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IN-VITRO ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE INHIBITORY ACTIVITIES OF POLYHERBAL FORMULATION

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Keywords:

Alpha amylase, Alpha glucosidase, Diabetic complications, IC_{50}

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ABSTRACT: Diabetes mellitus is a chronic disease identified as hyperglycemia that occurs either when the pancreas does not produce enough insulin or when the body cannot metabolize its insulin. Insulin is a hormone that regulates blood sugar levels. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and, over time, leads to serious damage to many of the body's systems. Alpha-amylase and alpha-glucosidase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. In the present study, the methanolic extract of leaves of *Azadirachta indica*, *Murraya koenigii* and *Psidium guajava* were prepared by maceration. The methanolic extracts of leaves of *Azadirachta indica*, *Murraya koenigii* and *Psidium guajava* were mixed in equal proportions to form a polyherbal extract and studied for *in-vitro* alpha (α)-amylase and alpha (α)-glucosidase inhibitory activities. In alpha-amylase activity, alpha-amylase solution (0.5 mg/mL) and substrate, 1% starch was used and the absorbance was measured at 540nm. In Alpha-glucosidase activity, alpha-glucosidase (0.5 mg/mL) and substrate, 5 mM p-nitrophenyl-alpha-D-glucopyranoside was used and the absorbance was recorded at 405 nm. Different concentrations of the polyherbal formulation were assessed for alpha-amylase and alpha-glucosidase inhibitory activities with an IC_{50} value 5.2 µg/ml and 5.98 µg/ml extract, respectively, and were well comparable with the standard drug, acarbose. The polyherbal formulation exhibited significant alpha-amylase and alpha-glucosidase inhibitory activities in dose-dependent manner and was comparable to that of standard drug, acarbose.

INTRODUCTION: Diabetes mellitus is a primary metabolic disorder of carbohydrate metabolism characterized by elevated blood glucose levels (hyperglycemia). Different classes of diabetes have been identified type 1, type 2, gestational diabetes, and specific types of diabetes due to other causes *e.g* monogenetic diabetes syndrome.

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Diabetes results from insufficient production of the hormone insulin (type1 diabetes) or an ineffective response of cells to insulin (type 2 diabetes). This disarray in metabolism leads to micro-and macro-vascular changes causing secondary complications including heart attack, stroke, kidney failure, leg amputation, vision loss, and nerve damage [1].

Among 7.7 billion total populations (2019), around 463 million adult people have diabetes, with a global prevalence of 9.3%, and may rise to 10.9% by 2045 [2]. The proportion of people with type 2 diabetes is increasing in most countries and 374 million people are at increased risk of developing

type 2 diabetes. Both women and men are prone to diabetes at any age [3]. The only therapy for type 1 diabetes is the substitution of insulin. Many and diverse therapeutic strategies for treating type 2 diabetes are known. The conventional treatments for diabetes include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of the degradation of oligo- and disaccharides [4]. One such group of drugs introduced in the management of type 2 diabetes is represented by the inhibitors of α-glucosidase. The enzyme α-glucosidase is responsible for breaking oligo- and/or disaccharides into monosaccharides. The inhibitory action of this enzyme leads to a decrease in blood glucose level because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine [5]. Another effective method to control diabetes is to inhibit the Alpha amylase enzyme activity of the α-amylase enzyme, which is responsible for the collapse of starch to more simple sugars (dextrin, malt triose, maltose, and glucose), resulting in increased glucose levels [6].

Pancreatic alpha-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine. Inhibition of these enzyme systems reduces the rate of digestion of carbohydrates. Less glucose is absorbed because the carbohydrates are not broken down into glucose molecules [7]. Some inhibitors currently in clinical use are acarbose, miglitol, voglibose, *etc*. However, many of these synthetic hypoglycemic agents have their side effects and fail to elevate diabetic complications.

The main side effects of these inhibitors are gastrointestinal *viz*., bloating, abdominal discomfort, diarrhea, and flatulence. In the current trend, herbal medicines are gaining more importance in the treatment of diabetes as they are free from side effects and economical when compared to synthetic hypoglycemic agents [7]. *Azadirachta indica,* commonly known as neem, belongs to the Meliaceae family and is native to the Indian subcontinent and most countries in Africa. The leaves are used to treat leprosy, eye disorders, bloody nose, intestinal worms, stomach upset, skin ulcers, heart and blood vessels (cardiovascular disease), fever, diabetes, gum disease (gingivitis), and liver problems [8]. The curry leaves (*Murraya koenigii*) are tropical to sub-tropical plants in Rutaceae. The leaves are used for inflammation, itching, fresh cuts, dysentery, bruises and diabetes [9]. *Psidium guajava* has been used as traditional medicine by many cultures throughout Central America, Africa and Asia. Guava leaves are used for stomach and intestinal conditions, pain, diabetes, and wound healing [10]. The present study was carried out to investigate the *in-vitro* α-glucosidase and α-amylase inhibitory activities of methanolic extract of the polyherbal extract made by mixing equal proportions of methanolic PHE of *Azadirachta indica, Murraya koenigii* and *Psidium guajava*.

MATERIAL AND METHODS:

Chemicals and Reagents: Porcine pancreatic α-amylase (EC 3.2.1.1) (PPA) and α –glucosidase, 3,5-Dinitrosalicylic acid (DNSA color reagent), Soluble starch, p-nitro phenyl- α -D-glucopyranoside (p-NPG), were obtained from SRL Laboratories (Hyderabad, India). Acarbose from Glucobay (Hyderabad, India), sodium potassium tartrate, dimethyl sulfoxide, sodium carbonate (Na_2CO_3), sodium dihydrogen phosphate, di-sodium hydrogen phosphate and other chemicals are of analytical grade.

Collection of Plant Material: Fresh leaves of *Azadirachta indica, Murraya koenigii and Psidium guajava* (1 kg) were collected from the local area of Hyderabad, Telangana (India). Dr. Mustafa, Professor, Department of Botany, authenticated the collected plant material, Kakatiya University, Warangal, Telangana, India. The voucher specimens of the plants (OU/SNVPMV/54, OU/SNVPMV/55, OU/SNVPMV/56) were being maintained in the herbarium of the Department of Pharmacognosy and Phytochemistry, Sarojini Naidu Vanitha Pharmacy Maha Vidyalaya, Tarnaka, Hyderabad. The leaves were washed thoroughly under tap water to remove all impurities. Then they were cut into pieces and dried under shade for three weeks. The dried leaves were powdered separately using a grinder.

Preparation of Plant Extract: The air-dried and coarse powdered sample of leaves of *Azadirachta indica, Murraya koenigii and Psidium guajava* were macerated separately with methanol in a

round bottom flask for 7 days with intermittent stirring and filtered after seven days and concentrated under reduced pressure to yield a dark green semi-solid mass.

The percentage yields of the extracts of *Azadirachta indica*, *Murraya koenigii* and *Psidium guajava* were 8.4%, 9.1%, and 9.5%, respectively. The extracts were kept in desiccators to remove moisture and used. The three extracts were mixed in equal proportions to form a polyherbal extract. It is given code as PHE (Polyherbal methanolic extract*)*

Preliminary Phytochemical Screening of the Extract PHE: The methanolic extract of leaves of polyherbal extract (PHE) was subjected to various test tube reactions to detect the different classes of phytoconstituents present in it [11].

Determination of *In-vitro* Alpha-amylase Enzyme Inhibitory Activity of PHE: The inhibition of α -amylase activity was determined according to method [12] described in the literature with minor modifications. A stock solution of extract was prepared by dissolving up to 100mg of each extract in 10ml of dimethyl sulfoxide.

A total of 250µl of extracts of varying concentrations (1, 2, 4, 8,10µg/ml) was placed in a tube and 250µl of 0.02M sodium phosphate buffer (pH-6.9) containing α-amylase solution (0.5mg/ml) was added.

This solution was pre-incubated at 25°C for 10min. After which 250µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at particular time intervals and then further incubated at 25°C for 10min.This reaction was terminated by adding a 500µl dinitro salicylic acid (DNS) reagent.

The tubes were kept in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was diluted with 5ml of distilled water, and the absorbance was measured at 540nm using UV- Visible spectrophotometer (Shimadzu UV-1800). Acarbose was used as a positive control. A control or blank was prepared using the same procedure replacing the extract with distilled water. The concentration of the extract or standard required to inhibit 50% of α -amylase activity under the assay conditions was defined as the IC_{50} value.

The percentage inhibition was calculated using the formula:

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

Determination of Alpha-glucosidase Enzyme Inhibitory Activity of PHE: The inhibition of α -glucosidase activity was determined according to the method [12] described in the literature with minor modifications. Aliquot of α -glucosidase was dissolved in 100 ml of phosphate buffer (pH 6.8).

To 100 µl of PHE of varying concentrations (1, 2, 4, 8, 10 µg/ml), 200 µl α -glucosidase were added, and the mixture was incubated at 37ºC for 20 min. 100 µl of 3mM p–nitrophenyl-α -D-glucopyranoside (p-NPG) was added to the reaction mixture and incubated at 37 ºC for 10 min.

The reaction was terminated by adding 2ml of 0.1M Na_2CO_3 solution and the α-glucosidase activity was determined spectrophotometrically at 405 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800) by measuring the quantity of p-nitrophenol released from *p*-NPG.

Acarbose was used as a positive control for α -glucosidase inhibitory activity. A control or blank was prepared using the same procedure replacing the extract with distilled water. The concentration of the extract required to inhibit 50% of α -glucosidase activity under the assay conditions was defined as the IC_{50} value.

The percentage inhibition was calculated using the formula:

% Inhibition = Absorbance (control) – Absorbance (extract) / Absorbance (control) × 100

RESULT:

Preliminary Phytochemical screening of the Extract PHE: The methanolic extract of leaves of polyherbal extract (PHE) has been found to contain various phytoconstituents of medicinal importance like flavonoids, polyphenols, saponins, steroids, alkaloids and glycosides *etc*. Preliminary phytochemical screening of the extract, PHE is presented in **Table 1.**

TABLE 1: PHYTOCHEMICALSCREENING OF METHANOLIC EXTRACT OF LEAVES OF POLYHERBAL EXTRACT

Classes of Phytoconstituents	PHE
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids/Triterpenoids	+
Carbohydrates	+
Glycosides	+
Tannins	+

(+) indicates presence and (-) indicates absence of the Phyto-constituents

In-vitro alpha-amylase enzyme inhibitory activity of PHE: The results of the study are presented in **Table 2** and **Fig. 1**. In the present study, methanolic extract of Polyherbal extract inhibited the catalysis of alpha-amylase at all concentrations (1, 2, 4, 8, 10 µg/ml), and the percentage inhibition was dose-dependent.

Among all the test doses, PHE has shown remarkable alpha-amylase enzyme inhibition, i.e., 79.5% at 10 µg/ml concentration, and it was comparable with the standard drug, acarbose (95.3% inhibition at 10 µg/ml concentration). The IC_{50} value of the extract (PHE) and standard (Acarbose) was found to be 5.20 µg/ml and 3.12 µg/ml, respectively.

TABLE 2: SHOWS *IN-VITRO* ALPHA-AMYLASE ENZYME INHIBITORY ACTIVITY OF METHANOLIC EXTRACT OF POLYHERBAL EXTRACT (PHE) AND STANDARD DRUG ACARBOSE

S. no.	Name of sample	Concentration (µg/ml)	% Inhibition	IC_{50} value (µg/ml)
1.	PHE	1	22.3±0.02	5.20
		2	31.8±0.04	
		4	42.5±0.06	
		6	54.4±0.08	
		8	68.52±0.05	
		10	79.5±0.41	
2.	Acarbose	1	34.6±0.44	3.12
		2	42.3±0.86	
		4	56.7±0.07	
		6	71.1±0.05	
		8	84.8±0.03	
		10	95.3±0.25	

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-amylase inhibitor.

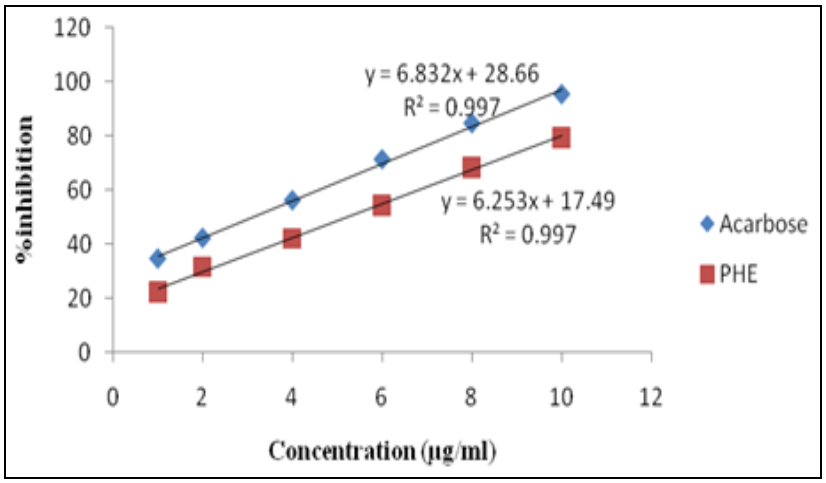

FIG. 1: *IN-VITRO* A-AMYLASE INHIBITORY ACTIVITY OF PHE AND ACARBOSE PHE: METHANOLIC EXTRACT OF POLYHERBAL EXTRACT

In-vitro alpha-glucosidase enzyme inhibitory activity of PHE: The results of the study are presented in **Table 3** and **Fig. 2**. The extract, PHE, was assessed for alpha-glucosidase enzyme inhibitory activity at different concentrations ranging from (1-10 µg/ml), and it exhibited potent α-glucosidase inhibitory activity in a dose-dependent manner comparable with that of the standard drug, acarbose. The IC_{50} value of the extract (PHE) and standard (Acarbose) was found to be 5.98 µg/ml and 2.91 µg/ml, respectively.

TABLE 3: *IN-VITRO* ALPHA-AMYLASE ENZYME INHIBITORY ACTIVITY OF METHANOLIC EXTRACT OF POLYHERBAL EXTRACT (PHE) AND STANDARD DRUG ACARBOSE

S. no.	Name of sample	Concentration (µg/ml)	% Inhibition	IC_{50} value (µg/ml)
1.	PHE	1	22.2±0.29	5.98
		2	29.1±0.87	
		4	38.5±0.39	
		6	49.2±0.58	
		8	59.4±0.14	
		10	74.3±0.25	
2.	Acarbose	1	38.3±0.12	2.91
		2	44.7±0.04	
		4	56.8±0.11	
		6	68.6±0.59	
		8	77.4±0.48	
		10	86.2±0.67	

The values are expressed as means ± SD of triplicate determinations. Acarbose is the standard α-amylase inhibitor.

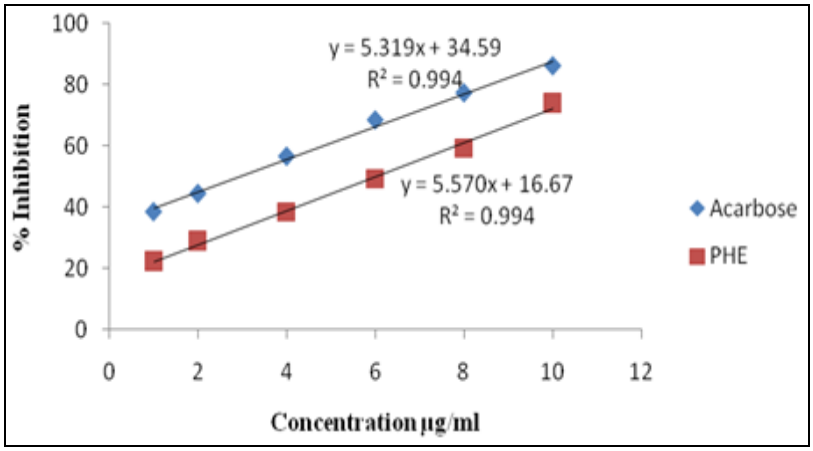

FIG. 2: *IN-VITRO* A-GLUCOSIDASE INHIBITORY ACTIVITY OF PHE AND ACARBOSE PHE: METHANOLIC EXTRACT OF POLYHERBAL EXTRACT

DISCUSSION: The methanolic extract of leaves of polyherbal extract has been found to contain various phytoactive constituents of medicinal importance like flavonoids, polyphenols, saponins, steroids, alkaloids, and glycosides *etc*. of which flavonoids and polyphenols are responsible for antioxidant activity [13]. The levels of oxidative stress are greater in diabetics resulting in greater requirements of antioxidants to combat oxidative stress [14].

Alpha-amylase is a prominent enzyme found in the pancreatic juice and saliva which breaks down large insoluble starch molecules into absorbable molecules. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides, leading to an increase in blood glucose levels. The α-amylase inhibitors delay the glucose absorption rate, decreasing the serum blood glucose levels in hyperglycemic individuals [7]. The polyherbal extract has shown dose-dependent inhibition of alpha-amylase enzymatic activity, indicating the antidiabetic potential of the extract. On the other hand, mammalian -glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in the human diet. α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia [5]. The extract, PHE, has shown significant inhibition of alpha-glucosidase enzymatic activity, indicating antihyperglycemic activity of the extract. α-amylase and α-glucosidase inhibitors play an important role in controlling high blood glucose levels.

Inhibitors of alpha-amylase and alpha-glucosidase delay the breaking down of carbohydrates in the small intestine and diminish the postprandial blood glucose excursion and hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate

digestion, which consequently reduces the postprandial plasma glucose level [3]. The extract, PHE has shown dose-dependent inhibition of both alpha-amylase and alpha-glucosidase enzymatic activities, indicating the antidiabetic potential of the extract.

CONCLUSION: In the present study, the polyherbal extract has shown dual inhibition of alpha-amylase and alpha-glucosidase enzymes, which reduces blood glucose levels, making the extract effective in managing diabetes and in turn, reduces diabetic complications. The antidiabetic activity may be attributed to various phytoactive constituents present in the extract like flavonoids, polyphenolic compounds, etc. Hence, the Polyherbal extract can be used as an adjuvant for the management of diabetes and thus prevent the complications associated with diabetes mellitus.

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CONFLICTS OF INTEREST: All authors declare no conflict of interest.

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