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Antioxidant and anti-diabetic activities of crude ethanolic extract from the banana inflorescence of *musa* (ABB group) namwa maliong

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ABSTRACT

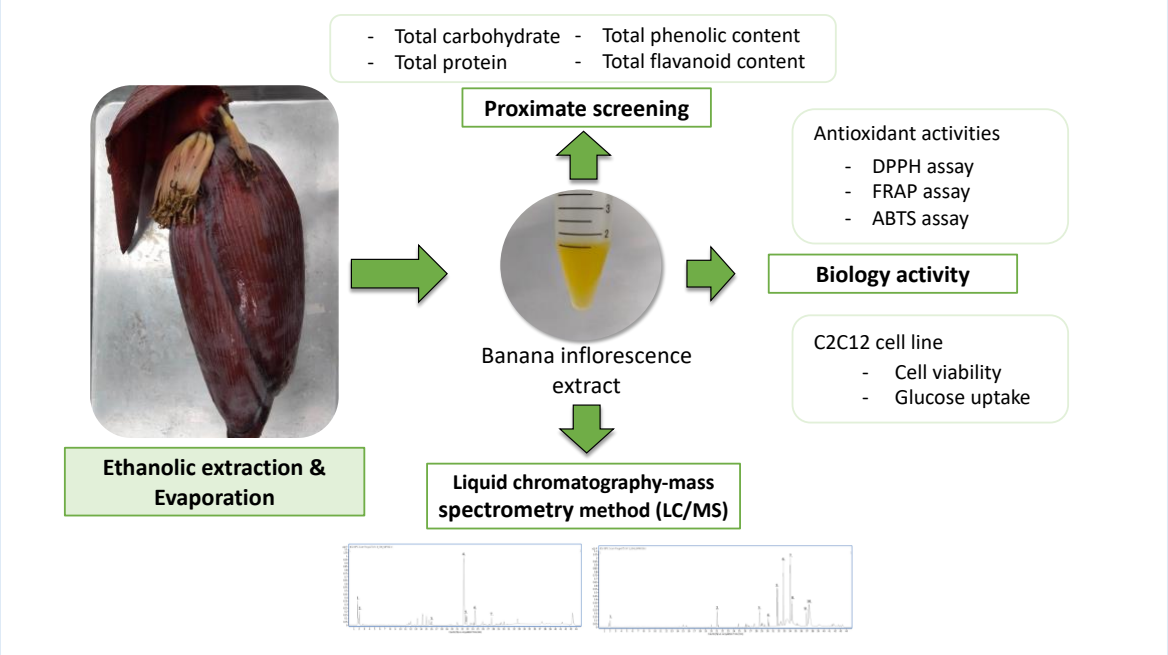
Background: Banana inflorescence is one of the edible parts of banana. It is well-known to have antioxidants and anti-diabetic potential health benefits.

Methods: In this study, the banana inflorescence from *Musa* (ABB group) Namwa Mali-Ong was prepared using ethanol extraction and investigated for its biochemical compositions and biological activities. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity assays were performed. The phytochemical composition of the extract was analyzed using the ultra-high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (UHLC-ESI-QTOF/MS) technique. Additionally, the ability of the extract to stimulate glucose in C2C12 myotube was investigated.

Results: The ethanolic extract of banana inflorescence contained carbohydrate, protein, phenolic, and flavonoid compounds. The results show that the extract exhibited low-level antioxidant activities. For example, the half-maximal inhibitory concentration (IC_{50}) in the DPPH and ABTS assays was at 27.89 ± 0.054 and 21.33 ± 0.87 mg/mL, respectively. Although the extract possesses low-level antioxidant activities, it stimulated glucose uptake in C2C12 myotubes in a dose-

and time-dependent fashion. Consistently, the LC-ESI-QTOF/MS analysis in both positive and negative electrospray ionization modes reveals several components in the extract such as phytosphingosine and α-linolenic acid that have previously been shown to exhibit an anti-diabetic activity.

Conclusion: The results show that the inflorescence ethanolic extract possesses antioxidant and anti-diabetic activities and may potentially be developed into a health-promoting product such as an anti-diabetic drug.



Keywords: antioxidant activity; banana inflorescence; glucose uptake; anti-diabetic; C2C12 myotubes

INTRODUCTION

Banana is an herbaceous flowering plant with an underground stem and a pseudostem. Bananas are widely consumed around the world. Banana inflorescence, which is also known as “banana blossom”, “banana flower”, or “banana heart” can be found at the end of the plant, extended from the pseudostem. Each plant develops only one inflorescence, which is a dark purple-red heart-shaped structure [1]. It is one of the edible by-products from bananas and is consumed as a vegetable, or used as an ingredient. Banana inflorescence is a good source of nutrients as it contains carbohydrates, proteins, lipids, vitamins, fibers and phytonutrients [1]. Also, banana inflorescence possesses many biological activities such as anti-microbial, anti-inflammatory, cardiovascular-protective, antioxidant, and anti-diabetic activities [1].

Among those biological activities, antioxidant and anti-diabetic activities of the banana inflorescence are the focus of this study. Antioxidants are usually small molecules that prevent the oxidizing damage done by free radicals and reactive oxygen species (ROS) from normal metabolic processes in the cells, or from external sources such as pollutants or chemicals [2]. These antioxidants can be found in various parts of the plant, including banana flowers and the entire inflorescence [3–5]. Likewise, anti-diabetic activity was found in both banana flowers and inflorescence. Diabetes mellitus is a chronic disorder characterized by an inability of the body to regulate glucose concentration in the blood, causing hyperglycemia or high blood glucose concentration. To date, several investigators have demonstrated an anti-

diabetic activity of extracts from banana flowers or inflorescence toward the inhibition of α-glucosidase and α-amylase enzyme, inhibition of diabetes in animals, or stimulation of glucose uptake in muscle cells [6–9]. Together, those findings illustrate the health benefits of banana flowers and inflorescence.

Musa (ABB group) Namwa Mali-Ong is an edible banana that belongs to the ABB banana cultivar group. This cultivar is well-known throughout Thailand as Thai people utilize all parts of the tree. The inflorescence of this banana is mainly used as a vegetable in many Thai dishes. Although many investigations have shown an anti-diabetic activity of banana inflorescence [6–9], the anti-diabetic activity of *Musa* (ABB group) Namwa Mali-Ong inflorescence has not been illustrated. Therefore, this study used an ethanolic extract of the entire inflorescence from *Musa* (ABB group) Namwa Mali-Ong to analyze its anti-diabetic activity. Phytochemical composition and the antioxidant activities of the extract are also discussed in this report.

MATERIALS AND METHODS

Preparation of fluorescence: The inflorescences from *Musa* (ABB group) Namwa Mali-Ong were obtained from a farm in Phatthalung, Thailand. The inflorescence was cut from the stem after the last banana comb was produced and within the 3 consecutive bracts discarded. The whole banana inflorescence was chopped into small pieces and dried in a hot air oven at 70°C. The dried inflorescence (approximately 80-90 g) was ground with a blender and stored in a humidity-controlled chamber or a desiccator until needed.

Ethanolic extraction: Dried banana inflorescence was placed in a 500 mL, or 1 L Duran bottle. Two hundred milliliters of absolute ethanol were added to the bottle. Extraction was performed by stirring a mixture for 12-16 hours at room temperature. The ethanolic extract was filtered through a Whatman No. 1 filter paper, and the residue was reextracted with ethanol nine more times or until the extract became colorless. Each filtrate was combined and evaporated to reduce the volume. The extract was stored in a -20°C freezer until needed, whereas the residue of the inflorescence was oven-dried and kept at -20°C for future use.

Total carbohydrate determination: Total carbohydrate determination method was determined using the phenol sulfuric acid assay [10]. Briefly, standard glucose was prepared by serially diluting to 3.125-100 µg/mL concentrations. A sample or standard glucose was mixed with a 5% aqueous solution of phenol (200 µL sample or standard: 200 µL 5% phenol) in a glass tube. The solution was mixed well with a vortex mixer. Subsequently, 1 mL of concentrated sulfuric acid was added to the reaction mixture. After a 30-minute incubation at room temperature, the absorbance at 470 nm was measured. The amount of carbohydrates was calculated based on the standard glucose.

Total protein determination: Bradford's assay was used to determine the concentration of a solubilized protein [11]. In brief, standard bovine serum albumin (BSA) was prepared by serially diluting to 100-1600 µg/mL concentrations. Fifty microliters of the sample, or standard were mixed with 2.5 mL Bradford solution in a glass tube. After a 20-minute incubation at room temperature, the mixture was transferred to a plastic cuvette and the absorbance at 595 nm was measured. The amount of protein was calculated based on the standard BSA protein.

Total phenolic content assay: The total phenolic content of the extract was determined using the Folin-Ciocalteau method [12] with some modifications. A solution of the ethanolic extract at 10-40 mg/mL was prepared by dissolving in absolute ethanol. Then, 20 µL of the extract and 40 µL of 10% of the Folin-Ciocalteau reagent were added and incubated for 6 minutes. The mixture was neutralized with 40 µL of 7.5% sodium carbonate solution (Na_2CO_3) and 100 µL of distilled water. After 1 hour of incubation at room temperature, the absorbance at 765 nm was immediately measured. The standard absorbance value was compared with gallic acid in the range between 25-100 µM. The results were expressed

as mg gallic acid equivalents (GAE)/g of extract.

Total flavonoid content assay: The total flavonoid content of the extract was determined using a modified colorimetric method [13]. In brief, 20 µL of extract, or a standard solution was added to 40 µL of 5% sodium nitrite ($NaNO_2$) solution and followed by 40 µL of 10% aluminum chloride ($AlCl_3$). Then, 100 µL of 1 M sodium hydroxide (NaOH) was added to the mixture. All reagents were mixed and incubated for 30 minutes at room temperature. After incubation, the absorbance at 510 nm was measured using a microplate reader. A calibration curve was prepared using quercetin in a concentration range from 25-300 µM. The results were expressed as mg of quercetin equivalents (QE)/ g of extract.

DPPH free radical scavenging assay: The DPPH scavenging activity of the extract was determined using the method previously described [14]. Briefly, 20 µL of the extract, or a standard solution were mixed with 180 µL of DPPH reagent in a 96-well plate. The mixture was incubated for 40 minutes at room temperature and protected from light. After incubation, the absorbance at 517 nm was measured using a microplate reader. Trolox was used as a positive control and to prepare a standard curve in a concentration range from 100-400 µM. The percentage of antioxidant activity was calculated as the percentage of the capture of the DPPH radicals, using the following equation:

$$\%\text{DPPH radical scavenging effect} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Then the scavenging activity was plotted with %DPPH scavenged versus concentration of standard antioxidant and the half-maximal inhibitory concentration (IC_{50}) value of the extract was calculated using linear regression analysis.

FRAP assay: The ferric reducing antioxidant power (FRAP) assay was performed as described by [15]. Briefly, 20 µL of the extract, or a standard solution were added directly to the 96-well microplate followed by 180 µL of working FRAP solution (a mixture of 300 mM acetate buffer pH 3.6: 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl):20 mM ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) at a ratio of 10:1:1, respectively, and warmed at 37°C for 10 minutes before using). The mixtures were incubated in the dark for 30 minutes. After incubation, the absorbance was measured at 593 nm using a microplate reader. Trolox solution in a concentration range from 100-700 µM was used to create a standard curve. The results were expressed as mM of $\pm$ equivalent (TE)/ g of extract.

ABTS radical scavenging assay: The method of ABTS radical scavenging activity was determined using the method described previously [16]. The $ABTS^{\cdot+}$ formation was prepared by mixing ABTS reagent with potassium sulfate in the ratio of 1:1 and then stirring overnight. Briefly, 20 µL of the extract was added to 180 µL of $ABTS^{\cdot+}$ working reagent in 96-well plate. All reagents were mixed and incubated for 15 minutes at room temperature in the dark. After incubation, the absorbance at 734 nm was measured with a microplate reader. A calibration curve was prepared using Trolox in a concentration range from 100-500 µM. The percentage of scavenging activity was calculated, using the following equation:

$$\%\ ABTS^{\cdot+}\text{ radical scavenging effect} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Then the scavenging activity was plotted with % $ABTS^{\cdot+}$ scavenged versus concentration of standard antioxidant and the IC_{50} value of the extract was calculated using a linear regression analysis.

Liquid chromatography-mass spectrometry method (LC/MS): The composition of banana inflorescence extract was analyzed using the ultra-high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (UHLC-ESI-QTOF/MS) technique with the 1290 Infinity II LC-6545 Quadrupole-TOF system (Agilent technologies). Briefly, the sample was resolved with Zorbax Eclipse Plus C18 Rapid Resolution HD column chromatography (150 mm

length × 2.1 mm inner-diameter, particle size 1.8 µm, Agilent technologies), which was connected to the ultra-high-performance liquid chromatography (UHPLC). The fractionated sample was subsequently subjected to the positive and negative charged ions. Mass spectrometry detection was formed with heated electrospray ionization (ESI) source, which is coupled with the UHPLC column. The mobile phase consisted of water and 0.1% formic acid (solvent A) and acetonitrile (solvent B). The sample was subjected to a gradient condition at a flow rate of 0.2 mL/min. The gradient elution was carried out in the following order: 95% of A for the first 4 minutes, 55% of A for 16 minutes, 5% of A for 20 minutes, and 95% of A for 5 minutes, respectively. The results were reported based on the molecular formula, mass to charge ratios (m/z) peak, retention time (RT). The specific compounds were identified by comparing the m/z ratio with that of the known compounds in the library and database.

Cell culture: C2C12 mouse myoblasts cell line was purchased from ATCC (CRL-1772) and grown in a 37°C incubator with 5% CO_2 using low-glucose, complete Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin antibiotics. Differentiation into myotubes and subsequent experiments were performed in a 96-well plate. Briefly, cells were seeded at a density of 5.0 x 10^3 per well and allowed to grow for two days or until they reached approximately 70-80% confluency. Then, the medium was changed to 2% horse serum (HS)-containing complete DMEM, which was replenished every two days. On the sixth day, the cells were differentiated into myotubes, which could easily be observed under a light microscope.

Cell viability assay: The viability of the treated cells was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) reduction assay [17]. Briefly, after washing cells with PBS, 0.5 mg/mL working solution of MTT dissolved in the complete DMEM supplemented with 2% HS was added to the cells. After a 2-hour incubation at 37°C and 5% CO_2, the medium was removed, and DMSO was added to the cells to solubilize the insoluble formazan crystals. The absorbance at 570 nm was measured and cell viability was determined using the following equation:

$$\% \text{ cell viability} = (Abs_{sample} / Abs_{control}) \times 100$$

Glucose oxidase assay: To access the glucose uptake ability of the cells, we measured the amount of glucose left in the medium after treatment. Thus, a low concentration of glucose in the medium suggests a high glucose uptake activity of the cells. The amount of glucose in the medium was measured using a glucose assay kit (Sigma-Aldrich) and the assay was performed as recommended by the manufacturer's instructions. The medium was collected for the glucose assay 24 hours after treatment. Insulin and metformin were included as positive controls. The percentage of stimulation was calculated using the following equation:

$$\% \text{ stimulation of glucose} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Statistical analysis: The experiments were performed at least three times independently and each data set was performed in triplicate. Statistical analysis was analyzed by using One-Way analysis of variance (ANOVA) with Duncan's multiple range test. Two datasets with different letters are significantly different with $p < 0.05$.

RESULT AND DISCUSSION

Ethanolic extraction of the inflorescences and characterization: In this study, we focused on the ethanolic extraction of 'Mali-Ong' banana inflorescence. We analyzed the ethanolic extracts from 5 inflorescences. The average percent dry weight of inflorescence, the weight of the extract, the percent total solid of the extract, and the percent yield of the extract are shown in Table 1. The variation of the wet weight of the inflorescence (1067-1508 g, data not shown) affects the amount of the extract. Because the percent total solid and the percent yield of the extract were

comparable, we used the extract from one inflorescence for all subsequent analyses and experiments.

Table 1. Percent dry weight of inflorescence, weight, total solid, and yield of the inflorescence extract. The value is shown as an average ± S.D. (n = 5)

Categories	Average amount
Dry weight of inflorescence (g/100 g)	8.30 ± 0.38
Weight of the extract (g)	7.30 ± 1.29
Total solid of the extract (g/100 g)	85.66 ± 1.90
Yield of the extract (g/100 g)	6.74 ± 0.62

The amount of total carbohydrate, total protein, total phenolic, and total flavonoid in the extract is shown in Table 2.

Table 2. Total carbohydrate, total protein, total phenolic, and flavonoid contents of the banana inflorescence extract. The value is shown as an average ± S.D. (n = 3)

Components	Amount
Total carbohydrate (g/100 g extract)	38.53 ± 3.00
Total protein (g/100 g extract)	7.90 ± 0.23
Total phenolic (mg GAE/ g extract)	8.06 ± 0.70
Total flavonoid (mg QE/ g extract)	4.79 ± 0.07

The extract shows a high composition of total phenolic and flavonoid compounds that are considered secondary metabolites and important sources of natural antioxidants. Both total phenolic and total flavonoid contents have been used as an indicator of antioxidant properties to inhibit oxidation reactions. A similar study that performed the ethanolic extraction from Kluai Namwa inflorescence has been done by Thaweesang and colleagues [4]. This study showed that the average total phenolic compounds extracted from florets and bracts were 1235.94 and 741.79 μg GAE/g extract, respectively. Therefore, the concentration of total phenolic compounds reported in this study is much higher than that reported by Thaweesang and colleagues. Nonetheless, there were many discrepancies between this study and the previous study. For example, Thaweesang and colleagues separated florets and bracts for the extraction. In addition, they did not perform the experiment to analyze the total flavonoid content. We noticed that the total phenolic compound in our extract was higher than the total flavonoids. This observation is consistent with another study that analyzed the ethanolic extract from the inflorescence of *Musa balbisiana* Colla [18].

Antioxidant activities of the extract: Many studies analyzed the antioxidant activities in many banana parts and in different species or cultivars [19–21]. DPPH, FRAP, and ABTS assays were used for the determination of antioxidant activities of the extract. The results of antioxidants from banana inflorescence extract are shown in Table 3 and Table 4. The % inhibition of DPPH and ABTS radical scavenging effect showed the antioxidant activity values quantified to the exact concentration in the range of 10-40 mg/mL. DPPH and ABTS assays have been widely used to estimate the free radical scavenging activity of plants and are considered a rapid method for investigating the antioxidant capacity. Antioxidant activities were calculated IC_{50} value for representing the concentration of the extract at 50% inhibition of free radical scavenging. The IC_{50} values of the extract were found to be 27.89 ± 0.054 and 21.33 ± 0.87 mg/mL for DPPH and ABTS assays, respectively. It was noticed that the antioxidant values of the extract by ABTS

were higher than the DPPH evaluation.

In FRAP assay, the reducing power of an antioxidant reacts with the Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+}-tripyridyl triazine (blue colored complex). FRAP activity of the extract was found to be 15.04 ± 1.03 µmole TE/ g of the extract.

Several studies have demonstrated the antioxidant activities of ethanolic extract from banana flowers or inflorescences. For example, a study showed that the IC_{50} values of the ethanolic extract from fresh and blanched floret extracts in the DPPH free radical scavenging assay were 1.27 and 1.30 mg/mL, respectively [4]. Another study showed the IC_{50} values of ethanolic extracts from the flowers of *Musa spp*. (Baxijiao) and *Musa Paradisiaca* in the DPPH, ABTS, and FRAP assays were within the concentration ranging from 2.12 and 5.84 µg/mL [22]. Thus, the antioxidant activities of the extract in this study are very low. Nonetheless, these results demonstrate the antioxidant activities of the ethanolic extract from the banana inflorescence.

Table 3. Antioxidant activities of the banana inflorescence extract. The values shown are the average ± S.D. (n = 3).

Concentration (mg/mL)	DPPH assay (%inhibition)	ABTS assay (%inhibition)	FRAP assay (µM TE)
10	18.70 ± 6.50	35.57 ± 0.80	197.50 ± 25.63
20	37.48 ± 4.51	48.85 ± 1.86	275.97 ± 10.34
40	70.50 ± 5.76	73.32 ± 1.45	463.35 ± 52.86

Table 4. IC_{50} values of the banana inflorescence extract. The values of the IC_{50} and the FRAP activity are shown as the average ± S.D. (n = 3).

Extract	Component analyzed		
	IC_{50} (mg/mL)		µmol TE/ g extract
	DPPH assay	ABTS assay	FRAP assay
Banana inflorescence	27.89 ± 0.54	21.33 ± 0.87	15.04 ± 1.03

Investigation of the chemical composition of the extract using LC/MS analysis: The chemical profiling of the extract was analyzed using the UHPLC-ESI-QTOF/MS technique. The sample was resolved in a C18 reverse phase column chromatography and subjected to the analysis in both positive and negative electrospray ionization modes. Base peak chromatograms from both modes are shown in Figure 1. Eleven and ten major compounds were identified from the positive and negative electrospray ionization modes, respectively (Table 5 and 6). Several types of compounds such as alcohol, amino alcohol, alkaloid, and fatty acids were identified. Some of those compounds were previously shown to have biological activities. For example, kolanone, a monoterpenoid, was shown to have antimicrobial activity [23,24]. In addition, α-linolenic acid, which was identified in the negative electrospray ionization mode, was shown to have an anti-diabetic activity [25]. Interestingly, the majority of the identified compounds that were reported to have an anti-diabetic activity were fatty acids. Thus, we hypothesized that our extract may have an anti-diabetic activity.

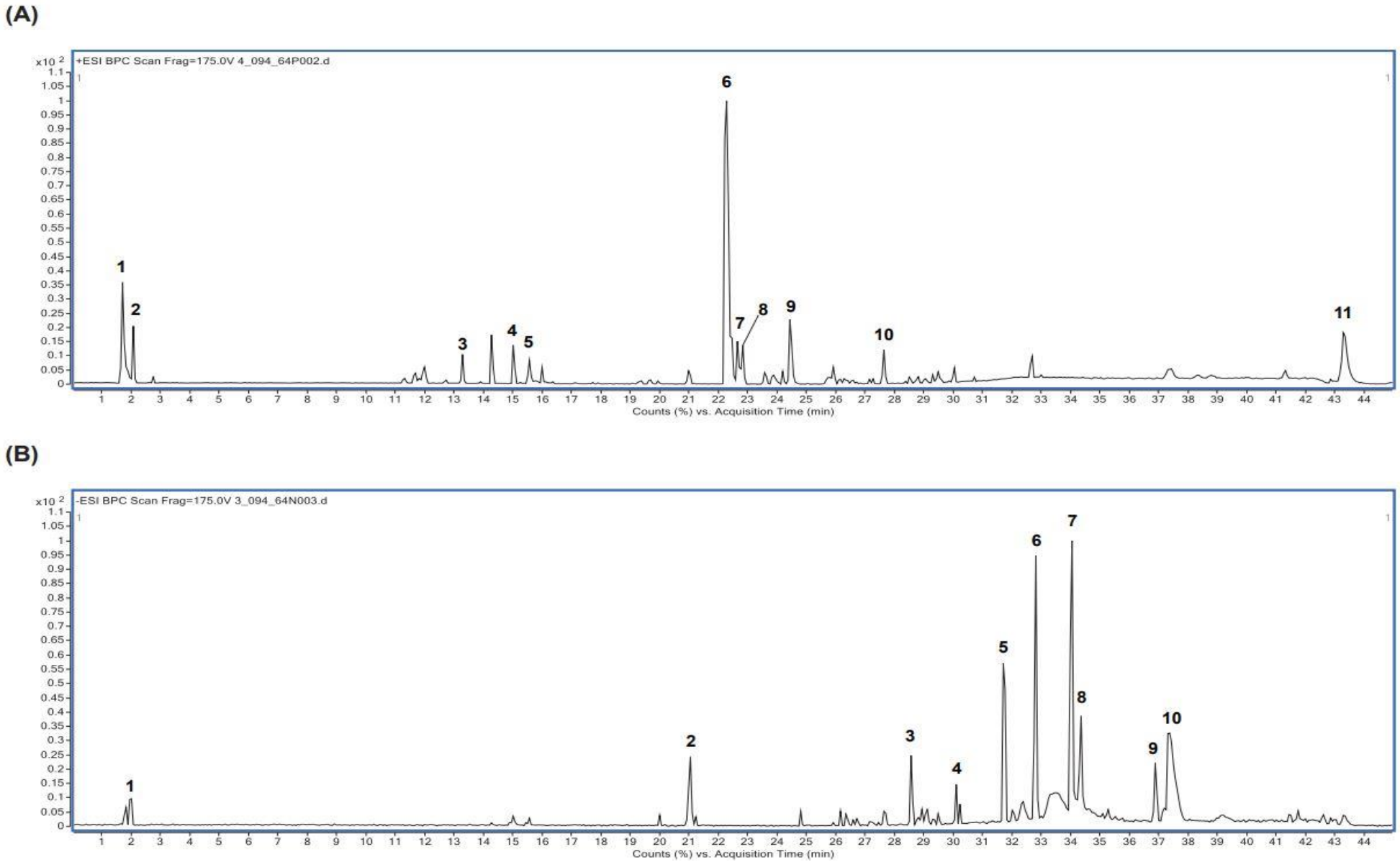


Figure 1. Base peak chromatogram (BPC) scan of the ethanolic extract from banana inflorescence. The analysis was done using the UHPLC-ESI-QTOF/MS. (A) shows BPC scan from the positive electrospray ionization mode. (B) shows BPC scan from the negative electrospray ionization mode.

Table 5. Compound, retention time, mass to charge ratio (m/z), type, and biological activities of the compounds identified in the mass spectrometry from the positive electrospray ionization mode UHPLC-ESI-QTOF/MS.

No.	Compound	Retention time(min)	Mass to charge ratio(m/z)	Type of the compound	Biological activities	References
1.	2-Amino-3-methyl-1-butanol	1.734	104.1073	amino alcohol	Antidiabetic?	[26]
2.	Adenine	2.126	136.062	purine nucleobase	Antidiabetic	[26]
3.	Solanocapsine	13.342	453.3438	steroid alkaloid	Antibacterial, Cytotoxic properties against HeLa cell lines	[27,28]
4.	L-4-Hydroxy-3-methoxy-a-methylphenylalanine	15.143	248.0896	aromatic L-alpha-amino acid	Not reported	-
5.	PI(O-16:0/16:0)	15.605	814.5788	phosphatidylinositol	Not reported	-
6.	Xestoaminol-C	22.497	230.2487	1,2-aminoalcohols	Antidiabetic?	[26]
7.	2-Hydroxyhexadecanoic acid	22.713	290.27	hydroxy fatty acid	Not reported	-
8.	3-Ethyltridecan-2-one	22.859	244.2644		Not reported	-
9.	Phytosphingosine	24.455	318.3013	amino alcohol	Antidiabetic	[26,29]
10.	Kolanone	27.695	520.3412	monoterpenoid	Antimicrobial	[23,24]
11.	1-Palmitoyl-2-linoleoyl PE	43.009	716.523	phosphatidylethanolamine	Not reported	-

Table 6: Compound, retention time, mass to charge ratio (m/z), type, and biological activities of the compounds identified in the mass spectrometry from the negative electrospray ionization mode UHPLC-ESI-QTOF/MS.

No.	Compound	Retention time(min)	Mass to charge ratio(m/z)	Type of the compound	Biological activities	References
1.	(R)-(+)-2-Pyrrolidone-5-carboxylic acid	2.091	128.0356	amino acid	Not reported	-
2.	11,12,13-trihydroxy-9-octadecenoic acid	21.068	329.2338	long-chain fatty acid	Not reported	-
3.	(±)12(13)-EpOME	28.537	295.2281	monounsaturated epoxy fatty acid	Not reported	-
4.	13(S)-HODE	30.148	295.2283	long-chain fatty acid	Not reported	-
5.	α-Linolenic Acid	31.825	277.2177	polyunsaturated fatty acid	Antidiabetic, Antioxidant	[25,30–33]
6.	Chaulmoogric acid	32.898	279.2337	monounsaturated long-chain fatty acid	Not reported	-
7.	Isopalmitic acid	34.122	255.2339	methyl-branched-chain fatty acid	Antioxidant	[33]
8.	Vaccenic acid	34.393	281.2487	trans fatty acid	Suppress intestinal inflammatory diseases, Antioxidant	[34]
9.	Stearic acid	36.94	283.2643	saturated long-chain fatty acid	Antidiabetic, Reduce cholesterol levels	[32,35]
10.	3R-hydroxy-eicosanoic acid	37.396	327.2906	long-chain fatty acid	Preventing chronic disease	[36]

An antidiabetic effect of the inflorescence extract: Identification of the compounds from the UHPLC-ESI-

QTOF/MS technique prompted us to investigate the anti-diabetic activity of the extract. To test the hypothesis, we analyzed whether the extract could stimulate glucose uptake in C2C12 muscle cells. Therefore, we first analyzed whether the extract has any effect on the viability of the cells by performing the MTT assay. The results show that C2C12 myotubes treated with the extract (0.125-0.5 mg/mL) had a viability greater than 95% (Figure 2). Insulin and metformin, which were included as positive controls, also allowed at least 95% cell viability (Figure 2). Thus, these results suggest that the inflorescence extract up to 0.5 mg/mL concentration does not affect the viability of the C2C12 myotubes.

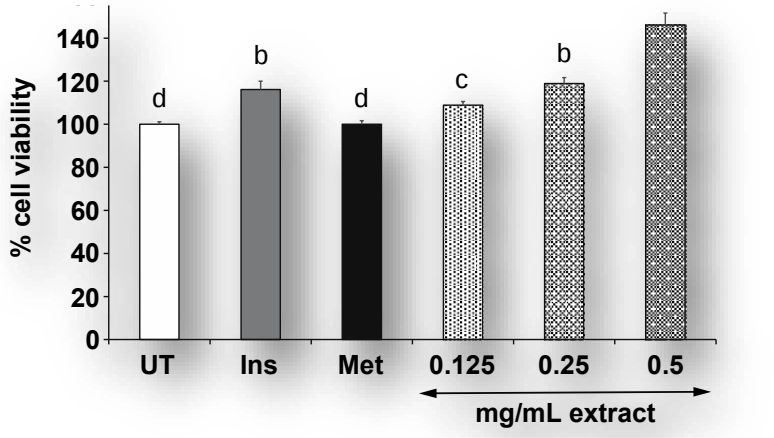


Figure 2. The ethanolic inflorescence extract does not affect the viability of C2C12 myotubes**.** The chart shows a representative dataset for the viability of C2C12 myotubes after treating the banana inflorescence extract. UT, Ins, and Met are abbreviated for untreated, insulin, and metformin, respectively. Ins and met were included as positive controls and used at 100 nM and 500 µM, respectively. Data are presented as mean values ± standard deviation (n =3).

The dose- and time-dependent effects of banana inflorescence extract on C2C12 myotubes were analyzed. We show that as the concentration of the extract increases, the glucose uptake activity of the cells also increases, suggesting the dose-dependent effect of the extract (Figure 3). Insulin and metformin, which are positive controls, also robustly stimulate glucose uptake into the cells (Figure 3). In addition, we analyzed the kinetics of glucose uptake activity induced by the extract. The results show that the extract at all concentrations used induced a significant stimulation of glucose uptake at 18 hours onward and continued to stimulate the glucose uptake until at least 24 hours (Figure 4), suggesting the time-dependent effect of the extract on the glucose uptake activity of the cells. Therefore, these data illustrate the anti-diabetic effect of the ethanolic extract from the banana inflorescence.

The effect of an ethanolic extract from banana inflorescence on C2C12 has not been illustrated. However, the effect of a methanolic extract from banana inflorescence on L6 myotubes has been shown. A study by Arun and colleagues showed that the methanolic extract from *Musa paradisiaca* stimulated 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino-2-deoxyglucose (2-NBDG) uptake by the L6 myotubes in a dose-dependent fashion [8], suggesting an anti-diabetic activity of the extract. This study also analyzed the phenolic compounds using high-performance liquid chromatography (HPLC) in the extract and found several phenolic compounds such as gallic acid, catechol, and chlorogenic acid [8]. Another study tested the ability of acetone, methanol, ethanol, and aqueous extracts from

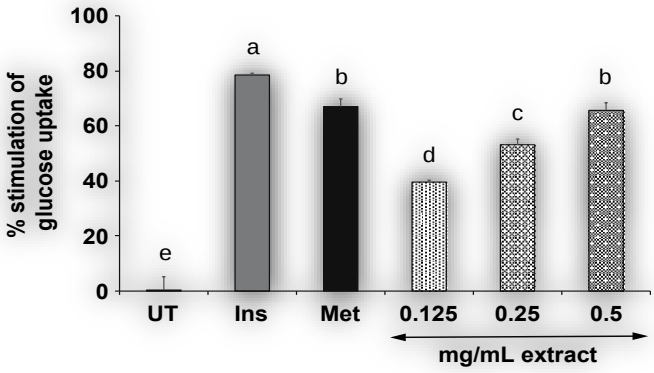


Figure 3. Banana inflorescence extract dose-dependently stimulate glucose uptake in C2C12 myotubes. The chart shows a representative dataset for the percent stimulation of glucose uptake in C2C12 myotubes after treating the banana inflorescence extract. UT, Ins, and Met are abbreviated for untreated, insulin, and metformin, respectively. Ins and Met were included as positive controls and used at 100 nM and 500 µM, respectively. Data are presented as mean values ± standard deviation (n =3)

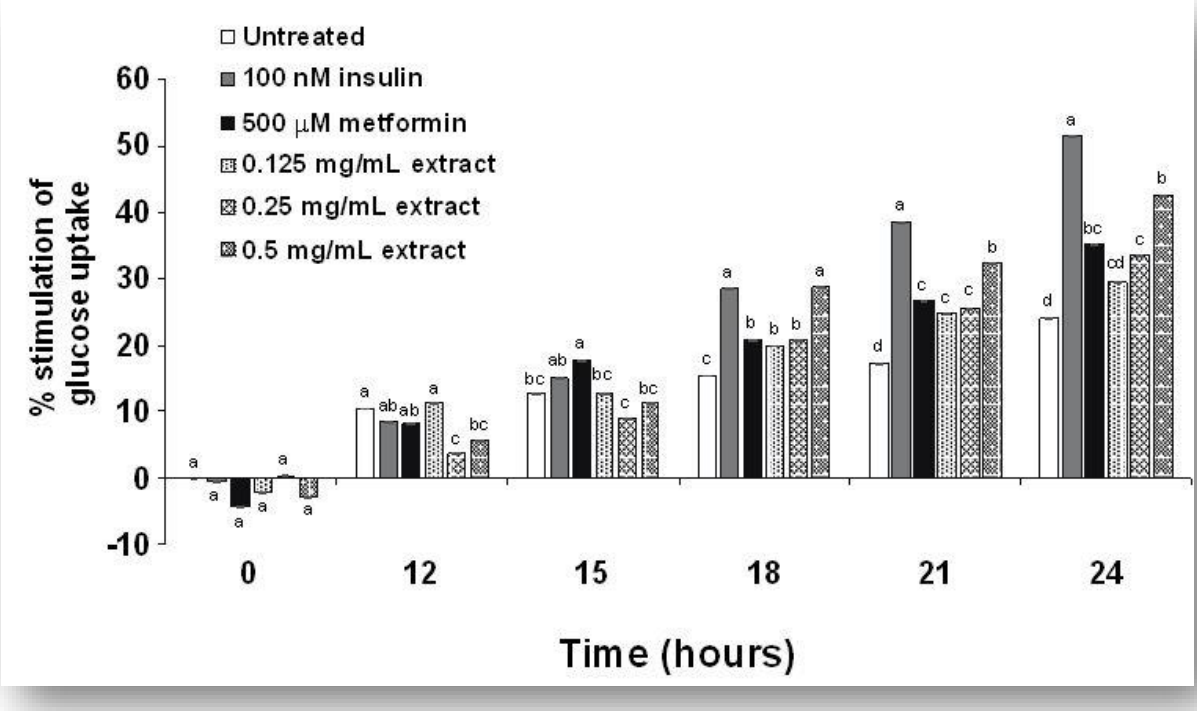


Figure 4. Banana inflorescence extract time-dependently stimulate glucose uptake in C2C12 myotubes. The chart shows a representative dataset for the percent stimulation of glucose uptake in C2C12 myotubes after treating the banana inflorescence extract at various time point. Statistical differences were analyzed among different treatments within the same time point. Data are presented as mean values ± standard deviation (n =3).

both flowers and stem of *Musa sp*. (cv. elakki bale) and found that all the extracts were able to stimulate 2-NBDG uptake in the Ehrlich ascites tumor (EAT) cells, suggesting that all the extracts possess an anti-diabetic activity [37]. Like the study by Arun and colleagues [8], Bhaskar and colleagues performed an analysis using the HPLC. They showed the common phenolic acids found in all the extracts are gallic acid and gentisic acid [37]. Gallic acid, the common phenolic compound found in both studies, has been shown to possess an anti-diabetic activity [38].

Therefore, it is highly likely that those phenolic acids might stimulate glucose uptake in those cells and could be the active compounds. Despite the findings, both studies have not performed the experiment to confirm that those phenolic acids were the *bona fide* anti-diabetic compounds in their extracts.

In contrast to the findings from those studies, the LC/MS analysis done in this study did not reveal the phenolic acid as the major component in the extract. However, some of the compounds identified in this study, including phytosphingosine and α-linolenic acid, were reported to have an anti-diabetic activity [25,29]. Therefore, phenolic acid and other compounds may contribute to the anti-diabetic activity of the extract. In this study, the actual anti-diabetic compounds in the extract might not be the phenolic acids. Identification of the anti-diabetic compounds in our extract remains to be explored in future experimentation.

CONCLUSION

In conclusion, the ethanolic extract of banana inflorescence from *Musa* (ABB group) Namwa Mali-Ong contains several phytochemicals including carbohydrate, protein, phenolic, flavonoid contents, and antioxidant activities. The phytochemical profiling of the LC/MS technique also showed that the extract found several compounds. Moreover, the extracts dose- and time-dependently stimulated glucose uptake in the C2C12 myotubes cell line. These results suggest that the crude ethanolic extract possesses the antioxidant and anti-diabetic activities. Identification of anti-diabetic component of the extract is of interest and will be investigated in future experimentations.

Competing Interests: The authors declare that there are no conflicts of interest.

Author's Contributions: P.A. performed all experiments, analyzed data, drafted the manuscript, and prepared figures and tables. S.W. helped with the LC/MS data interpretation, the antioxidant assays, and the phytochemical composition experiments. D.S. conceived the project, designed the experiments, acquired the research grant, analyzed and interpreted the data, revised all the figures, wrote, and edited the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Lau BF, Kong KW, Leong KH, et al. Banana inflorescence: its bio-prospects as an ingredient for functional foods. *Trends in Food Science & Technology*. 2020;97:14-28. https://doi.org/10.1016/j.tifs.2019.12.023
2. Kumar S, Sharma S, Vasudeva N. Review on antioxidants and evaluation procedures. *Chinese Journal of Integrative Medicine*. Published online October 6, 2017. https://doi.org/10.1007/s11655-017-2414-z
3. Chiang SH, Yang KM, Lai YC, Chen CW. Evaluation of the in vitro biological activities of banana flower and bract extracts and their bioactive compounds. *International Journal of Food Properties*. 2021;24(1):1-16. https://doi.org/10.1080/10942912.2020.1856134
4. Thaweesang S. Antioxidant activity and total phenolic compounds of fresh and blanching banana blossom (*Musa* ABB CV.Kluai "Namwa") in Thailand. *IOP Conference Series: Materials Science and Engineering*. 2019;639(1):012047. http://dx.doi.org/10.1088/1757-899X/639/1/012047

5. Amornlerdpison D, Choommongkol V, Narkprasom K, Yimyam S. Bioactive compounds and antioxidant properties of banana inflorescence in a beverage for maternal breastfeeding. *Applied Sciences*. 2020;11(1):343. https://doi.org/10.3390/app11010343
6. Ramu R, Shirahatti P, Dhanabal S, Zameer F, Dhananjaya B, Nagendra Prasad M. Investigation of antihyperglycaemic activity of banana (*Musa* sp. Var. Nanjangud rasa bale) flower in normal and diabetic rats. *Pharmacognosy Magazine*. 2017;13(51):417. https://dx.doi.org/10.4103%2F0973-1296.216331
7. Vilhena R, Fachi MM, Marson BM, et al. Antidiabetic potential of *Musa* spp. inflorescence: a systematic review. *Journal of Pharmacy and Pharmacology*. 2018;70(12):1583-1595. https://doi.org/10.1111/jphp.13020
8. Arun KB, Thomas S, Reshmitha TR, Akhil GC, Nisha P. Dietary fibre and phenolic-rich extracts from *Musa paradisiaca* inflorescence ameliorates type 2 diabetes and associated cardiovascular risks. *Journal of Functional Foods*. 2017;31:198-207. https://doi.org/10.1016/j.jff.2017.02.001
9. Borah M, Das S. Antidiabetic, antihyperlipidemic, and antioxidant activities of *Musa balbisiana* Colla. in Type 1 diabetic rats. *Indian J Pharmacol*. 2017;49(1):71-76. https://doi.org/10.4103/0253-7613.201030
10. Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F. A colorimetric method for the determination of sugars. *Dreywood, R, Indust Enq Chem, Anal Ed*. 1951;18(4):476. https://doi.org/10.1038/168167a0
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 1976;72:248-254. https://doi.org/10.1016/0003-2697(76)90527-3
12. Abdelhady MIS, Motaal AA, Beerhues L. Total phenolic content and antioxidant activity of standardized extracts from leaves and cell cultures of three Callistemon species. *American Journal of Plant Sciences*. 2011;02(06):847-850. https://doi.org/10.4236/ajps.2011.26100
13. China R, Dutta S, Sen S, et al. In vitro antioxidant activity of different cultivars of banana flower (*Musa paradicicus* L.) extracts available in India. *Journal of Food Science*. 2011;76(9):C1292-C1299. https://doi.org/10.1111/j.1750-3841.2011.02395.x
14. Mahmood A, Ngah N, Omar MN. Phytochemicals constituent and antioxidant activities in *Musa x paradisiaca* flower. *European Journal of Scientific Research*. 2011;66(2):311-318.
15. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "'antioxidant power'": the FRAP assay. *Analytical Biochemistry*. 1996;239:70-76. https://doi.org/10.1006/abio.1996.0292
16. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. Published online 1999:1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*. 1983;65:55-63. https://doi.org/10.1016/0022-1759(83)90303-4
18. Revadigar V, Al-Mansoub MA, Asif M, et al. Anti-oxidative and cytotoxic attributes of phenolic rich ethanol extract of *Musa balbisiana* Colla inflorescence. *Journal of Applied Pharmaceutical Science*. Published online 2017. https://doi.org/10.7324/JAPS.2017.70518
19. Adedayo BC, Oboh G, Oyeleye SI, Olasehinde TA. Antioxidant and antihyperglycemic properties of three banana cultivars (*Musa* spp.). *Scientifica (Cairo)*. 2016;2016:1-7. https://doi.org/10.1155/2016/8391398
20. Joseph J, Paul D, Kavitha MP, et al. Preliminary phytochemical screening and in vitro antioxidant activity of banana flower (*Musa paradisiaca* AAB Nendran variety). *Journal of Pharmacy Research*. 2014;8(2):144-147.
21. Apriasari ML, Iskandar, Suhartono E. Bioactive compound and antioxidant activity of methanol extract Mauli bananas (*Musa* sp) stem. *International Journal of Bioscience, Biochemistry and Bioinformatics*. 2014;4(2):110-115. https://doi.org/10.7763/IJBBB.2014.V4.321
22. Sheng ZW, Ma WH, Gao JH, et al. Antioxidant properties of banana flower of two cultivars in China using 2,2-diphenyl-1-picrylhydrazyl (DPPH,) reducing power, 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulphonate (ABTS) and inhibition of lipid peroxidation assays. *African Journal of Biotechnology*. 2011;10(21):4470-4477. https://doi.org/10.5897/AJB10.1659
23. Hussain R, Owegby A, Parimoo P, Waterman P. Kolanone, a novel polyisoprenylated benzophenone with antimicrobial properties from the fruit of *Garcinia kola*. *Planta Medica*. 1982;44(02):78-81. https://doi.org/10.1055/s-2007-971406
24. Madubunyi II. Antimicrobial activities of the constituents of *Garcinia kola* seeds. *International Journal of Pharmacognosy*. 1995;33(3):232-237. https://doi.org/10.3109/13880209509065369
25. Kato M, Miura T, Nakao M, Iwamoto N, Ishida T, Tanigawa K. Effect of alpha-linolenic acid on blood glucose, insulin and GLUT4 protein content of type 2 diabetic mice. *Journal of*

Health Science. 2000;46(6):489-492. https://doi.org/10.1248/jhs.46.489

26. Sandikapura MJ, Nyamathulla S, Noordin MI. Comparative antioxidant and antidiabetic effects of *Syzygium polyanthumleaf* and *Momordica charantia* fruit extracts. *Pak J Pharm Sci*. 2018;31(2(Suppl.)):623-635. https://dx.doi.org/10.1186%2Fs12906-019-2599-0
27. Fadl Almoulah N, Voynikov Y, Gevrenova R, et al. Antibacterial, antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species. *South African Journal of Botany*. 2017;112:368-374. https://doi.org/10.1016/j.sajb.2017.06.016
28. Dongre S.H., Badami S., Ashok G., Ravi S., Kottani R. In vitro cytotoxic properties of O-methylsolanocapsine isolated from *Solanum pseudocapsicum* leaves. Published online 2007:208. https://doi.org/10.4103/0253-7613.36542
29. Snel M, Sleddering MA, Pijl H, et al. The effect of dietary phytosphingosine on cholesterol levels and insulin sensitivity in subjects with the metabolic syndrome. *European Journal of Clinical Nutrition*. 2010;64(4):419-423. https://doi.org/10.1038/ejcn.2009.154
30. Comlekcioglu N. Bioactive compounds and antioxidant activity in leaves of endemic and Native *Isatis* spp in Turkey. *Brazilian Archives of Biology and Technology*. 2019;62. https://doi.org/10.1590/1678-4324-2019180330
31. Savych A, Marchyshyn S, Basaraba R. Determination of fatty acid composition content in the herbal antidiabetic collections. *Pharmacia*. 2020;67(3):153-159. https://doi.org/10.3897/pharmacia.67.e51812
32. Smorowska AJ, Żołnierczyk AK, Nawirska-Olszańska A, Sowiński J, Szumny A. Nutritional properties and in vitro antidiabetic activities of blue and yellow corn extracts: a comparative study. *Journal of Food Quality*. 2021;2021:1-10. https://doi.org/10.1155/2021/8813613
33. Khoo HE, Azlan A, Abd Kadir NAA. Fatty acid profile, phytochemicals, and other substances in *Canarium odontophyllum* fat extracted using supercritical carbon dioxide. *Frontiers in Chemistry*. 2019;7. https://doi.org/10.3389/fchem.2019.00005
34. Jacome-Sosa M, Vacca C, Mangat R, et al. Vaccenic acid suppresses intestinal inflammation by increasing anandamide and related N-acylethanolamines in the JCR:LA-cp rat. *Journal of Lipid Research*. 2016;57(4):638-649. https://doi.org/10.1194/jlr.M066308
35. Ahmad Z, Zamhuri KF, Yaacob A, et al. In vitro anti-diabetic activities and chemical analysis of polypeptide-k and oil isolated from seeds of *Momordica charantia* (bitter gourd). *Molecules*. 2012;17(8):9631-9640. https://doi.org/10.3390/molecules17089631
36. Maheshwari G, Ringseis R, Wen G, et al. Branched-chain fatty acids as mediators of the activation of hepatic peroxisome proliferator-activated receptor alpha by a fungal lipid extract. *Biomolecules*. 2020;10(9):1259. https://doi.org/10.3390/biom10091259
37. Bhaskar JJ, Mahadevamma S, Vishwanatha S, Salimath PV. Effect of banana (*Musa* sp. cultivar elakki bale) flower and stem on enzyme activities of intestinal and renal disaccharidases in streptozotocin-induced diabetic rats. *Journal of Food Biochemistry*. Published online March 2010. https://doi.org/10.1111/j.1745-4514.2009.00299.x
38. Xu Y, Tang G, Zhang C, Wang N, Feng Y. Gallic acid and diabetes mellitus: its association with oxidative stress. *Molecules*. 2021;26(23):7115. https://doi.org/10.3390/molecules26237115