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ORIGINAL ARTICLE **OPEN ACCESS**

A New Approach of Plant Biotechnology for Prevention and Treatment of Diabetes

Aparna J Joshi[1], Renuka K. Ghodke[2], Mahadev A. Jadhav[3]
[1, 2, 3] Department of Biotechnology, Deogiri College, Station Road, Aurangabad, 431005
Corresponding Author: Email- joshiaparna74@yahoo.in
Contact No.-9579095718

ABSTRACT

According to definition given by World Health Organization (WHO) Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism that result from defects in insulin secretion, insulin action, or both. The treatment of diabetes is complicated due to unavailability of safe and efficient drugs. Currently the diabetic patients are treated with various oral antidiabetic drugs and insulin. In developing countries, these drugs are either not easily available or expensive. Apart from these drawbacks, prolonged insulin treatment may not prove to be effective against the various complications. An alternative approach of herbal drugs can be a relatively new, ecofriendly, readily available and an economic strategy to overcome the complications and problems of conventional diabetes treatment. In this mode of treatment, fresh aqueous extracts of different plant parts mostly included in regular Indian diet are employed singly or in combination. In present work fresh aqueous extract of fruits of Abelmoschus esculentus (Bhindi) is estimated for its antidiabetic activity. When different assays such as alfa amylase inhibition, glucose uptake activity using yeast cells as well as hemoglobin glycosylation were used for estimating its antidiabetic activity promising results were obtained. It was also showing antioxidant activity that was confirmed by free radical scavenging assay.

Keywords: *Abelmoschus esculentus, Antidiabetic, alfa amylase inhibition, glucose uptake activity, hemoglobin glycosylation*
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INTRODUCTION

In the *Malvaceae* family, ***Abelmoschus esculentus*** is the only significant vegetable crop that is very popular in the IndoPak subcontinent. In India, it ranks number one in its consumption but its original home is Ethiopia and Sudan, the northeastern African countries. It is one of the oldest cultivated crops.
Biological name: *Hibiscus esculentus, Abelmoschusesculentus*.
The high fiber content of Okra "helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract". Significant features of Okra pods are mucilaginous nature, low calories content and rich in nutrition and a good source of edible fiber. It is a good vegetable for weak, exhausted, and people suffering from depression. It is used in ulcers, lung inflammation, sore throat as well as irritable bowel.It has been proved from the studies that the okra pod contains important bioactive compounds such as carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid and amino acids [6]. Okra is also found to be useful for minimizing blood sugar levels within the body simply by slowing down sugar assimilation through the intestines. The frequent usage of okra might help avoid kidney disease. Okra polysaccharide possesses anti complementary and hypoglycemic activity in normal mice [1].
It also lowers cholesterol level in blood and may prevent cancer by its ability to bind bile acids. Additionally, Okra seed possess blood glucose normalization and lipid profiles lowering action in diabetic condition
Two kinds of α-amylase are produced by mammals, salivary α-amylase from the parotid gland and pancreatic α-amylase from the pancreas. The salivary α-amylase in the mouth initiates digestion of food in mouth while the low pH of the stomach stops it.When the food passes from the stomach into the small intestine it is neutralized and the digestion of starch is completed by α-amylase secreted into the small intestine from the pancreas.
Thus, any drug or chemical that inhibits these enzymes will delay or prolonged the time period of carbohydrate digestion. While this will not decrease the actual amount of glucose formed, it does reduce the rate of glucose formation and subsequently slows the rate of absorption leading ultimately to a decrease in the maximum post-prandial plasma glucose.

In this study, fresh fruits of Okra used to prepare aqueous extract which was further used for antidiabetic activity profiling of Okra by different assays.

MATERIAL AND METHODS

I. Preparation of Aqueous Plant Extract

5g. Okra fruits were weighed, cut vertically and imbibed overnight in phosphate buffer(40mM containing 0.006M NaCl) then squeezed and filtered by using muslin cloth.The fresh filtrate collected, was used for further work.

2.Estimation of Antidiabetic Activity of Aqueous Plant Extract by

A. α amylase inhibition assay Using DNSA reagent

The α amylase inhibition assay was performed using Chromogenic DNSA method. The total assay mixture composed of 0.02M sodium phosphate Buffer (pH 6.9 containing 6mM sodium chloride), alpha amylase and fresh aqueous okra fruit extract at concentration 0.3-1.5 mg/ml(w/v)were incubated at 37ºC for 10 minutes. After pre-incubation, 1%(w/v)starchsolution was added to each tube and incubated at 37ºC for 15 min.The reaction was terminated by adding DNSA reagent and then placed in boiling water bath for 5 min,cooled to room temperature and the absorbance were measured at 540nm.The control without plant extract represent 100%enzyme activity. The absorbance produced by plant extract was eliminated,appropriate extract controls with the extract in the reaction mixture except for the enzyme were also included. The %of alpha amylase inhibition was calculated as follows;

% Relative Enzyme activity=(enzyme activity of test/enzyme activity of control)*100

% Inhibition in the amylase activity=(100-Relative Enzyme activity) [4].

B. Estimation of glucose uptake activity of aqueous plant extracts using yeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3000× g, 5 min) until supernatant fluids were obtained and10%(v/v) of the suspension was prepared in distilled water. Various concentration of aqueous plant extract (50-2000µg/ml) were added to 1ml of glucose solution (5,10,25mM) and incubated together for 10 min at 37ºC. Reaction was started by adding 100µL of yeast suspension followed by vertexing and further incubation at 37ºC for 60 min, the tubes were centrifuged(2500×g,5min) supernatant collected and terminated reaction by using DNSA reagent. the absorbance was measured at 54nm.amount of glucose was estimated in the supernatant. Metronidazole was used as standard drug [5,9].

The percentage increase in glucose uptake by yeast cells was calculated using following formula:

$$\text{Increases glucose uptake}(\%)\frac{\text{Absorbance of sample} - \text{Absorbance of control}}{\text{Absorbance of sample}} \times 100$$

C. Evaluation of hemoglobin glycosylation

1. Preparation of hemoglobin

The blood was collected from a healthy human volunteer and transferred into a blood bottle containing an anticoagulant. As per the principle of cell lysis in hypotonic solution, here hemolysate was prepared. 0.14MNacl solution was used for three times washing of red blood cells collected. Then lysis of one volume of red blood cells suspension was carried with two volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volume of CCl4. The hemolysate was then freed from debris by centrifugation at 2300rpm for 15 min at room temperature. The hemoglobin rich fraction the upper layer was separated and dispensed into sample bottle for storage and refrigerated until required for use

2. Effect of Extract on Hemoglobin Glycosylation

To 1ml of hemoglobin solution,5µl of Gentamycin and 25 µl of the plant extract (30µg/mL) were added.The reaction was started by the addition of 1ml of2% glucose in 0.01Mphosphate buffer(pH7.4)and incubated in the dark at room temperature. The concentration of glycatedhemoglobin at the incubation period of 0,24, 48and 72hours were estimated spectrophotometrically at 443nm [5,9].

3. To study antioxidant activity of different aqueous plant extract under study

The antioxidant activity of the aqueous plant extract was determined in terms of its ability to scavenge hydrogen peroxide. A solution of hydrogen peroxide (40mM)was prepared in phosphate buffer(pH7.4)extract (100µg/mL)in distilled water were added hydrogen peroxide solution (0.6mL,40mM).Absorbance of hydrogen peroxide at 230nm was determined 10 min. later using a blank solution containing the phosphate buffer without hydrogen peroxide. Ascorbic acid used as standard. The percentage of hydrogen peroxide scavenging of both extract and standard compounds were calculated.

% Of scavenged [H_2O_2] =[(Ac-As)/Ac] ×100

Ac –Absorbance of the control and As- Absorbance in the presence of sample [7,8].

RESULTS AND DISCUSSION:

I. Preparation of Aqueous Plant Extract:

All the respective aqueous extracts under study were freshly prepared and used for further study.

2.Estimation of Antidiabetic Activity of Aqueous Plant Extract by

A. α amylase inhibition assay Using DNSA reagent

a-Amylase (EC 3.2.1.1) randomly cleaves the a-(1,4) glycosidic linkages of amylose to yield dextrin, maltose, or malt triose. Incubation of the a-amylase with the substrate leads to the generation of maltose; however, the addition of the respective plant extract significantly inhibited the liberation of maltose in a dose-dependent manner, compared with acarbose. α amylase inhibition assay was performed using DNSA reagentWhen *Abelmoschus esculentus*(okra) aqueous fruit extract was tested for their antidiabetic activity it was showing significant percent inhibition of α amylase 86% inhibition with 600(µl/ml)dilution, compared with standard drug Acarbose.

B.Estimation of glucose binding activity of aqueous plant extracts using yeast cells

If glucose level in the blood of the diabetic patient is regulated then it can prevent the various complications associated with the disease. With the variety of dietary conditions for long term, the plasma glucose concentration is one of the most important and closely regulated processes observed in the mammalian species. The i*n vitro* assays of the present study indicated that all the five fresh aqueous extracts possess good anti diabetic activity. In Yeast (*Saccharomyces cerevisiae)* glucose transport occurs by the mechanism of facilitated diffusion.

After the treatment of the yeast cells with the respective fresh aqueous plant extracts, the glucose uptake was found to increase in a dose dependent manner. From the observation tables and graphs it is clear that the percent increase in glucose uptake by the yeast cell at different glucose concentrations, 25mM, 10mM and 5mM vary respectively with the sample and its dilution. *Abelmoschus esculentus*(okra) aqueous fruit extract was shoeing efficiency in increasing the glucose uptake by yeast cells.

C.Evaluation of hemoglobin glycosylation

Increased concentration of glucose in the blood leads to its binding to hemoglobin which may result in the formation of the reactive oxygen species. Glycosylation end products are inhibited by plant extracts. With the increasing concentration of the glucose (2mg, 10mg and 20mg) over a period of 72hrs. an increase in the glycosylation was observed on incubation of hemoglobin however, the plant extracts significantly inhibited the hemoglobin glycosylation which is indicated by the presence of increasing concentration of hemoglobin. The plant extract also displayed the inhibition of hemoglobin glycosylation at different physiological concentration of the glucose over the period 72hrs,indicating that the plant extract decreases the formation of the glucose-hemoglobin complex and thus amount of free hemoglobin increases.

3.To study antioxidant activity of different aqueous plant extract under study

The ability of the extracts to scavenge hydrogen peroxide was determined to study antioxidant activity.The percentage of hydrogen peroxide scavenging of both extract and standard compounds were calculated.The aqueous fruit extract of *Abelmoschus esculentus*(okra) was showing 69.15% H_2O_2 scavenging.

Table1.α amylase inhibition assay using DNSA assay method

Dilution (µl/mL)	*Abelmoschus esculentus*(okra) aqueous fruit extract			Standard (Acarbose)		
	Enzyme Activity	Relative Enzyme Activity	% Inhibition	Enzyme Activity	Relative Enzyme Activity	% Inhibition
300	7.92	21%	79%	28.6	76%	24%
600	5.28	14%	**86%**	21.12	53%	44%
900	10.56	28%	72%	14.08	37%	**63%**
1200	6.6	17%	83%	27.72	73%	27%
1500	7.92	21%	79%	25.08	66%	34%

Table2. Estimation of glucose binding activity of aqueous plant extracts using yeast cells

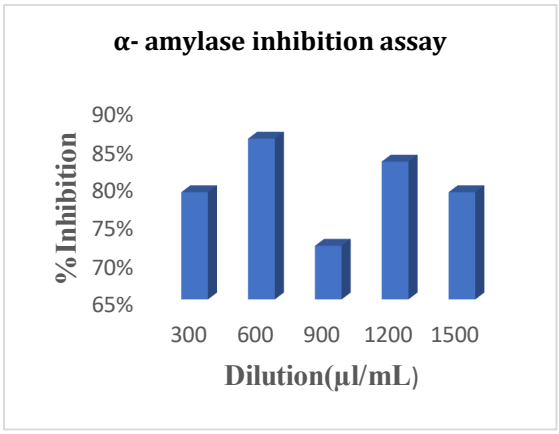
Dilutions (µL)	5mM		10Mm		25mM	
	Amt. of Glucose	%	Amt. of Glucose	%	Amt. of Glucose	%
Control	0.81	00	0.59	00	1.27	00
100	0.79	20%	0.56	5%	1.06	16%
500	0.38	**110%**	0.46	28%	1.11	11%
1000	0.46	76%	0.30	**96%**	0.86	**47%**
1500	0.53	52%	0.56	5%	1.12	13%
2000	0.52	55%	0.55	7%	1.59	20%

Table 3. Effect of Extract on Hemoglobin Glycosylation

Incubation Time period (Hrs.)	Control Gentamycin	*Abelmoschus Esculentus*
0hrs	0.9852	0.9896
24hrs	0.9568	0.7645
48hrs	2.1334	1.9119
72hrs	2.2866	2.1830

Table 4.Antioxidant activity of different aqueous plant extract under study

TIME	Blank	*Abelmoschus esculentus*
0 minutes (WithoutH_2O_2)	0.0744	0.0333
10 minutes	0.0778	0.0240
%H_2O_2scavanging	---	69.15%



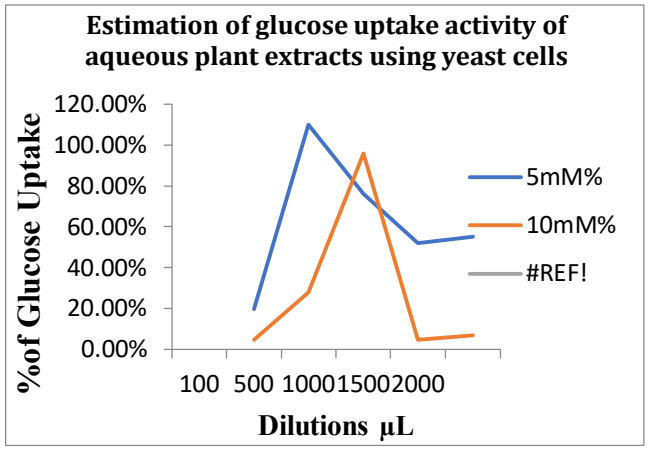


Figure 1. α amylase inhibition assay, using DNSA reagent

Figure 2. Estimation of glucose uptake activity of aqueous plant extracts using yeast cells

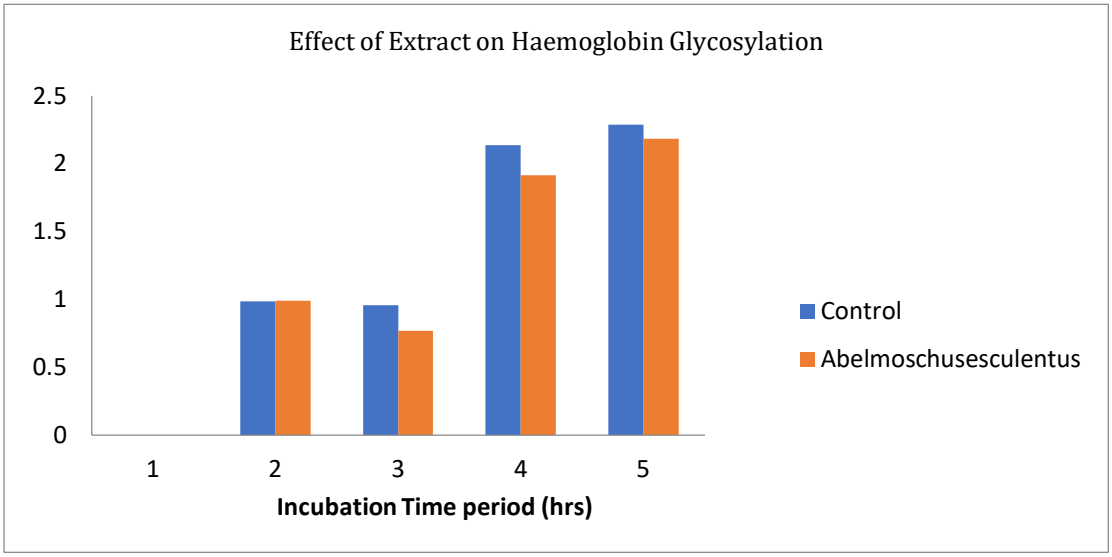


Figure3. Effect of Extract on Hemoglobin Glycosylation

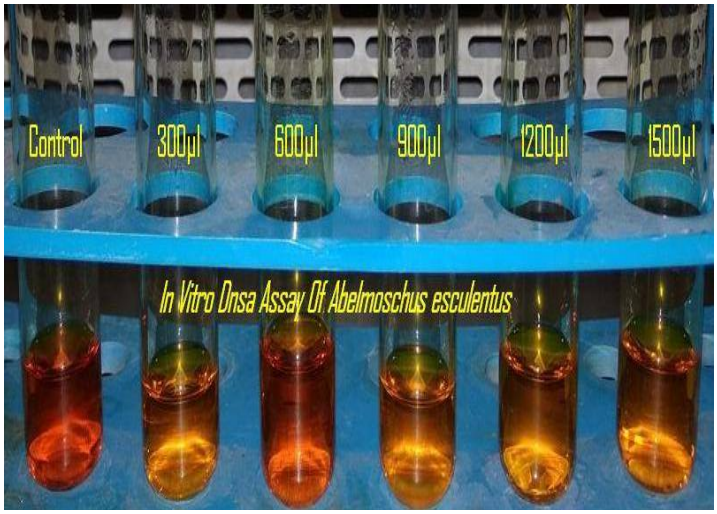
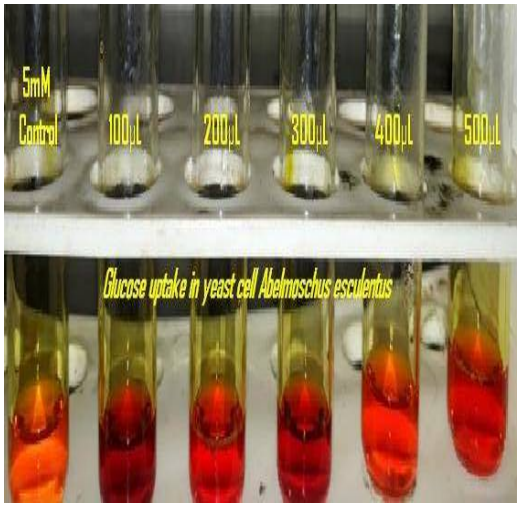


Figure 1.α amylase inhibition assay, using DNSA reagent



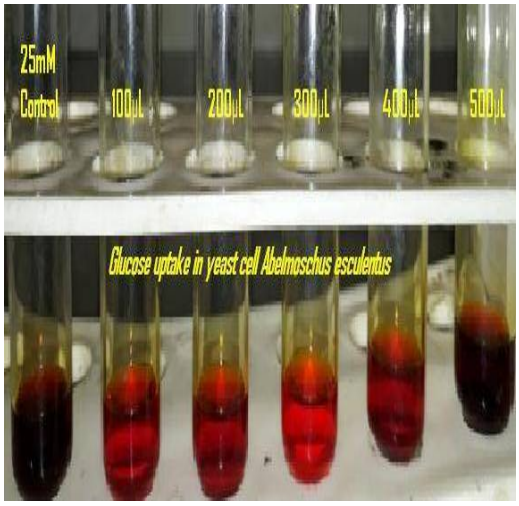


Fig 2 **Fig 3**

Figure: 2 Estimation of glucose uptake activity yeast cells

Figure :3 Estimation of glucose uptake activity of aqueous plant extracts using activity of aqueous plant extracts using yeast cells

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CONTRIBUTION OF AUTHORS

Ms. Aparna J. Joshi help to design research plan, supervision and guidance for result interpretation. Ms. Renuka K. Ghodke, has taken endless efforts for conducting practical work and significant contribution in experimental work. Mr. M. A. Jadhavhas provided consistent support, permission to conduct all experimental work in the laboratory of Department of Biotechnology and encouragement to us. Manuscript writing was completed by correspondent authorand significant contribution and their special interest in the appropriate presentation of the data and preparation of manuscript.

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