INVITRO ANTIDIABETIC ACTIVITY OF MOMORDICA CHARANTIA

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Abstract:

The effectiveness of organic bitter gourd as an in vitro antidiabetic was examined in this study using a number of biochemical tests (Momordicacharantia). Diabetes was extensively treated using the medicinal plant bitter gourdin conventional medicine. In this research, the organic extract of bitter gourd was made using ethanol as a solvent. A variety of in vitro assays were performed on the extract, including the promotion of glucose absorption in L6 myotubes and the suppression of alpha-glucosidase and alpha-amylase.According to the study's results, the organic extract of bitter gourd considerably reduced the activity of alpha-amylase,. The extract also showed potent alpha-glucosidase inhibitory action, with percentage of inhibition is 5.55/100µg. Moreover, the extract increased anti-diabetic activity. In our present study reported that ethanol extract of organic bitter gourd showed Inhibition of Alpha Amylase activity and Glucosidase.

Keywords: Organicbittergourd; Anti-diabetic activity; Inhibition; Assays; Amylase; Glucosidase.

INTRODUCTION

Throughout the world, millions of people battle the chronic metabolic disease known as diabetes mellitus. It is characterized by hyperglycemia, which is brought on by abnormalities alterations in either insulin action, secretion, or both. Diabetes includes a multitude of adverse outcomes that can significantly reduce a person's quality of life, including cardiovascular disease, neuropathy, and retinopathy.Momordicacharantia.has a lengthy history of therapeutic use across many cultures, particularly due to its hypoglycemic properties. The bitter gourd contains a number of bioactive compounds, such as vicine, polypeptide-p, and charantin, which have insulin-like properties and are assumed to be the reason for its hypoglycemic effects.The tropical and subtropical vine Momordicacharantia, sometimes called Cucurbitaceae is the family that

includes bittermelon and bittergourd.Several researchers have used it for medical purposes in the past, most notably for its hypoglycemic effects. The bitter gourd's hypoglycemic effects are likely to be caused by a number of bioactive compounds, including vicine, charantin, and polypeptide-p, which have insulin-like qualities.For its therapeutic benefits, especially for its hypoglycemic effects, it has historically been employed in a variety of civilizations. Bitter gourd is thought to have hypoglycemic qualities because it contains a number of bioactive substances, including vicine, polypeptide-p, and charantin, which have insulin-like properties.

It has a lengthy history of therapeutic use across many cultures, especially due to its hypoglycemic properties. The bitter gourd's hypoglycemic effects are likely to be caused by a number of bioactive compounds, including, charantin, and polypeptide-p, which have insulin-like qualities. The bitter gourd has drawn The adoption of natural medicines to manage diabetes has seen a surge in attention in recent years.which has brought its potential antidiabetic characteristics a lot of attention.. There has been little research on the antidiabetic properties of organic bitter gourd, despite the fact that many studies have examined the hypoglycemic effects of bitter gourd extracts.

The hypoglycemic qualities of the medicinal plants from the Peruvian Amazon used to treat diabetes that have been published in the literature over the past 20 years using several bibliographic databases. Indigenous people in the Amazonian region claim to use 77 medicinal plants to treat diabetes, and 46.75 percent of those species exhibit hypoglycemic activity, proving that traditional knowledge is a valuable resource for discovering new anti-diabetic medications as well as a viable alternative to further study.(Gabriel Vargas-Arana *etal.*,2023). The medicinal plants from the Peruvian Amazon used to cure diabetes are discussed in this article, along with a thorough analysis of their hypoglycemic effects as documented in the literature during the past 20 years and used in several bibliographic databases. Native Amazonians have reported using 77 medicinal plants to treat diabetes, with 46.75% of species reporting hypoglycemic action. This data shows that traditional knowledge is an excellent place to start when looking for antidiabetic medications and is also a viable alternative for further study(Fatma Hussain etal., 2022). Their findings demonstrated that the safety and great therapeutic potential of M. charantia. The methanolic extract showed the hypoglycemic and anti-neurodegenerative among the seven

fractions or extracts utilised in the invitro experiment, M. charantia had the most potent inhibitory capacity of alpha-glucosidase and acetylcholinesterase. The current study co-administered alloxan and M. charantia extract to diabetic rats at a level of 80 mg/kg body weight. This significantly raised the levels of insulin, glycatedhaemoglobin, and fasting blood glucose. By employing structural elucidation and HPLC analysis, it was possible to prove the existence of flavonoids, phenols, tannins, and saponins. FTIR can be used to identify a number of significant functional groups, including hydroxyl groups, esters, alkanes, alkenes, alkynes, ketones, alcohols, amines, and carboxylic acids.Clinical research using an animal model of diabetes showed encouraging results(Ngan Tranetal.,2020).As they have been in use for so long, traditional medicines are essential as alternative therapies. Also, compared to oral hypoglycemic drugs now used in clinical therapy, several recently discovered bioactive medicines made from plants have shown superior efficacy in treating diabetes. In terms of clinical practise, traditional medicine has a solid track record and a promising future in management of diabetes mellitus. According to the WHO, preventing diabetes and its complications is crucial to achieving universal health as well as a serious challenge for the future. As a result, this study provides a brief overview of a few well-known plants that have been widely utilized to treat diabetes in terms of their active components and pharmacological effects. Traditional medicine has been practised for a very long time and is essential as an alternative to modern medicine. Additionally, in recent years, a few brand-new bioactive medications made from plants have been developed. The major use of bitter gourd fruit will lows the sugar levels in blood.

Despite the fact that terpenoid-rich extract inhibited -glucosidase, it is clear from the study's findings that further purification of the extract is necessary to increase its effectiveness as a -glucosidase inhibitor. Momordicacharantia's possible mode of action for reducing blood sugar levels was shown by the extract's impact on glucosidase(Sallau et al., 2018). Certain selected edible vegetables in tropical regions have anti-diabetic potential.There are numerous natural sources where substances with inhibitory effects against glucosidase and -amylase can be found. Plants of the Cucurbitaceae family are abundant in cucurbitanes that have the ability to inhibit -glucosidase and can be used in clinical settings to treat diabetes.Both medications were determined to be compatible with one another, and they pass every test under different circumstances. For diabetic patients, this is the greatest nutritional diet and supplementation. The

bitter melon, M. charantia L., fruit is used to lower blood sugar levels. If Charantin's efficacy is improved and is backed by adequate clinical data, this plant-based chemical could take the lead among hypoglycemic medicines for treatment of diabetes.Possible anti-diabetic qualities of medicinal plants used widely in Sri Lanka's traditional medical system.The bitter melon, M. charantia L., fruit is used to lower blood sugar levels. If Charantin's efficacy is improved and is backed by adequate clinical data, this plant-based chemical could take the lead among hypoglycemic medicines for the treatment of diabetes(KeddagodaGamagePiyumiWasana 2021).Ethiopian medicinal herbs' possible ant diabetic propertiesFurther research is suggested to support the usage of these therapeutic herbs as an antidiabetic drug.(ZemeneDemelash Kifle etal., 2022). Renowned plants whose therapeutic properties have been heavily utilized in the management of diabetes chemicals and pharmacological effects. For plant identification, morphological information from the V-herb database was also incorporated for each species(Ngan Tran etal.,2020).

Momordicacharantia, sometimes referred to as bitter melon, is a tropical vine that is frequently used in traditional medicine to treat numerous illnesses, including diabetes. Momordicacharantia's in vitro ant diabetic efficacy has been the subject of numerous studies.One study, published in the Journal of Ethnopharmacology in 2003, looked at how awater extract of bitter melon affected the rate of glucose absorption in rat skeletal muscle cells.. The study discovered that the extract markedly and dose-dependently boosted glucose absorption, showing that it can lower blood sugar levels. Another investigation into the antidiabetic effects of several bitter melon fruit extracts on pancreatic beta cells was reported in the Journal of Medicinal Food in 2018. The research discovered that the ethyl acetate extract had the strongest anti-diabetic effects, leading to appreciable rises in insulin secretion and glucose absorption.A third research project examined the in vitro anti-diabetic efficacy of bitter melon seed extract in human adipose tissue-derived stem cells. It was published in the Journal of Traditional and Complementary Medicine in 2015.All things considered, these results indicate that bitter melon has strong in vitro antidiabetic action and may hold potential as a natural diabetes therapy option. To ascertain its efficacy and safety in human clinical trials, more study is necessary.

MATERIALS AND METHODS

MATERIALS

In our research, we used the chemicals α-amylase, sodium phosphate buffer, sodium phosphate ,buffer, 3, 5 dinitrosalicylic acid, p-nitrophenyl alpha- D-glucosidase, 0.1 M sodium carbonate, ethanol and distilled water were purchased from Krishna Raman ChemicalsPvt. Ltd, Chennai,Tamilnadu. We used all AR grade chemicals to carry out the research and consolidate the results.

Sample collection and sample preparation:

To perform the experiment, we collected organic bitter gourd, and this sample was brought to lab.Using the tap water and double distilled water, the samples were washed thoroughly and allowed to dry the sample.The dried sample was weighed and made into pieces and prepared extraction using the ethanol. This ethanol sample extract as used to perform the anti-diabetic assays for in-vitro analysis.

Inhibition of Alpha Amylase

Procedure

The standard medication was taken at various concentrations. After that, each tube received 1 ml of -amylase in 0.2 M sodium phosphate buffer (pH 6.9), which was then incubated at 25°C for 30 min. Each tube was then filled with 1 ml of a 1% starch solution in 0.2 M sodium phosphate buffer (pH 6.9). Afterwards, the reaction mixtures were incubated for 3 minutes at 25°C. With the help of 1 ml of 3, 5-dinitrosalicylic acid, the process was stopped. To the reaction mixture was added 9 cc of distilled water. At 540 nm, absorbance was measured..

$$\% \text{ of alpha amylase inhibition} = \frac{\text{OD sample} - \text{OD control}}{\text{OD sample}} \times 100$$

Tables 1 and 2 show the concentration vs percentage of inhibition for control 0.14 and blank 0.14 w.r.talpha amylase.

Table 1 Concentration vs percentage of inhibition for control 0.14

Concentration(µg)	100	200	300	400	500
Standard O.D	0.15	0.21	0.25	0.30	0.46
% of inhibition	6.6	33.3	44.0	53.3	69.5

Table 2 Concentration vs percentage of inhibition for blank 0.14

Sample Concentration (µg)	100	200	300	400	500
Bitter gourd	0.18	0.21	0.23	0.25	0.28
% inhibition	22.2	33.3	39.1	44	50

Fig. 1 shows the graphical representation of % of concentration vs % of inhibition w.r.t alpha amylase.

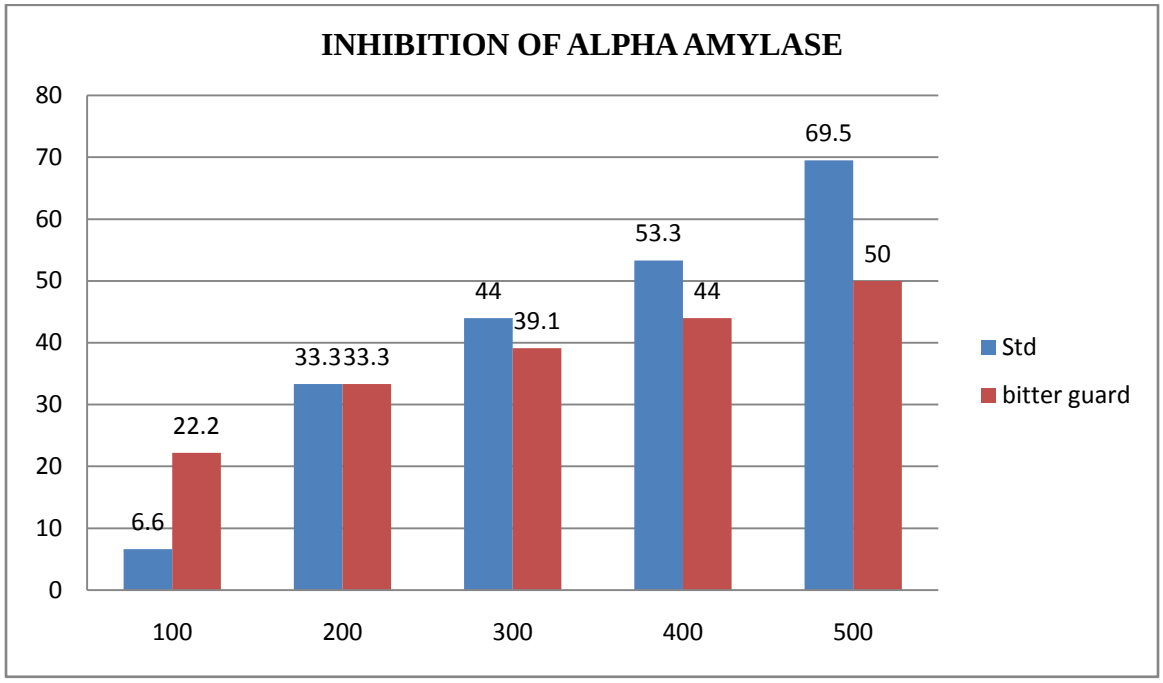

Fig. 1 Percentage of concentration vs percentage of inhibition w.r.t alpha amylase.

Inhibition of Alpha glucosidase

Procedure

Different tubes each contained 100 l of 0.1 U glucosidase. This was then combined with 50 l of sample and standard at various concentrations (do not mix), and incubated at 25 °C for 10 min. After adding 50 l of p-nitrophenyl alpha-D-glucosidase, vortexed, and incubating for 5 min at 25°C. 800 l of sodium carbonate 0.1 M stop solution were added. At 405 nm, absorbance was measured.

OD control – OD sample

$$\% \text{ of alpha glucosidase inhibition} = \frac{\text{OD control}}{\text{OD control}} \times 100$$

Tables 3 and 4 show the concentration vs percentage of inhibition for control 0.18 and blank 0.18 w.r.talpha glucosidase.

Table 3 Concentration vs percentage of inhibition for control 0.18

Concentration(µg)	100	200	300	400	500
Standard O.D	0.12	0.09	0.07	0.05	0.03
% of inhibition	33.3	50.0	61.1	72.2	83.3

Table 3 Concentration vs percentage of inhibition for blank 0.18

Sample Concentration (µg)	100	200	300	400	500
Bitter gourd	0.17	0.14	0.12	0.09	0.06
% inhibition	5.55	28.5	33.3	50	66.6

Fig. 2 shows the graphical representation of % of concentration vs % of inhibition w.r.t alpha glucosidase.

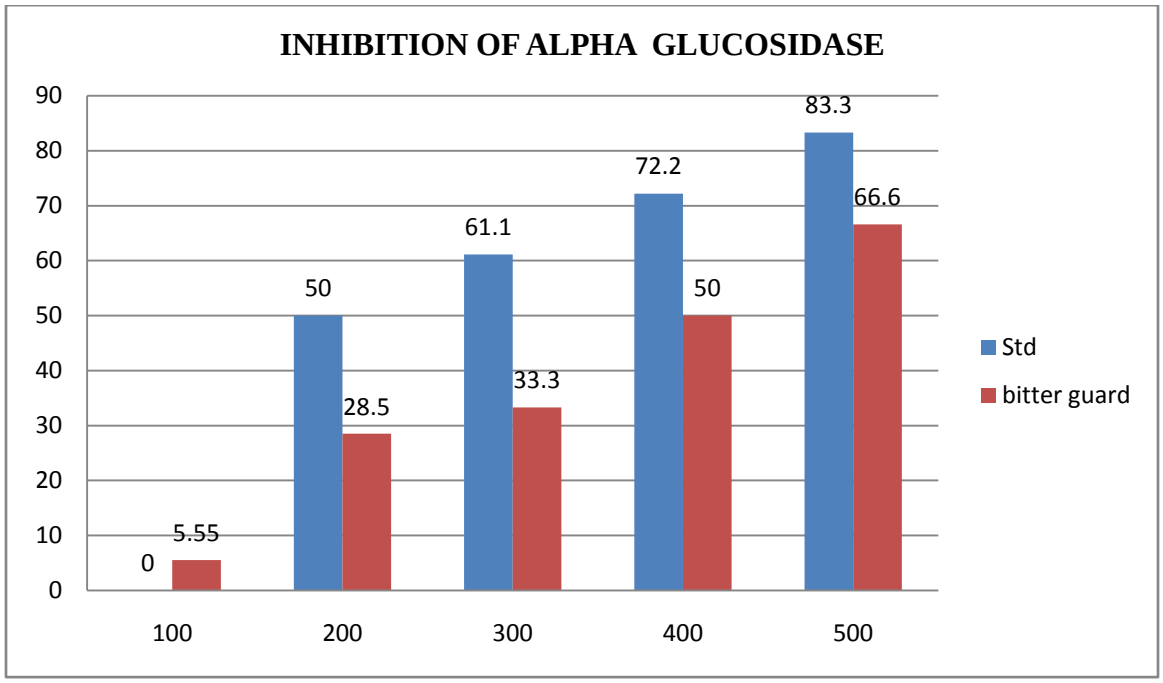

Fig. 2 Percentage of concentration vs percentage of inhibition w.r.t alpha glucosidase

Fig. 3 shows the typical organic sample of bitter gourd, sample preparation and storing of the sample.

Fig. 3 Typical organic sample (MOMORDICA CHARANTIA), preparation and storing

Fig. 4 shows the ethanol extract preparation of sample to perform the anti-diabetic activity and glucosidase can be seen in Fig. 5.

Fig. 4 Ethanol extract

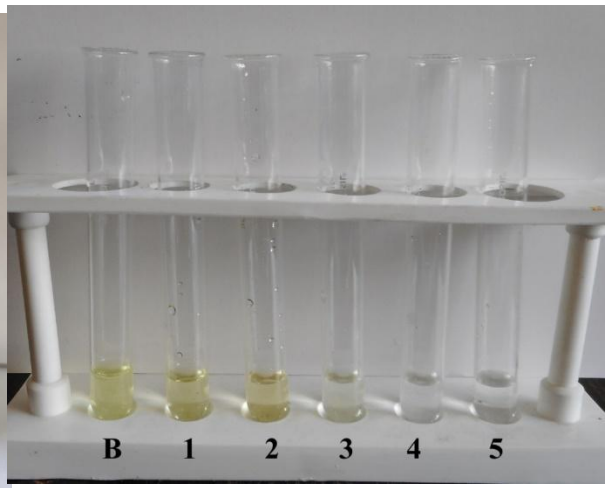

Fig. 5 Glucosidase STD

Figures 6 to 8 show the glucosidase inhibition reaction, amylase STD and amylase inhibition reaction respectively.

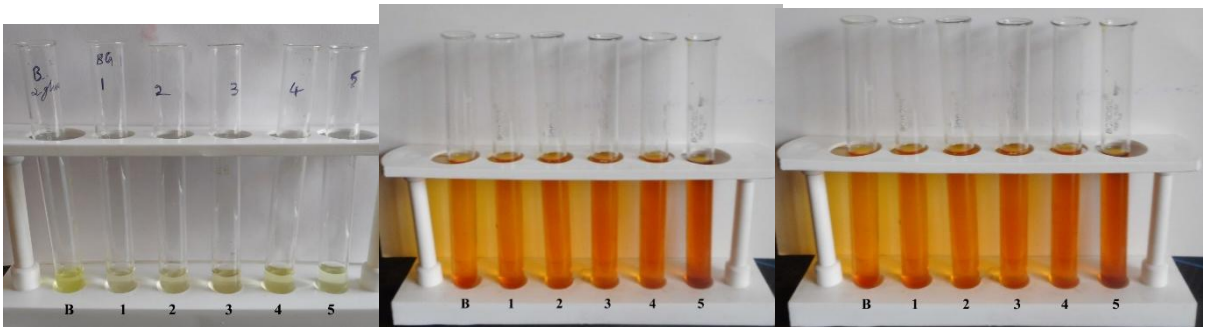

Fig. 6 Glucosidase inhibition reaction Fig. 7 Amylase STD Fig. 8 Amylase inhibition reaction

RESULTS AND DISCUSSION

The medical benefits of bitter melon, which may have ant diabetic effects as well, have long been appreciated by various cultures. Promising findings have been reported from numerous researches that examined the ant diabetic efficacy of bitter gourd extracts in vitro and in vivo.Studies carried out in a lab environment without a living organism are known as in vitro experiments. Before a chemical is tested on animals or people, such studies can assist identify the potential efficacy and safety of the substance. It is crucial to remember that the outcomes of in vitro experiments do not always predict how a chemical will act in a living being.In our research we collected bitter gourd and prepared the samples to evaluate the antidiabietic activity using Amylase Inhibition Reaction and Glucosidase Inhibition reaction.KrishnaveniNagappan(2018)according to reports, a major obstacle for current scientific study is the development of active and safe blood glucose reducing agents. Momordicacharantia fruit juice has often been used for a sizable amount of time to cure diabetes. Momordicacharantia has an unique steroidal glycoside called charantin, which has been discovered to have medicinal potential for decreasing blood sugar levels. Nevertheless, this substance was not clinically tested for the treatment of diabetes. This study condenses the science, the method of action, and the clear tactics for charantin.Gabriel Vargas-Arana (2023) reported the antidiabetic efficacy of the bitter melon. For its medical benefits, including potential antidiabetic effects, bitter melon has long been valued in many cultures. Promising findings have been reported from numerous researches that examined the antidiabetic efficacy of bitter gourd extracts in vitro and in vivo.Studies carried out in a lab environment without a living organism are known as in vitro experiments. Before a chemical is tested on animals or people, such studies can assist identify the potential efficacy and safety of the substance. It is crucial to remember that the outcomes of in vitro experiments do not always predict how a chemical will act in a living being (Gabriel Vargas-Arana, 2023).

Sabina BibiJhaumeerLaullooetal.(2021) found that the anti-diabetic medications and as a backup plan for future research, knowledge is a fantastic

resource.KeddagodaGamagePiyumiWasana(2021)reported the treatment of diabetes mellitus using medicinal plant extracts.The natural world provides a variety of substances that have inhibitory effects on -glucosidase and -amylase.

Plants of the Cucurbitacins family are rich in cucurbitane, which has the capacity to block -glucosidase and can be utilized in clinical settings to treat diabetes. Throughout the past few decades, there has been a marked growth in the use of isolated bioactive components and plant extracts for the treatment of diabetes mellitus. Several of the described medicinal plants' bioactive substances and ant diabetic mechanism(s) have not yet been isolated, nor have their structures been thoroughly characterized. The presented anti-diabetic medicinal plants offer new sources for the development of anti-diabetic drugs with a wide range of therapeutic targets thanks to additional features like antioxidant and anti-hyperlipidemia activity's

Bitter gourd formulations may be helpful in the treatment of diabetes, according to long-standing traditional use and primarily favorable in vivo study results. Studies on cucurbitane-type triterpenoids are ongoing, and several of the compounds are showing substantial effects in glucose absorption experiments at nanomolar levels, pointing to this chemical class as the active Beneficial Benefits and MomordicaCharantia Juice's Mechanism of Action in the Treatment of Rats With Streptozotocin-Induced Diabetes Mellitus. to low micromolar concentrations. Moreover, some of the examined substances shown effects on insulin sensitization equivalent to those of thiazolidinediones as well as increased insulin secretion (Serhat S. Çiçek, 2022).

In our research we examined the organic bitter gourd activity against two assays and proved that these ethanol extract sample showed the inhibition activity of Amylase and Glucosidase activity. These activities showed that ethanol extract of bitter gourd sample showed invitro antidiabetic activity.

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