COMPARATIVE EVALUATION OF THE ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF DIFFERENT PARTS OF AEGLE MARMELOS AND ITS CHEMICAL PROFILING USING HPLC AND HPTLC TECHNIQUE.

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ABSTRACT

Aegle marmelos L. Correa commonly known as Bael belonging to the family Rutaceae has been widely used in Indian medicine due to its various medicinal properties. The roots of the plant are widely used in many ayurvedic formulations and hence trade data collected over years has indicated that the demand has exceeded the supply. Therefore it is necessary to screen the bioactive phytocompounds present in all other parts of this plant so as to explore a possibility to replace the roots by substitution with other parts. Hence a comparative evaluation viz chemical and biological screening is hereby attempted to find out whether there exist any identical bioactive compounds in various parts of this plant. The major focus of the present study is to investigate on the in vitro antioxidant and α-glucosidase inhibitory activity of aqueous extracts prepared from various parts of Aegle marmelos and also to perform the chemical profiling by HPLC and HPTLC techniques. A number of methods were employed for this investigation, including DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging, total polyphenol content and inhibition of α-glucosidase enzyme. Results showed that aqueous leaf extract showed antioxidant and anti-diabetic activities similar to root extract and chemical profiling showed the presence of Umbelliferone in the bark extract as well as root extract. Thus we conceded out a systematic record on the comparative antioxidant study and chemical profiling of different parts of Aegle marmelos.
KEYWORDS: *Aegle marmelos*, antioxidant, α-glucosidase, HPLC, HPTLC, Umbelliferone

INTRODUCTION

Medicinal plants are the local heritage with global importance. India has a rich heritage of traditional knowledge and is a birth place to several important time-honored systems of health care like Ayurveda. *Aegle marmelos*, commonly known as bael, is a spiny tree belonging to the family Rutaceae.\(^1\) All the parts of this tree including stem, bark, root, leaves and fruit at all stages of maturity have medicinal virtues and have been used as traditional medicine for a long time. In fact, as per Charaka (1500 B.C) no drug has been longer or better known or appreciated by the inhabitants of India than the Bael.\(^2\) The plant is proven to have a number of pharmacological activities such as antifungal, antibacterial, antiprotozoal, antispermatogenic, anti-inflammatory, anthelmintic, antidiabetic, laxative, febrifuge and expectorant.\(^3\) An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect.\(^4\) *Aegle* leaf extract has been reported to regenerate damaged pancreatic s-cells in diabetic rats.\(^5\)

The roots of the plant are widely used in many major ayurvedic formulations including Dasamoola preparations and the trade data collected over years has indicated that the demand has exceeded the supply.\(^6\) Also the plant has been stated to be in the red list in vulnerable status.\(^7\) Therefore it is necessary to screen the phytochemicals in all parts of the plant so as to find out whether there exist similar active compounds in one or the other parts to be used instead or along with the root and there is a need to control and assure the quality through systematic scientific studies including chemical characterization, biological assays and validated clinical trials. With the help of marker compound the screening is made more efficient by determining the identical phytochemicals in all the parts of the plant. The marker compound used for our study is Umbelliferone and it is a widely occurring phenolic compound of plant origin, for which many biological activities against chronic diseases have been reported.

According to many reviews, *Aegle marmelos* is used in the treatment of type-II diabetes mellitus (DM), a chronic metabolic disease characterized by major imbalances in glucose metabolism and abnormalities in fat and protein metabolism. About 346 million people worldwide suffer from diabetes and this number has been estimated to be doubled in 2030.\(^8\) Oxidative stress is known to play a significant role in the development and progression of Diabetes.\(^9\) Reactive oxygen species (ROS) are typically generated as the byproduct of
cellular metabolic processes and are carefully controlled by cellular antioxidants or scavengers. Oxidative stress is considered the main cause for several chronic diseases, including diabetes. Oxidative stress occurs in the cell when the generation of ROS overwhelms the cells’ natural antioxidant defense.

Very few and sporadic works are there in the literature regarding the comparative antioxidant, anti-diabetic and chromatographic fingerprint profile evaluation of the plant parts. The present study involves the inhibitory effect of various extracts prepared from Aegle marmelos plant parts on α-glucosidase for anti-diabetic property and its reaction on ROS. HPLC and HPTLC quantification of the active constituents present in the extracts was also carried out.

MATERIALS AND METHODS

Collection of plant samples
The various parts (Root, Bark, Leaf and Fruit) of A. marmelos were purchased from AVS, Kottakal, Tirur.

Preparation of aqueous extracts
The various parts (Leaf, Root, Bark, Fruit peel, Fruit pulp and Seeds) were individually washed and shade dried for 3 days. The dried powdered plant parts (100 mg) was extracted with 1 liter of distilled water for 72 hours in a round bottom flask, by placing on water bath, attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract was obtain.

Standard and Chemicals
1,1-Diphenyl-2-picrylhydrazyl (DPPH), α-glucosidase, gallic acid, Standard Umbelliferone was procured from Sigma-Aldrich (St.Louis MO,USA). Butylated hydroxyl toluene (BHT), Trichloroacetic acid (TCA), ferric chloride, Folin–Ciocalteu’s reagent was from Sisco Research laboratories (India). All solvents used for HPLC were of HPLC grade. Methanol (purity 99.00%), were obtained from Spectrochem Pvt. Ltd., Mumbai, India and Acetonitrile (purity 99.8%) was procured from Merck, India. All other chemicals and solvents used were of standard analytical grade.

Phytochemical analysis
The aqueous extracts of Aegle marmelos plant parts were studied for their phytoconstituents using different phytochemical tests.\(^{[10]}\)
**Total phenols estimation**

TPC of extracts were determined by using Folin–Ciocalteu’s reagent.\[^{[11]}\] Briefly, 100 µl of different aqueous extracts of *Aegle marmelos* (three replicates), 500 µl of Folin–Ciocalteu’s reagent and 1 ml sodium carbonate (20%) were added and incubated at ambient temperature (25-27\(^{0}\)C) for 90 min. The color developed was measured at 760 nm using UV–VIS spectrophotometer (UV-2450 PC, Shimadzu, Japan). Total phenol values are expressed in terms of gallic acid equivalent (mg/ g of dry mass), which is a common reference compound or Percentage Total phenolic content.

**DPPH Radical Scavenging assay**

The antioxidant activity of different *Aegle marmelos* extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method.\[^{[12]}\] Three milliliters of reaction mixture contains 2.8 ml methanolic DPPH and 0.2 ml extract at various concentrations. The contents were mixed well immediately and incubated for 30 min at room temperature (25–29\(^{0}\)C). The degree of reduction in absorbance was recorded in UV–VIS spectrophotometer (UV-2450PC, Shimadzu, Japan) at 517 nm. The radical scavenging activity was calculated as a percentage of DPPH scavenging activity using the equation.

\[
\text{\% inhibition} = \frac{A_0 - A_i}{A_0} \times 100; \text{ Where } A_0 \text{ is the absorbance of the control and } A_i \text{ is the absorbance in the presence of sample.}
\]

**Assay for alpha Glucosidase inhibition**

\(\alpha\)-Glucosidase inhibition was assayed using different concentrations of sample stock solution (100–500 µg/ml), 100 ml of 0.1M phosphate buffer (pH 6.9) containing \(\alpha\)-glucosidase solution (1.0 U/ml), and was incubated in 96-well plates at 25\(^{0}\)C for 10 min. After pre-incubation, 50 ml of 5mM p-nitrophenyl-a-D-glucopyranoside solution in 0.1Mphosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25\(^{0}\)C for 5min. Before and after incubation, absorbance readings were recorded at 405nm by a Synergy 4 Biotek multiplate reader (Biotek Instruments Inc., Highland Park, PO Box 998, Winooski, Vermont-0504-0998, USA) and compared with a control that had an adequate amount of buffer solution in place of the extract.\[^{[13]}\] Acarbose was used as the standard. The \(\alpha\)-glucosidase inhibitory activity was expressed as the inhibition percentage and was calculated as follows.

\[
\text{\% Inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]
where Acontrol is the absorbance of control without sample and Asample is the absorbance of the sample. The concentration of the extract having 50% inhibition (IC50) was calculated from the concentration inhibition response curve.

Chemical Profiling of Aegle marmelos Using HPLC and HPTLC Technique

Preparation of Mobile Phase

The mobile phase used in quantification of Umbelliferone from Aegle marmelos by HPLC method, was prepared by Acetonitrile and water 5:5. The mobile phase used in quantification of Umbelliferone from A marmelos by HPTLC method, was prepared by mixing toluene: ethyl acetate: formic acid, methanol (17:10:2:1, v/v/v).

Preparation of stock solution of Umbelliferone

For HPLC, the stock solution of umbelliferone was prepared by dissolving 5mg of Umbelliferone standard in 10mL of methanol in a 10mL standard volumetric flask, followed by shaking. The contents of the flask were then diluted up to the mark with methanol. For HPTLC, the stock solutions of umbelliferone (0.0229g/ 10mL) were prepared in methanol.

Sample Preparation

Aegle marmelos parts were dried and extracted with distilled water to obtain various aqueous extracts. Then 1g of each extract were prepared in 10mL HPLC grade methanol. Then the sample was sonicated using ultrasonicator for 10 min. Then extract was filtered and injected into the HPLC column using suitable mobile phase. For HPTLC each extracts were weighted accurately (1 gm) and sonicated for 30 min and then extracted with methanol under reflux on water bath at 60°C and finally made upto 10ml. Each extracts were then filtered through filter paper Ashless circles 110mm (Whatman). 20ul sample were applied to the plates in duplicates.

Chromatographic conditions

HPLC was conducted in a column of C18 (reversed phase column Lichrospher 100 : RP18) length 4.6 mm x 25 cm, equipped with a pump (LC -10AT VP1), SIL-6A automatic injector furnished with a 50 Dl loop, detector (SPD - 10AVP) set at 370 nm and C- R6A chromatography data station software. 10 Dl of the standard Umbelliferone was injected into the loop and the temperature was maintained at 40°C. The solvents were used at a constant flow rate of 0.6 ml/min. All the solvents used were of HPLC grade.
The HPTLC densitometric scanning was performed on CAMAG TLC scanner III connected to PC running WINCATS software under MS Windows, connected with microlitre syringe and linked to a nitrogen tank. The samples were spotted on pre-coated silica gel aluminium plate 60 F254 (20 cm × 10 cm with 0.2 mm thickness) using a CAMAG Linomat V (Switzerland) applicator. The plates were prewashed by methanol and activated at 60°C for 5 min, prior to chromatography. The mobile phase consisted of toluene: ethyl acetate: formic acid, methanol (17:10:2:1, v/v/v) used for chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (CAMAG, Switzerland) saturated for 30 min with the development solvent system allowed to start at the position 10 mm and migrate up to a height of 80 mm from the lower edge at room temperature (25±2 °C) and relative humidity of 60±5%. The chromatogram was developed for 30 min to a distance of 80 mm and dried in a current of hot air using an air dryer. Deuterium and tungsten lamps, in the absorbance mode at 190 and 400 nm emitting a continuous UV spectrum was used as the source of radiation. A constant application rate of 150nL/sec was used. The scanner was set for maximum light optimization and with the settings of slit dimension, scanning speed 20 mm/sec and data resolution speed 100 m/step. The scan started at position 10 mm and ended at position 80 mm. Calibration curve of umbelliferone were prepared by plotting peak area versus concentration.

Statistical Analysis
The experimental results were expressed as mean ± standard deviation (SD) of triplicate measurements. The data were subjected to one way analysis of variance and the significance of differences between means were calculated by Duncan’s multiple range test using SPSS for Windows, standard version 7.5.1, and the significance accepted at $P < 0.05$.

RESULTS
Preliminary Phytochemical assay
The results of preliminary phytochemical screening of various aqueous extracts of Aegle marmelos are shown in Table 1.

<table>
<thead>
<tr>
<th>Method</th>
<th>AMLE</th>
<th>AMFpE</th>
<th>AMFPE</th>
<th>AMSE</th>
<th>AMBE</th>
<th>AMRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids Mayers test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins Foams test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Reducing sugars | + | + | + | - | + | + 
Terpenoids | - | + | + | - | + | + 
Steroids | - | + | - | + | + | +

AMLE-Aegle marmelos leaf extract, AMFpE-Aegle marmelos fruit pulp extract, AMFPE-Aegle marmelos fruit peel extract, AMSE-Aegle marmelos seed extract, AMBE-Aegle marmelos bark extract, AMRE-Aegle marmelos root extract.

Total phenolic content

Total phenolic contents were determined using the Folin-Ciocalteu reagent and expressed as % Total phenolic content. Total phenolic content assay revealed that the leaf extract of *A. marmelos*, which hold maximum amount of phenolic compounds, followed by fruit pulp extract (Table 2). *Aegle marmelos* bark aqueous extract also have considerable amount of phenolic compounds. Aqueous seed extract showed minimum phenolic content. Various studies have established a positive correlation between the antioxidant activity and total phenolic content.

**TABLE 2: Total phenolic content of different parts of Aegle marmelos**

<table>
<thead>
<tr>
<th>Plant Parts</th>
<th>% TPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>10±3.1</td>
</tr>
<tr>
<td>Bark</td>
<td>6±0.9</td>
</tr>
<tr>
<td>Root</td>
<td>5±0.4</td>
</tr>
<tr>
<td>Fruit Pulp</td>
<td>8.3±5.2</td>
</tr>
<tr>
<td>Fruit Peel</td>
<td>6.4±2.5</td>
</tr>
<tr>
<td>Seeds</td>
<td>3±0.9</td>
</tr>
</tbody>
</table>

Each value in the table is represented as mean ± SD (n = 3).

DPPH radical scavenging assay

DPPH is one of the powerful free radical which is used to evaluate the electron donating capacity of antioxidants.[14] All the extracts were able to reduce the stable pink colored free radical DPPH to yellow colored diphenyl picrylhydrazine. Percentage inhibition of different extracts of *Aegle marmelos* is given in Figure 1. BHT was used as positive control. The effect of antioxidants on DPPH is due to their hydrogen-donating ability. DPPH, a stable nitrogen centered free radical, has been used to evaluate natural antioxidants for their radical quenching capacities in a relatively short time, compared with other methods. Phenolic compounds in plants are viewed as powerful *in vitro* antioxidants due to their ability to donate hydrogen or electron, and to form stable radical intermediates. The method is based on the reduction of the absorbance of methanolic DPPH solution at 517 nm in the presence of proton donating substance due to the formation of the diamagnetic molecule by accepting an
electron or hydrogen radical.[15] All extracts showed inhibition dose dependently but *Aegle marmelos* leaf extract showed 50% inhibition at a lower concentration i.e 25 µg.

![Graph showing antioxidant capability of different extracts of *Aegle marmelos*](image)

**FIGURE 1: Determination of antioxidant capability of different extracts of *Aegle marmelos***

*AMLE*-Aegle marmelos leaf extract, *AMFpE*-Aegle marmelos fruit pulp extract, *AMFPE*-Aegle marmelos fruit peel extract, *AMSE*-Aegle marmelos seed extract, *AMBE*-Aegle marmelos bark extract, *AMRE*-Aegle marmelos root extract.** Statistically significant compared to other extracts. Each value is expressed as mean ±SD of triplicate measurements and the significance accepted at *p*<0.05

**α-Glucosidase assay**

Currently glycosidase inhibitors attract considerable attention due to their promising therapeutic potential in the treatment of diabetes. Different extracts prepared from *Aegle marmelos* were evaluated for its alpha-glucosidase inhibitory potential and was compared with that of standard compound acarbose. The results showed that *Aegle marmelos* leaf aqueous extract was able to inhibit the enzyme with an *IC*₅₀ value of 280±0.96 µg/ml and *Aegle marmelos* root aqueous extract was able to inhibit the enzyme with an *IC*₅₀ value of 300 ±0.42 µg/ml. *Aegle marmelos* fruit pulp aqueous extract also inhibited the enzyme with an *IC*₅₀ value of 45±0.51 µg/ml. A sudden rise in blood glucose levels causing hyperglycemia in type 2 diabetes happens due to hydrolysis of carbohydrates by pancreatic alpha-amylases and intestinal alpha-glucosidase.[16] Inhibition of alpha glucosidase can provide an alternative way to manage hyperglycemia in type 2 diabetes, which is one of reasons for the production of free radicals and oxidative stress.
Table 3: IC₅₀ values of different extracts of Aegle marmelos against α-glucosidase

<table>
<thead>
<tr>
<th>Number</th>
<th>Sample</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acarbose</td>
<td>45±0.51 µg</td>
</tr>
<tr>
<td>2</td>
<td>Aegle marmelos leaf aqueous extract</td>
<td>280±0.96 µg</td>
</tr>
<tr>
<td>3</td>
<td>Aegle marmelos root aqueous extract</td>
<td>300±0.42 µg</td>
</tr>
<tr>
<td>4</td>
<td>Aegle marmelos Bark aqueous extract</td>
<td>------</td>
</tr>
<tr>
<td>5</td>
<td>Aegle marmelos fruit pulp extract</td>
<td>400 µg ±0.58</td>
</tr>
<tr>
<td>6</td>
<td>Aegle marmelos fruit peel</td>
<td>------</td>
</tr>
<tr>
<td>7</td>
<td>Aegle marmelos seed aqueous extract</td>
<td>------</td>
</tr>
</tbody>
</table>

% inhibition of yeast alpha glucosidase enzyme by Aegle marmelos extracts and reference alpha glucosidase inhibitor, Acarbose (values are expressed as mean ± SD, n = 3)

HPLC profiling of Umbelliferone in Aegle marmelos extracts

The retention time of umbelliferone in the standard solution was found to be 4.085 min. Area corresponding to umbelliferone standard was found to be 60422733 (Figure 2). Amount of umbelliferone present in the sample solution was determined from the calibration curve by using the peak area of umbelliferone in the sample solution. To ascertain the repeatability of the method, the assay experiment was repeated three times. Leaf, Fruit pulp, Fruit peel and seed did not show the presence of umbelliferone. Bark showed a peak 4.224 and an area corresponding to 8500383 (Figure 3) and it was found out that Aegle marmelos bark extract contains 0.07g umbelliferone in 100g extract. Root extract showed a peak at 4.181 minute and an area corresponding to 7482459 (Figure 4) and was found out that 0.06 g umbelliferone in 100g root extract.

FIGURE 2: A Typical chromatogram of standard Umbelliferone
FIGURE 3: A Typical HPLC chromatographic pattern for determination of umbelliferone from aqueous extract of *Aegle marmelos* bark

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.57</td>
<td>25642069</td>
<td>40.42</td>
</tr>
<tr>
<td>3.37</td>
<td>6674923</td>
<td>10.24</td>
</tr>
<tr>
<td>4.234</td>
<td>830583</td>
<td>12.63</td>
</tr>
<tr>
<td>6.398</td>
<td>19360127</td>
<td>30.42</td>
</tr>
<tr>
<td>11.381</td>
<td>1999982</td>
<td>3.31</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>6029299</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

FIGURE 4: A Typical HPLC chromatographic pattern for determination of umbelliferone from aqueous extract of *Aegle marmelos* root extract.

<table>
<thead>
<tr>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.368</td>
<td>4935148</td>
<td>46.28</td>
</tr>
<tr>
<td>3.963</td>
<td>1875575</td>
<td>1.99</td>
</tr>
<tr>
<td>3.442</td>
<td>1976591</td>
<td>1.13</td>
</tr>
<tr>
<td>3.563</td>
<td>156341</td>
<td>0.17</td>
</tr>
<tr>
<td>4.191</td>
<td>7482459</td>
<td>7.96</td>
</tr>
<tr>
<td>5.708</td>
<td>39472918</td>
<td>40.51</td>
</tr>
<tr>
<td>8.203</td>
<td>744126</td>
<td>0.79</td>
</tr>
<tr>
<td>11.837</td>
<td>741446</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>94851813</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

HPTLC profiling of Umbelliferone in Aqueous extracts of *Aegle marmelos*

The identity of the umbelliferone band in the sample solution was confirmed by comparing the UV absorption spectra of the sample solution with that of the reference standard solution. The presence of umbelliferone was confirmed in the bark and root aqueous extract. It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The use of markers ensures that the
concentration of the specific component in the extracts is present in reproducible levels. In this way use of markers and chromatographic fingerprinting can give promising information.

FIGURE 5: High performance thin layer chromatography separation of standard umbelliferone and various extracts of Aegle marmelos aqueous extracts

DISCUSSION

In the present study different aqueous extracts prepared from Aegle marmelos were explored for its antioxidant and antidiabetic activity. In an effort to find out the phytochemical compounds phytochemical screening was done and the result showed significant amount of alkaloids, saponins, tannins and flavanoids were present in Aegle marmelos leaf, root and bark aqueous extract which could be responsible for the antioxidant and antidiabetic activities. Recent interest in plant polyphenols has focused on their potential benefits to human health. The polyphenols are capable not only of reducing oxidative stress but also of inhibiting carbohydrate hydrolyzing enzymes to prevent hyperglycemia. The findings found in the present study clarified the polyphenol contents, the α-glucosidase inhibitory effect, and the antioxidant activity of different extracts prepared from Aegle marmelos.

Antioxidants eradicate damaging chemicals in the body and protect against heart disease, arthritis, cancer and many other chronic diseases. Hence sufficient intake of antioxidants daily protects the cells from decomposition. The free radicals mediated toxicity can be effectively eliminated by plant derived antioxidant compounds and many of these activities have been already reported for a wide range of plants. DPPH scavenging activity has been
used by various researchers as a quick and reliable parameter to assess the in vitro antioxidant activity of crude aqueous plant extracts.\textsuperscript{[19]} All extracts inhibited the DPPH radical in a dose dependent manner and the \textit{Aegle marmelos} leaf extract showed maximum inhibition.

The ability of various \textit{Aegle marmelos} aqueous extracts to inhibit $\alpha$-glucosidase was measured using five different dosages. $\alpha$-Glucosidase inhibitory activity was measured using concentrations of 100, 200, 300,400 and 500 $\mu$g/ml. \textit{Aegle marmelos} leaf aqueous extract showed the highest activity (Figure 2) among the extracts and expressed in terms of IC$_{50}$ value. A lower IC$_{50}$ value indicates higher inhibition. The IC$_{50}$ value of standard acarbose was 45 $\mu$g/ml and that of leaf aqueous extract was 280 $\pm$0.96 $\mu$g/ml. The digestion of carbohydrates can be slowed by consuming herbal medicines containing components that inhibit carbohydrates hydrolyzing intestinal enzymes, a property comparable to that of drugs used for inhibiting these enzymes in the treatment of postprandial hyperglycemia, including acarbose, miglitol, and voglibose.

Standardization and quality of control of raw materials and herbal preparations need to be permanently carried out. In the cases, when the active component is unknown, marker substance(s) can be established for analytical purposes during quality evaluation. In this current work, we tried to focus on complete validation of detection and quantitation of umbelliferone with HPLC and HPTLC as per ICH Guidelines.\textsuperscript{[20]} The HPLC conditions for the analysis of umbelliferone were optimized with different mobile phases, in different ratios. A well separated peak of umbelliferone is observed in the plant sample. The results of HPLC and HPTLC quantification of different extracts indicate that root and bark aqueous extract contains an active compound, umbelliferone.

Since root is of main importance due to its demand, HPLC and HPTLC profile of root is compared with other parts of the plant. The root and stem bark have identical compound and so they have same pharmacological activities and therefore stem bark could be substituted or used along with root in any of drug preparation where root is of important. The marker compound umbelliferone is present in root and bark. \textit{Aegle marmelos} leaf aqueous extract exhibited more significant antioxidant and free radical scavenging activity than the other fractions, which is attributed to the presence of high phenolic content, since a linear relation was observed between the phenolic content and the antioxidant parameters. Umbelliferone was not present in the leaf extract but various studies reports the presence of active components like marmelosin, quercetin in \textit{Aegle marmelos} leaf extract.\textsuperscript{[21]} However, there is
a need for further studies regarding the substitution of other parts of *Aegle marmelos* extract as natural antioxidants, possible food supplements/additives and pharmaceutical agents.

**CONCLUSION**

*Aegle marmelos* is ethnically used in various diseases in humans and animals. In conclusion, the results obtained in the present study have shown that the phenolic compounds from aqueous extract of *Aegle marmelos* can effectively scavenge free radicals under *in vitro* conditions. The antioxidant and antidiabetic activity of *Aegle marmelos* leaves and bark extract showed promising activity compared with that of standard compound and root extract. From our chromatographic studies, results showed the presence of umbelliferone in the bark extract as well as root extract. Thus through this preliminary screening we concluded that the presence of some of the active compounds present in the roots and other substitute parts mostly bark of *Aegle marmelos* tree. Also the aqueous extracts from the other parts showed antioxidant and antidiabetic activities. However more systematic investigation is needed to establish the correlation between the isolated bio marker compounds from the plant parts responsible for their respective bio activity.

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**DECLARATION OF INTEREST**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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