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In Vitro Studies on α-Amylase And α-Glucosidase Inhibitory Activity Of *Antigonon leptopus* (Hook Et. Arn) Leaf Extract and its Fractions

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Abstract: *Antigonon leptopus* leaves and their methanolic extracts and fractions were tested for their ability to inhibit α-amylase and α-glucosidase activity *in vitro*. Fractions of *Antigonon leptopus* leaves were obtained by dispersing evenly in distilled water and subjected to solvent fractionation with toluene, ethyl acetate and butan-2-one under decreased pressure and concentrated. These extracts were tested *in vitro* for their inhibitory effects on α-amylase and α-glucosidase inhibitory activities, as well as for several antioxidant characteristics.. A dose-dependent inhibition of α-amylase and α-glucosidase enzymes was found in the methanolic extract and fractions thereof. It was shown that the ethyl acetate fraction of *Antigonon leptopus* had the greatest enzyme inhibitory activity (IC_{50} values of 3.95 mg/ml for α-amylase, and of 3.4 mg/ml for α-glucosidase) and that its potency compared well to that of the standard medication Acarbose. Research on this plant's chemistry showed that it is rich in a wide range of chemicals including alkaloids, flavonoids, carbohydrates, tannins and saponin. Total phenolic and total flavonoid contents were also calculated. *Antigonon leptopus* has been used in traditional medicine to treat diabetes by lowering postprandial blood sugar levels. *Antigonon leptopus* ethyl acetate fraction has strong α-amylase and α-glucosidase enzyme inhibitory capabilities. The glucose absorption may be regulated by high TPC and TFC levels, which may be the cause of enzyme inhibition.

Keywords: Antigononleptopus, α-amylase, α-glucosidase, Acarbose, Flavonoids, Polyphenols, Alkaloids

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Introduction

Diabetes and hyperglycemia may be prevented by controlling blood glucose levels. Digestive enzymes like α-glucosidase and α-amylase break down carbs into sugars in the bloodstream (Digantakalita *et al*., 2018). α-glucosidase is an enzyme that hydrolyzes the terminal glycosidic bonds at the non-reducing end of saccharide polymers to liberate -glucose, which is essential for carbohydrate metabolism. The pharmaceutical industry has paid close attention to α-glucosidase

since inhibiting its catalytic activity reduces glucose absorption and, as a consequence, blood sugar levels after meals. New anti-diabetic drugs should focus on α-glucosidase as a primary target. One of the most important enzymes in the digestive tract, pancreatic amylase begins the breakdown of starch into maltose and then glucose. Post-prandial hyperglycemia is exacerbated as a consequence of fast starch degradation. Controlling the activity of human pancreatic α-amylase in the gut is an essential part of the therapy of diabetes because it raises post-prandial glucose levels. The prevention of glucose absorption by inhibiting enzymes like α-amylase and α-glucosidase would thus be critical in the management of diabetes (Martha *et al*., 2012).

One in every four people has diabetes mellitus, making it the most prevalent hormonal illness. Around 2.8% of the world's population was impacted, and that number is expected to rise to 5.4% by the year 2025 (Patel *et al*., 2012). Diabetes is one of the most common and rapidly spreading illnesses in the world. Diabetic incidence is expected to rise by 35 per cent in the near future according to the World Health Organization. More than 150 million people in the globe already have diabetes, which is expected to rise to 300 million by the year 2025, according to the World Health Organization. The number of diabetics in India is expected to rise from 15 million in 1995 to 57 million by 2025, making it the world's best-performing diabetic population (IDF *et al*., 2013).

As a result, the digestive system's α-amylase and α-glucosidase inhibitors are considered therapeutic approaches for diabetes management. To the best of our knowledge *Antigonon leptopus* has not been previously studied for its α-amylase and α-glucosidase enzyme inhibitory properties. Therefore, this study was conducted to determine the possible mechanism underlying its hypoglycemic action.

Materials and Methods

Collection of plant specimens:

Plant specimens of *Antigonon leptopus* were obtained from Kakatiya University campus and validated by Dr. V. S. Raju (Taxonomist), Department of Botany at Kakatiya University, Warangal, Telangana, India. Warangal University's department of Pharmacognosy and Phytochemistry has a specimen of this plant (KU/UCPSC/No.63), which serves as the plant's "voucher specimen."

Extracting methanolic compounds from natural sources:

For the maceration with methanol, the *Antigonon leptopus* leaves were cleaned and dried under shade before being ground into a coarse powder. Rotavapour was used to concentrate the methanolic extracts, which had been extracted to their fullest extent, into a green mass. Fractionation of the methanolic extract of *Antigonon leptopus* (MEAL) was carried out using toluene (TLAL), ethyl acetate (EAAL), and butan-2-one (BNAL). Reduced pressure was used to extract the solvents from the fractions, resulting in the desired fraction.

Chemicals:

Sodium carbonate, Sodium potassium tartarate, Sodium dihydrogen phosphate, Potassium dihydrogen phosphate, Di-Sodium hydrogen phosphate, Di-potassium hydrogen phosphate, and Sodium hydroxide were purchased from Gamut Scientifics (SRL), Secunderabad, India. The enzymes α-Amylase (from porcine pancreas), α-Glucosidase (from *Saccharomyces cerevisiae* and Folin-Ciocalteu reagent were provided by the Hi-media (Mumbai, India). Chemicals and solvents used in this experiment were of analytical quality.

Antigonon leptopus leaf extract was tested for the presence of organic components such as alkaloids and flavonoids, steroids/triterpenes and carbohydrates. Tannins, saponins and other organic compounds were also found to be present in dried samples (Kokate *et al*. 2005).

Total phenolic content and total flavonoid content determination:

Folin-Ciocalteu colorimetric technique was used to determine the extract's total phenolic content (Praneetha *et al*., 2014). In a 25 ml volumetric flask, the extract/fractions (100-1000 g/ml) or the standard solution of gallic acid (10-100 g/ml) was added. Reagent blank was created by substituting the sample with distilled water. Reagent for Folin-Ciocalteu phenol was added and mixed into the mixture. Sodium carbonate solution was added to the mixture after 5 min. The solution was diluted with double distilled water to a volume of 25 ml and well mixed before use. Absorption against a prepared reagent blank was determined at 760 nm using an ELICO SL159 UV-Visible Spectrophotometer after 90 min of incubation at room temperature. In order to calculate the gallic acid equivalent (GAE) per gram of extract, we used the standard gallic acid as a reference.

The extract's total flavonoid concentration was determined using a colorimetric aluminium chloride technique (Swarooparani *et al*., 2019). In a 10 ml volumetric flask, a standard solution (10-100 g/ml) of Rutin or an extract/fractions (100-1000 g/ml) was added to 4 ml of double distilled water. A solution of 5 per cent sodium nitrite was added to the flask, followed by 0.3 ml of a solution of 10 per cent aluminium chloride, which was added at the end of 5 min and 2 ml of 1M NaOH at the end of 6 min. The final volume was 10 ml of double-distilled water. The solution was well mixed, and the absorbance was measured at 510 nm by using UV-Visible Spectrophotometer against a prepared reagent blank. The total flavonoid content was expressed in mg of Rutin equivalents per gram of extract.

α-Amylase inhibition test:

A modified McCue and Shetty (2004) technique was used to conduct this experiment. To make a stock solution of the extracts, 10 mg of each extract was dissolved in 1 ml of DMSO. It took a total of 250 ml of extract (1.25-10 mg/ml) to make 250 ml of buffer (0.02M Sodium Phosphate Buffer, pH 6.9) with α-amylase solution (0.5 mg/ml). At regular intervals, 250 L of 1 per cent starch solution in pH 6.9 buffer solution was added to the solution, which was pre-incubated for 10 min at 25 ℃. This solution was then re-incubated for another 10 min at 25℃. Following a 5 min incubation in boiling water, the tubes were cooled to room temperature and 500 µl of Dinitrosalicylic acid (DNS) reagent was added to end the reaction. The absorbance at 540 nm was measured using a UV-Visible Spectrophotometer after diluting the reaction liquid with 5 ml of distilled water. The same process was used to make a control, except distilled water was substituted for the extract.

Per cent Inhibition= [(Absorbance control-Absorbance extract)/Absorbance control] x100

α-Glucosidase inhibition assay:

According to Apostolidis' approach, the glucosidase inhibitory activity was determined using certain changes (Apostolidis *et al*., 2007). Stock extract solutions were produced by dissolving up to 10 mg of each extract or fraction in 1ml of DMSO.. Extracted samples (1.25-10 mg/ml) were incubated at 25 ℃ for 10 min with 100 L of yeast α-glucosidase solution and 50 ml of p-nitrophenyl—D-glucopyranoside solution (0.1M phosphate buffer, pH 6.9). Afterwards, the reaction was stopped by adding 3 ml of 100 mM sodium carbonate solution into the mixture and the absorbance was measured at 405 nm by using UV-Visible Spectrophotometer at the end of the experiment.

As a standard, Acarbose was utilised to determine the inhibitory activity of α-amylase and α-glucosidase. Inhibition as a percentage equals 100 times the ratio of absorption control to absorption extract. To get IC_{50} values (inhibitory concentrations at which 50% inhibition of enzyme activity occurs), the experiments as described above were performed with various concentrations of the test samples ranging from 1.25 to 10 mg/ml. Per cent inhibition vs concentration was used to get the IC_{50}. We performed the experiment three times.

Results

Screening of methanolic extracts for preliminary phytochemical content:

Table 1: Phytochemical constituents identified in methanolic leaf extract of *Antigonon leptopus* and its fractions toluene, ethyl acetate, butan-2-one

Class of chemical constituents and the tests	**Methanolic extract**	**Toluene fraction**	**Ethylacetate fraction**	**Butanone fraction**
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Steroids/Terpenoids	+	+	+	+
Carbohydrates	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phenolic compounds	+	+	+	+

+ Present; - Absent

Table 2: Total phenolic and flavonoid content of methanolic extract of *Antigonon leptopus* and its fractions

Extract/fraction	Total phenolic content (mg of GAE/g of extract)	Total flavonoid content (mg of RE/g of extract)
Methanol extract	13.94±0.24	7.83±0.13
Toluene fraction	20.17±0.35	9.53±0.54
Ethylacetate fraction	26.64±0.29	9.92±0.42
Butanol fraction	14.47±0.18	8.53±0.26

Values represent mean ± standard deviation of triplicate experiments (n=3). GAE; Gallic acid equivalents, RE; Rutin equivalents

Methanolic leaf extracts from *Antigonon leptopus* were subjected to preliminary phytochemical screening and found to contain a variety of phytoconstituents including alkaloid, flavonoid, carbohydrate, steroid, triterpenoid as well as their glycosides (Table 1),

Total phenolic and flavonoid content:

An acid standard curve was used to determine the content of polyphenols in the extracts and its fractions. Each of the four extracts studied had a gallic acid equivalent concentration of 13.94 mg/g, 20.17 mg/g, 26.64 mg/g and 14.47 mg/g, respectively.

Extracts and fractions of flavonoid content were determined using a standard curve based on rutin's flavonoid content. Each gram of extract included 7.830.13 mg of total flavonoid, 9.530.54 mg of total flavonoid equivalent, 9.920.42 mg and 8.530.26 mg of total flavonoid.

Among the methanolic extract of *Antigonon leptopus* and its fractions, total phenolic and total flavonoid content was found to be in the order of EAAL>TLAL>BNAL>MEAL. Results are shown in Table 2.

In vitro α-amylase and α-glucosidase inhibitory activity:

In this study, the *in vitro* inhibitory impact of methanolic extracts of *Antigonon leptopus* and its fractions on α-amylase and α-glucosidase enzymes was examined. The methanolic extracts of *Antigonon leptopus* and its fractions (toluene, ethyl acetate and butanone) and standard drug acarbose at a concentration of 10mg/ml exhibited 52.21%, 63.34%, 83.01%, 58.83%, 88.03% for α-amylase inhibitory activity shown in Table 3 and Figure 1. This is in comparison to acarbose at 10 mg/ml, which showed a 50% inhibition. The inhibitory activity of α-glucosidase was 60, 64.18, 85.01, 66.8, and 88.03 per cent. IC_{50} values of 4.23 mg/ml for α-amylase inhibition and 4.23 mg/ml for α-glucosidase inhibition were utilised as a standard reference for acarbose. The enzyme inhibitory activity of *Antigonon leptopus* ethyl acetate fraction was equivalent to that of standard medication acarbose with IC_{50} values of 3.95

Table 3: α-Amylase inhibitory activity and IC_{50} values by *Antigononleptopus*leaf extract, fractions and Acarbose

Test sample	Concentration(mg/ml)	% Inhibition	IC_{50} Value
Methanolic extract	1.25 2.5 5 10	19.01±0.06 23.48±0.08 35.20±0.05 52.21±0.04	7.88±0.01
Toluene fraction	1.25 2.5 5 10	32.69±0.01 39.13±0.03 45.30±0.02 63.34±0.01	6.04±0.02
Ethylacetate fraction	1.25 2.5 5 10	34.60±0.05 42.12±0.03 58.60±0.02 83.01±0.03	3.95±0.01
Butanol fraction	1.25 2.5 5 10	37.81±0.01 43.30±0.02 49.76±0.02 58.83±0.01	6.16±0.03
Acarbose	1.25 2.5 5 10	34.04 ±0.05 41.04±0.04 57.30±0.02 88.03±0.03	4.23±0.01

Data expressed as mean ± Standard deviation of triplicate experiments

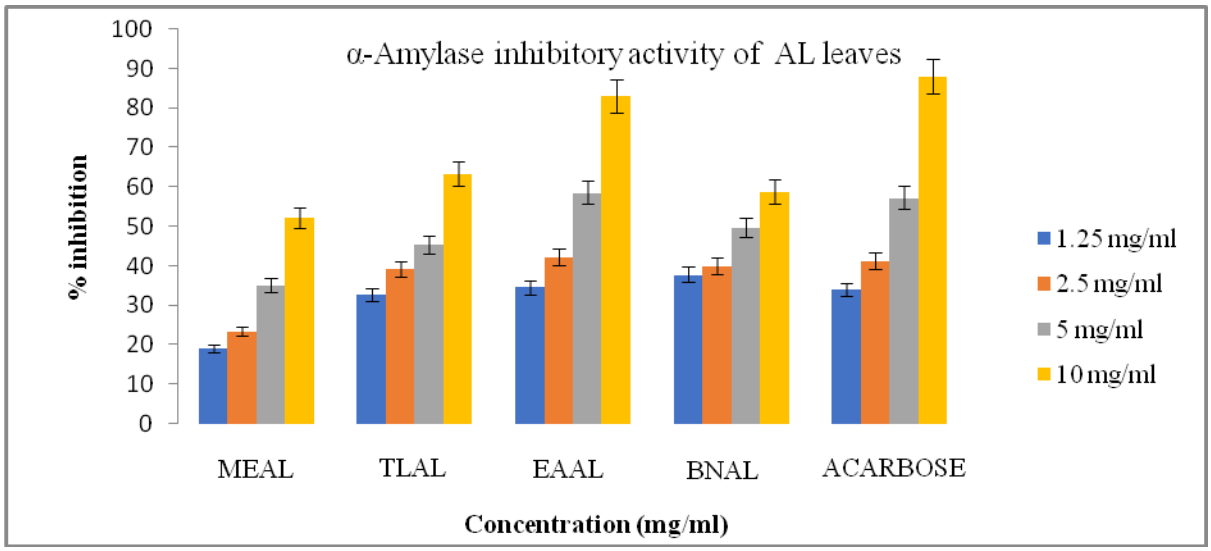

Fig. 1: Effect of methanolic extract (MEAL) and different fractions (TLAL, EAAL and BNAL) of *Antigonon leptopus* and Acarbose on α-amylase enzyme inhibition.

Table 4: α-Glucosidase inhibitory activity and IC_{50} values by *Antigononleptopus*leaf extract, fractions and Acarbose

Test sample	Concentration(mg/ml)	%Inhibition	IC_{50} value
Methanol extract	1.25 2.5 5 10	22.30±0.01 29.36±0.03 38.01±0.04 60.30±0.04	7.35±0.01
Toluene fraction	1.25 2.5 5 10	29.05±0.01 39.83±0.03 48.43±0.01 64.18±0.01	4.06±0.05
Ethylacetate fraction	1.25 2.5 5 10	43.20±0.06 51.03±0.02 63.02±0.01 85.01±0.03	3.44±0.03
Butanol fraction	1.25 2.5 5 10	27.81±0.01 33.05±0.02 47.99±0.02 66.80±0.01	6.04±0.07
Acarbose	1.25 2.5 5 10	34.04 ±0.05 41.04±0.04 57.30±0.02 88.03±0.03	4.23±0.05

Data expressed as mean ± Standard deviation of triplicate experiments

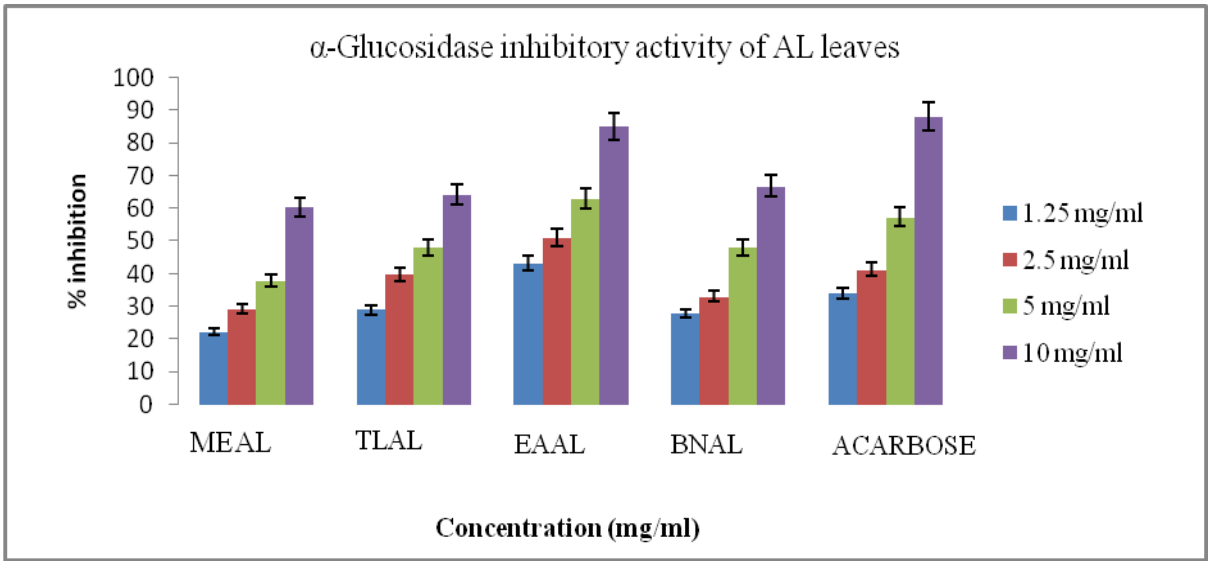

Fig. 2: Effect of methanolic extract (MEAL) and different fractions of (TLAL, EAAL and BNAL) *Antigonon leptopus* and Acarbose on alpha glucosidase enzyme inhibition.

mg/ml (α-amylase) and 3.44 mg/ml (α-glucosidase) (Table 4; Fig. 2).

Discussion

Diabetes mellitus is one of the major public health challenges of the 21st century. In this quickly contagious metabolic endocrine disorder, both carbohydrate and lipid metabolism are affected in a negative way. It is possible that the improper metabolism is the result of either impaired insulin synthesis and/or impaired insulin action.

Both the enzymes α-amylase and α-glucosidase have been linked to postprandial

elevations in blood glucose levels (BGL). α-Amylase is responsible for the hydrolysis of polysaccharides into their constituent disaccharides and oligosaccharides. The enzyme known as α-glucosidase is responsible for the breakdown of disaccharides and polysaccharides into glucose monomers. Postprandial blood glucose levels may be lowered by controlling carbohydrate digestion by enzyme inhibition. This, in turn, has a significant influence on the treatment of diabetes (Oboh *et al*.. 2013).

Diabetes may be treated with any one of a number of different medications; however, the risk of adverse effects and the high cost of these medications prevents widespread use. Oral hypoglycemic treatments (also known as pharmaceuticals) and insulin have been demonstrated to be inefficient in effectively controlling and curing this illness. Natural herbal medications have a significant therapeutic potential but few or no adverse effects have recently been the centre of attention for scientists (Kwon *et al*., 2007; Patel *et al*., 2012).

When analysing the inhibitory effects of a methanolic extract of *Antigonon leptopus* on porcine pancreatic α-amylase and yeast α-glucosidase, the anti-diabetic medicine acarbose was used. An early phytochemical examination of methanolic leaf extracts and its fractions revealed the presence of a variety of substances including alkaloids, flavonoids, carbohydrates, tannins and saponins, steroidal, and phenolic compounds.

Antigonon leptopus' ethyl acetate fraction has IC_{50} values of 3.95 and 3.4 mg/l for its inhibitory effects on amylase and glycosidase, respectively. These values were determined using the anti-amylase and anti-glycosidase activities of *Antigonon leptopus.* The standard reference medication, acarbose had IC_{50} values of 6.04 mg/ml and 4.03 mg/ml, respectively, for a toluene fraction that had moderate inhibitory effects on amylase and glucosidase, respectively. These values were determined by measuring the concentration at which 50% of the enzyme's activity was inhibited. The butan-2-one component of *Antigonon leptopus* displayed moderate inhibitory effects for both α-glucosidase and α-amylase, with IC_{50} values of 6.16 and 6.04 mg/ml, respectively. An antifungal methanolic extract of *Antigonon leptopus* was demonstrated to have minor inhibitory effects on α-amylase (IC_{50} 7.88 mg/ml) and α-glucosidase (IC_{50} 7.35 mg/ml). It has been shown that the methanolic extract of *Antigonon leptopus* has a significant percentage of inhibitory influence on both α-amylase and α-glucosidase enzymes. This could be attributed to the fact that the extract has a high total phenolic and flavonoid content. It was revealed that extracts of methanolic leaves and fractions of those extracts might block the enzymes α-amylase and α-glucosidase.

We determined the total amount of flavonoid present in the methanolic extract of *Antigonon leptopus* as well as its fractions by the use of aluminium chloride colorimetry. In terms of the abundance of flavonoids, EAAL was found to have the most, followed by TALAL, BNL, and MEALA in that order. The methanolic extract of *Antigonon leptopus* and its fractions were put through an analysis using the Folin-Ciocalteu reagent so that the total phenolic content could be determined. It was discovered that EAAL, TLAL, BNAL, and MEAL all had the greatest quantities of phenols. There is a possibility that the capacity of the plant to block the enzymes amylase and glucose oxidase is due to the presence of flavonoids and phenolic compounds inside the plant itself.

Conclusion

According to the findings of this study, the leaves of *Antigonon leptopus* may be useful in the treatment of diabetes via a mechanism that is based on their ability to block the enzymes amylase and glucose oxidase. Additional study is required in order to demonstrate the existence of these effects in living organisms.

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