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Original Article

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_{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_{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* ($p<0.001$).

Key words: α-Amylase inhibitory activity, *Basella rubra*, Diabetes mellitus, Postprandial hyperglycemia.

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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 hypoglycemic 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,[1] larvicidal,[2] hypoglycemic,[3] anti-ulcer,[4] analgesic, anti-pyretic, diuretic,[5] and anti-oxidant properties.[6] 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.[7] The hypoglycemic effect of *Basella rubra* in streptpzotocin induced diabetic albino rats was reported.[8]

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 authentified 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 get various plant extracts namely *Basella rubra* Leaf Extract (BRLE), *Basella rubra* Stem Extract (BRSE), *Basella rubra* Flower Extract (BRFLE) and *Basella rubra* Fruit Extract (BRFRE).

Phytochemical Screening

Different extracts of *Basella rubra* were subjected to various phyto-chemical tests to find out the presence of sterols, terpenoids, carbohydrates,

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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 method[13] 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 *Kazeem 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 dinitro salicylic acid (DNS) reagent and further incubated in boiling water bath for 5 min and cooled to room temperature. The content of each test tube was diluted up to 5ml with distilled water and the absorbance was measured at 540 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.

$$\% \text{ Inhbition} = \frac{\text{Control absorbance} - \text{Corrected test absorbance}}{\text{Control absorbance}} \times 100$$

Alpha Glucosidase Inhibitory Assay

In vitro α-Glucosidase Inhibitory activity of hydroalcoholic extract of various plant parts (leaf, stem, fruit, and flower) of BR was carried out by using *Kazeem et al* 2013 method. 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 100 µl of α-Glucosidase (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-glucosidase was used as blank for correcting the background absorbance. The percentage inhibition of α-glucosidase enzyme was calculated using the following formula.

$$\% \text{ Inhbition} = \frac{\text{Control absorbance} - \text{Corrected test absorbance}}{\text{Control absorbance}} \times 100$$

Where

Corrected test absorbance = Sample absorbance – Blank absorbance

The concentration of extract resulting in 50% inhibition of enzyme activity (IC_{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_{50} values in the scavenging of DPPH, Nitric acid and NBT assay. All the results were comparable with the standard. The IC_{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 α amylase with IC_{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_{50} value of 71.66 µg/ml. BRSE, BRFRE, BRFLE (at concentration 100 µg/ml) exhibited 56.84 ± 2.47, 63.1 ± 3.07, 61.03 ± 1.16% of α-amylase inhibitory activity respectively with IC_{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.

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 α-glucosidase with IC_{50} value 24.85 µg/ml (Table 6). BRLE (at concentration 100 µg/ml) exhibited 97.63 ± 1.16% of α-glucosidase inhibitory activity with an IC_{50} value of 26.97 µg/ml. Percentage inhibition for BRSE, BRFRE, BRFLE (at concentration 100 µg/ml) exhibited 92.79 ± 1.08, 82.17 ± 2.26, 92.71 ± 2.92% of α-glucosidase inhibition with an IC_{50} value as in Table 4 (28.53,

Table 1: Phytochemical analysis for Hydroalcoholic extract of various plant parts of *Basella rubra*

Name of the test	Leaf Extract	Stem Extract	Flower Extract	Fruit Extract
Alkaloids	-	-	-	-
Glycosides	+	+	-	-
Terpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	+	+	+
Flavonoids	+	+	+	+
Sterols	+	+	+	+
Tannins	+	+	+	+
Anthraquinone	-	-	-	-
Saponins	+	+	-	-
Mucilage	+	+	-	-

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

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

Name of the part	Total Phenolics GAE mg/g	Total Flavonoids RE mg/g
BRLE	25.9	4.27
BRSE	23.8	3.89
BRFRE	18.5	2.97
BRFLE	20.5	3.34

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.

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

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

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

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

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

Table 5: Alpha Glucosidase Inhibitory Assay of various extracts of *Basella rubra* and Acarbose

Conc mcg/ml	% Inhibition				
	Acarbose	BRLE	BRSE	BRFRE	BRFLE
20	50.57 ± 0.94	44.42 ± 1.25	42.91 ± 2.61	41.01 ± 1.85	40.39 ± 1.73
40	60.7 ± 0.86	66.91 ± 1.66	65.03 ± 1.77	50.91 ± 1.01	55.01 ± 1.91
60	77.13 ± 1.71	73.38 ± 2.64	71.84 ± 1.83	62.8 ± 2.93	63.83 ± 0.33
80	92.31 ± 2.66	90.47 ± 0.96	85.81 ± 2.49	71.62 ± 1.46	81.92 ± 1.26
100	97.67 ± 0.89	97.63 ± 1.16	92.79 ± 1.08	82.17 ± 2.26	92.71 ± 2.92

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

Table 6: IC50 Values of various extract of *Basella rubra* and Acarbose

Name	IC_{50} Values of α-amylase	IC_{50} Values of α-glucosidase
Acarbose	64.97	24.85 µg/ml
BRLE	71.66	26.97 µg/ml
BRSE	89.69	28.53 µg/ml
BRFRE	73.68	41.30 µg/ml
BRFLE	80.37	38.80 µg/ml

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

41.30, 38.80 µg/ml) respectively. Results obtained from the above investigation showed that BRLE showed more potent inhibitory activity of α-glucosidase when compared with BRSE, BRFRE, BRFLE.

DISCUSSION

Diabetes mellitus is a common metabolic disorder that increases the postprandial glucose level which may lead to multiple organ damage and increase the risk of cardiovascular diseases which are the most common causes of death among people with diabetes. Management of blood glucose level is critical in the early treatment of diabetes mellitus and its complication. Sharp rise in the blood glucose level after food intake is aided by α-Amylase and α- Glucosidase enzymes, which break the carbohydrate to simple absorbable sugars.[16] Synthetic enzyme inhibitors such as acarbose, voglibose, miglitol, are useful as oral hypoglycemic drugs for the control of post prandial hyperglycemia especially in type II diabetes patients.[17] These inhibitors delay carbohydrate digestion and prolong the time taken for glucose absorption in intestine.[18] Chronic usage of these inhibitors in conjunction with other antidiabetic drugs leads to GI side effects like abdominal discomfort, flatulence and diarrhea.[19] Several plants have been identified as potential source of drugs in Indian system of medicine for the treatment of diabetes.

Phenolic compounds, whose formation is associated with the normal metabolism of aerobic cells can protect the human body from free radicals. Such compounds are strong in antioxidants and can remove free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocophenol radicals and inhibit oxidases.[20] Derivatives of flavonoids have been found in many fruits and vegetables. Further, numerous studies have shown that majority of the antioxidant activities maybe from compounds such as flavonoids, isoflavones, flavones, anthocyanins, catechin and isocatechin rather than from vitamins C, E and β-carotene.[21] Flavonoids have antioxidant activity and could therefore lower cellular oxidative stress.[22]

In the present study, BRLE showed a significant ($p<0.001$) inhibition of α–amylase and α–glucosidase activity at all concentrations tested and hence it can be used for the management of diabetes mellitus.

CONCLUSION

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.

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CONFLICT OF INTEREST

The authors have no conflict of interest

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