



Bioactivity and Plant Growth Stimulation Studies using *Mangifera indica* L. Gum

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Abstract

The potential of plant gum as a bioactive agent and plant growth enhancer has not been exploited well and plant gums are suitable for such purposes as they are non-toxic and biodegradable. Therefore, the aim of this study was to verify the potential of *Mangifera indica* (MI) gum as a bioactive agent and plant growth enhancer. Plant gum was collected from the bark of MI and polysaccharides were extracted, purified and characterized with ultraviolet-visible (UV-Vis) spectroscopic, Fourier-transform infrared spectroscopy and gas chromatography (GC) analyses. Crude and purified polysaccharides were tested for their antibacterial and antioxidant activity. The crude gum was subjected to plant growth stimulation study like germination percentage, shoot length, root length and wet weight of chilli (*Capsicum frutescens*). The effect of MI gum on soil porosity and water holding capacity (WHC) was also tested. UV-Vis and GC analyses of gum polysaccharide showed the presence of several types of monosaccharides in MI gum. The plant gum did not show any antibacterial activity against *Escherichia coli*, *Pseudomonas* sp., *Bacillus* sp. and *Staphylococcus aureus*, but was found to exhibit low antioxidant activity. The gum was found to enhance the seed germination and seedling growth *in-vitro* and *in-vivo*.

Keywords: *Mangifera indica* L, Gum, Bioactivity, plant growth

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INTRODUCTION

Gums are biomaterials composed of polysaccharides produced naturally either by plants, marine organisms or microorganisms.¹ Gums of plant origin can be found in seed or branches and bark of woody plants² and are produced in response to injury or environmental stress.² Among all the gum-producing woody plants, higher production of gum was seen in trees of families Fabaceae, Combretaceae and Sterculiaceae, as they produce good quality gum suitable for commercial use.³ These gum derived polysaccharides are with excellent biocompatibility, biodegradability and non-toxic, they are suitable to be applied onto living organisms including plants.⁴ Their role as a plant growth enhancer is supported by existing literatures.⁵⁻⁷ Polysaccharides when added to soil, it is depolymerized by soil microorganisms and converted into essential nutrients readily absorbed by plants.⁸ Mango (*Mangifera indica*, MI) gum was chosen for our study because mango trees are found abundantly in Malaysia. They can be found growing wild or on roadsides and even growing at home.⁹ MI gum was found to contain nutrients required in plant growth such as nitrogen, phosphorus, potassium, calcium and iron.¹⁰ In this study, MI gum was tested for its antioxidant and antibacterial activity, and also checked for its phytostimulation study *in-vitro* and *in-vivo*.

MATERIALS AND METHODS

Materials, Chemicals and Reagents

F1 hybrid new green eagle chilli seeds (*Capsicum frutescens*) (Sin Seng Huat

Seeds), Absolute ethanol (System Chemicals), Trichloroacetic acid (Merck Chemicals), Acetone (SRL Chemicals), Nutrient broth (Himedia Chemicals), Nutrient agar (SRL Chemicals), Ascorbic acid (SRL Chemicals), DPPH reagent (SRL Chemicals), Glucose (Sigma-Aldrich) were used in this study. All the reagents used in this study are analytical grade.

Apparatus and Instruments

Weighing balance (A&D Company Ltd), Magnetic stirrer (Lab Tech), Centrifuge (Thermo Scientific), Dialysis tubes (Hi Media), UV-Vis Spectrophotometer (Geneva IOS UV-Vis-Thermo Scientific), FTIR (Perkin Elmer Spectrum Two), Biological Safety Cabinet (ESCO Class II BSC), Refrigerator (Thermo Fisher Scientific), Incubator (Thermo Scientific), Hot air oven were used in this study.

Collection of Plant Gum

Plant gum was collected from the bark of *Mangifera indica* tree found at Jalan Dato Abdul Hamid 3, Taman Sentosa, Klang, Malaysia after incisions were made (Fig. 1). The collected gum was then transferred to a clean glass container and dried at room temperature.

Extraction and Purification of Polysaccharide

Crude polysaccharide extract was prepared by dissolving 0.5 g of gum in 50 ml of distilled water under heat and then filtered. Purification of polysaccharide started with defatting procedure by soaking the gum (20 g) in 50 ml absolute ethanol and left overnight at room temperature. The sediment was collected and dried in a hot air oven (58 °C). 10 g of defatted gum was measured and dissolved in 100ml of



Fig. 1. Collection of plant gum from *Mangifera indica* tree; A- Plant, B- Gum collected in a silica cup.

distilled water under continuous stirring for 4 h using a magnetic stirrer. The solution was filtered using muslin cloth to remove particles that do not dissolve in water. Then, 100ml of distilled water was added to the filtrate, and stirred under 100 °C for 1 h. Once solution was cooled to room temperature, it was again filtered. To the filtrate, equal volume of 10% trichloroacetic acid was added to precipitate out proteins. The solution was then centrifuged at 10,000 rpm for 10 minutes. Supernatant was transferred into a beaker, and acetone was added in 1 to 0.5 ratio (supernatant: acetone). The solution was centrifuged at 10,000 rpm for 10 min. Supernatant was transferred to dialysis tubes and dialyzed against distilled water for 5 days. Purified polysaccharide was obtained after 5 days of dialysis process.¹¹

Characterization of Polysaccharide

Crude and purified polysaccharide was subjected to UV-Vis spectroscopic analysis. Absorbance was measured at wavelength range from 200 nm to 800 nm. Purified polysaccharide alone was subjected to FTIR and GC analyses as described earlier.¹¹

Bioactivities of Polysaccharide

Antibacterial Activity

Antibacterial activity of polysaccharide was determined using agar well diffusion method.^{12,13} For both crude and purified polysaccharide, they were tested against two Gram positive bacteria (*Staphylococcus aureus* and *Bacillus sp.*) and two Gram negative bacteria (*Pseudomonas sp.* and *Escherichia coli*). It started by punching holes using a cork borer on the

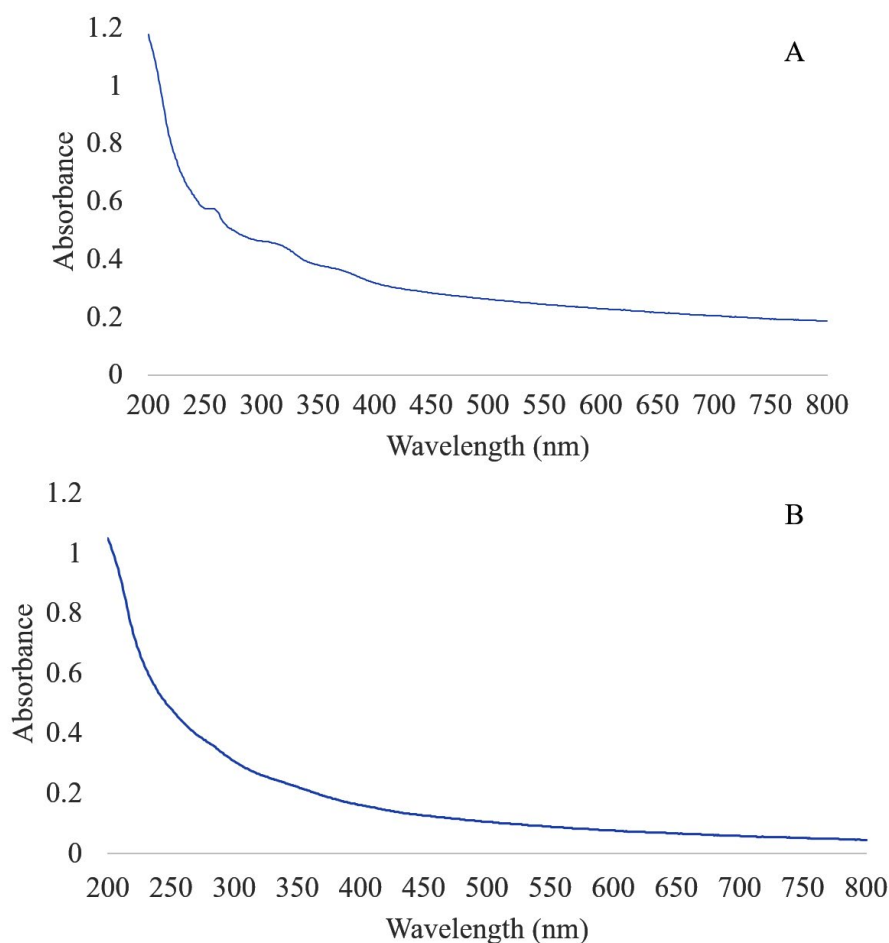


Fig. 2. Results of UV-Vis analysis a) Crude Polysaccharide, b) Purified Polysaccharide.

nutrient agar, followed by the swabbing of 24 h broth culture over the entire agar. Then, four different concentrations (100 µg, 200 µg, 300 µg and 400 µg) of crude / purified polysaccharide were added into the wells. One well was loaded with distilled water as negative control, and 5 µg ciprofloxacin antibiotic disc was used as the positive control. The agar plates were incubated at 37 °C overnight. Zone of inhibition was measured 24 hours later.

Swarming Motility Assay

Nutrient agar plates were prepared with its base layer composed of 1.5% agar. An overlay was prepared by adding 500µl polysaccharides solution (crude or purified) and 1ml of 1% glucose to 20ml of warmed 0.5% nutrient agar. The mixture was mixed well and poured on the pre-cooled nutrient agar plates. Agar was allowed to solidify, forming a substrate layer for motile microorganisms. The crude and purified polysaccharides were tested for its ability to inhibit swarming motility of *Bacillus* sp. and

Escherichia coli. Similar procedure was followed in preparing control plates, with 0.5% nutrient agar (supplemented with glucose) as substrate layer but without addition of polysaccharides. 10 µl of 24 hours bacterial culture was spot inoculated at the centre of substrate layer. Agar plates were then incubated at 37 °C for 24 h. After incubation, radius (r) of biofilm was measured from the spot of inoculation and results were reported in diameter (d=2r).¹⁴

Antioxidant Activity

Antioxidant activity of polysaccharide was determined using DPPH assay¹⁵. 0.1 mM of DPPH reagent was prepared along with the preparation of various concentrations of crude and purified polysaccharide (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml and 500 µg/ml). In a test tube, 1ml of polysaccharide solution was added to 2ml of DPPH solution and was shaken vigorously. The tubes were allowed to stand at room temperature in dark for a duration of 30 min. After that, absorbance was measured at 517nm

Table 1. Antibacterial activity of polysaccharide

Organism	Zone of Inhibition (mm)											
	Crude Polysaccharide					Purified Polysaccharide						
	PC	NC	Concentration (µg)				PC	NC	Concentration (µg)			
			100	200	300	400			100	200	300	400
<i>Escherichia coli</i>	24	-	-	-	-	-	23	-	-	-	-	-
<i>Pseudomonas</i> sp.	33	-	-	-	-	-	23	-	-	-	-	-
<i>Staphylococcus aureus</i>	27	-	-	-	-	-	33	-	-	-	-	-
<i>Bacillus</i> sp.	24	-	-	-	-	-	25	-	-	-	-	-

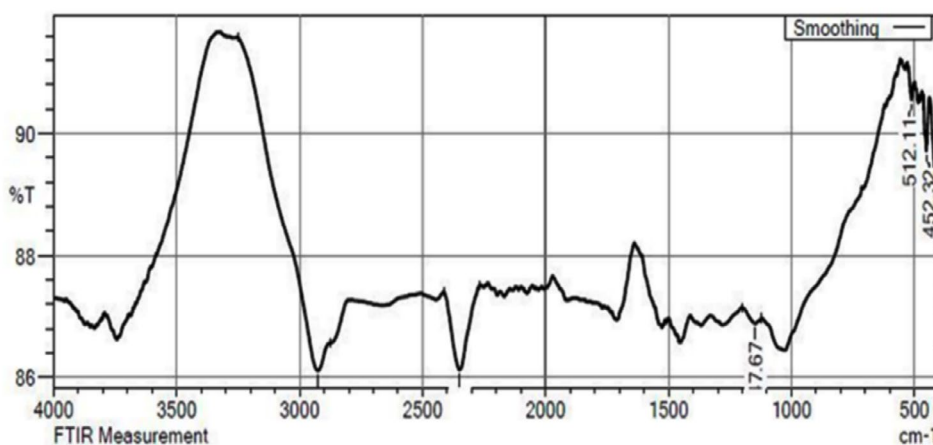


Fig. 3. FTIR spectrum of purified polysaccharide.

using UV-Vis spectrophotometer. The standard solution used in this assay was ascorbic acid, and it was prepared in different concentrations same as

the test samples. Methanol was used as blank and control was prepared by adding 1ml of methanol to 2ml of DPPH solution. DPPH scavenging activity was calculated using the following formula:

Table 2. Results of swarming motility assay

Organism	Diameter of Biofilm (mm)		
	Control	Crude	Purified
<i>Bacillus</i> sp.	10	10	6
<i>Escherichia coli</i>	12	12	6

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample / standard}}{\text{Absorbance of control}} \times 100\%$$

Seed Germination and Seedling Growth Study

Total of six plastic round dishes were prepared. 3 control dishes labelled as C1, C2 and

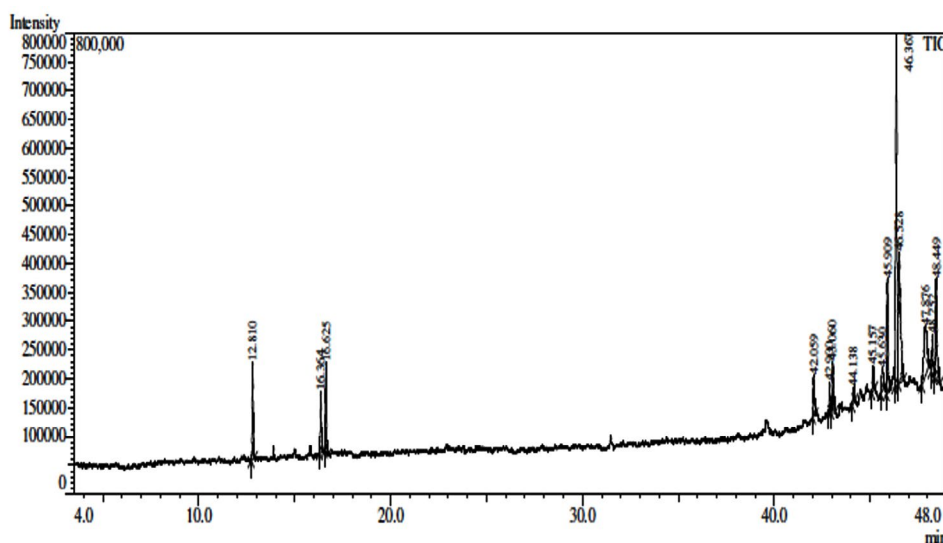


Fig. 4. GC chromatogram of purified polysaccharide.

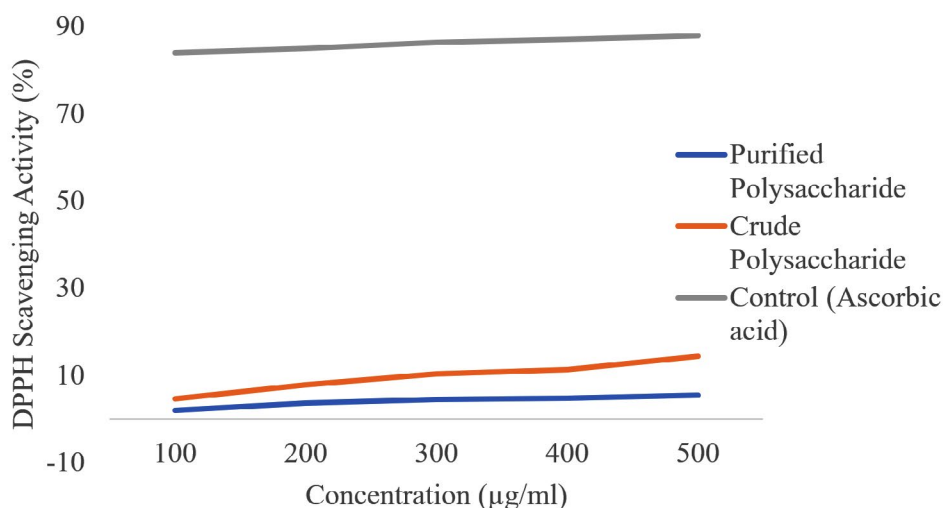


Fig. 5. A comparison of DPPH scavenging activity between purified polysaccharide, crude polysaccharide and ascorbic acid.

C3, another 3 were test dishes labelled as T1, T2 and T3. A layer of cotton was laid on the dish and moisten with distilled water. 5 chilli seeds were placed into each dish. Each seed from the control group received 10 drops of distilled water once per day, and seeds from the test group received 10 drops of 10% gum solution for three days, followed by distilled water for the remaining days. All dishes were kept in a humid chamber and placed in dark. Number of seeds germinated was recorded every day. With these data, germination percentage was calculated using formula below¹⁶:

$$\text{Germination percentage} = \frac{\text{No. of germinated seeds}}{\text{Total No. of seeds}} \times 100\%$$

Measurement on root length, shoot length and wet weight were done on 18th, 21st and 24th day.

Effects of Plant Gum on Soil Quality

Unfertile soil collected was used as negative control, and organic soil was used as positive control. Collected unfertile soil was added

Table 3. Soil porosity of the three different types of soil: positive control, negative control and test

Types of Soil	Soil Porosity (%)	WHC (%)
Positive Control	47.5	98
Negative Control	22.0	32
Test (Gum-Treated Soil)	27.0	70

with 10% gum solution and called as test soil. The soil was first spread over a tray. To allow better mixing, gum solution was poured uniformly over the soil followed by thorough mixing. The soil was left undisturbed for two days. The three types of soil were subjected to soil porosity testing and water holding capacity (WHC) determination.

Soil Porosity

A 250 ml beaker was filled with soil until it reached the 200ml mark. Then, 100ml of water in a beaker was slowly poured until water just starts to pool up on the soil surface. The volume left in the beaker was recorded and used in the calculation for volume of water poured. Soil porosity was measured using formula:

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

Water Holding Capacity (WHC)

10g of soil was measured and transferred into a funnel with filter paper at its base. Then, 100ml of distilled water was poured onto the soil. The water drained by gravity was collected in a measuring cylinder. WHC in percentage was calculated using formula below¹⁷:

$$\text{WHC (\%)} = \frac{\text{Volume of water poured} - \text{Volume of water collected in measuring cylinder}}{\text{Weight of soil (g)}} \times 100\%$$

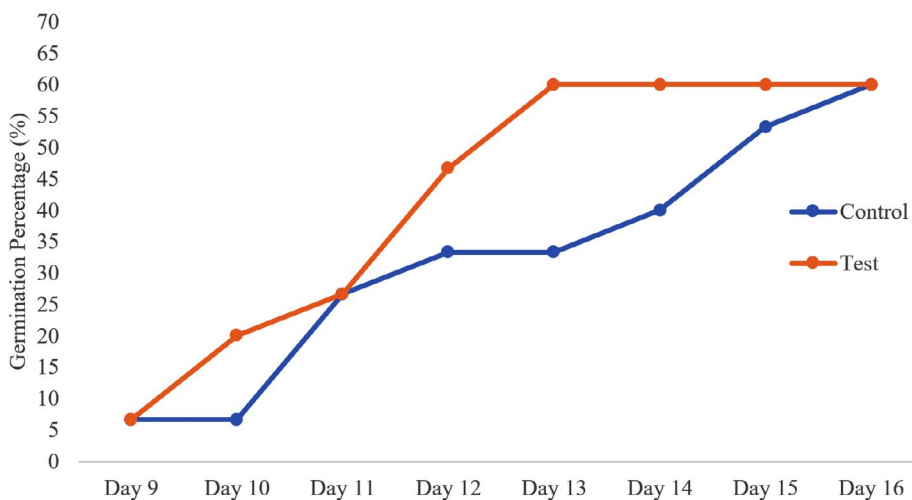


Fig. 6. Germination percentage of chili seeds from day 9 to day 16.

Plant Study

Nine pots were prepared and equally divided into three sets as positive control (PC), negative control (NC) and test. All pots were filled with respective soil until three fourth full. To each pot, 5 chilli seeds were sowed and were watered in the morning. Shoot length, root length and wet weight of plant were measured on 20th day, 25th day and 110th day.

Statistical Analysis

In this study, data were collected thrice and are reported as mean \pm standard deviation.

RESULTS

Characterization of Polysaccharides

UV-Vis Spectroscopic Analysis

Absorbance values were obtained at wavelength ranges from 200 to 800 nm, with a peak formed at 254 nm for crude polysaccharides. For purified polysaccharide, high absorbance was found between 200 nm and 300 nm (Fig. 2). In UV-Vis analysis, a peak formed around 254nm (crude polysaccharide) might indicate the presence of xylose Oliver et al.¹⁸ observed the maximum absorbance of xylose at 245 nm to 255 nm. However, peak at 210 nm was not observed, which is another wavelength of maximum absorbance for xylose. Purified polysaccharide (Fig. 2 B) showed high absorbance between 200 nm to 300 nm might indicate presence of xylose and glucose.¹⁸

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Fig. 3 shows the spectrum obtained for purified polysaccharide. It showed the formation of band at about 2350 cm⁻¹, 2900 cm⁻¹, and also one band in the range between 1200 cm⁻¹ and 1100cm⁻¹. In FTIR spectrum, the band at around 2900cm⁻¹ represents the asymmetric CH stretch of the methylene group and the band at wavenumbers in the range between 1200 and 1100cm⁻¹ can be attributed to C–O stretching of alcohol group or C–O stretching vibrations in C–O–C glycosidic linkages of oligosaccharides.¹⁹ There was one band observed at about 2350cm⁻¹, which can be assigned to NH component.²⁰

Gas Chromatography (GC) Analysis

GC analysis confirms the presence of xylose and D-allose (RT at 12.810 and 16 respectively). Samrot et al.¹¹ reported these

Table 4. Plant growth on day 20, 25 and 110

category	20 days			25 days			110 days		
	Positive control	Negative control	Test	Positive control	Negative control	Test	Positive control	Negative control	Test control
Shoot	32.33 \pm 2.31	31.67 \pm 18.15	39.33 \pm 9.02	37 \pm 8.16	36.33 \pm 18.18	43.33 \pm 17.48	114.33 \pm 10.21	84.33 \pm 16.4	120.21 \pm 14.50
Root	25 \pm 7	17 \pm 7.55	30.33 \pm 14.57	28 \pm 13.11488	19.33 \pm 8.14	34.67 \pm 27.59	183 \pm 7.2	154.67 \pm 19.28	187.24 \pm 17.79
Biomass (wet weight)	0.05 \pm 0.006	0.04 \pm 0.021	0.06 \pm 0.006	0.07 \pm 0.046	0.06 \pm 0.051	0.1 \pm 0.025	2.76 \pm 2.36	0.7 \pm 0.97	2.86 \pm 0.10

monosaccharides in *A. indica* gum too. *Araucaria heterophylla* has been reported to have galactose and xylose. The peaks of various sugar moieties have been determined using GC-MS analysis.²¹⁻²³

Bioactivities of Polysaccharides

Antibacterial Activity

The absence of zone of inhibition signified the inability of both crude and purified polysaccharide in inhibiting four organisms used in this study (Table 1). This study demonstrated that polysaccharides extracted from MI have no antibacterial activity, probably due to the lack of bioactive compounds whereas leaves of MI has been reported to have antibacterial activity.²⁴ As according to a study by Yahia et al.²⁵ they showed that the leaves extract of *Ziziphus lotus* and *Ziziphus mauritiana* L. possess high antimicrobial activity mainly due to their rich contents of bioactive compounds such as polyphenols, flavonoids and tannins. In contrast to the results obtained in this study, gum of several plant species were demonstrated to exhibit antibacterial activity. Polysaccharide extracted from *Azadirachta indica* gum can inhibit the growth of *E. coli* at a concentration of 20µg/ml.¹¹ In a study by Samrot et al.²⁶ antibacterial activity against *Bacillus* sp. was found in purified polysaccharide of *Araucaria heterophylla* L. and *Prosopis chilensis* L., with their minimal inhibitory concentration reported as 8mg. Also, in another study performed using *Ficus iyrata* plant gum, there were absence of zone of inhibitions for all the tested organisms (*S. aureus*, *Bacillus* sp, *E. coli* and *Klebsiella* sp.) tested with water extract and purified polysaccharide. However, chloroform extract showed antibacterial activity against *Bacillus* sp. at concentration of 4mg/ml, 6mg/ml and 8mg/ml.²⁷

Swarming Motility Assay

Diameter of biofilm obtained for crude polysaccharide was the same as the control for both organisms. In contrast, the diameter of biofilm measured for purified polysaccharide was lower than control for both organisms (Table 2), which indicated better inhibition of swarming motility of microorganisms.²⁸ Compared with other studies using different types of plant gum, purified polysaccharide of *Terminalia catappa* L. gum was found to inhibit swarming motility of *Pseudomonas aeruginosa*.²⁹

In another study, Samrot et al.²⁶ found that purified polysaccharide of *A. heterophylla* and *P. chilensis* was able to inhibit swarming motility of *Bacillus* sp.

Antioxidant Activity

DPPH scavenging activity increases with the increase in concentration of both crude and purified polysaccharides. The antioxidant activity of ascorbic acid was notably higher than polysaccharides. Higher scavenging activity was observed in crude polysaccharide when compared to purified polysaccharide (Fig. 5). However, the scavenging effect of crude polysaccharide was not significant because of the huge difference in percentage scavenging compared to ascorbic acid (standard). Similar results were obtained by Samrot et al.¹¹ who tested on *Azadirachta indica* gum, in which the extracted polysaccharide had scavenging activity significantly lower than the control. In contrast to the findings of this study, purified polysaccharide of *Terminalia catappa* L., *A. heterophylla* and *P. chilensis* showed significant antioxidant property as their percentage scavenging was comparable to the standard.^{26,29} Moreover, Samrot et al.²⁷ found that water extract of *Ficus iyrata* gum exhibit significant free radical scavenging effect.

Seed Germination and Seedlings Growth Study Germination Percentage

Control and test group had 60% of germination (No more germinated seeds observed after day 16). However, test group reached to this percentage at day 13, 3 days before the control group. Germination did not occur earlier in the test group as both had their first germinated seeds observed on Day 9. Germination Percentage was a higher in the test group, with exception seen on day 9 and 11 (Fig. 6). Gum-treated seeds also showed increased pattern of all parameters i.e. shoot length, root length and wet weight of seedlings. As sugars are an important factor in plant growth and development, the presence of polysaccharides in plant gum makes it a possible growth promoter. According to Ciereszko³⁰, germination and other plant development stages are affected by changes in sugars concentration. Hernandez-Herrera et al.⁷ carried out a study on seed germination and seedling growth using extract of brown seaweed *Ulva lactuca* and *Padina gymnospora* which contain polysaccharides. Both extracts prepared in

alkaline conditions slightly enhanced germination of tomato seeds, but showed adverse effect when used in high concentration (10mg/ml).

Seedlings Growth

Fig. 7a-c showed that test groups have mean shoot length, root length and wet weight greater than the controls in every observation day. It was noticed that all the parameters showed an increase trend over the three observations days. Polysaccharide of microalgae has been reported to improve the growth of *Solanum lycopersium* and *Capsicum annum*.³¹ Polysaccharides of *Chlorella*

vulgaris was reported to enhance the seedling growth and also found to increase the protein and carbohydrate content.³²

Soil Testing

Positive control soil had the largest porosity, with a value of 47.5%. For negative control, soil porosity was determined to be 22%, slightly lower than the test soil i.e. 27%, (Table 3). WHC was found to be 98% for positive control soil, which is the highest among the three types of soil. Test soil (70%) has field capacity higher than the negative control soil (32%) (Table 3).

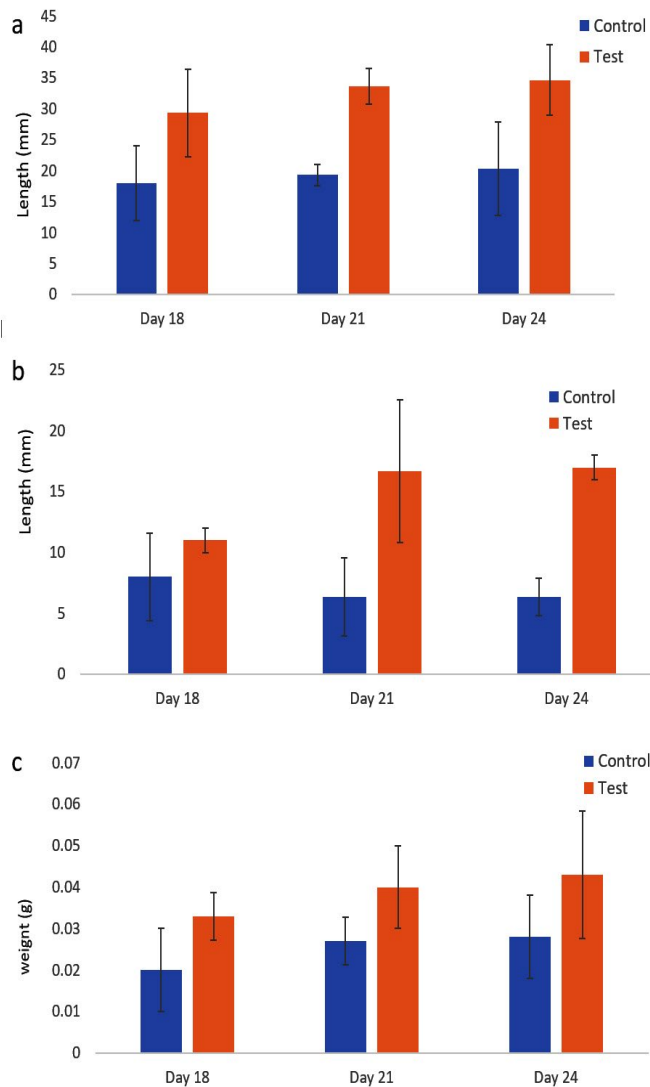


Fig. 7. Parameters measured on day 18, 21 and 24; a-shoot length, b- root length; c- wet weight.

Results obtained in this study showed increased soil porosity and WHC after addition of plant gum. Soil porosity directly affects WHC, as an increased in soil porosity will lead to enhanced WHC. The results obtained are in consonance with Vengadaramana and Jashothan³³, who reported significantly increase in WHC of soil treated with cow dung and compost organic fertilizer when compared to the untreated soil. As plants can only absorb nutrients in soluble form, soil with larger WHC supports plant growth better because of its ability to retain more nutrients.³⁴ Available water holding capacity (AWHC), which is the amount of water in soil that is available to plants, is a more precise determinator than WHC alone.³⁵ Minasnay and McBratne.³⁵ demonstrated that AWHC only increased by 1.16% averagely with a 1% increase in soil organic carbon.²⁹ Similarly, Libohova et al.³⁶ showed 1% increase in AWHC.

Plant Study

The Mean shoot and root length of test group was the highest (greater than positive control) for day 20, 25 and 110 (result not shown here). The increased growth parameters could be contributed by the high polysaccharides content of MI gum, in the presence of proteins and lipids.³⁷ Biopolymers are found in all forms of manure, including cow and poultry manures, which are well-known for its fertilizing properties.³⁸ Naishima et al.¹⁶ demonstrated the plant to grow larger than control with greater height and stem diameter. Another factor contributes and supported better plant growth is the nutritional supply. MI gum was proven for its nutritional contents of nitrogen, phosphorus, potassium, calcium and iron¹⁰. All are nutrients required in large quantities for plant to grow and develop, with iron as the only micronutrient.³⁹

CONCLUSION

In conclusion, polysaccharide was extracted from MI gum and purified. Crude and purified polysaccharides were characterized using UV-Vis, FTIR and GC. Both forms of polysaccharide showed weak antioxidant activity and neither of them exhibit antibacterial property. MI gum was found to enhance seed germination, seedling growth and vegetative growth. In this study,

addition of MI gum to soil increased its porosity and WHC.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors designed the experiments. AVS and LSJ performed the experiments. AS, ERR, DS, PP, SS, SN, analyzed and helped in writing the paper. All the authors read and approved the work.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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