

Evaluation of anti-diabetic activity of leaves of *Convolvulus Pluricaulis* by enzyme inhibition

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ABSTRACT

Objective: To investigate the therapeutic effects of Ethanolic leaf extract and its fractions of *Convolvulus pluriacualis* were evaluated for their on alpha-amylase and alpha-glucosidase inhibition by using *in vitro* assay.

Methods: Leaves of *Convolvulus pluriacualis* extracted with ethanol and its fractions are prepared by Ethanolic extract of *Convolvulus pluriacualis* was dispersed in 1L of distilled water separately and was fractionated with toluene, ethyl acetate and butan-2-one in succession. The solvents were removed from the fractions under reduced pressure to yield the corresponding extracts are concentrated under reduced pressure. The inhibitory effect of these extracts on α -amylase and α -glucosidase inhibitory activities as well as some antioxidant parameters was determined *in vitro*.

Results: The results revealed that these ethanolic extracts and its fractions inhibited α -amylase and α -glucosidase activities in a dose dependent manner. The plant extracts and their fractions were used at varying concentrations to ensure which concentration of the extract and its fractions causes the most inhibition. Among all fractions, Ethyl acetate fraction of *Convolvulus pluriacualis* has shown the prominent enzyme inhibitory activity (for α -amylase and α -glucosidase inhibitory activities with IC₅₀ values of 4.95 mg/ml and 4.4 mg/ml) as well as comparable with standard drug acarbose (for α -amylase IC₅₀ 2.32 mg/ml and for α -glucosidase IC₅₀ 2.00mg/ml) respectively. Phytochemical analysis of the extracts indicated the presence of Alkaloids, flavonoids, steroids/triterpenoids and their glycosides, saponins, phenolic compounds. Further, the total phenolic and total flavonoid contents were estimated. These results substantiate the use of *Convolvulus pluricaulis* in traditional medicine for the treatment of diabetes by controlling postprandial hyperglycemia.

Conclusion: The antioxidant properties and enzyme inhibition could be part of the mechanism by which they are used in the treatment/prevention of type-2 diabetes. These findings suggest that the plants may be a potential source for the development of new oral hypoglycemic agent.

Keywords: *Convolvulus pluricaulis*, α -amylase, α -glucosidase, Acarbose, Total flavonoids, Total phenolics.

1. Introduction

Diabetes mellitus is one of the most common systemic disease in the world and it occurs when the pancreas does not enough insulin or when the body cannot effectively use that insulin. Hyperglycemia (Evaluation of blood glucose concentration) is a common effect of uncontrolled diabetes and over time this leads to damage to, and dysfunction and failure of many of the body's organs including the eye. [1]. One of the most effective methods to prevent diabetes and hyperglycemia is to control the glucose level in blood. Sugars in blood originates from the hydrolysis of carbohydrates and is catalyzed by digestive enzymes, such as α -glucosidase and α -amylase [2]. α -glucosidase is an enzyme that plays a central role in carbohydrate metabolism by hydrolysing the terminal glycosidic bonds at the non-reducing end of saccharide polymers to release α -glucose. Much attention has been given to α -glucosidase in the pharmaceutical community because inhibition of its catalytic activity leads to impaired glucose absorption and a decrease in postprandial blood glucose levels. Therefore, α -glucosidase is currently the preferred target for the development of new antidiabetes agents. Pancreatic α -amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to maltose and finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated post prandial hyperglycemia. It has been shown that activity of human pancreatic α -amylase in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of diabetes. Hence retardation of starch digestion by inhibition of enzymes such as α -amylase would play a key role in the control of diabetes [3].

India has the 3rd largest diabetic population in the world. According to a report by IDF (International diabetes federation 2015), 1 in 7 births is effected by gestational diabetes, 1 in 11 adults have diabetes (415 millions), by 2040, 1 adult in 10 (642 million) will have diabetes, every 6 seconds a person dies from diabetes (5 million deaths). Diabetes mellitus is the most common endocrine disorder. It affected around 2.8% of the world's population and is anticipated to cross 5.4% by the year 2025 [4].

Diabetes is one of the most prevalent diseases in the world which is rapidly increasing worldwide. According to the WHO, the occurrence of diabetes might increase by 35% in the near future. Currently, over 150 million populations in the world are affected by diabetes, which is likely to increase over 300 million or more by the year 2025. In India, the number of diabetic people will increase from 15 million in 1995 to 57 million in the year 2025, which is considered to be the highest number of diabetics in the world [5].

Therefore, inhibition of these enzyme activities in digestive organs is considered to be a therapeutic approach and a strong option for managing diabetes. For the best of my knowledge, previous studies have not investigated the α -amylase and α -glucosidase

enzyme inhibitory activities of *Convolvulus pluricaulis*. Therefore, this work was undertaken to evaluate the probable mechanism behind its hypoglycemic activity.

2. Materials and methods

2.1. Collection of plant material

The leaves of *Convolvulus pluricaulis* was collected in Feb 2022 from Suryapet, Telangana, India and authenticated by Dr. V. S. Raju (taxonomist), Department of Botany, Kakatiya University, Warangal. The Voucher specimen of the plant is deposited in the Department of Pharmacognosy, Pratishta Institute of Pharmaceutical Sciences, Suryapet, Telangana, India.

2.2. Preparation of methanolic extracts

The leaves of *Convolvulus pluricaulis* was collected, washed under running tap water, dried under shade ground into coarse powder and then Extracted with Soxlet apparatus. After extraction, the ethanolic extracts were concentrated under reduced pressure (Rotavapour, Switzerland) to yield green coloured mass. The ethanolic extract of *Convolvulus pluricaulis* was dispersed in 1L of distilled water separately and was fractionated with toluene, ethyl acetate and butan-2-one in succession. The solvents were removed from the fractions under reduced pressure to yield the corresponding extracts.

2.3. Chemicals

α -amylase (porcine pancreas), α -Glucosidase (*Saccharomyces cerevisiae*), P-nitro phenyl α -D-glucopyranoside, 3,5-Dinitrosalicylic acid, acarbose, sodium carbonate, sodium potassium tartarate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, di-sodium hydrogen phosphate and di-potassium hydrogen phosphate, sodium hydroxide were procured from Gamut Scientifics (SRL), Secunderabad, India. Folin-ciocalteu reagent from Hi-media (Mumbai, India). All other chemicals and solvents used are of analytical grade.

2.4. Phytochemical screening

The ethanolic leaf extracts of *Convolvulus plucaulis* was subjected to phytochemical screening using dried samples for the presence of different classes of organic compounds like alkaloids, flavonoids, steroids/triterpenoids, carbohydrates, tannins, saponins, phenolic compounds etc.

2.5. Determination of total phenolic content and total flavonoid content

2.5.1. Total phenolic content

The total phenolic content of the extract was determined using Folin-ciocalteu colorimetric method as described in the literature [6]. The extract (100-1000 μ g/ml) or standard solution of gallic acid (10-100 μ g/ml) was added to 25 ml volumetric flask containing 9 ml of distilled water. A reagent blank was prepared using distilled water instead of a sample. 1 ml of Folin-ciocalteus phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% aqueous sodium carbonate solution was added to the mixture. The solution was diluted to 25 ml with double distilled water and mixed. After incubation for 90 min at room temperature, the absorption against prepared reagent blank was determined at 760 nm (ELICO SL159 UV-Visible Spectrophotometer). Quantification was done concerning the standard gallic acid and expressed as gallic acid equivalent (GAE) in mg per gram of extract.

2.5.2. Total flavonoid content

The total flavonoid content of the extract was measured by the aluminum chloride colorimetric method as described in the literature [7]. Extract (10-100 μ g/ml) or standard solution of Rutin (10-100 μ g/ml) was added to 10 ml volumetric flask containing 4 ml double distilled water. To the flask 0.3 ml of 5%, sodium nitrite solution was added. After 5 min, 0.3 ml of 10% aluminum chloride solution was added at 6th min, 2 ml of 1M NaOH was added, and the total volume was made up to 10ml with double distilled water. The solution was mixed well, and the absorbance was measured against prepared reagent blank at 510 nm (ELICO SL159 UV-Visible Spectrophotometer). The total flavonoid content was expressed as Rutin equivalent in mg per gram of extract.

2.6. α - Amylase inhibition assay

This assay was carried out by using a modified procedure of McCue and Shetty [8]. Stock solution of extracts was prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 250 μ L of extracts (1.25-10mg/mL) was placed in a tube and 250 μ L of 0.02M sodium phosphate buffer (pH6.9) containing α -amylase solution (0.5mg/ml) was added. This solution was pre-incubated in at 25 $^{\circ}$ C for 10 min, after which 250 μ L of 1% starch solution in 0.02M sodium phosphate buffer (pH6.9) was added at particular time intervals and then further incubated at 25 $^{\circ}$ C for 10min. The reaction was terminated by adding 500mL of Dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5min and cooled to room temperature. The reaction mixture was diluted with 5mL of distilled water and the absorbance was measured at 540nm using (ELICO SL159 UV-Visible Spectrophotometer). A control was prepared using the same procedure replacing the extract with distilled water.

$$\% \text{ Inhibition} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}})}{\text{Absorbance}_{\text{control}}} \times 100$$

2.7. α - Glucosidase inhibition assay

The alpha-glucosidase inhibitory activity was determined according to the method described by Apostolidis with some modifications [9]. Stock solutions of extracts were prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 50 μ L of extracts (1.25-10mg/mL) and 100mL of yeast alpha glucosidase solution in phosphate buffer (pH-6.9) were incubated at 25 $^{\circ}$ C for 10 minutes followed by the addition of 50mL of 5Mmol/L p-nitrophenyl- α -D-glucopyranoside solution in 0.1M phosphate buffer (pH6.9). The reaction mixture was then incubated at 25 $^{\circ}$ C for 5min the reaction was terminated by adding 3mL of 100Mm sodium carbonate solution into the mixture to stop the reaction and absorbance of liberated p-nitrophenol was read at 405nm using (ELICO SL159 UV-Visible Spectrophotometer).

Acarbose was used a positive control (standard) and the inhibitory activity of alpha-amylase and alpha-glucosidase were calculated by using the following formula

$$\% \text{ Inhibition} = [(Absorbance_{\text{control}} - Absorbance_{\text{extract}}) / Absorbance_{\text{control}}] \times 100$$

The IC₅₀ values (inhibitory concentration at which 50% inhibition of the enzyme activity occurs) of the test samples were determined by performing the assays as above with varying concentration of the test samples ranging from 1.25 to 10 mg/ml. The IC₅₀ values were determined from plots of percentage inhibition vs. concentration. The total experiment was done in triplicate.

3. Results

3.1. Preliminary phytochemical screening of methanolic extracts

The preliminary phytochemical screening showed the presence of different Phytoconstituent such as, Alkaloids, carbohydrates, flavonoids, phenolic compounds, steroidal/triterpenoid compounds, and their glycosides, saponins, in the ethanolic leaf extracts of *Convolvulus pluricaulis*. Results are shown in Table 1.

TABLE 1

Phytochemical constituents identified in methanolic leaf extracts of *Convolvulus pluricaulis* and its fractions toluene, ethyl acetate, butan -2-one.

Class of chemical constituents and the tests	EECL	TLCL	EACL	BNCL
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Steroids/triterpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	+	+
Phenolic compounds	+	+	+	+

+: Present, -: Absent

3.2. Total phenolic content and total flavonoid content

The concentration of polyphenols present in the extracts and its fractions is calculated by using a standard curve prepared with Gallic acid. The total phenolic content of EEAL, TLCP, EACP and BNCP were found to be 19.17 mg, 13.94mg, 28.64 mg, 16.47 mg equivalent per gram of extract respectively.

The concentration of flavonoid content present in the extracts and its fractions is calculated by using a standard curve prepared with Rutin. The total flavonoid content of EECP, TLCP, EACP and BNCP were found to be 8.53mg, 7.83mg, 10.92mg, 8.53mg of Rutin equivalents per gram of extract respectively.

Among the ethanolic extracts of *Convolvulus pluricaulis* and its fractions, total phenolic and flavonoid contents were found to be more in order EACP>EECP>BNCP>TLCP. Results are shown in Table 2.

TABLE 2

Total phenolic and flavonoid content of ethanolic extract of *Convolvulus pluricaulis* and its fractions

Ethanolic plant extracts & its fractions	Total phenolic content (mg of GAE/gm of extract)	Total flavonoid content (mg of RE/gm of extract)
EECP	19.17	8.53
TLCP	13.94	7.83
EACP	28.64	10.93
BNCP	16.47	8.53

Values represent mean ± standard deviation of triplicate experiments

3.3. In vitro α-amylase and α-glucosidase inhibitory activity

In the present study ethanolic extract of *Convolvulus pluricaulis* and its fractions were inhibitory effect on α-amylase and α-glucosidase enzymes by the *in-vitro* method. The ethanolic extracts of *Convolvulus pluricaulis* and its fractions and standard drug Acarbose at a concentration of 10mg/ml exhibited 62.21%, 65.39%, 86.01%, 57.83%, 91.56% α-amylase inhibitory activity shown in Figure 1 and 61.3%, 65.18%, 89.01%, 68.8%, 97.53% α-glucosidase inhibitory activity shown in Figure 2 respectively. Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC₅₀ value 2.32 mg/ml and α-glucosidase inhibitory activity with an IC₅₀ value 2.00 mg/ml. Among all the test samples, ethyl acetate fraction of *Convolvulus pluricaulis* has shown the prominent enzyme inhibitory activity with IC₅₀ 3.44mg/ml (α-amylase), 3.95mg/ml (α-glucosidase) which were well comparable with that of standard drug Acarbose.

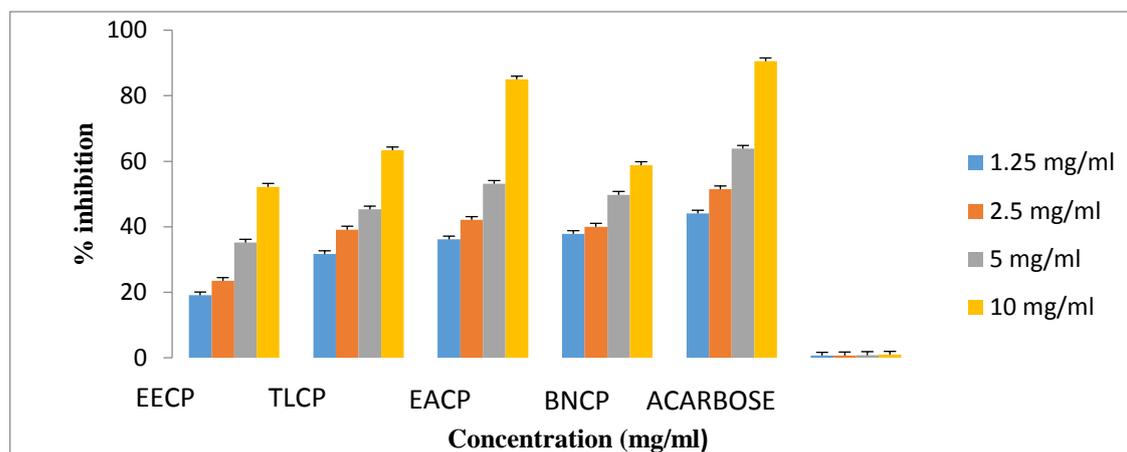


Figure 1. Porcine pancreatic α -amylase inhibition by ethanolic leaf extracts of *Convolvulus pluricaulis* (EECP, TLCP, EACP and BNCP) and Acarbose. Data expressed as mean \pm SD, (n=3).

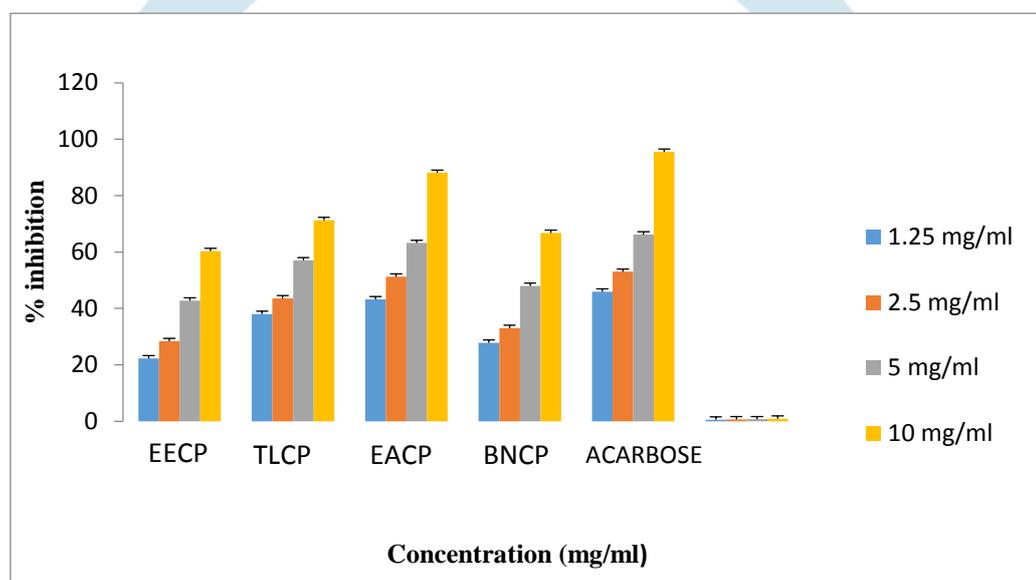


Figure 2. Yeast α -glucosidase inhibition by ethanolic leaf extracts of *Convolvulus pluricaulis* (EECP, TLCP, EACP and BNCP) and Acarbose. Data expressed as mean \pm SD, (n=3).

4. Discussion

Diabetes mellitus is one of the international health crises of the 21st century [10]. It is the world's fastest-growing metabolic endocrine disorder with compromised carbohydrate and lipid metabolism. The defected metabolism can be attributed to the impaired insulin secretion, insulin action or both.

The enzymes α -amylase and α -glucosidase are related to post prandial high blood glucose levels (BGL). α -amylase is connected to breaking the polysaccharides into disaccharides and oligosaccharides. α -glucosidase works on the disaccharides and polysaccharides to break them into glucose monomers aiding carbohydrate digestion. Inhibition of these enzymes can lead to a control on postprandial BGL by controlling carbohydrate digestion and hence controls diabetes significantly [11].

Even though plenty of medications are available for the treatment of diabetes, they are having limitations due to their adverse side effects and high costs. Hence it was found to be very difficult to manage and cure this disease effectively by using the available oral hypoglycaemic agents (drugs) or insulin. Therefore, the focus of scientists shifted towards the development of the natural herbal medicine with high therapeutic potential and less or no toxic effect [12].

The inhibitory effects of ethanolic extract of *Convolvulus pluricaulis* against porcine pancreas alpha-amylase and yeast alpha-glucosidase were evaluated in comparison with the anti-diabetic drug Acarbose.

Preliminary phytochemical screening of ethanolic leaf extracts and its fractions revealed the presence of alkaloids, flavonoids, steroids/triterpenoids and their glycosides, carbohydrates, saponins and phenolic compounds.

Ethyl acetate fraction of *Convolvulus pluricaulis* showed most active alpha-amylase and alpha-glucosidase inhibitory activities with IC_{50} values of 4.95 mg/ml and 4.4 mg/ml respectively. Toluene fraction showed moderate inhibitory effect against alpha-amylase and alpha-glucosidase when compared with that of standard reference drug, Acarbose, with IC_{50} values of 6.04 mg/ml and 4.03 mg/ml respectively. Butanone fraction of *Convolvulus pluricaulis* showed moderate inhibition against alpha-amylase and alpha-glucosidase with IC_{50} values of 6.16 mg/ml against alpha-amylase and 6.04 mg/ml against alpha-glucosidase. The methanolic extract of *Convolvulus pluricaulis* showed weak inhibition against alpha-amylase and alpha-glucosidase, when compared with that of standard drug, Acarbose.

The total flavonoid content of the ethanolic extract and fractions of *Convolvulus pluricaulis* was determined using Aluminium chloride colorimetric method. Highest amount of flavonoid were found to be in the order EACP>EECP>BCPL>TLCP.

Total phenolic content of the ethanolic extract and its fractions was determined by using Folin-ciocalteu reagent. Highest amount of phenolic content were found to be in the order EACP>EECP>BNCP>TLCP.

flavonoids and phenolic compounds present in the plant may be responsible for alpha amylase and alpha glucosidase enzyme inhibitory activity.

The results of the study elaborated scientific support regarding the use of ethanolic extracts and its fractions to treat diabetes through a mechanism based on its alpha amylase and alpha glucosidase enzyme inhibitory activity and this therapeutic potentiality could be exploited in the management of postprandial hyperglycaemia in the treatment of type 2 diabetes mellitus. However, further studies are needed to confirm these findings to characterize and determine the bioactive components responsible for this effect.

Conflict of interest statement

We declare that there are no conflicts of interest.

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