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*In vitro*Free Radical Scavenging and α-Amylase Inhibitory Activity of Ethanolic Extract of *Basella alba*

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Abstract

Basella alba is an edible perennial plant in the family Basellaceae. It is native to the Indian Subcontinent, widely spread in Asia and Africa, and used as a leafy vegetable.Worldwide the number of people with diabetes is increasing every year. Due to the several risk factors inherent to the disease, the treatment of type II diabetes is complicated. Many plants with hypoglycaemic effects can be usedto develop drugs for diabetes mellitus. Decreasing the postprandial hyperglycemia is one of the aspectsof treating type II diabetes mellitus. This is possible by inhibiting certain carbohydrate hydrolysing enzymes like α-amylase and α-glucosidase. The objective of the present study was to evaluate *in-vitro* antioxidant and α-amylase inhibitory activity of the ethanolic extract of *Basella alba*. The extract showed significant radical scavenging activities like DPPH, ABTS, Super oxide, Nitric oxide and Hydroxyl radical scavenging activities with IC_{50} of 83.0 µg/mL, 87.5µg/mL, 91.0µg/mL, 96.5µg/mL and 70.0µg/mL. The extract also showed strong α-amylase inhibition with IC_{50} 77.8 mg/mL. These results suggest the possible use of *Basella alba* in the management of diabetes mellitus.

Keywords:*Basella alba*; *Antioxidant; α-Amylase; DPPH; ABTS and Diabetes mellitus*

INTRODUCTION

Diabetes mellitus is a metabolic disease of the human race with numerous clinical complications and abnormally high levels of blood glucose. It is a clinical syndrome characterized by hyperglycemia due to defects of insulin secretion, insulin action, or both. In 2021, according to the International Diabetes Federation (IDF), it was estimated that 537 million people had diabetes, and it is projected to reach 643 million by 2030 and 783 million by 2045. On an average, India accounts for 1 in 7 adults living with diabetes worldwide. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas and biguanides used to achieve better glycemia regulation. Many of the oral antidiabetic drugs have serious adverse effects and thus, the management of diabetes without side effects is still a challenge(Patel *et al*., 2012).

Medicinal plants are used by traditional methods to treat a variety of ailments and symptoms, including diabetes. Indian system of medicine uses plants source for treating a number of aliments. Many plants provide useful sources for the development of antidiabetic drugs, which can be used to treat diabetes mellitus without any side effects.*Basella alba* is an edible perennial plant in the family Basellaceae. It is widespread in tropical Asia and Africa,and it is widely used as a leaf vegetable. *Basella alba*has various common names, such as vine spinach, red vine spinach, malabar spinach, climbing spinach, and creeping spinach. Ithas been used for the treatment of many diseases like dysentery, diarrhea, wound healing, anemia, cancer etc. (Roshan *et al*., 2012).

Based on these facts of biochemical data available for *B. alba*, it was thought worthwhile to evaluate in-vitro antioxidant and α-amylase inhibitory activity of ethanolic extract of its aerial parts.The present study has been undertaken to evaluate *in-vitro* free radical scavenging activityandα-amylase inhibitory activity of the ethanolic extract of *B. alba*.

METHODS AND MATERIALS

Preparation of Plant Extract

Fresh aerial parts of *B. alba* were purchased from the market, and the leaves of *Basella alba* were spread out and dried at room temperature for 8-10 days. Then they were ground to fine powder using electric blender and stored in a closed container at room temperature until required. The powdered plant materials were taken and subjected to

successive solvent extraction. The extraction was carried out for 16 h with the solvents in the increasing order of polarity.

The plant powder was extracted with solvent in the ratio of 1:5 (w/v) for 16 hours by using soxhlet apparatus with the following solvents in the increasing order of polarity. The extracts were filtered using Whatman filter paper No.2 on a Buchner funnel, and the solvent was removed under reduced pressure using a rotary evaporator at 40^{o}C. The extract was placed in pre-weighed flasks before drying for quantitative determinations. Sequential solvent extraction was done with plant material. The ethanolic extract of *B. alba* was used for the analyses.

DPPH free radical scavenging assay

DPPH radical scavenging assay by the method of Blois (1958). Antioxidants scavenge DPPH radicals by donation of a proton that forms the reduced DPPH. The change in colour frompurple to yellow can be measured by reading the absorbance at 517 nm. Various concentrations (20, 40, 60, 80, and 100 µg/mL) of samples were taken in different test tubes. Ascorbic acid is used as the positive control, which is also taken in different concentrations, and final volume was adjusted to 500 µLµLusing methanol. Then to these tubes 5 mL of 0.1 mM methanolic DPPH was added, vortexed, andincubated at room temperature for 20 min. The control was prepared as above without any extract, and methanol was used for the baseline correction. Changes in the optical density of the samples were measured at 517 nm. The inhibition percentage was calculated using the following formula, Percentage of radical scavenging activity= [(Control OD - Sample OD)/ Control OD] x 100.

ABTS radical scavenging activity

ABTS radical scavenging assay was determined by the method of Re *et al.* (1999). The test was based on the relative activity of antioxidants to quench the radical cation $ABTS^{+}$. ABTS decolourisation assay involves the generation of the $ABTS^{+}$ chromophore by the oxidation of ABTS with ammonium per sulphate. The scavenging activity of the plant extract on ABTS radical cation was measured at 734 nm. The reaction was initiated by the addition of 1.0 mL of diluted ABTS to 10 µLof different concentrations of aqueous extract of the sample or 10 µLof methanol served as control. The absorbance was read at 734 nm. The

inhibition percentage was calculated using the following formula, Percentage of radical scavenging activity = [(Control OD - Sample OD)/ Control OD] x 100.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging assay was determined by the method of Klein *et al.* (1991). Ferrous ammonium sulphate and EDTA were used to generate hydroxyl radicals. This was detected by their ability to react with ascorbic acid to produce a yellow color complex measured at 412 nm. Extract in the concentrations of 20, 40, 60, 80 and 100 µg/mL were added to 1 mL of Fe-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1 mL of DMSO. By adding 0.5mL of ascorbic acid (0.22%) the reaction was initiated and then it is incubated for 15 min at 80-90^{o}C. The reaction was terminated by adding 1 mL of ice-cold TCA (17.5% w/v) after incubation, and the 3 mL of Nash reagent was added and kept at room temperature for 15 min. A control without sample was also used. The Optical Density was measured at 412 nm. The percentage of hydroxyl radical scavenging activity is calculated by the following formula, Percentage of radical scavenging activity = [(Control OD - Sample OD)/ Control OD] x 100.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging assay was determined by Garrat (1964) method. The reaction mixture (3 mL) contains 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffered saline (PBS) and 0.5 mL of extract or standard solution and incubated at 25^{o}C for 150 min. After incubation, 0.5 mL of the reaction mixture containing nitrite was pipetted out and mixed with 1mL of sulphanilic acid reagent and allowed to stand for 5 min for completing diazotization. It is incubated for 30 min at 25^{o}C after addition of 1 mL of naphthyl ethylene diamine dihydrochloride and a pink coloured chromophore was formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Percentage inhibition was calculated by the formula, Percentage of radical scavenging activity = [(Control OD - Sample OD)/ Control OD] x 100.

Super oxide radical scavenging activity

Superoxide radical scavenging assay was done by the method of Liu *et al.* (2000). Superoxide anions were created in the reactions of PMS, NADH and O_2, then reduction of nitro blue tetrazolium (NBT) was assayed. Various concentrations (20, 40, 60, 80 and 100 µg/mL) of standard ascorbic acid solutions were taken in different test tubes. 20, 40, 60, 80 and 100 µg/mL of plant sample solutions weretaken in another set of test tubes. All the tubes were made up to 3 mL with acetone. 3 mL of alcohol was taken as blank. 1mL of each NBT, NADH and PMS were added in all the tubes and were incubated for 5 min. at room temperature. The absorbance was taken at 560 nm. The percentage of radical scavenging activity was calculated by the following formula, Percentage of radical scavenging activity = [(Control OD - Sample OD)/ Control OD] x 100.

α-Amylase inhibitory activity

The *α*-amylase inhibitory activity was determined accordingto the method described by Miller, 1959.The assaymixture containing 200 µLof 0.02 M sodium phosphatebuffer, 20 µL of enzyme (PPA), and the plant extracts in therange 10-100 µg/mLconcentration were incubated for 10min at room temperature followed by addition of 200 µL of1% starch in all the test tubes. The reaction was terminatedwith addition of 400 µL of 3,5 dinitrosalicylic acid (DNSA)colour reagent, placed in boiling water bath for 5 min, cooledto room temperature and diluted with 15 mL of distilledwater and the absorbance measured at 540 nm (Shimadzu-UV-VIS Spectrophotometer). The control tubes were prepared without any plant extracts andwere compared with the test samples. The results were expressed as % inhibitioncalculated using the formula:Inhibition activity (%)= [(Control OD - Sample OD)/ Control OD] x 100.

Statistical Analysis

All the experimental results were centred using three parallel measurements of the mean ± the standard deviation.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability, the plants with higher hydrogen donating capacity have shown higher DPPH free radical scavenging activity. *In vitro*assessment of antioxidant potential was estimated using DPPH radical. The free radical scavenging activity of the ethanolic extract of *Basella alba*was assessed by DPPH scavenging assay. Table 1, illustrates the scavenging activity. The 50% inhibition concentration (IC_{50}) of *Basella alba* ethanolic extract is found to be 83.0 µg/mL. The activity of the ethanolic extract of *Basella alba* was comparable to that of the standard ascorbic acid.The DPPH free radical scavenging of antioxidants is due to their hydrogen donating ability, the plants with higher hydrogen donating capacity have shown higher DPPH free radical scavenging activity (Chen and Ho, 1995). DPPH radical is a common assessment method for antioxidant potential (Zhou and Yu, 2004). DPPH is known to abstract labile hydrogen (Matsubara *et al.,* 1991) and the ability to scavenge the DPPH radical is related to the inhibition of lipid peroxidation (Rekka and Kourounakis, 1991).

ABTS radical scavenging activity

In this assay ABTS radical cation was generated directly in stable form using potassium persulphate. The generation of radical before the antioxidants are added prevents interference of compounds, which affect radical formation. The IC_{50} for ABTS radical scavenging activity of *Basella alba* ethanolic extract is found to be 87.5µg/mL. ABTS radical cation has often been used to evaluate the antioxidant activity of single compounds and complex mixtures of various origins (body fluids, foods, beverages, plant extracts). In this assay ABTS radical cation was generated directly in stable form using potassium persulphate. The generation of radical before the antioxidants are added prevents interference of compounds, which affect radical formation (Sanchez- Moreno, 2002).

Hydroxyl radical scavenging activity

Hydroxyl radical is an extremely reactive species thatis capable of damaging living cells by initiating lipid peroxidation and abstracting hydrogen atoms of unsaturated fatty acids(Hochestein and Atallah, 1988). The IC_{50} for hydroxyl radical scavenging activity of

Basella alba ethanolic extract is found to be 70.0µg/mL. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition, hydroxyl radical is considered to be one of the quick initiators of thelipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Kappus, 1991).

Nitric oxide radical scavenging activity

Nitric oxide (NO) radical is said to be a potential pleiotropic mediator of many physiological processes. The IC_{50} for nitric radical scavenging activity of *Basella alba* ethanolic extract is found to be 96.5µg/mL.Nitric oxide is a free radical which plays key roles in biological systems including vasodilation and antitumor activitiesas an effector molecule (Hagerman *et al.,* 1998).

Super oxide radical scavenging activity

The IC_{50} for nitric radical scavenging activity of *Basella alba* ethanolic extract is found to be 91.0µg/mL. Superoxide is the first reduction product of molecular oxygen, a highly toxic radical, the most abundantly produced in all aerobic cells by several enzymatic and non-enzymatic pathways, attacks a number of biological molecules and leads to unfavourable alterations of biomolecules including DNA (Waris *et al.*, 2000). It also forms an important source of other deleterious radicals such as hydroxyl and hydroperoxides, which initiate free radical chain reactions (Halliwell and Gutteridge, 1990).

α-Amylase inhibitory activity

The ethanolic leaf extract of *Basella alba* revealed α-Amylase inhibitory effectto some extend when compared with standard alpha amylase inhibitor drug acarbose. Acarbose was used as a positive control. It is an alpha amylase inhibitor that decreases intestinal absorption of carbohydrates and is used as an adjunctive therapy in managing type II diabetes. Alpha-amylase is an important enzyme for starch hydrolysis and determines the amount of glucose released in the digestive system. Inhibition of amylase is an importantmethod to control starch digestion and regulate postprandial rise in blood glucose in diabetic patients (Akwam and Salah *et al.*, 2022).

Table 1.DPPH radical scavenging activity

Concentration (µg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Ascorbic acid**
20	6.8 ± 0.8	20.1 ± 1.2
40	16.3 ± 0.7	45.7 ± 2.3
60	31.5 ± 1.2	70.2 ± 1.6
80	45.7 ± 1.9	82.6 ± 1.9
100	69.8 ± 1.1	90.3 ± 2.4

Values are taken as a mean ±SD of three individual experiments

Table 2: ABTS radical scavenging activity

Concentration (µg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Ascorbic acid**
20	5.4 ± 0.6	16.2 ± 0.7
40	13.1 ± 1.2	35.8 ± 1.4
60	28.3 ± 1.3	59.8 ± 1.1
80	42.2 ± 0.9	75.2 ± 0.8
100	62.8 ± 1.3	86.1 ± 1.5

Values are taken as a mean ±SD of three individual experiments

Table 3: Hydroxyl radical scavenging activity

Concentration (µg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Ascorbic acid**
20	10.2 ± 0.5	25.2 ± 0.8
40	20.1 ± 0.9	47.2 ± 0.6
60	42.3 ± 0.7	69.1 ± 1.3
80	58.3 ± 1.2	81.3 ± 0.9
100	64.2 ± 0.4	93.2 ± 1.2

Values are taken as a mean ±SD of three individual experiments

Table 4: Nitric oxide radical scavenging activity

Concentration (µg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Ascorbic acid**
20	7.1 ± 0.6	15.2 ± 1.2
40	15.3 ± 1.2	32.2 ± 1.3

60	27.6 ± 0.8	59.4 ± 0.6
80	34.8 ± 0.9	73.1 ± 0.9
100	52.9 ± 1.2	82.9 ± 1.2

Values are taken as a mean ±SD of three individual experiments

Table 5: Super oxide radical scavenging activity

Concentration (µg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Ascorbic acid**
20	6.8 ± 0.9	13.2 ± 1.1
40	16.5 ± 1.1	28.5 ± 1.2
60	29.5 ± 1.3	46.9 ± 0.9
80	42.8 ± 0.6	67.2 ± 1.2
100	57.4 ± 0.8	79.8 ± 0.8

Values are taken as a mean ±SD of three individual experiments

Table 6: α-Amylase inhibitory effect of *Basella alba* leaf extract

Concentration (mg/mL)	% of inhibition	
	***B. alba* Ethanolic extract**	**Acarbose**
10	09.46 ± 0.35	30.17 ± 0.81
20	16.05 ± 1.07	43.67 ± 0.75
30	21.43 ± 0.27	54.21 ± 0.92
40	26.90 ± 0.92	63.26 ± 1.02
50	30.80 ± 0.91	72.65 ± 1.06

Values are taken as a mean ±SD of three individual experiments

Conclusion

The ethanolic extract of *Basella alba* has been studied for its potential *in vitro* antidiabetic and antioxidant activities. It showed an excellent antioxidant and radical scavenging activity. The extract also showed a very good *in vitro* α-amylase inhibitory activity. The IC_{50} values of*B. alba* ethanolic extract are comparable to thestandard drug acarbose and thus these extracts might helpin identification of new molecules for natural amylaseinhibitors. However, further studies on isolation and characterization of theactive principles with amylase inhibitory activity has to be carried out to confirm these observations.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research

Originality and plagiarism

This research is done by me and standard methods and previous work cited are appropriately cited in the reference

Consent for publication

All the authors agreed to publish the content

Competing interests

There were no conflict of interest in the publication of this content.

REFERENCES

Akwam M. Abd Elkader, Salah Labib, Taha F. Taha, Fayez Althobaiti, Adil Aldhahrani, Heba M. Salem, Ahmed Saad, Faten M. Ibrahim. 2021. Phytogenic compounds from avocado (*Persea americana* L.) extracts; antioxidant activity, amylase inhibitory activity, therapeutic potential of type 2 diabetes. *Saudi Journal of Biological Sciences*.**29.** 1428–1433.

Blois, M.S. 1958. Antioxidant determinations by the use of stable free radical. *Nature*,**1**: 1199-2000.

Chen, C.W., Ho, C.T. 1995. Antioxidant properties of polyphenols extracted from green and black tea. *Journal of Food Lipids*, **2:** 35-46.

Garrat, D.C. 1964. The Quantitative Analysis of Drugs. Chapman and Hall Ltd., Japan **3**: 456-458.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., Riechel, T.L. 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *Journal of Agricultural and Food Chemistry*, **46:** 1887-1892.

Halliwell, B., Gutteridge, J.M.C. 1990. Role of oxygen free radicals and catalytic metal ions in human disease: an overview. *Methods of Enzymology*, **186:** 1-85.

International Diabetes Federation, Diabetes Atlas 2021, 10th edition (www.diabetesatlas.org)

Kappus, H. 1991. In Aruoma, O., Halliwell, B. (Eds.), Lipid peroxidation: Mechanism and biological relevance in the book free radicals and food additives London, Taylor and Francis Ltd. p. 59-75.

Klein, S.M., Cohen, G., Cederbaum, A.I. 1991. Production of formaldehyde during metabolism of dimethyl sulphoxide by hydroxyl radical radical generating system. *Biochemistry*,**20**: 6006-6012.

Liu, F., Ng, T.B. 2000. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life Sciences*, **66**: 725–735.

Matsubara, N., Fuchimoto, S., Iwagaki, H., Nonaka, Y., Kimura, T., Kashino, H., Edamatsu, R., Hiramatsu, M., Orita, K. 1991. The possible involvement of free radical scavenging properties in the action of cytokines. *Research Communication in Chemical Pathology and Pharmacology*,**71:** 239-242.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem.* ;**31**:426–8.

Patel D, K., Kumar R,, Laloo D,, and Hemalatha S. 2012. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac J Trop Biomed.***2(5)**: 411–420.

Re, R., Pelligrini, N., Proteggeenate, A., Yang, M., Rice-Evans, C. 1999. Antioxidants activity of applying an improved ABTS radical cation decolorisation assay. *Free Radical Boilogy and Medicine*,**26**: 1231-1237.

Rekka, E., Kourounakis, P.N. 1991. Effect of hydroxyethyl rutenosides and related compounds on lipid peroxidation and free radical scavenging activity. Some structural aspects. *Journal of Pharmacy and Pharmacology*, **43:** 486-491.

Roshan Adhikari, Naveen KumarH. N.,Shruthi S. D. 2012. A Review on Medicinal Importance of Basella alba L. *International Journal of Pharmaceutical Sciences and Drug Research* **4(2):**110-114.

Sanchez-Moreno, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Science and Technology International*, **8:** 121-137.

Waris, G., Ahsan, H., Alam, K. 2000. Evidence for the possible involvement of $O_2^{\cdot -}$ modified-DNA in the etiopathogenesis of systemic *Lupus erythematosus*. The *Journal of Biochemistry,Molecular Biology and Biophysics*, **4:** 9-16.

Zhou, K., Yu, L. 2004. Effects of extraction solvent on wheat antioxidant activity estimation. *Lebensmittel-Wissenschaft and Technologie*, **37:** 717-721.