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IN-VITRO STUDIES ON α-AMYLASE, α-GLUCOSIDASE AND β-GALACTOSIDASE INHIBITORY ACTIVITY OF METHANOLIC EXTRACT OF *THERIOPHONUM SIVAGANGANUM* TUBERS AND ITS FRACTIONS

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ABSTRACT

To investigate the therapeutic effects of *Theriophonum sivaganganum* methanolic extract and its fractions for α-amylase, α-glucosidase and β-galactosidase inhibitory activity by *in vitro* assays. Tubers of *Theriophonum sivaganganum* (TS) were extracted with methanol by maceration. And its fractions were prepared by dispersing uniformly in distilled water and subjected to solvent fractionation with toluene, ethyl acetate and butanol. The fractions were concentrated under reduced pressure. The inhibitory effect of these TS methanolic extract and its fractions on α-amylase, α-glucosidase and β-galactosidase enzymes was determined by *in-vitro* assays. The results revealed that the methanolic extract and its fractions of *Theriophonum sivagnganum* were inhibited α-amylase, α-glucosidase and β-galactosidase enzymes in a dose dependent manner. Among all these ethyl acetate fraction of *Theriophonum sivagnganum* has shown the prominent α-amylase, α-glucosidase and β-galactosidase enzyme inhibitory activity with IC_{50} values of 5.75±0.18, 5.64±0.74 and 10.86±0.13mg/ml and it was well comparable with standard IC_{50} value 5.29±0.59, 5.15±0.48 and 10.16±0.57mg/ml respectively. And Phytochemical reports of TS revealed that it contains alkaloids, carbohydrates, steroids, saponins, flavonoids and phenolic compounds. These results substantiate the use of *Theriophonum sivaganganum* as medicine for the treatment of diabetes by controlling

postprandial hyperglycemia. The results suggest that ethyl acetate fraction of tubers of *Theriophonum sivaganganum* shown significant α-amylase, α-glucosidase and β-galactosidase enzyme inhibitory activity, which may be helpful to reduce the postprandial blood glucose levels. However, the chemical compounds responsible for the inhibitory action of these enzymes need to be further identified and characterized. This may be useful for the development of new antidiabetic agents from plant sources.

Keywords: *Theriophonum sivaganganum,* α-Amylase, α-Glucosidase AND β-Galactosidase

INTRODUCTION:

Theriophonum sivaganganum (Ramam. & Sebastine) Boger, commonly called as Sivaganga Voodoo lily. [Synonym: Pauella sivaganganum (Ramam. & Sebastine] belongs to family Araceae and it is endemic to India. And it is distributed in Piranmalai Hills (Sivagangai district, Tamil Nadu State). It is also found in Peninsular India, known only from South Tamilnadu **[1]**.

Vernacular names of TS: In Tamilnadu it is called as Thiruchillavalli. In maharastra it is called as are doda and tangya. The Koyas of Andhra Pradesh called *Theriophorum sivagangaum* as 'adavi Champak'**[2]**.

Description: It is a stemless herb, with corm 2.5cm across. It is named after Sivaganga Forest where it was first found. Leaves are up to 20 x 0.6cm, oblong, lanceshaped, pointed at tip; leaf-stalk up to 15cm long, sheathing at the base.

Flowering & Fruiting: November-January.

Traditional uses of TS: Leaves edible (to treat diarrhea), tubers are used as antidote for poisonous snake bite along with black pepper **[3]**.

Ethnomedicinal knowledge reveals that the tubers of *Theriophonum* species are boiled with tamarind leaves, washed and then cooked as vegetable by Konda reddis and Koyas of East Godavari district of Andhra Pradesh, and by the Koyas of Lankapalli Forest Reserve in Khammam district of Telangana state **[4]**. Aroids are used as ornamental indoor plants, some species of Amorphophallus, Colocasia, Alocasia, and Xanthosoma are very rich source of carbohydrate. Many of the aroids possesses medicinal properties, such as *Agalonema treubii* is a valuable source for glycosidase inhibitors that are antidiabetic, antimetastatic, antiviral, and immunomodulatory agent **[2]**.

Literature review of Some of the species of theriophonum sivaganganum like *Theriophonum minutum* (Willd.) Baillon, Root pounded together with 2 ½ black pepper (golmirch) is applied externally for 3-4 days as an antidote to poisonous snake bite **[3]**. Tubers of *Theriophonum divaricatum* are used against diarrhea and tubers of *Theriophonum minutum* are dried

and eaten after steaming, also used in confectionary by locals. Leaves were used as wild vegetable and also in treating common head ache **[5]**.

In-vitro antioxidant activity of acetone extract of leaves and methanol extract of tubers of *Theriophonum minitum* through DPPH and Hydrogen peroxide scavenging activity. Both the extracts showed potent DPPH activity though the methanol extract of tubers showed greater potent Hydrogen peroxide scavenging activity than the acetone extract of leaves **[6]**. The dried plant petroleum ether, ethyl acetate, ethanol, hydro-alcoholic and Aqueous extracts shown invitro anticancer activity **[7]**.

Diabetes Mellitus is the world's fastest growing metabolic endocrine disorder with compromised carbohydrate, lipid and fat metabolism. India has an estimated 77million people with diabetes, which makes it the second most affected in the world, after China. The number is projected to grow by 2045 to become 134million per the international diabetes federation (IDF**) [8]**. Traditionally, a number of plants have been used in the treatment of diabetes and only few of them have been proven scientifically.

Currently available anti-diabetic drugs exert their activity through various mechanisms. One such is the controlling of post-prandial glucose in blood. Plants have been reported to reduce the absorption of glucose by retarding the digestive enzymes i.e. alpha amylase and alpha glucosidase from pancreas and gut which in-turn helps preventing the increasing of postprandial glucose in the blood **[9]**. Inhibition of the aforementioned enzymes has been positively linked with antioxidants specially flavonoids and phenolic compounds. Natural antioxidants like phenolic compounds and flavonoids have been shown to prevent oxidative stress, thereby, reducing the onset and prognosis of diseases like diabetes mellitus. The natural antioxidants, apart from preventing oxidative stress can also display digestive enzymes inhibitory activities **[10]**. Hence the present study is focused on the invitro antidiabetic activity by inhibition activity of α-amylase, α-glucosidase and β-galactosidase enzymes of roots *Theriophonum sivaganganum* root extract and its fractions *in-vitro.*

MATERIALS AND MATERIALS:

Collection of Plant material: For the present investigation, the TS tubers was collected from Station Ghanpur, after proper identification by an expert taxonomist, Dr. V.S. Raju, Department of Botany, Kakatiya University, Warangal. And a specimen of the plant (20190720) was deposited in the Department of Pharmacognosy, University College of Pharmaceutical Sciences, Warangal, India. The collected plant material was thoroughly checked for foreign organic matter and removed. For preparation

of crude drug powder, aerial parts of plant were taken separately and dried under shade. The completely shade dried tubers were powdered and extracted with methanol by maceration in a round bottom flask at room temperature. Then methanolic extract of TS (METS) was dispersed uniformly in distilled water and subjected to solvent fractionation with with toluene (TLTS), ethylacetate (EATS) and butanol (BLTS). The fractions were concentrated under reduced pressure and phytochemical investigations were done.

DRUGS & CHEMICALS: α-amylase (Porcine pancreas), α-glucosidase (*Saccharomyces cervisiae*) and β-galactosidase (*E.coli*), O-nitrophenyl-β-galactosidase (ONPG), P-nitro phenyl β-D-glucopyranoside (PNPG), Acarbose, Quercetin, 3,5-Dinitro salicylic acid, Sodium dihydrogen phosphate, Potassium dihydrogen phosphate, Di sodium hydrogen phosphate, Sodium potassium tartarate, and Di potassium hydrogen phosphate, Sodium nitrate, Dimethyl sulfoxide, Aluminium chloride, reagents and buffers were procured from Gamut Scientifics (SRL), Secunderabad, Telangana, India. All other chemicals and solvents used are of analytical grade.

PHYTOCHEMICAL SCREENING: The dried extract and fractions of TS were subjected to phytochemical screening for the presence of different classes of organic compounds like alkaloids, carbohydrates, steroids, saponins, flavonoids, tannins, steroids, proteins and phenolic compounds according to standard methods **[11]**. Any change of colours or the precipitate formation was used as indicative of positive response to these tests.

INVITRO ANTIDIABETIC ACTIVITY

α-AMYLASE ENZYME INHIBITORY ASSAY: It was carried out by using a modified procedure given by McCue and Shetty **[12]**. Stock solution of TS methanolic extract and its fractions were prepared by dissolving up to 10mg of extract/fractions in 1ml of DMSO. A total of 250µL of extract/fractions (1.25-10mg/mL) was placed in a tube and 250µL of 0.02M sodium phosphate buffer (pH6.9) containing α-amylase solution (0.5mg/ml) was added. This solution was pre-incubated at 25^0C for 10min, after which 250µL of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at particular time intervals and then further incubated at 25^0C for 10min. The reaction was terminated by adding 500µL 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 distilled water and the absorbance was measured at 540nm using ELICO SL159 UV-Visible Spectrophotometer. The assay was performed in triplicates. Acarbose was used

a standard and the inhibitory activity of α-amylase were calculated by using the following formula.

%Inhibitory = {[Absorbance (control) - Absorbance (sample)] /Absorbance (control)} X100.

A control was prepared using the same procedure replacing the extract with distilled water.

α - GLUCOSIDASE ENZYME INHIBITORY ASSAY:

It was determined according to the method explained by Apostolidis with some modifications **[13]**. Stock solutions of extracts were prepared by dissolving up to 10mg of each extract in 1ml of DMSO. A total of 50µL of extracts (1.25-10mg/mL) and 100µL of yeast alpha glucosidase solution in phosphate buffer (pH6.9) were incubated at 25^0C for 10minutes followed by the addition of 50µL of 5Mmol/L P-nitrophenyl-β-D-glucopyranoside solution in 0.1M phosphate buffer (pH 6.9). The reaction mixture was then incubated at 25^0C for 5min and the reaction was terminated by adding 3mL of 100Mm sodium carbonate solution into the mixture and absorbance of liberated p-nitrophenol was observed at 405nm using ELICO SL159 UV-Visible Spectrophotometer. The assay was performed in triplicates. Acarbose was used a standard and the inhibitory activity of α-glucosidase were calculated by using the following formula.

% Inhibition = {[Absorbance (control) – Absorbance (extract)]/ Absorbance (control)} X100.

β - GALACTOSIDASE ENZYME INHIBITORY ASSAY:

β-galactosidase solution was adjusted to 0.2 U/mL by 0.1M phosphate buffer (pH 7.3). 200µl of enzyme solution, 100µl of different concentrations of TS extract/fractions and 2.4mL of 0.1M phosphate buffer (pH 7.3) were mixed and incubated at 37ºC for 15 min. After pre-incubation, 100µl of 30Mm, 2- Nitro Phenyl *β*-D-galactopyranozide (ONPG) solution in 0.1M phosphate buffer (pH 7.3) was added and incubated at 37ºC for 30 min. The reaction was terminated by 2mL of 0.2M sodium carbonate solution. The absorbance of the mixture solution was measured by at spectrophotometer at 410 nm **[14]**. The assay was performed in triplicates. The % inhibitory of TS extract and its fractions were calculated according to the following equation:

%Inhibitory = {[Absorbance (control) - Absorbance (sample)] /Absorbance (control)}X100.

The IC_{50} values defined as the concentration of the extract that inhibited 50% of the enzyme activity were determined from plots of % inhibition versus log inhibitor concentration and calculated by logarithmic regression analysis from the mean inhibitory values.

RESULTS:

Table 1: Preliminary Phytochemical screening of methanolic extract of TS and its fractions

S. No.	Phytoconstituents	METS	DIFFERENT FRACTIONS OF EXTRACT		
			TLTS	EATS	BLTS
1	Alkaloids	+ve	+ve	+ve	+ve
2	Carbohydrates	+ve	+ve	+ve	+ve
3	Steroids/triterpenoids	+ve	+ve	+ve	+ve
4	Saponins	+ve	+ve	+ve	+ve
5	Flavonoids	+ve	+ve	+ve	+ve
6	Proteins & Amino acids	-ve	-ve	-ve	-ve
7	Phenolic compounds	+ve	+ve	+ve	+ve

+; Present, -; Absent

Table 2: Effect of METS and its fractions on α-amylase

Extract/fractions	Concentration (mg/ml)	%inhibition α-amylase	IC_{50}
METS	1.25	12.34±0.27	8.19±0.48
	2.5	21.27±1.31	
	5	35.61±2.34	
	10	58.31±3.54	
TLTS	1.25	10.36±0.54	7.45±0.51
	2.5	19.68±1.32	
	5	35.67±1.95	
	10	65.34±3.11	
EATS	1.25	13.24±0.67	5.75±0.18
	2.5	23.54±0.56	
	5	45.79±2.22	
	10	83.24±1.97	
BLTS	1.25	11.38±0.38	6.42±0.27
	2.5	20.37±1.56	
	5	38.67±2.07	
	10	79.25±3.14	
Acarbose	1.25	14.01±0.35	5.29±0.59
	2.5	24.56±0.87	
	5	46.83±0.57	
	10	92.67±0.71	

Data expressed as mean ±SD (n=3)

Methanolic extract and its fractions of *Theriophonum sivaganganum* inhibited α-amylase catalysis at concentrations of 1.25, 2.5, 5 and 10mg/ml, with the percentage inhibition being dose dependent. METS showed percentage inhibition ranging from 12.31% to 58.31% with IC_{50} value 8.19mg/ml, TLTS showed percentage inhibition ranging from 10.36% to 65.34% with IC_{50} value 7.45mg/ml, and EATS showed percentage inhibition ranging from 13.24% to 85.64% with IC_{50} value 5.75mg/ml. BLTS inhibited the enzyme from 11.38% to 79.25% with an IC_{50} value of 6.42mg/ml. Among all fractions, EATS inhibited α-amylase enzymes by 86.64% with an IC_{50} value of 5.64mg/ml at 10mg/ml concentration, and it was close to the standard drug acarbose by 92.67% at 10mg/ml with an IC_{50} value of 5.29mg/ml **(Table 3, Figure 1)**.

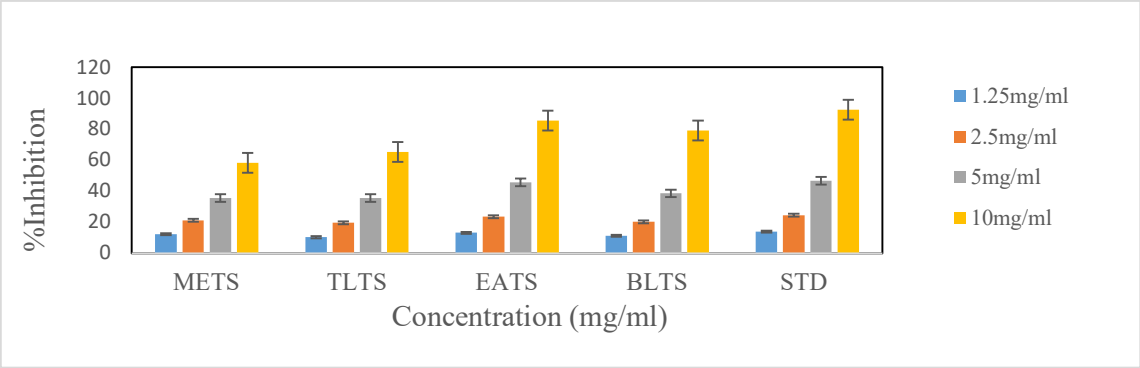

Figure 1: Effect of METS and its fractions on α-amylase

Table 3: Effect of METS and its fractions on α-glucosidase

Extract/fractions	Concentration (mg/ml)	%Inhibition	IC_{50}
METS	**1.25** **2.5** **5** **10**	**11.24±0.37** **17.85±1.28** **30.25±1.89** **54.32±3.54**	**9.08±0.48**
TLTS	**1.25** **2.5** **5** **10**	**8.69±0.87** **15.87±1.32** **32.16±1.95** **59.84±3.11**	**8.24±0.16**
EATS	**1.25** **2.5** **5** **10**	**12.35±0.51** **23.57±0.48** **43.51±2.77** **87.61±1.73**	**5.64±0.74**
BLTS	**1.25** **2.5** **5** **10**	**9.35±0.38** **18.34±1.26** **35.12±2.37** **70.51±2.67**	**7.08±0.77**
Acarbose	**1.25** **2.5** **5** **10**	**13.26±0.46** **24.65±0.37** **48.32±0.34** **96.28±0.25**	**5.15±0.48**

Data expressed as mean ±SD (n=3)

Methanolic extract of TS and its fractions inhibited the catalysis of α-glucosidase at concentrations of 1.25, 2.5, 5 and 10mg/ml, with the percentage inhibition being dose dependent. METS showed percentage inhibition ranging from 11.245 to 54.32% with IC_{50} value 9.08mg/ml, TLTS showed percentage inhibition ranging from 8.69% to 59.84% with IC_{50} value 8.24mg/ml, and EATS showed percentage inhibition ranging from 12.35% to 87.61% with IC_{50} value 5.64mg/ml. BLEC inhibited the enzyme from 9.35 to 96.28% with an IC_{50} value of 5.51mg/ml. Among all fractions, EATS demonstrated remarkable α-glucosidase enzyme inhibition, 87.61 with IC_{50} value 5.64mg/ml at 10mg/ml concentration, and it was closer to the standard acarbose 96.28 percent with IC_{50} value 5.15mg/ml at 10mg/ml concentration **(Figure 2)**.

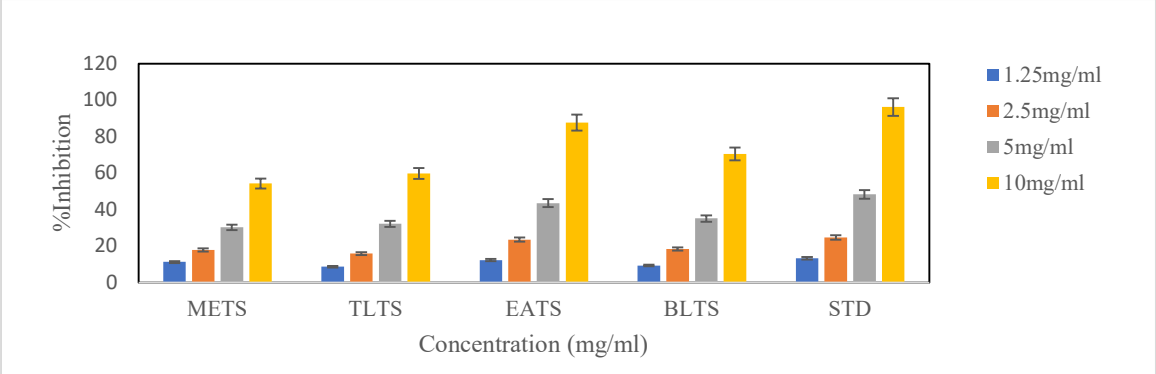

Figure 2: Effect of METS and its fractions on α-glucosidase

Table 4: Effect of METS and its fractions on β-galactosidase

Extract/fractions	Concentration (mg/ml)	%Inhibition	IC_{50}
METS	**1.25**	**6.81±0.97**	**13.30±0.48**
	2.5	**9.88±1.59**	
	5	**20.47±1.66**	
	10	**39.23±2.74**	
	20	**73.69±1.34**	
TLTS	**1.25**	**5.99±0.59**	**12.14±0.28**
	2.5	**10.86±0.98**	
	5	**19.84±1.65**	
	10	**41.23±2.68**	
	20	**82.31±1.95**	
EATS	**1.25**	**6.09±0.46**	**10.86±0.79**
	2.5	**13.24±0.53**	
	5	**25.39±2.57**	
	10	**47.65±1.67**	
	20	**92.34±2.24**	
BLTS	**1.25**	**8.12±0.68**	**11.36±0.68**
	2.5	**12.63±1.86**	
	5	**23.87±2.08**	
	10	**43.69±1.97**	
	20	**86.54±1.54**	
Quercetin	**1.25**	**7.26±0.38**	**10.16±0.57**
	2.5	**13.24±0.58**	
	5	**24.51±0.59**	
	10	**48.72±0.18**	
	20	**97.65±2.54**	

Data expressed as mean±SD (n=3)

Methanolic extract of TS and fractions of inhibited β-galactosidase catalysis at concentrations of 1.25, 2.5, 5, 10 & 20mg/ml, with the percentage inhibition being dose dependent. METS showed percentage inhibition ranging from 6.81 to 73.61% with an IC_{50} of 13.31 mg/ml, TLTS showed percentage inhibition ranging from 5.99 to 82.31% with an IC_{50} of 12.14mg/ml, and EATS showed percentage inhibition ranging from 8.12% to 86.54 percent with an IC_{50} of 11.36mg/ml. EATS inhibited the β-galactosidase enzyme with a remarkable 92.34% with an IC_{50} of 10.63mg/ml at a concentration of 20mg/ml, which was similar to the standard drug acarbose, which inhibited the enzyme with an IC_{50} of 10.16mg/ml **(Table 4, Figure 3)**.

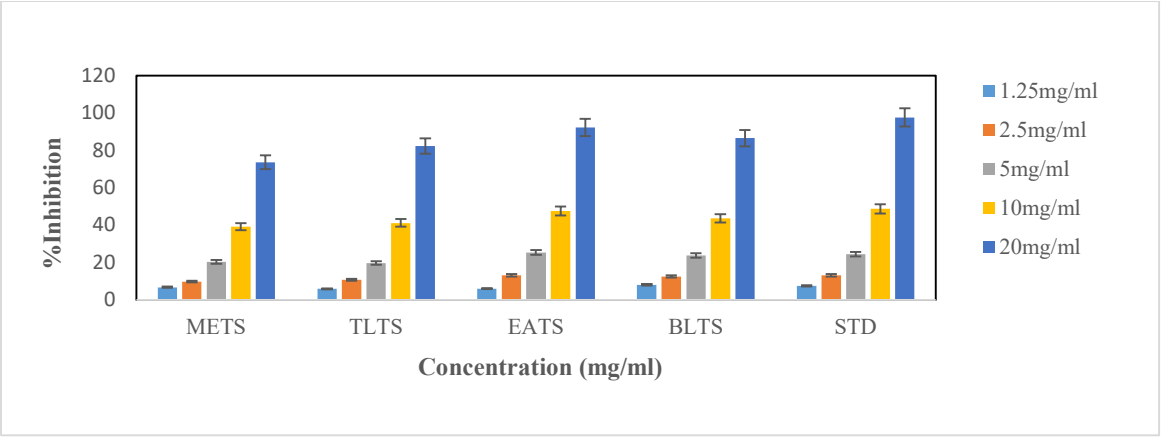

Figure 3: Effect of METS and its fractions on β-galactosidase inhibition

DISCUSSION:

The enzymes α-amylase and α-glucosidase are related to postprandial high blood glucose levels. α-Amylase is responsible for the conversion of polysaccharides to disaccharides while α-glucosidase catalyses the hydrolysis of disaccharides to glucose. The inhibition of these enzymes delay the breakdown of carbohydrates in the small intestine, thereby controlling the blood glucose level and decreasing the postprandial hyperglycaemia in diabetic patients **[15]**. Agents with α-amylase and α-glucosidase inhibitory activity have been useful as oral hypoglycaemic agents for the control of hyperglycaemia in patients with diabetes. There are many natural sources with α-amylase and α-glucosidase inhibitory activity and preventing an excessive postprandial rise of blood glucose level by α-amylase and α-glucosidase inhibition from natural resources is effective in real life as well **[16]**.

The enzymes α-amylase, α-glucosidase and β-galactosidases 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. β-galactosidase or lactase (glycoside hydrolase enzyme) that catalyses the hydrolysis of β-galactosides in to monosaccharides **[17]**. The inhibitory effects of methanolic extract of *Theriophonum sivaganganum* against porcine pancreas α-amylase, yeast α-glucosidase and *E.coli* β-galactosidase were evaluated in comparison with the anti-diabetic drugs. Preliminary phytochemical screening of methanolic extracts and its fractions of TS revealed the presence of alkaloids, carbohydrates, steroids, saponins, flavonoids and phenolic compounds.

Among all the TS methanolic extract and its fractions, EATS showed significant

against α-amylase, α-glucosidase and β-galactosidase inhibitory activity, with IC_{50} values of 5.75±0.18, 5.64±0.74 and 10.86±0.79mg/ml, BLTS showed mild inhibition IC_{50} values of 6.42±0.27, 7.08±0.77 and 11.36±0.68mg/ml, TLTS showed moderate inhibitory effect IC_{50} values of 7.45±0.51, 8.24±0.16 and 12.14±0.28mg/ml and METS showed weak enzyme inhibition IC_{50} values of 8.19±0.48, 9.08±0.48 and 12.14±0.28mg/ml, against α-amylase, α-glucosidase and β-galactosidase when compared with standard drug (acarbose) IC_{50} value 5.29±0.59, 5.15±0.48mg/ml and (quercetin) 10.16±0.57mg/ml respectively. Ethylacetate fraction of methanolic extract of tubers of *Theriophonum sivaganganum* has shown a significant percentage of inhibitory effect on α-amylase, α-glucosidase and β-galactosidase enzymes.

The inhibitory effect of TS extract and fractions on α-amylase, α-glucosidase and β-galactosidase enzymes were found to be in the order EATS>BLTS>TLTS>METS.

CONCLUSION:

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

CONFLICTS OF INTEREST

We declare that there are no conflicts of interest.

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