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In Vitro Evaluation of Alpha-Amylase Inhibitory Activity of Ethanolic Leaf Extracts of Aegle Marmelos and Turnera Ulmifolia

Satyasuma Sunkara[1], Yanagala Swathi[2], Vipparthi Kavya Sri[2], Yamala Sai Meghana[2], Yalla Jyothi Vijaya Sai[2], Veliventi Siva Kalyani[2], Abhinav VKS Grandhi*[3], Devanaboyina Narendra[4]

[1]Assistant Professor, Department of Pharmacology, VJ's College of Pharmacy, Diwancheruvu, Rajahmundry - 533296, Andhra Pradesh, India.
[2]IV/IV, Bachelor of Pharmacy, VJ's College of Pharmacy, Diwancheruvu, Rajahmundry - 533296, Andhra Pradesh, India.
[3]Assistant Professor, Department of Pharmacy Practice, VJ's College of Pharmacy, Diwancheruvu, Rajahmundry - 533296, Andhra Pradesh, India.
[4]Principal and Professor, Department of Pharmacognosy, VJ's College of Pharmacy, Diwancheruvu, Rajahmundry - 533296, Andhra Pradesh, India.

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Abstract

Aegle marmelos (Bael), a medicinal plant widely used in traditional Ayurvedic medicine, has shown promising antidiabetic, antioxidant, and hepatoprotective effects. Their leaves contain many bioactive constituents such as flavonoids, tannins, alkaloids, and coumarins, contributing to their therapeutic potential. Similarly, Turnera ulmifolia, commonly known as yellow alder, is an ethnomedicinal plant exhibiting antihyperglycemic, antioxidant, and anti-inflammatory activities. This study investigates the in vitro α-amylase inhibitory potential of ethanolic leaf extracts of Aegle marmelos and Turnera ulmifolia. The extracts were prepared by cold maceration and assessed using the DNS assay. Among the tested samples, Aegle marmelos demonstrated notable inhibitory activity, while Turnera ulmifolia showed minimal effect. These findings support the traditional use of Aegle marmelos in diabetes management and highlight the need for further studies to isolate active constituents and evaluate their in vivo efficacy.

Keywords: Aegle marmelos, Turnera ulmifolia, Alpha-amylase inhibition, Postprandial hyperglycemia, Plant-based enzyme inhibitors.

***Corresponding Author**
Dr. Abhinav V.K.S. Grandhi,
Assistant Professor,
VJ's College of Pharmacy,
Rajahmundry - 533296, Andhra Pradesh, India.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder marked by persistent hyperglycemia due to impaired insulin secretion or insulin action or both. It disrupts the normal metabolism of carbohydrates, fats, and proteins, and it is also associated with severe complications, including nephropathy, neuropathy, retinopathy, and cardiovascular diseases [1]. The International Diabetes Federation estimates that there were over 537 million adults globally living with diabetes by 2021, and this number may rise to 643 million by 2030 [2]. India alone is expected to have over 69 million diabetic individuals by 2025, highlighting the urgent need for effective and safe therapeutic options for their cure [3, 27].

Controlling postprandial hyperglycemia is a crucial strategy in the management of type 2 diabetes mellitus. This can be achieved by inhibiting key carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase, which break down complex carbohydrates into glucose [4]. Inhibiting these enzymes may delay carbohydrate digestion, reduce glucose absorption, and ultimately lower post-meal blood glucose spikes [5].

Currently available α-amylase inhibitors, such as acarbose, voglibose, and miglitol, are effective, but their use is often limited due to gastrointestinal side effects such as bloating, flatulence, and diarrhea [6]. Therefore, the search for natural alternatives with fewer side effects has led researchers to explore plant-based inhibitors.

Aegle marmelos (Bael), a medicinal plant widely used in traditional Ayurvedic medicine, has shown promising antidiabetic, antioxidant, and hepatoprotective effects. Its leaves contain several bioactive constituents such as flavonoids, tannins, alkaloids, and coumarins, which contribute to its therapeutic potential [7]. Similarly, Turnera ulmifolia, commonly known as yellow alder, is an ethnomedicinal plant that exhibits

antihyperglycemic, antioxidant, and anti-inflammatory activities [8, 25, 26].

Our present study is designed to evaluate the in vitro α-amylase inhibitory activity of ethanolic extracts of Aegle marmelos and Turnera ulmifolia leaves. The findings from this study may support the development of herbal formulations for managing postprandial hyperglycemia and provide a basis for further pharmacological investigation.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh leaves of Aegle marmelos and Turnera ulmifolia were collected from botanical sources in Andhra Pradesh, India, and authenticated by the Department of Pharmacognosy, VJ's College of Pharmacy. The leaves were washed with distilled water, shade-dried for 7 days, and then powdered using a mechanical grinder. The powdered material was sieved (mesh #60) and stored in airtight containers under cool, dry conditions to preserve phytochemicals [9,10].

Table 01: Phytochemical Screening of Ethanol Extract Of Aegle Marmelos And Turnera Ulmifolia

S.No	Secondary Metabolites	Turnera Ulmifolia Ethanol Leaf Extract	Aegle Marmelos Ethanolic Leaf Extract
1.	Alkaloids	+	+
2.	Flavanoids	+	+
3.	Glycosides	+	+
4.	Phenols	+	+
5.	Saponins	-	+
6.	Steroids	+	+
7.	Tannins	+	+
8.	Triterpenoids	-	+
9.	Reducing sugars	+	+

EXTRACTION PROCEDURE

The dried powders of Aegle marmelos and Turnera ulmifolia were subjected to cold maceration using 95% ethanol as solvent. Specifically, 50 g of A. marmelos and 25 g of T. ulmifolia leaf powders were macerated in 200 mL of ethanol for 48 hours with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper and then evaporated under reduced pressure using a rotary evaporator at 40–50°C. The resultant semisolid extracts were dried and stored in a desiccator over anhydrous calcium chloride until use [11, 12].

Figure 01: Extraction procedure

PHYTOCHEMICAL SCREENING

The qualitative phytochemical analysis is carried out on the ethanolic extracts to identify the presence of secondary metabolites such as alkaloids, flavonoids, phenols, glycosides, tannins, steroids, and saponins. Standard phytochemical procedures were followed, including Dragendorff's test for alkaloids, the Shinoda test for flavonoids, and the Liebermann-Burchard test for steroids [13,14].

ALPHA-AMYLASE INHIBITORY ASSAY

The α-amylase inhibitory activity was evaluated using the dinitrosalicylic acid (DNS) method with slight modifications from the procedures described by Bernfeld and Ademiluyi et al.15,16. In a 96-well plate, 50 µL phosphate buffer (100 mM, pH 6.8), 10 µL porcine pancreatic α-amylase (2 U/mL), and 20 µL plant extract at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 mg/mL) were pre-incubated at 37°C for 20 minutes. Next, 20 µL of 1% soluble starch was added as the substrate, and the mixture was incubated for another 30 minutes at 37°C. The reaction was terminated by adding 100 µL of DNS reagent and heating in a boiling water bath for 10 minutes. After cooling at room temperature, absorbance was measured at 540 nm using a microplate reader. Acarbose served as the standard inhibitor at identical concentrations. All experiments were carried out in triplicate. The percentage inhibition was calculated using the formula:

$$\text{Inhibition } (\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100$$

Where,

As is the absorbance in the presence of the sample

Ac is the absorbance of the control [16, 17].

Procedure for Bael Leaf Extract

Sample (The Bael extract of 1g was dissolved in 10 ml of ethanol), to this add 25 ml of 4 % potato starch (4g in 100 ml)

↓

To this, add 100 mg of α α-amylase and starch solution.

↓

Stir and incubate at 37°C for 60 minutes.

↓

After 0.1M NaOH (To terminate enzyme activity)

↓

Then this solution was observed under UV spectrometry for its absorbance at 540 nm.

↓

Readings were noted

Procedure for Turnera Leaf Extract

Sample (The Turnera leaf extract of 1g was dissolved in 10 ml of ethanol), to this add 25 ml of 4 % potato starch (4g in 100 ml).

↓

To this, add 100 mg of α α-amylase and starch solution.

↓

Stir and incubate at 37°C for 60 minutes.

↓

After 0.1M NaOH (To terminate enzyme activity)

↓

Then this solution was observed under UV spectrometry for its absorbance at 540 nm.

↓

Readings were noted

Procedure for Combination [Aegle + Turnera]

Sample (Aegle marmelos + Yellow alder leaf extract 1g was dissolved in 10 ml of ethanol), to this add 25 ml of 4 % potato starch (4g in 100 ml).

↓

To this, add 100 mg of α α-amylase and starch solution.

↓

Stir and incubate at 37°C for 60 minutes.

↓

After 0.1M NaOH (To terminate enzyme activity)

↓

Then this solution was observed under UV spectrometry for its absorbance at 540 nm.

↓

Readings were noted

Results

The α-amylase inhibitory activity of ethanolic extracts of Aegle marmelos, Turnera ulmifolia, and their combination was assessed using the DNSA method. The absorbance values were recorded at 540 nm, and the per cent inhibition was calculated.

Table 02: Absorbance Values

SAMPLE	ABSORBANCE
Blank	0.003
Bale	1.471
Bale + α amylase	1.540
Yellow alder	1.653
Yellow alder + α amylase	1.667
Combination	1.560
Combination + α amylase	1.574

The percentage inhibition of α-amylase was calculated using the formula:

$$\text{Inhibition } \% = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

Where:

- Acontrol = Absorbance of the control (enzyme + DMSO)
- Asample = Absorbance of the test sample (enzyme + plant extract)

Among the three test groups, Aegle marmelos showed the highest inhibitory activity (4.4%), while Turnera ulmifolia and the combination extract displayed lower inhibition (0.83% and 0.88%, respectively).

These findings suggest that Aegle marmelos has greater potential for inhibiting α-amylase activity compared to Turnera ulmifolia, with no synergistic enhancement observed in the combined extract.

Calculation

% Inhibition = [(Absorbance of Control - Absorbance of Sample) / Absorbance of control] ×100

Calculation for Aegle marmelos

% Inhibition = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] ×100

= (1.540 – 1.471) ÷ 1.540 × 100

= 0.069 ÷ 1.540

= 0.044 × 100

= 4.4

Calculation for Turnera ulmifolia

% Inhibition = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] ×100

= (1.667 – 1.653) ÷1.667× 100

= 0.014 ÷ 1.667 × 100

= 0.0083 × 100

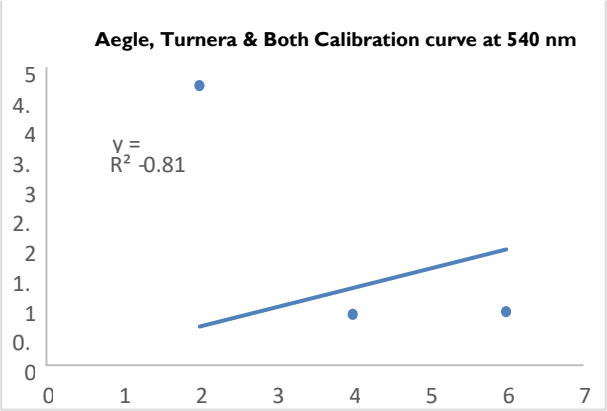
= 0.83

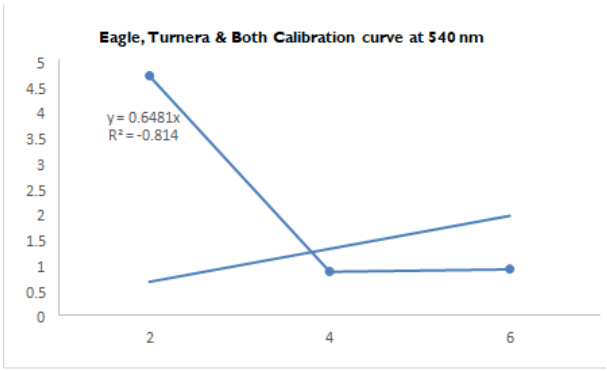
Calculation for Combination

% Inhibition = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] ×100
= (1.574– 1.560) ÷ 1.574 × 100
= 0.014 ÷ 1.574 × 100
= 0.0088× 100
= 0.88

Calibration Curve

The calibration curve generated at 540 nm for absorbance vs. concentration was used to confirm linearity in colourimetric response:





Aegle marmelos, Turnera ulmifolia, and the combination yielded linear responses with an equation of the form:

y = 0.6481x, R² = 0.814.

DISCUSSION

The present study was performed to evaluate the in vitro α-amylase inhibitory activity of ethanolic extracts of Aegle marmelos, Turnera ulmifolia, and their combination. The results indicate that Aegle marmelos extract demonstrated the highest percentage inhibition (4.4%) compared to Turnera ulmifolia (0.83%) and the combined extract (0.88%). These findings suggest that A. marmelos possesses more potent enzyme-inhibitory constituents compared to T. ulmifolia, and that their combination did not exhibit any synergistic effect.

The inhibition of α-amylase is a well-established mechanism for managing postprandial hyperglycemia in type 2 diabetes mellitus by delaying carbohydrate digestion and glucose absorption [18]. Acarbose, a commonly used α-amylase inhibitor, is effective but often associated with gastrointestinal side effects such as flatulence and diarrhoea [19]. The search for safer plant-based alternatives has gained momentum.

The moderate α-amylase inhibitory activity exhibited by A. marmelos in this study aligns with earlier reports highlighting its antidiabetic and enzyme-inhibiting potential20. This activity is attributed to the presence of flavonoids, tannins, and alkaloids, which can interact with the enzyme's active site and inhibit its catalytic function21. In contrast, T. ulmifolia, although traditionally used for managing diabetes and inflammation, exhibited negligible α-amylase inhibition in this study. This discrepancy may be due to the lower concentration or absence of potent inhibitory phytoconstituents in the ethanolic extract [22].

Interestingly, the combination of the two extracts did not enhance the inhibitory effect. This could be due to potential antagonistic interactions between certain phytochemicals or dilution of the active constituents of A. marmelos when mixed with the less active T. ulmifolia extract [23]. A similar lack of synergism has been observed in other plant extract combinations where the interaction of secondary metabolites reduces bioactivity [24].

Although the inhibition values were modest, especially when compared to standard drugs, they indicate potential for further optimisation. Fractionation of the extracts, isolation of bioactive compounds, and structural characterisation may yield more potent α-amylase inhibitors suitable for therapeutic use. Additionally, further in vivo studies are warranted to establish pharmacokinetic and pharmacodynamic profiles.

CONCLUSION

The present study demonstrated that the ethanolic extract of Aegle marmelos leaves possesses notable in vitro α-amylase inhibitory activity compared to Turnera ulmifolia and their combination. The moderate inhibitory effect of A. marmelos supports its traditional use in the management of diabetes and highlights its potential as a source of natural enzyme inhibitors. In contrast, T. ulmifolia exhibited only minimal inhibitory activity under the test conditions, and the combination extract did not enhance the overall effect, indicating a possible lack of synergistic interaction. These findings provide a scientific rationale for the antidiabetic use of A. marmelos and emphasise the need for further studies, including bioassay-guided isolation of active compounds, mechanistic studies, and in vivo evaluations, to fully explore its therapeutic potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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AUTHOR'S CONTRIBUTION

S.S Conceptualisation, methodology, and supervision; V.K.S, Y.S.M, Y.S, Y.J.V.S, V.S.K, Experimental work, data collection, and preliminary analysis; A.V.K.S.G Manuscript writing, critical revision, and correspondence; D.N Review, validation, and final approval of the manuscript

ETHICAL STATEMENT

The study did not involve human participants or animal experiments. All plant materials used in the research were collected under institutional, national, and international guidelines. The study complies with ethical standards in laboratory research involving medicinal plant extracts.

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