



***In vitro*, combined anti-diabetic potential of *S. oblonga* linn. and *E. littorale* blume. extract**

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Abstract

The objective of the study was to evaluate the combined *in-vitro* inhibitory potential of Indian medicinal aqueous plants extract of *S. oblonga* Linn. Stem and *E. littorale* Blume. Aerial part using α -amylase and α -glucosidase enzymes. The both plants parts were extracted with water at 50°C for 30 minutes. The extracted material was concentrated in water bath and allows drying naturally. The various concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) of both extracts individually and in combination (1:1 ratio) were subjected to α -amylase and α -glucosidase inhibitory assay. The standard drug was Acarbose with which comparison was made. The absorbance of mixture was measured at 540 and 405 nm. The percentage inhibition of α -amylase and α -glucosidase and IC₅₀ values of individual extract and the combined extracts were calculated. The results of the present study indicate aqueous extract of both the plants exhibited potential against diabetes mellitus in a dose dependent manner. Aqueous extract of *E. littorale* showed more inhibitory potential against α -glucosidase than α -amylase enzyme with IC₅₀ values 0.31±2.12 mg/ml and 0.40±1.09 mg/ml respectively. However, the aqueous extract of *S. oblonga* showed appreciable α -amylase and α -glucosidase inhibitory activity with an IC₅₀ values 0.42±2.01 mg/ml and 0.46±3.17 mg/ml, respectively, where as the acarbose showed IC₅₀ value of 0.17±1.21 mg/ml and 0.19±1.75 mg/ml against α -amylase and α -glucosidase enzyme respectively. The combined effect of the extract at (1:1 ratio) showed potential with IC₅₀ value of 0.28±2.11 mg/ml and 0.21±1.14 mg/ml against α -amylase and α -glucosidase enzyme respectively. The present study results indicate that, both the Indian medicinal plants have potential to decrease the postprandial blood glucose level, which may due to phytochemical such as triterpenoids, flavonoids and phenolics active constituents in the extracts. Hence, this study supports the Ayurvedic concept of plants, especially in the management of type 2 diabetes. The efficacies of the combined extract indicate the stronger synergistic effect, when compared with individual inhibitory activity. This showed a beneficial aspect of a combination of herbal medicine formulation to reduce the postprandial hyperglycemia.

Keywords: anti-diabetes, *S. oblonga*, *E. littorale*, α -amylase inhibitors, α -glucosidase inhibitors

Introduction

Diabetes mellitus is a serious chronic metabolic disorder due to inherited or acquired deficiency of insulin secretion and by decreased sensitivity of the organs to insulin; results in an increased blood glucose level. This is due to reduction of physical activity, healthy diets, obesity, increased in blood glucose and lipid profile [1]. Further, if not controlled may lead to chronic microvascular and macrovascular complication include kidney, blood vessels and nerves [2]. One of the approaches is to reduce the postprandial hyperglycemia by slowing down the absorption of glucose by inhibiting the enzymes, such as α -amylase and α -glucosidase [3]. α -amylase is a critical digestive enzyme that catalyses the breakdown of starch to maltose in the beginning and then converts it to glucose in the end, so reducing its activity can lead to a reduction in blood glucose levels [4].

The Indian system of medicines has played an important role in the protection and treatment of human health from centuries. The records of the Indian traditional medicine date from before 5000 years ago [5, 6]. However, throughout the ages, humans have relied on herbal medicines and phytonutrients to fight against diseases and about 6 billion people worldwide trust herbal medication for the treatment of various diseases [7, 8], despite many synthetic medicines are discovered, but sometimes they can produce many adverse effects. Herbal plants therapeutic activity is due to the availability of potentially active phytochemical constituents [9].

There are several medicinal plants, which are known for their contribution to traditional as well as modern medicines. The plants *E. littorale* and *S. oblonga* are among them and plays a vital role in human healthcare. In folk medicine *E. littorale* is used to treat rheumatism, diabetes mellitus, hernia, abdominal ulcers, insect poisoning, itching, swelling, inflammation, hypoglycemic and cancer [10]. *S. oblonga* roots & stem are extensively used in the Indian system of medicine for treating diabetes, hyperlipidemia, gonorrhoea, rheumatism, itching, asthma, ear diseases, leukemia, inflammation [11]. Although many believe herbal plant formulations are cheap and safe. They are often used singly or in combination. Polyherbal formulations are plant-based medicinal agents that, due to their various active principles, can have synergistic, potentiative, and agonistic effects [12].

Efforts have been undertaken in this direction to evaluate the combined inhibitory effect of herbals from natural origin with an aim to develop more effective and safer alternative to treat diabetes mellitus. Therefore the aim of the present study is to screen combined inhibitory effects of *S. oblonga* and *E. littorale* herbal extract, which are used in the folk medicine to control blood glucose level.

Material and Methods

The plants were collected from the local market (herbalist shops) in a dried form and authenticated by Dr. A. Singh, Dhanvantri Ayurvedic College, Mohali. Purified porcine pancreatic α -amylase α -glucosidase and other chemicals were obtained from HIMEDIA laboratory, Mumbai, India. Distilled water was used for the preparation of the different solutions.

Preparation of the extracts

The dried plant was grinding by mechanical grinder and pass through a 40-mesh sieve. The 100 g powder material was extracted with water for 30 minutes at 50°C. The extract was centrifuged, filtered, and crude powder extract were dried on the water bath, which was further used for the evaluation of α -amylase and α -glucosidase inhibitory activity.

α -amylase inhibitory activity

The extracts were tested for α -amylase inhibitory action using a conventional method with slight alteration^[13]. A mixture prepared by addition of 50 μ l phosphate buffer (100 mM, pH = 6.8), 10 μ l α -amylase (2 U/ml), and 20 μ l of various concentrations of extracts (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were incubated at 37°C for 20 minutes. Then, as a substrate, 20 μ l of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added and incubated for another 30 min at similar temperature conditions. Further, 100 μ l of DNS colour reagent was added and maintained in boiling water for 10 minutes. A spectrophotometer was used to measure the absorbance of the mixture at 540 nm. The Standard mixture was prepared using acarbose in 0.1-0.5 mg/ml concentration. The blank mixture was prepared with all the substances except test/standard. The experiment was performed in triplicate. The results were given as percentage inhibition.

$$\text{Inhibitory activity (\%)} = (1 - \text{As}/\text{Ac}) \times 100$$

α -glucosidase inhibitory activity

The extracts were tested for α -glucosidase inhibitory action using a conventional method with slight modifications^[14]. A mixture prepared by addition of 50 μ l phosphate buffer (100 mM, pH = 6.8), 10 μ l α -glucosidase (1 U/ml), and 20 μ l of various concentrations of extracts (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml). The mixture was incubated for 15 minutes at 37°C. Then, as a substrate, 20 μ l P-NPG (5 mM) was added and incubated for another 20 minutes at similar temperature conditions. The reaction was quenched by the addition of 50 μ l of Na₂CO₃ (0.1 M). A spectrophotometer was used to measure the absorbance of mixture at 405 nm. The Standard mixture was prepared using acarbose in 0.1-0.5 mg/ml concentration. The blank mixture was prepared with all the substances except test/standard. The experiment was performed in triplicate. The results were given as percentage inhibition.

$$\text{Inhibitory activity (\%)} = (1 - \text{As}/\text{Ac}) \times 100$$

Results and Discussion

The regulation of hyperglycemia is a major strategy in the control of diabetes and its later complications^[15]. In diabetes glycemic control is dependent on adequate regulation postprandial hyperglycemia^[16]. The agents that reduce postprandial blood glucose level by inhibiting the hydrolysis of starch, found to be useful in the management of diabetes^[17, 18].

In the present study, *in-vitro* combined antidiabetic effect of aqueous extracts of *S. oblonga* stem and *E. littorale* aerial part was evaluated for α -amylase and α -glucosidase inhibitory activity. Both the extracts found to have of capacity better inhibition of α -amylase and α -glucosidase, but the combined extract showed inhibition which is comparable to standard drug Acarbose. The aqueous extract of *S. oblonga* (at a concentration of 0.1 to 0.5 mg/ml) exhibited 20.12±0.15, 26.04±1.02, 32.42±0.96, 50.25±1.05 and 61.45±0.23 α -amylase inhibitory activity, and 17.34±0.27, 21.72±0.85, 30.49±1.14, 43.55±0.47 and 57.02±0.59 α -glucosidase inhibitory activity, respectively. A concentration dependent response was observed with varying the concentrations, suggesting a competitive type of inhibition.

Whereas, *E. littorale* aqueous extract (at a concentration of 0.1 to 0.5 mg/ml) exhibited 29.22±0.14, 32.34±1.23, 36.42±1.38, 47.25±1.55 and 63.45±2.27 α -amylase inhibitory activity, and 24.21±0.81, 31.44±2.12, 48.33±2.41, 56.59±1.37 and 70.41±1.36 α -glucosidase inhibitory activity, respectively. The combined effect of extract at the (1:1 ratio & dose 0.1-0.5 mg/ml) produces α -amylase inhibitory effect 34.04±2.15, 41.37±0.76, 51.21±1.28, 57.31±2.04 and 78.55±1.57, and α -glucosidase inhibitory effect 32.47±1.39, 48.11±0.85, 59.28±2.24, 65.51±1.66, and 84.22±1.72, which was comparable to standard drug. The plants have the ability to block α -amylase and α -glucosidase, they could be used as a safe and effective treatment for postprandial hyperglycemia^[19]. Phytochemical analysis of *E. littorale* revealed the presence of anti-oxidative chemicals includes two sterols,

alkaloids, saponins, phenolic acid, triterpenoids, catechins, xanthenes and flavonoids, responsible for the biological activities of plant [20, 21].

This is also instated by Pinto [22], which demonstrated that meals or herbal supplements with the capacity to block the α -amylase and α -glucosidase enzymes can be used to regulate blood glucose levels in diabetic patients. The IC_{50} value of *S. oblonga*, *E. littorale*, combined extract and Acarbose was 0.42, 0.40, 0.28 and 0.17 mg/ml for α -amylase inhibition, respectively. The IC_{50} value of *S. oblonga*, *E. littorale*, combined extract and Acarbose was 0.46, 0.31, 0.21 and 0.19 mg/ml for α -glucosidase inhibition, respectively. The combined extract exhibited an IC_{50} value comparable to standard positive control drug Acarbose for α -glucosidase, where as IC_{50} value of combined extract was less than Acarbose in case of α -amylase. Two carbohydrate hydrolyzing enzymes (α -amylase and α -glucosidase) are responsible for the rise in blood glucose levels after a meal. α -amylase catalyses the hydrolysis of 1, 4-glycosidic bonds of polysaccharides to disaccharides, while α -glucosidase catalyses the conversion of disaccharides to monosaccharides, resulting in postprandial hyperglycemia [2, 23, 24]. As a result, inhibitors of -amylase and -glucosidase are effective in lowering postprandial hyperglycemia by interfering with carbohydrate digestion.

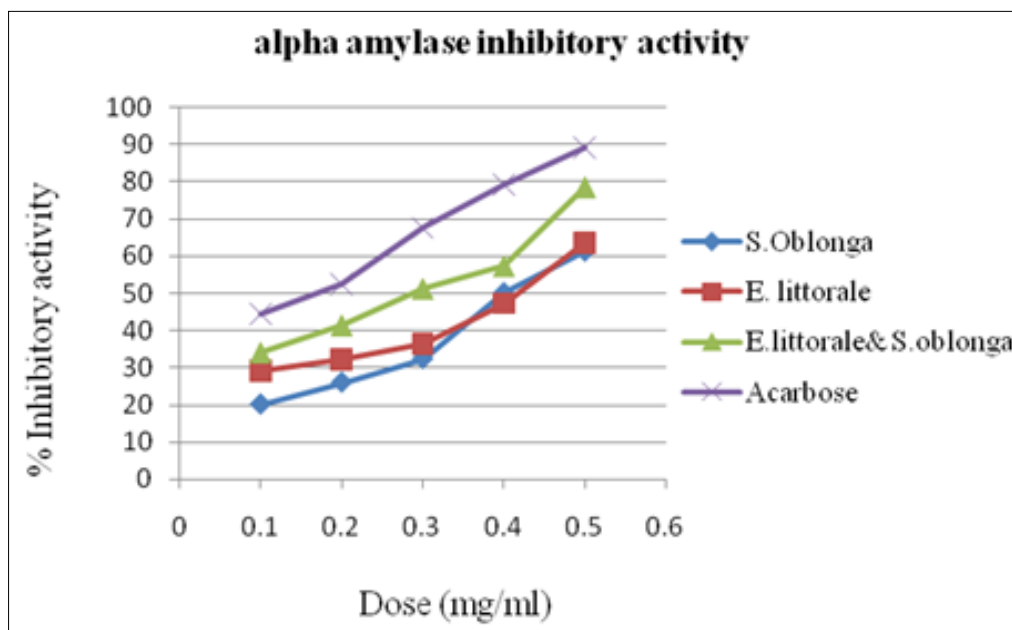


Fig 1: The percent α -amylase inhibition of extracts and acarbose (standard drug) at varying concentrations. The data is expressed as the Mean \pm SD; (n = 3).

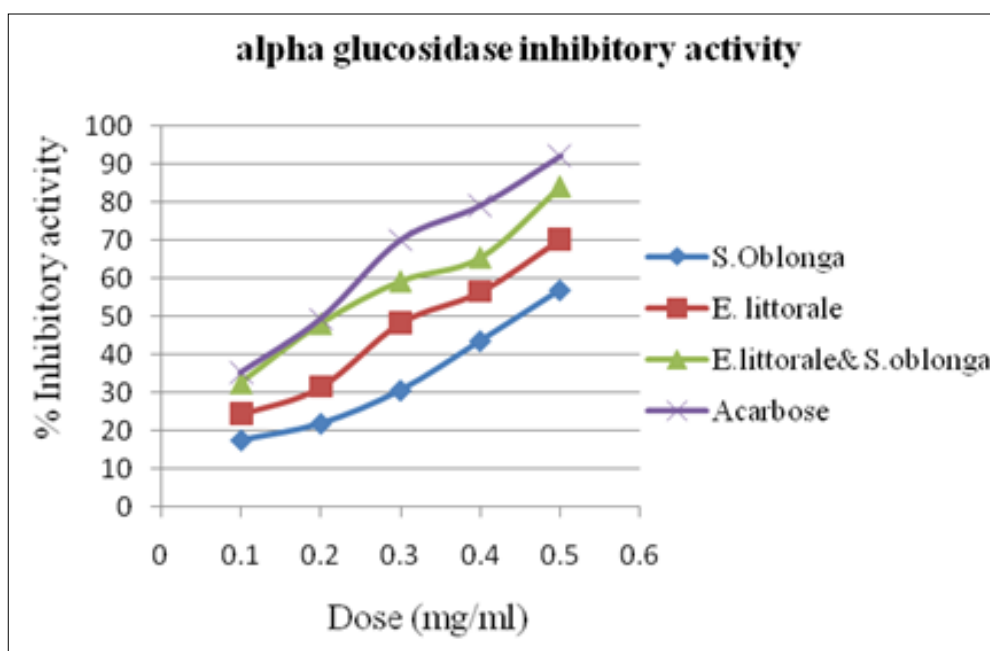


Fig 2: The percent α -glucosidase inhibition of extracts and acarbose (standard drug) at varying concentrations. The data is expressed as the Mean \pm SD; (n = 3).

Conclusion

According to the present study the combined effect of aqueous extract of *S. oblonga* stem and *E. littorale* aerial part showed potential against α -amylase and α -glucosidase. Hence, the present study supports the traditional uses of plants. It is suggested that phenols and flavonoids are the phytochemicals of plants extracts had showed antioxidant activity by virtue of which they has potential to treat diabetes. The result indicted that when the extract given in combination, they produced synergistic effect, which showed significantly difference from the individual plant extract. Furthermore, this study has opened opportunities for preparation of combined dosage form, which is effective for treatment of diabetics.

Acknowledgements

Authors acknowledge Department of Pharmaceutical Sciences, RIMT University, Mandi Gobindgarh, Punjab, India for providing research facilities.

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