

## Anti-diabetic Activities of the Fruit *Aegle marmelos*

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### Abstract

**Objective:** The objective of this study is pharmacological activities investigation of *Aegle marmelos* fruit.

**Methods:** After getting the sample (fruit) as dried powder, some sample was used for lactin isolation by ammonium sulphate precipitation method and some were used for methanolic extraction. Vacuum liquid chromatography (VLC), Thin layer chromatography (TLC) were used to detect the presence of various types of compound in rind. Presence of carbohydrate and proteins were investigated by thin layer chromatography. *In vitro* anti-diabetic assay was carried out by glucose uptake in yeast cells. Disc diffusion assay was performed to show the antibacterial effect using gram positive and gram negative strains of bacteria.

**Result:** Two extracts were used. One was methanolic extract and another was lectin extract. VLC fraction of methanolic extract of the *Aegle marmelos* fruit contains flavonoids and other biologically active compounds. The extract showed antibacterial activities against several bacteria. The lectin extract also showed antibacterial and anti-diabetic activity.

**Conclusion:** Therefore, *Aegle marmelos* may be considered as a plant of various health benefits.

**Keywords:** *Aegle marmelos*; Vacuum liquid chromatography (VLC); Ammonium sulphate; Jaundice; Diarrhoea

### Introduction

Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal plants. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal plants" [1].

Bael (*Aegle marmelos* (L.) Corr.) is important medicinal plant which available in Bangladesh and are reported to have various medicinal properties in traditional medical systems which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant [2,3]. It is commonly known as wood apple plant. *A. marmelos* is belonging to Rutaceae family, the family of flowering plants. It grows up in summer season [4]. Various phyto-constituents have been isolated from the fruit of *Aegle marmelos*, such as- marmelosin, luvangetin, aurapten, psoralen, marmelide, tannin etc., [5].

The different parts of Bael are used for various therapeutic purposes, such as for treatment of asthma, anaemia, fractures, healing of wounds, swollen joints, high blood pressure, jaundice, diarrhoea healthy mind and brain typhoid troubles during pregnancy [6]. *Aegle marmelos* has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in Bangladesh [7]. The main usage of the parts of this tree is for medicinal purposes. Various proved therapeutic values of *Aegle marmelos* are anti-diabetic activity, hepato-protective activity, anti-microbial activity, analgesic activity, anti-inflammatory, anti-pyretic activity and anti-cancer activity etc., [8].

### Materials and Methods

The fruit (Bael) was collected from Tangail, Dhaka, Bangladesh during April, 2015.

#### Extraction of the fruit portion of *Aegle marmelos*

Sun-dried and powdered fruit portion of *Aegle marmelos* (2000

g) was extracted with methanol. The extracts were concentrated with a rotary evaporator (IKA, Germany) at low temperature (40-50°C) and reduced pressure. The extracts were run into Vacuum Liquid Chromatography (VLC). Different fractions were obtained by using vacuum liquid chromatography apparatus. A sintered glass Buckner funnel attached to a vacuum line was packed with TLC grade silica gel. The silica gel was compressed under vacuum in order to achieve a uniform layer in order to get a better separation. The methanol extract was added to the amount (200 mg) of silica gel in order to make a smooth paste. n-hexane, dichloromethane, n-butanol, Ethyl Acetate and methanol were used as mobile phase in different ratios of increasing polarity from hexane to ethanol. The mixture to be separated according to the polarity of solvents. Each fraction was collected in a separate 100 ml beaker. The fractions were monitored by thin layer chromatography. The most active fractions having the similar thin layer chromatography profile were pooled together. For Lectin extraction 100 grams of powder of *Aegle marmelos* fruit was extracted overnight with 700 ml of PBS, pH 7.4, at 4°C. The suspension was centrifuged at 12,000 g for 30 min. The clear supernatant (crude extract) was subjected to 60% ammonium sulphate fractionation and the protein pellets were collected by centrifugation as described above. The pellet was re-suspended in PBS, pH 7.4 and dialyzed exhaustively against the same buffer for a period of 48 h. The resulting suspension was centrifuged at 12,000 g for 10 min and the supernatant was used for further analysis [9]. The VLC fraction of methanolic extract and lectin extract were stored at 4°C until used.

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## TLC analysis

VLC fraction of methanolic extracts were analyzed by performing TLC to determine the composition of extract. TLC was done using three solvent systems. The best result was obtained from solvent system-2 (chloroform: ethyl acetate: formic acid-5:4:1). After development of TLC plates, they were exposed to UV light. For charring, the plates were sprayed with 10% sulphuric acid solution, dried and then heated to 80-90°C. This allowed the spots to be visible. For detection of flavonoids the plates were dipped into 0.04% DPPH solution and dried while keeping in a dark place.

## Glucose uptake in yeast cells

Yeast cells were prepared according to the method of Yeast cells briefly, commercial baker's yeast was washed by repeated centrifugation (3,000 × g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of lectin extracts (5, 2.5, 1.25, 0.625, 0.3125 µg/mL) were added to 1mL of glucose solution and incubated together for 10 min at 37°C. Reaction was started by adding 100 µL of yeast suspension, vortex and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. Metformin was taken as standard drug [10]. The percentage increase in glucose uptake by yeast cells was calculated using the following formula: Increase in glucose uptake (%) = (Abs sample – Abs control) × 100/Abs sample, where Abs control is the absorbance of the control reaction (containing all reagents except the test sample) and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates [11].

## Antimicrobial screening of VLC fraction of methanolic and lectin extract of *aegle marmelos* (fruit)

The antibacterial activity was carried out by the disc diffusion method [12] using 100 µL of suspension containing ~103 CFU/mL of microorganism spread on nutrient agar medium (Himedia, India). Five different bacterial strains of gram positive, five different strains of gram negative bacteria were used to carry out this assay. Dried and sterilized filter paper discs (6 mm diameter), Four VLC fraction of methanolic extract of *Aegle marmelos* fruit, stocks solution of 3 mg/mL was prepared and discs was soaked with solutions of 10 µL of test samples and dried placed [13]. Standard disc of ciprofloxacin (30 µg/disc) was used as positive control. After incubation at 37°C for 24 hours, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The results were expressed as mean ± standard deviations.

## Determination of protein and carbohydrate concentration

The presence of carbohydrate and protein was detected using TLC method as previously described method with little modifications [14,15]. For detection of proteins, TLC tank was saturated with mobile phase having the composition of 1-butanol, glacial acetic acid and water in the ratio of 4:1:1 respectively. To the saturated tank the previously spotted plate was placed and the mobile phase was allowed to run through the spots until the solvent line was reached. The plate was then taken out of the tank, dried and then visualized under UV light in dark room. After marking the florescent compounds, the plate was sprayed with 2% ninhydrine in ethanol solution, dried and then heated using heat gun to make the protein or amino acid component spots visible. For detection of carbohydrate, the TLC tank was saturated with mobile phase having the composition of 1-butanol, acetone and phosphate buffer in the ratio of 4:5:1 respectively. To the saturated tank the previously spotted plate

was placed and the mobile phase was allowed to run through the spots until the solvent line was reached. The plate was then taken out of the tank, dried and then visualized under UV light in dark room. After marking the florescent compounds, the plate was sprayed with mixture of anisaldehyde with 0.5% sulphuric acid, dried and then heated using heat gun to make the carbohydrate component spots visible [16].

## Results

### TLC analysis

TLC analysis was done as described in materials and methods. The plate was observed under UV light (indicated as 2). After charring of the TLC plate with sulfuric acid (indicated as 3). After being soaked into DPPH (indicated as 4) showed moderate yellow color (Figure 1) which indicated the presence of flavonoids in the separated fraction of the extract. Four spots are visualized for each fraction. The Retention factor (RF) value of DCM = 0.1, 0.37, 0.84, 0.44; n-butanol = 0.14, 0.39, 0.5, 0.81; Ethyl acetate = 0.13, 0.39, 0.56, 0.81; and Methanol = 0.33, 0.83, 0.89 in polar solvents. The RF value of DCM = 0.08, 0.25, 0.5, 0.9; n-butanol = 0.35; Ethyl acetate = 0.18, 0.23, 0.37, 0.48 and Methanol = 0.37 in non-polar solvents. The sky blue, dip purple, light purple spot present in the plate indicates the presence of valuable compound in the different VLC fractions.

### Glucose uptake in yeast cells

The lectin extract of *Aegle marmelos* increased the glucose uptake in yeast cell 71.1% at the highest concentration (5 µg/mL) and 2.65% at the lowest concentration (0.313 µg/mL) whereas the standard (metformin) increased the glucose uptake in yeast cell 4.6 % at the highest concentration (5 µg/mL) Table 1. This result indicated that lectin extract of *Aegle marmelos* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin. It's IC<sub>50</sub> = 3.36 µg/mL.

### Antimicrobial screening of VLC fraction of methanolic extract

The antimicrobial activity of the VLC fraction of methanolic extract of *Aegle marmelos* fruit were studied against five Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Candida albicans*, *Staphylococcus aureus* and *Sarcina lutea*) and five Gram-negative (*Vibrio parahaemolyticus*, *Vibrio mimicus*, *Salmonella paratyphi*, *Salmonella typhi*, *Shigella dysenteriae*). Antibacterial potential of extracts was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 2 and 3. From the result it is observed that *Aegle marmelos* possess antibacterial activities.

### Antimicrobial screening of lectin extract

The antimicrobial activity of lectin extract of *Aegle marmelos* fruit were studied against four Gram-positive (*Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus* and *Sarcina lutea*) and two Gram-negative

Standard (Metformin)		% of glucose uptake	No. (fruit)	Concentration(µg/ml)	% of glucose uptake
No.	Concentration(µg/mL)				
M1	5	4.6 ± 0.7	F1	5	75.1 ± 6.9
M2	2.5	2.5 ± 1.3	F2	2.5	36.75 ± 10.25
M3	1.25	1.7 ± 0.8	F3	1.25	15.2 ± 2.5
M4	0.625	1.05 ± 0.6	F4	0.625	7.95 ± 2.3
M5	0.3125	0.5 ± 0.4	F5	0.3125	2.65 ± 1.3
IC <sub>50</sub> = 3.36(µg/mL)					

**Table 1:** Glucose uptake in yeast cells by lectin extract of fruit (*Aegle marmelos*).

Gram positive bacteria	Zone of Inhibition(mm)					
	DCM	n-butanol	Ethyl acetate	Methanol	Standard	Control
<i>Bacillus subtilis</i>	7 ± 0	11.5 ± 0.15	7.5 ± 0.5	11 ± 0.1	24 ± 1.0	0 ± 0
<i>Bacillus sereus</i>	0 ± 0	12.5 ± 0.15	7.5 ± 0.5	13 ± 0.2	28.5 ± 0.5	0 ± 0
<i>Candida albicans</i>	0 ± 0	7 ± 0	0 ± 0	0 ± 0	29.5 ± 0.5	0 ± 0
<i>Staphylococcus aureus</i>	7.5 ± 0.05	8.5 ± 0.05	7 ± 0	8.5 ± 0.05	27 ± 0.3	0 ± 0
<i>Sarcina lutea</i>	18 ± 0.04	9.5 ± 0.05	10.5 ± 1.5	7 ± 0	24.5 ± 4.5	0 ± 0

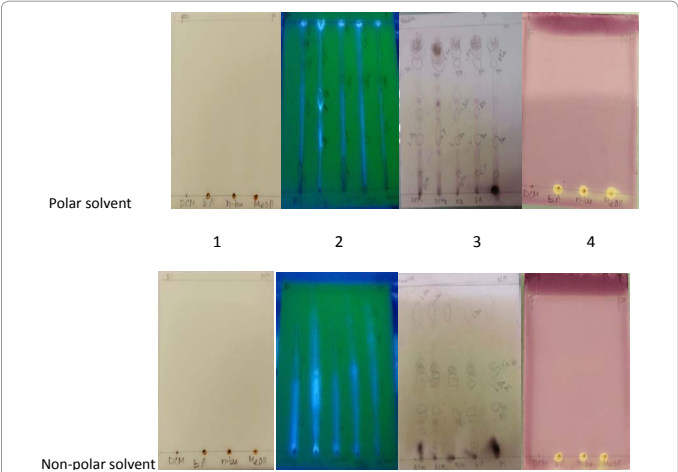
**Table 2:** Antibacterial activity of the methanolic extract of *Aegle marmelos* (fruit), VLC fraction of Dicloro methen, n-butanol, Ethyl acetate , methanol against gram positive bacteria.

Gram negative Bacteria	DCM(mm)	n-butanol(mm)	Ethyl acetate (mm)	Methanol (mm)	Standard (mm)	Control
<i>Vibrio parahemolyticus</i>	7 ± 0	7 ± 0	0 ± 0	7 ± 0.5	26.5 ± 3.5	0 ± 0
<i>Vibrio mimicus</i>	7 ± 0	7.5 ± 0.5	7.5 ± 0.05	7.5 ± 0.5	25.5 ± 0.5	0 ± 0
<i>Salmonella paratyphi</i>	0 ± 0	7.5 ± 0.5	0 ± 0	7.5 ± 0.5	28.5 ± 0.5	0 ± 0
<i>Salmonella typhi</i>	0 ± 0	7 ± 0	12 ± 0.1	8 ± 0	30 ± 0	0 ± 0
<i>Shigella dysenteriae</i>	0 ± 0	0 ± 0	0 ± 0	7 ± 0	29 ± 0	0 ± 0

**Table 3:** Anti-bacterial activity of the methanolic extract of *Aegle marmelos* (fruit), VLC fraction of Dicloro methen, n-butanol, Ethyl acetate, methanol against gram negative bacteria.

Name of Bacteria	Zone of inhibition of Lectin (Fruit) (mm)	Standard(mm)	Control
<i>Bacillus sereus</i> (+)	7 ± 0	29 ± 0.1	0 ± 0
<i>Bacillus subtilis</i> (+)	9.5 ± 1.5	26.5 ± 0.5	0 ± 0
<i>Staphylococcus aureus</i> (+)	0 ± 0	26.5 ± 3.5	0 ± 0
<i>Sarcina lutea</i> (+)	7 ± 0.5	29 ± 0	0 ± 0
<i>Escherichia coli</i> (neg)	7.5 ± 0.5	28 ± 1	0 ± 0
<i>Shigella dysenteriae</i> (neg)	0 ± 0	30 ± 0	0 ± 0

**Table 4:** Anti-bacterial activity of lectin of *Aegle marmelos* fruit against gram positive and gram negative bacteria.



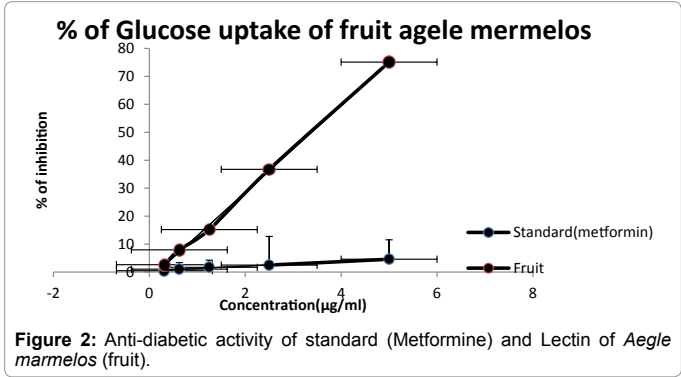
**Figure 1:** (1) TLC plate in naked eye view, (2) TLC plate Under UV light, (3) TLC plate after charring with H<sub>2</sub>SO<sub>4</sub>, (4) TLC plate after application of DPPH.

(*E.coli* and *Shigella dysenteriae*). Antibacterial potential of extracts was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 4. From the result it is observed the *Aegle marmelos* possess antibacterial activities.

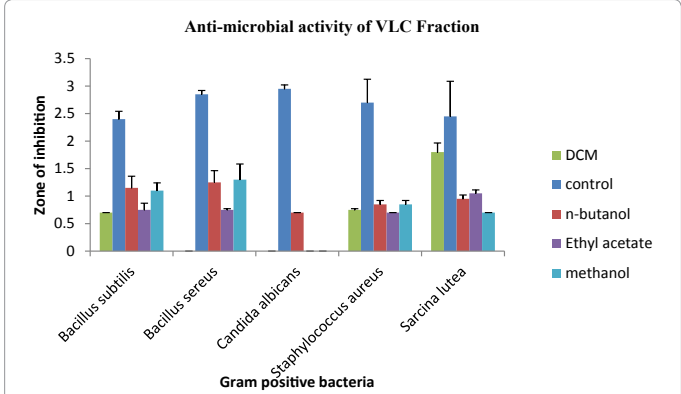
### Determination of carbohydrate and protein

We separated the carbohydrate like molecules and protein like molecules present in *Aegle marmelos* by TLC as described in the

method section. Then, we examined the plates under ultra-violet light and normal light (Figures 1-5). For detection of carbohydrate, the dried plates were sprayed with anisaldehyde with 0.5% sulphuric acid and heated. In the plate of carbohydrate test, the blue spot is present. The RF value of 1<sup>st</sup> spot = 0.4 and 2<sup>nd</sup> spot = 0.9. As shown in Figure 6, the sky blue, dip purple, light purple spot present in the plate indicates the presence of valuable protein compound in the lectin extract. The RF value of 1<sup>st</sup> spot = 0.5, 2<sup>nd</sup> spot = 0.6, 3<sup>rd</sup> spot = 0.72 and 4<sup>th</sup> spot = 0.81 in lectin extract. Charring the dried plates with 0.2% ninhydrin solution formed a brown spot on the TLC plate (Figure 7), which indicates the presence of protein in lectin extract of *Aegle marmelos*.



**Figure 2:** Anti-diabetic activity of standard (Metformine) and Lectin of *Aegle marmelos* (fruit).

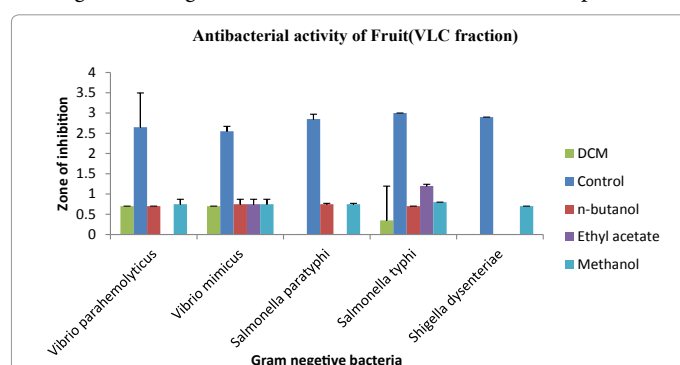


**Figure 3:** Zone of inhibition of different fraction of methanolic extract of *Aegle marmelos*(fruit) against Gram positive bacteria.

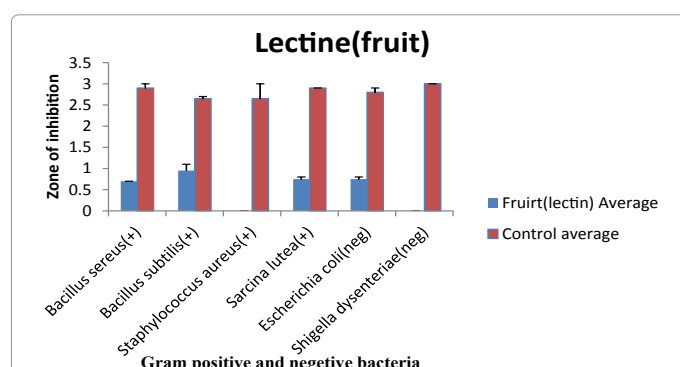
## Discussions

TLC plates were seen under UV light and found that different compounds were separated in the plates. After charring with  $H_2SO_4$  in high temperature, the separated compound transformed into black color. Staining the plate with DPPH solution the color of the separated compounds changed into yellow color. Such a result indicated the presence of flavonoids and many valuable compounds present in the separated fractions of the extract in the polar and nonpolar mobile phase. The formation of sky blue and purple spot on the plate (VLC fraction) indicates the extensive presence of valuable compounds from which we can have a preliminary idea of the valuable compounds that may be present in the methanolic extract of *Aegle marmelos* fruit.

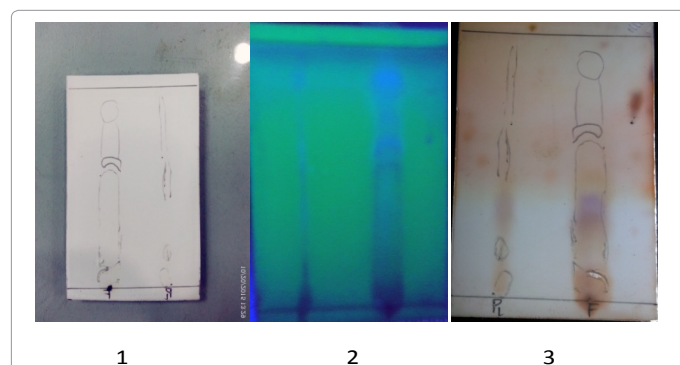
Regulation of glucose level in the blood of the diabetic patient can



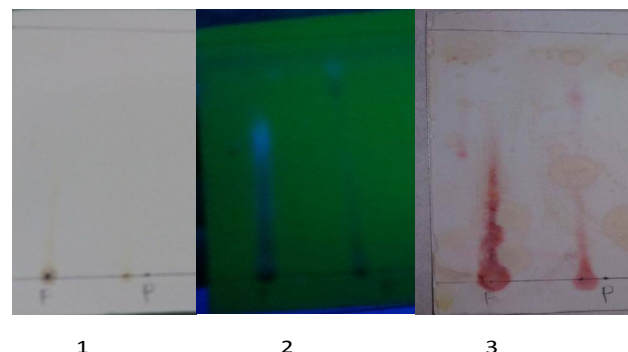
**Figure 4:** Graph of antibacterial activity of the methanolic extract of *Aegle marmelos* (fruit), VLC fraction of Dichloro methen, n-butanol, Ethyl acetate, methanol against gram negative bacteria.



**Figure 5:** Anti-bacterial activity of lectine extract of *Aegle marmelos* (fruit).



**Figure 6:** Thin layer Chromatography of Lectine extract of *Aegle marmelos* Fruit for protein test. (1) TLC plate in naked eye view, (2) TLC plate Under UV light, (3) TLC plate after charring with 2% ninhydrine in ethanol solution.



**Figure 7:** Thin layer Chromatography of Lectin extract of *Aegle marmelos* fruit for carbohydrate test. (1) TLC plate in naked eye view, (2) TLC plate under UV light, (3) TLC plate after charring with anisaldehyde with 0.5% Sulfuric acid.

prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species [17]. The *in vitro* assays of the present study indicated that lectin extract possess good anti-diabetic activity. The rate of glucose transport across cell membrane in yeast cells system is presented in Table 1. In Yeast (*Saccharomyces cerevisiae*), glucose transport takes place through facilitated diffusion. Type 2 Diabetes is characterized by the deficiency of insulin, causing increased amount of glucose in blood. After the treatment of the yeast cells with these extracts, the glucose uptake was found to increase in a dose dependent manner. The results show that the  $IC_{50}$  of lectin extract of *Aegle marmelos* fruit is 3.36  $\mu$ g/mL. Results also indicated that *Aegle marmelos* fruit had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin.

The antibacterial activity of the methanol extract of *Aegle marmelos* fruit was evaluated by disc diffusion method against gram positive and gram negative bacteria using ciproxacin as standard. Different VLC fraction of methanolic extract of *Aegle marmelos* (DCM, Ethyl acetate, n-butanol and methanol) shows varying degrees of antibacterial activities with zone of inhibition ranging from 7-11.2 mm respectively, while the highest antibacterial activity was seen against with, *Bacillus subtilis*, *Sarcina lutea*, *Salmonella typhi*.

The anti-bacterial activity of the lectin of *Aegle marmelos* fruit was evaluated by disc diffusion method against gram positive and gram negative bacteria using ciproxacin as standard. This lectin of *Aegle marmelos* fruit shows varying degrees of antibacterial activities with zone of inhibition ranging from 7-9 mm respectively, while the highest anti-bacterial activity was seen against with, *Bacillus subtilis*, *E.coli*.

TLC analysis of *Aegle marmelos* showed the presence of carbohydrate and protein molecules in the isolated crude lectin.

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