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PHYTOCHEMICAL SCREENING AND *IN VITRO* EVALUATION OF ANTIDIABETIC EFFICIENCY IN BIOACTIVE EXTRACTS OF *ABELMOSCHUS ESCULENTUS* AND *MOMORDICA CHARANTIA*

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Abstract

Diabetes mellitus (DM) is a severe, protracted, and complicated metabolic illness with various etiologies and life-altering short-term and long-term repercussions. People in both developed and developing countries are impacted by DM, which poses a serious socioeconomic problem. Pakistan has the third highest frequency of diabetes in the world after China and India. In response to the rising number of diabetic patients in Pakistan, the current study was designed to determine the in vitro antidiabetic efficiency of Abelmoschus esculentus and Momordica charantia, using inhibition of α-amylase and their phytochemical constituents. Extractions of these plants showed gradual increase in the α-amylase inhibition. The highest inhibition percentage of α-amylase obtained at highest concentration (5mg/ml) was 22% in A.esculentus and 26% in M. charantia. IC_{50} value obtained was 10.1mg/ml in A.esculentus and 10.5mg/ml in M. charantia. M. charantia has a slightly better inhibition percentage of α-amylase than. A. esculentus. These two must function as an affordable medication for DM with fewer side effects. Most of the tests performed to test phytochemicals in both plants came positive, indicating that phytochemicals may also have some relationship to antidiabetic action because of their capacity to scavenge free radicals.

Key words: *Alpha-amylase inhibition, Antidiabetic efficiency, Bioactive extracts, Bitter Gourd, Diabetes Mellitus, Okra, Phytochemicals Screening*

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolism of proteins, carbohydrates, lipids, water and electrolytes. It falls within the category of metabolic illnesses known as hyperglycemia, in which blood sugar levels rise either because of insufficient insulin production by the pancreas or when cells fail to respond to the insulin that is generated (1, 2). Diabetes is rising at an alarming rate, affecting nearly 10.5% of the global population. By 2045, it is anticipated that 783.2 million individuals will be diagnosed with diabetes (3, 4). Nearly 60% of the world's diabetic population lives in Asia, which is home to six of the top 10 nations with the highest prevalence of the disease. With the highest growth rate, Type 2 diabetes mellitus is more common in China and India. It also predominates in Pakistan approximately 11.77%. Pakistan is expected to have 33 million people with diabetes, making it the country with the third-largest population worldwide (5).

As the prevalence of diabetes mellitus has increased, the need for plant-based diabetes medications is rising globally. Practitioners of traditional medicine served as inspiration for the creation of around 75% of the herbal medicine. Due to their inherent strength and safety, medicinal plants are preferred for use in a variety of treatments such as, cosmetics, perfumes, food and beverage sector. Traditional medicinal plants include biologically active elements that result in a variety of medications with therapeutic benefits, and they continue to play a significant role in the quest for novel treatments. According to the World Health Organization (WHO) 80% of people worldwide, both in industrialized and developing nations, as well as in undeveloped nations, rely on traditional medical practices for their primary health care needs (6-8).

In traditional medicine *Abelmoschus esculentus*, commonly known as okra, reportedly has the capacity to control elevated blood glucose levels (9). The only vegetable crop of any economic importance in Malvaceae family is grown worldwide in tropical and subtropical climates and have become particularly popular in the Indo-Pak region (10). Similar to okra, *Momordica charantia*, which is additionally referred to as bitter melon, karela, balsam pear, or bitter gourd, is a popular vegetable that native populations in Asia, South America, India, the Caribbean, and East Africa frequently use to cure diabetes-related illnesses (11).

To check the antidiabetic potency scientifically one of the most significant in vitro methods is inhibition of α-amylase. α-amylases break down starch glycosidic bonds to produce glucose, maltose, maltotriose, and dextrin (12, 13). It assists in intestinal absorption and act as the primary digestive enzymes. α-amylase is a potential target for the creation of new diabetic treatments (14). The activity of these enzymes is also decreased when bioactive chemicals or phytochemicals are present (15). Therefore, the aim of this study is to screen different phytochemicals and identify the in vitro antidiabetic activity of okra (*Abelmoschus esculentus*) and bitter gourd (*Momordica charantia*) using inhibition of alpha-amylase.

MATERIALS AND METHODS

PLANT MATERIALS

In order to prepare the plant extract, 1 ½ kg of complete, fresh, green-colored, 2-3-inch-long okra (*A. esculentus*) pods of the same maturity as well as 1 ½ kg of fresh, complete, 5–6-inch-long, green-colored, and mature bitter gourds (*M. charantia*) were used.

EXTRACT PREPARATION

The vegetables were thoroughly cleaned and then given a distilled water rinse. Samples were kept until they were completely dry, at 45ºC in a hot air oven (Thermo Scientific-HERATHERM, Germany). Dried vegetables were finely powdered and then kept in airtight boxes to keep out ambient moisture.

PLANT EXTRACTION

Plant extracts were made as explained by (16) with slight modifications taking 30g of each sample powder (bitter gourd and okra) separately in flasks and dissolved in 250ml of 80% v/v ethanol. The mixture was placed in shaking incubator (Stuart SI500 (US)) for about 28 hours at 200 rpm and 24 hours without shaking overnight. Solutions were filtered separately using Whatmann filter paper 1 then ethanol was removed by using rotary evaporator (Sturat RE300-US). Both extracts were placed in the lyophilized freeze dryer (Laboqust-USA) to gently freeze the product. Freeze-dried powdered bitter gourd and okra were kept cool at 4 °C in refrigerator.

ANTI-DIABETIC ACTIVITY OF BITTER GOURD AND OKRA IN INHIBITION OF α-AMYLASE

Anti-diabetic activity of bitter gourd and okra in inhibition of α -amylase is determined by following with slight modification (16). Test tubes containing 250 µl of extracts of bitter gourd and okra (1.25–10 mg/ml) were then filled with a same volume of sodium phosphate buffer that contained an α -amylase (*Aspergillus oryzae*) (BDH Lab, England) solution at a concentration of 0.5 mg/ml. At 25 °C, these solutions were incubated for 10 min. A 0.02 M sodium phosphate buffer with a pH of 6.9 and 250 µl of a starch (1%) solution was added at specified intervals. The mixes were then incubated for a further 10 minutes at 25 °C. 500 µl of the di-nitro-salicylic acid (DNS) reagent was added to halt the reaction. The tubes were then cooled to room temperature after being incubated at boiling temperatures in a hot water bath (Thermo SCIENTIFIC PRECISION SWB 27). The reaction mixtures were then mixed with 5 ml of distilled water, and the absorbance was measured at 540 nm with a T80+UV/VIS Spectrometer (British company, pg instruments). By substituting distilled water for the extract throughout the same technique, a control was created. Using the provided equation, the α-amylase activity was determined.

$$\text{Amylase inhibitory activity} = \frac{((Ac+) - (Ac-) - (As- Ab))}{(Ac+)- (Ac-)} \times 100$$

Where; Ac+ = the absorbance of 100% enzyme activity (only solvent with enzyme)

Ac- = the absorbance 0% enzyme activity (only solvent without enzyme)

As = A test sample (with enzyme) and Ab = A blank (a test sample without enzyme)

PRELIMINARY PHYTOCHEMICALS SCREENING

Analysis of phytochemicals was done using the procedures recommended by (15) (17) and (18). The phytochemicals that were examined included alkaloids, amino acids, carbohydrates, cardiac glycoside, flavonoids, phenols, proteins, saponins, steroids, tannins and terpenoids. When certain detecting reagents were added to the test solutions of bitter gourd and okra, changes in color and/or the development of precipitate were seen. These observations were used to record the results as present (+) or absence (-). For confirmation, each qualitative phytochemical test was duplicated three times.

TEST FOR ALKALOIDS

A total of 0.5g of powdered bitter gourd and okra were added to separate test tubes. Chloroform was added to each test tube first, followed by an ammonia solution, and mixed well. Combinations were filtered and the chloroform was eliminated by evaporating over a water bath (Thermo Scientific Precision SWB 27). In the last stage, each test tube was combined with 2 ml of Mayer's reagent; the formation of a cream-colored precipitate indicated the presence of alkaloids.

TEST FOR AMINO ACIDS

Plant extracts (okra) and (bitter gourd) were dissolved in different test tubes then few drops of phenolphthalein were mixed, and dilute solution of sodium hydroxide was added drop-wise until the appearance of the color pink which suggested the existence of free amino acids.

TEST FOR ANTHRAQUINONES

Before filtering, 6g of dried plant powder was dissolved in 10 ml of benzene for 10 minutes. The filtrate was filled with 10 ml of a 10% ammonia solution and vigorously shaken for 30 seconds. The presence of anthraquinones was detected by the formation of pink, violet, or red hue in the ammonia phase.

TEST FOR CARBOHYDRATES

In 2ml extracts of okra and bitter gourd, 2 drops of alcoholic solution of α-naphthol was added and mixed well. Then, conc. H_2SO_4 was mixed in each test tube. The violet ring that formed was a sign of carbohydrates.

TEST FOR COUMARINS

Okra and bitter gourd powder 0.5g was taken in 5ml hot distilled water and cooled. The test samples were mixed with 0.5ml of 10% ammonium hydroxide was subsequently tested under UV light (λmax= 365 nm). A bright fluorescence that appeared indicated the presence of coumarins.

TEST FOR FLAVONOIDS

A Shinoda test was conducted using 5g of powder that was boiled in 20 ml of distilled water before being filtered. A tiny amount of magnesium ribbon and 2 ml of filtrate were combined with alcohol, and then heated conc. HCL was added. Flavonoids were indicated by their pink, orange or red to purple coloration.

TEST FOR GLYCOSIDES

FLAVONOL GLYCOSIDES

Dried plants powder (5mg) was dissolved with alcohol. A few fragments of magnesium ribbon or zinc powder were added to the test tube. Drop wise conc. HCL was added, and the color changed from pink to red, indicating the presence of flavonol glycosides.

TEST FOR STEROIDAL GLYCOSIDES

The plant extracts of bitter gourd and okra were dissolved separately in test tubes, and 2 ml of concentrated H_2SO_4 was added to each to identify the presence of steroidal glycosides. Reddish-brown results suggested the presence of steroidal glycosides.

TEST FOR PHENOLICS

In 5ml of distilled water, 50mg of plant powders were dissolved. In each plant solution, a few drops of 5% ferric chloride were added. The blue-black hue suggested the presence of phenolic chemicals.

TEST FOR PROTEINS

The presence of protein was determined using the Biuret test. A small amount of 1% $CuSO_4$ solution and 4% NaOH solution were added to 3 ml of each extract. The appearance of a violet or pink color suggested the presence of proteins.

TEST FOR REDUCING SUGAR

Dried 1g of each plant powder dissolved in 10mL distilled water. The mixture boiled for 5 min and filtered while hot. After cooling, the filtrate was made alkaline by adding 20% NaOH solution and checked with litmus paper. The resulting solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The presence of reducing chemical was shown by the production of a brick-red precipitate.

TEST FOR SAPONINS

20ml of distilled water and 0.5 g of powder were combined, shaken for 15 minutes, and the test tubes top was checked for foam development.

TEST FOR STEROIDS

Okra and bitter gourd 5ml extract added in test tubes then 2ml of chloroform was added. Conc. H_2SO_4 (2ml) was mixed and when steroids are present, the color turns red.

TEST FOR TANNINS

Powdered vegetables were dissolved in 20ml distilled water and boiled for few minutes then filtered. In the filtrate a few drops of 0.1% ferric chloride was added and brownish green color appeared that indicate presence of tannins.

TEST FOR TERPENOIDS

Powdered 0.5g plants were dissolved in 2ml chloroform separately, then 3ml conc. H_2SO_4 was added. Terpenoids were present at the junction as evidenced by the formation of a reddish-brown coloration there.

RESULTS AND DISCUSSION

INHIBITORY EFFECT OF ALPHA-AMYLASE (%)

According to the inhibition percentage (Fig. 1) of α-amylase there is gradual increase in percentage inhibition by using low (0.625mg/ml) to high (5mg/ml) concentration of bioactive extracts. The lowest inhibition % of α-amylase was found at 0.625mg/ml concentration, 4% and 6% in okra and bitter gourd respectively. The α-amylase inhibition activity of okra and bitter gourd, at highest concentration (5mg/ml) showed highest inhibition percentage of 22% and 26% respectively

INHIBITION PERCENTAGE (%) OF ALPHA-AMYLASE IC_{50} VALUE

The extracts of bitter gourd and okra showed the most effective α-amylase inhibition (Fig. 2). The results presented a nearly competitive reduction of enzyme activity, which can be used to evaluate the mode of α -amylase activity in bitter gourd and okra. The half–maximal inhibitory concentration (IC_{50})

indicates how much drug is needed to inhibit a biological process. IC_{50} value was obtained 10.1 and 10.5 of bitter gourd and okra respectively. The extracts must work as a cost effective medicine with lesser side effect due to less IC_{50} value.

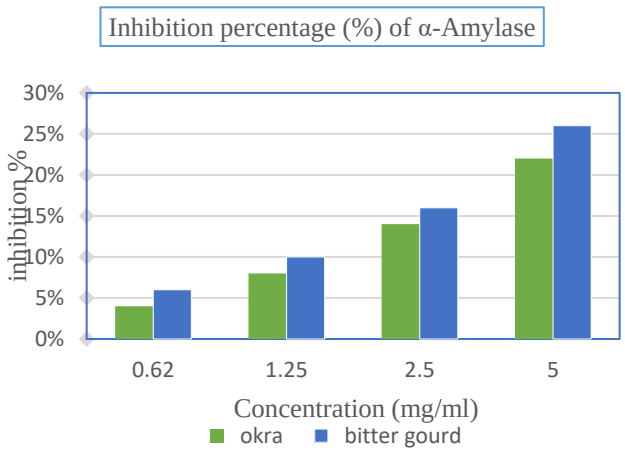

Fig. 1. Inhibition percentage (%) of α-amylase

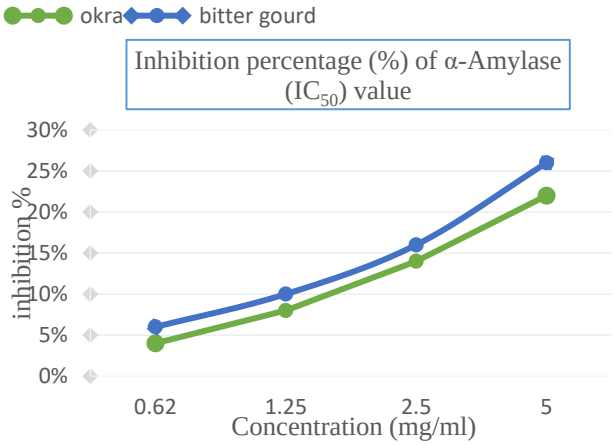

Fig. 2. Inhibition percentage (%) of Alpha-Amylase IC_{50} value

Traditional medicinal plant extracts that inhibit α -amylase function similarly to hypoglycemic drugs, are considered as a potent treatment for diabetes mellitus and a way to reduce postprandial glucose levels (19). Low density lipoprotein (LDL) oxidation and pro-atherogenic processes, which can harm microvascular structures, have been associated to elevated postprandial glucose levels. A diet high in carbohydrates causes a significant increase in blood glucose levels because dietary carbohydrates are quickly broken down in the intestine with the help of the enzymes α-amylase and α-glucosidase. To control hyperglycemia in type 2 diabetes mellitus, medications have been used to increase the duration of overall carbohydrate digestion processes and digest carbohydrates more slowly (15, 20). In traditional practices, medicinal plant extracts block the excessive inhibition of pancreatic α-amylase that cause abnormal bacterial growth on undigested carbohydrates in the colon (21). The use of plant alternatives has gradually lessened the adverse effects of synthetic hypoglycemic drugs like acarbose, miglitol and voglibose used in conjunction with other anti-diabetic medications (15). Therefore, these extracts need to function as a medication with low cost and few to no side effects (21).

In account of above benefits our study includes the two plants okra and bitter gourd and their bioactive ethanolic extracts (Amole and co-workers (2010) also suggest that ethanol functions as a better extract agent than water (22) inhibiting the α-amylase. The α- amylase inhibition activity of okra and bitter gourd, at highest concentration (5mg/ml) showed highest inhibition percentage of 22% and 26% obtained respectively. IC_{50} value obtained was 10.5 mg/ml and 10.1 mg/ml in okra and bitter gourd respectively. Almost similar results were obtained using okra pod by Anand and co-workers (2021) that α-amylase activity of okra aqueous was measured by graph using α-amylase inhibitor and IC_{50} value obtained was 11.4mg/ml at 5mg/ml, and it was shown that the extract demonstrated a nearly competitive suppression of enzyme activity. Kulkarni and co-workers, (2021) also worked with the inhibition of α -amylase using *M. charantia* and obtained IC_{50} value 15.86µg/ml (16, 23).

In vivo antidiabetic activity is also found in bitter gourd and okra. *M. charantia* (Cucurbitaceae) was tested for its ability to treat T2DM in KK-Ay mice, an animal model with hyperinsulinemia. It showed significantly decreased KK-Ay mice's serum insulin under comparable circumstances and reduced their blood glucose three weeks after oral administration (p0.01). In an insulin tolerance test, the blood glucose of MC-treated KK-Ay mice considerably dropped (24). According to Anand and co-workers (2021), okra extract includes a wide variety of bioactive components. Both diabetic people and animals can benefit from their hypoglycemic effects. In diabetic rats given STZ, okra powder has anti-hyperlipidemic and anti-diabetic effect (16). Saha and co-workers (2021) also found in their results that okra extract was tested for their ability to lower blood sugar levels in a mouse model of diabetes induced by alloxan. The outcomes showed that when

Glibenclamide was utilized as a standard, the effects of the aqueous extract of the powdered okra medication were at their peak (25).

The *in vivo* and in vitro antidiabetic activity is present in the extracts of okra and bitter gourd. This implies that the active extract ingredients fight with the substrate to bind with the enzyme active site, preventing the conversion of oligosaccharides into disaccharides. Chemicals originating from plants have attracted a lot of attention due to their versatility this concept is also supported by Matsuda and co-workers (2002) that active components of the extract compete with the substrate for binding to the enzyme active site, inhibiting the conversion of oligosaccharides into disaccharides (26). These are richest bio resource for medications in traditional systems, folk remedies, recent drugs, nutraceutical, medicinal intermediates, and chemical entities for synthetic drugs. The leaves, fruit and roots of the plants under study were found to be highly concentrated in alkaloids, phenol, and tannins based on phytochemical screening (27).

A medicinal plant's therapeutic effect is ascertained by looking at its phytoconstituents, either separately or in combination. Alkaloids, steroids, glycosides, tannins, phenols, saponins, terpenes, reducing sugars, and amino acids were found in the ethanolic extracts of *M. charantia* fruits and *A. esculentus* after preliminary phytochemical screening. Although there are many modern techniques for determining phytochemicals, initial phytochemical screening of plants is still frequently done using old qualitative testing (28).

Table I. Results of phytochemical screening of Okra and Bitter gourd

Phytochemical tests	Okra	Bitter gourd	Color
Test for alkaloids	+ve	+ve	Cream color precipitates appeared
Test for amino acid	+ve	+ve	Pink color appeared
Test for anthraquinones	-ve	-ve	Red color absent in ammonia phase
Carbohydrates test	-ve	-ve	Absence of violet ring
Test for coumarins	+ve	-ve	Intense fluorescence suggested the presence of coumarins and derivatives
Test for Flavonoids	+ve	+ve	Pink color appeared in okra while orange in bitter gourd.
Test for glycosides i. Flavonol glycosides	-ve	-ve	Pink to crimson color appeared that indicates flavonol glycosides.
ii. Test for steroidal glycosides	+ve	+ve	Reddish brown color indicates steroidal glycosides
Test for phenolics	+ve	-ve	Appearance of bluish black color indicate the presence of phenolics
Test for proteins	-ve	-ve	Pink/ violet color absent
Test for Reducing Sugar	+ve	+ve	Brick-red precipitate formed
Test for Saponins	+ve	+ve	Appearance of foam layer on the top of each test tubes
Test for Steroids	+ve	+ve	Red color appeared
Test for tannins	+ve	+ve	Brownish green color appeared
Test for terpenoids	+ve	+ve	Reddish brown color appeared

According to Ncube and co-workers (2008) plants may operate as an anti-microbial agent since they contain phenolic compounds. Because of their antioxidant properties, phenolic chemicals have been linked to a lower risk of heart and circulatory disorders (15, 27). Akinsulire and co-workers (2007) explained that tannins have astringent qualities and speed up the recovery of wounds and inflamed mucous membranes (29). The polyphenolic group of flavonoids is recognized for having health-promoting qualities like anti-allergic, anti-inflammatory, antibacterial, strong water-soluble R antioxidants, free radical scavengers, and anticancer activities. It prevents oxidative cell damage and has strong anti-tumor effects (15) (28). Hassan and co-workers (2010) also discussed the antibacterial properties of saponins, which are a component of plants defense mechanisms, are well documented (30). Sodipo and co-workers (2000) discussed that blood cells can precipitate and clump together when exposed to saponins. Since these phytochemicals are widely used as a therapeutic agent to treat a variety of ailments (31). But to fully understand the structure of these compounds, additional advanced hyphenated spectroscopic studies are required. Saxena and co-workers (2011) referred terpenoids as antibiotics, insecticides, anthelmintic, and antiseptics in the context of pharmacy (32). All

fractions found to contain alkaloids, apart from aqueous, shown to have analgesic, antispasmodic, antibacterial, antimalarial, and analgesic properties (33). Xiao and co-workers (2016) explained that glycosides were useful in cases of congestive heart failure (34).

Singh and co-workers (2014) suggested that the purified phytochemicals from medicinal plants have recently demonstrated promising antidiabetic potential (35). Oxidative stress is a result of increased extracellular and intracellular glucose concentrations, according to Anjum and Tripithi (2019) as well as Akinsulire and co-workers (2007) discussed that phenolics, flavonoids, tannins and their derivatives, are considered antioxidants or free radical scavengers. Therefore, phytochemicals are advantageous in diabetes and its associated problems (15, 29).

CONCLUSION

The present study contains two vegetables *Abelmoschus esculentus* and *Momordica charantia*. These have medicinal properties and can be used traditionally to treat diabetes, easily grown in Pakistan and economically feasible. The in vitro antidiabetic activity was found in both these vegetables. Bitter gourd has slightly better inhibition percentage of α-amylase than Okra. *Abelmoschus esculentus* and *Momordica charantia* these both must work as a cost-effective medicine with lesser adverse effects. Phytochemical screening was also done and most of the tests were positive in both plants, so it was concluded that phytochemicals also have some sort of association with antidiabetic activity due to free radical scavenging activity. However, the study recommends more investigation into the extraction and identification of specific chemical components in bitter gourd and okra that are responsible for their ability to decrease enzyme activity.

Conflict of interest:

Authors have no conflict of interest.

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