Jabirian
Journal of Biointerface Research in Pharmaceutics and Applied Chemistry
ISSN: 2584-2536 (Online)
Vol.1(6), Jan 2025, pp, 9-14
Journal homepage: https://sprinpub.com/jabirian

Research article

Phytochemical Profiling and Biological Activities of Musa acuminata Peel with Antioxidant and α-Amylase Inhibitory Effects

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ARTICLE INFO

Keywords:
Musa acuminata,
Phytochemicals, DPPH free radical, α-Amylase

Article History:
Received: 23-06-2024
Accepted: 20-01-2024
Published: 28-01-2025

ABSTRACT

Peels of Musa acuminata were used as healing medicine in Nepal, especially against gastrointestinal ailments and diabetic patients. However, the scientific evidence towards its use is still lacking. Therefore, the main aim of this research is to bridge the gap between traditional knowledge and scientific inquiry, offering evidence-based insights into the medicinal efficacy of Musa acuminata. The antioxidant activity of ripe peel extract was estimated spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH: 60 µM). Similarly, α-amylase inhibitory activity was analyzed using a modified starch iodine protocol. The percentage yield of the hydroethanolic extract of Musa acuminata ripe peel was found to be 16 %(w/w). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and reducing sugars in the extract, highlighting its diverse chemical composition. The DPPH scavenging activity percentage of ripe peel extract revealed good antioxidant activity with 54.58 ± 3.27 %, but showed 5.989± 1.96 % of α-amylase inhibition activity at 0.5 µg/ml concentration of extract, which was very low when compared with voglibose with 97.660 ± 98 % at same concentration. The findings suggest that Musa acuminata peels may possess antioxidant and mild amounts of α-amylase inhibition properties. Further exploration of its in-vivo pharmacological studies and clinical trials are needed to confirm their therapeutic potential.

Cite this article:
Chauhan S, K.C. Sindhu, Bhandari M, Poudel P, Yadav R, Ranabhat P, Shrestha S, Shrestha A. Phytochemical Profiling and Biological Activities of Musa acuminata Peel with Antioxidant and α-Amylase Inhibitory Effects. Jabirian J. Biointerface Res. Pharmaceut. Appl. Chem. 2025;1(6):9-14. https://doi.org/10.55559/jjbrpac.v1i6.443

1. INTRODUCTION

Diabetes mellitus is a worldwide disease, either inherited or acquired, that arises from an insulin deficiency or when the body's organs become insensitive to insulin, or both [1]. This state often leads to elevated glucose levels in the bloodstream, damaging the body's systems. Hyperglycemia can impair the body's natural antioxidant defense system, leading to oxidative stress.(1) High glucose levels induce oxidative stress through various mechanisms like activating the NADPH oxidase enzyme, protein kinase C and mitochondrial dysfunction. Oxidative stress has also been responsible for the development of diabetes, so while leading to the development of an antioxidant system a potential strategy in the health care system [2]. As of now, diabetes has been classified into type 1 and type 2 diabetes mellitus, caused by various mechanisms. Among them, type 2 diabetes mellitus is considered a global concern. Accounting for about 90% of cases,in 2023 more than 4 million people were affected by this disease. As increasing in the cases of diabetic patients has also led to raise the cost of treatment [3]. Various medications have been developed to manage type 2 diabetes mellitus including acarbose, voglibose and miglitol (a kind of α-glucosidase inhibitor), to reduce postprandial hyperglycemia by slowing the absorption of glucose into the bloodstream. However, some of these medications are leading to various side effects like gastrointestinal discomfort, diarrhea, abdominal pain and bloating [4]. α-Amylases are the enzymes, found in saliva and pancreatic juice that function in breaking down the carbohydrate into glucose and maltose [5]. Inhibiting α-amylase can be achieved by binding polyphenols to the α-amylase enzymes through various bonding. Due to increasing cases of side effects of conventional medications, many of the patients are inclined towards natural products as alternative forms of health care. Medicinal plants are considered safer and more effective than existing pharmaceutical products,despite limited clinical evaluation of their efficacy [6].

Musa acuminata belongs to the Musaceae family. Native to is cultivated in Terai region of Nepal (Chitwan, Jhapa, Sunsari, Saptari, Siraha and Dhanusha), which is situated in the southern lowland plain having tropical and subtropical climate along with fertile soil. This species has been noted for its various pharmacological activities including antioxidant activity, anti-inflammatory, antimicrobial activity, gastroprotective activity and antihypertensive activity [7]. However, there is limited scientific study on its ability to have antioxidant and α-amylase inhibition activity. This study aims to perform a phytochemical analysis of the peels extract of the *Musa acuminata* along with other studies including potential in vitro antioxidant and α-amylase inhibitory

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https://doi.org/10.55559/jjbrpac.v1i6.443

activity for scientific justification for the traditional use of peel of *Musa acuminata* as therapy for diabetic patients.

Graphical abstract

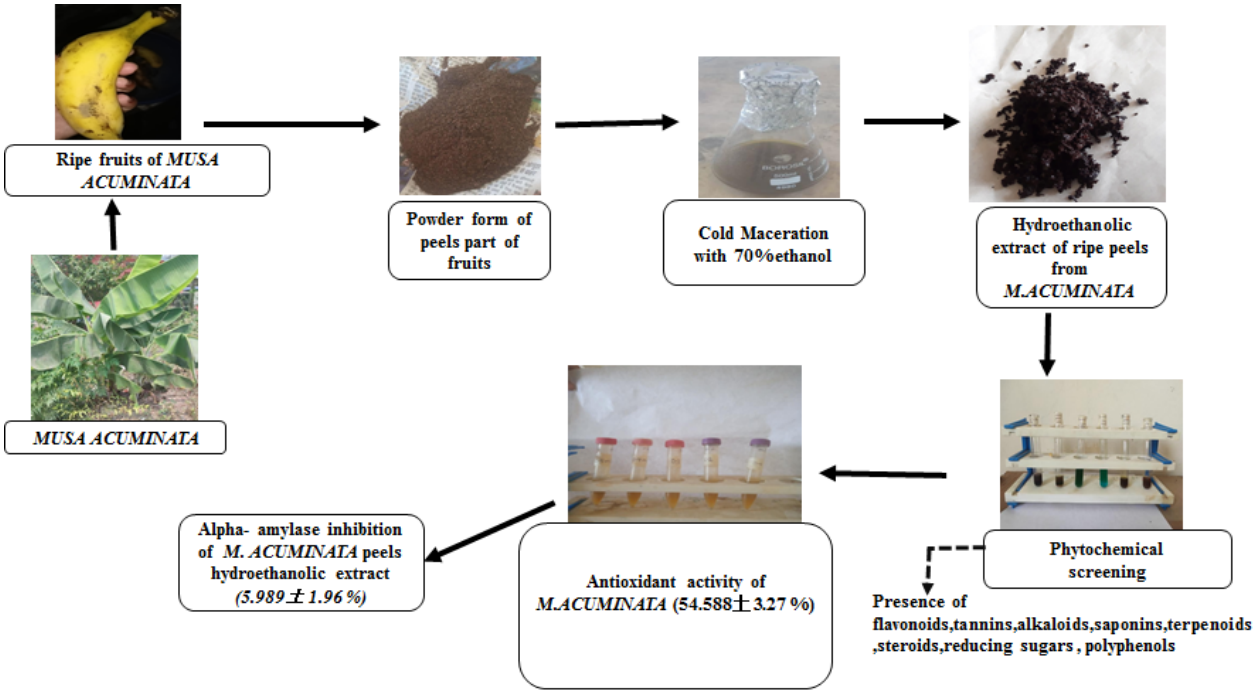

Figure 1. Antioxidant and α-Amylase Inhibitory Activities Evaluation, Phytochemical Profiling of Musa acuminata *Peel of Nepal*

2. Materials and Methods

2.1 *Chemicals*

2,2-diphenyl-1- picrylhydrazyl (DPPH) (HiMedia, India), α-amylase (Antozyme Biotech), starch (Qualigens Fine Chemicals), ascorbic acid (Ingredi), voglibose standard (Manus Aktteva Biopharma LLP) were used for investigation.

2.2 Plant Collection and Authentication

Musa acuminata was acquired from Bharatpur-7 Chitwan and then herbarium was prepared. After that, it was identified and authenticated at Birendra Multiple Campus by Botanist Dr.Manoj K. Lal Das . The herbarium of *Musa acuminata* was then kept in the Pharmacognosy lab at Chitwan Medical College with reference number CMC.HER.3.2024.

2.3 Extraction

Musa acuminata ripe fruit was collected from Bharatpur, Chitwan in the month of May 2024 based on ethnobotanical aspect and then the peel was separated, and shade-dried for 2 weeks.The 50gm dried peel of *Musa acuminata* was powdered and macerated in 70% ethanol (chosen for its efficacy in extracting both polar and non-polar compounds, maximizing the yield of phytochemicals constituents) and filtered out using Whatman filter paper (to separate out the marc and filtrate); Furthermore, the obtained hydroethanolic filtrate was concentrated in a rotary evaporator at 48±2 °C (temperature carefully selected to prevent the degradation of heat-sensitive compounds). The concentrated extract was stored in a vacuum desiccator to maintain stability, and then completely dried out extracts % yield was determined using formula [8,9]:Yield (%) = (weight of extract / weight of dried peel powder) x 100

2.4 *Qualitative phytochemical analysis*

The phytoconstituents were analyzed using various standard chemical test including Mayer's reagent test for alkaloid, Lead acetate test for flavonoids, Ferric chloride test for tannins, Benedict's test for reducing sugar, Molisch's test for carbohydrate, Salkowski's test for glycosides and terpenoids, Foam test for saponins, Liebermann-Burchard test for steroids [8].

2.5 In-vitro antioxidant assay

The DPPH free radical scavenging assay with slight modification was used to determine the antioxidant activity of ripe peel extract. The process is explained as follows: 2 ml of hydroethanolic extract of ripe *Musa acuminata* peel solution (1% DMSO+Distilled water) at various concentrations (100µg/ml,200 µg/ml ,300 µg/ml ,400 µg/ml, 500 µg/ml) as well as independently 2 ml of Ascorbic acid solution (1%DMSO + Distilled water) as standard at various concentration (100 µg/ml ,200 µg/ml,300 µg/ml,400 µg/ml, 500µg/ml) were combined with 2 ml of 60 µM DPPH solution. After blending, the mixture was incubated in a dark chamber for 30 minutes. Utilizing a UV spectrophotometer, the absorbance at 517 nm was determined. The subsequent equation was applied to calculate the scavenging activity of the sample against DPPH free radical.

Scavenging Activity (%) = [(Ac - As) / Ac] x 100.

Where, As represents sample's absorbance and (Ac) is control's absorbance (1% DMSO+Distilled water). All the tests were done three times to ensure accuracy and reliability.

2.5 *In-vitro α-amylase activity*

The modified starch iodine protocol was utilized to conduct the α-amylase inhibitory assay [10-12]. 1 ml of 0.02M phosphate buffer (pH 6.9 with 0.006M NaCl) containing α-amylase (1 mg/ml in phosphate buffer) solution was mixed with 1 ml of

hydroethanolic extract of ripe *Musa acuminata* peel at various concentration (0.1 µg/ml, 0.2 µg/ml, 0.3 go/ml, 0.4 µg/ml, 0.5 µg/ml) as well as independently with 1ml of standard drug Voglibose at various concentration (0.1 µg/ml, 0.2 µg/ml, 0.3 µg/ml, 0.4 µg/ml, 0.5 µg/ml) incubated for 10 minutes at 37 °C. Each test tube was then filled with 1 ml of 1% starch solution (normal saline as solvent). Following, the mixture was incubated at 37°C for 1 hour. After 1 hour incubation 0.04ml prepared 1 M HCL was added along with 0.1ml of 1% iodine solution. A UV-spectrophotometer was used to measure the absorbance at 565 nm [11-13]. The subsequent equation was: Percentage inhibition = [(As - Ac)/As] x 100

Where, as represents test sample absorbance and Ac represents control's absorbance.

All the tests were done in triplicate.

2.6 *Statistical analysis*

IBM SPSS (version 20) was used for statistical analysis of experimental results. Results of in-vitro antioxidant and in-vitro α-amylase were expressed as mean± standard deviation. Charts were developed to represent the results. Here all experiments for each concentration were performed in triplicate.

3. Results

3.1 *Extractive yield*

The total hydroethanolic extract of ripe *Musa acuminata* peel yield was 16%.

3.2 *Qualitative screening of phytochemicals*

The qualitative phytochemical screening of *Musa acuminata* peel revealed the presence of several bioactive compounds. Positive results were observed for alkaloids, flavonoid, tannin, saponin, terpenoid, steroid and reducing sugar. However, glycoside was not detected. The presence of polyphenols in the extract is of interest as it is reported in previous literature that these bioactive compounds are responsible for the oxidative radical and α-amylase inhibitory properties.

3.3 In-vitro antioxidant activity of hydroethanolic M. acuminata peel extract

Hydroethanolic extract of ripe *Musa acuminata* peel was subjected to the DPPH radical assay using ascorbic acid as the standard, adhering standard protocol and measuring absorbance at 517 nm. The radical scavenging capacity of hydroethanolic extract of ripe *Musa acuminata* peel increased with increasing concentration. The highest % DPPH radical scavenging was shown by ascorbic acid (76.879±3.65) at 500 µg/ml whereas hydroethanolic ripe peel extract of *Musa acuminata* showed highest % DPPH radical scavenging (54.588±3.27) at 500 µg/ml. This highest percentage inhibition of DPPH free radical corresponded to its higher antioxidant activity as shown **Table 1, Figure 1**.

Table 1. Percentage radical scavenging with different concentrations of hydroethanolic extract of ripe *Musa acuminata* peel and ascorbic acid.

S. N.	Concentration (µg/ml)	% DPPH radical by scavenging ascorbic acid (Mean±STD)	% DPPH radical scavenging by hydroethanolic extract of *Musa acuminata* (Mean ±STD)
1	100	19.102±1.07	8.547±2.26
2	200	34.563±3.87	13.760±2.56
3	300	48.345±2.86	21.301±3.67
4	400	56.764±4.47	33.025±1.45
5	500	76.879±3.65	54.588±3.27

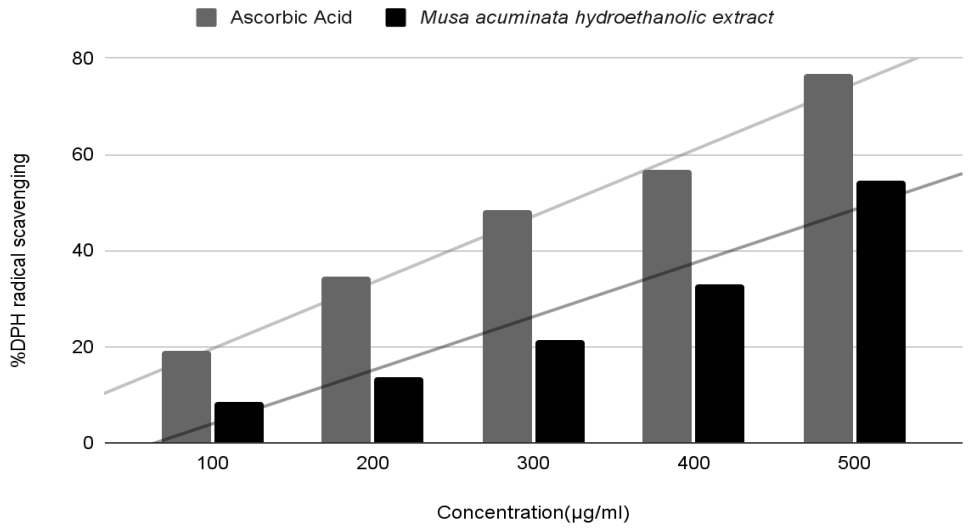

Figure 1. Percentage DPPH radical scavenging with different concentration of hydroethanolic extract of ripe *M. acuminata* peel and ascorbic acid.

3.4 Inhibition of α-Amylase enzyme using Musa acuminata extract

Table 2 and Figure 2, displays the percentage inhibition of α-Amylase by hydroethanolic extract of *Musa acuminata* peel solution and standard voglibose. Inhibition of α-Amylase was shown by hydroethanolic extract of ripe *Musa acuminata* peel in a dose dependent manner. The inhibition percentages varied at concentration between 0.1µg/ml to 0.5µg/ml; the maximum inhibition was detected at 0.5 µg/ml (97.660± 0.98%) by standard voglibose. Whereas hydroethanolic extract of ripe *Musa acuminata* peel showed 5.989± 1.96% of α-Amylase inhibition at 0.5 µg/ml.

Table 2. Percentage inhibition of by standard drug Voglibose and hydroethanolic extract of *Musa acuminata* peel

Concentration(µg/ml)	% Inhibition by standard drug voglibose	% Inhibition by hydroethanolic extract of ripe *Musa acuminata* peel
0.1	65.760± 0.78	3.101± 3.65
0.2	67.523± 1.2	4.481± 1.76
0.3	88.180± 3.45	5.050± 2.78
0.4	96.998± 0.99	5.505± 0.94
0.5	97.660± 0.98	5.989± 1.96

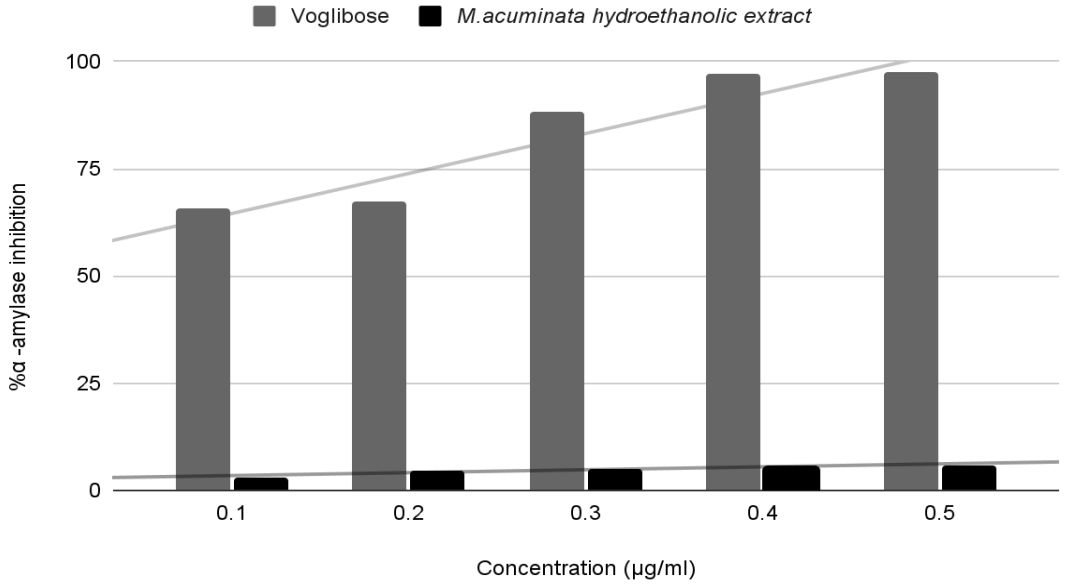

Figure 2. Comparative column chart illustrating inhibition of α- amylase by Standard drug voglibose and hydroethanolic extract of ripe *Musa acuminata* peel.

4. Discussion

In our study, the percentage yield of hydroethanolic extracts from the *Musa. acuminata* ripe peel powder was 16% (w/w). In the previous study, carried out in leaves extract of *Musa acuminata*, the peels extract yield was 13.03% using vacuum microwave method, which was very low when compared to our present study. So, this study could be helpful for further investigation to determine the high-quantity extract yield from *Musa* species through this procedure [9,10,12].

Mayers's test, lead acetate, ferric chloride, foam, Salkowski's, Liebermann-Burchard, Benedict's test confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, reducing sugars in our study. This study was further confirmed, from the previous study where methanol extract of pseudo stem from *M. acuminata* showed the positive test for the same phytoconstituents [14]. Hesperidin and amentoflavone from methanol extract present in *Musa sp.* have been reported to show the highest anti-α-glucosidase and anti-α-amylase activity with IC_{50}, 5.45 ± 0.39 mg/mL and 9.70 ± 0.29 mg/mL respectively [10,14-16].

Various studies have shown that multiple mechanisms and pathway activation are responsible to show the antioxidative action in living cells. In this regard, multiple screening tests were developed to quantify the antioxidant potency of the plant extract [10,12]. Hence, our study employed, DPPH free radical scavenging method to evaluate the antioxidant activity of the ripe peel hydroethanolic extract of *Musa acuminata*. Here through this DPPH scavenging study, the *M. acuminata* ripe peels hydroethanolic extract showed a concentration-dependent antioxidant activity which was parallel to ascorbic acid as reference standard.

This study revealed that the phytoconstituents present in *Musa acuminata* have the ability to reduce the absorption measured at 517 nm through UV spectrophotometry. As previous studies have reported that, phytoconstituents obtained from various species can lower the absorption measured, by scavenging the odd electron of the DPPH molecule and justifying itself as

lowering the free radical scavenging activity [8,15]. Further, our study showed that the percentage DPPH radical scavenging activity at higher dose 500 µg/ml was 76.879±3.65 and 54.588± 3.27 by Ascorbic acid and *Musa acuminata* ripe peel hydroethanolic extract respectively. This highest percentage inhibition of DPPH free radical corresponded to its higher antioxidant activity. This finding, aligned with another previous study, where butanol extract of fruit peels of *M. acuminata* showed concentration-dependent percentage inhibition of DPPH, where higher value of DPPH inhibition was at 500 µg/mL concentration assuring *Musa acuminata* can be a primary source of antioxidants [10].

The in-vitro α-amylase inhibition study is one of the effective models to study possible anti-diabetic action of the chemical compound. Here in the body α-amylase acts as a breakdown of carbohydrate into glucose, which is then absorbed in the bloodstream leading to increment in the blood glucose level, stating it to the diseases, a type 2 diabetic. It is a highly reliable study , to evaluate potential activity of compound or plant extract as an antidiabetic agent.(10) According,to previous study, presence of α-amylase enzymes in salivary gland, pancreas which are then released into small intestines leads to breakdown of carbohydrate into glucose [5]. In our study, α-amylase enzymes are pretreated with voglibose and ripe *Musa acuminata* peels hydroethanol extract at various doses independently. Additionally, all the dose from voglibose (reference standard) and *M. acuminata* peels hydroethanolic extract showed concentration dependent alpha amylase inhibition activity, but the percentage inhibition by *M. acuminata* peels is very low comparative to standard voglibose even at high concentration which is about 5.989± 1.96 %, suggesting that ripe *M. acuminata* peels have very mild percentage inhibition α-amylase to be used for treatment for type 1 diabetic ailment. In previous studies, *Musa acuminta* has shown positive result as an antidiabetic agent, in animal studies by reducing plasma glucose [8,15,16]. However, our study shows very low percentage of alpha-amylase inhibition activity compared to the previous work. It is suggested that *M. acuminata* ripe peel hydroethanolic extract exhibits a potential antioxidant activity against DPPH free radical, but have a minimum percentage of α-amylase inhibition activity, reporting that traditional use of ripe peel hydroethanolic extract of *M. acuminata* may not support effective diabetic treatment in individual. Naturally going on phytoconstituents, such as flavonoids, polyphenols, alkaloids, and saponins, play a crucial function in dealing with diabetes and other illnesses because of their numerous bioactive residences. In diabetes, these compounds inhibit enzymes like α-amylase and α-glucosidase, which slows glucose absorption and enables alter blood sugar tiers. They additionally beautify insulin sensitivity, lessen oxidative strain, and mitigate inflammation, all of which are essential in dealing with diabetes and its headaches [17-22]. Beyond diabetes, phytoconstituents offer extensive health blessings, consisting of antioxidant and anti-inflammatory results, cardiovascular protection, most cancers prevention, and neuroprotection. They additionally showcase antimicrobial homes and boost immune characteristics [23-26]. These herbal compounds, found in culmination, veggies, herbs, and other plant resources, are increasingly recognized for his or her potential to provide safe, powerful, and holistic answers to various chronic health situations.

5. Conclusions

The purpose of the study was to investigate the therapeutic potential of antioxidant and α-amylase inhibition from hydroethanolic extract of ripe *Musa acuminata* peel. The hydroethanolic extract of ripe *Musa acuminata* peel showed the potential antioxidant activity against DPPH free radical whereas, for α-amylase inhibition activity, it showed low α-amylase inhibition even at high concentration compared to standard drug voglibose. Overall, the result showed that the traditional use of *M. acuminata* peel can be used for the management of oxidative stress but may not be an effective anti-diabetic agent. Therefore, more research into the *Musa acuminata* plant is required in order to produce an effective potential pharmacological drug for antioxidant and diabetes-related disease.

Data availability

All the data are available in the main body.

Conflict of interest

The authors want to declare that they have no known financial interest or personal relationship that could have appeared to influence the work reported in this paper. All the authors have worked in this paper as per their interest.

Funding statement

No funding was provided to carry out these experiments.

Abbreviation

DPPH: 2,2-diphenyl-1-picrylhydrazyl
µM: Micro molar
µg /ml: Microgram per milliliter
w/w: Weight by weight
µg /ml: Microgram per milliliter
NADPH: Nicotinamide adenine dinucleotide phosphate hydrogen
A_c : Control's absorbance
A_s : Sample's absorbance
DMSO: Dimethyl Sulfoxide

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