IJBPAS, June, 2023, 12(6): 2649-2660

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF *ANTIGONON LEPTOPUS (HOOK ET. ARN)* ROOT EXTRACT AND FRACTIONS ON α-AMYLASE AND α-GLUCOSIDASE ENZYMES

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Received 15th July 2022; Revised 20th Aug. 2022; Accepted 5th Oct. 2022; Available online 1st June 2023

https://doi.org/10.31032/IJBPAS/2023/12.6.7187

ABSTRACT

To investigate the therapeutic effects of *Antigonon leptopus* root extract and its fractions of for α-amylase and α-glucosidase inhibitory activity by *in-vitro* assays. Roots of *Antigonon leptopus* were extracted with methanol and its fractions were prepared by dispersing uniformly in distilled water and subjected to solvent fractionation with ethyl acetate, butan-2-one and water. The extract and its fractions were concentrated under reduced pressure. The inhibitory effect of these extracts on these enzymes was determined by *in vitro* methods and some antioxidant parameters. The results revealed that the methanolic extract of roots and its fractions were inhibited α-amylase and α- glucosidase enzymes in a dose dependent manner. Among all these ethyl acetate fraction of *Antigonon leptopus* roots (EAAL) has shown the prominent α-amylase and α-glucosidase enzyme inhibitory activity with IC_{50} value 6.76±0.20 and IC_{50} value 7.58±0.21mg/ml α-amylase and α-glucosidase respectively and it was well comparable with standard acarbose (with IC_{50} values 5.7±0.29 and 6.95±0.80mg/ml respectively). Phytochemical reports of this plant revealed that it contains alkaloids, flavonoids, carbohydrates, tannins, saponins, steroidal and phenolic compounds. Further, the total phenolic and total flavonoid contents were also estimated. These results substantiate the use of *Antigonon leptopus* in traditional medicine for the treatment of diabetes by controlling postprandial hyperglycemia. The results suggest that ethyl acetate fraction (EAAL) of roots

shown significant α-amylase and α-glucosidase enzyme inhibitory activity. High total phenolic content and total flavonoid content of root may be responsible for enzyme inhibition by regulating the glucose absorption.

Keywords: *Antigonon leptopus*, α-amylase, α-glucosidase, Acarbose, quercetin, Total Phenolic Content, Total Flavonoid Content

INTRODUCTION

One of the therapeutic approach for treating diabetes is to decrease the post prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolysing enzymes α-amylase and α-glucosidase in the digestive tract. Inhibitors of these enzyme delay carbohydrate digestion and prolong the carbohydrate digestion time, causing a reduction in the rate of glucose absorption and consequently decrease the post-prandial plasma glucose rise. Therefore, inhibition of those enzymes in digestive organs is taken into account to be a therapeutic approach and a powerful option for managing diabetes **[1, 2]**.

Antigonon leptopus (AL) is a member of the family Polygonaceae. It is commonly known as coral vine, widely observed in the parks and gardens through out India. It is a tender perennial vine, can easily grow up to 30-40ft in length. It has heart shaped, green leaves as large as 4 inches long and 3 inches wide **[3]**. The stems are herbaceous and the flowers are tiny but the sepals are larger and provide the brilliant colours that range from white to pink to deep coral colour. Traditionally the leaves of this plant have been used to reduce swelling and tea made from the leaves can be used to treat diabetes, hypertension, and menstrual pains. The vine is used to treat cough and throat constriction, roots of this plant has analgesic and anti-inflammatory properties **[4]**. Tea made from the leaves and roots of *Antigonon leptopus* used to treat stomach ache **[5]**. In Jamaica, a hot tea prepared from the aerial portion of AL, is used in the treatment of cough, stomach ache and throat constriction and it is considered as one of the significant medicinal plant in their folklore medicine **[6]** and it is reported to contain phenolic aldehyde which is beneficial to health**[7]**.

Therapeutically, methanolic extract of *Antigonon leptopus* leaves reported for antithrombin activity **[8]**, cytotoxic **[9]**, antidiabetic **[10]**, analgesic **[11]**, hepatoprotective **[12, 13]**, anthelmintic **[14]**, antimicrobial **[9, 15]** and antioxidant activity **[16]**. Flowers were reported for antioxidant and antidiabetic activity **[17, 18]**. Aerial parts were reported for analgesic and anti-inflammatory activity **[9,**

11]. And leaves as well as roots were reported for anthelmintic **[14]** and antimicrobial activities **[15]** and antioxidant activity **[16, 19]**.

Though several studies showed the AL leaves and flowers shown antidiabetic activity, but no reports are found on the mechanism by which it exert antidiabetic activity of roots. Hence the present study was aimed to evaluate the effect of AL roots against two digestive enzymes α-amylase and α-glucosidase.

For the simplest of my knowledge, previous studies haven't investigated the α-amylase and α-glucosidase enzyme inhibitory activities of *Antigonon leptopus*. Therefore, this work was undertaken to know the probable mechanism behind its hypoglycemic activity.

MATERIALS AND METHODS

Collection of plant material

The roots of *Antigonon leptopus* was collected in the month of August 2019 from Kakatiya University campus, Warangal, Telangana, India and authenticated by Dr. V. S. Raju (Taxonomist), Department of Botany, Kakatiya University, Warangal. The voucher specimen (KU/UCPSC/No.63) of the plant was deposited in the Department of Pharmacognosy and Phytochemistry, University College of Pharmaceutical Sciences, Warangal, Telangana, India.

Preparation of methanolic extracts

The roots of *Antigonon leptopus* was collected and washed under running tap water dried under sun and leaves dried under shade, grinded into coarse powder and then macerated with methanol at room temperature separately for 5-7days. After exhaustive extraction, the methanolic extracts were concentrated under reduced pressure (Rotavapour, Switzerland) to yield concentrated extract. The methanolic extract (MEAL) of *Antigonon leptopus* were dispersed in 1L of distilled water separately and was fractionated with toluene (TLAL), ethyl acetate (EAAL) and butan-2-one (BNAL) in succession. The solvents were removed from the fractions under reduced pressure to yield the corresponding fraction.

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

Phytochemical screening

The methanolic extract and fractions of *Antigonon leptopus* roots were 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*. according to standard methods **[20]**.

Determination of total phenolic content and total flavonoid content.

Total phenolic content: The total phenolic content of the AL root extract and its fractions were determined using Folin-ciocalteu colorimetric method as described in the literature **[21]**. The extract/fractions (100-1000µg/ml) and standard solution of gallic acid (10-100µg/ml) was added to 25 ml volumetric flask containing 9 ml of distilled water. A blank was prepared using distilled water instead of a sample. 1ml of Folin-ciocalteu phenol reagent was added to the mixture and shaken. After 5 min, 10ml of 7% aqueous sodium carbonate solution was added to the mixture. The solution was diluted to 25 ml with double distilled water and mixed it properly. Incubate it for 90 min at room temperature, and then the absorption against prepared reagent blank was determined at 760nm (ELICO SL159) UV-Visible Spectrophotometer. Quantification was done concerning the quality of tannic acid and expressed as acid equivalent (GAE) in mg per gram of extract.

Total flavonoid content: The total flavonoid content of AL root extract and its fractions were measured by the aluminum chloride colorimetric method as described in the literature **[22]**. AL root extract and fractions at concentration (100-1000µg/ml) and standard solution of Rutin of concentration (10-100µg/ml) was added to 10 ml volumetric flask containing 4 ml of double distilled water. To the flask add 0.3ml of 5%, sodium nitrite solution and after 5 min add 0.3 ml of 10% aluminium chloride solution at 6th min then 2 ml of 1M sodium hydroxide 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 510nm (ELICO SL159) UV-Visible Spectrophotometer. The total flavonoid content was expressed as rutin equivalent in mg per gram of extract.

α-Amylase inhibition assay: This assay was administered by employing a modified procedure of McCue and Shetty **[23]**. Stock solution of extracts was prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 250µL of extracts (1.25-10mg/ml) was placed in a test tube and 250µL of 0.02M sodium orthophosphate buffer (pH6.9) containing α-amylase solution (0.5mg/ml) was added.

This solution was pre-incubated at 25^0C for 10 min, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH6.9), was added at particular time intervals and then incubated at 25^0C for 10min. The reaction was terminated by adding 500µL of Dinitrosalicylic acid (DNS) reagent and therefore the tubes were then incubated in boiling water for 5min and cooled to temperature. The reaction mixture was diluted with distilled water of 5ml and the absorbance was measured at 540nm using UV-Visible Spectrophotometer (ELICO SL159). A control was prepared by replacing the test sample with water.

% Inhibition = [(Absorbance$_{control}$-Absorbance$_{extract}$)/Absorbance$_{control}$] x100

α-Glucosidase inhibition assay: The α-glucosidase inhibitory activity was determined according to the method described by Apostolidis with some modifications **[24]**. Stock solutions of extracts and fractions of AL were prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 50µL of extracts and fractions (1.25-10mg/mL) and 100µL of yeast α-glucosidase solution in phosphate buffer (pH-6.9) were incubated at 25^0C 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 25^0C for 5min then the reaction was terminated by adding 3mL of 100Mm sodium carbonate solution into the mixture and absorbance was measured at 405nm using UV-Visible Spectrophotometer (ELICO SL159).

Acarbose was used a positive control (Standard) and the inhibitory activity of α - amylase and

α-glucosidase were calculated by using the following formula

% Inhibition = [(Absorbance$_{control}$-Absorbance$_{extract}$)/Absorbance$_{control}$] x100

The IC_{50} 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 10mg/ml. The IC_{50} values were determined from plots of % Inhibition *vs* concentration. The total experiment was done in triplicate.

RESULTS

Preliminary phytochemical screening of methanolic extracts: The preliminary phytochemical screening of the methanolic extract of AL roots and its fractions showed the presence of different phytoconstituents such as alkaloids, flavonoids, carbohydrates, tannins, saponins, phenols, steroidal/triterpenoid compounds and their glycosides.

Total phenolic content and total flavonoid content: The concentrations of

polyphenols present in the AL root extract and fractions were calculated by using a standard curve prepared with gallic acid. The total phenolic content of MEAL, EAAL, BNAL and AQAL were found to be 31.54±0.51mg, 32.34±0.23mg, 23.39±0.32mg, 15.92±0.41mg of gallic acid equivalent per gram of extract respectively.

The concentration of flavonoid content present in the extract and its fractions were calculated by using a standard curve prepared with rutin. The total flavonoid content of MEAL, EAAL, BNAL and AQAL were found to be 9.08±0.31mg, 9.98±0.27mg, 8.19±0.19 mg and 4.73±0.53mg of rutin equivalent per gram of extract respectively. The order of total phenolic and total flavonoid content of methanolic extract and its fractions of *Antigonon leptopus* roots is EAAL>MEAL>BNAL>AQAL. Results are shown in **Table 1**.

***In vitro* α-amylase and α-glucosidase inhibitory activity:** In the present study, methanolic extract and fractions of *Antigonon leptopus* roots were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by the *in-vitro* method **(Table 2)**.

The AL root methanolic extract, fractions (MEAL, EAAL, BNAL and AQAL) and standard drug (acarbose) at a concentration of 10mg/ml exhibited 66.4%, 70.50%, 66.7%, 63.5%, 85.6% α-amylase inhibitory activity with an IC_{50} value 6.99±18.9, 6.96±0.20, 7.48±0.20, 7.59±0.81, 5.7±0.29mg/ml shown in **Figure 1** and 59.7%, 64.3%, 63.8%, 52.2%, 70.6% α-glucosidase inhibitory activity with an IC_{50} value7.96±0.18, 7.58±0.21, 7.68±0.22, 8.13±0.36, 6.95±0.20mg/ml shown in **Figure 2** respectively.

Among all the test samples ethyl acetate fraction of *Antigonon leptopus* has shown the prominent enzyme inhibitory activity with IC_{50} 6.76±0.20mg/ml, and 7.58±0.21 mg/ml for for α-amylase and α-glucosidase respectively which was well comparable with that of IC_{50} value standard drug acarbose, i.e., 5.7±0.29 and 6.95±0.20 mg/ml for α-amylase and α-glucosidase respectively.

Table 1: Showing Total Phenolic Content and Total Flavonoid Content of methanolic extract *Antigonon leptopus* and its fractions

Extract/ Fractions of AL root	TPC (mg of GAE/gm of extract)	TFC (mg of RE/gm of extract)
MEAL	31.54±0.51	9.08±0.31
EAAL	32.34±0.23	9.98±0.23
BNAL	23.39±0.32	8.19±0.19
AQAL	15.92±0.41	4.73±0.53

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

Table 2: α-Amylase inhibitory activity and IC_{50} values by *Antigonon leptopus* root extract, fractions and Acarbose

Test samples	Concentration(mg/ml)	% Inhibition	IC_{50} values
MEAL	1.25 2.5 5 10	13.5±0.25 22.4±0.34 34.9±0.32 66.4±0.59	6.99±18.9
EAAL	1.25 2.5 5 10	12.5±0.23 20.3±0.46 35.8±0.60 70.5±0.69	6.76±0.20
BNAL	1.25 2.5 5 10	12.1±0.12 18.4±0.14 32.9±0.25 66.7±0.57	7.48±0.20
AQAL	1.25 2.5 5 10	12.8±0.28 16.7±0.25 30.9±0.31 63.5±0.64	7.59±0.81
Acarbose	1.25 2.5 5 10	14.1±0.11 22.6±0.20 44.2±0.39 85.6±0.70	5.7±0.29

Data expressed as mean ± Standard deviation of triplicate experiments

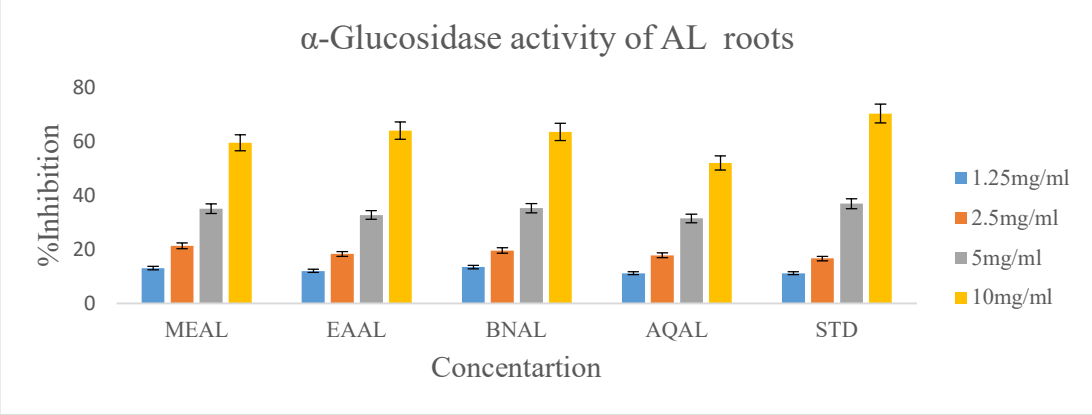

Figure 1: Effect of methanolic extract and different fractions of *Antigonon leptopus* and Acarbose on α-amylase enzyme inhibition. Data expressed as mean±SD (n=3)

Table 3: α-Glucosidase inhibitory activity and IC_{50} values by *Antigonon leptopus* root extract, fractions and Acarbose.

Test samples	Concentration(mg/ml)	% Inhibition	IC_{50} values
MEAL	1.25 2.5 5 10	13.1±0.6 21.4±0.18 35.2±0.57 59.7±0.69	7.96±0.18
EAAL	1.25 2.5 5 10	12.1±0.18 18.4±0.34 32.9±0.57 64.3±0.62	7.58±0.21
BNAL	1.25 2.5 5 10	13.5±0.11 19.7±0.17 35.4±0.28 63.8±0.62	7.68±0.22
AQAL	1.25 2.5 5 10	11.2±0.35 17.9±0.41 31.6±0.59 52.2±0.59	8.13±0.36
Acarbose	1.25	11.2±0.35	6.95±0.20

	2.5 **5** **10**	**16.7±0.39** **37.1±0.42** **70.6±0.80**	

Data expressed as mean ± SD, Standard deviation of triplicate experiments

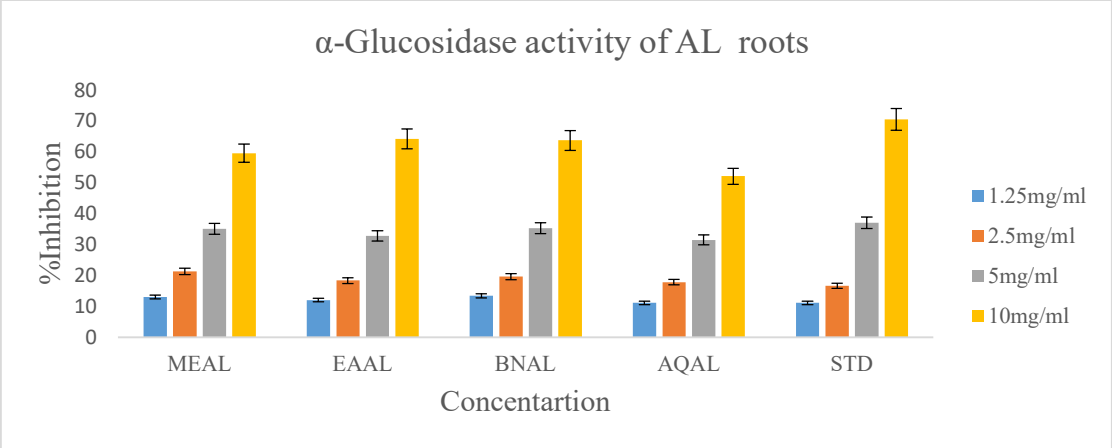

Figure 2: Effect of methanolic extract of roots and different fractions of *Antigonon leptopus* and Acarbose on alpha glucosidase enzyme inhibition. Data expressed as mean±SD (n=3)

DISCUSSION

Diabetes mellitus is one of the international health crises of the 21stcentury **[25]**. It is the world's fastest growing metabolic endocrine disorder with compromised carbohydrate and lipid metabolism. The imperfect metabolism can be attributed to the impaired insulin secretion, insulin action or both.

The enzymes α-amylase and α-glucosidase are linked to postprandial high blood glucose levels (BGL). α-amylase is linked to breaking the polysaccharides into disaccharides and oligosaccharides. α-glucosidase digest the disaccharides and polysaccharides and 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 **[24]**.

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 hypoglycemic 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 **[25]**.

The inhibitory effects of methanolic extract of *Antigonon leptopus* against porcine pancreas α-amylase and yeast α-glucosidase were evaluated in comparison with the anti-diabetic drug acarbose.

Preliminary phytochemical screening of methanolic leaf extracts and its fractions revealed the presence of alkaloids, flavonoids, carbohydrates, tannins,

saponins, steroidal and phenolic compounds **[16, 19]**.

Among all fractions, ethyl acetate fraction (EAAL) showed significant against α-amylase and α-glucosidase inhibitory activity i.e., IC_{50} 6.96±0.20mg/ml and 7.58±0.21mg/ml respectively, then methanolic extract (MEAL) was showed moderate inhibitory effect i.e., IC_{50} values of 6.99±1.89 mg/ml and 7.96±0.18 mg/ml, then butan-2-one fraction (BNAL) showed mild inhibition activity i.e., IC_{50} values of 7.48±0.20mg/ml and 7.68±0.22mg/ml respectively and aqueous extract (AQAL) of showed weak inhibition i.e., IC_{50} values of 7.59±0.81mg/ml and 8.13±0.36mg/ml respectively, when compared with that of standard drug acarbose i.e., IC_{50} value 5.7±0.29 and 6.95±0.20mg/ml respectively.

It is observed that significant percentage of inhibitory effect on both α- amylase and α-glucosidase enzymes by the ethylacetate extract of roots could be due to a major amount of total phenolic and flavonoid content. The inhibitory effect of α- amylase and α- glucosidase enzymes by AL roots were found to be in the order: EAAL>MEAL>BNAL> AQAL. The total phenolic content AL roots were determined by using Folin-ciocalteu reagent and were found in the order: EAAL>MEAL>BNAL>AQAL. The total flavonoid content of AL roots was determined using aluminium chloride colorimetric method and were found in the order: EAAL>MEAL>BNAL> AQAL.

CONCLUSION

The results achieved from this study elaborated scientific support regarding the use of *Antigonon leptopus* roots to treat diabetes through a mechanism based on its α- amylase and α-glucosidase enzyme inhibitory activity. However, further investigations are recommended to validate these effects *in-vivo.*

ACKNOWLEDGEMENT

We acknowledge Kakatiya University, Warangal (Telangana), India for providing all the facilities to perform experimental work.

AUTHORS CONTRIBUTION

The work was designed by Swaroopa Rani V and the experiment was done by Shirumalla Anuradha and Palle Swapna under the supervision of Swaroopa Rani V.

CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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