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In-Vitro Evaluation of comparable enzyme-inhibitory potentials of Aegeline and Charantin, in the context of Diabetes Mellitus management

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Abstract:

Diabetes mellitus is considered one of the five leading causes of death in the world affecting an estimated 537 million adults in the year 2021. By 2045, this number is expected to rise to 783 million worldwide. Herbal remedies have been used for centuries to manage diabetes, and many plants have been reported to have antidiabetic activity. Aegle marmelos and Momordica charantia are two plants that have been traditionally used to manage diabetes. The present comparative investigation clearly demonstrates that both Aegeline, an alkaloid from Aegle marmelos, and Charantin, a steroidal saponin from Momordica charantia, exhibit significant and concentration-dependent inhibition of α-amylase activity in vitro. The inhibition profiles of both compounds are remarkably similar, with IC_{50} values approximating 0.6 mg/mL, suggesting that both compounds have comparable enzyme-inhibitory potentials. Diabetes mellitus management, where regulation of postprandial blood glucose is a critical therapeutic target. α-Amylase inhibition delays the breakdown of complex carbohydrates, thereby slowing glucose absorption and reducing blood sugar spikes after meals. The results indicate that both Aegeline and Charantin can serve as natural α-amylase inhibitors, potentially offering a safer and more holistic alternative to synthetic drugs like acarbose. Moreover, the natural origin of both compounds supports their use in plant-based or herbal formulations, which are increasingly being explored for their lower toxicity, affordability, and multifactorial action. The traditional use of Aegle marmelos and Momordica charantia in Ayurvedic and folk medicine aligns with the current findings and reinforces their therapeutic credibility.

Key Words: Aegle marmelos , Momordica charantia, Diabetes mellitus, α-Amylase inhibition, Aegeline, Charantin

I. INTRODUCTION

Diabetes mellitus is considered one of the five leading causes of death in the world affecting an estimated 537 million adults in the year 2021. By 2045, this number is expected to rise to 783 million worldwide. There are two major types of DM, the first being type 1 diabetes (T1DM), characterized by

hyperglycemia due to the autoimmune destruction of pancreas beta cells, resulting in the overall decreased production of insulin. There are two major types of DM, the first being type 1 diabetes (T1DM), characterized by hyperglycemia due to the autoimmune destruction of pancreas beta cells, resulting in the overall decreased production of insulin. The more common version of this metabolic disorder is type 2 diabetes mellitus (T2DM),described by increased insulin release to compensate for insulin resistance and progressive decline in islet secretary function within the pancreas, thus causing overall insulin deficiency. Diabetes is caused by hormonal deregulation and defects in cellular capabilities that lead to improved fasting blood glucose leading to hyperglycemia and glucose intolerance. Type II is the most frequent type of diabetes in adult humans. Obesity is one of the major causes of Type II diabetes and 85-95% of diabetic patients suffer from Type II diabetes. The Several complications can result from diabetes, including nephropathy, retinopathy, neuropathy, and atherosclerosis. Aegle marmelos and Momordica charantia are two plants that have been traditionally used to manage diabetes. The Major bioactive Compounds in Momordica charantia are Polypeptide-p, Peroxidase, Saponins/Terpenoids, Charantin, Bioactive Functions includes antioxidant, antidiabetic, hypoglycaemic, anticancer, hypolipidemic, and antiviral. Aegle marmelous Pharmacological significance includes anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activities. Bael leaves contain compounds like ferulic acid and marmesin which may help enhance insulin secretion and improve glucose uptake by cells. Bael reduces oxidative stress, which plays a role in diabetes complications. Bael also helps maintain gut health, which indirectly supports better metabolic control.

II.PLANT PROFILE

Table 01 Taxonomical Information of Momordica charantia

Kingdom	Plantae	**Vernacular Names**	
Phylum	Streptophyta	**Hindi**	Karela
Class	Equisetopsida	**Punjabi**	Karele
Order	Cucurbitales	**Tamil**	Pavakka
Family	Cucurbitaceae	**Malayalam**	Paavakka
Genus	Momordica	**Marathi**	Karli
Species	charantia	**Telugu**	Kakarakaya
Momordica charantia		**Bitter Gourd, Bitter melon**	

Fig 01 Momordica charantia

Table 02 Taxonomical Information of Aegle marmelous

Kingdom	Plantae	**Vernacular Names**	
Class	Magnoliopsida	**Hindi**	Beal
Order	Sapindales	**Tamil**	Vilva Maram
Family	Rutaceae	**Sanskrit**	Bilva, Bilwa
Genus	Aegle	**Marathi**	Kaveeth
Species	marmelos	**Telugu**	Maredu
Aegle marmelous		**Wood/Stone apple**	

Fig 02 Aegle marmelous

III.MATERIAL AND METHODS:

These objectives highlight the potential anti diabetic effects of Bael and Bitter guard leaves, which may be useful in the management and treatment of diabetes mellitus. The leaves of Bael and Bitter guard were collected and shade dried, before proceedings the both plants were authenticated at Government institute of advanced study in Education, Kurnool, Andhrapradesh-518002.(Voucher No:442, 501). The leaves are milled and ground into a fine powder using a grinding machine This powdered form is stored for further use, such as extraction and In-Vitro Evaluations.

A) Procedure for Extraction of Aegle marmelous Leaf Powder: - 150gms of Bael leaves powder taken for Macerated with 150ml of ethanol and 1500ml water, completely covered the plant material solution with help of aluminum foil. Closed the container and keet it at room temperature for at least 3days, stirred the solution occasionally. After 3days cleaned the liquid extract by filtration. The filtered liquid is transferred in heating mantle to make extract into concentrate. Further the extract is evaluated for Physical and chemical methods of screening including, Moisture content, Ash content, The protein content and carbohydrate contents followed by standard procedures and reports were reported.

Fig 03 Maceration and Extraction of Aegle marmelous

B) Procedure for Extraction of Momordica charantia Leaf powder:

The Soxhlet method used for continuous solvent extraction of Momordica charantia Leaf powder, the solvent containing active constituents of the drug in the siphon tube siphon over runs into the flask, thus emptying the body of extractor. After extraction removed the flask and collected the filtrate and keept in hot air oven, extract is obtained. Further the extract is evaluated for Phytochemical screening Alkaloids and Flavonoids based on literatures by using standard methods and results were reported.

Fig 04 Soxhlet method Extraction of Momordica charantia

C) Procedure for in-vitro studies:

All the Reagents were prepared freshly by following procedures,

A. Preparation of DNS Reagent: Dissolved 0.2 g of 3,5-dinitrosalicylic acid in 20 mL of 40 mM sodium phosphate buffer (pH 6.9). Added 5.98 g of sodium potassium tartrate tetrahydrate and added 0.32 g of NaOH. Further Mixed until all components are fully dissolved.

B.Preparation of Starch Solution (1% w/v) : Dissolved 1 g of soluble starch in 100 mL of 40 mM sodium phosphate buffer (pH 6.9), Heated with constant stirring until fully dissolved further Cooled to room temperature.

C.Preparation of α-Amylase Solution: Dissolved 0.01 g of α-amylase in 10 mL of 40 mM sodium phosphate buffer containing 0.006 M NaCl.

D. Assay Procedure for Aegeline: Aegeline solutions at concentrations ranging from 0.2 to 1.6 mg/mL in distilled water prepared, Reaction Setup as followed , In each well of a 96-well microplate, added 50 µL of Aegeline solution , 150 µL of 1% starch solution and 10 µL of α-amylase solution. For the control, replaced Aegeline solution with 50 µL of distilled water. And for the blank, replaced α-amylase solution with 10 µL of buffer further Incubated the microplate at 37°C for 30 minutes. After incubation added 20 µL of 1 M NaOH to each well and 20 µL of DNS reagent to each well to stop reaction. Further sealed the microplate and place it in a boiling water bath (100°C) for 20 minutes and cooled the plates to room temperature it developed coloration and measured the absorbance at 540 nm. results were reported.

Fig 05 Assay Procedure for Aegeline

E.Assay Procedure for Charantin: solutions was prepared at concentrations ranging from 0.2 to 1.6 mg/mL in distilled water In each well of a 96-well microplate, added 50 µL of Charantin solution, 150 µL of 1% starch solution, 10 µL of α-amylase solution and For the purpose of control, replaced Charantin solution with 50 µL of distilled water and For the blank, replacedα-amylase solution with 10 µL of buffer and Incubated the microplate at 37C for 30 minutes, further added 20 µL of 1 M NaOH and, 20 µL of DNS reagent to each well and sealed the microplate and placed it in a boiling water bath (100°C) for 20 minutes and incubated after incubation Measured the absorbance at 540 nm., results were reported.

Fig 06 Assay Procedure for Charantin

F. Procedure for Aegeline and Charantin Assay in Combination: Reaction Setup as followed In each well of a 96-well microplate, added 50 µL of Charantin solution and Aegeline solution respectively concentrations ranging from 0.2 to 1.6 mg/mL in distilled water. Further added 150 µL of 1% starch solution, 10 µL of α-amylase solution, control is replaced Aegeline and Charantin solution with 50 µL of distilled water, For the blank, replaced α-amylase solution with 10 µL of buffer. Incubated the microplate at 37°C for 30 minutes Added 20 µL of 1 M NaOH and 20 µL of DNS reagent to each well further after Development of color Sealed the microplate and placed it in a boiling water bath (100°C) for 20 minutes. after cooling incubated and Measured the absorbance at 540 nm using a microplate reader.

IV.RESULTS AND DISCUSSION

Table 03 Phyto-Chemical screening of Aegle marmelous and Momordica charantia

Sl.no	Test for Alkaloids	Result	Test for Flavonoids	Results
1	Mayer's test	+ve	Shinoda Test	+ve
2	Dragendorff's Test	+ve	Lead Acetate Test	+ve
3	Wagner's Test	+ve	Sodium Hydroxide Test	+ve

Table 04 Assay Results for Aegeline (Aegle marmelous)

S.NO	Concentration	Absorbance	% Inhibition
1	**0.2**	**0.520**	**20**
2	**0.4**	**0.390**	**40**
3	**0.6**	**0.325**	**50**
4	**0.8**	**0.260**	**60**
5	**1.0**	**0.195**	**70**

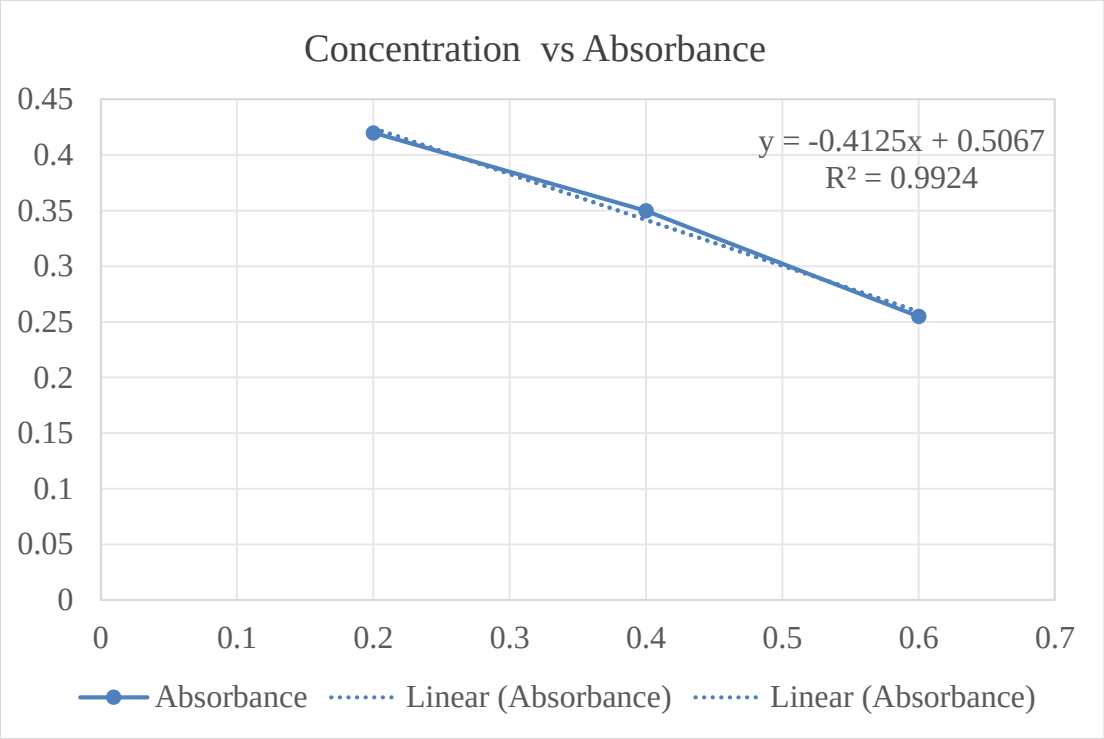

Fig 07 Assay of Aegeline (Aegle marmelous)

Table 05 Assay Results for Charantin (Momordica charantia)

S.NO	Concentration	Absorbance	% Inhibition
1	0.2	0.420	20
2	0.4	0.350	40
3	0.6	0.255	50
4	0.8	0.160	60
5	1.0	0.115	70

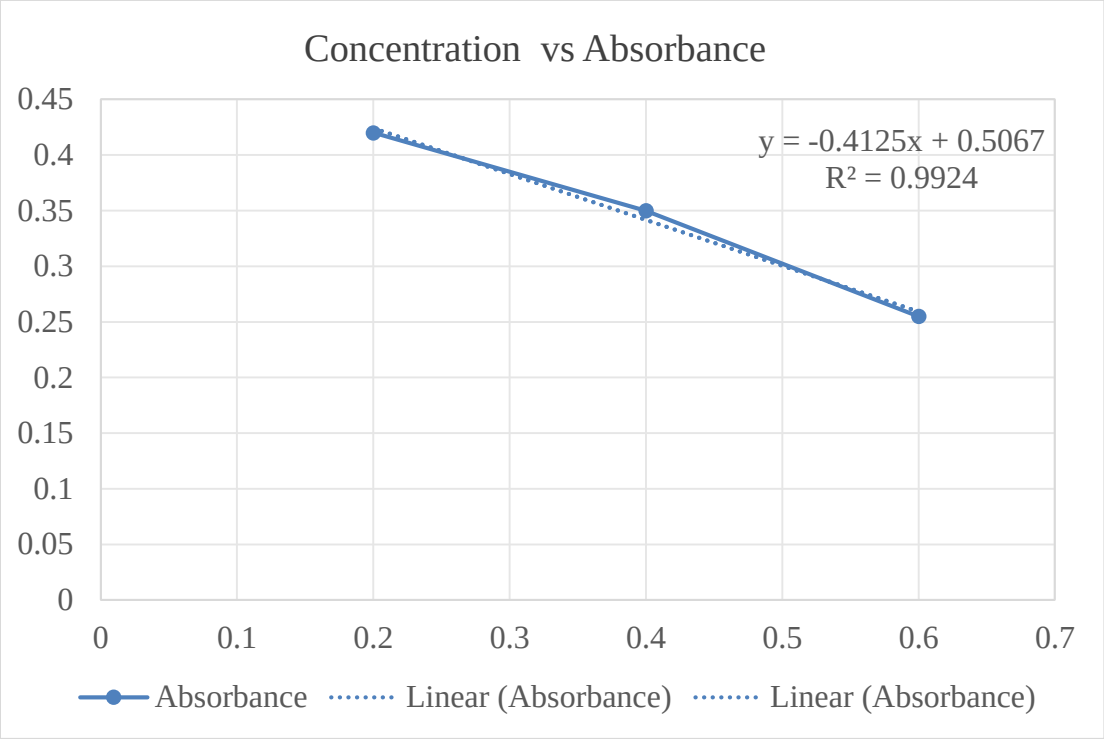

Fig 08 Assay of Charantin (Momordica charantia)

Table 05 Assay Results for Aegeline and Charantin

S.NO	Concentration	Absorbance	% Inhibition
1	0.2	0.38	25
2	0.4	0.31	45
3	0.6	0.215	55
4	0.8	0.12	65
5	1.0	0.075	75

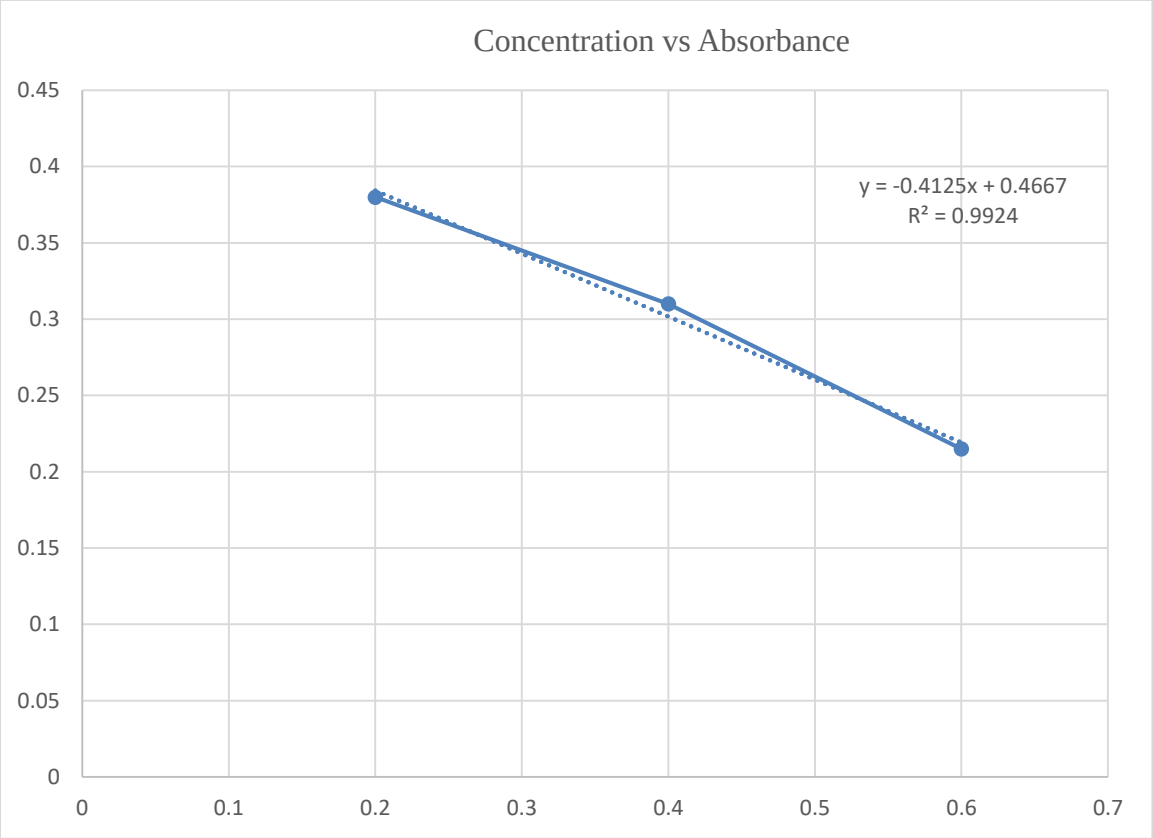

Fig 09 Assay of for Aegeline and Charantin

DISCUSSION:

In this study, **Aegeline** (from Aegle marmelos) and **Charantin** (from Momordica charantia) were evaluated for their α-amylase inhibitory activity using the 3,5-dinitrosalicylic acid (DNS) colorimetric assay. The in vitro inhibition of α-amylase by both agents supports their traditional use in diabetes management. Since aegeline is known for its safety and antioxidant properties, its dual role in enzyme inhibition and oxidative stress reduction could offer broader therapeutic benefits. Similarly, charantin, already known for its hypoglycemic effects in vivo, strengthens its case as a multi-mechanistic antidiabetic agent.

V.CONCLUSION:

The present comparative investigation clearly demonstrates that both Aegeline, an alkaloid from Aegle marmelos, and Charantin, a steroidal saponin from Momordica charantia, exhibit significant and concentration-dependent inhibition of α-amylase activity in vitro. The inhibition profiles of both compounds are remarkably similar, with IC_{50} values approximating 0.6 mg/mL, suggesting that both compounds have comparable enzyme-inhibitory potential. This outcome is particularly meaningful in the context of diabetes mellitus management, where regulation of postprandial blood glucose is a critical therapeutic target. α-Amylase inhibition delays the breakdown of complex carbohydrates, thereby slowing glucose absorption and reducing blood sugar spikes after meals. This study opens up promising

avenues for further mechanistic studies (e.g., molecular docking, enzyme kinetics) Formulation development using either or both compounds for Synergistic studies combining Aegeline and Charantin.

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