Anti-oxidant, Anti-diabetic and Anti-inflammatory Effect of *Muntingia calabura* (L.) Fruit Extract

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Abstract: The anti-oxidant, anti-diabetic and anti-inflammatory properties of *Muntingia calabura* fruit extracts were studied by *in vitro* models. Various solvent extraction of *Muntingia calabura* were primarily analyzed by phytochemical test, and the aqueous methanolic extract was found more potent in all the aspects of analysis. Phytochemical analysis showed the presence of phenol, flavonoids, and tannins. The fruit extract exhibited good antioxidant capacity and revealed an anti-inflammatory effect when compared to standard and the extract demonstrated anti-diabetic efficacy by enhancing glucose transport across the cell membrane in yeast cells without deleterious effects by *in vitro* model.

Keywords: Antioxidants, L6 cell line, Yeast cells, Antidiabetic, Anti-inflammatory, *In vitro*, *Muntingia calabura*


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Introduction

Universally people rely on traditional medicine for their fundamental health care requirements ever since ancient times (Gurjar et al., 2016). Plant phytochemicals are essential components of human nutrition. Phytochemicals have the ability to treat chronic diseases such as diabetes, cardiovascular diseases, and chronic inflammation-related disorders caused by oxidation and stress (Kwon et al., 2008). Stress-related illnesses cause an excess of free radicals to be produced by different external substances, and multiple endogenous metabolic processes oxidize biomolecules, resulting in tissue injury and cell death (Bhattacharyya et al., 2014). The capacity of phenolic compounds and flavonoids to scavenge free radicals is directly connected to their protective properties (Aruna Sindhe et al., 2013). Various studies support the antioxidant activity and health benefits of phenolic compounds found in fruits and vegetables. Especially, fruits have a...
variety of antioxidant compounds, including vitamin C and E, carotenoids, and phenolic compounds, which act as radical scavengers (Preethi et al., 2010). Numerous fruit extracts have demonstrated significant hypoglycemic and anti-inflammatory capabilities and antioxidant chemicals found in foods are an essential health-protecting element due to their nutritional and therapeutic benefits (Wang et al., 2013).

Local inflammation in the pancreatic islets can impair insulin production and induce apoptosis resulting in a reduction in islet mass, all of which are important events in the development of type 2 diabetes mellitus. Primary and secondary phytochemicals from fruit extracts have several hypoglycemic effects, including manipulation of glucose transporters, enhancement of insulin secretion, redevelopment of pancreatic β-cells, slow diffusion, and carbohydrate metabolizing enzymes are inhibited at the gut level, and diabetic people can benefit from a stimulating impact on glucose utilization as well as dietary adjuvants (Tiwari and Rao, 2002; Ahmed and Urooj, 2010; Sairam and Urooj, 2013; Revathi et al., 2015). Diabetes has been treated using a variety of synthetic medications, including meglitinides, biguanides, sulfonylureas, and thiazolidinediones (Ahmed et al., 2004; Anbu et al., 2012). Metformin has been shown to have anti-inflammatory effects in addition to lowering hyperglycemia and insulin resistance. Hypoglycemic medicines have anti-inflammatory benefits by either lowering hyperglycemia or directly acting on inflammatory pathways that are unrelated to glucose management however, they have certain adverse effects (Kothari et al., 2016). Currently, available medications have one or more side effects such as weight gain, stomach discomfort, diarrhea, hypoglycemia, drug resistance, and toxicity. Because the present medicines for the management of diabetes mellitus do not entirely meet our needs, the hunt for new medication still endures. As a result, managing diabetes without side effects remains a mystery and the quest continues for alternative medicines. This drives researchers to focus on natural resources with less or no side effects with increased affordability. Food supplements have become more appealing choices for preventing or treating hyperglycemia, particularly in patients with moderate hyperglycemia. Traditional foods and herbs have the ability of blood glucose management and prevent several metabolic disorders such as diabetes (Bhutkar and Bhise, 2012, 2013).

Muntingia calabura is a small tree that grows in sultry areas and belongs to the family Elaeocarpaceae. M. calabura is also known as the Jamaican Cherry tree. The plant grows all year; its fruits are berries that turn red when they mature. Jamaican Cherries are regarded as nutritional powerhouse fruits due to their low caloric content and high concentrations of anthocyanins, quercetin, carotenoids, hydroxycinnamates, potassium, fiber, melatonin, and vitamin C (Mahmood et al., 2014).

The M. calabura fruit extract contains some bioactive compounds that have hypoglycemic effects and anti-inflammatory activity without any negative side effects. The purpose of the study was aimed to examine the aqueous methanolic extract of M. calabura fruits for active phytochemicals and evaluate their antioxidant, anti-diabetic activity, and anti-inflammatory properties.

Materials and Methods

Collection of plant material:
The disease-free fruits of M. calabura were collected from various places, around Tirupattur District, Tamil Nadu, India. Collected fruit samples were identified and certified as M. calabura fruit by a taxonomist. The fruits were shade-dried, powdered, and utilized for analysis.

Preparation of fruit extract:
The soxhlet extraction technique was used to extract 25g of dry powder samples in 200 ml of solvent (180 ml of methanol and 20 ml of DW). The sample was extracted for 48 hours at room temperature and the crude sample collected was used for further analysis.
Qualitative analysis of phytochemical screening:

Screening of phytochemical constituents of *M. calabura* sample extract was assessed by standard methods described by Savithramma *et al.* (2011) and Selvaraj *et al.* (2014).

**In-vitro antioxidant activity:**

**Ferric ion reducing antioxidant power (FRAP) assay:**

Ferric ions reducing power of the fruit extract was determined by Oyaizu *et al.* (1986) with minor modifications. Aqueous methanolic fruit extract at various concentrations was mixed with 20 mmol/l phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min followed by addition of 10% TCA and 0.1% ferric chloride, which was then set aside for 10 min and the absorbance was measured at 700 nm. The assay was performed with appropriate positive controls (ascorbic acid) and negative control.

**Phosphomolybdenum assay:**

Total antioxidant activity of the aqueous methanol extracts was determined by phosphomolybdenum assay as described by Prieto *et al.* (1999). Aqueous methanolic fruits extract of *M. calabura* of various concentrations was diluted with distilled water. The dilutions of extracts were taken in the test tube, followed by the addition of 4X molybdate reagent and mixed gently. Further, the tubes were heated at 95°C for 90 min. The reaction mixture was then kept at room temperature for 20–30 min. Finally, the absorbance was measured at 695 nm. Simultaneously, 1 mM ascorbic acid was included in the assay as a positive control.

**Evaluation of in-vitro antidiabetic activity:**

**MTT-cytotoxicity assay:**

The L6 myogenic cell line was expanded in the DMEM with 10% fetal bovine serum. A day before the experiment, cells were trypsinized and seeded into the 96-well microtiter plate (1X10⁴ cells per well) and preincubated at 37°C for 24 h in 5% CO₂. After 24 h of preincubation, the spent media was flicked off without disturbing the monolayer and washed with fresh media. Cells were treated with varying concentrations of aqueous methanol extract diluted in the DMEM (with 10% FBS) for 24 h with suitable growth conditions (37°C for 24 h in 5% CO₂). After the end of treatment, 20 µl of MTT (2 mg/1 ml of MTT in PBS) was added and incubated for 4 h. Then, the supernatant was removed, and added 100 µl of DMSO (100%) to dissolve formazan crystals formed by the live cells. Absorbance was read at 570 nm using a microplate reader (Sodde *et al.*, 2015). The effect of fruit extract on the percentage of cell viability was calculated using the following formula:

\[
\text{% cell viability} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

**Glucose uptake assay:**

Glucose uptake assay was carried out by the procedure of Cirillo *et al.* (1963). The yeast, *Saccharomyces* spp., was suspended in distilled water and centrifuged three times (3000 ×g, 5 min) until clear supernatant fluids were formed and 10% (v/v) of the suspension was made in distilled water. Varying concentrations (1-7 mg/ml) of *M. calabura* fruit extract were mixed with 1 ml of glucose solution (5, 10, and 25 mM) and incubated at 37°C for 10 min. The reaction began with the addition of 100 µl of cell suspension, which was then vortexed and incubated at 37°C for 60 min. The tubes were then centrifuged (2500×g, 5 min) to quantify the amount of glucose in the supernatant. Absorbance was measured at 540 nm. The following formula was used to compute the percentage increase in glucose absorption by yeast cells:

\[
\text{Inhibition activity (\%)} = \frac{(\text{Sample absorbance} - \text{Control absorbance})}{\text{Sample absorbance}} \times 100
\]

**In-vitro anti-inflammatory activity:**

**Inhibition of albumin denaturation:**

The suppression of albumin denaturation technique proposed by Mizushima *et al.* (1968) and Sakat *et al.* (2010) was used to test the anti-inflammatory effects of *M. calabura* fruit extract. The reaction mixture was prepared with varying con...
concentrations of test extracts with a 1% albumin fraction, pH of the reaction mixture was adjusted with 1N HCl. The reaction mixture was exposed to 51°C for 20 min followed by incubation at 37°C for 20 min. Change in the reaction mixture turbidity denotes the denaturation of albumin, which was determined by recording the absorbance at 660 nm. Inhibition of protein denaturation was calculated using a formula:

\[
\text{Inhibition of protein denaturation (\%) = \left( \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \right) \times 100}
\]

**Results and Discussion**

*Qualitative phytochemical screening:*

Phytochemical screening of *M. calabura* fruit extracts revealed the presence of flavonoids, quinones, cardiac glycosides, terpenoids, phenols, steroids, coumarins, and, protein and amino acids. Flavonoids, tannins and polyphenolic chemicals present in fruit extracts have been found to offer a wide range of biological benefits, such as antioxidant activity and anti-diabetic properties (Rice-Evans *et al.*, 1996; Vinson *et al.*, 1995, Uppal *et al.*, 2012).

*In-vitro antioxidant activity:*

**Ferric ion reducing antioxidant power (FRAP) assay:**

The FRAP assay was performed on aqueous methanol extracts together with standard ascorbic acid. The reducing antioxidant power of the test sample was examined as a function of their concentration. The extract showed dose-dependent reducing power. *M. calabura* fruit extract showed higher reducing power activity (0.956 ± 0.005) compared to standard ascorbic acid (0.863 ± 0.005) though statistically not significant. Higher absorbance implies that the antioxidants have strong reducing ability. Figure 1 shows the reducing power (FRAP) of *M. calabura* increased with increasing concentration. This reductive test is based on the transformation of Fe³⁺, which is reduced to Fe²⁺ in the occurrence of a sample. Polyphenolic substances respond with free radicals by giving electrons to neutralization and converting them into more stable products. The existence of reductones, which were found to have antioxidant effects by donating electrons, is often associated with the presence of reducing characteristics (Preethi *et al.*, 2010).

**Total antioxidant capacity:**

Total antioxidant capacity was determined with phosphomolybdenum assay, which processes the reduction of metal Mo (VI) to Mo (V) by the test sample. The subsequent development of green phosphate Mo (V) complex at acidic pH, allow us to scrutinize the rate of reduction between antioxidant and molybdenum ligand. Absorbance is connected to antioxidant activity and demonstrates the ability of plant extracts to diminish metal ions. In this study, aqueous methanol extract exhibited good antioxidant activity (0.427 ± 0.01) compared to standard ascorbic acid (1.76 ± 0.03) (Fig. 2). An increased absorbance equates to an increased antioxidant ability of the fruit extract. The finding of present study implies that the extracts showed significant electron-donating ability contributing to the neutralization of antioxidant activity. Previous research has confirmed that the antioxidant activity of plant extracts is closely linked to their phenolic content (Simamora *et al.*, 2020). Bioactive components of fruit extracts have the considerable antioxidant capacity and radical-scavenging characteristics, which can contribute to anti-diabetes and anti-inflammatory benefits.

**Evaluation of in-vitro antidiabetic activity:**

**Effect of *M. calabura* on cell viability on L6 cell line:**

The cytotoxicity assays are based on the activity of mitochondrial cells and assess the number of active cells. The test technique 3-(4,5-Dimethylthiazol-2-yl) - 2, 5 - diphenyltetrazolium bromide (MTT) is reduced to form blue color formazan by mitochondrial dehydrogenases. The amount of formazan generated is related to number of viable cells (Asokan *et al.*, 2014). L6 cell line were treated with different concentrations (1.935-1000 µg) of *M. calabura* fruit extract and was assayed for their cytotoxic effect. The extract had no cytotoxic effect on the
cells. The concentrations of the extract used and the respective per cent cell viability (Fig. 3). The lowest concentration of *M. calabura* (1.935 µg) showed 94.2% viability and the highest concentration (1000 µg) showed 6.5% of viability after 24 h of exposure. The IC<sub>50</sub> value was found to be 105.6 µg. Thus, the result indicated that aqueous methanol extract of *M. calabura* fruit is not toxic to cells even at higher concentrations and could be used to analyze other parameters of *in vitro* and *in vivo* antidiabetic studies.

*Glucose uptake assay*:
The maintenance of plasma glucose concentration for a longer duration under varied dietary circumstances is one of the most essential and
Fig. 3: MTT based cytotoxicity assay. Normalized percentage of viability vs concentration of *M. calabura* fruit extract was analyzed with four PL variable slope.

Fig. 4: Glucose uptake by yeast cell. Glucose uptake percentage vs Concentration of *M. calabura* was analyzed with four PL variable slope.

carefully controlled processes identified in mammalian species (Ammayappan *et al.*, 2012). particularly type II diabetes, which is characterized by insulin deficiency causing an increase in blood glucose level and is dependent on glucose uptake by cells (Shori, 2015). Transport of glucose in yeast cells (*Saccharomyces cerevisiae*) has been discovered to be exceedingly intricate and generally, it is considered that glucose is transferred in yeast by facilitated diffusion. Facilitated transporters are specialized carriers that transport solutes across a gradient of concentration, with the emphasis on the fact that efficient transport is only possible if intracellular glucose is eliminated (Revathi and Ponniah, 2016). As a result, glucose transfer happens only when internal glucose levels are significantly reduced (utilized). The percentage of glucose absorption by
the extract in yeast cells was compared to different quantities of glucose solution (5, 10, and 25 mM). As the quantity of *M. calabura* extract increased, the percentage of glucose absorption increased in a dose-dependent manner (Fig. 4). The percentage improvement in glucose absorption by yeast cells was shown to be indirectly linked to glucose concentration and decreased the molar concentration of the enhanced glucose solution. The results indicated that the fruit extract improved glucose transport across the yeast cells. *M. calabura* fruit extract showed considerable moderate activity in all tested concentrations and confirmed that large amounts of extracts have substantial glucose absorption.

**In-vitro anti-inflammatory activity:**

**Inhibition of albumin denaturation:**

Protein denaturation is the source of inflammation. Denaturation of albumin protection contributes to anti-inflammatory action. As a reagent, BSA was utilized in the experiment and when exposed to heat, BSA becomes denatured. *M. calabura* fruit extract was tested for anti-inflammatory effects on protein denaturation. The *in vitro* anti-inflammatory efficacy of the extract was equivalent to that of a reference medication, diclofenac sodium. The standard drug IC\textsubscript{50} value was found to be 614.4µg and the fruit extract was found to be 1079 µg (Fig. 5). The impact of different concentrations of fruit extract prevented heat-induced BSA denaturation. The anti-inflammatory outcome may be due to the presence of terpenoids, flavonoids, tannins, steroid and, alkaloids in the *M. calabura* extracts (Rajesh *et al.*, 2019).

**Conclusion**

The present study clearly showed the biological properties of *M. calabura* fruit extract. The extract was endowed with antioxidant potential, anti-inflammatory properties and anti-hyperglycemic activity, which could be due to the presence of its active constituents. The findings of this study showed that *M. calabura* fruit had no cytotoxic effect on the cells. So, it can be utilized medicinally to prevent the onset and progression of a variety of illnesses especially, diabetes. More comprehensive research is needed to establish
their effectiveness as an adjuvant in the efficient management of diabetes mellitus using in vivo models and clinical studies.

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References


733


