

Research Article

Evaluation of Antidiabetic Potential of Selected Species of *Salacia* Leaf Extract

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Keywords:

Salacia beddomei,

Salacia chinensis,

Salacia fruticosa,

Salacia gambleana,

Salacia macrosperma,

Salacia malabarica,

Salacia oblonga,

α -amylase,

α -glucosidase,

Acarbose, IC 50

Abstract

The objective of this study was to determine anti – diabetic activity of selected seven species of *Salacia* such as *Salacia beddomei*, *Salacia chinensis*, *Salacia fruticosa*, *Salacia gambleana*, *Salacia macrosperma*, *Salacia malabarica* and *Salacia oblonga*. α -amylase and α -glucosidase enzymes are two enzymes responsible for diabetic condition. The methanolic extract of seven species of *Salacia* was found to be effective against these two enzymes. The inhibitory activity of α -amylase, *S. beddomei* shows IC 50 - 122 μ g/ml, *S. chinensis* IC 50 - 112 μ g/ml, *S. fruticosa*, IC 50 - 108 μ g/ml, *S. gambleana* IC 50 - 148 μ g/ml, *S. macrosperma* IC 50 - 125 μ g/ml, *S. malabarica* IC 50 - 121 μ g/ml, and *S. oblonga* IC 50 - 117 μ g/ml. Inhibition of α -glucosidase enzyme of seven species of *Salacia* such as *S. beddomei*, *S. chinensis*, *S. fruticosa*, *S. gambleana*, *S. macrosperma*, *S. malabarica* and *S. oblonga* showed IC 50 value of 107 μ g/ml, 105 μ g/ml, 80 μ g/ml, 110 μ g/ml, 109 μ g/ml, 107 μ g/ml and 108 μ g/ml respectively, whereas standard Acarbose of α -amylase and α -glucosidase shows IC 50 value of 196 μ g/ml.

1. Introduction

Diabetes mellitus is a chronic disorder in the metabolism of carbohydrates; proteins and fat due to absolute or relative deficiency of insulin secretion with / without vary in degree of insulin resistance. Diabetes mellitus is a metabolic disease which is found to be old as mankind and is seems to be high all over the world [1]. It is also a major cause of disability and hospitalization which results in significant financial burden [2].

Salacia is a valuable genus comprising of 407 species and is widely distributed in Sri Lanka, India, China, Vietnam, Indonesia, Brazil and other Asian countries [3,4] is belongs to the family Celastraceae, formerly called as Hippocrataceae [5] It is popularly called as “Saptrangi” in Ayurvedic medicines [6]. Medicinally importance of this genus *Salacia* is seems to increasing as it was initially used in Ayurvedha for the treatment of Madhumeha which was the ancient name for diabetes [7].

The number of people suffering from diabetes is increasing at an alarming rate worldwide [8] and the management of this disease is considered as a global problem and successful treatment is yet to be discovered. There are three types of diabetes a) Type I diabetes, b) Type II diabetes and c) Gestational diabetes. Of these three type II is most common and account in 90% cases. It is usually characterized by an abnormal increase in blood sugar especially after meal and is called as postprandial hyperglycemia [9].

Amylases (α -1,4-glucan-4-glucanohydrolase, EC3.2.1.1) are a group of enzymes which catalyze the hydrolysis of the (α -1,4) glycosidic linkages in starch and various other oligosaccharides [10]. Hence, the inhibition of α -amylase delays

carbohydrate digestion and prolongs the overall carbohydrate digestion time, causing a reduction in the rate of glucose absorption, consequently blunting increased post – prandial plasma glucose levels [11].

Alpha glucosidase is an important enzyme in the process of carbohydrate digestion. It catalyzes the hydrolysis of 1, 4-glucosidic bonds within carbohydrates with release of α -glucose and promotes the increase of blood glucose level after meal. Alpha – glucosidase inhibitors antagonize the activity of α -glucosidase, thereby delaying intestinal carbohydrate absorption and slowing the sharp rise in blood sugar levels that diabetic patients typically experience after meals [12].

Hence, the present study focussed to study the inhibitory activity of these two enzymes (i-e) α -amylase and α -glucosidase in seven species of *Salacia* such as *Salacia beddomei*, *Salacia chinensis*, *Salacia fruticosa*, *Salacia gambleana*, *Salacia macrosperma*, *Salacia malabarica* and *Salacia oblonga* using Acarbose as a standard positive control

2. Materials and Method

2.1 Collection and Extraction of Plant Materials

Leaves of seven species of *Salacia* such as *S.beddomei*, *S.chinensis*, *S.fruticosa*, *S.gambleana*, *S.macrosperma*, *S.malabarica* and *S.oblonga* were collected from Wayanad district of Kerala. They are washed thoroughly and shade dried. They are coarsely powdered. 100g of powdered plant material was soaked in 300 ml of Methanol for 48hrs. The extract was filtered using Whatmann Filter paper and the filtrate was concentrated under reduced pressure in vacuum at 40°C for 25min using a rotary evaporator. The percentage yield of extract was calculated using the formula,

$$\text{Percentage yield of extract} = \frac{\text{WE} - \text{WC}}{\text{WE}} \times 100$$

Where, WE – Weight of the extract

WC – Weight of the tube without extract

2.2 In vitro Anti diabetic Activity

a) Inhibition of alpha amylase enzyme

A starch solution of 0.1% w/v was prepared by stirring 0.g of potato starch in 100 ml of 16 mM of Sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100ml of distilled water. The calorimetric reagent was prepared by mixing sodium potassium tartarate solution and 3,5 di nitro salicylic acid solution (96 mM). The various concentrations of the plant extract (20 – 100 μ g/ml) were added to 1 ml of starch solution and left for 10 min. Further the reaction was initiated by the addition of the enzyme solution and allowed to react for 10 min under alkaline condition at 25°C. Finally the reaction was terminated by adding 1 ml of calorimetric reagent and then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in a similar way by replacing extract with DMSO. A similar experiment was conducted with the Standard drug Acarbose in triplicate.

b) Inhibition of alpha glucosidase enzyme

The inhibitory activity was determined by incubating a solution of Starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2M Tris buffer pH 8 and various concentrations of plant extracts for 5 min at 37°C. The reaction was initiated by adding 1ml of alpha glucosidase enzyme (1U/ml) to it followed by incubation for 40 min at 37°C. Then the reaction was terminated by the addition of 2 ml of 6N HCl. Then the intensity of colour was measured at 540 nm. Control experiment was done by replacing the extract with DMSO and also for a standard drug Acarbose in triplicate.

2.3 Statistical analysis

The data was statistically analyzed by one-way ANNOVA using SPSS 17.0. The difference was considered significant when $p < 0.005$. Triplicate assays were performed for each set of test conditions. All the values were expressed as Mean \pm SD (Standard Deviation). IC50 Value is also calculated for all test conditions.

3. Results

3.1 Yield Percentage of Plant Samples

Methanolic extract of leaves of seven species of *Salacia* such as *S.beddomei*, *S.chinensis*, *S.fruticosa*, *S.gambleana*, *S.macrosperma*, *S.malabarica* and *S.oblonga* were used for this study. Yield Percentage of above seven species of *Salacia* was *S.beddomei* (47.71%), *S.chinensis* (41.47%), *S.fruticosa* (58.46%), *S.gambleana* (51.86%), *S.macrosperma* (28.96%), *S.malabarica* (30.87%) and *S.oblonga* (52.75%).

The order of yield percentage for seven species of *Salacia* is as follows,

SF > SO > SG > SB > SC > SMAL > SMAC

3.2 In vitro Anti diabetic Activity

a) Inhibition of alpha amylase enzyme

This study was aimed to evaluate the inhibitory activity of this enzyme for Methanolic extract of seven species of *Salacia* such as. *S.beddomei*, *S.chinensis*, *S.fruticosa*, *S.gambleana*, *S.macrosperma*, *S.malabarica* and *S.oblonga*. IC₅₀ Value was calculated for seven species and also for standard Acarbose. IC₅₀ Value for seven species of *Salacia* was *Salacia beddomei* (122µg/ml), *Salacia chinensis* (112µg/ml), *Salacia fruticosa* (108µg/ml), *Salacia gambleana* (148µg/ml), *Salacia macrosperma* (125µg/ml), *Salacia malabarica* (121µg/ml), and *Salacia oblonga* (117µg/ml) (Table 1) (Figure 1 – 7). Alpha amylase inhibitory activity for seven species of *Salacia* can be arranged in ascending order was SF > SC > SO > SMAL > SB > SMAC > SG.

b) Inhibition of alpha glucosidase enzyme

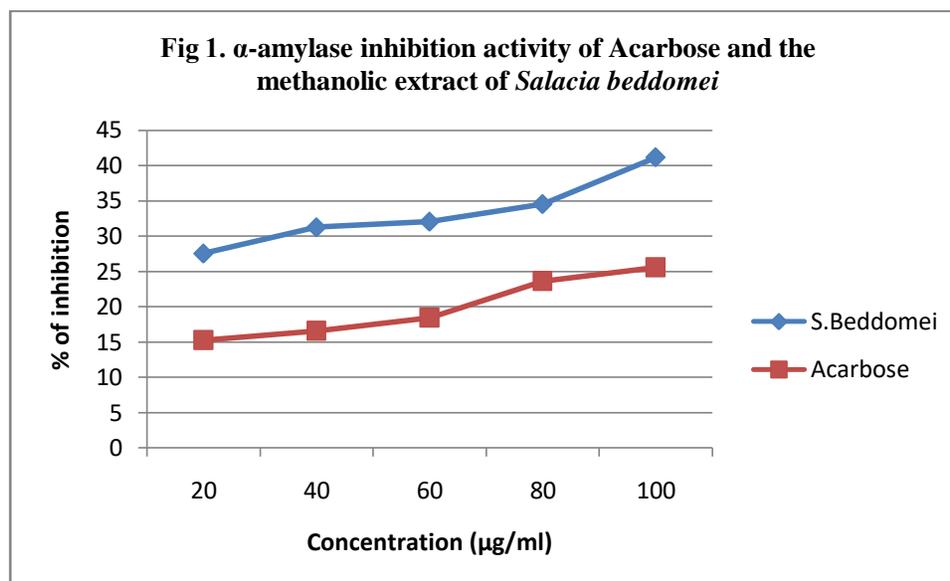
This study also explained the inhibitory activity of Alpha glucosidase enzyme for Methanolic extract of seven species of *Salacia*. The values are expressed as IC₅₀ Value for seven species and also for standard Acarbose. IC₅₀ Value for seven species of *Salacia* was *S.beddomei* (107µg/ml), *S.chinensis* (105µg/ml), *S.fruticosa* (80µg/ml), *S.gambleana* (110µg/ml), *S.macrosperma* (109µg/ml), *S.malabarica* (107µg/ml), and *S.oblonga* (108µg/ml) (Table 2) (Figure 8-14). The order wise arrangement of the Alpha glucosidase inhibitory activity was SF > SC > SB = SMAL > SO > SMAC > SG.

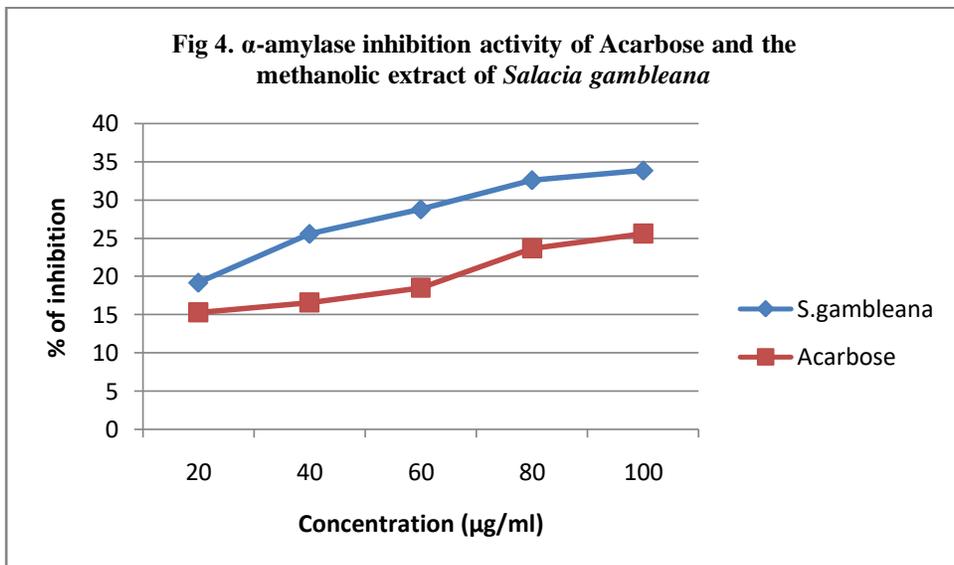
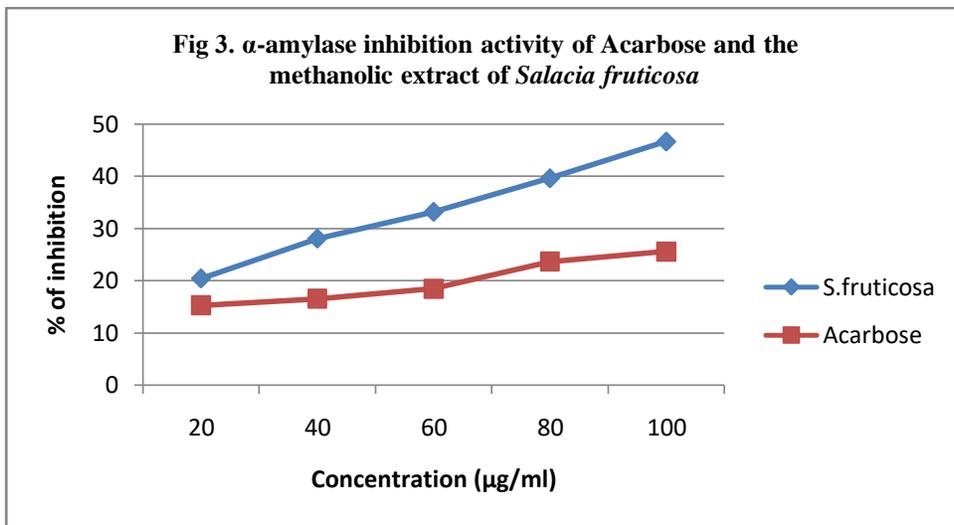
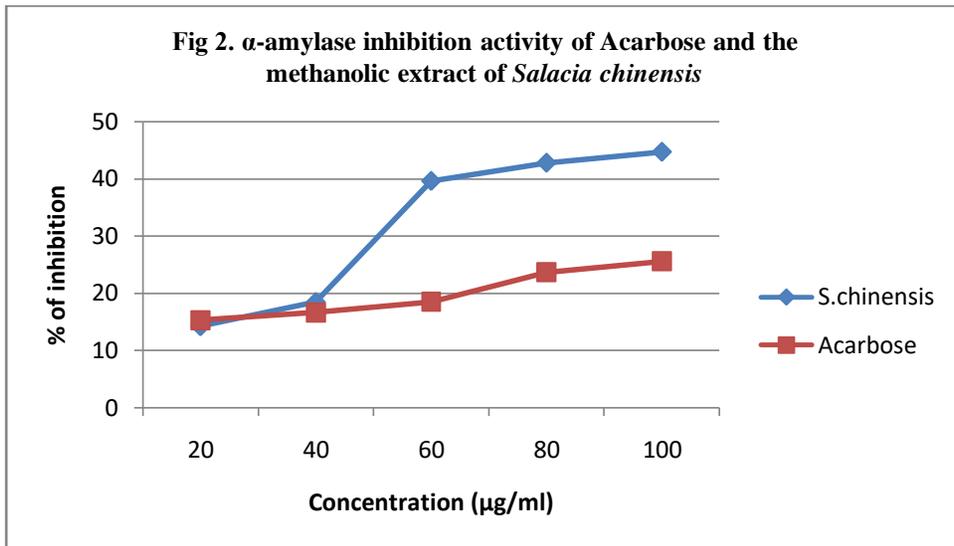
Table 1: Inhibition of Alpha Amylase Enzyme

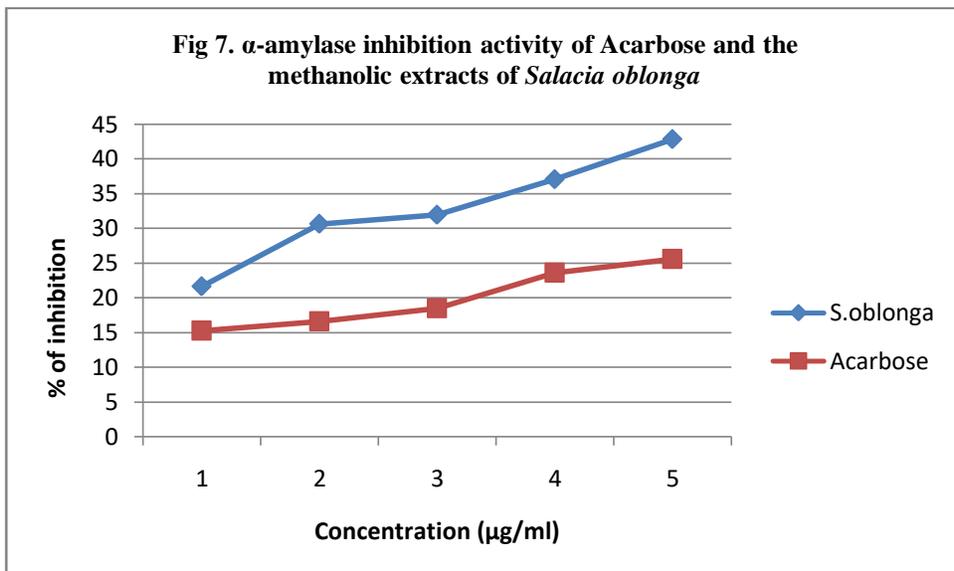
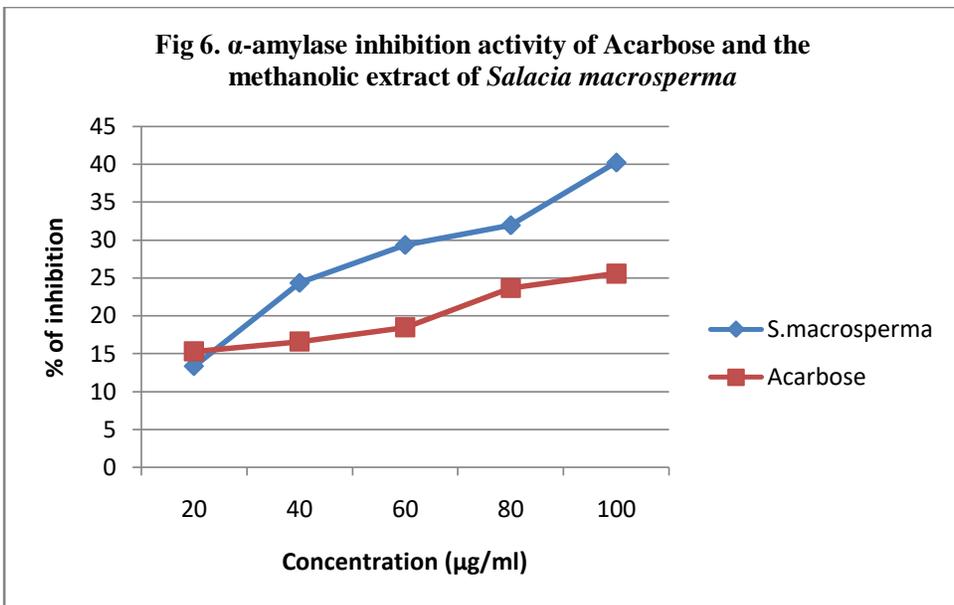
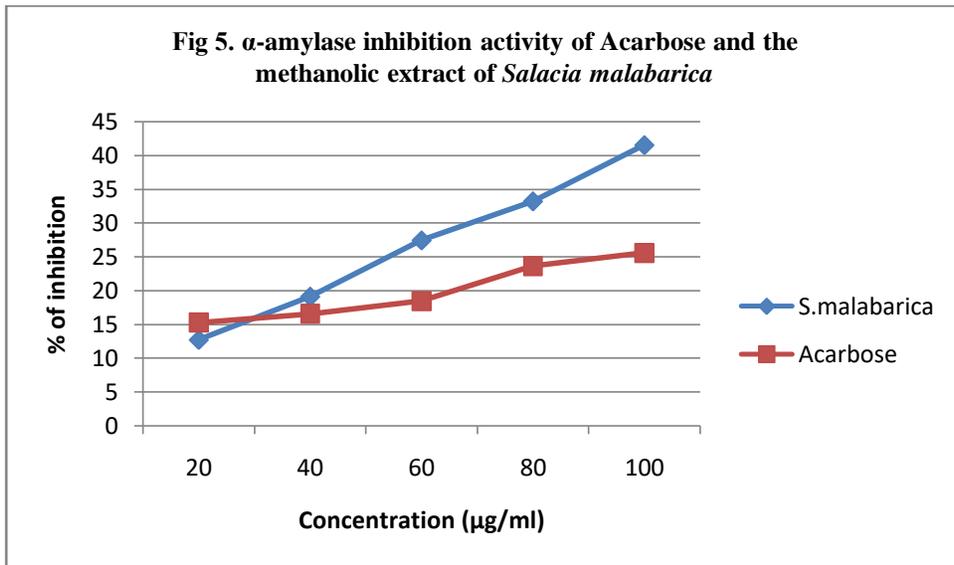
| S. No | Concentration (µg) | Acarbose (Mean±SD) | <i>Salacia beddomei</i> (Mean±SD) | <i>Salacia chinensis</i> (Mean±SD) | <i>Salacia fruticosa</i> (Mean±SD) | <i>Salacia gambleana</i> (Mean±SD) | <i>Salacia macrosperma</i> (Mean±SD) | <i>Salacia malabarica</i> (Mean±SD) | <i>Salacia oblonga</i> (Mean±SD) |
|-------|-------------------------|--------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------------------------|-------------------------------------|----------------------------------|
| 1. | 20 | 15.28±0.057 | 27.55±0.133 | 14.22±0.063 | 20.44±0.055 | 19.19±0.026 | 13.33±0.072 | 12.71±0.058 | 21.65±0.075 |
| 2. | 40 | 16.58±0.052 | 31.25±0.087 | 18.45±0.067 | 28.09±0.064 | 25.57±0.044 | 24.23±0.070 | 19.12±0.061 | 30.65±0.063 |
| 3. | 60 | 18.48±0.061 | 32.05±0.040 | 39.63±0.061 | 33.21±0.066 | 28.77±0.043 | 29.34±0.081 | 27.44±0.069 | 31.95±0.061 |
| 4. | 80 | 23.65±0.054 | 34.54±0.035 | 42.78±0.087 | 39.64±0.065 | 32.59±0.057 | 31.94±0.064 | 33.22±0.066 | 37.06±0.060 |
| 5. | 100 | 25.58±0.054 | 41.15±0.089 | 44.69±0.096 | 46.67±0.069 | 33.85±0.072 | 40.23±0.081 | 41.54±0.069 | 42.84±0.063 |
| | *IC ₅₀ Value | 196µg/ml | 122µg/ml | 112µg/ml | 108µg/ml | 148µg/ml | 125µg/ml | 121µg/ml | 117µg/ml |

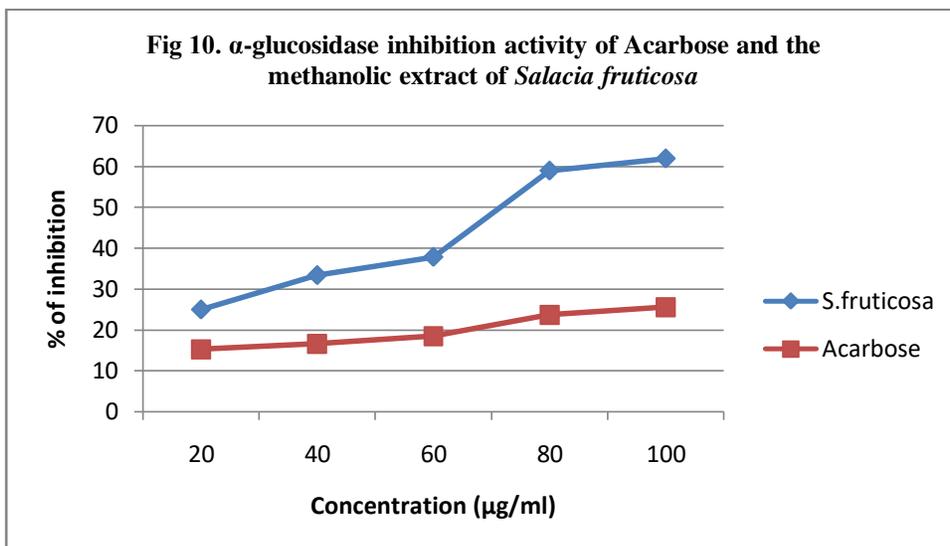
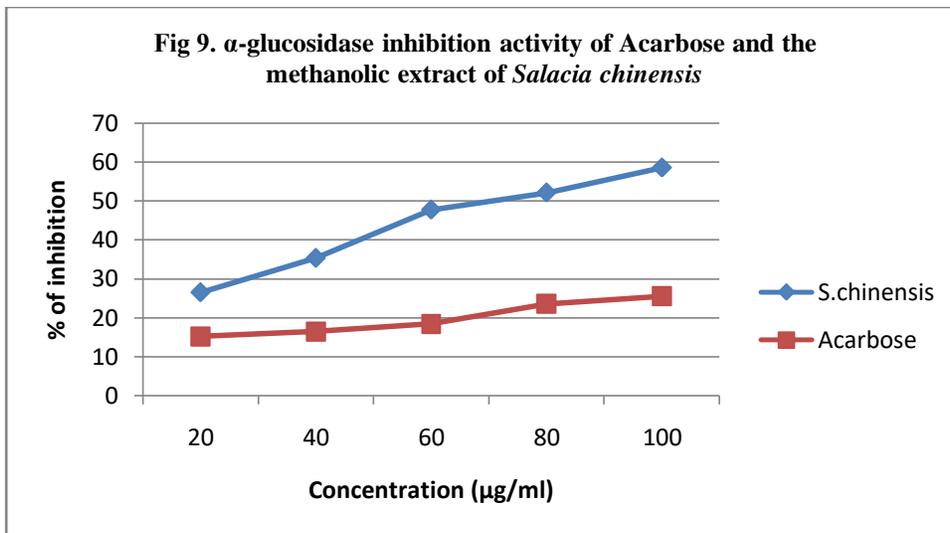
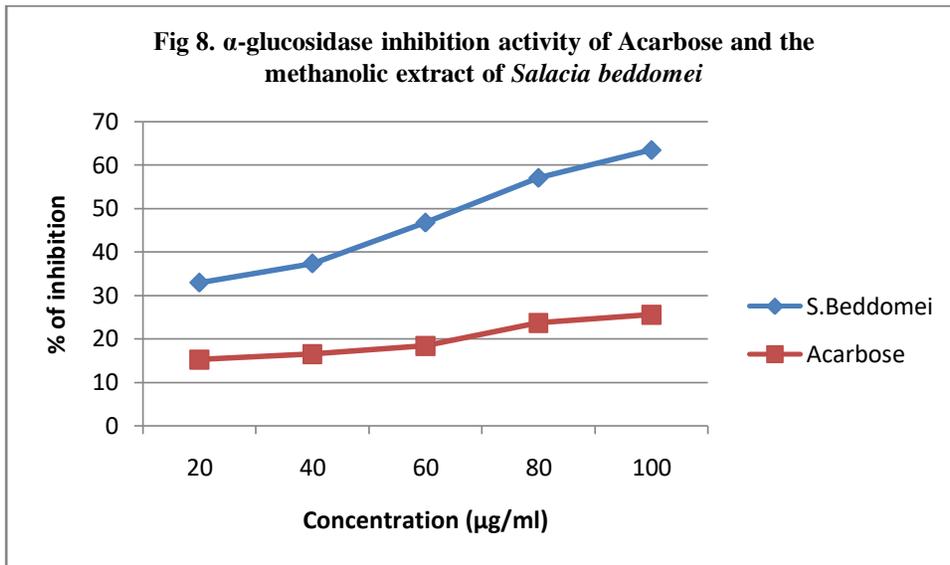
Table 2: Inhibition of alpha glucosidase enzyme

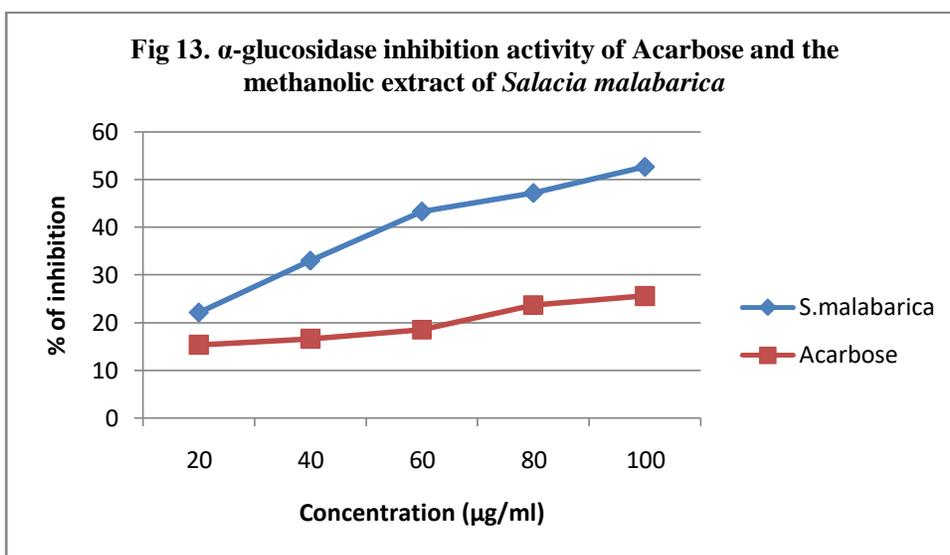
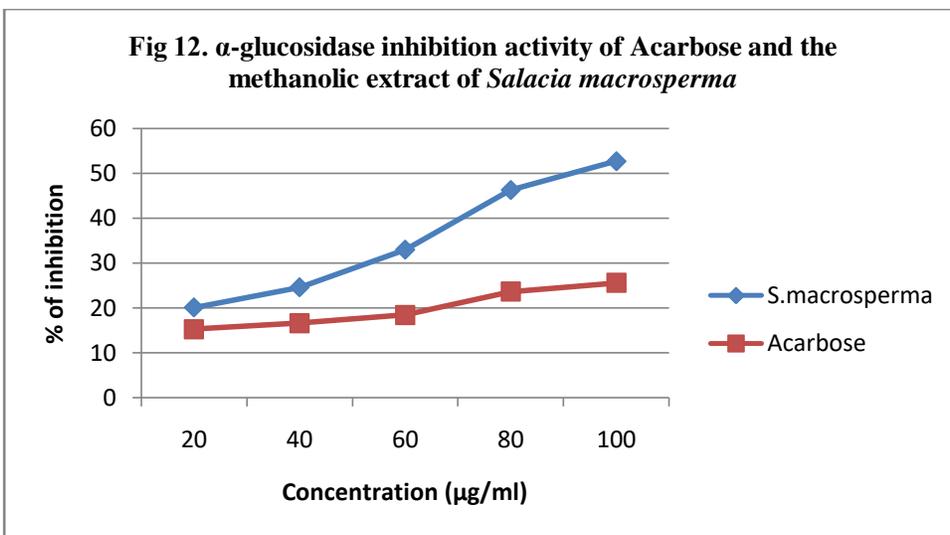
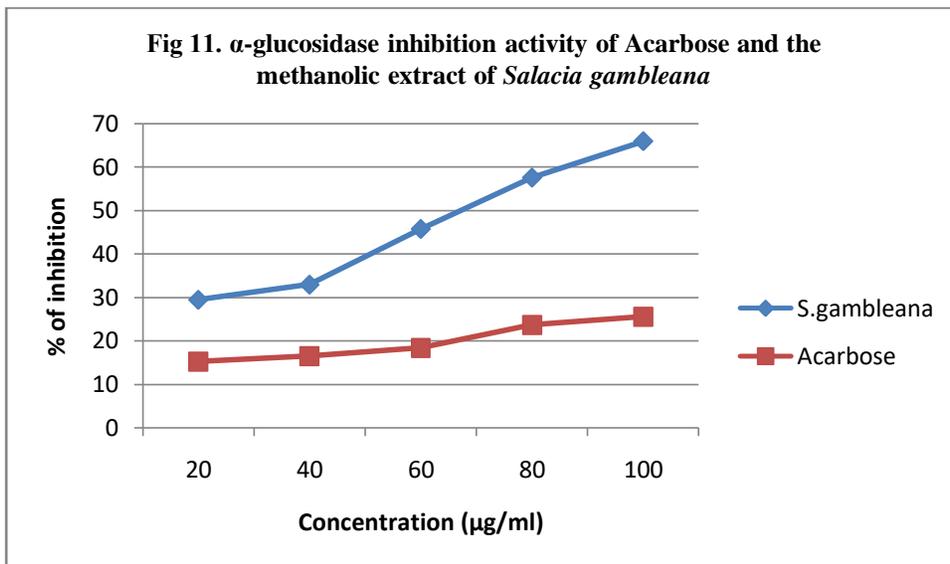
| S. No | Concentration (µg) | Acarbose (Mean±SD) | <i>Salacia beddomei</i> (Mean±SD) | <i>Salacia chinensis</i> (Mean±SD) | <i>Salacia fruticosa</i> (Mean±SD) | <i>Salacia gambleana</i> (Mean±SD) | <i>Salacia macrosperma</i> (Mean±SD) | <i>Salacia malabarica</i> (Mean±SD) | <i>Salacia oblonga</i> (Mean±SD) |
|-------|-------------------------|--------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|--------------------------------------|-------------------------------------|----------------------------------|
| 1. | 20 | 15.28±0.057 | 32.92±0.040 | 26.54±0.032 | 24.96±0.086 | 29.46±0.065 | 20.08±0.060 | 22.07±0.052 | 26.97±0.059 |
| 2. | 40 | 16.58±0.052 | 37.33±0.0058 | 35.35±0.053 | 33.37±0.062 | 32.94±0.034 | 24.56±0.047 | 32.95±0.033 | 32.94±0.035 |
| 3. | 60 | 18.48±0.061 | 46.74±0.066 | 47.76±0.063 | 37.84±0.062 | 45.74±0.047 | 32.95±0.040 | 43.27±0.047 | 46.68±0.057 |
| 4. | 80 | 23.65±0.054 | 57.04±0.055 | 52.14±0.052 | 58.97±0.070 | 57.54±0.055 | 46.24±0.034 | 47.15±0.087 | 57.56±0.044 |
| 5. | 100 | 25.58±0.054 | 63.44±0.064 | 58.55±0.043 | 61.94±0.069 | 65.91±0.046 | 52.65±0.033 | 52.64±0.031 | 59.97±0.061 |
| | *IC ₅₀ Value | 196µg/ml | 107µg/ml | 105µg/ml | 80µg/ml | 110µg/ml | 109µg/ml | 107µg/ml | 108µg/ml |

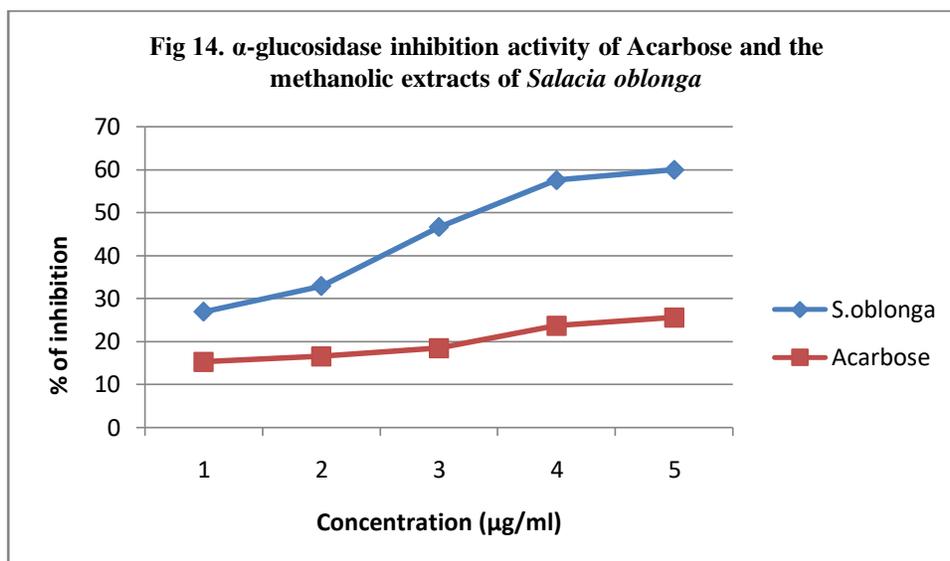












4. Discussion

Diabetes includes the development of micro and macro vascular diabetic complications [13]. In humans, glucose tolerance impairs prior to maturity – onset of hyperglycemia [14] and is widely used as a clinical index to predict the potentiality of developing diabetes [15].

Most of plants and their products are used as a valuable source of anti-diabetic drugs. In both Ayurvedic medicinal preparations and also Chinese medicinal preparations, includes various medicinal plants to treat diabetes [16, 17].

Alpha amylase is a salivary or pancreatic enzyme which plays an important role in earlier breakdown of starch to simpler sugars. The inhibitors of alpha amylase can delay the carbohydrate digestion and reduce the rate of glucose absorption. As a result there is a decrease in postprandial blood glucose level. Hence, they have been thought to improve glucose tolerance in diabetic patients [18, 19].

In normal or impaired individuals, glucose tolerance can be found along with hyperinsulinemia. In such cases, alpha glucosidase inhibitors decrease hyperinsulinemia and improve insulin sensitivity [20]. It can be induce fasting hyperglycemia and be associated with coagulation activation and lipid metabolism abnormalities [21]. Alpha glucosidase inhibitors can delay the action of alpha glucosidases to break complex carbohydrates into simple sugars thereby lowering the absorption of glucose [22].

Salacia is one of the valuable medicinal plants used in the treatment of diabetes. In the present study Methanolic leaf extracts of selected species of *Salacia* was used to evaluate both Alpha amylase and Alpha glucosidase inhibitory activities. They are considered as a rich source of active principles such as Salacinol and Kotalanol and used as alternative and less expensive therapies in diabetic management.

5. Conclusion

The present study investigates the anti diabetic property of seven species of *Salacia*. It is basically attributed to the inhibitory activity of intestinal enzymes such as Alpha amylase and Alpha glucosidase. The inhibitory property of this extract may be attributed to the presence of phytochemicals such as saponins and flavonoids. Hence, this is a valuable genus which should be protected in all aspects.

Acknowledgement

The authors are thankful to National Medicinal Plants Board, New Delhi for providing financial assistance to carry out this study.

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