

<https://doi.org/10.33472/AFJBS.6.8.2024.2321-2334>



African Journal of Biological Sciences



Research Paper

Open Access

Exploring Antioxidant and Antidiabetic Potential of *Muntingia calabura* Fruit Extract: *In Vitro* Analysis

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Article History

Volume 6, Issue 8, 2024
Received: 15 Apr 2024
Accepted: 29 May 2024
doi: 10.33472/AFJBS.6.8.2024.2321-2334

Abstract

Objective: The aim of our present study was to explore the Antioxidant and Antidiabetic potential of *Muntingia calabura* fruit extract by *in vitro* analysis.

Methods: *Muntingia calabura* fruits were clean dried, powered, sieved, soaked and kept for 2hr in ethanol and simultaneously aqueous extract was prepared. The samples were filtered and utilized for further analysis. Ferric reducing antioxidant power (FRAP) and nitric oxide radical scavenging activity was assayed to assess the antioxidant activity of *Muntingia calabura* fruit extract. In addition to assess the Antidiabetic activity, α -amylase and α -glucosidase inhibitory activity was measured, hydrolysis index and glycemic index were also assessed.

Results: The aqueous and ethanolic extract of *Muntingia calabura* fruit extract shows a very good free radical scavenging activity in a dose dependent manner. In addition to this the extracts were showed a gradual increase in the inhibition of both α -amylase and α -glucosidase in a dose dependent manner. It also showed a low glycemic index, hence it may play a vital role in preventing or managing diabetes mellitus.

Keywords: *Muntingia calabura*, antioxidant, Antidiabetic, glycemic index, *in vitro*

1. Introduction

Muntingia calabura is referred to as "Jamaican cherry" everywhere in the world; in Malaysia, especially among the Malay population, it is called "kerukup siam." It is the only species in the genus *Muntingia*. It is also extensively grown in warm regions of India and Southeast Asia, including Malaysia, Indonesia, and Philippines. In fact, *M. Calabura* are frequently grown as roadside trees in Malaysia.^{1,2,3} Throughout the world, medicinal plants are extensively dispersed and constitute a significant part of the flora. One well-established technique for identifying lead compounds that may lead to the creation of innovative and secure therapeutic medicines is the pharmacological examination of chemicals derived from plants. Medicinal plants are made up of certain organic compounds known as phytochemicals for short, which have specific physiological effects on the human body. These bioactive substances include tannins, alkaloids, terpenoids, steroids, and flavonoids, and others. A thorough study of medicinal plants is necessary for the creation of pharmaceutical goods in order to increase our understanding of their biological activities and the phytoconstituents that underpin them^{4,5}. Moreover, the paucity of thorough scientific examinations in this field is further evidenced by the fact that only a small number of species of medicinal plants have been thoroughly examined⁶.

Hyperglycemia and impaired glucose metabolism are hallmarks of type 2 diabetes (T2D), which is a major global source of morbidity and mortality as well as a significant economic burden⁷. The International Diabetes Federation estimates that 382 million people worldwide suffered from diabetes in 2013, and that figure is projected to double by 2035⁸. The majority of these newly diagnosed cases of diabetes (>95%) are type 2 diabetes (T2D), which is brought on by insulin resistance and malfunctioning pancreatic β -cells, which results in hyperglycemia⁹. It has been discovered that the development of T2D¹⁰ problems and their onset are significantly influenced by postprandial blood glucose levels. The World Health Organization predicts that by 2045, there will be 700 million more people living with diabetes. Inhibiting α -amylase and α -glucosidase is one treatment method for postprandial hyperglycemia; however, it might cause flatulence, vomiting, diarrhoea, and distention of the abdomen¹¹. Additionally, excessive non-enzymatic glycation of proteins and the production of advanced glycation end products (AGE) can be caused by hyperglycemia. By causing nephropathy, cataracts, vasculopathy, and atherosclerosis, the glycation changes can exacerbate the pathophysiology of diabetes¹². According to several reviews and research, phytochemicals such phenolics have the potential to be therapeutically beneficial in reducing the complications of diabetes and obesity as well as having inhibitory effects on α -amylase and α -glucosidase^{13,14,15,16,17}. Plants that include antioxidant chemicals have the ability to shield β -cells against reactive oxygen species (ROS), hence mitigating the risk of diabetes caused by ROS¹⁸. Thus, it is important to investigate the traditional medicinal plants that can be used to lower biomarkers associated with obesity as well as blood glucose levels.

2. Materials & Methods

2.1. Sample Collection and processing for extract preparation

The fruit, *Muntingia calabura*, was purchased from Thiruvottiyur, Chennai, and neighborhood. Fruits were washed and sun-dried. Each dried fruit was ground into a powder before being sieved. The fine powder was sieved and then put into a sanitised container. To extract the readily soluble bioactive components, 50% ethanol is then added to the fine powder, allowed to soak, and left for two hours. Water was used to create an aqueous extract simultaneously. Next, grade 1 Whatman paper was used to filter the samples. They were preserved and used for additional research. 2% extract was made.

2.2. Ferric reducing antioxidant power assay

The antioxidant capacity of the extract was estimated spectrophotometrically following the procedure of Benzie and Strain¹⁹. The method is based on the reduction of Fe³⁺ TPTZ complex (colorless complex) to Fe²⁺-tripirydyltriazine (blue colored complex) formed by the action of electron donating antioxidants at low pH. This reaction is monitored by measuring the change in absorbance at 593 nm.

The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml TPTZ in 40 mM HCl and 20 mM FeCl₃.6H₂O in the proportion of 10:1:1 at 37°. Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995 ml) and mixed with 5 µl of the appropriately diluted plant sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe³⁺ TPTZ) complex was reduced to ferrous (Fe²⁺) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent 5 µl distilled water) after 30 min incubation at 37°. All the determinations were performed in triplicates.

2.3. Determination of Nitric oxide radical scavenging activity

The Griess-Ilosvay reaction was used to measure the extract's ability to scavenge nitric oxide radicals using sodium nitroprusside²⁰.

In a typical experiment, 0.5 mL of samples, 0.5 mL of phosphate buffer (pH-7.4), and 2 mL of sodium nitroprusside (10 mM) were added to the reaction mixture. Vitamin C (standard) was then added, and the mixture was incubated for 150 minutes at 25 °C. Following the incubation period, 1 mL of sulfanilic acid reagent (0.33% sulfanilic acid in 2% glacial acetic acid) was added to 0.5 mL of nitrite and left for five minutes. After adding 1 mL of 1% naphthyl ethylene diamine dihydrochloride (NEDD), the mixture was let to stand at 25 °C for 30 minutes. At 540 nm, the solution's pink colour absorption was measured.

The following formula was used to get the proportion of nitric oxide inhibition:
Nitric oxide radical scavenging assay percentage (%) = $[(A_0 - A_1) / A_0] \times 100$, where A₀ is the control sample's absorbance and A₁ is the treatment sample's absorbance.

2.4. Estimation of Iron

2.4.1. Working standard preparation

Started by making the usual solutions (0.001 M Fe (NO₃)₃) and adding the right amounts to five test tubes. Using a stirring rod, the tubes were completely combined. After that, each test tube received 2.5 mL of 0.1 M KSCN, which was thoroughly mixed.

2.4.2. Preparation of the Food Samples

Around 2.5 g of the solid food were weighed out and put in a crucible. The food sample was then cooked in the crucible over a high burner flame until it turned to ash. This should take five to twenty minutes, depending on the type of food sample that is used. After taking the crucible off the flame, the ash was allowed to cool. Ash was moved to a little beaker once it had cooled. After that, add 10 mL of 2.0 M HCl, and stir thoroughly for a minute. 10 mL of distilled water were added. After thorough stirring and filtering, 2.5 mL of 0.1 M KSCN was added and well mixed with the filtrate. The content of iron in the rice samples was then calculated by plotting the absorbance of the standard and sample solutions against the concentration of 0.001M Fe (NO₃)₃ at a wavelength of 458 nm.

2.5. Estimation of Calcium content

2.5.1. Ashing and extraction

Weigh the item precisely three grams into a silica dish. Till the ash is white, or nearly so, gently char and continue the ashing in a muffle furnace at a temperature not to exceed 450°C. After cooling the ash, add 3 to 5 millilitres of strong hydrochloric acid drop by drop while moistening with a few millilitres of distilled water. In order to make silica insoluble, evaporate to dryness on a water bath and keep heating for an hour. After soaking the residue in 20 millilitres of distilled water, add two to three millilitres of strong hydrochloric acid. After a few minutes of heating on a water bath, pour the mixture through medium filter paper into a 250 ml volumetric flask. After thoroughly washing the filter paper in hot water, letting the filtrate cool and adding volume, give it a good shake.

Estimation: A 25 ml aliquot of the solution made according to the ashing technique should be transferred to a 400 ml beaker, diluted with water to make around 100 ml, and then two drops of methyl red indicator solution should be added. Drop by drop, add ammonium hydroxide solution until the mixture turns brownish-orange (pH 5.6). Two drops of hydrochloric acid (pH 2.5 to 3.0) should be added so that the solution turns pink. Dilute to roughly 150 ml, bring to a boil, and then gradually add 10 ml of hot ammonium oxalate solution while stirring constantly. Should the solution's red hue shift to orange or yellow, gradually add hydrochloric acid drops until the colour returns to pink. Give the precipitate time to settle by leaving it overnight. Use ash-free filter paper to strain the liquid supernatant, and then thoroughly wash the precipitate with a diluted ammonium hydroxide solution. After placing the paper with the precipitate in the beaker used for the precipitation, fill it with 125 millilitres of water and 5 millilitres of strong sulfuric acid. Heat the mixture to between 70 and 90 degrees Celsius, then titrate it using the usual potassium permanganate solution until the first hint of pink colour appears. Next, the calcium content was

calculated in milligrammes per kilogramme dry weight using the volume of potassium permanganate consumed and the potassium permanganate's normalcy.

2.6. Antidiabetic activity assay

2.6.1. *In Vitro* α -Amylase inhibitory activity

The procedure described²¹ was followed in the performance of this experiment. The sample solution (0.2 mL) was mixed with the substrate solution (0.2 mL) containing starch and the pancreatic amylase solution (0.1 mL at a concentration of 2 units/mL) at various concentrations ranging from 1000 μ g/mL to 3000 μ g/mL.

After the reaction reached its peak at 37 °C for 10 minutes, each tube was filled with 0.5 mL of 50% acetic acid to terminate it. A spectrophotometer set to 595 nm was used to measure the optic density of the supernatant after the tubes were centrifuged for five minutes at 4 °C at 3000× rpm. The positive control used in this test was acarbose, an inhibitor of α -amylase. The experiment was run three times for each concentration. The following formula was used to calculate the enzyme's inhibitory activity: activity inhibition = $[(A - B)/A] \times 100$, where A represents the sample absorbance and B the control absorbance.

2.6.2. *In Vitro* α -Glucosidase inhibitory activity

The procedure described²¹ was followed in the performance of this experiment. Samples ranging in concentration from 1000 to 3000 μ g/mL totaled 50 μ L, which were then combined with 10 μ L of α -glucosidase (maltase at a concentration of 1 unit/mL) and 125 μ L of 0.1 M phosphate buffer, and incubated for 20 minutes at 37 °C. After preparing 20 μ L of the substrate (pNPG) at 1 M, it was added to the mixture and left to incubate for 30 minutes. To halt the reaction, 50 μ L of Na₂CO₃ (0.1 N) was added. At 405 nm, optical density measurements were made. Three replications of the experiment were conducted at each concentration, with acarbose serving as the positive control.

The formula used to calculate the enzyme's inhibitory activity was,

activity inhibition = $[(A - B)/A] \times 100$, where A represents the absorbance of the control and B represents the absorbance of the sample.

2.6.3. Steps of Glycemic Response Determination System

Determining the glucose response was done after an *in vitro* simulation of the mouth, stomach, and small intestine²².

Step 1: Mouth digestion: In this investigation, *in vitro* mouth digestion was performed using a coffee grinder as opposed to mouth chewing. Samples were independently ground and homogenised in 0.5–1 minutes at room temperature using a coffee grinder.

Step 2: Digestive system: Every sample with 0.5 g of DC was placed in a 50 mL falcon tube, to which 5 mL of distilled water was added, and the mixture was vortexed for a minute. 0.5 g of pepsin and 0.5 g of guar gum were combined with 100 mL of 0.05 N HCl to create the pepsin-guar gum solution. After adjusting the pH to 1.5 and adding 10 mL of pepsin-guar gum solution to each sample individually, the samples were incubated for 30 minutes at 37°C in a shaking water bath.

Step 3: Small intestine digestion: We employed 136 mg/mL of pancreatin, 13.4 U/mL of amyloglucosidase, and 25.43 U/mL of invertase enzymes to perform *in vitro* small intestinal

digestion on each sample. 1.78 mL of amyloglucosidase and 0.00034 g of invertase were added to the supernatant of the combination containing 5.44 g of pancreatin and 36.28 mL of distilled water, which was centrifuged for five minutes at 3000 rpm to create the triple enzyme mixture. For every sample that was broken down in the stomach at 30, 60, 90, 120, and 180 minutes, 5 millilitres of sodium acetate and 5 millilitres of a triple enzyme mixture were added. The samples were then shaken in a shaking water bath for 30 minutes at 37 degrees Celsius. To make a final amount of 10 mL, 0.5 mL of each sample was taken, 2 mL of ethanol was added, and then distilled water was added to distilled water.

Determination of Glucose Produced after *in-Vitro* Digestion

D-glucose was quantified using a commercially available glucose oxidase/peroxidase (GOPOD) D-glucose assay kit (GOPOD format), which was based on enzymatic methods as colorimetric, in the final reaction medium following in vitro intestinal enzymatic digestion. After incubating for 30, 60, 90, 120, and 180 minutes, 3 mL of GOPOD reagent was added to 0.1 mL of each sample, which was then added and incubated at 50°C for 20 minutes. Following incubation, each sample's absorbance was measured individually using a UV spectrophotometer set to 510 nm. The final stage of in vitro digestion produced a graph with a glucose content between 0 and 180 minutes. The term "Hydrolyzed Curve" describes this curve. Using an Excel programme, the area under the hydrolyzed curve (AUHC) was determined.

During this phase, 0.1 mL of D-glucose was used in place of the sample from the GOPOD test kit for the conventional glucose assay. By using all processes, such as hydrolyzing each sample, the hydrolyzed curve and the area under the hydrolyzed curve (AUHC) of standard D-glucose were also determined.

2.6.4. Hydrolysis Index (HI) Procedure and Expression of HI Value

Using an Excel programme, the Hydrolyzed Index (HI) value of glucose was determined from the Hydrolyzed Curve between 0 and 180 minutes at 5 distinct intervals, based on the outcome of the in vitro digestion of the sample. The same methods were used to calculate the HI value of commercial glucose. The following formula was used to compute both HI values. HI is equal to $(\text{AUHC (Reference Carbohydrate)} / \text{AUHC (Sample)}) \times 100$. Following in vitro digestion, the hydrolyzed glucose curve was used to compute the HI values, which were then compared to those of commercial glucose.

2.6.5. Glycemic Index (GI)

The formula developed by Goni was utilized to compute the samples GI value: $\{\text{GI} = 0.7 \times [39.71 + (0.559 \times \text{HI})]\}$. (Goni and others, 1997).

2.7. Statistical analysis

Every experiment in this study was conducted in three separate biological replicates, and the results were presented as mean \pm standard deviation for every set of circumstances. Using one-way analysis of variance (ANOVA) and the least significant difference with $p < 0.05$, $p < 0.01$, and $p < 0.001$, the statistical significance of the data was examined.

3. Results

Figure 1: Antioxidant activity of Aqueous & Ethanolic extracts of *Muntingia Calabura* fruit

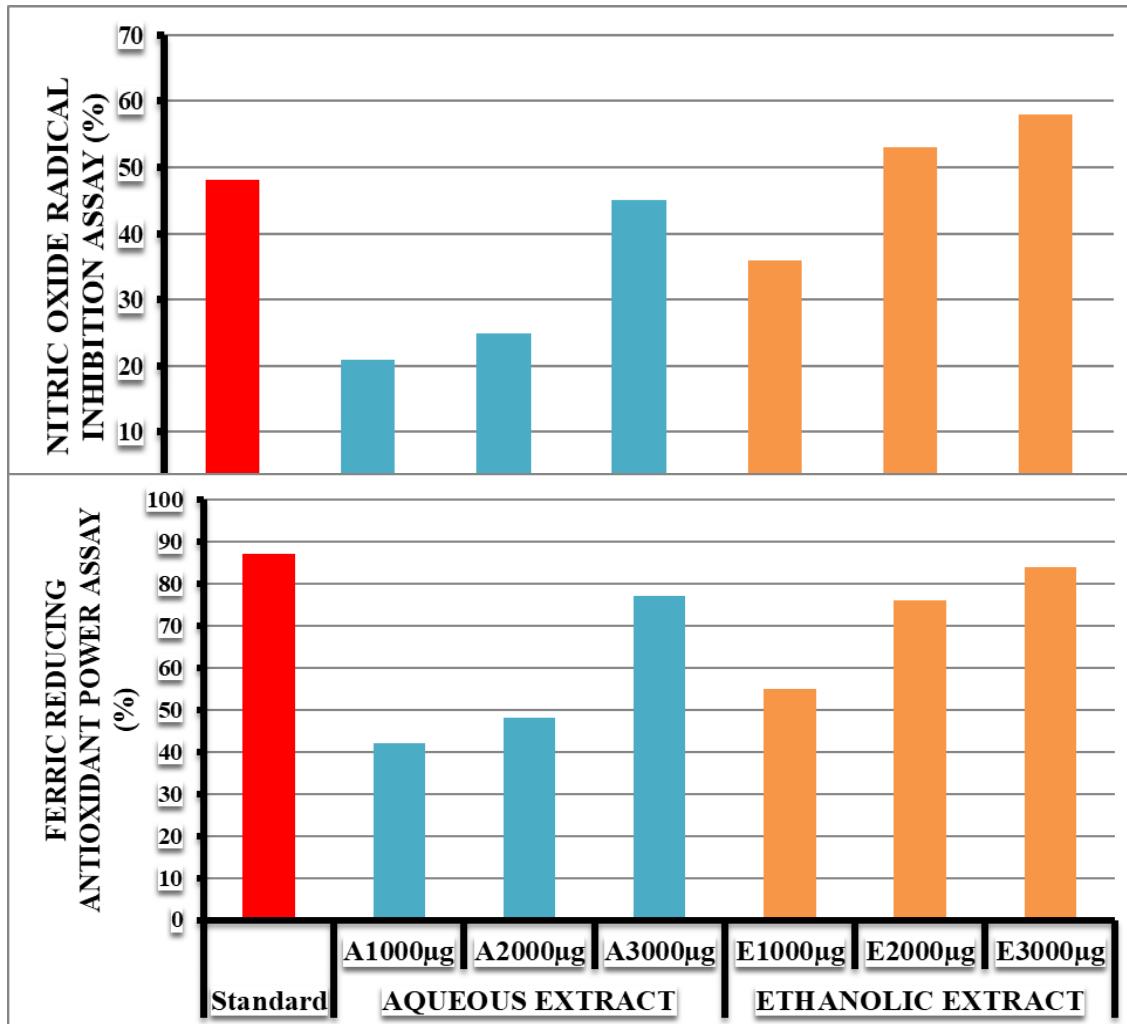


Figure 1A and 1B depicts the antioxidant activity of *Muntingia* fruit extracts which showed the gradual increase in antioxidant activity towards ferric ion and nitric oxide radical for both aqueous and ethanolic extracts. In addition, it showed the comparatively increased antioxidant activity for ethanolic extract compared to aqueous extract.

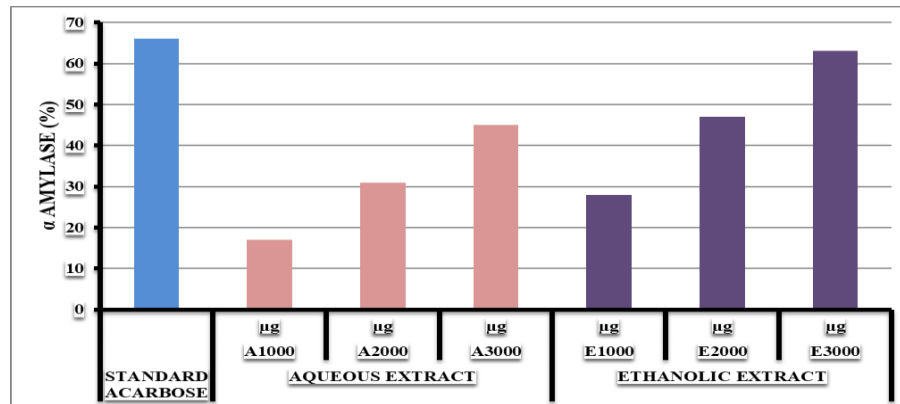
Table 2: Estimation of iron and calcium of *Muntingia calabura* fruit.

N=5; Values are Mean \pm SD

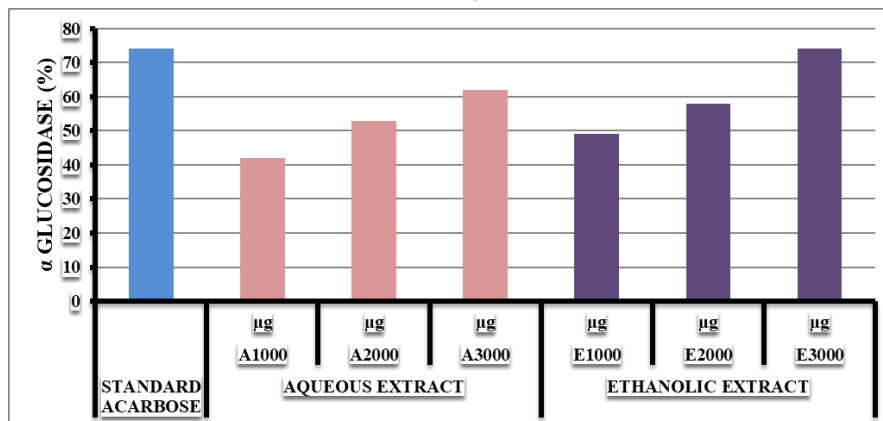
S.NO	MINERALS	<i>MUNTINGIA CALABURA</i> FRUIT
1	Iron content (mg/Kg dry weight)	1.32 \pm 0.062
2	Calcium content (mg/Kg dry weight)	123 \pm 0.127

Iron and calcium content were depicted in Table 2, showing their nutritional value as a source of calcium as well as iron.

Figure 2: Antidiabetic activity of aqueous & ethanolic extract of *Muntingia calabura* fruits.



2A: α-amylase inhibition



2B: α glucosidase inhibition

Figure 2A and 2B depicts the antidiabetic activity of *M. calabura* fruit extracts. It showed the gradual increase in the inhibition of both enzymes in a concentration dependent manner.

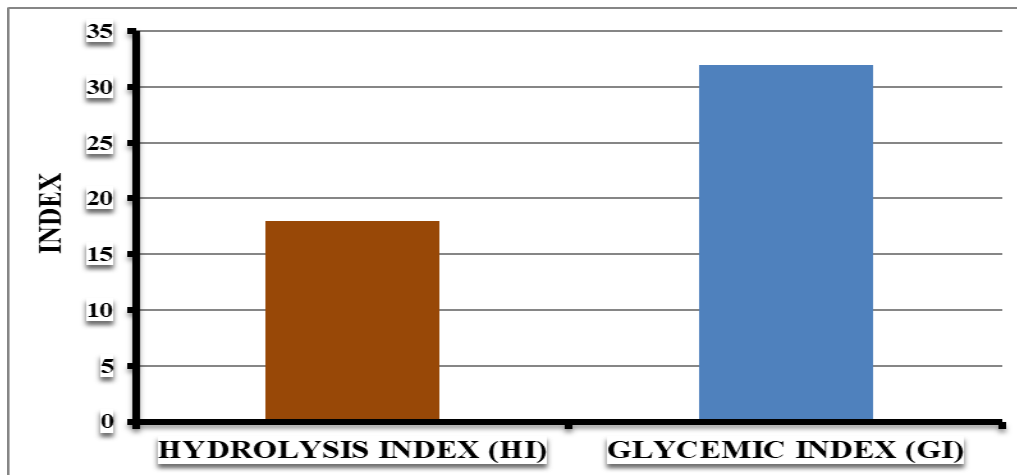
Figure 3: Hydrolysis index (HI) & Glycemic index (GI) of *Muntingia calabura* fruits.

Figure 3 depicts the hydrolysis and glycemic index of *M. calabura* which showed that it exhibits low GI, hence it may play a vital role in preventing or managing diabetes mellitus.

4. Discussion

Nearly all berries—aside from the Jamaican berry—have been further studied for their antioxidant potential. Consequently, we used nitric oxide and ferric ion radical scavenging tests in this work to examine the antioxidant activity of Jamaican berry extracts. Figures 1A and 1B illustrate the antioxidant activity of fruit extracts from *Muntingia*, demonstrating a progressive rise in antioxidant activity against nitric oxide radical and ferric ions for both ethanolic and aqueous extracts. Furthermore, it shown that the ethanolic extract had significantly higher antioxidant activity than the aqueous extract. Numerous studies indicate that the antioxidant activity of berries such as strawberries (*Rosaceae Fragaria*), blueberries (*Vaccinium corymbosum* L.), raspberries (*Rubus idaeus* L.), blackberries (*Rubus fruticosus*), resveratrol (*Polygonum cuspidatum*), and acai berries (*Euterpe Oleracea*) is generally high. This is associated with the fruit's levels of polyphenolic compounds like flavanoids, catechins, and anthocyanins. The results of these studies are similar to ours, where an increase in free radical quenching was noted (Figure 1A and 1B). It implies that the enhanced phytochemicals found in Jamaican berry fruit extracts could be the cause of this. Because of their high polyphenol content, a variety of berries have been shown to have strong antioxidant properties and to offer a number of health benefits. It is well recognised that oxidative stress has a significant role in disrupting normal physiological function, which can eventually lead to the development of inflammatory, cardiovascular, neurological, or metabolic problems, as well as the development of cancer²³. As a result, these antioxidant properties have been associated with numerous advantages for human health, including the prevention of allergies, inflammation, metabolic abnormalities, and diseases like diabetes, cardiopathies, and even cancer²⁴. After conducting both in vitro and in vivo investigations, these effects have been reported in a variety of tiny berry pomace species.

Mulberry polyphenols, for example, have been shown to have a variety of antioxidant and antidiabetic effects²⁵; mice treated with blueberry and mulberry juices demonstrated a reduction in the symptoms associated with obesity²⁶. It is important to note that consumption of black raspberries has been shown to reliably prevent the growth of colon adenocarcinomas and increase the survival of afflicted mice^{27,28}. Similarly, a few clinical trials have shown that little berry intake or extracts rich in polyphenols can reduce gut inflammation in patients with ulcerative colitis²⁹ or lower the risk of cardiovascular events in obese people³⁰. Because of their high polyphenol content, there is evidence to support the claim that these fruits have positive health effects. Consistently, *M. calabura* fruit extracts showed notable antioxidant activity against ferric ion radical and nitric oxide in both their ethanolic and aqueous extracts. Furthermore, the content of iron and calcium was examined and represented in Table 2, demonstrating their nutritional worth as sources of both calcium and iron. It is common practice to measure and compare the anthocyanin and other phenolic component contents of various berry species, both farmed and wild. Seldom has the concentration of vital elements—which are also significant constituents of blueberry fruits—been documented. The body's ability to function depends heavily on trace components. They primarily have regulatory activities or function as cofactors for different enzyme systems (usually redox-active metals). In this case, it was discovered that the *M. calabura* fruit extract had high amounts of iron and calcium.

To help avoid non-communicable diseases like heart disease and some types of cancer, the World Health Organisation advises consuming fruits and fresh vegetables on a daily basis. As a result, it's critical to measure the mineral content of fruits in order to evaluate their contribution to the suggested dietary requirements. In this case, the mineral contents of the *M. calabura* fruit extract were determined, including calcium and iron.

The skeleton contains 90% of the mineral calcium, with the remaining 10% found in the muscles and blood plasma. Calcium is the most prevalent mineral in the human body. Because it is involved in blood coagulation, bone and tooth development, hormone release, muscle fibre contractions, and nervous system function, this mineral is vital to human health (FDA, 2020). An essential component of the human body's operation is iron. Red blood cell creation, immunological function, growth and development, and energy production are the primary uses of this mineral (FDA, 2020). The fruit extract of *M. calabura* was discovered to have considerable amounts of both calcium and iron. Therefore, the findings imply that *M. calabura* fruit is an important source of minerals, able to contribute to the daily Dietary Reference intakes that the Food and Drug Administration recommends for adults.

The last few decades have seen a rise in the prevalence of diabetes due to changes in lifestyle that have resulted in higher caloric intake and decreased physical exercise. Numerous studies have indicated that the gastrointestinal tract may play a role in the anti-diabetic effects of berry fruits high in polyphenols. Specifically, findings from both in vitro and in vivo animal studies suggested that polyphenols could regulate the availability of nutrients, including glucose, by inhibiting the digestive enzymes that are involved in the breakdown and absorption of carbohydrates.

This could have an impact on blood glucose regulation. Figures 2A and 2B coherently illustrate the antidiabetic properties of fruit extracts from *M. calabura*. It demonstrated how the inhibition of both enzymes gradually increased in a concentration-dependent way. According to our research findings, other studies have shown that berries, with the exception of the Jamaican berry, which is the subject of this study, have antidiabetic potential. According to a study by Anastasia et al., low levels of polyphenols from berries—strawberries, black currants, arctic brambles, cloudberries, blueberries, and rowanberries—in vitro inhibit both α -amylase and α -glucosidase activity, suggesting that they may also modulate starch digestion³¹. It was demonstrated that the inhibitory effects of extracts obtained from red and yellow raspberries on α -amylase were comparable. Since anthocyanins are absent from yellow raspberry extracts, it has been hypothesised that they are not essential for amylase inhibition. According to a study by McDougall from 2005, raspberry ellagitannins were the primary active ingredients causing amylase suppression. Additionally, an extract from blackberries demonstrated α -glucosidase and α -amylase inhibitory action in vitro³². These findings suggest that enzyme inhibition may be used to regulate the gastrointestinal tract's starch digestion. According to the body of research, berry fruits high in polyphenols may offer a way to control the rate at which intestinal glucose is absorbed and digested, offering an additional means of managing diabetes. In the long run, this may also provide some protection against the development of type 2 diabetes.

The effects of polyphenolic compounds derived from berries in vivo, however, are not dependent on the direct effects of polyphenolic compounds upon intestinal digestion of carbohydrates and absorption of glucose; rather, they are based upon glycemia or glucose tolerance data. Congruently, Figure 3 shows the glycemic index and hydrolysis of *M. calabura*, indicating that it has a low GI and may be essential in the prevention or treatment of diabetes mellitus. Consistent with our investigation, a study found that the polyphenolic components in berries may have a positive impact on glycemic regulation. In general, GI is a measure of the quality of carbohydrates in meals and is a relative glycaemic response. Foods can be divided into three categories based on the GI classification system: high GI (>70), medium GI (55 to 70), and low GI (55).^{33,34} During the postprandial period, a high-GI diet produces a greater area under the glucose curve compared to a low-GI food that contains an equivalent amount of carbohydrates³⁴. Food makers find low-GI food to be an appealing target since many diabetics and health-conscious consumers favour low-GI food^{33,34}. The GI is a reliable method for classifying foods high in carbohydrates based on their postprandial glycemic response and is used to gauge the quality of carbohydrates³⁴. It is commonly known that while a low GI diet might enhance health status, high GI foods can raise the chances of diabetes, obesity, several types of cancer, and cardiovascular diseases³⁵.

It is commonly known that while a low GI diet might enhance health status, high GI foods can raise the chances of obesity, diabetes, several types of cancer, and cardiovascular diseases³⁵. Accordingly, it was demonstrated that *M. calabura* extract had a very low GI, indicating that it may have a function in both the therapy and prevention of diabetes.

To investigate the potential of *M. calabura* as an effective antidiabetic drug, more research is necessary to validate its antidiabetic properties.

5. Conclusion

The current investigation offers the first pharmacological understanding of *Muntingia calabura* fruit extract's antioxidant and antidiabetic properties. Additionally, the fruit extract dramatically decreased the activities of α -amylase and α -glucosidase when compared to the most commonly used medication, acarbose. This suggests that the polyphenols included in fruit extracts may be able to lower postprandial hyperglycemia by postponing the breakdown of carbohydrates. In vivo experimental models should be employed to investigate the antidiabetic potential of AGE, α -amylase, and α -glucosidase enzymes. These models will help to validate the results obtained in this work. Future study paths on the utilisation of traditional medicinal herbs in the creation of medicines and nutraceuticals would benefit from the current finding.

6. Conflicts of Interest

No conflict of interest

7. Author Contributions

H.R develop the topic, design, supervise, correct and approve the text. N.S. Wrote the manuscript. J.S. Statistical analysis. M.K. conducted investigation for antioxidant assay. P.R. and S.P. conducted investigation for Antidiabetic activity.

8. Reference

1. Morton JF. Jamaica cherry. In: Morton JF, ed. Fruits of Warm Climates, Vol. 2. Miami (FL): Timber Press. 1987; 2:65–9
2. Sani MH, Zakaria ZA, Balan T, et al. Antinociceptive activity of methanol extract of *Muntingia calabura* leaves and the mechanisms of action involved. *Evid Based Comp Altern Med* 2012; 890361.
3. Yusof MM, Teh LK, Zakaria ZA, Ahmat N. Antinociceptive activity of the fractionated extracts of *Muntingia calabura*. *Planta Med.* 2011; 77:PF21.
4. Edeoga H.O, Okwu D.E, Mbaebie B.O. Phytochemical constituents of some Nigerian medicinal plants. *A'fr.J. Biotechnol.* 2005; 4(7): 685-688
5. Jothy S.L, Torey A, Darah I, Choong Y.S, Saravanan D, Chen Y, Latha L.Y, Deivanai S; Sasidharan S. *Cassia spectabilis*: A promising traditional herb in health improvement. *Molecules.* 2012; (17) 6:10292– 10305.
6. Mazumder P.M, Percha V, Farswan M, Upaganlawar. A. *Cassia*: A wonder gift to medical sciences. *Int. J. Clin. Pharm.* 2008; 17(1): 16–38.
7. Upadhyay J, Polyzos SA, Perakakis N, Thakkar B, Paschou SA, Katsiki N, et al. Pharmacotherapy of type 2 diabetes: an update. *Metabolism.* 2018; 78:13-42
8. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract.* 2014; 103: 137-49.
9. Lacroix IME, Li-Chan ECY. Overview of food products and dietary constituents with Antidiabetic properties and their putative mechanisms of action: a natural approach to

- complement pharmacotherapy in the management of diabetes. *Mol Nutr Food Res.* 2014; 58: 61-78.
10. Chang AM, Smith MJ, Bloem CJ, Galecki AT, Halter JB. Effect of lowering postprandial hyperglycemia on insulin secretion in older people with impaired glucose tolerance. *AJP Endocrinol Metab.* 2004; 287:E906-11.
 11. Rupasinghe HPV, Sekhon-Loodu S, Mantso T, Panayiotidis MI. Phytochemicals in regulating fatty acid β -oxidation: Potential-underlying mechanisms and their involvement in obesity and weight loss. *Pharmacol Ther.* 2016; 165: 153-63.
 12. Vinson JA, Howard TB. Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients. *J Nutr Biochem.* 1996; 7:659-63.
 13. Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α -amylase and α -glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. *Exp Toxicol Pathol.* 2013; 65: 305-9.
 14. Ibrahim MA, Koorbanally NA, Islam MS. Antioxidative activity and inhibition of key enzymes linked to type-2 diabetes (α -glucosidase and α -amylase) by *Khaya Senegalensis*. *Acta Pharmaceutica.* 2014; 63: 311-24.
 15. Striegel L, Kang B, Pilkenton SJ, Rychlik M, Apostolidis E Effect of black tea and black tea pomace polyphenols on α -glucosidase and α -amylase inhibition, relevant to type 2 diabetes prevention. *Front Nutr.* 2015; 2:3.
 16. Buchholz T, Melzig MF. Medicinal plants traditionally used for treatment of obesity and diabetes mellitus – screening for pancreatic lipase and α -amylase inhibition. *Phytother Res.* 2016; 30:260-6.
 17. Rupasinghe HPV, Balasuriya N, Wang Y. Prevention of type2 diabetes by polyphenols of fruits. In: Kais H, Al-Gubory KH, Laher I, editors. *Nutritional Antioxidant Therapies: Treatments and Perspectives.* Springer 2017; 447-66.
 18. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having Antidiabetic activity. *Asian Pac J Trop Biomed.* 2012; 2:411-20.
 19. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as measurement of “antioxidant power” The FRAP assay. *Anal Biochem.* 1996; 239:70–6.
 20. Can Z, Keskin B, Üzer A, Apak R. Detection of nitric oxide radical and determination of its scavenging activity by antioxidants using spectrophotometric and spectrofluorometric methods. *Talanta.* 2022; 1.
 21. Telagari M, Hullatti K. In-vitro α -amylase and α -glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian J Pharmacol.* 2015; 47(4):425-9.
 22. Goni I, Garcia-Alonso A, Saura-Calixto F. A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research.* 1997; 17(3):427–437.

23. Jimenez-Lopez, C., Carpena, M., Lourenço-Lopes, C., Gallardo-Gomez, M., Lorenzo, J.M., Barba, F.J., Prieto, M.A., Simal-Gandara, J., Bioactive compounds and quality of extra virgin olive oil. *Foods*; 2020; 9(8),1014
24. Bouyahya, A., Omari, N. El, EL Hachlafi, N., Jemly, M. El, Hakkour, M., Balahbib, A., El Menyiy, N., Bakrim, S., Naceiri Mrabti, H., Khouchlaa, A., Mahomoodally, M. F., Catauro, M., Montesano, D., & Zengin, G. Chemical compounds of berryderived polyphenols and their effects on gut microbiota, inflammation, and cancer. *Molecules*. 2022; 27(10), 3286.
25. Li, F., Zhang, B., Chen, G., & Fu, X. The novel contributors of anti-diabetic potential in mulberry polyphenols revealed by UHPLC-HR-ESI-TOF-MS/MS. *Food Research International*. 2017; 100, 873–884.
26. Wu, T., Tang, Q., Gao, Z., Yu, Z., Song, H., Zheng, X., & Chen, W. Blueberry and mulberry juice prevent obesity development in C57bl/6 mice. *PLoS One*. 2013; 8(10), 2–8.
27. Dong, A., Huang, Y., Yearsley, M., Oshima, K., Chen, X., Yu, J., & Wang, L. Dietary supplementation with black raspberries prolongs survival in *Apc Min/+* mice. *Food Frontiers*. 2021; 2(3), 324–328.
28. Huang, Y., Mo, Y. Y., Echeveste, C. E., Oshima, K., Zhang, J., Yearsley, M., Lin, C., Yu, J., Liu, P., Du, M., Sun, C., Xiao, J., & Wang, L. Black raspberries attenuate colonic adenoma development in *Apc Min* mice: Relationship to hypomethylation of promoters and gene bodies. *Food Frontiers*. 2020; 1(3), 234–242.
29. Roth, S., Spalinger, M. R., Gottier, C., Biedermann, L., Zeitz, J., Lang, S., Weber, A., Rogler, G., & Scharl, M. Bilberry-derived anthocyanins modulate cytokine expression in the intestine of patients with ulcerative colitis. *PLoS One*, 2016; 11(5), 1–17.
30. Basu, A., Du, M., Leyva, M. J., Sanchez, K., Betts, N. M., Wu, M., Aston, C. E., & Lyons, T. J. Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. *Journal of Nutrition*. 2010; 140(9), 1582–1587.
31. Anastasia-Varvara Ferlemi and Fotini N. Lamari. Berry Leaves: An Alternative source of bioactive natural products of nutritional and medicinal value. *Antioxidant*. 2016; 5 (2): 17
32. Gordon J Mcdougall. Different polypehnnolic componens of soft fruits inhibit amylase and glucosidase. *Journal of agricultural and food chemistry*. 2005; 53 (7): 2760-6.
33. Kanter, M., Angadi, S., Miller-Jones, J., & Beals, K. A. Limitations of the glycaemic index and the need for nuance when determining carbohydrate quality. *Journal of Cardiovascular Research*. 2022; 118, E38–E39
34. Cairano, D. M., Tchuenbou, M. F. L., Condelli, N., Cela, N., Ojo, C. C., Radecka, I., et al. Glycaemic index of gluten-free biscuits with resistant starch and sucrose replacers: an in vivo and in vitro comparative study. *Foods*. 2022; 11(20), 3253.
35. Ni, C., Jia, Q., Ding, G., Wu, X., & Yang, M. (2022). Low-glycemic index diets as an intervention in metabolic diseases: A systematic review and meta-analysis. *Nutrients*, 14, 307 [PubMed].