



Phytochemical Screening, *Invitro* antidiabetic activity of *Muntingia calabura* leaves extract on alpha-amylase and alpha-glucosidase enzymes

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ABSTRACT

Muntingia calabura is a medicinal plant broadly used in conventional therapeutic preparation of many pharmacological activities. In the present study, the *Invitro* antidiabetic property of *Muntingia calabura* leaves extracts was analyzed by using standard methods. An in vitro anti-diabetic study was done by inhibition of α -amylase and α -glucosidase enzymes. The phytochemical screening of *Muntingia Calabura leaves* revealed that the extract is rich in the secondary metabolites such as alkaloids, polyphenols and tannins. The in vitro antidiabetic capability of extracts such as Petroleum ether, Chloroform, Methanol and aqueous through α -amylase enzyme and alpha-glucosidase enzyme inhibition studies. The results of the present study concluded that the methanolic extract of *Muntingia calabura* exhibits 80% in α -amylase and 60% in alpha-glucosidase activity while compared to acarbose. The phytochemistry study portrays the antidiabetic activity of *Muntingia calabura* is due to the presence of polyphenols

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INTRODUCTION

Diabetes mellitus (DM) is an inescapable and multifactorial incessant metabolic issue, is portrayed by deformities in endogenous insulin discharge or activity, or both, which brings about endless hyperglycemia, a clinical sign of diabetes, regularly joined

by glycosuria, polydipsia polyuria and weight reduction (Thangasami and Chandani, 2015; Vasanth S *et al.*, 2018).

Hyperglycemia, in the long run, prompts dynamic beta-cell brokenness, weakened insulin quality translation and perpetual-cell misfortune because of apoptosis (Kido *et al.*, 2000). Subsequently, in the diabetic condition, changes in insulin production and its activity lead to weakened glucose homeostasis and the event of perpetual hyperglycemia, which prompts a decreased number of glucose transporters, down guideline in the quantity of insulin receptors just as imperfections of tissue insulin signal transduction, all of which results in insulin obstruction (Prameswari and Widjanarko, 2013).

Management of DM at the present is immobile symptomatic and must be completed for a generation, so probably inflicting a range of aspect effects, like

hypoglycaemic, etc. (Sujono and Wahyuni, 2005). Hence it's essential in the direction of discovering the substitute treatment. This can stabilize blood sugar levels in traditional vary. At present, the plant is considered as a conceivable asset of bioactive mixes. The auxiliary metabolite substance in the plant has been distinguished to contain an amount of action.

Medicinal plants are wellsprings of a significant helpful guide for easing human illnesses. Around 80% of the general populations in the developing nations everywhere throughout the world rely upon the conventional drug for their essential health-care. Remarkably, about 85% of a traditional customary prescription includes the utilization of plant removes.

Enthusiasm for phytomedicine started over the most recent 20 years and with expanding familiarity with the wellbeing dangers and toxicities related with unsystematic utilization of manufactured medications and anti-infection agents, enthusiasm for the utilization of plants and plant-based medications has resuscitated all through the world (Mahmood et al., 2014; Sarojini and Mounika, 2018).

In any case, an enormous number of therapeutic plants stay to be explored for their conceivable pharmacological worth. One of the plants that have as of late picked up a therapeutic plant status is *Muntingia calabura* L. (Elaeocarpaceae). *M. calabura* leaves of have probable anti-diabetic and antioxidant activity (Zakaria et al., 2006; Zakaria, 2007).

It is indicated that, the leaves and fruits are revealed very suitable anti-diabetic properties. The previous studies exhibited that, the plant extracts shown good antioxidant and anti-diabetic activities. In observation of the above evidences, in the current work, we have conceded the phytochemical analysis and anti-diabetic action of extracts of *M. calabura* leaves through inhibition of α -amylase and α -glucosidase enzymes.

MATERIALS AND METHODS

Extracts Preparation

Identification and Authentication of Plant Material were obtained from PARK, Chennai, by Prof. P. Jayaraman. Fresh *M. calabura* Leaves were dried at Shado and powdered well utilizing a blender and put away for further use. The powdered (100 g) were taken for extraction (500 ml) with petroleum ether, chloroform, methanol and aqueous. The plant extracts were concentrated and stored in a vial for further studies.

Phytochemical screening

Preliminary phytochemical analysis, the freshly prepared crude with petroleum ether, chloroform, methanol and aqueous of the leaves were tested for the presence or absence of phytoconstituents such as reducing sugar, tannins, flavonoids, steroids, and alkaloids with standard procedures (Evans and Evans, 2003).

In vitro antidiabetic studies Inhibition of alpha-amylase enzyme

The inhibition alpha-amylase enzyme was determined by Malik and Singh (1980). Test mixture having 0.2ml of buffers (0.02 M), 20 μ l of the enzyme, and the range of plant extracts from 20-100 μ l was incubated for 10 min at room temperature followed by the addition of 200 μ l of 1% starch in all the test tubes.

Both control and plant extracts were added with starch solution and left to react with an alpha-amylase solution to the alkaline environment. Changes in the reaction were measured more than 3 min. The Production of maltose was quantified at 540 nm.

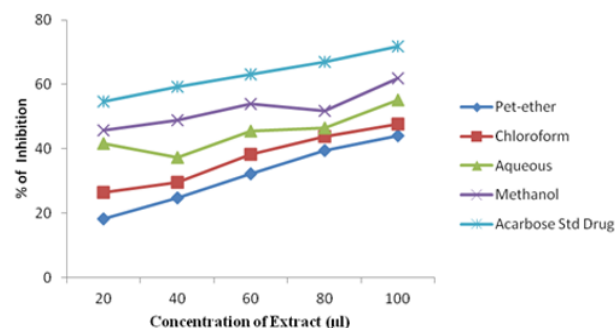


Figure 1: Alpha-amylase activity of *Muntingia Calabura*

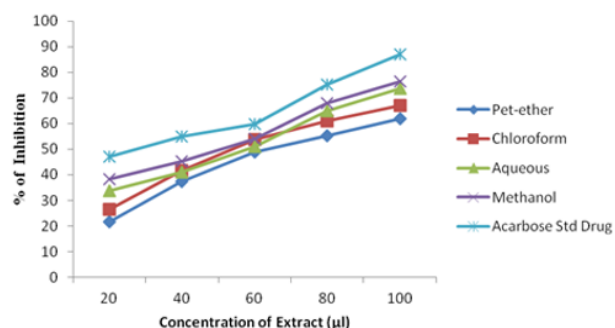


Figure 2: In vitro α -glucosidase inhibitory activity of *Muntingia calabura*

Inhibition of the alpha-glucosidase enzyme

The inhibition of alpha-glucosidase enzyme activity was determined (Krishnaveni et al., 1984). Incubating a solution to the substrate (2% w/v maltose) 1 ml with 0.2 M Tris buffer pH 8.0 and different concentrations (20-100 μ l) of plant extract were added

Table 1: Preliminary phytochemical screening of *Muntingia calabura*

S. No	Phytochemical constituents	Petroleumether	Chloroform	Methanol	Aqueous
1	Alkaloids	-	+	++	+
2	Carbohydrates	-	++	++	+++
3	Flavonoids	-	++	+	+++
4	Saponin	-	++	+	++
5	Steroids	-	+	+	++
6	Tannins	-	++	++	+++
7	Polyphenols	++	++	+++	+++

+++ = High, ++ = moderate, + = present, - = absent

incubation for 5 min at 37°C. The reaction was initiated by adding 1 ml of the alpha-glucosidase enzyme (1 U/ml) to it, followed by incubation for 40 min at 35°C. Then, the color development was measured at 540 nm.

Statistical analysis

The values were expressed as mean \pm standard error (n=5). Differences between groups were assessed by one-way analysis of variance using the Statistical Packages for the SPSS (version 16.0). Post-hoc testing was carried out for intergroup comparisons using the least significant difference test and the values of $p < 0.05$ were considered as significant statistically.

RESULTS AND DISCUSSION

Phytochemical analyses of all extract were tabulated in Table 1. The preliminary phytochemical screening of crude extracts indicated the presence of alkaloids, carbohydrates, flavonoids, saponin, steroids, tannins and phenolic compounds. The percentage yield of petroleum ether 7%, chloroform 9%, methanol 8% and aqueous 9%. In that maximum yield was found in chloroform and aqueous extract. One of these secondary metabolites, individually or in combination with others, might be reliable for the antidiabetic action of the plant.

Inhibition of alpha-amylase enzyme

In vitro α -amylase inhibitory activity of leaves (*Muntingia Calabura*) was studied as clarified in methods. We found that, there is an increase in the ratio of inhibitory activity with the rise in dosage against α -amylase. Acarbose was used as a standard drug, with related dosage to compare the inhibitory capacity of the *Muntingia Calabura* leaves using different extraction ratios of petroleum ether to water. Figure 1 showed that, the % inhibitory activity of *Muntingia Calabura* leaves ranges a minimum of 18.33 ± 0.42 (at $20 \mu\text{g/ml}$) to a maximum of 61.93 ± 0.21 (at $100 \mu\text{g/ml}$) where as the Acar-

bose showed % inhibitory activity ranges from 54.7 ± 0.18 (at $20 \mu\text{g/ml}$) to a maximum of 71.29 (at $100 \mu\text{g/ml}$).

Inhibition of the α -glucosidase enzyme

Medicinal Plant compounds are still the most accessible resource of α -glucosidase inhibitors. Therefore, we investigated biologically active compounds from *Muntingia calabura* using different extraction ratios of petroleum ether to water. Extracts under different concentrations of *Muntingia calabura* were tested for α -glucosidase inhibitory activity Figure 2.

The methanolic extract showed higher α -glucosidase inhibitory activity than aqueous extract, whereas petroleum ether and chloroform extract did show moderate inhibit α -glucosidase at all. Especially, neat alcoholic (methanolic or ethanolic) extracts exhibited stronger inhibitory effects than their corresponding aqueous mixtures.

In the current study, *Muntingia Calabura* extract was accessed the antihyperglycemic activity on in vitro enzymatic methods. The *Muntingia Calabura* showed that hypoglycemic activity at all extracts dose in contrast with acarbose.

The results presented in Figure 1 and Figure 2 indicated that *Muntingia Calabura* extract produced antihyperglycemic activity in a dose-dependent manner. At the concentration of $100 \mu\text{g/ml}$, the M.c extract indicated a significant hypoglycemic activity when compared with acarbose. This result was supported by (Ramadas et al., 2015), noted that the in vitro α -glucosidase inhibitory activity in *M. calabura* root extract (Ramadas et al., 2015). Further, the preliminary phytochemical compounds from the M.c revealed a potent inhibition of alpha-glycosidase and alpha-amylase. An earlier report has determined numerous key structural features required for single flavonoids to inhibit α -amylase and α -glucosidase activity (IOP, 2019). The antidiabetic effect is due to the presence of tannins have a significant effect against lowering inhibition activity of

α -glucosidase and alpha-amylase.

The present study demonstrated that M.c extracts effectively involved in controlling and management of hyperglycemia. In comparison with two antidiabetic enzyme activity, M.C revealed good inhibition in alpha-glucosidase than the alpha-amylase enzyme.

CONCLUSIONS

The conclusion from this investigation was demonstrated that the phytochemical analysis of *Muntingia Calabura* leaves extracts is rich in secondary metabolites. The study concluded that *Muntingia Calabura* has potent antidiabetic activity through alpha-glucosidase and alpha-amylase inhibition.

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