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Evaluation of *In-vitro* Antidiabetic Activities of Fruit Extracts of *Momordica cymbalaria*

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Abstract

Diabetes mellitus is an inescapable and multifactorial incessant metabolic issue which leads to hyperglycemia due to impairment of insulin secretion, insulin function or both. Till today, acarbose and voglibose are used either alone or in combination with insulin as an inhibitor of carbohydrate digestive enzymes. However, harmful effects of these compounds, such as liver disorders, flatulence, abdominal fullness and diarrhea, have been reported. Current study aimed to evaluate the antidiabetic potential of *Momordica cymbalaria* through *in-vitro* evaluation of inhibition of carbohydrate hydrolyzing enzymes such as, α-amylase and α-glucosidase. Results depicted that in an α-amylase inhibitory assay with aq. extract of fruits of *M. cymbalaria* at a concentration range of 50µg/ml, 100µg/ml, 150µg/ml, and 200µg/ml, shown inhibition effect of 18.66%, 30.29%, 42.59%, and 53.36% respectively with an IC_{50} value of 187.33 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 82.10 µg/ml. Similarly, in an α-glucosidase inhibitory assay with aq. extract of fruits of *M. cymbalaria* at a concentration range of 50µg/ml, 100µg/ml, 150µg/ml, and 200µg/ml, shown inhibition effect of 16.75%, 28.42%, 33.29%, and 47.40% respectively with an IC_{50} value of 192.55 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 106.61 µg/ml. Furthermore, the phytochemical analysis of aq. extract of fruits of *M. cymbalaria* revealed that *M. cymbalaria* fruits are rich in secondary metabolites such as flavonoids and phenolics. In conclusion, potent antidiabetic activity *M. cymbalaria*

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has been demonstrated through inhibition of activities of α-amylase and α-glucosidase enzymes.

Keywords: *Momordica cymbalaria*, Seed extract Diabetes mellitus, α-glucosidase, α-amylase, Antidiabetic potential

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Introduction

Diabetes mellitus is an inescapable and multifactorial incessant metabolic issue, is portrayed by deformities in endogenous insulin discharge or activity, or both, which brings about endless hyperglycemia, a clinical sign of diabetes, regularly joined by glycosuria, polydipsia polyuria and weight reduction. Hyperglycemia, in the long run, prompts dynamic beta-cell brokenness, weakened insulin quality translation and perpetual-cell misfortune because of apoptosis. Subsequently, in the diabetic condition, changes in insulin production and its activity leads to weakened glucose homeostasis and the event of perpetual hyperglycemia, which prompts a decreased number of glucose transporters, down guideline in the quantity of insulin receptors just as imperfections of tissue insulin signal transduction, all of which results in insulin obstruction.[1]

Management of diabetes mellitus at the present is immobile symptomatic and must be completed for a generation, so probably inflicting a range of aspect effects, like hypoglycaemic, etc.[2] Hence, it's essential in the direction of discovering the substitute treatment. This can stabilize blood sugar levels in traditional vary. At present, the plant is considered as a conceivable asset of bioactive mixes. The auxiliary metabolite substance in the plant has been distinguished to contain an amount of action.

Medicinal plants are wellsprings of a significant helpful guide for easing human illnesses. Around 80% of the general populations in the developing nations everywhere throughout the world rely upon the conventional drug for their essential healthcare. Remarkably, about 85% of a traditional customary prescription includes the utilization of plant removes. *Momordica cymbalaria* is a more prominent nutritious vegetable of South India. It is a perennial climber of Cucurbitaceae family (Figure 1 and Figure 2) which is available only during the monsoon season, found in the Indian states of Andhra Pradesh,

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Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. *M. cymbalaria* has been used as traditional medicine for diabetes mellitus and rheumatic disorders.

Figure 1: Showing *M. cymbalaria* plant

Figure 2: Showing *M. cymbalaria* fruits

M. cymbalaria is a close relative of bitter melon (*Momordica charantia*) which is better known for its antidiabetic activity and bitter in taste similar to bitter melon. Decreasing postprandial hyperglycemia by inhibition of carbohydrate hydrolyzing enzymes such as, α-amylase and α- glucosidase is one of the therapeutic approaches. Until now, acarbose and voglibose are used either alone or in combination with insulin as an inhibitor of carbohydrate digestive enzymes.[3] However, harmful effects of these compounds, such as liver disorders, flatulence, abdominal fullness and diarrhea, have been reported.[4]

With this scenario, the present study was designed to evaluate the antidiabetic potential of *M. cymbalaria* through *in-vitro* evaluation of inhibition of carbohydrate hydrolyzing enzymes such as, α-amylase and α- glucosidase.

Materials and Methods

Collection of *M. cymbalaria* Fruits

The fruits of *M. cymbalaria* were purchased from local market in my native place Bellary district head quarter, Karnataka, India. The fruits of *M. cymbalaria* were sprayed with ethanol, and then shade dried at room temperature for 10 days. The dried fruits were crushed

to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered fruits of *M. cymbalaria* were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of double distilled water. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use.[5]

Quantitative Estimation of Phytochemicals

Total phenolics

The concentration of total phenolics in the aqueous (aq.) extract of fruits of *M. cymbalaria* was determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[6] The phenolic content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent/g of extract powder.

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in aq. extract of fruits of *M. cymbalaria*.[7] The flavonoid content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg gallic acid equivalent/g of extract powder.

Inhibition Assays of Carbohydrate Hydrolyzing Enzymes

α-amylase inhibitory assay

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The α-amylase inhibition assay was carried out by the method of Miller, (1959).[8] Aq. extract of fruits of *M. cymbalaria*/acarbose (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) were incubated for 10 minutes at 25°C with 500 µl of 20 mM sodium phosphate buffer (pH 6.8) with 20 µl of amylase (1U/ml). After pre-incubation, each tube was added with 1 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 15 min. One ml DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10ml) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation of the control samples. The following formula was used to determine the percent inhibition of α-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

α-glucosidase inhibition assay

The α-glucosidase inhibition assay was carried out as described by Matsui et al (1996) with slight modifications.[9] The different concentrations of Aq. extract of fruits of *M. cymbalaria* and standard drug acarbose (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) were prepared. Phosphate buffer (1 ml; 100mM, pH 6.8) and 80 µl of test Aq. extract of fruits of *M. cymbalaria* / acarbose of concentrations (5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) were added to 20 µl of α-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG- 50µl (5mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5ml of 0.1 M Na_2CO_3. The absorbance was measured at 400nm to determine the activity of α-glucosidase activity. The following formula was used to determine the percent inhibition of α-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

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Results

Quantitative estimation of phytochemicals

Quantitative estimation of phytochemicals in aq. extract of fruits of *M. cymbalaria* was represented in Table 1. Results revealed that total flavonoids quantity was found to be highest (16.38 GAE/g extract) in aq. extract of fruits of *M. cymbalaria* when compared with total phenolic quantities (15.12 GAE/g extract).

Table 1: Quantitative analysis of phytochemicals of aq. extract of fruits of *M. cymbalaria*

Phytochemicals	**Aq. extract of fruits of *M. cymbalaria***
Total Phenolics	15.12 GAE/g extract
Total flavonoids	16.38 GAE/g extract

α-amylase inhibitory assay

The results of effect of aq. extract of fruits of *M. cymbalaria* on α-amylase inhibition activity was represented in Table 2 and plotted in Figure 3. Results depicted that aq. extract of fruits of *M. cymbalaria* at a concentration range of 50µg/ml, 100µg/ml, 150µg/ml, and 200µg/ml, shown inhibition effect of 18.66%, 30.29%, 42.59%, and 53.36% respectively with an IC_{50} value of 187.33 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 82.10 µg/ml.

Table 2: Effect of aq. extract of fruits of *M. cymbalaria* on α-amylase inhibition activity

Conc. of aq. extract of fruits of *M. cymbalaria* (µg/ml)	**Inhibition (%)**	**Conc. of Acarbose (µg/ml)**	**Inhibition (%)**
50	18.66 ± 0.08	50	37.17 ± 0.03

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100	30.29 ± 0.11	100	58.09 ± 0.08
150	42.59 ± 0.06	150	73.28 ± 0.05
200	53.36 ± 0.08	200	83.31 ± 0.04
IC_{50} (ug/mL) = 187.33		IC_{50} (ug/mL) = 82.10	

Values were expressed Mean ± SD; n=3

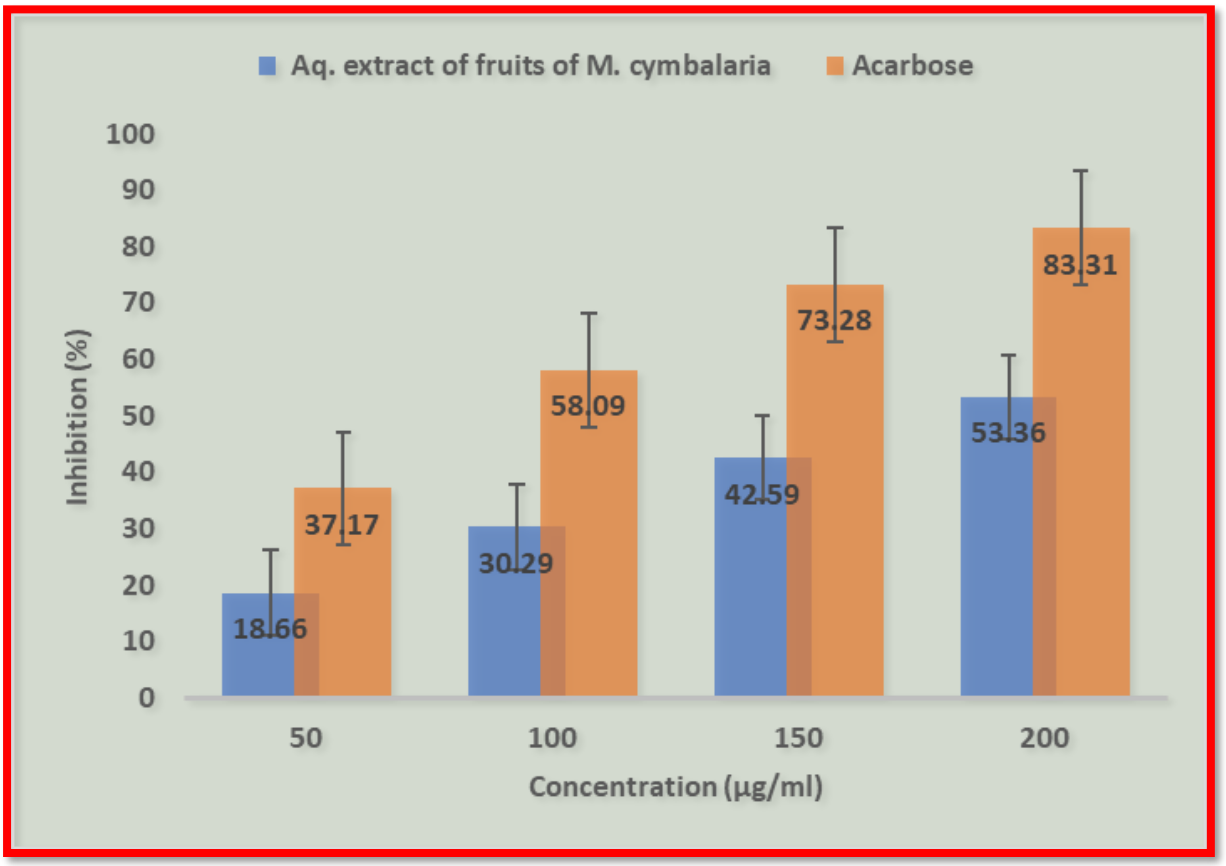

Figure 3: Effect of aq. extract of fruits of *M. cymbalaria* on α-amylase inhibition activity
Values were expressed Mean; n=3

α-glucosidase inhibitory assay

The results of effect of aq. extract of fruits of *M. cymbalaria* on α-glucosidase inhibition activity was represented in Table 3 and plotted in Figure 4. Results depicted that aq. extract of fruits of *M. cymbalaria* at a concentration range of 50µg/ml, 100µg/ml, 150µg/ml, and

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200µg/ml, shown inhibition effect of 16.75%, 28.42%, 33.29%, and 47.40% respectively with an IC_{50} value of 192.55 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 106.61 µg/ml.

Table 3: Effect of aq. extract of fruits of *M. cymbalaria* on α-glucosidase inhibition activity

Conc. of Aq. extract of fruits of *M. cymbalaria* (µg/ml)	**Inhibition (%)**	**Conc. of Acarbose (µg/ml)**	**Inhibition (%)**
50	16.75 ± 0.05	50	31.69 ± 0.03
100	28.42 ± 0.11	100	46.55 ± 0.08
150	33.29 ± 0.09	150	62.84 ± 0.05
200	47.40 ± 0.15	200	75.27 ± 0.04
IC_{50} (ug/mL) = 192.55		IC_{50} (ug/mL) = 106.61	

Values were expressed Mean ± SD; n=3

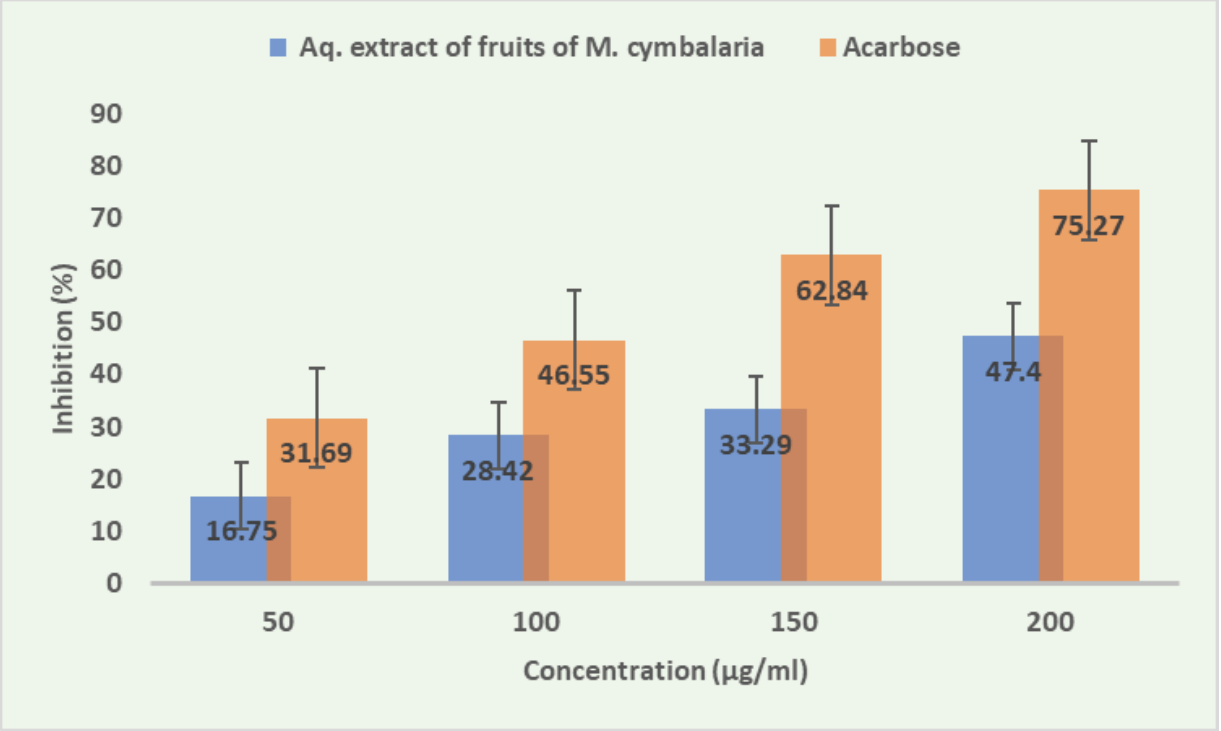

Figure 4: Effect of aq. extract of fruits of *M. cymbalaria* on α-glucosidase inhibition activity
Values were expressed Mean ± SD; n=3

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Discussion

Plants with potential therapeutic values have been used from time immemorial to cure various ailments and infectious diseases. Of late, scientific evidences have been provided on the potential therapeutic agent exhibited by certain traditionally used vegetable extracts. The *Momordica* species have been used in indigenous knowledge; wild plant foods play a vital role in the complex cultural system of tribal people for reducing various disorders. Research has shown that many edible wild plants are rich in specific constituents, referred as phytochemicals, which may have health promoting effects. Furthermore, α-amylase and α-glucosidase inhibitors have become a new treatment strategy to combat diabetes mellitus.[10] Therefore, in the present study we aimed to evaluate the antidiabetic potential of aq. extract of fruits of *M. cymbalaria* through *in-vitro* evaluation of inhibition of carbohydrate hydrolyzing enzymes such as, α-amylase and α- glucosidase.

Our study results revealed that that total flavonoid quantity was found to be highest (16.38 GAE/g extract) in aq. extract of fruits of *M. cymbalaria* when compared with total phenolic quantities (15.12 GAE/g extract). The aq. extract of fruits of *M. cymbalaria* exhibited IC_{50} value of 187.33 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 82.10 µg/ml in on α-amylase inhibition assay. Similarly, the aq. extract of fruits of *M. cymbalaria* exhibited an IC_{50} value of 192.55 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 106.61 µg/ml in α-glucosidase inhibition activity.

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The results of our study are in comparison with various other studies reported in literature. Firdous et al., reported potential antidiabetic activity of saponin present in *M. cymbalaria* in their study of type-2 diabetes in BALB/C mice was induced by single I.P injection of Streptozotocin (100mg/kg), 15 min after the I.P. administration of Nicotinamide (240mg/kg).[11] Koneri and Balaraman demonstrated that saponin-steroidal glycosidal fraction from the ethanolic extract of roots of *M. cymbalaria* showed antidiabetic activity.[12]

Plausible physiological action of *M. cymbalaria* accredited to antidiabetic activity may be due to presence of its hypoglycaemic ingredients polypeptide-p, plant insulin, phenolic acids, flavonoids, carotenoids, cucurbitane, triterpenoid, and phytosterol and glycosides improve blood sugar levels by increasing glucose uptake and glycogen synthesis in the liver, muscles, and fat cells. They also improve insulin release from pancreatic beta cells, and repair or promote new growth of insulin secreting beta cells. P-insulin, a polypeptide from the fruits and seeds rapidly decreased and normalized the blood sugar level.

Conclusion

The conclusion from this investigation was demonstrated that the phytochemical analysis of *Momordica cymbalaria* fruit extract is rich in secondary metabolites such as flavonoids and phenolics. The study concluded that *Momordica cymbalaria* has potent antidiabetic potential through inhibition of activities of carbohydrate hydrolyzing enzymes *viz.* α-amylase and α-glucosidase.

References

1. Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, Accili D. Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. The Journal of clinical investigation. 2000;105(2):199-205.
2. Sujono TA, Wahyuni AS. The influence decocta of *Lidah buaya* (*Aloe vera* L.) leaves to blood glucose level in glucose pre-loades Rabbits. Jurnal Penelitian Sains dan Teknologi. 2005;6(1):26-34.
3. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007;40(3):163-73.
4. Fujisawa T, Ikegami H, Inoue K, Kawabata Y, Ogihara T. Effect of two α-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism. 2005;54(3):387-90.
5. Shi Y, Burn P. Lipid metabolic enzymes: emerging drug targets for the treatment of obesity. Nature reviews Drug discovery. 2004;3(8):695-710.
6. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidative substrates by means of Folin-Ciocalteau reagent, Packer L. Methods in Enzymology. 1999; 299:152-78.
7. Ordonez AAL, Gomez JD, Vattuone MA, Lsla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chemistry. 2006;97(3):452-8.

8. Miller GL. Use of dinitro salicylic acid reagent for determination of reducing sugar. Analytical chemistry. 1959;31: 426-428.
9. Matsui T, Yoshimoto C, Osajima K, Oki T, Osajima Y. In vitro survey of α-glucosidase inhibitory food components. Bioscience, biotechnology, and biochemistry. 1996;60(12):2019-22.
10. Honda M, Hara Y. Inhibition of rat small intestinal sucrase and α-glucosidase activities by tea polyphenols. Bioscience, biotechnology, and Biochemistry. 1993;57(1):123-4.
11. Firdous M, Koneri R, Sarvaraidu CH, Shubhapriya KH. NIDDM antidiabetic activity of saponins of *Momordica cymbalaria* in streptozotocin-nicotinamide NIDDM mice. Journal of Clinical and Diagnosis Research. 2009; 3:1460-5.
12. Koneri R, Balaraman R. Antidiabetic mechanisms of Saponins of *Momordica cymabalaria*. Pharmacognosy Magazine. 2008; 4(15):197–206.