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Alpha-Amylase and Alpha-Glucosidase Inhibitory Potential of Overnight Soaked Aqueous Extract of *Abelmoschus esculentus*

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Abstract

Phytochemicals are secondary metabolites which act as antioxidants and possess various protective role.Alpha Amylase and Alpha Glucosidase enzymes catalyse hydrolysis of starch to simple sugars. Abelmoschus esculentus is a flowering plant of the Malvaceae family and is valued for its green seed pods. It naturally possesses anti-diabetic property, acts as a starch blocker as it prevents the absorption of starch in the body. Abelmoschus esculentus was cut vertically to open and added to 50ml of water. Soaked overnight and the aqueous extract was decanted and used for the phytochemical screening test followed by the evaluation of antioxidant and antidiabetic potential. The data were analysed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Overnight soaked extract of Abelmoschus esculentus exhibited a significant antioxidant potential (IC50=280 µg/ml) and compared with standard vitamin C. Alpha amylase and alpha glucosidase inhibitory potential of Abelmoschus esculentus was analysed (IC50=380µg/ml and 320µg/ml respectively) and compared with standard acarbose. Though Abelmoschus esculentus is a common vegetable, its efficiency increases when soaking overnight.

Keywords: *Abelmoschus Esculentus, Active Compounds, Alpha Amylase, Alpha Glucosidase, Innovative Technology, Interactions Assessment, Novel Method.*

Introduction

Abelmoschus esculentus is used in medicine for the treatment of diabetes mellitus. The monech seeds of *Abelmoschus esculentus* have potential in the development of a drug for diabetes due to their anti-diabetic activity [1]. Besides extensive research efforts, occurrence of diabetes is still increasing at a phenomenal rate with over 346 million people affected all over the world and the number is expected to increase to 544 million in 2030 [2]. Glycosidases catalyzing the hydrolysis of glycosidic bonds in polysaccharides and glycoconjugates play major role in numerous biological processes together with carbohydrate digestion ,lysosomal catabolism of glycoconjugates and post - transitional changes as cellular glycoproteins [3]. Alpha-amylase is a pre-eminent enzyme found in the pancreatic juice and saliva in which it breaks down large insoluble starch molecules into absorbable molecules. On the other hand, mammalian glucosidase in the mucosal brush border of the small intestine catalyses the end step of digestion of starch and disaccharides that are abundant in the human diet. Inhibitors of alpha-amylase and alpha-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion. [4].

Abelmoschus esculentus is the only crop which has more significance in the Malvaceae family. It is sensitive to feast . It is rich in vitamins and calcium . Its mature fruit and stems are used as crude fibre in the paper industry [5]. *Abelmoschus esculentus* works as an

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antidiabetic with the least side effects. If it is taken in large amounts it worsens the kidney gall bladder stone formation due to presence of oxalate crystals [6].

Diabetes mellitus is a most common metabolic disorder of glucose metabolism. The management of blood glucose level is necessary to treat this disease . This may be accomplished by the use of hypoglycemic drugs such as biguanides insulin secretagogues and Alpha - glucosidase inhibitors [7].

Okra is known for its good palatability among different places and its culinary uses are wide. Its immature fresh green seed pods are eaten as vegetables while the extract obtained from the fruit is used in various recipes including thicken stews, soups and sauces to improve their consistency [8],[9],[10]. In folk medicine, okra is regularly used to nurse gastritis. Pharmacological studies have spotlighted its antioxidant, antidiabetic, antihyperlipidemic, and anti-fatigue activities. Okra pods have formerly been shown to have high contents of polysaccharides, phenols, and flavonoids. The latter two possess strong antioxidant effects and emanate from the okra seeds while its skin extract hardly displayed such reactions, [11], [12], [13], [14]. The genus Abelmoschus has been used for several ethnomedicinal practices and has diverse pharmacological activities and possess several phyto- chemical and nutritional properties as well as having no adverse effect on living cells. Their pods, seeds and leaves are used as food, in pharmaceutical industries and as traditional remedies all over the world [14], [15]. The aim of the study is to assess Alpha - amylase and Alpha - glucosidase inhibitory potential of overnight soaked extract of *Abelmoschus esculentus.*

Materials And Methods

Chemicals

All chemicals and reagents used for this research work were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA

Collection of Plant Material

Abelmoschus esculentus fruits were collected from Chennai District, Tamil Nadu, India. The species were identified and authenticated at the Department of Centre for Advanced Study in Botany, University of Madras, Chennai, India. The fruit part of the plant was cut vertically open and soaked overnight. The aqueous extract was decanted and was used for extraction.

Preparation of Plant Extracts

50gms of *Abelmoschus esculentus* was cut vertically open and added to 50ml of water. Soaked overnight and the aqueous extract was decanted and used for further analysis.

Phytochemical Screening Test

The phytochemical screening was assessed by the method of [16] .

Test For Phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

Test for Alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test For Proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of Saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for Steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

Antioxidant Activity

Dpph Free Radical Scavenging Activity

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of [17]. DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%).

Alpha Amylase Inhibitory Activity of Overnight Soaked Aqueous Extract of Abelmoschus Esculentus

Alpha amylase inhibitory activity of extract was carried out according to the standard method by [18]. In a test tube a reaction mixture contains 500 mu/l phosphate buffer (100mM ; pH=6.8). 100 mu alpha amylase (2 mu/l) and varying concentration of extract (0.1 - 0.5 mg/ml) was Incubated at 37degree Celsius for 20 minutes. Then the 200 mu/l of 1% soluble starch(100 MM phosphate buffer 6.8) was added as a substrate and incubated further at 37 degree Celsius for 30 minutes, 1000 mu/l of the 3,5 Dinitrosalicylic Acid[DNS], DNS colour reagent was then added and boiled for 10 minutes. The absorbance of the resulting mixture was measured at 540 nm using a multi plate reader. Acarbose at various concentrations (0.1-0.5 mg/ml) was used as a standard.

Alpha Glucosidase Inhibitory Activity Overnight Soaked Aqueous Extract of Abelmoschus Esculentus

Alpha glucosidase inhibitory activity of extract was carried out according to the method of [18]. Reaction mixture containing 500 mu/l phosphate buffer(100mM pH 6.8), 100mu/l glucosidase (10 ml) and varying concentration of extract (0.1 to 0.5 mg /ml) was pre incubated at 37 degree Celsius for 15 minutes. Then 200 mu/l p-NPG(5mM) was added as a substrate and incubated further at 37degree Celsius for 30 minutes. The reaction was stopped by adding 50 mu/l sodium carbonate (0.1M). The absorbance of the released p- nitrophenol was measured at 405 nm using multiple readers. Acarbose at various concentrations (0.1-0.5mg/ml)was used as a standard.

Statistical Analysis

The data were subjected to statistical analysis using Two-way analysis of variance (ANOVA) and Tukey's multiple range test to assess the significance of individual variations between

the groups. In Tukey's test, significance was considered at the level of $p<0.05$

Results

Phytochemical Analysis

From the study, it was evident that the overnight soaked aqueous extract of *Abelmoschus esculentus* was found to be rich in phytochemicals such as Alkaloids, flavonoids, terpenoids, saponins and steroids (Table 1). The presence of these phytochemicals helps the extract to act as a good antioxidant.

Table 1. Phytochemical Analysis of Overnight Soaked Aqueous Extract of Abelmoschus Esculentus

SNO	PHYTOCHEMICALS	*Abelmoschus esculentus*
1	Alkaloids	+ +
2	Flavonoids	+ +
3	Terpenoids	+ +
4	Carbohydrates	+ +
5	Saponins	+ + +
6	Phenols	-
7	Proteins	-
8	Tannin	+
9	Steroids	+

Antioxidant Analysis

Antioxidant analysis of the overnight soaked aqueous extract of *Abelmoschus esculentus* was analysed and compared with the standard vitamin- C. Ic 50 of the extract was found to be 280µ g/ ml (Figure 1). Antioxidant potential of the extract increased in a dose dependent manner as compared to the standard (Vitamin C).

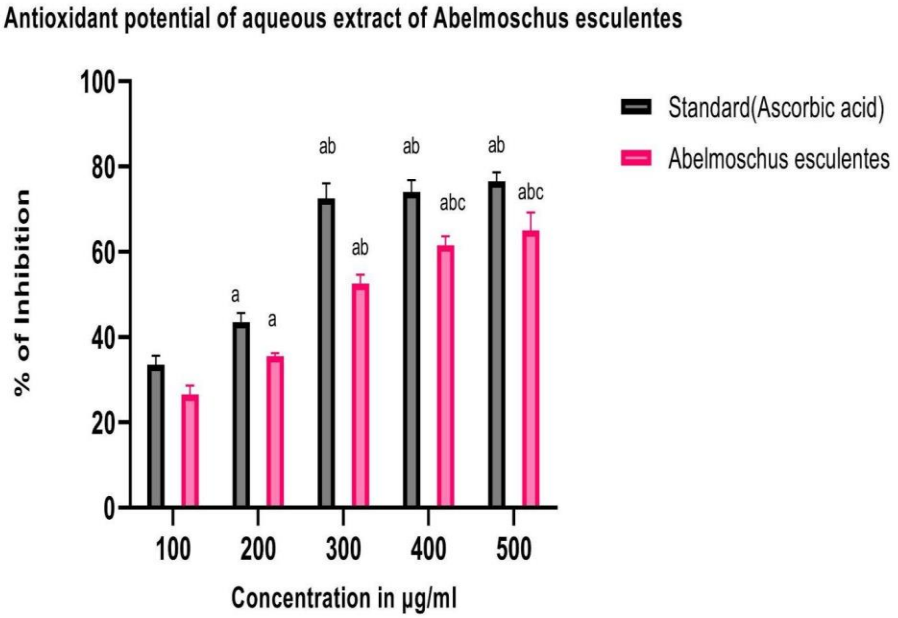

Figure 1. Antioxidant Potential of Overnight Soaked Extract of Abelmoschus Esculentus Compared with the Standard (Vitamin C)- Dpph Assay

In vitro Antidiabetic Activity

Antidiabetic potential of the extract was analysed by estimating the extract's α- Amylase and α- Glucosidase inhibitory potential and compared with the standard Acarbose. The enzymes amylase and glucosidase act on starch and release free glucose molecules. If the extract has significant inhibition of these enzymes it is proportional to its antidiabetic potential.The extract exhibited a significant α- Amylase (Figure 2)and α- Glucosidase inhibitory potential (Figure 3) with an IC_{50} of 380 and 320µg/ ml respectively. Antidiabetic potential of the extract increased in a dose dependent manner as compared to the standard- Acarbose.

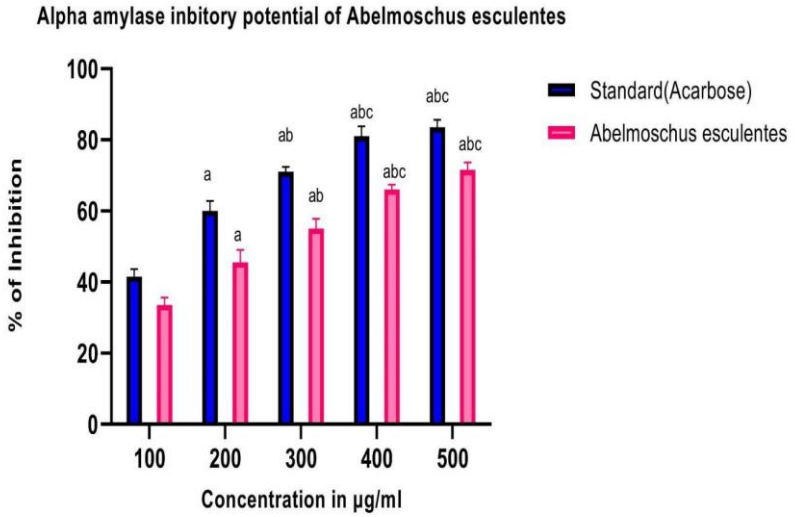

Figure 2. Alpha Amylase Inhibitory Potential of Abelmoschus Esculentus Compared with the Standard- Acarbose

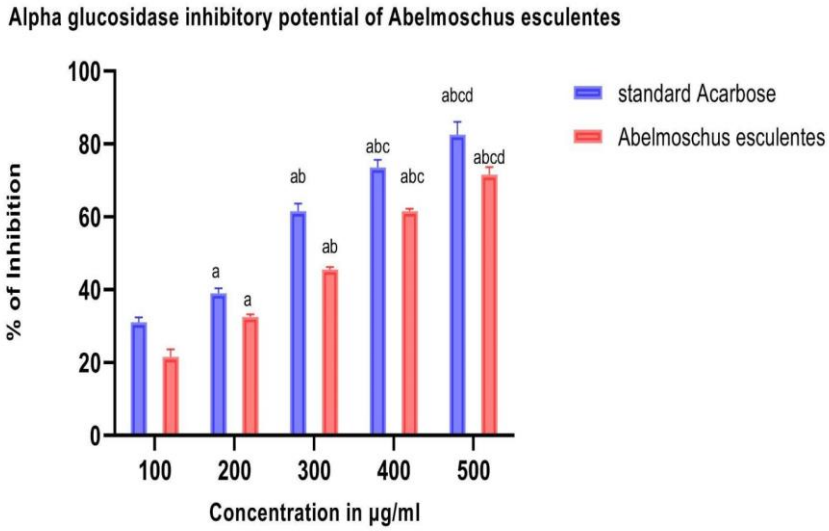

Figure 3. Alpha Glucosidase Inhibitory Potential of Abelmoschus Esculentus Extract Compared with the Standard- Acarbose

Discussion

Phytochemicals present in *Abelmoschus esculentus* were found to be alkaloids, flavonoids, terpenoids, carbohydrates, saponins, tannin, steroids.Phytochemicals are secondary metabolites which are present only in plants and are responsible for the medicinal value of plant extract. The aqueous extract due to the rich phytochemicals exhibited a

significant antioxidant potential. Presence of phenolic phytonutrients like flavonoids , alkaloids indicate that the extract can be a potential antioxidant and can also be tested for other medicinal properties. In the previous research done [19], it was analysed that the antioxidant potential of the extract was proportionately equivalent to the total flavonoid content of the extract.

The effect of antioxidants on DPPH free radical scavenging was considered to be due to hydrogen donating ability of the plant's phytonutrients. The result shows the significant antioxidant ability. A similar research [20], was done in which the antioxidant potential in tomato leaves increased in all varieties as temperature decreased. Anti-diabetic analysis of *Abelmoschus esculentus* was analyzed and compared with the standard acarbose. Acarbose is a standard drug used as a starch blocker since it prevents the absorption of starch into the body mainly by blocking the alpha 1-4 glycosidic linkage. The inhibitory activity would delay the degradation of carbohydrates which would in turn cause a decrease in glucose absorption as result a reduction in blood glucose level. In a similar study [21], antidiabetic potential of traditionally used medicinal plants were analysed by in vitro alpha amylase and glucosidase inhibitory potential.

Oxidative stress acts as a key factor in the etiology and development of several diseases such as atherosclerosis, liver cirrhosis, cardiovascular diseases, cancer and diabetes [12]. In diabetes, persistent hyperglycemia increases the generation of free radicals, can initiate lipid peroxidation, which results in the stimulation of non-enzymatic glycation of protein, alterations in structure and function of basement membrane and collagen and enzyme inactivation which collectively develops the complications of diabetes [22].

Diabetes is a metabolic disorder and has become a most prevalent disorder worldwide.Though many synthetic drugs help in the reduction of blood glucose, prolonged use of the same results in various side effects[23].A critical defect in type 2 diabetes is the impaired insulin signaling leading to defective glucose transport and metabolism in muscles and adipocytes thereby resulting in insulin resistance. Moreover there is just management which is available now, not a cure. Since plant extracts are rich in phytonutrients it can be a potential drug for various ailments [24]. More research is needed to study traditional use of the plant and for subsequent validation of activity and the mechanism of action.Therefore, the current study on *Abelmoschus esculentus* needs more attention by the scientific community, for the welfare of the society. Diverse groups of secondary metabolites exist in plants and the current technologies in analytical chemistry as well as biochemistry provide opportunities to develop efficient high throughput screening methods for these compounds [25]. A critical defect in type 2 diabetes is the impaired insulin signaling leading to defective glucose transport and metabolism in muscles and adipocytes thereby resulting in insulin resistance[26]. Naturally occurring compounds have been proposed to exert beneficial effects on health and have drawn attention because of their safety [27,28,29]. Thus in future, phytonutrients can be isolated, purified and studied for its increased pharmacokinetic potential.

Conclusion

Abelmoschus esculentus is a common vegetable, cost effective and easily available.From the study it was evident that the extract becomes rich in various phytonutrients after soaking overnight. Traditionally as a folk medicine overnight soaked aqueous extract is taken as a relief for diabetes, but research has not been done on this. From the current study it was found that the extract is a source of medicinally active compounds and its diverse pharmacological effects need to be analysed. Further in vivo study is needed to prove the

effective antidiabetic potential of overnight soaked aqueous extract of *Abelmoschus esculentus.*

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Conflict of Interest

The authors hereby declare that there is no conflict of interest in this study.

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