. Then, 200 ml of water was poured to the soils. The excess water was flowing into the measuring cylinder and the reading were recorded once the water stop from dripping. Water holding capacity was determined as below <sup>13</sup>.

$$\text{Water retained /100g of sample} = \text{Water added} - \text{Water in measuring cylinder}$$

Soil porosity was determined by preparing a beaker and filled the beaker with 200 g of soil sample. 1000 ml of water was filled into the measuring cylinder and poured into the beaker with soil until the soil are fully covered. The amounts of water poured were recorded and used for calculations with the equation below. The procedure is repeated with latex-treated soil.

$$\text{Soil Porosity (\%)} = \frac{\text{Volume of water poured}}{\text{Volume of soil}} \times 100$$

Soil texture was analyzed for both the latex added sample and control using SEM (scanning electron microscopy) analysis.

### Pot Study

5 seeds of long bean were placed into each pot containing either latex or not as prepared earlier at 1 cm of depth. It was watered every day. Germination rate was recorded and on the 10th day, 20th day and 30th day, the length of shoot, length of root and number of leaves were measured and recorded. One entire set was kept to study the crop yield.

## Insecticidal Activity

Different concentrations of latex extracts are prepared. 10% of latex extract were prepared by adding 4.5ml of water to 0.5 ml of latex while 20 % of latex extract were prepared by adding 1ml of latex to 4 ml of water. Total 3 sets of pests are prepared and each set with 10 mealy bugs. It was divided into control set, Test 1 and Test 2. Mealy bugs were stuck to the hibiscus leaf and put into petri dishes. Test 1 was poured with 1ml 10 % of latex extract while Test 2 is poured with 20% of latex extract. The number of pests that died after 48hours are recorded and used to calculate mortality rate. Mortality rate are calculated with the equation below

$$\text{Mortality Rate (\%)} = \frac{\text{Number of pests died after 48h}}{\text{Initial number of pests (10)}} \times 100$$

Histology of mealy bug also had been carried out by staining it with Haematoxylin &Eosin (H&E).

## RESULT AND DISCUSSION

### Characterization and bioactivity studies

UV-Vis analysis shows maximum absorbance peak around 240 nm (Figure 2) which might be because of presence of ester, even cafestol palmitate esters from coffee has shown the absorbance of 240 nm. Another absorbance peaks shown around 275 nm to 290 nm was due to presence of highly esters, where kahweol palmitate of coffee also showed around this absorbance<sup>14</sup>. TLC analysis showed the presence few metabolites with Rf value of 0.20 and 0.83 (Figure 3). Even Leon et al<sup>15</sup> also have shown separation of metabolites of latex of *Jatropha gaueri* L through TLC. Esters, cardanol, hydroxycardanol and solanesol are detected through GC-MS analysis (Figure 4). cardanol and hydroxycardanol which possessed antitumour, antileishmanial, antitumoral antifeedant and larvicidal activities<sup>16</sup>. Solanesol which is biosynthetic precursor of coenzyme Q10. Solanesol even possessed anti-inflammatory, antimicrobial, antiviral and neuroprotective activity. Pentacyclic triterpenoids also had been detected in sapodilla's latex. It provides wide range of bioactivities such as anti-inflammatory, hepatoprotective, anti-hypertensive, antiulcerogenic as well as anti-tumour<sup>17</sup>. The latex did not show any antioxidant or antibacterial activity (results not shown here).

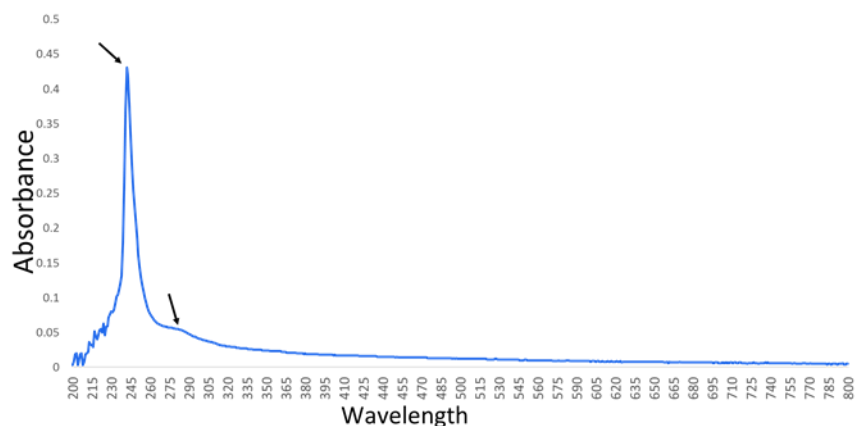
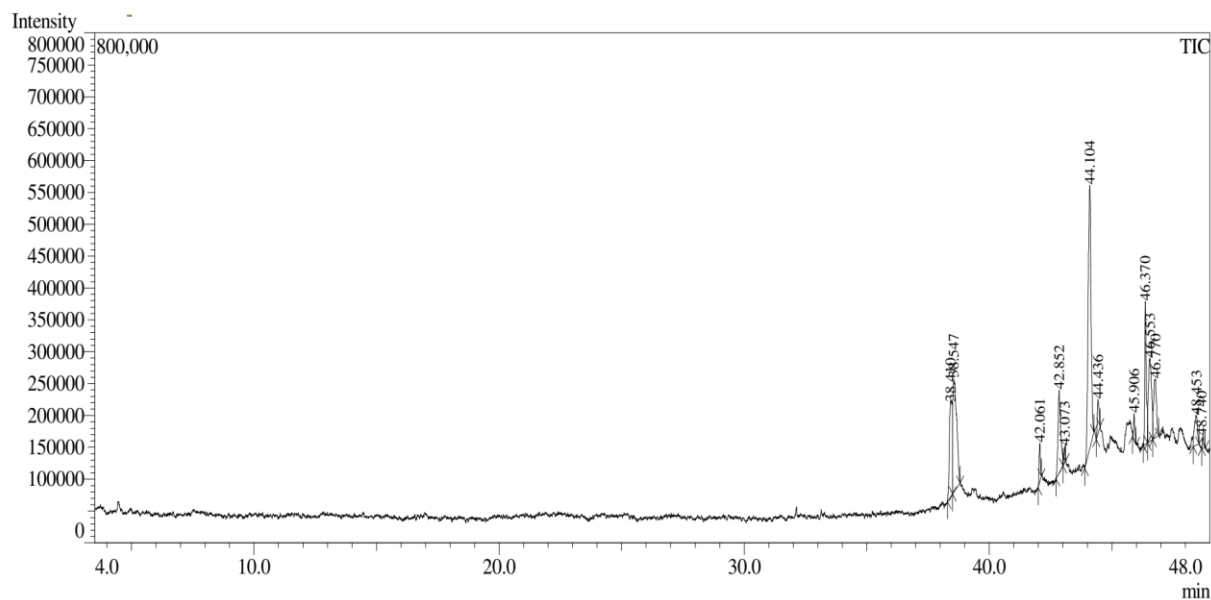


Figure 2. UV – Vis analysis of aqueous extract of *M. zapota*



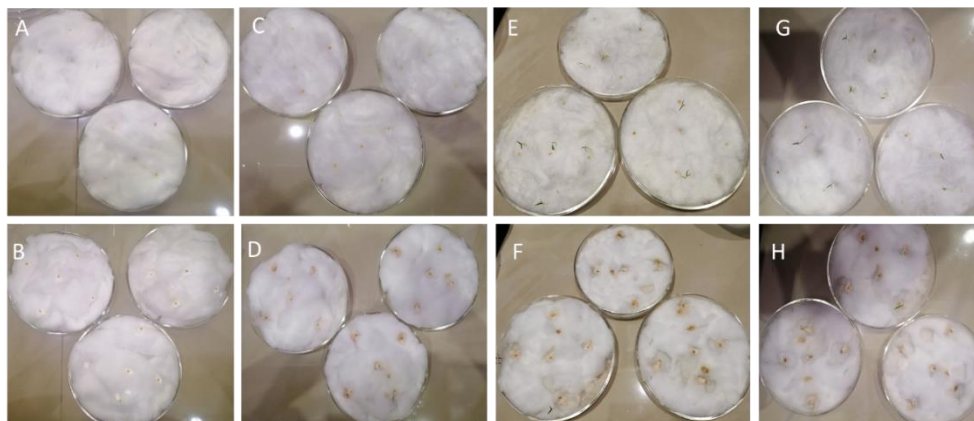
**Figure 3. TLC analysis of aqueous extract of *M. zapota***



**Figure 4. GC-MS analysis of aqueous extract of *M. zapota***

### Seed Germination Study

According to seed germination study, germination rate for control set was 66.67% but for test was 26.67% and the shoot length of the control was longer than the test (Table 1; Figure 5). However, the roots of the test were significantly longer than control and enhanced by 56.91% (figure. 6). Significant increase in germination percentage was found on *Calotropis* sp and *Euphorbia* sp latex treated *Vigna mungo* L seeds<sup>18</sup>.



**Figure 5. Seed germination study on (A) 1st day of Control (B) 1st day of Test (C) 5th day of control (D) 5th day of test (E) 10th day of control (F) 10th day of test (G) 20th day of control (H) 20th day of test**



**Figure 6. Comparison of shoot and root of control (A&C) and latex treated seed (B&D)**

**Table 1. Seed germination study**

Plant	Measurement			
	Shoot Length (cm)	Root length (cm)	No. of leaf	Germination Rate (%)
<b>Control (20<sup>th</sup> Day)</b>	1.81±0.79	0.71±0.27	1.6±0.84	66.67
<b>Test (20<sup>th</sup> Day)</b>	1.63±1.92	1.23±0.62	0.67±1.0	26.67

### Soil property

Non-treated soil water holding capacity was 5 ml/100g while latex-treated fertilized soil was 7ml/100g (Table 2). Latex from *M. zapota* increased the water holding capacity of soil by 4%. The soil porosity for non-treated soil was 45 %. Latex had enhanced the soil porosity of soil to 50 % (Table 2). Water holding capacity increase the water retention in soil as well as water available for plant growth via plant root systems<sup>19</sup>. Latex from sapodilla can be used as soil organic matters since it increased water holding capacity and soil porosity. However, latex improved the structure of fertilized soil by making it more regular (Figure 7). Biopolymers like xanthan gum was reported to increase particle cohesion<sup>20,21</sup>. Latex also enhanced the structure and texture of the soil by forming a more uniform environment which is better for rooting<sup>22</sup>.

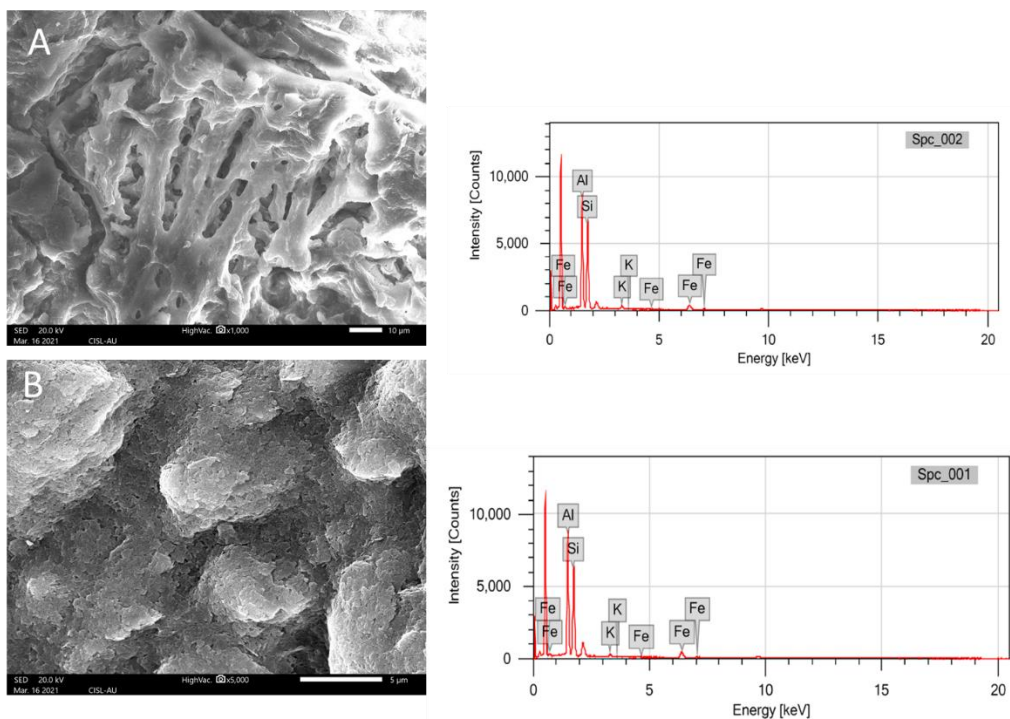


Figure 7. SEM-EDX analysis of (a) non-treated soil (b) latex-treated soil

Table 2. Water holding capacity for both non-treated fertilized soil and latex treated fertilized soil

Category	Water Holding capacity (ml/100g)	Soil Porosity
Non fertile soil (Control)	5	45 %
Latex-treated soil (Test)	7	50 %

### Pot Study

The root length of latex treated on Day 10 was 12.35 cm while for control was 11.86 cm. The root length for latex treated soil were longer than non-treated soil. On 20<sup>th</sup> day, the number of leaves for test increased by 35% than control set. On 30<sup>th</sup> day, test 3 (F) the roots of one of the shoot are visible longer than control. Fruits was yielded in only latex treated group (Table 3). Both the seed germination study and pot study shown that latex inducing the root formation but not for growth of shoot (Figure. 8). Latex from sapodilla also enhances flowering and crops yields. Rooting is normally regulated by plant hormone known as auxin. Latex of *M. zapota* might stimulate the secretion of auxins which in turns enhances the root growth<sup>23</sup>. The growth of root might also due to presence of tannins in latex which is antimicrobial and antibacterial which prevent the plant from plant disease. Flowering is controlled by a hormone known as florigens while fruiting are regulated by auxin, cytokinin and gibberellins<sup>24</sup>. Latex of sapodilla affects the regulation of florigens to fasten the flowering in plants. It might also involve in auxin, cytokinin or gibberellins regulation to increase the crop yields<sup>25</sup>. Mixture of para rubber latex and swine dung were found to increase seedling growth which was equivalent to chemical fertilizer<sup>26</sup>.



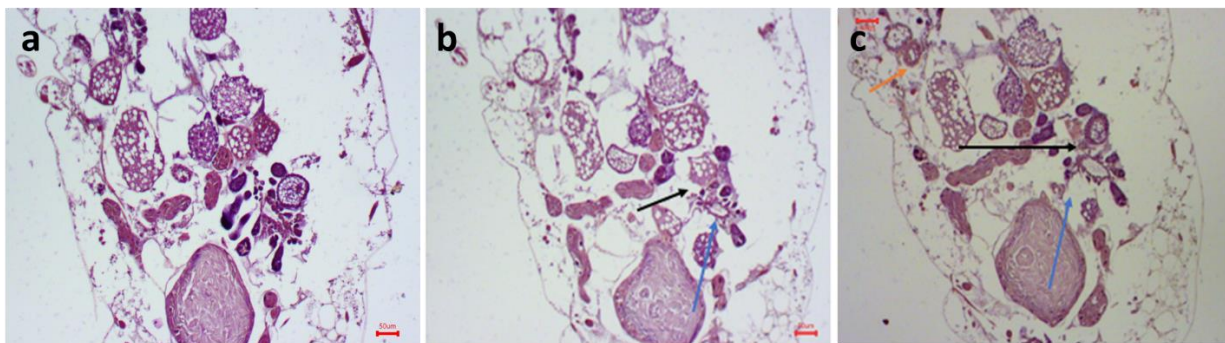
Figure 8. Comparison between (a) control on 10th day, (b) Test on 10th day, (c) control on 20th day (d) Test on 20th day (e) Control on 30th day and (f) Test on 30th day

Table 3. Pot study

Plant	Measurement (cm)				
	Shoot Length (cm)	Root length (cm)	No. of leaf	No of Flower	long bean length
Control (Day 10)	15.42± 1.47	11.86± 1.86	4.8±0.45	-	-
Test 1 (Day 10)	13.9±1.23	12.35±1.81	4.75±0.25	1±0	-
Control (Day 20)	26±0	18.93±0	5±0	-	-
Test 2 (Day 20)	22.97±3.84	15.7±2.31	7.67±0.33	1±0	-
Control (Day 30)	26±0	20.1±0	6.5±0.5	1±0	-
Test 3 (Day 30)	14.57±13.12	15.73±11.54	5.3±1.76	1±0	20.15±8.98

### Insecticidal activity

The number of pests after 48 h for control set and 10 % latex were remained as initial numbers. Mortality percentages of 20 % latex was 20 % (table 5). Mealy bugs treated with 10 % latex extracts demonstrated disintegration of gamma proteobacterial cells and disintegration of gut regions. 20 % of latex also disintegrate both gamma proteobacterial cells and gut regions. Its eventually caused nuclear damage in mealy bug and hence resulting in mortality (Figure 9). Its insecticidal activity may be due to the presence of cardanol and hydroxycardanol which exhibited Pest repulsion activity.



**Figure 9.** Histology of mealy bug after 48 hours (a) Control (b) 10% latex (c) 20% latex. Black arrow indicates disintegration of gamma proteobacterial cells, blue arrow indicates disintegration of gut regions and orange arrow indicates nuclear damage.

**Table 4. Mortality rate of latex extract**

Category	Initial Number of Pests	Number of Pest remained after 48 h	Mortality percentage (%)
Control	10	10	0
T1 (10%)	10	10	0
T2 (20%)	10	8	20

## CONCLUSION

In this study, latex from *M. zapota* were collected, extracted and characterized with TLC, UV-Vis analysis and GC-MS. The bioactive compounds like cardanol, hydroxycardanol etc has been determined by GC-MS analysis. However, it did not show any antioxidant and antibacterial activity. Latex from *M. zapota* improved water holding capacity and soil porosity of the soil. The structures of soil were also improved. Subsequently, latex from sapodilla also can be sources of organic fertilizer since it induced the root formations as well as increased flowering and crops yields. However, the germination rates were relatively low. Latex from *M. zapota* can be used as natural insect repellents since it possesses insecticidal activity as increasing the concentrations. Thus, chemical insecticides can be replaced and the pollutions to environment can be reduced. Consequently, the risk of food poisoning could significantly be reduced.

### Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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