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Research Article

The ethanol extract of *Muntingia calabura* exhibits in vitro antidiabetic potentials by inhibiting carbohydrate digestive enzymes

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Abstract

Objective: The present research study was conducted to investigate *in vitro* anti-diabetic potentials of ethanol extract of leaves of *Muntingia calabura* for α-glucosidase and α-amylase inhibitory activities.

Materials & Methods: The leaves of *Muntingia calabura* were collected and authenticated. dried and powdered. The powdered drug was defatted using petroleum ether and extracted with ethanol. The ethanol extract of *Muntingia calabura* was subjected to preliminary phytochemical investigation. The *in vitro* α-glucosidase inhibitory and α-amylase inhibitory properties were determined for the ethanol extract and concentrations of extract required to inhibit absorbance (IC_{50} values) EEMC were determined.

Results: Significant IC_{50} values were found in both tests that assessed the inhibitory activity of α-glucosidase and α-amylase, demonstrating the capacity of the ethanol extract of *Muntingia calabura* to block carbohydrate digesting enzymes and prevent postprandial blood glucose.

Conclusion: The results of the presents investigation recommends that ethanol extract of *Muntingia calabura* possess significant α-glucosidase and α-amylase inhibitory activity.

Keywords: Antidiabetic activity, *Muntingia calabura*, α-glucosidase and α-amylase and IC_{50}values.

INTRODUCTION

A non-infectious pathological ailment that could affect the entire world in this millennium is diabetes mellitus. According to estimates, India will account for half of all diabetes patients by 2025, making it the "Diabetic Capital of the World"[1].

Diabetes mellitus (DM), a chronic metabolic condition that affects the metabolism of carbohydrates, proteins, and lipids, is brought on by the body's inability to secrete insulin. Hyperlipidemia is one of the symptoms of diabetes mellitus in addition to the more common ones like hyperglycemia, weight loss, polyurea, and polydypsia. These symptoms are linked to the development of microvascular and macrovascular problems in diabetic patients and may result in death[1,2].

In most Western nations, 2-6% of individuals have type II diabetes mellitus, sometimes referred to as non-insulin dependent diabetes mellitus, which typically develops in middle or later life.[3]In the twenty-first century, diabetes mellitus (DM) is the most prevalent health issue worldwide. Additional than 366 million people worldwide currently have diabetes, and the World Health Organization predicts that by 2030, 552 million additional people will have the disease[4].Diabetes hyperglycemia is treated with oral hypoglycemic medications, food restriction, and exercise.For the treatment of diabetes mellitus, a variety of oral hypoglycemic medications are available, along with insulin, but due to the adverse effects of conventional therapeutic agents, there is increased interest in herbal therapies. Herbal medicines are frequently recommended even when their biologically active ingredients are unknown due to their perceived effectiveness, few side effects in clinical practice, and relative affordability[3].

Sulfonylurea, biguanide, thiazolidinedione, and glycosidase inhibitors are among the pharmacological and chemical treatments for type II diabetes currently on the market, but they are known to have a number of unfavourable side effects and have little effect on the progression of diabetic complications. Currently, insulin is the medicine of choice for treating insulin-dependent diabetes mellitus (Type I-IDDM), although sulfonylureas and insulin sensitizers are effective treatments for type II non-insulin-dependent diabetic mellitus (Type II-NIDDM). However, these medications have potentially fatal side effects such cardiotoxicity, nephrotoxicity, and others.[5]Therefore, despite enormous medical developments, there is now no truly effective medication for the treatment of diabetes mellitus. Because of this, there is always room for the development of drugs with plant origins that are already successfully used in Indian traditional medicine, such as Ayurveda, to treat diabetes mellitus. Additionally, WHO constantly promotes research using natural sources to stop the high prevalence of diabetes

as well as its long-term complications.[4]Herbal treatments have been utilised to treat diabetes mellitus since the dawn of time. In rural areas of developing nations, around 90% of the population only uses traditional medicines for primary healthcare[5].

Plants have a special place in the treatment of cancer, ulcerogenic, inflammatory etc. It is estimated that plant derived compounds having the property of Antibacterial Activity, Antifungal Activity, Antihyperglycemic and Antidiabetic Activities, Antioxidant Activity, Anti-inflammatory activity, and Antipyretic Activity[6,7]. The *Muntingia calabura* also known as belongs to the family *Muntingiaceae* native of western South America, including Bolivia and Argentina, as well as southern Mexico, the Caribbean, the Caribbean Sea and Western Ghat in South India. *Muntingia calabura* is a plant with excellent therapeutic properties. It was used to treat diabetes and other ailments in the traditional ancient medical system. The present study was created to assess the in vitro antidiabetic activity of ethanol extract via α-glucosidase and α-amylase inhibitory activity of *Muntingia calabura* because there is a lack of scientific evidence to support this activity[6,7].

MATERIALS & METHODS

Plant material

The leaves of *Muntingia calabura* was collected from local area in Bangalore and authenticated by Dr. V. Rama Rao, Research Officer (Botany), Central Ayurveda Research Institute. The authenticated leaves were separated from other plant parts, cleaned, washed and dried for further use.

Preparation of the ethanol extract

The plant's leaves are gathered and dried in the shade. The powdered dried leaves are next treated with petroleum ether to remove fat from the coarse powder. The defatted powdered drug will undergo 48 hours of ethanol extraction in a Soxhlet system, and the residual marc will undergo an aqueous extraction process using chloroform water.[8].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanol (EEMC) of *Muntingia calabura* was conducted as per procedure prescribed by Khandelwal[9].

Evaluation of in vitro antidiabetic activity of extract of *Muntingia calabura*

α-Glucosidase inhibitory assay

The test was conducted to investigate the potential inhibitory effects of TPME on the GIT's α-glucosidase enzyme for sucrose and maltase carbohydrate digestion. Despite the fact that α-glucosidase enzyme isolated from yeast is extensively utilised to assess α-Glucosidase inhibitor medicines, the outcomes may not always be consistent with those of mammal enzymes.

As a result, the small intestine homogenate of albino mice was chosen in the current research investigation since it was hypothesised that it would more accurately represent the physiological state occurring in vivo. The techniques utilised in earlier research investigations were somewhat modified in order to test the inhibitory activity of EEMC against glucosidase and amylase. After 20 hours of fasting, the small intestine of the experimental mouse duodenum and cecum was cut and removed. The portion of intestine that was removed was homogenised with 12 mL of maleate buffer (100 mM, pH 6), after being washed with ice-cold normal saline solution. The homogenate component was used as α-glucosidase solution for additional research. The assay's reaction mixture included 2% (w/v) sucrose and maltose substrate solution (100 ml), 100 mM maleate buffer (pH 6), and an ethanol extract of *Muntingia calabura* (20–640 g/mL). Raw α-glucosidase enzyme solution (1ml) was added to the reaction after a preincubation of 5 minutes at 37^{O}C and further10-minutes incubation at 37^{O}C. A glucose evaluation kit from Span Diagnostic Ltd. in Mumbai, India was used to measure the amount of glucose produced in the reaction under consideration. The amount of glucose released by the positive control (GCP), glucose generation blank value (GCB) and quantity of glucose produced by the addition of EEMC (GCT) were noted[10,11]. The amount of glucose produced when the carbohydrate was completely broken down was used to calculate the rate of carbohydrate breakdown. The rate of inhibition was determined by the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{GCP- GCT-GCB}}{\text{GCP}} \text{ X100}$$

α-Amylase inhibitory assay

The assay samples of ethanol extract of *Muntingia calabura* serial concentrations (6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, 100 mg/mL, 200 mg/mL) and reference standard nojirimycin (6.25-200 µg/mL)] of 500 ml were added to 500 ml of 0.02 M sodium phosphate buffer (at pH 6.9 with 0.006 M sodium chloride) containing 0.5 mg/mL porcine pancreatic enzyme alpha-amylase solution and were kept for incubation for 10 minutes at temperature 25^{O}C. After the pre-incubation, At specified intervals, 500 ml of a 1% starch solution in a 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) were added to each test. After that, the test mixtures were incubated for a further 10 minutes at 25^{O}C.A last step in the assay was adding 1 mL of the 3,5-dinitro-salicylic acid colour reagent. All test tubes were incubated for 5 minutes at boiling temperatures in a water bath before being cooled to room temperature using tap water. After that, the test was diluted by including10 mL of distilled water were used, and absorbance at 540 nm was measured[10,11].

$$\text{\% inhibition} = \frac{\text{Abs(Control)(540) - Abs (Extract)(540)}}{\text{Abs (Control)(540)}}$$

RESULTS

Evaluation of in vitro anti-diabetic activity

α-Glucosidase inhibitory activities

In order to determine the inhibitory potentials of the ethanol extract of *Muntingia calabura*, an in vitro α-glucosidase enzymes inhibitory test was conducted. The half of maximal concentration requires to inhibit sucrose and maltase enzymes (IC_{50}) were 354.17 µg/mL and 360.59 µg/mL, respectively. The evidence suggests that EEMC has significant, dose-dependent properties, making it a potent α-glucosidase inhibitor.[Table No .1 and Figure No.1 and 2].

α-Amylase inhibitory activities

To evaluate the inhibitory activity of ethanol extract of *Muntingia calabura* onpostprandial glucose rise, an in vitro α-Amylase inhibition test was performed. In this study, EEMC demonstrated potent inhibition of α-amylase equivalent to the reference standard. [Table No .1 and Figure No.3].

Table 1: Effect of ethanol extract of *Muntingia calabura*on α-Glucosidase (Sucrase and maltase) and amylase inhibitory activity

Treatment	IC_{50} Values (µg/ml)		
	α-Glucosidase (Sucrase)	α-Glucosidase (Maltase)	α-Amylase
EEMC	354.17	360.59	324.11
Acarbose	254.49	243.01	--
Nojirimycin	--	--	259.29

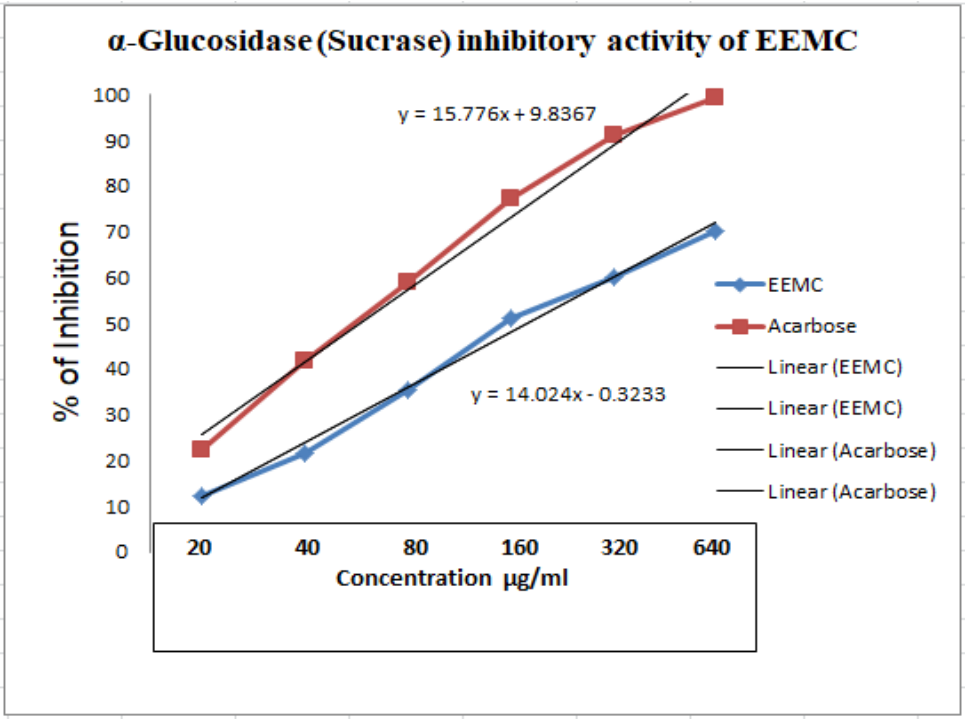

Figure 1: Effect of EEMC on α-Glucosidase (Sucrase) and amylase inhibitory activity

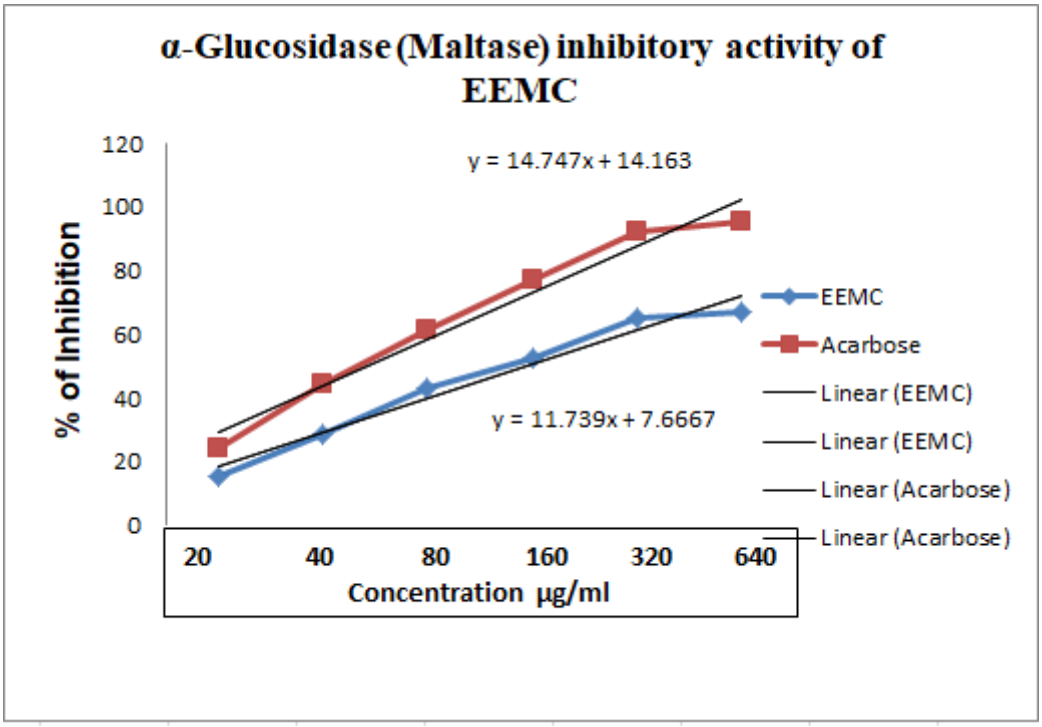

Figure 2: Effect of EEMC on α-Glucosidase (maltase) inhibitory activity

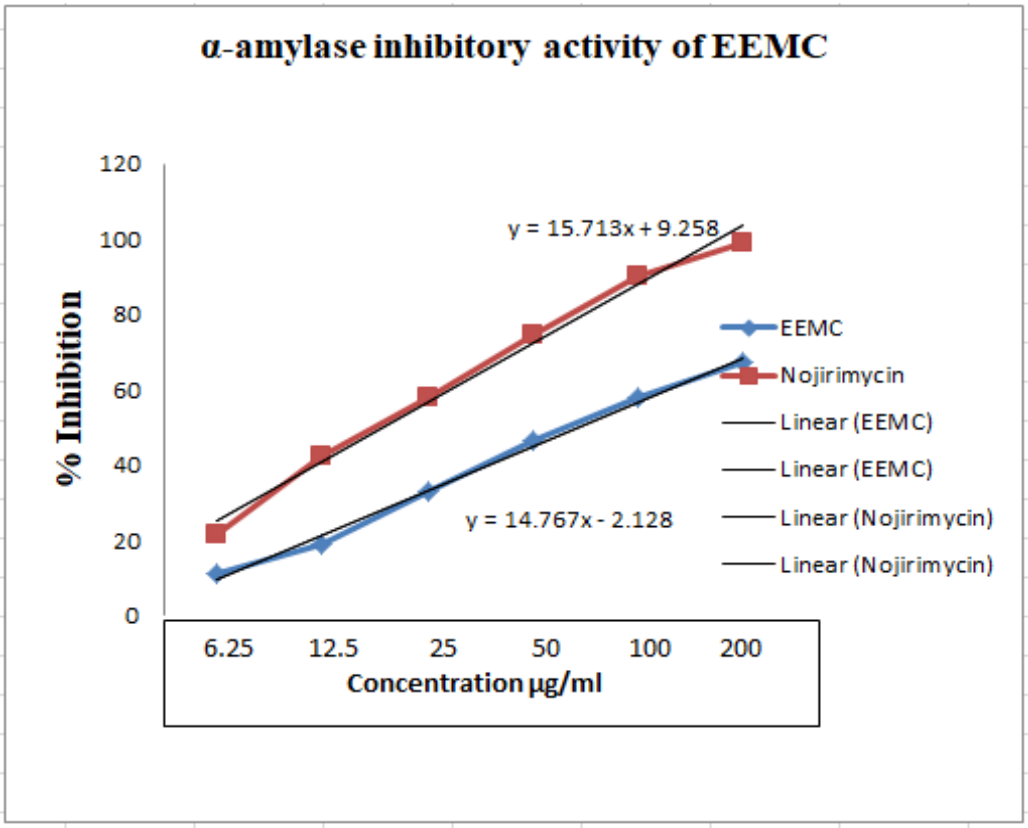

Figure 3: Effect of EEMC on amylase inhibitory activity

DISCUSSION

Inactivating carbohydrate hydrolyzing enzymes such α-amylase and α-glucosidase enzyme to prevent the absorption of glucose from GIT and so diminish postprandial hyperglycemia and its complications is a unique therapeutic method for managing diabetes mellitus[12,13].By employing 4-Nitrophenyl-b-D-glucopyranosiduronic acid (pNPG) as the reaction precursor and small intestine as a source of α-glucosidases, sucrase, and maltase, the α-glucosidase enzyme inhibition by EEMC was investigated.[14,15]. However, it is still unclear whether the inactivation of the α-amylase and α-glucosidase enzymes by EEMC is caused by competitive or noncompetitive inhibition mechanisms. In the current investigation, the EEMC have demonstrated significant α-glucosidase and α-amylase inhibitory properties indicate its usefulness to reduce postprandial glucose. While the inactivation rate for the enzyme α-glucosidase was close to that of the study's reference standard medication, acarbose, the rate for -amylase was significantly lower. This demonstrates that EEMC is a powerful inhibitor of α-glucosidase and has a weaker inhibitory effect than α-amylase. By reducing the entry of glucose into the circulation, the α-glucosidase and α-amylase enzyme inhibitory characteristics of EEMC can be regarded as a successful strategy for the prevention of diabetes mellitus. Diabetes patients frequently have significant post-meal hyperglycemia, which might be managed if the rate of glucose absorption from the GIT into the blood circulation could be slowed down by inhibiting carbohydrate hydrolysis.[16].

About 30–40% of the body's mass is made up of skeletal muscle, making it one of the most important target tissues for insulin's activity, which improves the utilization of glucose at the peripheral level. It is generally known that insulin and anti-diabetic medications promote the uptake of glucose by peripheral cells and tissues. The stimulation of glucose uptake from the rat's hemidiaphragm, which is made up of muscle tissue and is vital for insulin-regulated glucose discharge, is another key discovery of the current study. This is evidenced by the fact that EEMC have significant actions similar to those of insulin. Compared to insulin, the EEMC significantly increased the absorption of glucose by isolated rat muscle hemidiaphragm. The results of the normal group of glucose utilization by rat peripheral tissue seem to indicate that EEMC has an effect on those tissues, and they are consistent with past studies[17].

Despite the fact that the exact method by which alloxan causes pancreatic damage is unknown, research suggests that the substance kills pancreatic cells as a result of its free radical nature, which is followed by absolute insulin insufficiency and diabetes mellitus[18,19]. Although the precise mechanism by which alloxan damages the pancreas is unknown, research suggests that the chemical kills pancreatic cells as a result of its free radical nature, followed by complete insulin insufficiency and diabetes mellitus[21,22]. The ethanol extract was successful in the current in vivo test in stimulating insulin secretion and controlling the therapy groups' normal blood glucose levels. The antioxidant capabilities of EEMC, which are a potential mechanism of action in the current study and potentially protect pancreatic cells from alloxan-mediated damage and normalize insulin secretion, should be investigated. By boosting the utilization of glucose by peripheral tissues, the EEMC demonstrated its ability to combat insulin resistance. The extract also demonstrated its capability to inhibit GIT digestive enzymes to reduce the problems of post-prandial hyperglycemia.

CONCLUSION

The findings of this study suggest that *Muntingia calabura* ethanol extract has considerable α-glucosidase and α-amylase inhibitory action. But further examination is necessary to isolate and estimate the specific components present in methanol extract of *Muntingia calabura* that may be

responsible for these beneficial properties to improve the health conditions connected with diabetes mellitus.

CONFLICT OF INTEREST

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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