EFFECT OF BHAVANA WITH AMLA JUICE ON IN-VITRO ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF AMALAKI (Emblica officinalis Gaertn.)

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Abstract
Objective: Amalaki (Emblica officinalis Gaertn., Fam: Euphorbiaceae) is a well known and potent rasayanas drug and increases our defense mechanism. The drug is included in best antidiabetic drugs mentioned in Ayurvedic text. According to Acharya charaka mentioned bhavana (trituration) with fresh juice of itself increases drugs potency by many fold so that the dose of medicine reduces. The aim of present study was to explore the effects bhavana on in-vitro hypoglycemic activity and in-vitro antioxidant activity.

Methods: 500g of powder E. officinalis was triturated with fresh fruit juice of E. officinalis 21 times. 20g of the triturated material were collected after 5th, 10th, 15th and 21st trituration and hydroalcoholic extract (methanol:water::80:20) of each sample was used for experimental purpose.

Results: The result shows that the raw and processed possess α-glucosidase and α-amylase inhibition activity which was increases with the number of trituration and dose. The IC₅₀ value of 21th triturated amalaki was found to be 32.74 μg/mL and 48.22 μg/mL respectively and was comparable with standard drug acarbose. The in-vitro antioxidant potential of E. officinalis and effect of processing (bhawana) was investigated three in-vitro methods viz. DPPH free radical scavenging, scavenging of hydroxyl radical by deoxyribose method and nitric oxide scavenging. The results demonstrate that the free radicals were scavenged by the drug in dose dependent manner. Moreover the potency increases as number of trituration increases. The IC₅₀ values for E. officinalis were found to be 38.68 μg/mL, 43.04 μg/mL and 55.85 μg/mL respectively.

Conclusion: These findings reveal that bhavana increases in-vitro antidiabetes as well as in-vitro antioxidant activity of E. officinalis.

Keywords: Emblica officinalis, Bhavana, Hypoglycemic, Antioxidant, Free Radical.

Introduction: World’s most serious health concerns diabetes mellitus (DM) is a chronic disease the prevalence of which is rapidly increasing in the current scenario with the increase in obesity and advancing age in the general global population. It is estimated that the number of persons in the world suffering from Type 2 DM will reach at least 380 million in 2025. Type 2 DM is primarily caused by defective glucose absorption, insufficient insulin production and its resistance. It is considered to be a preventable disease. In DM, the postprandial phase is characterized by a rapid increase in plasma glucose levels and this postprandial “hyperglycemic spikes” play an important role in the progress of type 2 DM and leads to several micro- and macro-vascular, complications such as retinopathy, nephropathy, and neuropathy. Moreover, postprandial state also contributes in the development of atherosclerosis and cardiovascular disease. Controlling postprandial hyperglycemia plays an important role in delaying or preventing Type 2 DM and its micro- as well as macro-vascular complications. Dietary control is the best way to control the postprandial hyperglycemia and it has synergistic effect with oral hypoglycemic agents (OHA). However, it depends upon depend on types and quantity of food consumed and such type of dieatery control is not seem to be possible in the present life style. Another possible therapeutic approach for decreasing postprandial hyperglycemia involves the retardation of fast uptake of glucose in the intestine which is
possible by the inhibition of carbohydrate-hydrolyzing enzymes (especially pancreatic α-glucosidase and α-amylase) in the gastrointestinal tract[7]. Several synthetic α-glucosidase and α-amylase inhibitors, like acarbose are in clinical practices for reducing the sudden rise of blood sugar levels after taking food [8]. However, the continuous use of OHA may cause side effects such as flatulence, abdominal distention, vomiting, possibly diarrhea, renal tumors, serious hepatic injury and acute hepatitis[9,10]. Moreover, excessive inhibition of α-amylase may leads to abnormal bacterial fermentation of undigested carbohydrates in the colon [11]. Hence, in the search of effective α-glucosidase and α-amylase inhibitors with lesser side effect, numerous in vivo as well as in-vitro studies were carried out and still going on [12].

Emblica officinalis Gaertn. (Euphorbiaceae) is a well known and potent rasayasana drug which reputed to promote health and longevity by increasing defense against diseases [13]. The fruits, fresh, dried or stewed act as a tonic, a diuretic and a laxative. The fruits are useful in treating diabetes, cough, asthma, bronchitis, intermittent fevers and cardiac disorders [14]. E. officinalis has been reported to possess free radicals scavenging effect and is considered as a rich source of vitamin C. It consists of large amount of Vitamin C (ascorbic acid), tannins 30%, phyllemblic acid, phyllembin, gallic acid, ellagic acid in natural form and cytokine like substances identified as Zeatin, Zriboside, Z nucleotide [15]. Acharya charaka mentioned that if a powdered drug was triturated with its fresh juice, the potency of drugs increases [16]. Through the bhavana (trituration) one can reduced the dose of drug and enhance its effect. Bhavana is a special process describe in Ayurveda, commonly used in shodhana (detoxification with therapeutic enhancement) and marana (incineration) process of rasa (mineral origin) drugs. It was also described for many plant origin dugs too [17]. This study was designed to evaluate the effect of trituration on in-vitro anti diabetic and antioxidant activities of E. officinalis.

Materials and Methods

**Sample Preparation:** Dry fruit of E. officinalis were homogenised to a fine powder by using mechanical grinder and passed through mesh sieve (85#). The powder was stored in opaque screw-top jars at room temperature (20±2°C) until use. 500g of powder was then triturated with fresh fruit juice of E. officinalis in motor pestle until dry fine powder obtained. The process was repeated 21 times. 20g of the triturated material were collected after 5th, 10th, 15th and 21st trituration and stored in opaque screw-top jars at room temperature until use. The drug samples (20g), were extracted with hydroalcoholic solvent (methanol:water::80:20) (100 mL) using cold maceration process for 10 day. After 10 days the content was filtered and the filtrate obtained was concentrated under reduced pressure in rotary evaporator (Perfit India, Pvt. Ltd., India) below 60°C. The extracts were store at room temperature in air tied container.

**Drug and Chemical:** α-glucosidase (EC 3.2.1.20), pancreatic-amylase (EC 3.2.1.1), 4-nitrophenyl-a-D-glucopyranoside (pNPG) and DPPH were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). While acarbose tablet was purchased from local market. All reagents used in the experiment are of analytical grade.

**In-vitro anti-diabetic Activity**

**α-glucosidase Inhibition Assay:** Determination of α-glucosidase inhibitory activity has been done as per the method of Ranilla et al., 2010 [18]. 50 l of extract has been added at different concentrations (5, 10, 20, 40 and 80 g/mL in DMSO) with 1mL of 0.1M potassium phosphate buffer (pH 6.9) containing α-glucosidase solution. The mixture was then incubated at 25 °C. After incubation, 500 l of 5mM pNPG solution in 0.1M potassium phosphate buffer was then added to the mixture. It was then reincubated at 25 °C for 5min. The absorbance was then measured using a U.V. –Visible spectrophotometer (Varian-carry-100Bio), before and after the incubation period. The absorbance was compared to that of control, containing 500 L of buffer solution instead of extract. The percentage enzyme inhibition was calculated using the following expression:

\[
\text{Inhibition} \% = \frac{1 - (\Delta A_{\text{samp}} - \Delta A_{\text{std}}) \times 100}{\Delta A_{\text{std}}}
\]

Where,

- \(A_{\text{sample}}\) = Absorbance of sample drug
- \(A_{\text{std}}\) = absorbance of the standard drug

**α-amylase Inhibition Assay:** Determination of α-amylase inhibitory activity was done as per the method of Ranilla et al., 2010 [18]. 200 L of extract has been mixed at different concentrations (5, 10, 20, 40 and 80 g/mL in DMSO) with 1mL of sodium phosphate buffer (pH6.8) containing 400 L of α-amylase solution. The mixture was then incubated at 37
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In-vitro α-glucosidase and α-amylase Inhibition Activity: Figure 1 illustrates inhibitory property of the raw and processed amlaki as well as standard acarbose on α-glucosidase and α-amylase. The results confirm α-glucosidase and α-amylase inhibition activity of amlaki. Moreover, the inhibitory potential was increases with the number of trituration and dose. At the highest concentration of 80 g/mL, the % inhibition of α-glucosidase was increases from 40.54 to 74.78% after 21th trituration, while % inhibition of α-amylase was increases from 31.25 to 62.45%. The IC50 value of 21th triturated amlaki was comparable with standard drug acarbose. Therefore we can conclude that this fruit extract have moderate α-amylase inhibitory activity.

Figure 1. in-vitro antidiabetic activity of the raw and processed E. officinalis.
**In-vitro Antioxidant Activity:** The *in-vitro* antioxidant potential of *E. officinalis* and effect of processing (*bhawanar*) was investigated three *in-vitro* methods. The results demonstrate that the free radicals were scavenged by the drug in dose dependent manner. Moreover the potency increases as number of trituration increases. Results of *in-vitro* antioxidant activity were demonstrated in figure 2. **Fig 2A** shows that the *E. officinalis* possess DPPH radical scavenging ability. Maximum activity (95.68 %) was observed at 400 g/mL concentration and the IC$_{50}$ value for *E. officinalis* decreases as number of trituration increases. The IC value of 21$^{th}$ triturated amalaki and ascorbic acid were found to be 38.68 g/mL and 32.36 g/mL respectively (table. 1). **Fig. 2B** reveals that the drug also possesses hydroxyl radical scavenging activity in which increases with trituration. Maximum scavenging activity (90.25%) was observed at 400 g/mL concentration and the IC$_{50}$ value of 21$^{th}$ triturated amalaki and ascorbic acid were found to be 43.04 g/mL and 62.16 g/mL respectively (table 1). The nitric acid scavenging power measurements were shown in **Fig 2C**. In this assay, the hydro-methanolic extract of 21$^{th}$ triturated amalaki at dose 400 g/mL had maximum reductive potential (92.36%) with IC$_{50}$ of 55.85 g/mL.

**Table 1. IC$_{50}$ values of standard and different processed samples of *E. officinalis***

<table>
<thead>
<tr>
<th>Activity</th>
<th>Raw Amalaki</th>
<th>5$^{th}$ trituruation</th>
<th>10$^{th}$ trituruation</th>
<th>15$^{th}$ trituruation</th>
<th>21$^{th}$ trituruation</th>
<th>Std. Acarbose</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-glucosidase inhibition</td>
<td>88.62</td>
<td>69.78</td>
<td>60.02</td>
<td>47.43</td>
<td>32.74</td>
<td>39.56</td>
<td>----</td>
</tr>
<tr>
<td>α-amylase inhibition</td>
<td>120.25</td>
<td>104.98</td>
<td>83.89</td>
<td>66.35</td>
<td>48.22</td>
<td>46.10</td>
<td>----</td>
</tr>
<tr>
<td>Free Radical Scavenging by DPPH Method</td>
<td>159.21</td>
<td>136.63</td>
<td>120.20</td>
<td>97.29</td>
<td>38.68</td>
<td>----</td>
<td>32.36</td>
</tr>
<tr>
<td>Nitric Oxide Scavenging Activity</td>
<td>226.84</td>
<td>180.83</td>
<td>149.77</td>
<td>86.45</td>
<td>43.04</td>
<td>62.16</td>
<td>22.90</td>
</tr>
<tr>
<td>Scavenging of hydroxyl radical by deoxyribose method</td>
<td>216.73</td>
<td>154.14</td>
<td>118.75</td>
<td>77.73</td>
<td>55.85</td>
<td>----</td>
<td>22.90</td>
</tr>
</tbody>
</table>

**Discussion:** The conventional pharmaco agents aimed to reduce the blood sugar level toward normal. After meal a sudden rise in postprandial blood glucose occurs due to digestion of carbohydrates by α-amylase and absorption of glucose by α-glycosidase [21]. The clinically used OHA are having insufficient hypoglycemic effect on postprandial spike of glucose level [22]. Agents which have inhibitory effect on α-glucosidases and α-amylase, may be added in clinical practice along with other OHA such as metformin, glimepride. α-Glucosidase enzyme is present on the brush-border surface of intestinal cell membrane [23]. It is a exo-type carbohydrate enzyme [24] which catalyze the hydrolysis of the α- (1, 4) -glucosidic linkage of starch and
disaccharides by providing hydrogen \cite{24}. So hydrogen scavengers may act as α-glucosidase inhibitors like acarbose \cite{25}. Pancreas and salivary glands secrete α-amylase which catalyses the hydrolysis of α-1, 4-glucosidic linkages of starch, glycogen, and oligosaccharides \cite{26}. α-amylase inhibitors acts through two mechanisms. They either form a complex with enzyme and limit its activity \cite{27} or reduce the diffusion rate of glucose from the active site \cite{27, 28}. Therefore, scientific communities are still investigating natural origin drugs for their possible role in the inhibition of these enzymes \cite{12}.

Generation of oxidative stress in diabetes mellitus is well known. Use of antioxidants with OHA increases at present. Free radicals are produced in different oxidation reactions in the body, which participate in progress of diseases. Antioxidants terminate the oxidative chain reactions through free radicals scavenging activity and act as reducing agents like ascorbic acid and polyphenols \cite{29}. Production of reactive oxygen species (ROS) is a continuous process and different intracellular enzymatic and nonenzymatic antioxidants play important role in protection of cells from these ROS \cite{30}. Hence, Antioxidants possess reducing power, free radical scavenging power, metal chelating power, and may activate antioxidative defense enzyme system of body \cite{30}. Therefore, in-vitro antioxidant activity evaluation of herbal drugs may prove their therapeutic significance. Previouly a variety of plants and phyto-molecules were investigated for their protective role in oxidative stress \cite{31}.

However, *E. officinalis* has been used in Ayurveda for a number of disorders such as liver diseases, atherosclerosis and diabetes. It is also considered as one of the best rasayana (immunomodulatory) drug. Many researchers were reported its efficacy in diabetes mellitus in vivo as well as in-vitro \cite{32}. The present article deals with effect of trituration of *E. officinalis* with its juice on its antidiabetic and antioxidant activity in-vitro. The results of study reveal that the in-vitro anti-diabetic as well as antioxidant potency of *E. officinalis* was increases after successive trituration with its juice. The tannoid principles from fruits of *E. officinalis* were reported to be responsible for its antioxidant activity \cite{33}. Because of the antioxidant activity of *E. officinalis* extract and quercetin, they are also found to possess cytoprotective effects \cite{34}. *E. officinalis* significantly scavenges superoxide as well as inhibits its generation and aqueous *E. officinalis* has been found to be potent antioxidants in-vitro.

### References


