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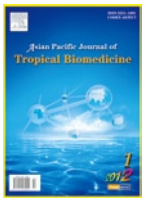

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In vitro α-glucosidase and α-amylase enzyme inhibitory effects in aqueous extracts of *Abelmoscus esculentus* (L.) Moench

V. Sabitha[1], K. Panneerselvam[1], S.Ramachandran[2*]

[1] *Department of Bioinformatics, Karunya University, Coimbatore-641 114 , Tamil Nadu, India*
[2] *Department of Pharmacology, KMCH College of Pharmacy, Coimbatore-641 048, Tamil Nadu, India*

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ABSTRACT

Objective: To provide *in vitro* evidence for antidiabetic activity through potential inhibition of α-glucosidase and α-amylase enzymes using the aqueous extracts of *Abelmoschus esculentus* (L.) Moench. (*A. esculentus*) peel (AAPP) and seed (AASP). **Methods:** The peel and seed of okra was dried under shade and powdered. The powdered peel and seed were used for the preparing the aqueous extract. The aqueous extract (AAPP and AASP) were used for the *in vitro* study. **Results:** The AAPP and AASP showed appreciable α-glucosidase [IC_{50} = (142.69 ± 0.32) μg/mL and (150.47 ± 0.28) μg/mL] and α-amylase [(IC_{50} = (132.63 ± 0.16) μg/mL and (147.23 ± 0.21) μg/mL] inhibitory effect in a concentration-dependent manner. Thus the present study confirms the hypoglycemic effect in the AAPP and AASP aqueous extracts of *A. esculentus*. **Conclusions:** The present study results, gives a clear evidence that *A. esculentus* has antidiabetic activity.

1. Introduction

Diabetes mellitus, a leading non-communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world [1]. It is a metabolic disorder affecting carbohydrate, fat and protein metabolism. A worldwide survey reported that diabetes mellitus is affecting nearly 10% of the population every year [2]. Diabetics are prone to many complications due to the nature of the disease. Long-standing diabetes can lead to heart, kidney and circulation problems, including stroke. In traditional world, nutrition and health care have connectivity for which many plants are consumed as food in order to benefit health[3]. The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables are regarded worldwide as an important area of the nutritional and phytotherapic research [4]. Motivation of people towards herbal medicines is increasing to avoid side effects of drugs prepared from synthetic materials. Medicinal plants are considered to be an important source of antidiabetic compounds and the therapeutic benefit of many medicinal plants is often attributed to their hypoglycemic activity. The present study was undertaken to investigate *invitro* assay of okra seed and peel aqueous extracts.

Abelmoschus esculentus (L.) Moench. (*A. esculentus*), Synonym of okra, known in many English-speaking countries as lady's fingers or gumbo is a flowering plant in the mallow family. It is one of the most important vegetables widely grown in Nigeria for its tender fruits and young leaves. It is distributed from Africa to Asia, Southern European and America [5]. In some regions, the leaves are also used for human consumption. Okra constitutes a combination of vitamins and mineral salts, including calcium; which are often lacking in diet of developing countries. The anti Physico chemical, hypolipidemic effective drugs [6] are a few earlier research work on *A. esculentus*. Okra is reported to have its hypolipidemic effect by decreasing absorption of cholesterol from diet[7]. It also possesses antidepressant activity[8]. The plant has a wide range of medicinal value and has been used to control of various diseases and disorders. Okra polysaccharide possesses anti-complementary and hypoglycemic activity in normal mice[8]. Okra's vitamin C is an antioxidant and anti-inflammatory, which curtail the development of asthma symptoms. *Invitro* studies of methanolic of Hibiscus esculentus seed was proven to act as an antioxidant

*Corresponding author: S.Ramachandran,Department of Pharmacology, KMCH College of Pharmacy, Coimbatore-641 048, Tamil Nadu, India.
Tel: +91-9626298693
E-mail: sabithav86@gmail.com

agent[9]. It is reported that the biological activity in this species are antioxidant potential [10]. A study from okra fruits has pointed to the effects of glycosylated compounds inhibiting the adhesion of Helibacter pylori to human gastric mucosa[11]. The *in vitro* study in okra diabetes basis has been reported relative to cholestyramine [12]. There is lacking scientific reported study on these plant properties based on antidiabetic activity despite its wide usage as medicinal plant. Present study aimed to investigate confirmatory test for antidiabetic activity through *in vitro* study by the inhibition of α-glucosidase and α-amylase, *A. esculentus* peel and seed aqueous extracts.

2. Materials and methods

2.1. Plant materials

Fresh okra pod was collected from the local farm in Coimbatore, Tamil Nadu, India. Botanical identification was carried out and was identified as *A. esculentus* (L.) Moench., authenticated by the Botanical Survey of India department in TNAU, Coimbatore. The authentication number of the plant is BSI/SC/5/23/2010-11/Tech.1907. The peel and seed were separated, cleaned, air dried under shade and powdered. The powdered material was stored in air-tight container. The dried powder was mixed in 1 liter of water, left undisturbed for an hour at room temperature. Boiled the solution for 30 minutes and filtered hot through muslin cloth. Filtrate was then dried in oven and stored in an airtight container. This was used as the aqueous extract for *in vitro* analysis.

2.2. Inhibition assay for α-glucosidase activity

α-Glucosidase (0.075 units) was premixed with the aqueous extract at various concentrations (50-250 μg/mL). 3 mM p-nitrophenyl glucopyranoside (pNPG) as a substrate was added to the reaction mixture to start the reaction [13]. The reaction was incubated at 37 ℃ for 30 min and stopped by adding 2 mL of Na_2CO_3. The α-glucosidase activity was determined by measuring the p-nitrophenol release from pNPG at 400 nm. The IC_{50} value was defined as the concentration of α-glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.

2.3. Inhibition assay α-amylase activity

α-Amylase was premixed with the aqueous extract at various concentrations (50-250 μg/mL) and starch as a substrate was added as a 0.5% starch solution to start the reaction. This was carried out at 37 ℃ for 5 min and terminated by addition of 2 mL of DNS (3,5-dinitrosalicylic acid) reagent. The reaction mixture was heated for 15 min at 100 ℃ and diluted with 10 mL of distilled water in an ice bath [13]. α-Amylase activity was determined by measuring spectrum at 540 nm. The IC_{50} value was defined as the concentration of α-amylase inhibitor to inhibit 50% of its activity under the assay conditions.

$$\% \text{ Inhibition} = \frac{\text{A540 Control} - \text{A540 Exp.}}{\text{A540 Control}} \times 100$$

3. Result

3.1. In vitro α-glucosidase inhibition study

The in vitro α-glucosidase inhibitory studies demonstrated that both AAPP and AASP had α-glucosidase inhibitory activity. The percentage inhibition at 50,100,150,200,250 μg/ml concentrations of AAPP showed a concentration-dependent reduction in percentage inhibition. Thus the highest concentration 250 μg/ml tests showed a maximum inhibition of nearly 88.7 %. The percentage inhibition varied from 88.7 - 30.8 % from the highest concentration to the lowest concentration of 50 μg /ml. AASP showed a inhibitory potential of 80.9% at the highest concentration of 250 μg /ml. AASP seems to be less potent in α-glucosidase inhibitory potential compared to AAPP. Table 1 represents the inhibitory activity of AAPP and AASD.

Table 1
α-glucosidase inhibition with IC_{50} value.

Sample	Concentration (μg)	% Inhibition	IC_{50}
AAPP	50	30.8 ± 0.6	142.69 ± 0.32
	100	42.0 ± 0.1	
	150	50.1 ± 0.4	
	200	63.8 ± 0.1	
	250	88.7 ± 0.2	
AASP	50	29.3 ± 0.2	150.47 ± 0.28
	100	39.2 ± 0.1	
	150	51.1 ± 0.5	
	200	62.1 ± 0.1	
	250	80.9 ± 0.4	

Table 2
α-amylase inhibition with IC_{50} value.

Sample	Concentration (μg)	% Inhibition	IC_{50}
AAPP	50	34.89 ± 0.1	132.63 ± 0.16
	100	47.73 ± 0.4	
	150	60.71 ± 0.1	
	200	71.58 ± 0.5	
	250	87.57 ± 0.3	
AASP	50	30.35 ± 0.3	147.23 ± 0.21
	100	41.48 ± 0.8	
	150	51.68 ± 0.6	
	200	66.33 ± 0.1	
	250	80.06 ± 0.2	

3.2. In vitro α-amylase inhibition study

The maximum inhibition of AAPP was 87.57% at a

concentration 250 μg/mL. The percentage inhibition ranged from 87.57% − 34.89 %. AASP produced a maximum inhibition of 80.06% at a concentration 250 μg/mL. At the lowest concentration 50 μg/mL, there was about 30.35% inhibition. AAPP showed a higher inhibitory potential than AASP. The IC_{50} values are tabulated. Table 2 represents the inhibitory activity of AAPP and AASD.

4. Discussion

Diabetes mellitus is a dreadful disorder and leads to various other metabolic disorder. It is estimated that its annual incidence rate will continue to increase in the future worldwide. Diabetes involves with the development of micro and macro vascular diabetic complications [14]. In human, glucose tolerance impairs prior to maturity-onset of hyperglycemia [15, 16] and is widely used as a clinical index to predict the potentiality of developing diabetes[17]. The objective of our study is to investigate the hypoglycemic effect in the aqueous extracts of okra seed and peel.

In the present study, *in vitro* α-glucosidase and α-amylase inhibitory studies demonstrated that both AAPP and AASP had inhibitory activity. The percentage inhibition at 50,100,150, 200, 250 μg/mL concentrations of AAPP and AASP on α-glucosidase and α-amylase showed a concentration-dependent reduction in percentage inhibition. The highest concentration of AAPP (250 μg/mL) tests showed a maximum inhibition of nearly 88.7% and 87.57 % of α-glucosidase and α-amylase respectively. The highest concentration of AASP (250 μg/ml) tests showed a maximum inhibition of nearly 80.97% and 80.06 % of α-glucosidase and α-amylase respectively. AASP seems to be less potent in α-glucosidase inhibitory potential compared to AAPP. Therefore, the antidiabetic effect of AAPP and AASD might attribute to its inhibitory effect against a-glucosidase that retarding the digestion of carbohydrate to delay the postprandial rise in blood glucose.

Our *in vitro* studies demonstrated an appreciable α-glucosidase and α-amylase inhibitory activity present in AAPP and AASP where further experiments can be performed on animal models to confirm the hypoglycemic activity. It is a safe and effective intervention for diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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