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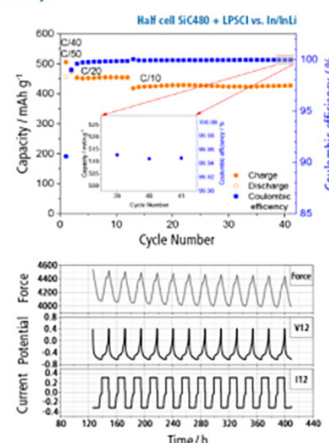
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Comparative Analysis Of In Vitro Antidiabetic Activity Of Wild And Micropropagated *Solanum Trilobatum* L

P. Rajasulochana¹, Abhigna Battineni², S. Fahira Tasneem³

Department of Genetic Engineering, Bharath Institute of Higher Education and Research,
Chennai – 600073, TN, India.

e-mail: ¹prsnellore@gmail.com

Abstract. Diabetes is metabolic disorder which is characterized by an increase in glucose level due to a relative deficiency of insulin. Recent decades have experienced a very high prevalence of diabetes mellitus. One way of treating diabetes is to reduce gastrointestinal glucose production by inhibiting carbohydrate digesting enzymes called pancreatic alpha-amylase and intestinal alpha-glucosidase. The present study aims to compare the efficacy of wild and micropropagated *Solanum trilobatum* for its in vitro antidiabetic property. *Solanum trilobatum* grown in vitro under aseptic conditions was compared with wild-grown, and the results have revealed that both wild and micropropagated plants have effective dose-dependent inhibition of alpha-amylase and alpha-glucosidase enzyme. For both alpha-amylase and alpha-glucosidase assay micropropagated plant has shown lesser efficacy at all concentrations when compared to that of wild grown. still micropropagated showed effective inhibition with nearly 90% similarity.

Keywords: *Solanum trilobatum*; In vitro propagation; α -amylase enzyme; α -glucosidase enzyme

1. Introduction

Diabetes which is often referred as diabetes mellitus is a group of metabolic diseases where the person is having high glucose levels in their blood (blood sugar) either because insulin production is inadequate, or the body's cells do not respond properly to the insulin produced, or both [1]. Patients with high blood sugar will typically experience Polyuria (frequent urination), Polydipsia (increasingly thirsty) and Polyphagia (hungry) [2]. Diabetes is of two types: Type-1 diabetes, which is a chronic condition where pancreas produces little or no amount of insulin because of the presence of autoantibodies against pancreas and Type-2 diabetes, which is also a chronic condition where the cells of the body are resistant to the insulin produced or the insulin produced is not functional [3]. There is yet another type of diabetes called gestational diabetes which occurs commonly in females during pregnancy. There are various types of therapies available which includes: hypoglycemic drugs, insulin and cell therapy, but these types of therapies have their own limitations [4,5] either because of side effects or they do not provide satisfactory results which created increasing demands for traditional drugs. One such medicinally important plant to treat diabetes is *solanum trilobatum* [6,7].

Solanum trilobatum which is distributed throughout South India, belongs to the family *Solanaceae* and order *Solanales*. It is known to have anti-oxidant activity, cytotoxic activity, hemolytic activity,



protective effect, immunomodulatory activity, anti-microbial activity, skin repellent activity, and Antidiabetic activity. Phytochemical screening has shown that, carbohydrates, saponins, phytosterols, tannins, flavonoids and cardiac glucosides are present as major metabolites [8]. Bioactive compounds such as sobatum, solasodine, tomatidine, disogenin, and solaine are reported to be present in various parts of the plant [9].

Increasing demands lead to decline and scarcity of plant resources, habitat destruction [10]. To overcome this problem in vitro mass production techniques are employed which can also provide higher selectivity, and yields in short period of time without causing any changes to plant habitat.

Partial inhibition of enzymes that are involved in the production of glucose can manage the blood glucose levels [11]. The present study focuses on testing and evaluating the efficiency of methanolic leaf extract of micropropagated and wild-grown *Solanum trilobatum* for its in vitro Antidiabetic activity.

2. Materials and Methods

Samples (stems) used in this study were obtained from Tambaram, Kanchipuram DST, Tamil Nadu and the chemicals and Enzymes used in the assays like Alpha-amylase and alpha-glucosidase were analytically graded and were purchased from HiMedia PVT ltd, Mumbai Starch was obtained from SRL Pvt ltd, Mumbai; MS media stock solutions were prepared using analytical chemicals. Fluorescent tubes used were 40W and Philips company. other chemicals used were DNS (Di-nitro Salicylic Acid), Na_2CO_3 , P-NGP (P-Nitrophenyl α -D- GlucoPyranoside).

2.1. Preparation of Culture Media

Isolated explants need a nutrient supplement for their growth. And these nutrients can be artificially prepared and supplied. Various types of media are available for various plants. Medium selected depends upon the type of culture and the type of plant. In this study, apical stems were used as explants; hence MS (Murashige and Skoog's, 1962) media is used [12]. The composition of chemicals in correct quantities is important for the successful growth of explants. Media is prepared for 500 ml as four stock solutions: Stock solution-1 (Major nutrients), Stock solution-2 (Micro nutrients), Stock solution-3 (Iron source) Stock solution-4 (Organic supplement). Carbon source used is Sucrose – 30g/l, and Agar – 8g/l is used as solidifying agent. Growth regulators used were 25 μ l of both IAA (Indole Acetic Acid) and GA (Gibberellic Acid). The working solution which is prepared from stock solution is taken for 250 ml of double-distilled water and 20ml of working solution is added to the flasks. All the flasks, media, which were autoclaved, taken into the laminar airflow and allowed to cool.

2.2. Sterilization of Explant

Actively growing nodal regions of the stem were selected for this study and prewashed with running tap water followed by distilled water then followed by tween 20 solution for 2 minutes and were surface sterilized using 0.1% mercuric chloride (HgCl_2) solution and finally rinsed with double distilled water to remove any traces of mercuric chloride as it can harm plant cells [13].

2.3. Inoculation of Explants

Sterilized explants were carefully inoculated in sterilized medium and the cultures were maintained at a regulatory temperature of $25 \pm 3^\circ\text{C}$ temperature under a photoperiod of 18 hrs light and 6 hrs dark with a light intensity of 30 μ mol/m²/S provided by 40 W fluorescent bulbs.

2.4 Preparation of Plant Extract

Leaves collected from both wild and micropropagated (15 days after the inoculation) plants were washed thoroughly and shade dried for two days and powdered mechanically using mortar and pestle. 2.7 gm of wild and 0.7 gm of micropropagated powdered extract were taken and added to 50 ml of methanol separately in two separate containers and kept in shaker overnight at 37°C . On the next day residue was removed through filtration process, and the filtrate is concentrated under reduced pressure

in hot air oven at 60°C for one day, and the crude extract was collected and stored in the refrigerator for further study.

2.5 Alpha-Amylase Inhibition Assay

A modified procedure of McCue and Shetty is used to carry out this assay where the enzyme solution was prepared by dissolving α -amylase in 20 Mm phosphate buffer having PH 6.9 at a concentration of 0.1 mg/ml. 1 ml of the extract of both wild and micropropagated were taken at various concentrations say, 50, 100, 150, 200, 250 separately in separate test tubes along with control that doesn't include any of the sample extract. To all these test tubes, 0.25ml of enzyme solution is added and incubated at 37°C for 5 minutes followed by 0.5 ml starch solution and further incubated at 37°C for 5 min. The reaction was stopped by adding 0.5 ml of DNS (Di Nitro Salicylic acid) and heated in water bath at 100°C for 5 min. After cooling, absorbance is measured at 540nm, and values were noted. The percentage of inhibition is calculated using the formula:

$$\text{Percentage of inhibition} = \frac{\text{Ab(sample)} - \text{Ab(control)}}{\text{Ab(sample)}} \times 100$$

2.6 Alpha-Glucosidase Inhibition Assay

This assay which is carried out using modified method published [14], started by adding 50 μ l of phosphate buffer having PH 6.9 in 96 well plates, to this 10 μ l of α -glucosidase enzymes is added followed by addition of extract of both wild and micropropagated plants separately at various concentrations say 20, 40, 60, 80, 100 μ l and then it is incubated at 37°C for 15 min. DHNP a substrate for β D-glucosidase is then added and further incubated for 20 min, after incubation the reaction is stopped by adding 50 μ l of Na_2CO_3 (0.1M), and the absorbance is measured at 405nm, and the percentage of inhibition is calculated using the formula:

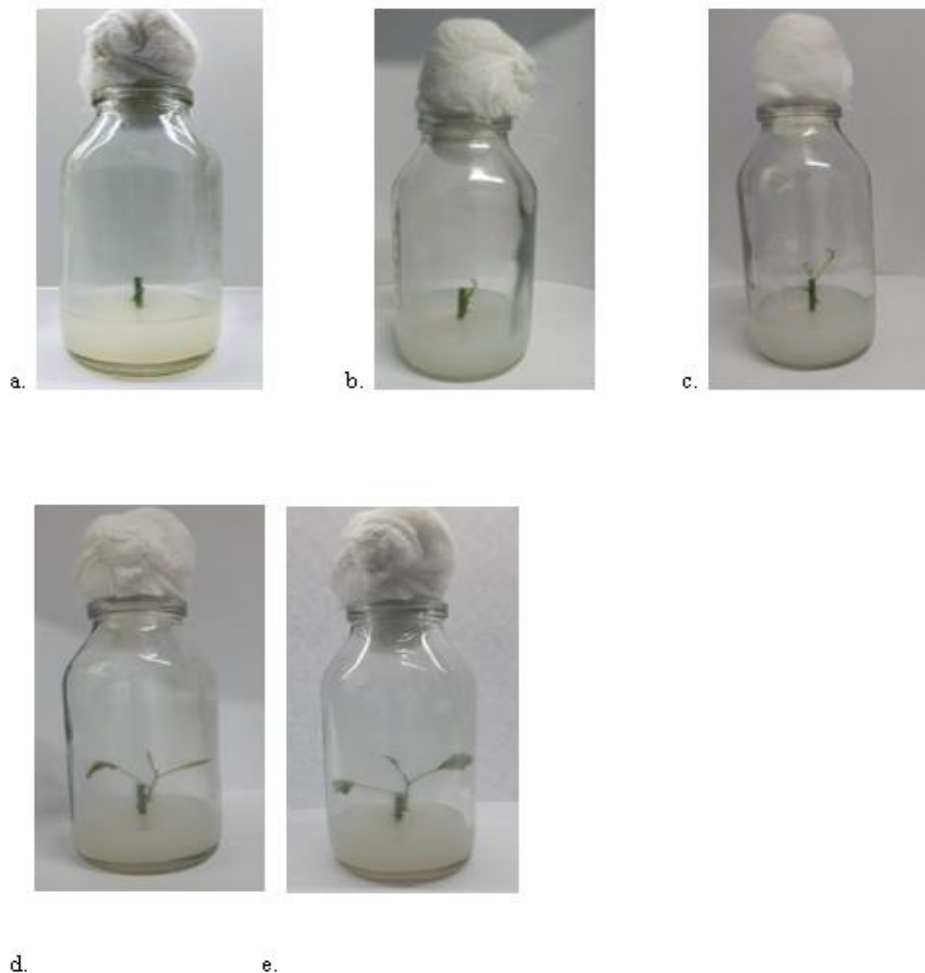
$$\text{Percentage of inhibition} = \frac{\text{Ab(sample)} - \text{Ab(control)}}{\text{Ab(sample)}} \times 100$$

3. Results

3.1. Preparation of Culture Media

Table 1: Growth of inoculated explants from the 0th day to the 15th day

Day	Result observed
0 th day	Inoculation
3 rd day	The appearance of growth of lateral shoot from the node
5 th day	Growth of lateral shoot
6 th day	The appearance of leaves from lateral shoots and formation of callus
7 th day	Growth of shoots and more leaves
10 th day	Leaves growing larger
11 th day	New leaves were grown
12 th day	Growth of lateral shoots and also leaves
13 th day	Growth of lateral shoots and leaves into much larger
15 th day	Sufficient growth of leaves and formation of roots.

Figure 1: Growth of inoculated explants from the 0th day to the 15th day.

a. 0th day; b. 5th day, growth of lateral shoots; c. 7th day, development of lateral shoot & leaves; d. 11th day, the formation of new leaves; e. 15th day, growth seen in lateral shoots and leaves.

3.2 Alpha Amylase Inhibition Assay

Table 2: Showing % of inhibition increased as the concentration increased in wild plant

Concentration ($\mu\text{g/ml}$)	(in % Inhibition of amylase)
50	61.32%
100	73.02%
150	79.08%
200	83.76%
250	87.42%

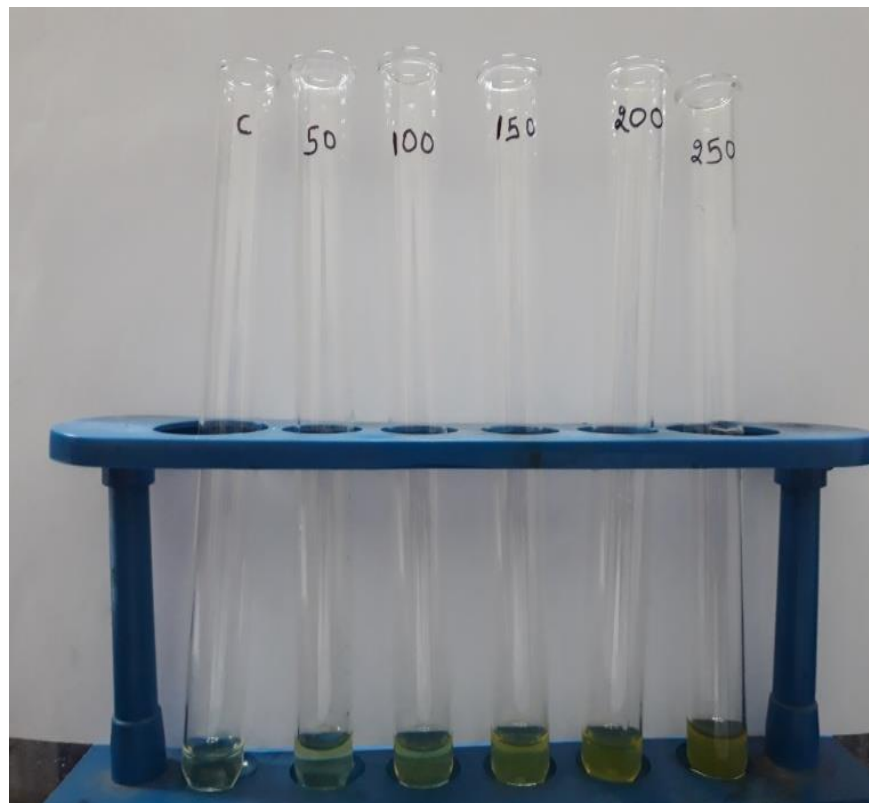


Figure 2: Alpha-amylase inhibition of wild *Solanum trilobatum*

Table 3: Showing % inhibition in wild micropropagated *Solanum trilobatum* plant

Concentration	% Inhibition of amylase
50	35.93%
100	45.33%
150	63.06%
200	82.32%
250	84.38%

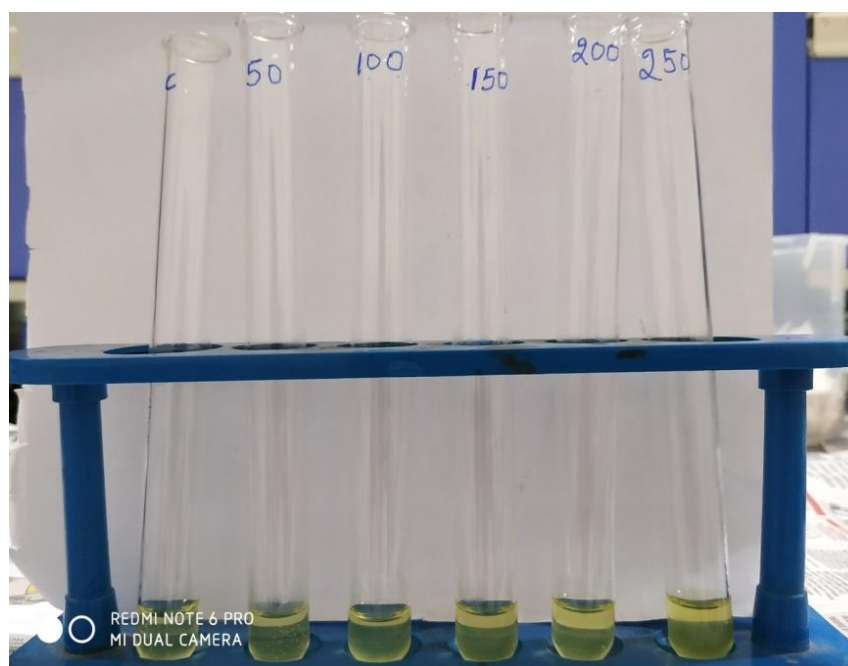


Figure 3: Alpha-amylase inhibition of micropropagated *Solanum trilobatum*

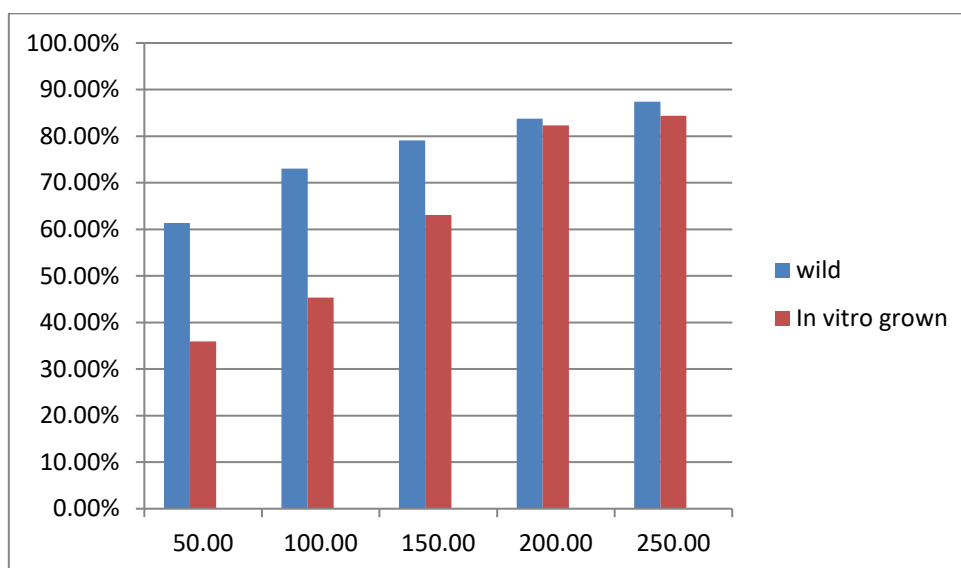


Figure 4: Graphical comparison of inhibition of amylase by wild and micropropagated *Solanum trilobatum*

3.3 Alpha Glucosidase Assay

Table 4: Inhibition of wild plant in alpha-glucosidase assay

Concentration in µg/ml	% inhibition of glucosidase
20	52.68%
40	70.86%
60	71.36%
80	82.98%
100	83.81%

Table 5: Inhibition of micropropagated plant in alpha-glucosidase assay

Concentration	% of inhibition
20	37.24%
40	58.52%
60	64.45%
80	67.10%
100	68.72%

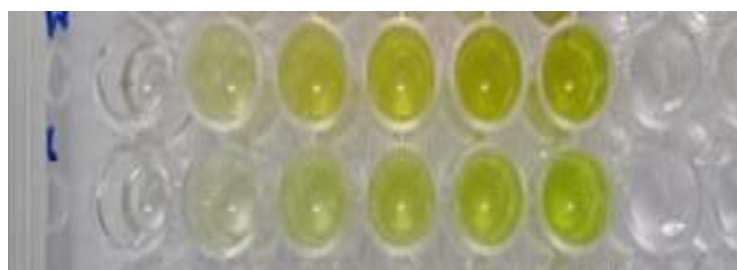


Fig 5: Alpha-glucosidase assay of both wild and in vitro propagated *Solanum trilobatum*

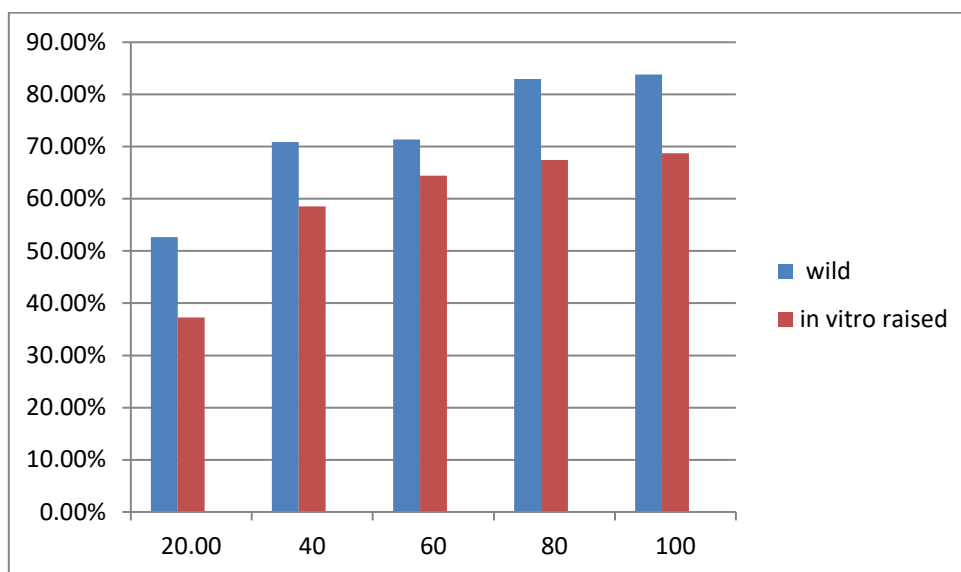


Fig 6: Graphical comparison of inhibition of alpha-glucosidase by wild and micropropagated plants.

4. Discussion

In vitro micropropagation: *Solanum trilobatum*, which is a climbing herb, is very easy to be grown in vitro. The growth is fast hence, a large number of plants can be obtained in short time. Growth factors supplemented (IAA, GA) were effective in growing lateral shoots, leaves and also roots. Fungal contamination is seen in some flasks, but overall growth is effective.

Inhibition of amylase and glucosidase: This study is an attempt to find the efficacy of wild and micropropagated *solanum trilobatum* on enzymes acting on carbohydrates. The effect of methanol extract of *Solanum trilobatum* (wild and micropropagated) was evaluated on the activity of alpha-amylase and alpha-glucosidase. And both the plant extract showed a dose-dependent potential inhibition. For both alpha-amylase and alpha-glucosidase the in vitro plant showed lesser efficacy when compared to wild; this may be due to the presence of high amounts inhibiting compounds in wild plants than in micropropagated plant at the same concentrations or may be due to the effect of environmental that make the wild plant produce more metabolites that are inhibiting glucose producing enzymes.

Previous studies on wild *Solanum trilobatum* for antidiabetic activity concluded that the leaf extract is having glucose-lowering properties in diabetic rats without any side effects [15]. The present study has shown that even micropropagated plants are still having potential inhibition. As *Solanum trilobatum* is having a wide range of phytochemicals, any of the classes could inhibit alpha-amylase and alpha-glucosidase enzymes. Future work should aim at integrating the study with membrane processes [16] and lab-on-a-chip technology [17,18].

5. Conclusion

It can be concluded that both wild and micropropagated *Solanum trilobatum* plants showed Antidiabetic activity effectively in dose-dependent manner. This study suggests when *Solanum trilobatum* is rare in distribution then micropropagated *Solanum trilobatum* can replace the need

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