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Effect of *Muntingia calabura* L. fruit and leaf extracts on hypoglycemia and hypocholesterolemia indices in albino Wistar rats

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Abstract

A pioneer species, the Singapore or Jamaican cherry tree (*Muntingia calabura* L.; Family - Muntingiaceae) thrives best in open ground, is accustomed to poor soil and can with stand acidic, alkaline and dry conditions. The influence of fruit and leaf extracts on blood sugar and lipid was examined in an animal model. According to the results, normal control rats showed no significant changes in their blood parameters during the study period. Over a 28 day period, the diabetic control rats had reduced body weight or elevated blood glucose levels. *M. calabura* fruit and leaf extracts allowed diabetic-induced rats to maintain body weight and maintain near normal blood glucose levels (fruits (F)-156.48 ± 4.72 mg/dl; leaves extract (LE)-46.40 ± 7.26 mg/dl). The level of total hemoglobin (F-12.40 g/dl; LE-12.65 g/dl), glycosylated hemoglobin (F-0.50%; LE-0.48%) and plasma insulin (F-30.82 µU/ml; LE-29.90 µU/ml) in comparison to the toxic control, diabetic rats treated with *M. calabura* fruits or leaves extracts experienced a return to a near normal range. Compared to streptozotocin induced diabetic control rats, diabetic rats cured with extracts from fruits and leaves of *M. calabura* displayed significant reductions of triglycerides, total and LDL cholesterol, phospholipids but increase in highdensity lipoprotein. The liver glycogen content of the treatment control was reduced by 79.89%. Treatment with glipizide and *M. calabura* fruit and leaf extracts increased liver glycogen levels by 74.47%, 66.05% and 68.79% respectively.

1. Introduction

Muntingia calabura L. also known as the singapore cherry, is the only species in the genus *Muntingia* is a flowering plant. Parts of the tree have several documented medicinal uses, meaning they possess antiproliferative, antioxidant, antinociceptive, cardioprotective and antipyretic effects (Zakaria *et al*., 2011; Siddiqua *et al*., 2010 and Preethi *et al*., 2010). The native of the tree is Mexico to South Tropical America (POWO, 2024) cultivated widely in tropical and subtropical regions of the world (Fathima and Bhat, 2025). It thrives best in hot and humid low lands and stands in poor soils, alkaline and acidic environments but eludes saline conditions. It has wider environmental uses as medicine, food, animal food and fuel. In India, the tree is scattered in the states like Tamil Nadu, Kerala, Karnataka, Uttar Pradesh, Maharastra and Gujarat (Rao and Kumar, 2024; Chaurasiya *et al*., 2024).

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The fruits are round, have smooth, tender, red skin, juicy pulp with a unique flavor and sweet taste, packed with tiny, yellowish seeds. The fruits have good nutritional quality (Einbond *et al*., 2004) and have many health benefits. They are eaten by children due to their sweet taste and also used to prepare tart and jam (Aruna *et al*., 2013). It was hypothesized that the fruits and leaves would reduce blood cholesterol and glucose levels in the human body. Upadhye *et al*. (2021) stated the proven pharmacological activities like antihypertensive, antidiabetic, antioxidant, antibacterial, antiinflammatory and cardioprotective activity of *M. calabura.*

Although the fruits are unique in taste, they are underutilized, unfamiliar and the toxicity research was conducted on *M. calabura* fruits or leaves and reported by different authors. Sridhar *et al*. (2011) found that up to a dose of 2000 mg/kg methanol extract of *M. calabura* leaf extract displayed no signs of toxicity and no mortality was recorded. Ibrahim *et al.* (2012) also reported that ethanol extract of leaves (EEMCL) has been administered orally at doses of 2000 and 5000 mg/kg to animals for up to fourteen days and observed that there was no mortality and no visible clinical signs after extract administration, except for general weakness of the animals.

Diabetes mellitus is a metabolic disorder in the body fails to produce and utilize insulin effectively. It results in complications involving micro angiopathy, retinopathy, nephropathy as well as disturbances in protein, carbohydrate and lipid metabolism. The World Health Organization (WHO) approximates that 422 million people globally are affected by diabetes with the majority residing in low or middle income countries. Diabetes is primarily responsible for 1.5 million deaths annually. Before the age of 70, nearly half of all fatalities associated with elevated blood glucose levels occur. It is estimated by the World Health Organization that diabetes was the seventh most common cause of mortality in 2016. Similarly, 17.9 million lives per year are lost globally due to cardiovascular diseases (WHO, 2024). CVDs are a cluster of conditions of blood vessels or the heart, which involve cerebrovascular disease, rheumatic heart disease, coronary heart disease, *etc*.

Complications of those noncommunicable diseases and their cost of management are increasing over time. Though, there are medicines available to manage these conditions, people are interested in natural remedies. Therefore, a study was conducted on the hypoglycemic and hypocholesterolemic effects of *M. calabura* fruits and leaves in an animal model.

2. Materials and Methods

2.1 Plant materials

Muntingia calabura L. leaves and fruits were collected from the college orchard, Agricultural College and Research Institute, Madurai (Figure 1). The plant was authenticated and deposited (Voucher Number: SI/SC/5/23/0910/Tech132) at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The leaves were cleaned, washed, shade dried and ground into powder. Leaf extract was prepared with two per cent leaf powder in aqueous solution, boiled and filtered. The fruits were used as such without further processing and chemicals used in the study were of analytical grade.

Figure 1: *Muntingia calabura* L. leaves and fruits.

2.2 Animals

The experiment has been performed at KM College of Pharmacy, Madurai, during the year 2019 (IAEC proposal no. IAEC/E. Tamilselvi/Ph.D/TNAU/004KMCP/75/2019). In the present study, male albino wistar rats weighing 180 to 220 g have been utilized for animal experiments. The animal experimental paradigm was chosen to satisfy the following criteria, *viz.,* animals develop hyperglycemia rapidly, undergo pathological changes at the site of induction that outcome in pancreatitis as well as damage to β-cells and that signs be improved by effective drug treatment. In addition to being fed commercial pellets or given unlimited access to water *ad libitum* animals have been kept in spacious cages. Over the course of the trial, animals were acclimated to the typical 12 h light dark cycles, temperature (22 ± 5°C) and humidity (55 ± 5%). Due to streptozotocins capacity to kill pancreatic β-cells, it was used to cause diabetic mellitus in rats (S.D. Fine. Chem. Ltd, Mumbai).

2.3 Experimental methodology

2.3.1 Induction of diabetes mellitus

After 12 h single intraperitoneal injection of newly made streptozotocin solution (25 mg/kg BW) in physiological saline has been utilized to develop diabetes mellitus in wistar rats (Al-Shamoany *et al*., 1994). Development of hyperglycemia in rat was verified by measuring their plasma glucose 72 h after receiving a streptozotocin injection. For this investigation, rats having fasting plasma glucose levels between 180 and 220 mg/dl were employed. Out of a total of 30 rats, 24 diabetic induced rats and six normal rats have been employed in the experiment with six rats per group. Three days before to start of the experiment, rats were given diabetes. The treatment protocol is given in Table 1.

2.3.2 Biochemical analysis

The biochemical values like the plasma insulin, haemoglobin, blood glucose, glycosylated haemoglobin, glycogen content, body weight, lipid profiles were determined initially and after 28 days of treatment. For the purpose of estimating blood parameters, blood has been extracted from eyes (venous pool) *via* sino-ocular puncture (Waynforth, 1980) in plasma tubes coated with EDTA. A commercially available glucose kit (one touch ultra) from johnson and johnson was used to estimate blood glucose using the glucose oxidase method (Trinder, 1969). By utilizing an ES300 Boehringer analyzer (Mannheim, Germany) and Boehringer-Mannheim kit (Anderson *et al*., 1993), plasma insulin has been measured by the ELISA technique. Drabkin and Austins (1932) method was used to measure total hemoglobin, whereas Sudhakar Nayak and Pattabiramans (1981) approach was used to measure glycosylated hemoglobin.Total cholesterol (Parkeh and Jung, 1970), HDL cholesterol (Gidez and Webb, 1950), phospholipids (Zilversmith and Davis, 1950) and triglycerides (Rice, 1970) were measured using an auto analyzer. Glycogen content has been determined colorimetrically (Morales *et al*., 1973).

Table 1: Treatment protocol

Experimental groups	Treatments
Group 1	Normal control consists of healthy rats given with 10 ml/kg of normal saline, orally.
Group 2	Toxic control consists of diabetes induced rats dosed with 25 mg/kg of streptozotocin through I.P.
Group 3	Diabetes induced rats treated with glipizide at a dose of 10 mg/kg orally for 28 days.
Group 4	Diabetes induced rats treated with *M. calabura* fruit at a dose of 100 mg/kg orally for 28 days.
Group 5	Diabetes induced rats treated with leaf extract of *M. calabura* at a dose of 2.5 ml/kg orally for 28 days.

2.4 Statistical analysis

ANOVA (analysis of variance) has been used to examine the biochemical parameter data and NKMRT (Newman-Keuls Multiple Range test) has been utilized to compare group means. At $p<0.01$, values have been deemed statistically significant.

3. Results

Heart disease and diabetes are dangerous, complex conditions that require consistent self-care. If issues develop, they can drastically reduce life expectancy and quality of life. Diabetes cannot be permanently cured but by managing it correctly one can led to happy life (Suchitra *et al.,* 2023). Hyperlipidemia is clinically confirmed by low levels of high levels low density lipoprotein (LDL), high level density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG) in the blood. Antihyperglycemic and antihyperlipidemic drugs are available now, but they may have adverse effects. *M. calabura* is one of the medicinal plants with antihyperglycemic and antihyperlipidemic effects. The hypoglycaemic and hypocholesterolaemic effects of *M. calabura* fruits and leaves were analysed through biochemical analysis and the results are given in Figure 2 and Tables 2, 3 and 4.

Table 2: Effect of *M. calabura* fruits and leaves extract on body weight and blood glucose in normal and treated animals

Treatment groups	Body weight (g)		Blood glucose (mg/100 ml)	
	Initial	Final	Initial	Final
Group 1	242 ± 6.20*	245 ± 6.25	90.68 ± 4.45	92.90 ± 3.25
Group 2	230 ± 5.65	175[a] ± 7.30**	89.30 ± 3.75	220.40[a] ± 5.80**
Group 3	230 ± 7.50	240 ± 7.35	90.70 ± 4.35	126.55[b] ± 4.35*
Group 4	232 ± 7.30	246 ± 7.45	88.80 ± 3.75	146.40[b] ± 7.26**
Group 5	238 ± 7.40	242 ± 7.48	90.45 ± 3.90	156.48[b] ± 4.72**

*Mean ± SEM is used to express values; ANOVA (analysis of variance) or Newman-Keul's Multiple Range tests have been used to compare values; [a] and **At $p<0.001$, values differ considerably from normal control Group 1; [b] and ** At $p<0.001$, values differ considerably from diabetic control Group 2.

3.1 Hypoglycemic effect of *M. calabura* fruits and leaves

3.1.1 Body weight and blood glucose

One of the most significant clinical indicators of the prevalence of diabetes mellitus in people is body weight. In the current investigation, diabetic rats (Group 2) had a considerably lower body weight (Table 2) than normal control rats. However, when compared to normal control animals, the body weight of diabetic control rats treated with extracts of *M. calabura* fruits or leaves at doses of 100 mg/kg or 2.5 ml/kg, respectively, was elevated but the rise was not statistically significant.

Rats with diabetes had a considerably higher fasting blood glucose level than normal rats, measuring 220.40 ± 5.80. However, diabetic rats given extract of *M. calabura* fruits and leaves at doses of 100 mg/kg and 2.5 ml/kg, respectively. Shown a return to the near normal range of fasting blood glucose indicating that they have a beneficial effect on lowering blood glucose levels.

Additionally, according to Zolkeflee *et al.* (2022) freeze dried *M. calabura* leaves that were extracted with 50% ethanol showed strong inhibitory effects on α-glucosidase or α-amylase with IC_{50} values of 0.46 ± 0.05 or 26.39 ± 3.93 µg/ml correspondingly. Using UHPLC-ESI-MS/MS, they also discovered sixty one bioactive chemicals including daidzein, geniposide, 6-hydroxyflavanone, quercitrin, kaempferoland formononetin, which may be used to treat diabetes.

Suchitra *et al.* (2023) analyzed in *in vitro* antidiabetic activity in *M. calabura* ethanolic extracts and the results revealed that 53% in the ethanolic extract, which exhibited substantial action. When compared to acarbose, ethanolic flower extracts of *M. calabura* significantly inhibited the activity of the enzyme α-amylase. It could be as a result of the ethanol and hexane extracts having higher concentrations of chemical components such as lignans (phyllanthus and hypophyllanthin), terpenes, tripenes, flavonoids (quercetin and rutin), alkaloids and flavonoids.

Solikhah and Solikhah (2021) evaluated the consequence of *M. calabura* leaf extract on blood glucose level or weight of alloxan induced diabetic mice or outcomes revealed significant reduction in blood glucose level or increase of weight on 7th and 14th day in mice treated with *M. calabura* leaf extract (100 or 300 mg/kg) and glibenclamide (600 µg/kg) compared to model control group. The study recommended that *M. calabura* leaf extract have significant antidiabetic results besides controlling the weight of alloxan induced diabetic mice.

Table 3: Effect of *M. calabura* fruits and leaves extract on plasma insulin, haemoglobin and glycosylated haemoglobin in normal and treated animals

Treatment groups	Plasma insulin (µU/ml)	Hemoglobin (g/100ml)	Glycosylated hemoglobin HbA_1 (%)	Liver tissue glycogen content (mg/g tissue)
Group 1	33.30 ± 2.80	13.95 ± 1.40*	0.45 ± 0.06	44.32 ± 3.55*
Group 2	13.65^a ± 1.65**	6.55^a ± 0.50**	0.92^a ± 0.15**	12.25^a ± 0.75*
Group 3	32.40^b ± 2.40**	14.35^b ± 1.65**	0.46^b ± 0.07**	36.52^b ± 1.80*
Group 4	30.82^b ± 2.65**	12.40^b ± 0.90**	0.50^b ± 0.09**	28.40^b ± 1.32*
Group 5	29.90^b ± 2.60**	12.65^b ± 1.36**	0.48^b ± 0.05**	30.65^b ± 1.52*

*Mean ± SEM is used to express values; ANOVA or Newman-Keul's Multiple Range tests have been used to compare the values; ^{a} and ** at $p<0.001$, values differ considerably from normal control Group 1; ^{b} and ** at $p<0.001$, values differ considerably from diabetic control Group 2.

3.1.2 Plasma insulin, haemoglobin, glycosylated haemoglobin and liver tissue glycogen content

The Table 3 shows the levels of total glycosylated haemoglobin, haemoglobin, plasma insulin, liver tissue glycogen content in normal or treatment control animals in every group. Findings showed a significant drop in both plasma insulin levels and total hemoglobin levels. In contrast, rats in the toxic control had far higher amounts of glycosylated hemoglobin than rats in the normal control. However, diabetic rats given extract of *M. calabura* fruits or leaves at doses of 100 mg/kg or 2.5 ml/kg, respectively, shown a return to nearly normal levels of glycosylated hemoglobin, total hemoglobin and plasma insulin. This could be because the extracts improved glucose metabolism.

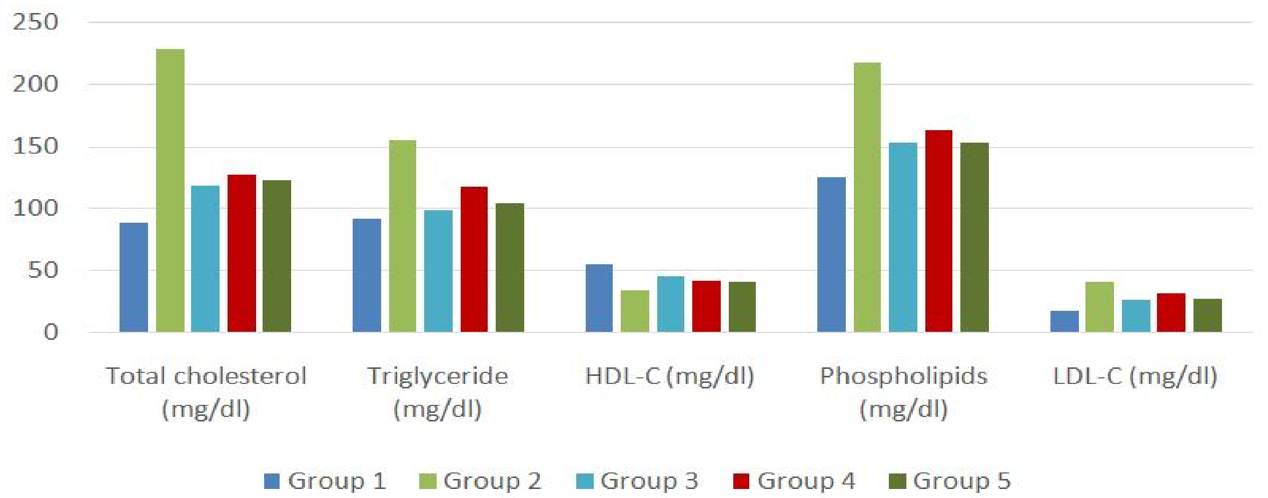
On the 28th day, the amount of glycogen in each group's liver tissue was calculated and is displayed in Table 3. When comparing the diabetic control rat to the non-diabetic control, liver glycogen content dropped by 79.89%. Treatment with glipizide, *M. calabura* fruits and leaf extracts at a dose of 100 mg/kg or 2.5 ml/kg led to an increase in liver glycogen content to 74.47%, 66.05% and 68.79% correspondingly, when compared to diabetic control, which means fruits and leaves of *M. calabura* have positive glucose metabolism.

The antidiabetic effect of *M. calabura* might be due to the presence and mechanisms of bioactive compounds like phenolics, triterpenoids, tannins, alkaloids and flavonoids.

The impact of *M. calabura* leaves on the hematological condition of *Claria* ssp. Fish was investigated by Febrianti (2021). Commercial feed and egg white were combined with *M. calabura* leaf powder to create the feed, which was given three times a day for two weeks at dosages of 0 g/kg (KN), 5 g/kg (M_1), 20 g/kg (M_3), 10 g/kg (M_2), 40 g/kg (M_4). Findings demonstrated that during treatments, there was a change in the levels of hematocrit, hemoglobin, RBC and total white blood cells (WBC).The highest WBC, RBC, haemoglobin and hematocrit levels observed in treatment M_2 (169 ± 21.378 x 10 w cells ml^{-1}), M_1 (365 ± 118.216 x 10 w cells ml^{-1}), M_3 (8.60 ± 0.265 g dl^{-1}) and treatment M_4 (65.333 ± 9.609%), correspondingly.

3.2 Hypocholesterolemic effect of *M. calabura* fruits and leaves

Lipid profile includes triglycerides, total cholesterol, phospholipid, HDL and LDL in the blood. High level of all parameters except HDL is stated as hyperlipidemia, which affects human health. There are drugs available for the treatment of hyperlipidemia but they may have adverse effects.



Group 1-normal control; Group 2-diabetic control; Group 3-diabetic rats received glipizide;Group 4-diabetic rats received *M. calabura* fruits and Group 5-diabetic rats received *M. calabura* leaves

Figure 2: Effect of *M. calabura* fruits and leaves on lipid profile of normal and experimental groups.

Triglycerides, total cholesterol, LDL, HDL and phospholipid levels in serum of both normal and experimental animals are displayed in Figure 2. As compared to normal rats, streptozotocin induced diabetic rats had considerably higher levels of total cholesterol, high density lipoprotein, LDL, triglycerides and phospholipids, while their levels of HDL-C were lower. When singapore cherry fruit and leaf extracts have been administered to normal or streptozotocin induced diabetic rats for 28 days at doses of 100 mg/kg and 2.5 ml/kg, respectively. triglycerides, LDL, phospholipids and total cholesterol significantly decreased, while HDL increased in comparison to streptozotocin induced diabetic rats. From this study, it is clearly noted that the fruits and leaves of *M. calabura* had a very good role in the elevation of good cholesterol and the reduction of bad cholesterol. The hypocholesterolemic effect of *M. calabura* might be due to the synergistic action of phytochemicals like phytol, saponins, flavonoids, triterpenoids, *etc*.

In addition, Solikhah and Solikhah (2023) found that giving mice with diabetes glibenclamide or *M. calabura* fruit may significantly lower their total cholesterol, triglycerides or low-density lipoprotein while raising their high-density lipoprotein levels in comparison to the diabetes control group ($p<0.05$). Additionally, granulation and cell density of pancreatic langerhans cells are greatly increased in mice with *M. calabura* fruit extract.This leads to enhancements in the lipid profile or the regeneration of pancreatic langerhans cells as well as the possibility of an antihyperlipidemic medication.

4. Discussion

Metabolic diseases are important public health threatening problems due to the high incidence, disability and mortality. Overweight, obesity, type 2 diabetes mellitus, hypertension and dyslipidaemia are the most prevalent and have a high social and economic cost for the patient, the family and the health systems. Diabetes is a complex and multifactorial metabolic disease, characterized by the abnormal and chronic increase in blood glucose levels (hyperglycemia) and lipid metabolism disorders are associated with alterations in the concentrations of triglycerides, phospholipids, total cholesterol, HDL and LDL which leads to cardiovascular diseases. There are different treatment alternatives, lifestyle changes to control or reverse these conditions. Despite the presence of identified antidiabetic medicine in the pharmaceutical market several traditional plant based treatments are used throughout the world and there is an increasing demand by patients to use the natural products with hypoglycemic and hypolipidemic activity. Phytochemicals are also having the promising role in counteracting those diseases. Plants with medicinal properties in its roots, bark, leaves, flowers, fruits are used in healthcare systems from ancient civilizations in India to treat ailments and improving human wellbeing. These plants are pools of bioactive compounds with alkaloids, terpenes and flavonoids that address various health challenges ranging from minor infections to chronic illnesses.

In the present investigation, the hypoglycemic and hypo cholesterolemic activities of *M. calabura* fruit and leaf extracts were explored. The *M. calabura* tree is a pioneer species that thrives best in open country adapted to poor soil and able to withstand acidic, alkaline and drought conditions. The results revealed that consuming fruit and leaf extract has a positive effect on blood glucose and cholesterol levels in the animal model. Thirty numbers of male albino wistar rats weighing 180 to 220 g were divided into five groups and each group receives different treatments including diabetic control (diabetes induced through giving streptozotocin), Diabetes induced rats treated with glipizide, diabetes induced rats treated with *M. calabura* fruits at a dose of 100 mg/kg, diabetes induced rats treated with *M. calabura* leaves extract at a dose of 2.5 ml/kg besides normal control (10 ml/kg of normal saline given orally) for 28 days. The body weight of animals and blood profiles were assessed to determine the effect of different treatments.

The results revealed that no significant changes in blood parameters were observed in the normal control rats during the study period. Whereas the diabetic control rats have reduced body weight and increased blood glucose value after 28-day period. Similar to the normal control rats, the diabetic induced rats treated with *M. calabura* fruits and leaves extract maintained the body weight and the blood glucose values returned to near normal range in fruits (F) 156.48 ± 4.72 mg/dl and leaves extract (LE) in 146.40 ± 7.26 mg/dl. The level of total hemoglobin (F-12.40 g; LE-12.65 g), glycosylated hemoglobin (F-0.50%; LE-0.48%) and plasma insulin (F-30.82 µU/ml; LE-29.90 µU/ml) returned to near normal range in diabetic rats treated with *M. calabura* fruits and leaves extracts as compared to diabetic control. Treatment of diabetic rats with *M. calabura* fruits and leaves extracts resulted in a marked decrease in total cholesterol, triglycerides, low density lipoprotein (LDL), phospholipids levels and an increase in high density lipoproteins (HDL) levels as compared to diabetic control rats. In diabetic control, rat liver glycogen content decreased significantly by 79.89% as compared to the normal control. Treatment with glipizide *M. calabura* fruits and leaves extracts led to 74.47%, 66.05% and 68.79% increase in liver glycogen content in comparison to diabetic control. From this study, it is suggested that the fruits and leaves of *M. calabura* have hypoglycemic and hypocholesterolemic effects.

5. Conclusion

The *M. calabura* tree is very common in nurseries, streets, residential areas and it is used as a shade tree on roadsides. Since fruits are an outstanding source of vitamin C, carotene or other bioactive compounds, they could be considered as a poor man's source of the vital nutrients. Also, the fruits and leaves of the plant were reported to possess antiproliferative, antinociceptive, antioxidant, cardio-protective and antipyretic effects. From this study, it was found that the *M. calabura* fruits and leaves have hypoglycaemic and hypocholesterolemic effects. The findings may further be validated and the consumption of *M. calabura* fruits and leaves could be encouraged to reap the health benefits.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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