Phytochemicals, Nutritional Constituents, Anti-bacterial and Hypoglycemic Activity of Aegle Marmelos Lin. Leaf Extract in Alloxan Induced Diabetic Rats

Asaduzzaman M1,2, Lutfun Nahar2, Fazley Rabbi M1, Mahadi Hasan3, Anowara Khatun2, Zinat Tamanna1, Rasel Molla M1, Abdur Rashid Mia1, Noor Rahman Dastagi2, Shyam Sundar Shaha1, Maniruzzaman M1, Manobendro Nath Ray7, Najem Uddin M1 and Mobassirul Islam M3

1Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh
2Gonoshasthaya Samaj Vittik Medical College and Hospital, Dhaka, Bangladesh
3Department of Biophysical Chemistry, Kyoto Pharmaceutical University, Kyoto, Japan
4Department of Pharmacology, Kyoto Pharmaceutical University, Kyoto, Japan
5Department of Public Health and Informatics, Jahangirnagar University, Savar, Dhaka, Bangladesh
6Department of Pharmacy, Varendra University, Rajshahi, Bangladesh
7Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh

*Corresponding author: Asaduzzaman M, Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh, Tel: 8801719248007, E-mail: asad99mb@gmail.com

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Abstract

Aegle marmelos is a popular medicinal plant in the Ayurvedic and Siddha systems of medicine and folk medicines used to treat a wide variety of ailments. Aegle marmelos is locally known as “Bael” found everywhere in Bangladesh and many other countries of the world. The nutritional composition of Aegle marmelos leaves was determined by standard method. The proximate composition of leaves revealed moisture (infant 37.80 gm%, mature 40.01 gm% and ripen 32.50 gm%), ash (infant 0.012 gm%, mature 0.82 gm% and ripen 0.75 gm%), pH (infant 6.30, mature 6.00 and ripen 6.15), total sugar (infant 0.9 gm%, mature 1.9 gm% and ripen 1.7 gm%), reducing sugar (infant 0.30 gm%, mature 0.90 gm% and ripen 0.75 gm%), non-reducing sugar (infant 0.57 gm% mature 0.95 gm% and ripen 0.90 gm %), starch (infant 0.8 gm%, mature 2.5 gm% and ripen 2.0 gm%), crude fiber (infant 8.1 gm%, mature 11.0 gm% and ripen 9.5 gm%). Vitamin C for (infant, mature and ripen leaves contain 3.5 gm%, 7.1 gm%, 6.0 gm%) respectively. Total phenol present in (1.9 gm%, 5.01 gm%, 3.99 gm% for infant, mature and ripen) respectively. The leaves also contain negligible amount of cholesterol. The leaves also contain sodium (infant 2.5%, mature 7.0% and ripen 6.5%), Potassium (infant 1.52%, mature 5.001% and ripen 3.99%), Calcium (infant 0.001%, mature 0.25% and ripen 0.21%), Phosphorous (infant 3.5%, mature 7.82% and ripen 7.11%) etc. This experiment also showed significantly reduction of blood glucose level of diabetic rats (P<0.001). A large number of populations in Bangladesh have been suffering from malnutrition. For the ignorance of people, they don’t know the nutritive value of different kinds of foods. It has important role as a source of vitamins, minerals and other nutrients in human diet, which are necessary. The present study was investigated the Phytochemical properties, Nutritional Properties, Antibacterial Activity and hyperglycemic effect of aqueous extract of A. marmelos leaves on diabetic rats.

Keywords: Aegle marmelos, Alloxan; Antidiabetic; Vitamin C; Minerals and antibacterial activity

Introduction

The wood Apple (Aegle marmelos) or popularly known as Bael tree (In Bengali) is native to the Indo-Malayan region [1] and is currently cultivated in India, Pakistan, Bangladesh, Sri Lanka, Burma and Thailand [2]. The tree is slender, aromatic perennial 6.0-7.5 m tall and 90-120 cm girth. It flower from May to July and yields an annamval) average of 300-400 fruits (200-250 kg) per tree. Aegle marmelos is a popular medicinal plant in the Ayurvedic and Siddha systems of medicine and folk medicines used to treat a wide variety of ailments. Various parts of tree including the fruit, possess medicinal properties. The roots are useful for treating diarrhoea, dysentery and dyspepsia [3]. The leaf is used for ophthalmia, diabetes and asthmatic complaints. Unripe fruit is useful for treating diarrhoea, dysentery and dyspepsia [3]. The leaf is useful for ophthalmia, diabetes and asthmatic complaints. Unripe fruit is useful for treating diarrhoea, dysentery and stomachalgia. It also used to treat malaria, fever, jaundice and skin diseases such as ulcers, urticaria and eczema [4]. In pharmacological trials it showed antimoebic and hypoglycemic activities [5,6]. It is rich in alkaloids, among which aegeline, marmesin, marmina and marmelosin are the major ones. Aqueous leaf extract should preventive effect myocardial diseases [7,8]. The compound luvangetin and pyranocoumarin showed significant antiallergy activity. Essential oil isolated from leaf has an antifungal activity [9].

A large number of populations in Bangladesh have been suffering from malnutrition. There are many kinds of fruits available in Bangladesh, which are rich in nutrients. For the ignorance of people, they do not know the nutritive value of different kinds of foods. A. marmelos leaves have important role as a source of vitamins, minerals and other nutrients in human diet, which are necessary for maintaining proper health and nutrition [10,11]. There are many varieties of Wood Apple in our country but their nutritive values are not known clearly. The concentration of these nutrients also varies with...
different varieties. This study was therefore undertaken to find out whether in case of *Aegle marmelos* extracts are more effective than those from ordinary leaf materials is the management of diabetes (Figure 1).

**Extract preparation**

The powder of *Aegle marmelos* leaves (1 kg) was mixed with ethanol in a 600 mL flask with mild shaking. The flask was closed with cotton plug and aluminum foil at 48 hours at room temperature. The extract was filtered through Whatman filter paper (No. 1), concentrated using a rotary evaporator at low temperature (40-50°C). The extract was preserved in airtight container and kept at 4°C until further use.

**Phytochemical activity**

The different extracts obtained were subjected to phytochemical properties for the presence of flavanoids, tannins, alkaloids, Cardiac Glycosides (Keller-Kiliani test), phytosterols, triterpinoids, saponins according to standard procedures [12-14].

**Nutritional analysis**

*pH*: The pH of *A. marmelos* leaves extract was determined by the conventional procedure using a pH meter [15].

**Moisture content**: About 5 gm of each of three stages of *Aegle marmelos* (infant, mature and ripen) leaves were weighed in a porcelain crucible (which was previously cleaned, heated to 100°C, cooled and weighed). The crucible with the sample was heated in an electrical oven for about six hours at 100°C. It was then cooled in desiccators and weighed again [16].

**Ash content**: About 5 grams of infant mature and ripen *Aegle marmelos* leaves were weighed in a porcelain crucible (which was previously cleaned and heated to about 100°C, cooled and weighed). The crucible was placed in a muffle furnace for about four hours at about 600°C. It was then cooled in a desiccator and weighed. To ensure completion of burning, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights were the same and the ash was almost white in color [17].

**Total sugar, reducing, non-reducing sugar and starch content of *Aegle marmelos***: The sugar content of *A. marmelos* leaves sample was determined based on the refractometric method (Atago handheld refractometer, ATAGO, N-1α, Japan). Briefly, the *A. marmelos* samples were suspended in milliQ water to make a solution of 20% (w/v) concentration. The percentage of sucrose content was measured in g/mL *A. marmelos* leaves [18]. The starch content of *C. Cordifolia* leaves was determined by the Anthrone method [19].

**Protein content**: The total protein content of *A. marmelos* leaves was determined by Lowry's method [20] of protein estimation, which is based on the formation of a copper-protein complex and the reduction of phosphomolybdate and phosphotungstate present in Folin-Ciocalteau reagent to hetero polymolybdenum blue and tungsten blue, respectively. Bovine serum albumin (BSA) (0-100 μg/ml) was used as a standard for preparing the calibration curve.

**Fat content**: Fat content was determined by extracting 2 g dried *A. marmelos* leaves sample with petrol in a Soxhlet extractor, heating the flask on a heating mantle for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petrol, the residual petrol was filtered using Whatman no. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave crude fat [21].

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**Materials and Method**

**Collection and identification of plant materials**

Wood Apple (*Aegle marmelos*) leaves were collected during the stages of infant, mature and ripen. These leaves were collected from Rajshahi University Campus. Identification of the samples was further confirmed with the Department of Botany, Rajshahi University, Rajshahi-6205, Bangladesh.
**Crude fiber content:** The crude fiber content was determined to be reported along with the nutritive value. For determination of crude fiber, the estimation was based on treating the moisture and fat-free material with 1.25% dilute acid, then with 1.25% alkali, thus imitating the gastric and intestinal action in the process of digestion. Then, 2 g of moisture and fat-free material was treated with 200 ml of 1.25% H2SO4. After filtration and washing, the residue was treated with 1.25% NaOH. It was filtered, washed with hot water and then 1% HNO3 and again with hot water. The residue was ignited, and the ash weighed. Loss in weight gave the weight of crude fibre [22].

**Carbohydrate content:** Carbohydrate contents of the A. marmelos leaves were determined by calculation (by difference) as follows:

\[
\% \text{Carbohydrate} = 100\% - (\% \text{Moisture} + \% \text{Crude Fat} + \% \text{Crude Protein} + \% \text{Ash})
\]

**Determination of vitamin C (Ascorbic Acid)**

Determination of ascorbic acid content was done following the method described by Ferreira et al. [23]. Briefly, the A. marmelos leaves sample (100 mg) was mixed with 10 ml 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml 2,6-dichlorophenolindophenol (DCPIP) 0.005%, and the absorbance was measured within 30 min at 515 nm against a blank. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (50, 100, 200 and 400 μg/ml; Y=3.2453; X=0.0703; r²=0.9440) and the results were expressed as mg ascorbic acid/kg A. marmelos leaves.

**Determination of minerals content**

Atomic absorption spectrometry (Analyt 200, Perkin Elmer, and Waltham, MA, USA) was used to determine mineral content of A. marmelos leaves extract solution (K, Na, Mg, P, Ca) in central science Laboratory, Rajshahi University [24-26]. The content of selected metals copper (Cu), nickel (Ni), iron (Fe), zinc (Zn), Lead (Pb) and cobalt (Co) were determined at 324.8, 232.0, 248.3, 279.5, 213.9, 237.4, 357.9, 240.7 and 228.8 nm, respectively, and using air-acetylene flow. The absorbance was measured within 30 min at 515 nm against a blank.

**Antimicrobial activity of Aegle marmelos Lin**

The Aegle marmelos leaves extracts mentioned above were tested against five pathogenic bacterial strains three gram-positive (S. aureus, B. cereus and B. subtilis) and three gram-negative (E. coli, s. dysenteriae and S. sonnei). Antibacterial screening was done using agar well diffusion method [30]. For this 20 mL of sterile Mueller Hinton Agar (Hi-media) was poured in sterile autoclaved Petri plates. After solidification, the sterile cotton swab was dipped into the bacterial culture. The entire agar surface of each plate was evenly inoculated by swabbing. The seven uniform wells were prepared with the help of sterile 6 mm diameter cork-borer. Each well was filled with the various concentrations of both the aqueous and methanol extract (10, 20, 25, 30, and 40 mg/mL), respectively, whereas, in case of aqueous: Ethanol, (40, 80, 100, and 120 mg/mL) concentrations were used and allowed for diffusion for 45 minutes. The plates were then incubated at 37°C for 24 hrs. Triplicate plates were prepared for each experiment and the average zone of inhibition excluding well was recorded. 9% DMSO was used as negative control. Turbidity of bacterial culture was maintained up to 1 x 10⁸ CFU/mL. The antibacterial potential of extracts was compared with standard antibiotic Ampicillin (10 g/disc) with paper disc (Hi-media) method.

**Hypoglycemic activity of Aegle marmelos (animal studies)**

**Animals care:** Test animals were collected from International Cholera and Dysentery Disease Research, in Bangladesh (icddr). Albino rats (wistar strain) of both sexes weighing 175 g (average) were used for the study and also recruited both gender. They were individually housed in polypropylene cages in well-ventilated rooms, under hygienic conditions. Feeding of animals was done a libitum, along with drinking water and maintained at natural day night cycle.

**Induction of diabetic:** Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of 5% solution of Alloxan monohydrate (110 mg/kg body weight) (Sigma Chemical Co., USA) in a 0.1 M sodium citrate buffer (pH 4.5). The age-matched control rats received an equivalent amount of citrate buffer. Food and water intake were closely monitored daily after Alloxan administration. The development of hyperglycaemia in rats was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood, 48 hours after Alloxan administration, with a Portable glucometer (Accu-Chek, Roche, Germany). The animals with fasting blood glucose level ≥11.0 mmol/L with other symptoms of diabetes mellitus such as polyphagia, polydipsia, polyuria, and weight loss were considered diabetic and included in the study.

**Blood collection:** Blood samples from all groups were collected on days 1, 3, 6, 9, 12, 15, 18 and 21 in a fasting state from rat's marginal ear vein by 26 G needle and syringe [9]. Blood glucose level was determined by "Humylazer 2000" analyser (Human, Germany). The values was expressed as mean ± SEM, Statistical analyses were performed by SPSS-16 one-way analysis of variance (ANOVA), followed by post-hoc Tukey's test for multiple comparisons. P<0.05, P<0.001 were considered as significant.

**Experimental animals grouping and treatment:** The animals were randomly divided into six groups. Each group contain six rats (n=6). The treatment of animals began on the initial day after Alloxan injection and this was considered as 1st day of treatment. The animals were treated for 3 weeks as follows:

**Group-1:** Control rats feed with standard pellet diet and water.

**Group-2:** The rats were made diabetic by an intra-peritoneal injection of single dose of 110 mg/kg body weight followed by of 5% solution of Alloxan monohydrate. Animals whose blood glucose level exceeded 11.0 mmol/L at 72 h after treatment were considered diabetic. These animals served as untreated diabetic control.

**Group-3:** The diabetic rats treated with A. marmelos leaves extract solution at a dose of 2.0 g/kg body weight for 21 days.

**Group-4:** Diabetic rats were treated with Glibenclamide at a dose of 0.5 mg/kg b.wt.

**Results**

pH of three stages of Aegle marmelos leaves are shown in Table 1. The pH of Aegle marmelos leaves showed in the acidic range of the pH scale. The results indicate that the acidity of Aegle marmelos leaves increases gradually with the advancement of maturity. Moisture is necessary for most of the physiological reaction in plant tissue and if it is lack, life does not exist. The moisture contents were found to be

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varied in three stages of *Aegle marmelos* leaves (37.8%, 40.01% and 32.50%) as shown in Table 1. The results reveal that the moisture content of *Aegle marmelos* leaves decreases with the advancement of maturity. Most of the inorganic constituents or minerals are present in ash. The ash content of three-stages of *Aegle marmelos* leaves is given in the Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infant</th>
<th>Mature</th>
<th>Ripen</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.15 ± 0.04</td>
<td>6.00 ± 0.01</td>
<td>6.30 ± 0.02</td>
</tr>
<tr>
<td>Moisture (gm%)</td>
<td>37.8 ± 0.21</td>
<td>40.01 ± 0.34</td>
<td>32.50 ± 0.02</td>
</tr>
<tr>
<td>Ash (gm%)</td>
<td>0.012 ± 0.03</td>
<td>0.82 ± 0.04</td>
<td>0.75 ± 0.01</td>
</tr>
</tbody>
</table>

Table 1: pH, moisture and ash content of *Aegle marmelos* leaves.

Table 2 showed the phytochemical investigation revealed the fact that the hypoglycemic activity could be due to the presence of flavonoids, terpenoids or alkaloids.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Ethanol</th>
<th>Chloroform</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tanins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpinoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Phytochemical screening of *Aegle marmelos* leaves.

Table 3 showed the Total sugar, reducing, non-reducing sugar, Starch content, protein, Vitamin C, fat, carbohydrate and crude fiber contents of *Aegle marmelos* leaves in three different stages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infant</th>
<th>Mature</th>
<th>Ripen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sugar content (gm%)</td>
<td>0.9 ± 0.003</td>
<td>1.9 ± 0.001</td>
<td>1.7 ± 0.005</td>
</tr>
<tr>
<td>Reducing sugar (gm%)</td>
<td>0.30 ± 0.003</td>
<td>0.90 ± 0.004</td>
<td>0.75 ± 0.003</td>
</tr>
<tr>
<td>Non-reducing sugar (gm%)</td>
<td>0.57 ± 0.004</td>
<td>0.95 ± 0.003</td>
<td>0.90 ± 0.009</td>
</tr>
<tr>
<td>Starch (gm%)</td>
<td>0.8 ± 0.002</td>
<td>2.5 ± 0.007</td>
<td>2.0 ± 0.001</td>
</tr>
<tr>
<td>Fat (gm%)</td>
<td>0.001 ± 0.002</td>
<td>0.05 ± 0.005</td>
<td>0.02 ± 0.004</td>
</tr>
<tr>
<td>Protein (gm%)</td>
<td>0.15 ± 0.001</td>
<td>0.26 ± 0.003</td>
<td>0.21 ± 0.002</td>
</tr>
<tr>
<td>Vitamin C (mg%)</td>
<td>3.5 ± 0.001</td>
<td>7.1 ± 0.002</td>
<td>6.0 ± 0.005</td>
</tr>
<tr>
<td>Crude fiber (gm%)</td>
<td>8.1 ± 0.003</td>
<td>11.0 ± 0.004</td>
<td>9.5 ± 0.005</td>
</tr>
<tr>
<td>Carbohydrate (gm%)</td>
<td>22.5 ± 0.15</td>
<td>10 ± 0.013</td>
<td>8.6 ± 0.017</td>
</tr>
</tbody>
</table>

Table 3: Total sugar, reducing sugar, non reducing sugar, starch, fat, protein, vitamin C, crude fiber and carbohydrate content of *Aegle marmelos* leaves.

Minerals content were shown in Table 4. All minerals content significantly increased up to the mature stage and then decreased in ripen stage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infant</th>
<th>Mature</th>
<th>Ripen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (%)</td>
<td>1.52</td>
<td>5.001</td>
<td>3.99</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>2.5</td>
<td>7</td>
<td>6.5</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.001</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>3.5</td>
<td>7.82</td>
<td>7.11</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.79</td>
<td>2.17</td>
<td>0.46</td>
</tr>
<tr>
<td>Iron (%)</td>
<td>3.32</td>
<td>1.98</td>
<td>3.23</td>
</tr>
<tr>
<td>Zinc (%)</td>
<td>1.5</td>
<td>0.78</td>
<td>1.5</td>
</tr>
<tr>
<td>Copper (%)</td>
<td>1.29</td>
<td>0.009</td>
<td>0.46</td>
</tr>
<tr>
<td>Lead (%)</td>
<td>0.056</td>
<td>0.038</td>
<td>0.67</td>
</tr>
<tr>
<td>Nickel (%)</td>
<td>0.0042</td>
<td>0.086</td>
<td>0.023</td>
</tr>
<tr>
<td>Cobalt (%)</td>
<td>0.019</td>
<td>0.454</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Table 4: Mineral contents of three different stages of *Aegle marmelos* leaves.

Table 5 showed Antibacterial activity of *A. marmelos*. The antibacterial activity of the diluted honey (100%) was tested against three gram-positive (*S. aureus, B. cereus* and *B. subtilis*) and three gram-negative (*E. coli, S. dysenteriae* and *S. sonnei*) bacterial strains. The methanolic mixture of *A. marmelos* was found with best antimicrobial effect against *S. aureus* (9.3 ± 0.04 mm zone of inhibition), *B. cereus* (7.3 ± 0.05 mm zone of inhibition), and *B. subtilis* (11.8 ± 0.19 mm zone of inhibition). Another antimicrobial effect against *E. coli* (9.8 ± 0.95 mm zone of inhibition), *S. dysenteriae* (6.3 ± 0.15 mm zone of inhibition), *S. sonnei* (8.6 ± 0.07 mm zone of inhibition). The reference antibiotic, Azithromycin, was taken as control which showed maximum inhibition of *B. subtilis* (30 ± 1.33 mm zone of inhibition). The highest antibacterial activity of methanolic mixture of *A. marmelos* against *B. subtilis* attributed to its good therapeutic value against infection diseases.

<table>
<thead>
<tr>
<th>Bacterial Strain</th>
<th>Zone of Inhibition (mm)</th>
<th>Methanol (Aegle marmelos)</th>
<th>Azithromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>7.3 ± 0.05</td>
<td>28 ± 0.78</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>11.6 ± 0.19</td>
<td>30 ± 1.33</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>9.3 ± 0.04</td>
<td>22 ± 0.95</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>9.8 ± 0.95</td>
<td>23 ± 0.72</td>
<td></td>
</tr>
<tr>
<td><em>Shigella dysenteriae</em></td>
<td>6.3 ± 0.15</td>
<td>26 ± 0.53</td>
<td></td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>8.6 ± 0.07</td>
<td>27 ± 0.37</td>
<td></td>
</tr>
</tbody>
</table>

Each value is the mean ± SD of triplicate measurements.

Table 5: Antibacterial activity of *Aegle marmelos* leaves.
Figure 2 showed the hypoglycemic activity of A. marmelos. In 21 days the level of glucose decreases significantly. Comparing the blood sugar level in Streptozotocin induced diabetic rats, A. marmelos administered subject showed significant reduction of blood glucose level which is as near as glibenclamide administered subject at (P<0.001) (Table 1) 3rd, 6th, 9th, 12th, 15th, 18th, and 21th days. A. marmelos supplementation group’s glucose levels maintained 16.50-48.57% lower than the diabetic control group where as in case of glibenclamide it was 14.31-61.63% lower than significantly diabetic control group (P<0.05).

![Blood Glucose Graph](Image)

Figure 2: Effect of A. Marmelos on the blood glucose level of experimental rats in 21 days (mmol/L).

Discussion

Diabetes mellitus is the most common endocrine disorder which has affected several millions of population worldwide. There are several oral hypoglycemic agents that are the primary forms of treatment for diabetes. However, such drugs have prominent side effects and the number of people seeking for alternative therapies that may have less severe or no side effects is increasing. Thus, plant based herbal drugs or botanicals are emerging as the primary components of holistic approaches to diabetes management. Plant extract contains phenolics and Flavonoids, vitamin C, Vitamin A, and the methanol extract showed the presence of saponins, flavonoids, terpenoids, tannins and Gylcosides [31,32]. *Aegle marmelos* (Linn) correa, commonly known as badol (or bel), belonging to the family Rutaceae, is a moderately sized, slender and aromatic tree. A number of chemical constituents and various therapeutic effects of leaves of *A. marmelos* have been reported by different workers. Extensive investigations have been carried out on different parts of *Aegle marmelos* and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and amino acids have been isolated from its different parts. Broadly, *Aegle marmelos* leaves contain alkaloids, Phenylpropanoids, terpenoids and other miscellaneous compounds whereas potential pharmacological activity of the leaves are hypoglycemic, anti-inflammatory, antimicrobial, anticancer, radioprotective, chemopreventive and anti-oxidative activity. Anhydroasegeline can be used as marker to standardize the plant material with respect to its potential anti diabetic activity [15]. Various studies have been done to know the proximate composition of the leaves, pulp of fruit and seed powder of *Aegle Marmelos*. A study was conducted to analyze values for proximate composition of *Aegle Marmelos* leaf, pulp and seed powder using standard methods found that bael leaf, pulp and seed powder are good source of protein, fat, minerals, crude fiber and energy, rich source of available carbohydrates, dietary fiber and also contain antinutrient content which help in controlling blood sugar [33]. The antibacterial activity of this variety using methanol, aqueous, and aqueous: Ethanol as the solvent has been investigated. The activity was further confirmed by performing GC-MS which revealed the presence of different phytochemicals. These medicinally bioactive components exert antimicrobial action through different mechanism. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with proline rich protein [34]. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell [35]. Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue [36]. Diabetic is a group of metabolic disease characterized by hyperglycemia high blood sugar level. Noninsulin dependent diabetes mellitus is the commonest form of globeries as well as in India. Hereditary factor obesity sedentary life style and aging have been shown to raise the risk for diabetes. The proper medical care and a regular monitoring of diabetes are essential not only to keep the disease and the management. To prevent the varieties of other Diabetes related problems because no were cure has been identified. Hence, management of Diabetes with diet exercise and drug has been established [37]. *Aegle marmelosis* widely used Ayurvedic medicine for the treatment of diabetes mellitus [38]. Oral as well as intraperitoneal administration of the aqueous extract of various parts of *Aegle marmelos* exhibited hypoglycemic effect against streptozotocin-induced diabetic rats [39]. Antidiabetic mode of action is of multidirectional as the extract can significantly lower the levels of blood glucose and glycosylated hemoglobin and increased the plasma insulin as well as liver glycogen in diabetic rats [39]. The leaf extract at a dose of 250 mg/kg exhibited to be more effective than glibenclamide, a well-known hypoglycemic drug. This antidiabetic effect is probably due to the presence of euugenol and marmesin in bael leaves extract suggesting antioxidant potential of the leaves which potentiate the insulin secretion from existing beta cells of the islets of Langerhans [40]. It was further proved that aqueous leaf extract of *Aegle marmelos* have anti hypoglycemic activity, as the aqueous extract of the *Aegle marmelos* leaves were found to inhibit primarily the uptake of glucose across rat inverted gut sacs [39]. With further research on cell viability tests and in vivo studies, this finding may have important implications in the treatment of cardiovascular diseases which is increasing at an alarming rate. Since the drugs used for the cardio vascular diseases are not economical and not accessible to the greater section of the society, application of this study may be a soon for them [41].

Conclusion

Wood Apple (*Aegle marmelos*) leaves have great medicinal importance which serve as a food supplements and medicines of various life threatening disease. It is also a popular medicinal plant in the Ayurvedic and siddha systems of medicine and folk medicines used to treat a wide variety of ailments. From nutritional analysis it was found that *Aegle marmelos* leaves contained some important nutrients like sugar, protein, vitamin C, crude fiber, phenol compounds, and some essential minerals, which are important for public health. From the antidiabetic activity studies it was found that crude ethanol and methanol extracts of *Aegle marmelos* leaves showed significant activity against alloxan induced diabetic rats. Finally we can conclude that, leaves of this plant have almost some nutritional, antimicrobial and...
antidiabetic values. Therefore the leaves may be used in traditional medicine system for different types of ailments.

Conflict of Interests
The Authors declare that they have no competing interests.

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References