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Research Article

EVALUATION OF *INVITRO* ANTI DIABETIC ACTIVITY OF *SEENDHIL* HERBAL FORMULATION

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ABSTRACT

Objective: The main aim of the study was to screen the ethanolic extract of *seendhil* polyherbal formulation for its *invitro* antidiabetic activity and its efficacy in inhibiting alpha amylase and alpha glucosidase enzymes as inhibition of these enzymes prevents raise in postprandial glucose level in blood.

Methods: Both solvent and aqueous extract were prepared and assayed for the presence of phytochemicals. *In vitro* α-amylase and α-glucosidase inhibitory activity of the formulation were determined according to standard method using acarbose as control.The pre substrate and post substrate addition absorbance was measured at 405 nm on a microplate reader. The increase in absorbance on substrate addition was obtained. Each test was performed three times and the mean absorption was used to calculate percentage enzyme inhibition.

Results: The preliminary phytochemical screening of both aqueous and ethanolic extract showed the presence of significant secondary metabolites.Inhibition percentage of alpha amylase was 75.3% with a IC50 value of 2.90mg/ml and for alpha glucosidase inhibition percentage was 79.8% with a IC50 value of 2.78mg/ml.

Conclusion :At the concentration of 10mg/ml the extract showed significant and higher inhibitory activity against α-amylase and α-glucosidase enzymes.The present study indicated that *Seendhil* formulation could be useful in management of postprandial hyperglycemia.

Keywords: Diabetes mellitus, hyperglycemia, alpha amylase, glucosidase, inhibition, *Seendhil* formulation.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1].Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially eyes, kidneys, nerves, heart, and blood vessels. Symptoms of marked hyperglycemia include polyuria, polydypsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome [2].

According to WHO, it is estimated that 3% of the world's population have diabetes and the prevalence is expected to double by the year 2025 to 6.3% [3].Therefore one therapeutic approach for treating diabetes is to control the postprandial hyperglycemia by retarding the absorption of glucose.Alpha amylase and glucosidase inhibitors are drug-design targets in the development of compounds for the treatment of diabetes, obesity and hyperlipaemia. Alpha amylase is an enzyme (EC 3.2.1.1) that hydrolyses alpha bonds of large, alpha-linked polysaccharides, such as starch and glycogen, yielding glucose and maltose [4].Enzyme alpha-glucosidase (EC 3.2.1.20), present in the epithelial mucosa of small intestine cleaves glycosidic bonds in complex carbohydrate to release absorbable monosaccharides [5].Inhibition of alpha-glucosidase in the digestive tract delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial blood glucose levels [6].

Plants have long been used for the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment. Because of the possible importance of these inhibitors in plant physiology, animal and human nutrition, extensive research has been conducted on their properties and biological effects [7].

It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine [8].

Herbal medicine also called botanical medicine or phytomedicine refers to the use of plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease [9].

Siddha medicine is one of the most ancient medicinal system practiced in southern parts of India.The World Health Organisation has documented that the vast majority of people mostly living in the developing countries and significant number in the developed industrialized nations, prefer and preferring traditional medicine for treating common ailments and chronic diseases as they have no side effects [10].*Siddha* medicine employs a variety of herbs and minerals, many of which were developed in the ancient past under advanced scientific techniques, even by todays standards. The development of medicine has been a continuous process in Asia although it has always taken a more natural approach of healing than western medicine.

Seendhil formulation is a polyherbal siddha medicine consisting of 10 different herbs [Table 1] and used to treat diabetics mellitus and other chronic diseases.This formulation controls diabetes by inhibiting the enzyme alpha amylase and alpha glucosidase. The use of alchocol or water as solvent is efficient in extracting a wide variety of active components. Therefore, we analysed the *Seendhil formulation* for the presence of bioactive components and evaluated *invitro* anti diabetic activity using the ethanolic extract of the formulation.

Table 1: Composition of Seendhil Polyherbal Formulation

S.No	Siddha Name	Botanical Name
1.	Seendhil	*Tinospora cordifolia*
2.	Kadukkai thol	*Terminalia chebula*
3.	Nellikkai vattral	*Ribes uvacrispa*
4.	Kariveppillai elai	*Murraya koenigii*
5.	Vilvam	*Aegle marmelos*
6	Manjal	*Curcuma longa*
7	Vendhayam	*Trigonellafoenum-graecum*
8	Kovai elai	*Coccinia grandis*
9	Sirukurinjaan elai	*Gymnema sylvestre*
10	Maramanjal	*Berberisaristata*

MATERIALS AND METHODS

Preparation of Formulation

The *Seendhil* formulation in the form of tablet were procured from reputed commercial Siddha supplier (SKM Siddha Pharmacy,Erode) and authenticated**.** The tablet was made in to fine powder mixture. The mixture was further boiled in distilled water at 100ºC for 1 hr and filtered. The filtrate was evaporated to dryness, and used for subsequent experiments.

Solvent extract preparation

10 grams of the powdered formulation was extracted with 100ml of Organic solvent(Ethanol) and kept on rotary shaker at 190-220 rpm for 24 hours. The supernatant was collected and solvent was evaporated to make the final volume one – fourth of the original volume and stored at 40C in air tight bottles [11].

Aqueous extract preparation

The aqueous extract is prepared by soaking 100grams of the powdered formulation in 200 ml of distilled water for 12hours.The extracts were filtered using Whatmann filter paper (125 mm) [12].

Phytochemical screening

The qualitative tests were carried out in both the extract of *Seendhil* formulation using standard procedures [13-15].Both the extracts were analysed for the presence of significant secondary metabolites *viz* Flavonoids, Alkaloids, Saponins, Cardiac glycosides, Tannins and Steroids [Table 2].

Table 2: Phytochemical Analysis of *Seendhil* Polyherbal Formulation

Compounds	Result
Tannins	+
Saponins	+
Cardiac glycosides	+
Steriods	+
Flavaniods	+
Anthroquinone	-
Catachol	-
Alkaloids	+
Phenols	+

\- : indicates negative results
\+ : indicates positive results

In vitro alpha amylase and α-glucosidase inhibition study

The α-amylase and α-glucosidase inhibitory effect of the formulation extracts was determined according to the standard method [16].For alpha glucosidase inhibition, yeast α-glucosidase was dissolved in 100 mM phosphate buffer, pH7.0, containing bovine serum albumin 2 g/liter and sodium azide 0.2 g/liter which was used as enzyme source. Paranitrophenyl- α-d-glucopyranoside was used as substrate. The ethanolic extract of *Seendhil* formulation were made in serial dilutions of 1, 2.5, 5, 10, mg/ml were made up to equal volumes by dimethylsulfoxide and distilled water. 10µl of extract dilutions was incubated for 5 min with 50µl enzyme source. After incubation, 50µl of substrate was added and further incubated for 5 min at room temp. The pre substrate and post substrate addition absorbance was measured at 405 nm on a microplate reader. The increase in absorbance on substrate addition was obtained. Each test was performed three times and the mean absorption was used to calculate percentage α-glucosidase inhibition. Acarbose was used as positive control with various concentrations 1,2.5,5,10mg/ml. Percentage α-glucosidase inhibition was calculated according to the following formula[17]

Percentage of inhibition = $\frac{[(\text{Control 405} - \text{Extract 405})] \times 100}{\text{Control 405}}$

The alpha amylase inhibition activity of the formulation was assayed by the same method used for alpha glucosidase inhibition activity.

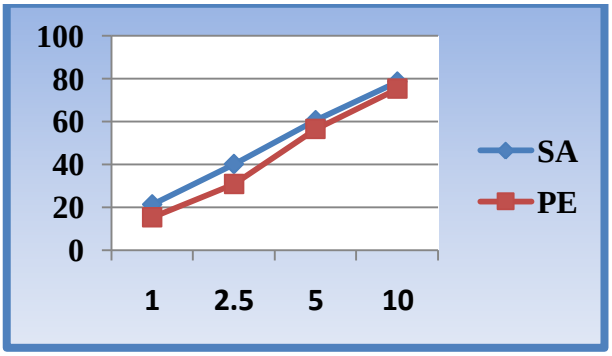
For alpha amylase inhibition assay the enzyme porcine pancreatic amylase and substrate Paranitro phenyl alpha D- malto pentoglycoside were used and absorbance was measured at 540 nm on a microplate reader [17].

Percentage of inhibition = $\frac{[(\text{Control 540} - \text{Extract540})] \times 100}{\text{Control 540}}$

RESULTS

The *Seendhil* formulation was extracted with aqueous and ethanolic solvents. The preliminary phytochemical screening of both aqueous and ethanol extract showed the presence of maximum compounds like Alkaloids, Flavonoids, Tannins, Steroids, Saponins and Cardiac Glycosides. Hence the ethanolic extract of *Seendhil* formulation were assayed for *in vitro* alpha amylase and alpha glucosidase inhibitory activity. Four different concentration were tested, the extract showed good inhibitory effect at all the tested concentrations (1, 2.5,5 and 10 mg/ml). Acarbose enzyme was used as standard for various concentrations. At a concentration of 10 mg/ml the maximum inhibitory effect of ethanolic extract of the formulation was observed. Inhibition percentage of alpha amylase was 75.3% with a IC50 value of 2.90mg [Figure 1] and for alpha glucosidase inhibition percentage was 79.8% with a IC50 value of 2.78mg [Figure 2]. The experiment was repeated for three times and results were expressed as mean value of inhibition activity percentage.

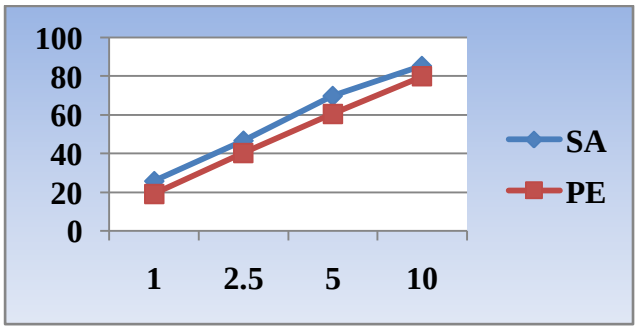
Fig.1: Alpha amylase inhibition activity of *Seendhil* Formulation



X-Axis-Conc(mg) Y Axis-Inhibition %

SA-Standard Acarbose PE-Plant Extract

Fig.2: Alpha Glucosidase inhibition activity of the Formulation



X-Axis-Conc(mg) Y Axis-Inhibition %

DISCUSSION

Diabetes mellitus is a debilitating and often life threatening disorder with increasing incidence throughout the world. There is a steady raise in the rate of incidence of Diabetes mellitus and estimated that 1 in 5 may be diabetic by 2025 [18].With the distinctive traditional medical opinions and natural medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a bright future in the therapy of diabetes mellitus and its complications[19].Traditional medicine formulations are aimed for holistic health care. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity[20].It is believed that different ingredients of one formulation produce synergistic effect by acting in multiple targets and also nullify the adverse effect caused by each other[21].

Since, the main concern of the general public and science is in finding new natural and therapeutically active agents; scientists all over the globe have started screening plants for searching new phytochemicals.[22].The numerous polyphenolic compounds, triterpenoids and other chemical compounds present in the plant may account for the observed antidiabetic effects of the extracts. Tannins, Flavonoids, Saponins, Alkaloids and other chemical compounds present in the formulation are speculated to account for the observed hypoglycemic effect [23]. In this study, the preliminary phytochemical screening of ethanolic extract of the formulation showed the presence of compounds that are reported as anti diabetic [24-26].Hence, *invitro* effect of different concentrations of ethanolic extract of *Seendhil* formulation was evaluated. At the concentration of 10mg/ml the extract showed significant and higher inhibitory activity. The present study indicated that *Seendhil* formulation could be useful in management of postprandial hyperglycemia. The results indicates that ethanol extract of *Seendhil* formulation showed appreciable inhibition activity.

CONCLUSION

In this present study we evaluated *in vitro* alpha amylase and alpha glucosidase inhibitory activity of ethanolic extract of *Seendhil* formulation.The extract showed significant inhibition activity, so further the compound isolation, purification and characterization which is responsible for the inhibition activity has to be elucidated for proper usage of this formulation as anti-diabetic agent.

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