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In-Vitro Antioxidant and Antidiabetic Activity of Ethanolic Extract of *Trema orientalis* (L.) Blume Leaves

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ABSTRACT

Trema orientalis (L.) Blume is a species of flowering tree in the hemp family Cannabaceae. This common tree is widely used in African folk medicine for many diseases, for example, asthma, cough, dysenteria and hypertension. The objective of the study was to evaluate the antidiabetic activity and antioxidant effects of ethanolic extract of *Trema orientalis* (L.) Blume (EETO) leaves by in-vitro methods. The leaves of *Trema orientalis* were dried under shade and then powdered, and extracted with ethanol by hot continuous extraction method using Soxhlet apparatus. Preliminary phytochemical studies were carried out on extract. Anti-oxidant potential was evaluated by Nitric oxide scavenging assay using ascorbic acid as standard and hypoglycemic properties were evaluated by Alpha-glucosidase and Alpha-amylase enzyme inhibition assays using Acarbose as standard drug. In vitro evaluations showed significant free radicle scavenging and hypoglycemic effects of EETO as compared with the corresponding standard drugs used for the study thus offered a potential effect for use of dietary intervention in the clinical management or control of postprandial hyperglycemia associated with type-2 diabetes. Both *in-vitro* effects were produced in a dose-dependent manner. The hypoglycemic activity of EETO may be due to the presence of its phytoconstituents, especially due to flavonoids. Further experiment should be carried out for isolating the possible hypoglycemic compounds and then to explain the actual mechanism of antidiabetic actions of this plant extract.

INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. **[1]**

Epidemiology suggesting that diabetics worldwide number is likely to increase to 300 million or more by the year 2025. **[2-3]** Statistical projection about India suggest that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025 making it the country with highest number of diabetics in the world. **[2]**

Diabetes is a chronic medical condition, it can be controlled by medication and lifestyle modification throughout the life span. The clinical manifestation leading damage to the small blood vessels, referred to as microvascular disease. Diabetes is also an important factor in accelerating the hardening and narrowing the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel diseases. This is referred to as macrovascular diseases. **[4]**

This dreadful disease is found in all parts of world and is becoming a serious threat to mankind. A lot of synthetic chemical agents are available to control and treat diabetic patients, but total recovery from diabetes has not been reported till date. Plants provide a potential source of hypoglycemic drugs and are widely used in several traditional system of medicine. The effects of these plants may delay the development of diabetic complications and correct the metabolic abnormalities by various mechanisms. **[5]**

Currently available oral anti-hyperglycemic agents in clinical use have characteristic profile of side effects. Management of diabetes with agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with anti-hyperglycemic activity having fewer side effects. **[6]** It is therefore imperative that the larger number of crude drugs which are in use as complementary and alternative medicine claiming to be useful in diabetes, be subjected to scientific evaluation.

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of

medicinal herbs and is called as botanical garden of the world. **[7]** Many traditional treatments have been recommended in the complementary and alternative system of medicine for treatment of diabetes mellitus.

Traditional antidiabetic plants might provide a new oral hypoglycemic compound, which can counter the high cost and poor availability of the current medicine present day drugs for many rural populations in developing countries. **[8-9]**

The WHO Expert-Committee on diabetes recommended further evaluation of the folkloric methods of managing these diseases because of the high mortality and morbidity arising from its attendant complications and problem associated with the use of conventional antidiabetic agent. **[10]** Diabetes is still not completely curable by the present antidiabetic agents. Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy and fatty liver in chronic treatment. The major advantages of herbal medicine seem to be their efficacy, low incidence of side effects, and low cost. Several indigenous medicinal plants are employed in the traditional management of diabetes mellitus but there is a need to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic values. The medicinal plant might a useful source for the management of diabetes and its complications for development of new pharmaceutical entities or as a dietary adjunct to existing therapies. Few of the plants used for the treatment of diabetes have received scientific or medical scrutiny even the WHO expert committee on diabetes recommends that this area warrant further attention. **[11]**

Trema orientalis(L.) Blume is a species of flowering tree in the hemp family *Cannabaceae*. This common tree is widely used in African folk medicine for many diseases, for example asthma, cough, dysenteria and hypertension. **[12]** The root of this plant has been used for treatment of trauma, blood stasis, hematuria and bleeding of intestine and stomach. **[13-14]** The root, bark and leaves are used in epilepsy. The stem bark is used as a poultice for muscular pain. **[15]**

The study aims to scientifically evaluate the in vitro antioxidant and anti-diabetic properties of ethanolic leaf extract of *T. orientalis* L. Blume leaves.

MATERIALS AND METHODS

Chemicals & Apparatus

All the chemicals and reagents used for the study were of analytical grade and were purchased from Nice Chemicals Pvt Ltd. Kochi. α-amylase enzyme and α-glucosidase enzyme were purchased from Sigma Aldrich – Merck, Bengaluru, India. Apparatus/ instruments specifications -UV/VIS spectrophotometer (AGPPE Diagnostics, SEAC RADIM Company, MISPAPLUS MODEL), pH meter (Eutech Instruments India), Digital balance (Shimadzu. Japan), Micropipette (Rivera Glass Pvt. Ltd, India).

Collection, Extraction and Preliminary Phytochemical Evaluation

Collection and authentification of the sample

Trema orientalis (L.) Blume was collected from Kottayam M G University Campus and authenticated by the botanist, Mr. Joby Paul, School of Environmental science, M.G University, Kottayam. A voucher specimen (SES.M.G.UTY NO. 1458) is preserved at the Herbarium of School of Environmental science, M.G University, Kottayam.

Preparation of *Trema orientalis* leaves extract:

Fresh leaves were collected and shade dried at room temperature to remove moisture, then coarsely powdered by using electric grinder. The powdered materials stored in an airtight container and used for further extraction.

Extraction procedure:

Extraction of leaves of *Trema orientalis* was carried out using ethanol by hot continuous extraction method using soxhlet apparatus. 500g of shade dried leaves were taken and size reduced, extracted with 2L of ethanol in the round bottom flask and extraction was continued for 10 hours. The extract obtained was collected and concentrated by gentle heating followed by using vacuum evaporator. The concentrated extract was then weighed, stored and calculated the percentage yield. Preliminary phytochemical screening of the extract was performed. **[16-17]**

***In-vitro* Antioxidant studies -Nitric oxide scavenging assay [18-19]**

Nitric oxide free radical is generated from the sodium nitroprusside in aqueous solution at physiological pH. This nitric oxide is spontaneously interacting with the oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The antioxidant molecules or the free radical scavengers compete with the oxygen leading to the reduced production of nitrite.

Preparation of standard

The standard used here was ascorbic acid. 10, 20, 30, 40, 50 and 60mcg/ml solution of standard in ethanol was used for assay.

Preparation of sample

Sample solution was prepared from ethanolic extract of *T.orientalis* leaves dissolved in ethanol. 10mg of extract was dissolved in ethanol and solutions of 10, 20, 30, 40, 50 and 60mcg/ml concentration were prepared.

Preparation of phosphate buffered saline solution (pH 7.4)

8g of Sodium chloride, 1.44g of Potassium dihydrogen phosphate and 2.38g of Disodium hydrogen phosphate was dissolved in 800ml of distilled water and made up to 1L volume.

Preparation of Griess reagent

Sulphanilamide (1%) was prepared by dissolving 500mg of Sulphanilamide in 50ml of distilled water, O-phosphoric acid (2%) was prepared by dissolving 1g or 0.59ml of O-phosphoric acid (d=1.6850) in 50ml of water, Napthyl ethylene diamine dihydrochloride (0.1%) was prepared by dissolving 50mg of Napthyl ethylenediamine dihydrochloride in 100ml of distilled water.

Preparation of Sodium nitroprusside (100mM)

Sodium nitroprusside (100mM) was prepared by dissolving 2.98g of Sodium nitroprusside in 100ml of Phosphate buffer saline solution (pH7.4).

Estimation procedure

In this assay 0.5 ml Sodium nitroprusside (5mmolL^{-1}) in phosphate buffered saline pH 7.4, was mixed with different concentration of the extract and incubated at 25°C for 180 minutes. A control without the test compound, but an equivalent amount of ethanol was taken. After 3 hrs, 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride) was added and incubated for 30 minutes for color development. Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphathyl ethylene diamine dihydrochloride was measured at 546nm and the percentage scavenging activity was calculated with reference to the standard. The scavenging activity on the nitric oxide was expressed as inhibition percentage using the following equation:

$$\% \text{ Nitric oxide Scavenging} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

Alpha-Glucosidase and Alpha-Amylase Enzyme Inhibition Assays

Inhibition of enzymes (α-glucosidase and α-amylase) involved in the metabolism of carbohydrates is one of the therapeutic approaches for reducing postprandial hyperglycemia. Inhibition of, these enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in the management of PPBS level in type 2 diabetic patients and borderline patients.

Alpha- glucosidase enzyme inhibition assay [20]

Incubation of starch substrate solution with α-glucosidase enzyme solution will liberate glucose. The amount of liberated glucose is measured by glucose oxidase peroxidase method. The molecule having enzyme inhibiting capacity will inhibit α-glucosidase enzyme leading to the reduced production of glucose.

Glucose is oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. Hydrogen peroxide in presence of enzyme peroxidase oxidizes phenol which combines with 4-amino antipyrine to produce a red colored quinoneimine dye. The intensity of the red colour so developed is measured at 505 nm and is directly proportional to glucose concentration.

Preparation of standard

The standard drug used was Acarbose. 100, 200, 300, 400, 500 and 600mcg/ml solution of standard in DMSO was used for assay.

Preparation of sample

Sample solution was prepared from ethanolic extract of *T.orientalis* leaves dissolved in DMSO. 10mg of extract was dissolved in DMSO and solutions of 100, 200, 300, 400, 500 and 600mcg/ml concentration were prepared.

Estimation procedure

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract or standard drug (100 - 600mcg/ml) for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath. The amount of liberated glucose is measured by glucose oxidase peroxidase method using glucose diagnostic kit. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle. The percentage inhibiting activity was calculated with reference to the standard. The inhibiting activity on the α-glucosidase enzyme was expressed as inhibition percentage using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

Alpha-amylase enzyme inhibition assay [21]

Incubation of starch substrate with α-amylase enzyme will liberate glucose. The amount of liberated glucose is measured by using 3, 5 dinitrosalicylic acid colour reagent. The molecule having enzyme inhibiting capacity will inhibit α-amylase enzyme leading to the reduced production of glucose.

Preparation of standard

The standard drug used was Acarbose. 100, 200, 300, 400, 500 and 600mcg/ml solution of standard in DMSO was used for assay.

Preparation of sample

Sample solution was prepared from ethanolic extract of *T.orientalis* leaves dissolved in DMSO. 10mg of extract was dissolved in DMSO and solutions of 100, 200, 300, 400, 500 and 600mcg/ml concentration were prepared.

Estimation procedure

Test and standard drug of varied concentration in 500 µl were added to 500 µl of 0.20 M sodium phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.20 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle. The percentage inhibiting activity was calculated with reference to the standard. The inhibiting activity on the α-amylase enzyme was expressed as inhibition percentage using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

The tests were carried out in triplicate. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against extract concentration.

Statistical analysis

Data were statistically evaluated using Mann Whitney t-test using GraphPad Prism version 6 computer software.

RESULTS AND DISCUSSION

The percentage yield obtained after the ethanolic extraction of dried leaves of *Trema orientalis* (L.) Blume was 10.02 % w/w. Result of preliminary phytochemical studies of ethanolic extract of *Trema orientalis* is presented in table 1.

Table No. 1: Preliminary phytochemical screening of ethanolic extract of *Trema orientalis* (L.) Blume leaves

Sl No.	Test	Extract	Inference
I	**Test for carbohydrates**		
	Molisch's test	-	Absence of carbohydrates.
	Benedicts test	-	Absence of carbohydrates.
	Fehlings test	-	Absence of carbohydrates.
II	**Test for tannins and phenolics**		
	Lead acetate test	+	Presence of phenolics and tannins.
	Ferric chloride test	+	Presence of phenolics and tannins.
III	**Test for steroids**		
	Salkowski's test Libermann Burchard test	+ +	Presence of steroids. Presence of steroids.
IV	**Test for triterpenoids** Isoprenoid test	+	Presence of triterpenoids.
V	**Test for flavones and flavonoids**		
	Shinoda test	+	Presence of flavonoids.
	Aqueous sodium hydroxide test	+	Presence of flavonoids.
VI	**Test for alkaloids**		
	Mayer's test	+	Presence of alkaloids.
	Hager's test	+	Presence of alkaloids.
	Dragendroff's test	+	Presence of alkaloids.
	Wagner's test	+	Presence of alkaloids.
VII	**Test for Glycosides**		
	Legal test	+	Presence of glycosides.
	Baljet test	+	Presence of glycosides.
VIII	**Test for Proteins**		
	Millon's test	-	Absence of proteins.
	Biuret test	-	Absence of proteins.
	Ninhydrin test	-	Absence of proteins.
IX	**Test for Saponins**		
	Foam/Froth test	+	Presence of saponins.

Nitric oxide scavenging assay

The percentage inhibition obtained in the different concentrations of sample extract were compared to that of standard and it is tabulated in Table 2 and Figure 1.

Table No. 2: Percentage inhibition of Nitric oxide radical by ethanolic extract of *Trema orientalis* (L.) Blume leaves and ascorbic acid

Sl.no	Concentration (µg/ml)	OD Std	% Inhibition Std	OD EETO	% Inhibition EETO
1	Control	0.95± 0.01		0.95± 0.01	
2	10	0.60±0.02	36.82±2.77	0.76±0.01	20.00±0.21
3	20	0.52±0.01	45.27±0.48	0.69±0.01	27.37±0.29
4	30	0.44±0.04	53.71±3.73	0.57±0.02	40.01±1.48
5	40	0.37±0.02	61.07±1.70	0.53±0.03	44.23±2.57
6	50	0.27±0.01	71.57±1.36	0.46±0.02	51.59±1.60
7	60	0.17±0.02	82.12±1.92	0.39±0.01	58.95±0.63

Values are mean ± S.D of triplicate.

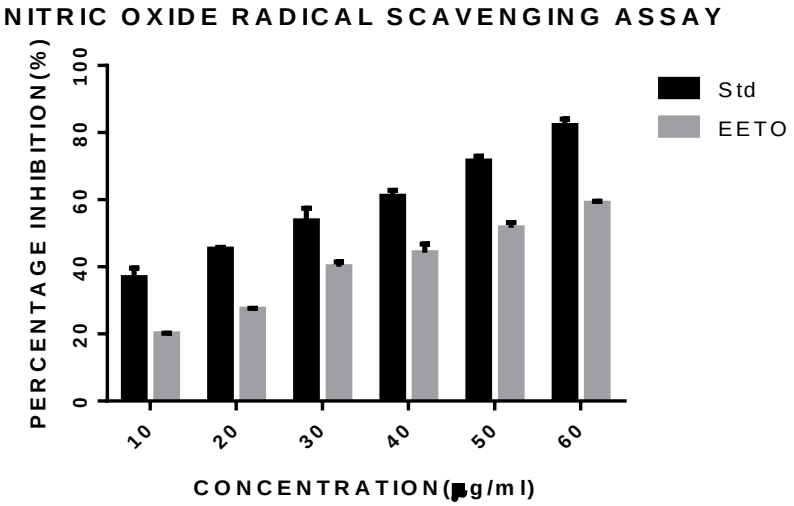

Figure No. 1: Nitric oxide scavenging assay

Alpha- glucosidase enzyme inhibition assay

The EETO revealed a significant inhibitory action on α-glucosidase enzyme. There was a dose-dependent increase in percentage inhibitory activity of EETO against α-glucosidase enzyme. The percentage inhibition (EETO) varied from 26.78±3.84 to 68.05±1.73 for lowest concentration (100µg/ml) to the highest concentration of 600µg/ml. The concentration required for 50% inhibition (IC_{50}) was found to be 368.81µg/ml whereas the positive control acarbose produced inhibitory percentage of 38.15±0.39 for 100µg/ml and 78.36±0.81 for 600µg/ml. The IC50 value of standard drug acarbose against α-glucosidase was found to be 250.99 µg/ml. The percentage inhibition obtained in the different concentrations of sample extract were compared to that of standard and it is tabulated in Table 3 and Figure 2.

Table No. 3: Percentage inhibition of α-glucosidase enzyme by ethanolic extract of *Trema orientalis* (L.) Blume leaves and acarbose.

Sl. No.	Concentration (µg/ml)	OD Std	% Inhibition Std	OD EETO	% Inhibition EETO
1	Control	0.97±0.01		0.97±0.01	
2	100	0.6.±0.01	38.15±0.39	0.71±0.03	26.78±3.84
3	200	0.53±0.01	45.38±2.53	0.64±0.02	34.03±1.39
4	300	0.45±0.06	53.63±2.62	0.51±0.01	47.43±0.49
5	400	0.36±0.04	62.91±3.06	0.45±0.01	53.60±1.51
6	500	0.29±0.02	69.87±1.40	0.37±0.03	61.87±2.70
7	600	0.21±0.01	78.36±0.81	0.31±0.02	68.05±1.73

Values are mean ± S.D of triplicate.

Statistical evaluation showed that there was no statistical significant difference between percentage inhibitions of Std and extract, corresponding to each concentrations. i.e. EETO possess significant alpha glucosidase enzyme inhibition activity as compared with acarbose.

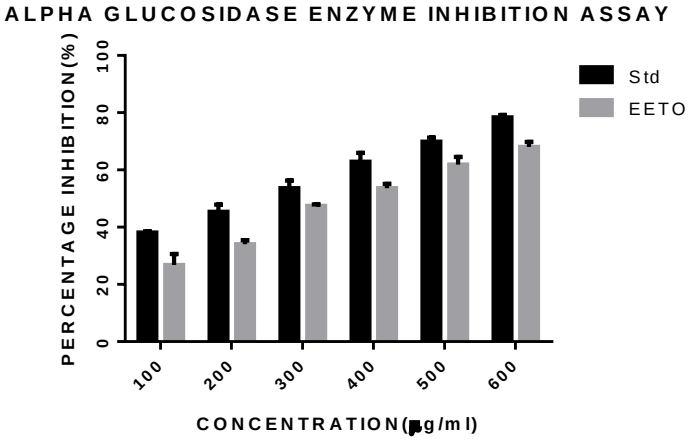

Figure No. 2: Alpha- glucosidase enzyme inhibition assay

Alpha-amylase enzyme inhibition assay

There was a dose-dependent increase in percentage of inhibitory activity against α-amylase enzyme. At a concentration, 100µg/ml of extract showed a percentage inhibition 19.38±4.51 and for 600µg/ml it was 63.46±2.83. The extract gave an IC_{50} value of 448.09µg/ml. The IC_{50} value of standard drug acarbose was found to be 333.33µg/ml. The percentage inhibition obtained in the different concentrations of sample extract were compared to that of standard and it is tabulated in Table 4 and Figure 3.

Table No. 4: Percentage inhibition of α-glucosidase enzyme by ethanolic extract of *Trema orientalis* (L.) Blume leaves and acarbose

Sl.no	Concentration (µg/ml)	OD Std	% Inhibition Std	OD EETO	% Inhibition EETO
1	Control	0.93±0.01		0.93±0.01	
2	100	0.63±0.03	32.28±2.50	0.75±0.05	19.38±4.51
3	200	0.55±0.02	40.87±1.52	0.66±0.02	29.04±1.39
4	300	0.49±0.01	47.32±0.51	0.62±0.01	33.34±0.36
5	400	0.42±0.02	54.85±1.67	0.48±0.02	48.4±1.60
6	500	0.35±0.01	62.36±1.48	0.42±0.02	54.85±1.67
7	600	0.27±0.02	70.65±2.36	0.34±0.03	63.46±2.83

Values are mean ± S.D of triplicate.

Statistical evaluation showed that there was no statistical significant difference between percentage inhibitions of Std and extact, corresponding to each concentrations. i.e. EETO possess significant alpha-amylase enzyme inhibition activity as compared with acarbose.

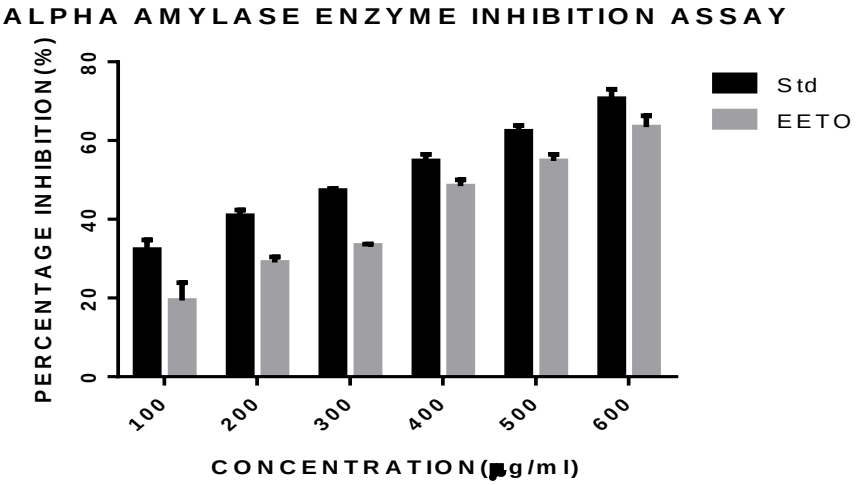

Figure No. 3: Alpha-amylase enzyme inhibition assay

Persistent hyperglycemia, the common characteristic of diabetes can cause most of diabetic complications. In all patients, treatment should aim to lower elevated blood glucose level to near-normal levels. **[22]**

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels. The hyperglycemia is known to be associated with an increased incidence of microvascular complications in patients with type 2 diabetes. **[23]** The studies suggested that postprandial hyperglycemia could induce the nonenzymatic glycosylation of various proteins, resulting in the development of chronic complications in diabetes. **[24-25]** Therefore, control of postprandial plasma glucose levels is critical in the early treatment of diabetes and in reducing chronic vascular complications. **[24]** One of the therapeutic approaches for reducing postprandial hyperglycemia is to prevent absorption of carbohydrates after food intake. Complex polysaccharides must be digested by the enteric digestive enzymes including alpha-glucosidase, alpha-amylase since only monosaccharides can be absorbed from the intestinal lumen and transported into blood circulation. Therefore, alpha-glucosidase inhibitors such as acarbose, voglibose, and miglitol are widely used either alone, or in combination with insulin secretagogues in patient with type 2 diabetes. **[25]**

In addition, several alpha-glucosidase inhibitors have been recently developed from natural sources. **[26-28]** Synthetic inhibitor causes side effects such as abdominal pain, diarrhea and soft faeces in the colon. Inhibition of α-glucosidase and α-amylase, enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet and therefore it can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients. **[29]**

Intestinal alpha-glucosidase acting as a key enzyme for carbohydrate digestion, located at the epithelium of the small intestine. Alpha-glucosidase has been recognized as a therapeutic target for modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in Type 2 DM. **[29]**

Alpha-Amylases are considered as endo-acting enzymes which randomly attack internal β-1, 4-glycosidic linkages, except for those adjacent to the ends of the substrate and those near the branch points, the products of this reaction are maltose, maltotriose and β-dextrins. Comparatively, β-amylases are classified as exo-acting hydrolysing β1, 4 bonds from the non-reducing ends of the substrate thus producing β-products. In monosaccharide glucose can be readily absorbed from the GI tract into the bloodstream after the hydrolysis of glycosidic bonds in digestible carbohydrate foods containing starch by the enzyme α-amylase. The inhibition of these enzymes reduces the high post parandial blood glucose in diabetics. **[30]** This has led to the generation of anti-diabetic and obesity treatment in humans by controlling postprandial blood-glucose levels. Present study shows that EETO having good inhibitory activities against key enzymes linked to type-2 diabetes, namely, α-amylase and α-glucosidase. Thus offers a potential use for dietary intervention in the management or control of postprandial hyperglycemia associated with type-2 diabetes.

Higher amounts of ROS have been shown to play role in the development of diabetic complications as well as in a number of other disease states. Oxidative stress has been shown to play a role in the causation of diabetes 1 and 2 and as such, antioxidants may have a role in the alleviation of diabetes. Streptozotocin (STZ) produces free radicles which produce hyperglycemia in rats which EETO has been proven for effective against STZ induced hyperglycemia **[31]** Data of Nitric oxide scavenging activity shows that EETO had strong protective effect against free radical toxicity.

The phytochemical screening result showed that EETO contains high amounts of phenolics, tannins, flavonoids and terpenoids. Other constituents like saponins and alkaloids were present in low amounts. These phytochemical constituents are known to be hypoglycemic. Various studies on medicinal plants have reported promising antidiabetic activity of these phytochemical constituents. Petroleum ether fraction of *C.roseus* contains tannins, flavonoids and alkaloid compounds (catharanthin, leurosine, lochnerine, tetrahydroalstonin, vindoline and vindolinine) produced varying degree of blood sugar reduction, **[32]** Inhibition of α-amylase and α-glucosidase by flavanoids. **[33]** Several authors reported that saponins, flavonoids, phenolics compounds, glycosides and triterpenoids have hypolipidemic and hypocholesterolemic effects. **[34-35]** In the light of our pharmacological studies it can assume that the antidiabetic activity of EETO may be due to the presence of these phytoconstituents, especially due to flavonoids.

This study would be helpful for the human subject to produce herbal formulation with less side effects and cost effective treatment for diabetes. Further work at biomolecular level of the extracts and formulations will ensure that the formulation meets the global standard and acceptability.

As the *Trema orientalis* is safe, more details models are required and it will be useful for fix the optimized dose in herbal formulation. The findings of the bioactive molecule from this herbal plant would be effective drug target against diabetes.

CONCLUSION

Present *in vitro* studies showed that ethanolic extract of *Trema orientalis* (L.) Blume leaves (EETO) having good inhibitory activities against key enzymes linked to type-2 diabetes, namely, α-amylase and α-glucosidase. Thus offered a potential effect for use of dietary intervention in the clinical management or control of postprandial hyperglycemia associated with type-2 diabetes. Nitric oxide radical scavenging assay revealed that EETO has strong protective effect against free radical toxicity. The hypoglycemic activity of EETO may be due to the presence of its phytoconstituents, especially due to flavonoids. Further experiment should be carried out for isolating the possible hypoglycemic compounds and then to explain the actual mechanism of antidiabetic actions of this plant extract.

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