In Vitro Free Radical Scavenging Activity and Alpha Amylase Inhibitory Activity of *Croton tiglium* Extract

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ABSTRACT

Introduction and Aim: Diabetes mellitus is a metabolic disorder which has emerged as a global public health threat in the present century. The present oral hypoglycemic agents produce undesirable side effects and oxidative stress. Thus, there is a need for a safe, cost-effective and complementary therapy for Diabetes mellitus. To evaluate the free radical scavenging activity and Alpha amylase inhibitory activity of *Croton tiglium* extract.

Materials and Methods: Different concentrations of the plant extract (1.5, 3, 7, 15.30, 60, 125, 250, 500 and 1000 µg/ml) were prepared in ethanol and subjected to α amylase inhibitory and free radical scavenging activity DPPH assay. The absorbance was read at 540nm and 546nm respectively using a spectrophotometer. The ability of ethanolic extract of *Croton tiglium* to scavenge free radical by DPPH assay was determined according to the method of Chang et al (2001). Alpha-amylase inhibitory activity was evaluated according to the method of Bernfield (1955).

Results: % Inhibition of DPPH by *Croton tiglium* extract at 20, 40, 60, 80, 100µg/ml was 14.24±0.70, 29.37±0.13, 45.52±0.97, 65.97±0.36, 84.29±0.27 respectively. % inhibition of Alpha amylase by extract of *Croton tiglium* at 20, 40, 60, 80, 100µg/ml are 23.52±0.28, 32.54±0.12, 42.82±0.46, 55.09±0.09 and 74.15±0.86 respectively.

Conclusion: Based on the results we conclude that *Croton tiglium* as strong in vitro antioxidant and alpha amylase inhibitory activity. Thus, *Croton tiglium* is a potential antidiabetic agent.

Key Words: In vitro, DPPH assay, Alpha amylase, *Croton tiglium.*

INTRODUCTION

Living cells produce free radicals as a by-product of biochemical processes. Free radicals are the cause of a number of disorders in humans like diabetes, tumour, cardiovascular accidents, arthritis, etc. Diabetes has emerged as a major health problem worldwide (1). Oral hypoglycaemic agents produce adverse effects like hypoglycaemia, hypersensitivity, lactic acidosis, weight gain, GI side effects, etc. The transition to herbal drugs seems necessary considering their efficacy, safety, and cost (2).

The antioxidant property of plants products is mainly attributed to the phytochemical constituents present in the plant. The treatment of diabetes these days is focused on decreasing the glucose level by inhibiting α-amylase (3). Therefore ideal antidiabetic properties in herbs are high flavonoids, tannins contents, and presence of amylase inhibitory activity. The aim of the present study is to evaluate the free radical scavenging activity and alpha amylase inhibitory activity of *Croton tiglium* extract.

MATERIALS AND METHODS

Material

The plant material (*Croton tiglium*) was procured from Green Chem. Herbal Extract & Formulations, Bangalore. The Hydrogen peroxide was purchased from Sisco Research Laboratories Pvt. Ltd, Pallikaranai, Chennai.

Figure 1: *Pterocarpus marsupium* plant

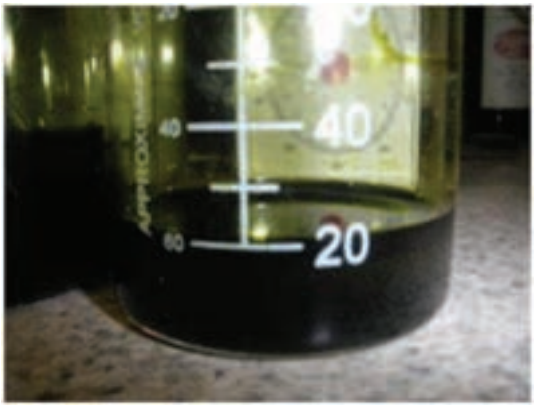
Preparation of Plant Extract

Leaves were washed with running tap water thoroughly, rinsed with distilled water, sun-dried, powdered and made as extract with chloroform, ethanol, petroleum ether, ethyl acetate and water using soxhlet equipment.

The obtained extract was filtered using whatman no:1 filter paper, condensed using rotary flash vaporator and stored in an airtight container (4).

Figure 2: Dried leaves of Pterocarpus marsupium

Figure 3: *Croton tiglium* extract



Extraction of Wheat α-Amylase

500mg of wheat flour was added to 1 litre of 0.2% calcium acetate solute at room temperature and was continuously stirred for a while. The suspension was then centrifuged. The obtained extract was stored at 3oC. β-amylase activity was inactivated by heating the extract at 70^{o}C for 10 minutes at a pH of 6.6. The extract was cooled to 4^{o}C until use (5).

Figure 4: Wheat α-amylase extract

DPPH Assay (2, 2-DIPHENYL-1-PICRYLHYDRAZYL)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as a reference (6).

Principle:

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as,

$$(DPPH) + (H\text{-}A) \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (7).

Reagent preparation

0.1mM DPPH solution was prepared by dissolving

4mg of DPPH in 100ml of ethanol.

Working procedure

Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in a dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\% \text{ RSA} = \frac{\text{Abs Control-Abs Sample}}{\text{Abs Control}} \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract (8).

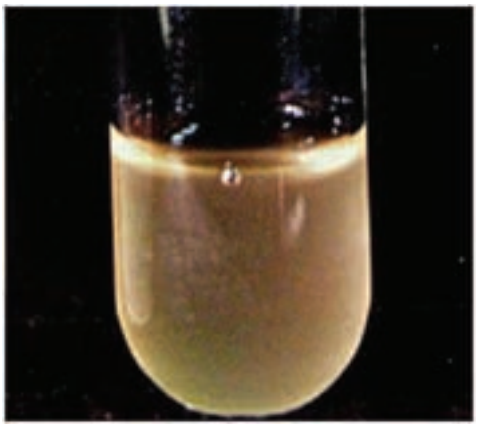
Determination of α-Amylase Inhibitory Activity

The mixture containing 20µl of enzyme, 200µl of 0.02M sodium phosphate buffer and plant extract of concentration ranging between 20-100µg/ml was incubated in room temperature for 10minutes followed by adding 200µl of starch in all dilution. The reaction is terminated by adding 400µl of DNS. Absorbance is measured at 540nm. Acarbose was used as a reference.

$$\text{Percentage inhibition} = \frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Acarbose was used as the reference α-amylase inhibitor (9).

Figure 5: α-amylase inhibitory activity



RESULTS

Table 1: Hydrogen peroxide scavenging activity

Concentration (µg/ml)	% Inhibition Quercetin	% Inhibition *Croton tiglium*
20	**49.86 ± 1.14**	**14.24±0.70**
40	**61.29 ± 0.14**	**29.37±0.13**
60	**74.09 ± 0.26**	**45.52±0.97**
80	**84.04 ± 2.48**	**65.97±0.36**
100	**94.14 ± 1.07**	**84.29±0.27**

Table 2: Amylase inhibitory activity

Concentration (µg/ml)	% Inhibition Acarbose	% Inhibition *Croton tiglium*
20	**46.66 ± 0.10**	**23.52±0.28**
40	**59.54 ± 0.52**	**32.54±0.12**
60	**68.25 ± 0.12**	**42.82±0.46**
80	**76.14 ± 1.01**	**55.09±0.09**
100	**82.69 ± 1.02**	**74.15±0.86**

Figure 6: % Inhibition DPPH *Croton tiglium* Vs Quercetin

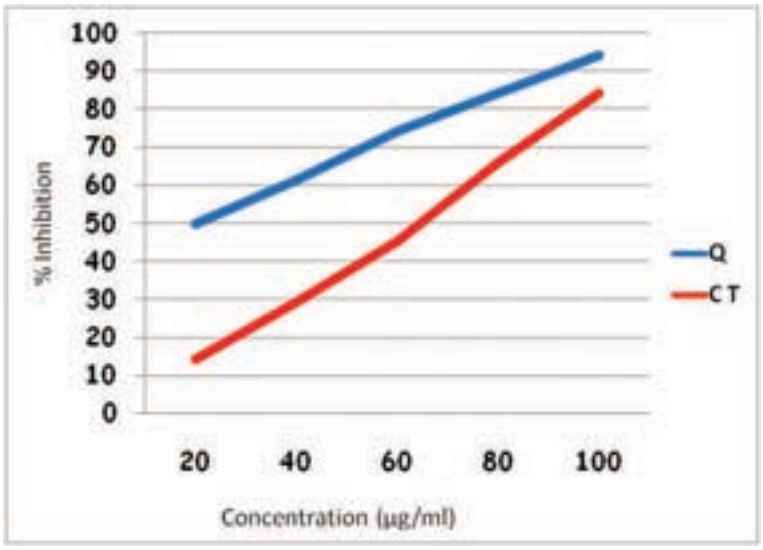
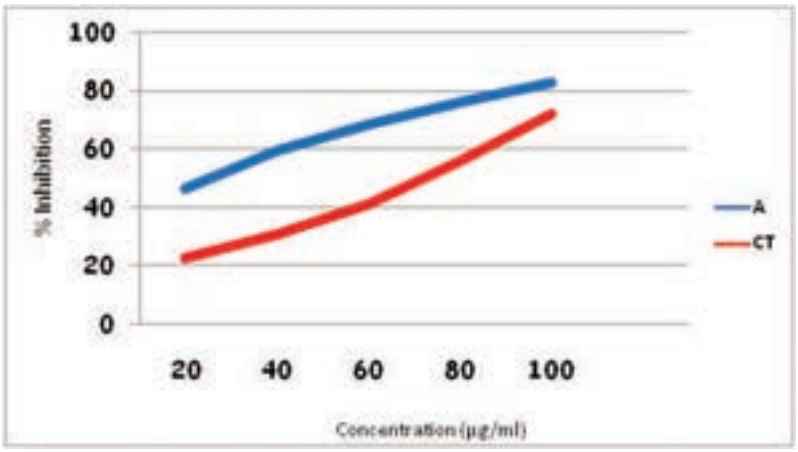


Figure 7: % Inhibition α-amylase *Croton tiglium* Vs Acarbose



DISCUSSION

Antioxidant Activity: Antioxidants present in herbal plants are accountable for the prevention of damage caused by free radicals. Flavonoids and tannins present in plants are potent free radical scavengers (10). DPPH is a widely used chemical compound for assessing free radical scavenging activity. The above results show that *Croton tiglium* has a significant antioxidant property under in vitro condition. Moreover, the graph indicates a dose-dependent inhibition of DPPH.

α-Amylase Inhibition Activity: α-amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategies of treating diabetes. Inhibiting α-amylase will lower post-prandial blood sugar (11). The result suggests that ethanolic extract of *Croton tiglium* exhibit good αamylase activity under in vitro condition. Dose-dependent % inhibitory activity against α-amylase was noted.

Our study indicates that *Croton tiglium* could be useful in the treatment of post-prandial hyperglycaemia. The Antioxidant and anti-diabetic activity may be attributed to the presence of flavonoids, tannins & anti-α-amylase activity.

CONCLUSION

Based on the above results we conclude that ethanolic leaf extract of *Croton tiglium* exhibit potent α-amylase inhibitory activity and could be exploited in the management of post-prandial hyperglycemia in the treatment of Type 2 diabetes mellitus. However pharmacokinetic and safety profile of *Croton tiglium* requires pre-clinical testing prior to its application on humans

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