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Evaluation of Alpha-Amylase Inhibition by *Catharanthus roseus* and *Azadirachta indica* Leaf Extracts: An *In Vitro* Approach

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Abstract
Background: Type II diabetes mellitus specifically impairs glucose metabolism and poses severe health risks, with its prevalence rapidly rising in India. Conventional drugs like metformin and enzyme inhibitors manage blood sugar but often cause gastrointestinal side effects. α-Amylase inhibition has emerged as a targeted approach to control postprandial glucose spikes. *Catharanthus roseus* and *Azadirachta indica*, long used in traditional medicine, offer rich phytochemical profiles with proven antidiabetic potential.
Methodology: *Catharanthus roseus* and *Azadirachta indica* fresh leaves were washed, shade dried, powdered and extracted with ethanol through Soxhlet extraction. Rotary evaporator was used to concentrate crude extracts to be analyzed further. Phytochemical screening revealed tannins, alkaloids, flavonoids and glycosides. The starch-iodine colorimetric method was used in the assessment of alpha-amylase inhibition. Extracts and acarbose were assayed at 200-1000 µg/mL with the reaction incubated for 1 hour at 37°C. The enzyme inhibition was measured at 565 nm as a comparison between plant extracts and the standard drug acarbose.
Results: This study confirmed that the two plant extracts contained bioactive compounds including glycosides, alkaloids, flavonoids and tannins. All samples exhibited dose-dependent inhibition of alpha-amylase, with the polyherbal blend achieving the highest efficacy (71.85% at 1000 µg/mL; IC_{50} = 534.39 µg/mL), approaching that of Acarbose (74.7%; IC_{50} = 513.97 µg/mL). The single plant extracts showed moderate inhibitory properties, and this shows the enhanced efficacy of a combination of these extracts in a synergistic manner.
Conclusion: The current research demonstrates the strong antidiabetic activity of a polyherbal combination of *Catharanthus roseus* and *Azadirachta indica*. The starch-iodine method of quantifying alpha-amylase inhibition was dose dependent with the combined extract being more effective than the individual extracts. The findings indicate that such synergistic treatment can be effective in improving the treatment of type 2 diabetes with the possible reduction of side effects associated with standard treatment.

Keywords: *Catharanthus roseus*, *Azadirachta indica*, Glycemic activity, Polyherbal formulation.

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by high blood glucose levels due to the deficiencies of insulin production. Heart disease, kidney failure, nerve damage, and premature death are serious side effects that may be caused by it. Type II diabetes is the most common type and it currently afflicts 77 million individuals in India and it is estimated that it will rise to 134 million by 2045. Treatment involves insulin, lifestyle changes, and oral medications. Metformin helps by reducing sugar production in the liver and increasing absorption in the gut. Other drugs like Acarbose, Miglitol, and Voglibose slow carbohydrate digestion by blocking enzymes like α-glucosidase and α-amylase, which helps control blood sugar after meals, though they may cause stomach issues like gas and diarrhea.[1]
α-Amylases are key endoenzymes that hydrolyze α-D-(1→4) glycosidic bonds in dietary carbohydrates, breaking them down into absorbable monosaccharides. Inhibiting α-amylase slows carbohydrate digestion, reducing postprandial blood glucose levels making it a promising approach for diabetes management. However, current α-amylase inhibitors often lack specificity

and can cause adverse effects, limiting their clinical utility. Interestingly, ethnobotanical studies highlight nearly 800 medicinal plants with potential antidiabetic properties, offering a natural alternative for enzyme inhibition.[2]

Catharanthus roseus, or periwinkle, is a perennial herb from the Apocynaceae family, prized for both its ornamental beauty and medicinal value. Native mostly to Madagascar, with one species each from India, Sri Lanka, and China, it has a rich history in global traditional medicine. Introduced to India by the Portuguese, it has been used in Ayurveda, Unani, Siddha, and Chinese practices to manage ailments like muscle pain, oral ulcers, wasp stings, and emotional distress.

This plant which has a rich phytochemical content has a wide spectrum of therapeutic effects such as antidiabetic and antihypertensive effects. It still remains an important part of ethnopharmacology. This review gives a brief overview of its medicinal significance as a useful tool to the researcher.[3]

Neem (Azadirachta indica) is a very important medicinal plant with a wide range of biological properties. It has been traditionally used in medical care of various diseases, including malaria, neurological and muscular pain, skin infections, diabetes, cancer, and cardiovascular disorders. Although its antidiabetic prospective is well recognized, comprehensive information regarding its precise mechanism of action remains limited.[4]

Catharanthus roseus

Fresh leaves

Shade dried leaves

Powder

Azadirachta indica

Fresh leaves

Shade dried leaves

Powder

MATERIALS AND METHODS

Sample Preparation and Extraction

Healthy, fresh leaves of Catharanthus roseus and Azadirachta indica were handpicked and meticulously rinsed with clean water to eliminate any adhering dust or surface contaminants. The leaves were then shade-dried to prevent degradation of heat-sensitive phytoconstituents. An electric grinder was used to ground the leaves into a coarse powder when they had completely dried.

The resulting powders underwent individual extraction processes using the Soxhlet apparatus, a highly efficient method known for minimizing solvent usage. For each extraction, 50 grams of powdered leaf material were carefully packed into a cellulose thimble and extracted using 500 mL of ethanol, adhering to a standardized 1:10 solid-to-solvent ratio. For eight hours, the extraction process was carried out at temperature slightly below ethanol's boiling point. After extraction, the crude extracts were concentrated using a rotary evaporator to remove the solvent, yielding the final concentrated plant extracts for further analysis.

Soxhlet extractor system

Rotary evaporator

Preliminary Phytochemical Screening

A preliminary analysis was conducted on the leaf extracts of *Catharanthus roseus* and *Azadirachta indica* to identify the presence of different phytochemical constituents. The screening procedures followed established protocols as outlined in standard pharmacognosy texts (Kokate, 2008; Khandelwal, 2008).

Inhibition of Alpha-Amylase Enzyme Assay

Pancreatic α-amylase, classified as an α-1,4-glucanohydrolase, plays an important function in carbohydrate digestion by initiating the breakdown of starch as smaller sugar molecules such as maltotriose and maltose. These sugars are subsequently hydrolysed by α-glucosidases into glucose, which is then absorbed into bloodstream. In the therapeutic landscape of managing diabetes, inhibition of α-amylase represents a well-established intervention aimed at attenuating postprandial glycemic excursions an essential objective in the regulation for type 2 diabetes mellitus. Phytochemicals possessing α-amylase inhibitory properties have exhibited substantial potential in mitigating postprandial hyperglycemia, thereby offering a compelling, naturally derived alternative to synthetic pharmacological agents.

Materials

Instrument:
"Shimadzu UV-Visible Spectrophotometer (Model 1800)"
Reagents Used:
Alpha-amylase solution: This was prepared by dissolving 27.5 mg of the enzyme in 100 ml of distilled water.
Substrate solution: 1% soluble starch - enzymatic substrate.
Colour indicator: Iodine–potassium iodide solution- residual starch.
Buffer system: 0.1 M sodium acetate buffer.

Procedure

Starch-iodine colorimetric approach was utilized to identify the inhibitory effect of plant extracts on the alpha-amylase activity. The stock solutions were made by mixing the plant extract and the reference drug, acarbose in 1:1 ratio, 1 mg/mL each, in 0.1 M sodium acetate buffer as a solvent. These stock solutions were diluted to give a series of dilutions to give final concentrations of 200-1000 µg/mL. An enzymatic reaction was performed on each of the concentrations to identify the degree of inhibition for alpha-amylase. The inhibitory potency was then analyzed and compared across the different concentrations to determine the concentration-dependent efficacy of extract of plant in combination with acarbose. Alpha-amylase enzyme (0.1 mL), 0.1 mL of 1% soluble starch, and 2mL of 0.1 M sodium phosphate buffer (pH 7.2) were added to each test tube. For one hour, the mixture was incubated at 37°C. After incubation, 0.1 millilitres of the iodine-iodide indicator were introduced. A UV-Visible spectrophotometer was used to detect the resultant color intensity at 565 nm. The blank was sodium acetate buffer, while the controls were reactions devoid of plant extracts. Included as a reference inhibitor was acarbose.

RESULTS AND DISCUSSION

Phytochemical constituents present in extracts of *Catharanthus roseus* and *Azadirachta indica* leaves.

Tab: Details of qualitative phytochemical tests:

Sl. No.	Test	Result	
		CREE	AIEE
1	**Test for Carbohydrates**		
	Molisch's test	+	+
	Fehling's test	+	+
	Barfoed's test	+	+
2	**Test of starch**	+	+
3	**Test for Proteins and amino acids**		
	Million's test	+	+
	Biuret test	+	+
	Ninhydrin test	+	+
4	**Test for Phenolic compounds and tannins**		
	Ferric chloride test	+	+
	Test with Lead acetate Solution	+	+
	Gelatin test	+	+
5	**Test for Phytosterols**		
	Salkowski test	+	+
	Libermann – Burchards test	+	+
6	**Test for Fixed oils and fats**		
	Spot test	+	+
	Saponification	+	+

7	**Test for Alkaloids**		
	Mayer's test	+	+
	Dragendroff's test	+	+
	Wagner's test	+	+
	Hager's test	+	+
8	**Test for Glycosides**		
	Legal's test	+	+
	Balget's test	+	+
	Borntrager's test	+	+
	Modified Borntrager's test	+	+
9	**Test for Flavonoids**		
	Ferric chloride test	+	+
	Shinoda's test	+	+
	Fluorescence test	+	+
	Reaction with alkali and acid	+	+
	Zinc, HCl reduction test	+	+
	Test for Flavonoids	+	+
10	**Test for Saponins**	+	+

INHIBITION OF ALPHA AMYLASE ENZYME ASSSAY

The table and the graphical depiction in Fig. show the findings of the α-amylase enzyme test inhibition.

Tab: Inhibition of alpha amylase enzyme assay

Conc. µg/ mL	CREE		AIEE		Polyherbal Combination (1:1)		Acarbose	
	Absorbance at 565nm*	% Inhibition	Absorbance at 565nm*	% Inhibition	Absorbance at 565nm*	% Inhibition	Absorbance at 565nm*	% Inhibition
200	0.06±0.0007	26.22±0.63	0.07±0.0008	28.23±0.83	0.091±0.001	39.89±0.18	0.094±0.001	41.44±1.08
400	0.083±0.001	38.87±0.55	0.094±0.002	40.87±0.45	0.108±0.003	44.77±1.42	0.113±0.003	45.89±1.64
600	0.113±0.001	53.52±0.31	0.124±0.001	55.63±0.41	0.142±0.005	61.26±0.16	0.147±0.001	62.14±0.32
800	0.123±0.002	57.04±0.10	0.134±0.003	59.05±0.10	0.176±0.005	68.71±1.80	0.184±0.002	70.36±0.26
1000	0.152±0.001	65.38±0.44	0.163±0.002	66.37±0.55	0.195±0.002	71.85±0.41	0.224±0.001	74.70±0.58
IC_{50}	619.73 µg/mL		639.83 µg/mL		534.39 µg/mL		513.97µg/mL	

*** *Mean of three readings ±SEM**

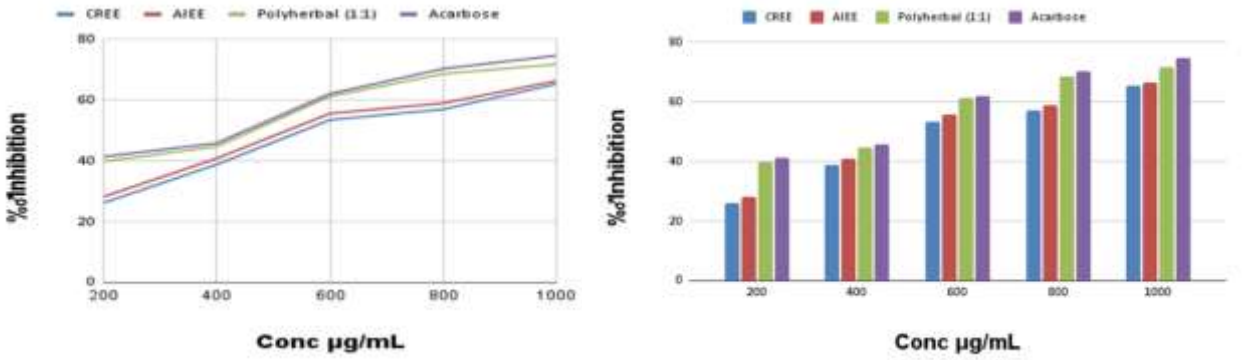

Fig: Inhibition of alpha amylase enzyme assay

Analysis of Table indicates that the polyherbal combination (1:1) demonstrated notably higher antidiabetic activity compared to the individual extracts of *Catharanthus roseus* ethanol extract (CREE) and *Azadirachta indica* ethanol extract (AIEE). In the process of in vitro α-amylase inhibition assay both CREE and AIEE showed inhibitory effects on the enzyme, with all samples exhibiting a concentration-dependent increase in inhibition across the tested range (200–1000µg/mL).

At 1000 µg/mL, the maximum concentration tested, the polyherbal combination (1:1) achieved a maximum inhibition of 71.85±0.41%, while CREE and AIEE recorded 65.38±0.44% and 66.37±0.55% inhibition, respectively. For comparison, the standard drug acarbose showed 74.7±0.58% inhibition at the same concentration. At the lowest concentration (200µg/mL), the polyherbal combination (1:1), CREE, AIEE, and acarbose showed minimal inhibition values of 39.89±0.18%, 26.22±0.63%, 28.23±0.83%, and 41.44±1.08%, respectively.

The polyherbal combination (1:1) consistently showed stronger α-amylase inhibitory activity than the individual extracts. The IC_{50} values, signifying the focus necessary to inhibit 50% of α-amylase activity, were 534.39 µg/mL for the polyherbal combination, 619.73 µg/mL for CREE, 639.83 µg/mL for AIEE, and 513.97 µg/mL for acarbose. These results suggest that the polyherbal combination is more effective than either extract alone, though slightly less potent than acarbose, the standard reference inhibitor.

Alpha-amylase is in charge of breaking down alpha-bonds in big polysaccharides like starch and glycogen to create glucose and maltose. Inhibitors of this enzyme attach to the polysaccharide substrate, preventing its breakdown into simpler sugars. In this study, the polyherbal combination (1:1) demonstrated significant inhibition of α-amylase activity, surpassing the effects observed with the individual extracts.

CONCLUSION

This study illustrates the significant antidiabetic potential of a polyherbal formulation comprising *Catharanthus roseus* and *Azadirachta indica*. The alpha-amylase inhibition test by the starch-iodine method was used to determine the antidiabetic activity and all the tested extracts had significant inhibitory effects. The synergistic effect of C. roseus and A. indica was improved and dose-dependent in the alpha-amylase inhibition and the polyherbal mixture was more active than the individual extracts. These findings imply that a mixture of plant extracts can be utilized to enhance the antidiabetic effect and can offer therapeutic benefits in the treatment of type 2 diabetes, which may reduce side effects of the traditional drugs.

SUMMARY

The aim of this in vitro experiment was to determine the antidiabetic effect of a polyherbal mixture of Catharanthus roseus and Azadirachta indica. The Soxhlet extraction was carried out with ethanol to extract the plant extracts and the phytochemical analysis revealed that the plant extracts have significant bioactive compounds such as tannins, alkaloids, flavonoids and glycosides. It is vital to mention that the formulation was capable of inhibiting the reactions of alpha-amylase, which is a pointer that it can be of therapeutic benefit in the treatment of diabetes.

Starch-iodine colorimetric method was used to determine the alpha-amylase inhibitory activity that exhibited dose dependent response. The polyherbal extract had improved inhibitory ability as compared to the single plant extracts. Its IC_{50} was 534.39 µg/mL, nearly the same as the standard antidiabetic compound acarbose that had an IC_{50} of 513.97 µg/mL.

The findings indicate that there was a synergistic effect between the two plant extracts and the antidiabetic effect was potentiated. The polyherbal preparation is a potential alternative in management of type 2 diabetes with the benefit of containing fewer side effects than the standard pharmaceutical drugs.

References

1. Joseph N, Jiji MG. In Vitro alpha amylase inhibitory effect of selected medicinal plants. Int J Pharm Res Allied Sci. 2023;12(4):47–54. doi:10.51847/Vh0CtKQ2WS
2. Ishnava KB, Motisariya DM. In-vitro study on α-amylase inhibitory activity of selected ethnobotanical plant extracts and its herbal formulations. *Int J Pharmacogn Chinese Med.* 2018;2(3):136. doi:10.23880/IPCM-16000136.
3. Chaturvedi V, Goyal S, Siddiqui MM, Meghani M, Patwekar F, Patwekar M, Khan SK, Sharma GN. A comprehensive review on *Catharanthus roseus* L. (G.) Don: clinical pharmacology, ethnopharmacology and phytochemistry. *J Pharmacol Res Dev*. 2022;4(2):17–36. doi:10.46610/JPRD.2022.v04i02.003.
4. Kazeem MI, Dansu T, Adeola SA. Inhibitory effect of *Azadirachta indica* A. Juss leaf extract on the activities of α-amylase and α-glucosidase. *Pak J Biol Sci*. 2013;16(21):1358–62. doi:10.3923/pjbs.2013.1358.1362.
5. Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). Current Science, 82(11), 1336-1345.
6. Kumar, S., Kumar, D., Deshmukh, R. R., Lokhande, P. D., & More, S. N. (2012). Antidiabetic potential of *Catharanthus roseus* Linn. and its effect on biochemical parameters in streptozotocin induced diabetic rats. Asian Journal of Pharmaceutical and Clinical Research, 5(3), 114-117.
7. Sudha, P., Zinjarde, S. S., Bhargava, S. Y., & Kumar, A. R. (2011). Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine, 11(1), 5.
8. Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Medicinal & Aromatic Plants, 4(3), 196. https://doi.org/10.4172/2167-0412.1000196
9. Kokate, C.K. (2008). Practical Pharmacognosy (4th ed.). Vallabh Prakashan.
10. Khandelwal, K.R. (2008). Practical Pharmacognosy: Techniques and Experiments (19th ed.). Nirali Prakashan.
11. Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2011). α-Glucosidase and α-amylase inhibitory activities of *Salvia officinalis* L. and *Salvia fruticosa* Mill. extracts. Food Chemistry, 126(2), 664-670.
12. Abo Baker, S. M., et al. (1988). A simple and rapid method for the determination of alpha-amylase activity. Analytical Biochemistry, 174(2), 536-53