In vitro Studies on Basella rubra Different Extracts as Inhibitors of Key Enzymes Linked to Diabetes Mellitus

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ABSTRACT

Enzyme, inhibiting carbohydrate metabolism and thereby decreasing glucose level is a class of drugs helpful in the management of type 2 Diabetes mellitus. Naturally existing α-amylase and α-glucosidase inhibitors from medicinally significant plants are shown to be effective in the management of postprandial hyperglycemia. In this investigation, leaf extract (BRLE), stem extract (BRSE), fruit extract (BRFRE) and flower extract (BRFLE) of Basella rubra were subjected to evaluate their antioxidant potential and their possible inhibitory effects on α-amylase and α-glucosidase. BRLE, BRSE, BRFRE, BRFLE (at concentration 100µg/ml) exhibited 65.78, 56.84, 63.1, 61.03% of α-amylase inhibitory activity respectively with IC\textsubscript{50} values of 71.66, 89.69, 73.68, 80.37 µg/ml respectively. In the same way BRLE, BRSE, BRFRE, BRFLE (at concentration 100 µg/ml) exhibited 97.63, 92.79, 82.17, 92.71 % of α-glucosidase inhibition with an IC\textsubscript{50} value of 26.97, 28.53, 41.30, 38.80 µg/ml respectively. Among the samples, the leaf extract of B. rubra registered higher content of total phenolics and flavonoids and also higher antioxidant activity in DPPH, nitric oxide and NBT radical scavenging assays. Though all the parts had shown potent inhibitory effects on α-amylase and α-glucosidase, the highest inhibitory potency was observed in the leaf extract of Basella rubra (IC\textsubscript{50} = 0.001).

Key words: α-Amylese inhibitory activity, Basella rubra, Diabetes mellitus, Postprandial hyperglycemiam.

INTRODUCTION

Diabetic Mellitus (DM) is a disease which is caused due to the metabolic disorder of carbohydrate metabolism. The management of DM was mostly carried out by the treatment of oral hypoglycemiac agents or antihyperglycemic agents and insulin. However due to their undesired effect the use of medicinal plants in the management of DM gained considerable interest. Thus the management of DM can be effected by inhibiting pancreatic enzymes like α-amylase and α-glucosidase which is responsible for the hydrolysis of carbohydrate and thereby causing postprandial hyperglycemia. Basella rubra (BR) belonging to the family Basellaceae is commonly called Malabar spinach and is native to the East Indies. It is a vigorous, climbing tropical vine that may be grown as leafy vegetable for its edible spinach-like stems and leaves or as an ornamental foliage vine. Leaves and stems are a good source of Vitamins A and C, calcium and iron. Basella rubra has been reported to have anti-microbial, larvicidal, hypoglycemiac, anti-ulcer, analgesic, anti-pyretic, diuretic, and anti-oxidant properties. Phytochemical investigation of this plant yielded different phyto-constituents like cardiac glycosides, saponins, tannins, flavonoids, terpenoids, carbohydrates, reducing sugars and basellasaponins A, B, C, and D. The hypoglycemic effect of Basella rubra in streptozotocin induced diabetic albino rats was reported.

Natural inhibitors of carbohydrate enzymes from plant sources put forth an attractive strategy for the control of postprandial hyperglycemia. This effort has been taken to investigate the α-amylase and α-glucosidase inhibitory activity of the leaf, stem, flower and fruit extracts of Basella rubra in the management of diabetes mellitus.

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected from Hudco Colony, Peelamedu, Coimbatore and authenticated by Mr. MURTHY G.V.S, Scientist, Botanical Survey of India, Tamilnadu Agriculture University Campus, Coimbatore. A voucher specimen was prepared in the research laboratory and the voucher with no. PSGCP/DPC/03, is maintained for further reference.

Preparation of plant extracts (Leaf, Stem, Fruit and Flower)

The shade dried parts of the plant were powdered and subjected to defatting with petroleum ether. The marc is then subjected to cold maceration with 70% Hydroalcohol for 48 hr. Finally all the extracts were filtered and concentrated under reduced pressure to laboratory and the voucher with no. PSGCP/DPC/03, is maintained for further reference.

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Phytochemical Screening

Different extracts of Basella rubra were subjected to various phyto-chemical tests to find out the presence of sterols, terpenoids, carbohydrates, and other phytochemicals.
flavonoids, proteins, alkaloids, glycosides, saponins, tannins, volatile oils and mucilage.9

Estimation of Total Phenols
Total phenolic content was determined in all the extracts by Folin–Ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation, a green–blue complex is formed, which is measured at 750 nm. The total phenol content of a tested material can be related to the antioxidant activity shown by it.10

Estimation of Total Flavonoids
Flavonoids present in the extracts were estimated by their characteristic absorption in the UV region and by their specific reaction with aluminium chloride and potassium acetate.11

Antioxidant Assay
Antioxidant potential of different parts of Basella rubra was assessed by DPPH method,12 Nitric oxide method13 and Nitro blue tetrazolium method.14

Alpha Amylase Inhibitory Assay
In vitro α-Amylase Inhibitory activity of alcoholic extract of various plant parts (leaf, stem, fruit, flower) of BR was carried out by using Kazem et al 2013 method.15 In this assay various concentrations (20, 40, 60, 80, 100 µl) of different plant parts of alcoholic extract of BR was allowed to react with 250 µl of 0.02 m sodium phosphate buffer (pH 6.9) which contains α-amylase solution (0.5 mg/ml) (Hi media Rm 638 - α-Amylase from fungi). The content of the tubes was pre incubated at 25°C for 10 min after which 250 µl of 1% starch solution was added in 0.02 M sodium phosphate buffer (pH 6.9). The reaction mixture was incubated at 25°C for 10 min. The reaction was terminated by adding 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) which contains α-amylase (RM7067 α-Glucosidase from Saccharomyces species) (1 Unit/ml) in 20 mM phosphate buffer (pH 6.9). The content of the tubes was pre incubated at 25°C for 10 min. 50 µL of p-nitropheynyl glucopyranoside was added to start the reaction. The reaction mixture was incubated at 25°C for 20 min. The reaction was terminated by adding 2 ml of 0.1 M sodium carbonate solution and finally made up to 5ml with distilled water. Then enzyme inhibition was (absorbance) measured at 405 nm by Spectrophotometer. The reaction system without plant extracts was used as control and the system without α-amylase was used as blank for correcting the background absorbance. The percentage inhibition of α-amylase enzyme was calculated using the following formula.

\[
\text{% Inhibition} = \frac{\text{Control absorbance} - \text{Corrected test absorbance}}{\text{Control absorbance}} \times 100
\]

Where
Corrected test absorbance = Sample absorbance – Blank absorbance
The concentration of extract resulting in 50% inhibition of enzyme activity (IC\text{50}) was determined graphically using Microsoft excel.

Statistical analysis
Statistical analysis was performed using GraphPad Prism statistical package (GraphPad Software, USA). The data were analyzed by One-way Analysis of Variance (ANOVA) method followed by Tukey's multiple comparison. The results were considered to be statistically significant when the \(P<0.05\). All the results were expressed as mean ± SD for triplicate determinations.

RESULTS

Phytochemical study
Preliminary phytochemical screening of various hydro alcoholic extracts of BRLE, BRSE, BRFRE, BRFLE exhibited the presence of glycosides, terpenoids, carbohydrates, proteins, falvanoids, sterols, tannins, saponins and mucilage (Table 1).

Secondary metabolite
Estimation of the secondary metabolites showed significant content of total phenolic (25.9 GAE mg/g) and total flavonoid content (4.27 RE mg/g) in BRLE (Table 2).

Antioxidant potential
Four extracts namely BRLE, BRSE, BRFRE, BRFLE were subjected to in vitro antioxidant activity. Among the four extracts evaluated, hydro alcoholic extract of BRLE showed potent antioxidant property with the minimum IC\text{50} values in the scavenging of DPPH, Nitric acid and NBT assay. All the results were comparable with the standard. The IC\text{50} values of hydro alcoholic extract of BRLE were found to be 10.98, 62.5 and 48.24 against DPPH, Nitric oxide and NBT methods respectively (Table 3).

In vitro α− amylase assay
The % inhibition of α-amylase activity of hydro alcoholic extracts of BRLE, BRSE, BRFRE, BRFLE at the concentrations of 20, 40, 60, 80 and 100 mg/ml as shown in Table 4 was studied in comparison with standard Acarbose. The standard Acarbose showed 70.06 ± 3.73% (at concentration 100 µg/ml) inhibitory activity of an α-amylase with IC\text{50} value 64.97 µg/ml (Table 6). BRLE (at concentration 100µg/ml) exhibited 65.78 ± 1.51% of α-amylase inhibitory activity with an IC\text{50} value of 71.66 µg/ml. BRSE, BRFRE, BRFLE (at concentration 100 µg/ml) exhibited 65.78 ± 2.47, 63.1 ± 3.07, 61.03 ± 1.16% of α-amylase inhibitory activity respectively with IC\text{50} values 89.69, 73.68, 80.37 µg/ml respectively (Table 4). Results obtained from the above investigation showed that BRLE showed more potent inhibitory activity of α-amylase when compared with BRSE, BRFRE, BRFLE.

In vitro α− glucosidase assay
The % inhibition of α-glucosidase activity of hydro alcoholic extracts of BRLE, BRSE, BRFRE, BRFLE at the concentrations of 20, 40, 60, 80 and 100 mg/ml as shown in Table 4 was studied in comparison with the standard drug i.e. Acarbose. The standard acarbose showed 97.67 ± 0.89% (at concentration 100 µg/ml) inhibitory activity of an α-glucosidase with IC\text{50} value 89.69 µg/ml (Table 6). BRFLE (at concentration 100 µg/ml) exhibited 92.71 ± 2.92% of α-glucosidase inhibition with an IC\text{50} value in Table 4 (28.53,
Table 1: Phytochemical analysis for Hydroalcoholic extract of various plant parts of *Basella rubra*

<table>
<thead>
<tr>
<th>Name of the test</th>
<th>Leaf Extract</th>
<th>Stem Extract</th>
<th>Flower Extract</th>
<th>Fruit Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mucilage</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) indicates positive reaction  (-) indicates negative reaction

Table 2: Estimation of Phenolic and Flavonoid Content of different parts of *Basella rubra*

<table>
<thead>
<tr>
<th>Name of the part</th>
<th>Total Phenolics GAE mg/g</th>
<th>Total Flavonoids RE mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRLE</td>
<td>25.9</td>
<td>4.27</td>
</tr>
<tr>
<td>BRSE</td>
<td>23.8</td>
<td>3.89</td>
</tr>
<tr>
<td>BRFRE</td>
<td>18.5</td>
<td>2.97</td>
</tr>
<tr>
<td>BRFLE</td>
<td>20.5</td>
<td>3.34</td>
</tr>
</tbody>
</table>

Data are mean ± SD or % ± SD for triplicate measurements.
GAE – gallic acid equivalent, RE – Rutin equivalent.

Table 3: *In vitro* antioxidant studies of different extracts of *Basella rubra* by DPPH, Nitric acid and NBT and methods.

<table>
<thead>
<tr>
<th>Different extracts / Standard</th>
<th>IC50 values (mg/ml)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH</td>
<td>Nitric oxide</td>
<td>NBT</td>
</tr>
<tr>
<td>BRLE</td>
<td>10.98 ± 0.72</td>
<td>62.5 ± 0.42</td>
<td>48.24 ± 0.32</td>
</tr>
<tr>
<td>BRSE</td>
<td>14.78 ± 0.54</td>
<td>125.4 ± 0.82</td>
<td>104.36 ± 0.49</td>
</tr>
<tr>
<td>BRFRE</td>
<td>11.74 ± 0.36</td>
<td>75.2 ± 0.36</td>
<td>94.38 ± 0.54</td>
</tr>
<tr>
<td>BRFLE</td>
<td>16.72 ± 0.62</td>
<td>82.4 ± 0.52</td>
<td>71.59 ± 0.69</td>
</tr>
</tbody>
</table>

Data are mean ± SD or % ± SD for triplicate measurements.

Table 4: Alpha Amylase Inhibitory Assay of various extracts of *Basella rubra*

<table>
<thead>
<tr>
<th>Conc mcg/ml</th>
<th>% Inhibition</th>
<th>Carboase</th>
<th>BRLE</th>
<th>BRSE</th>
<th>BRFRE</th>
<th>BRFLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>22.50±0.90</td>
<td>20.46±0.98</td>
<td>16.79±0.57</td>
<td>17.21±0.65</td>
<td>12.99±0.36</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>36.25±0.69</td>
<td>30.33±0.85</td>
<td>25.33±0.30</td>
<td>30.48±0.96</td>
<td>21.61±0.98</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>46.63±0.79</td>
<td>40.62±0.99</td>
<td>32.03±0.55</td>
<td>35.34±1.14</td>
<td>38.56±1.32</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>62.47±0.69</td>
<td>51.88±0.80</td>
<td>43.85±0.72</td>
<td>52.59±1.19</td>
<td>50.54±0.69</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>70.83±1.48</td>
<td>65.53±1.18</td>
<td>56.39±0.90</td>
<td>63.05±0.55</td>
<td>61.35±1.10</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD or % ± SD for triplicate measurements.
In the present study, BRLE showed a significant (p<0.001) inhibition of α-glucosidase when compared with BRSE, BRFRE, BRFLE. Results obtained from the above investigation showed that BRLE showed more potent inhibitory activity of α-glucosidase than 41.30, 38.80 µg/ml respectively. The percentage inhibition of leaf extract of *Basella rubra* is more than that of the extracts of stem, flower and fruit. It is concluded by the study that the percentage inhibition of leaf extract of *Basella rubra* is more than that of the extracts of stem, flower and fruit. The IC_{50} value of leaf extract is less than that of stem, fruit and flower extracts of *Basella rubra* and hence it shows high alpha amylase and high alpha glucosidase inhibitory action. This activity may be due to the significant antioxidant property which may be due to high levels of phenolic and flavonoid content. However, further study is needed to isolate the active principle(s) in this plant which is responsible for this activity.

**REFERENCES**

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