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Evaluation of In vitro Antidiabetic Potentials of a Polyherbal Formulation Using In Vitro Alpha-Amylase Inhibition Assay

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ABSTRACT

The current study investigates the synergistic antidiabetic potential of a polyherbal formulation (PHF) comprising six medicinal plants: *Gymnema sylvestre*, *Phyllanthus emblica*, *Tinospora cordifolia*, *Ocimum tenuiflorum*, *Withania somnifera*, and *Coleus forskohlii*. The plants were selected based on their ethnomedicinal use, authenticated, and subjected to aqueous and ethanolic extraction. The antidiabetic activity was assessed in vitro using the porcine pancreatic alpha-amylase inhibition assay, with acarbose as the standard. Results revealed the hot water extract exhibited significant inhibition (IC50 = 41.84 µg/ml), outperforming the ethanolic extract (IC50 = 66.62 µg/ml) and the standard (IC50 = 83.19 µg/ml). These findings highlight the potential of the PHF for therapeutic use in managing diabetes mellitus, justifying further in vivo and clinical evaluation.

Keywords: Polyherbal formulation, Antidiabetic activity, *Gymnema sylvestre*, *Phyllanthus emblica*, *Tinospora cordifolia*, *Withania somnifera*, *Coleus forskohlii*

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global prevalence of DM continues to rise, demanding effective, safe, and affordable therapeutic strategies [1-3]. Medicinal plants offer a rich source of bioactive compounds with potential antidiabetic activity, often acting synergistically when combined in polyherbal formulations [4-6].

Polyherbal formulations (PHFs) leverage the pharmacological synergy of multiple plant species, providing enhanced efficacy and reduced side effects compared to single-herb preparations [7-9]. In traditional medicine systems, such as Ayurveda, plants like *Gymnema sylvestre*, *Phyllanthus emblica*, *Tinospora cordifolia*, *Ocimum tenuiflorum*, *Withania somnifera*, and *Coleus forskohlii* are well-documented for their antidiabetic properties [10-12]. These plants act through various mechanisms, including modulation of carbohydrate metabolism, inhibition of carbohydrate-digesting enzymes, and antioxidant activity [13-15].

Alpha-amylase is a key enzyme in carbohydrate digestion, catalyzing the hydrolysis of polysaccharides into glucose. Its inhibition can effectively reduce postprandial hyperglycemia, making alpha-amylase inhibitors a promising therapeutic target for DM management [16-20]. This study aims to evaluate the antidiabetic efficacy of a PHF prepared from the selected plants through an in vitro alpha-amylase inhibition assay. The results will contribute to the development of cost-effective herbal therapeutics for DM.

MATERIALS AND METHODS

Collection of Plant Material:

- The present study is aim to screen the synergetic antidiabetic potential of six selected medicinal plants. Initially screen by the taxonomist of Institute of trans-disciplinary health sciences and technology (Transdisciplinary University) and

Once again in 2015 the plants were re- authenticated after taxonomical screening before submission of the thesis (Final authentication is attached with the copy of the thesis). The plant materials were collected from western ghat and Bangalore rural zone. In the polyherbal preparation, leaves of *Tinnospora cordifolia, ocimum sanctum, Gymnema sylvestre* were selected, where as in the plants like *Withania somnifera* and *Coleus forskohlii*, the root part of the plant was selected, and the plant like *Phyllanthus emblica*, the fruit portion of the plant was selected to prepare the polyherbal formulation and to screen the antidiabetic and antioxidant therapeutic value through in vitro and in vivo studies.

Table 1: Authenticated plant species

S. No.	Botanical Name	Family	Part Traded
1	*Gymnema sylvestre* (Retz.). ex Sm.	Apocynaceae	Leaves
2	*Phyllanthus emblica* L.	Phyllanthaceae	Fruits
3	*Tinospora cordifolia* (Wild.) Miers	Menispermaceae	Leaves
4	*Ocimum tenuiflorum* L.	Lamiaceae	Leaves
5	*Withania somnifera* (L.) Dunal	Solanaceae	Roots
6	*Coleus forskohlii* (willd.) Briq.	Lamiaceae	Roots

Alpha-Amylase Inhibition Assay

Initially, preliminary digestion by the salivary amylase results in the degradation of polymeric starch substrates into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic α-amylase into maltose, maltriose and malto- oligosaccharides. The gut enzyme (α-amylase) is responsible for the hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of α-amylase can lead to reduction in post prandial hyperglycaemia in diabetic condition.

Principle:

This enzyme activity can be measured in-vitro by hydrolysis of starch in the presence of α-amylase enzyme. This process is further quantified by using iodine, which develops blue color with starch. The reduced intensity of blue color indicates the enzyme induced hydrolysis of starch into monosaccharides. If the extract possesses α- amylase inhibitory activity, the intensity of blue colour will be more. In other words, the intensity of blue color in test sample is directly proportional to α-amylase inhibitory activity to assess the antidiabetic efficacy.

Procedure:

The hot water and ethanolic extract of polyherbal formulation were serially diluted to obtain required concentration to prove the alpha amylase inhibition assays.

Preparation of working solutions:

Phosphate buffer (0.1 M,pH 7, 25◦C):

It was prepared using 39.0 ml of 0.2 M monobasic sodium phosphateand 61.0 ml of 0.2 M dibasic sodium phosphate and diluting to a total volume of 200 ml, to make a 0.1 M phosphate buffer of the required pH 7.0 at room temperature.

Enzyme (0.48412units/ml): 2.65mg of □-amylase is made up to 100 ml with 0.1 M phosphate buffer pH 6.9. (Enzyme units are lot specific).

Starch 1%: 1% of starch solution was prepared by dissolving 1.0 gm soluble starch, in 100 ml 0.02 M sodium phosphate buffer and pH 6.9 with 0.006 M sodium chloride and gentle boil to dissolve. Cool and bring volume to 100 ml, using water, if necessary. Then incubate at 25°C for 4-5 minutes prior to assay.

DNS (3, 5 Dinitrosalicylic acid): 1.0 gm of 3, 5-dinitrosalicylic acid was dissolved in 50 ml of reagent grade water to this 30.0 gms sodium potassium tartrate tetrahydrate was added slowly. Add 20 ml of 2 N NaOH. Dilute to a final volume of 100 ml with reagent grade water. Precaution was followed to protect from carbon dioxide and store no longer than 2 weeks.

Positive control:

Stock 1: (1mg/ml): 50mg of Acarbose dissolved in 50ml of 0.1M Phosphate buffer, pH 6.9.

Stock 2: Diluted to a concentration of 2.5µg/ml with 0.1M Phosphate buffer, pH 6.9.

Working stock: Diluted to a concentration of 0.25µg/ml with 0.1M Phosphate buffer, pH 6.9.

Sample preparation:

A sample stock of 310µg/ml was prepared for the sample by dissolving 3.1mg of the sample in 10ml with 0.1M Phosphate buffer, which was adjust for pH 6.9. Further dilutions were made as required with 0.1M Phosphate buffer and pH 6.9 was maintained.

Positive control: Stock- 50 mg of Acarbose in 50 ml was prepared to set 0.1M phosphate buffer.

Procedure

DNS (3, 5 Dinitrosalicylic acid) method it was performed to screen the α-Amylase inhibitory activity, by quantifying the reducing sugar which is liberated in the assay. The enzyme inhibitory efficacy was expressed as decrease in units of liberated glucose. Plant extract concentrations from 10-100µg were incubated with 1ml of 1unit Porcine Pancreatic Alpha-Amylase enzyme for 30minnutes at 37°C. After incubation 1ml of 1% buffered starch was added and the mixture was further incubated for 10minutes duration at room temperature. The reaction was made to stop by adding 1ml DNS reagent and the contents were heated in boiling water bath for duration of 5minutes. Blank was prepared without adding plant extract and enzyme which was replaced with equal quantity of 0.1M phosphate buffer. Control representing 100% enzyme activity. The absorbance was read at 540nm using UV Spectrophotometer. The standard antidiabetic drug Acarbose was utilised as positive control. The antidiabetic property was determined through inhibition of alpha amylase which is expressed as % of inhibition. % of inhibition was calculated as below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test} \times 100}{\text{Absorbance of control}}$$

IC50 values of Acarbose and Polyherbal extracts of hot water and ethanolic solvents were determined to plots the percentage inhibition versus concentration (µg/ml).

RESULTS AND DISCUSSION

Polyherbal formulation (PHF) extracts of two different organic solvents exhibited significant reduction in alpha-amylase activity. Acarbose standard antidiabetic drug which was used as positive control at concentrations 10-100µg/ml showed PPA inhibitory activity from 18.75% to 58.60% with an IC50 value of 83.19µg/ml. Polyherbal formulation of hot water extract at concentration10-100µg/ml showed maximum inhibitory effects on alpha-amylase activity from 23.44% to 88.22%with an IC50 value of 41.84µg/ml also carried out. Polyherbal formulation of Ethanolic extracts at concentrations 10-100µg/ml showed moderate inhibitory effects on Alpha-Amylase activity from 17.05% to 70.05% with anIC50 value of 66.62µg/ml. Out of two selected solvent the hot water extract showed maximal inhibition which indicates its maximal therapeutic efficacy.

Table 2: Percentage inhibition and IC50 Values of Acarbose on Alpha- Amylase

	Concentration (µg/ml)	% Inhibition	IC50 Value
Standard durg (Acarbose)	10	18.75	83.19 µg/ml
	20	22.41	
	40	29.73	
	60	38.74	
	80	48.27	
	100	58.60	

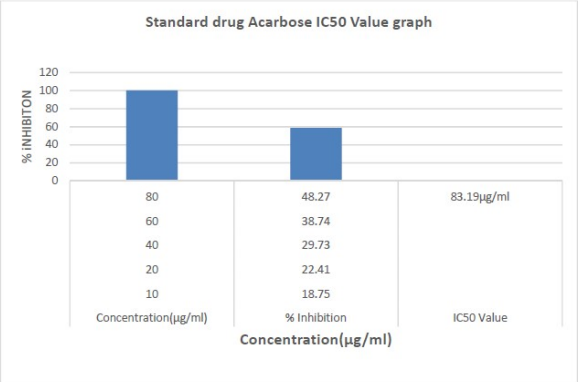

Fig. 1: Acarbose standard drug IC50 Value graph

Table 3: PHF showing maximum inhibitory effects on alpha-amylase activity

	Extract	Concentration (µg/ml)	% Inhibition	IC50 Value
Polyherbal formulation	Hot water	10	23.44	41.84 µg/ml
		20	41.23	
		40	50.51	
		60	61.62	
		80	73.64	
		100	88.22	

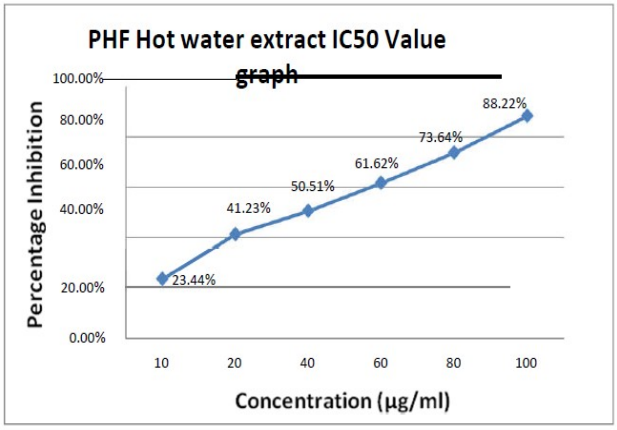

Fig.2: PHF Hot water extract IC50 value graph

Table 4: PHF showing moderate inhibitory effects on alpha-amylase activity

	Extract	Concentration (µg/ml)	% Inhibition	IC50 Value
Polyherbal formulation	Ethanol	10	17.05	66.62 µg/ml
		20	26.01	
		40	32.33	
		60	41.10	
		80	61.11	
		100	70.05	

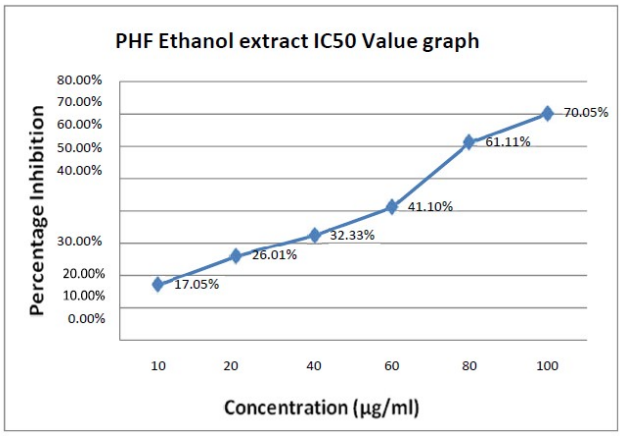

Fig. 3: PHF Ethanol extract IC50 Value graph

CONCLUSION

The study demonstrated that the polyherbal formulation (PHF) possesses significant alpha-amylase inhibitory activity, particularly in its hot water extract, indicating strong antidiabetic potential. The findings support the traditional use of the selected plants and highlight the therapeutic promise of PHFs in diabetes management. Further in vivo and clinical studies are warranted to validate these in vitro findings and explore the formulation's pharmacokinetics and long-term safety.

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