Antioxidant and Antidiabetic Activities of Ethanolic Extract from the Inflorescence of *Musa* ABB cv. 'Kluai Namwa'

Piyapat Aiemcharoen

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Functional Food and Nutrition

Prince of Songkla University

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Author Miss Piyapat Aiemcharoen

Major Program Functional Food and Nutrition

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This is to certify that the work here submitted is the result of the candidate's own investigations. Due acknowledgement has been made of any assistance received.

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I hereby certify that this work has not been accepted in substance for any degree, and is not being currently submitted in candidature for any degree.

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ชื่อวิทยานิพนธ์ ฤทธิ์ต้านอนุมูลอิสระและต้านเบาหวานของสารสกัดเอทานอลจากปลีกล้วยน้ำว้า
ผู้เขียน นางสาวปิยาพัชร เอี่ยมเจริญ
สาขาวิชา อาหารสุขภาพและโภชนาการ
ปีการศึกษา 2565

บทคัดย่อ

ปลีกล้วยหรือหัวปลี จัดอยู่ในวงศ์ Musaceae เป็นที่ทราบกันดีว่ามีความสามารถในการลดปริมาณน้ำตาลในเลือดหรือควบคุมโรคเบาหวานได้ดี อย่างไรก็ตามยังไม่มีการศึกษาความสามารถของสารสกัดเอทานอลจากปลีกล้วยเพื่อกระตุ้นการใช้กลูโคสในเซลล์กล้ามเนื้อลาย การวิจัยนี้จึงทำการสกัด ศึกษาคุณลักษณะของสารสกัดเอทานอลจากปลีกล้วย และศึกษาฤทธิ์ต้านอนุมูลอิสระและฤทธิ์ต้านเบาหวานของสารสกัด โดยใช้ปลีกล้วยกล้วยน้ำวามะลิอ่อง ผลการทดลองแสดงให้เห็นว่าสารสกัดปลีกล้วยมีปริมาณองค์ประกอบของคาร์โบไฮเดรต โปรตีน สารประกอบฟีนอลิก ฟลาโวนอยด์ และกรดไขมัน อีกทั้งสารสกัดยังมีประสิทธิภาพในการต้านอนุมูลอิสระโดยใช้วิธี DPPH FRAP และ ABTS ค่าความเข้มข้นที่สารสกัดมีประสิทธิภาพในการยับยั้งที่ที่ 50% (IC_{50}) ในการทดสอบโดยวิธี DPPH และ ABTS แสดงให้เห็นที่ 27.89 ± 0.54 และ 21.33 ± 0.87 mg/mL. ตามลำดับ นอกจากนี้ ฤทธิ์ต้านอนุมูลอิสระที่วัดโดยใช้วิธี FRAP แสดงออกที่ 752.21 ± 17.09 mM TE/ g extract การทำโปรไฟล์ทางชีวเคมีของสารสกัดด้วยเครื่องลิควิดโครมาโตกราฟี พร้อมแมสสเปคโตมิเตอร์ ชนิดอิเล็คโตสเปรย์ควอดดรูโพล ไทม์ออฟไฟล์ท (UHLC-ESI-QTOF/MS) ที่มีประสิทธิภาพสูงในอิเล็กโทรสเปรย์โหมดไอออนบวกและไอออนลบ สารประกอบหลักทั้งหมดสิบและสิบเอ็ดชนิดสามารถระบุในโหมดไอออนบวกและไอออนลบ นอกจากนี้ สารสกัดยังแสดงให้เห็นความสามารถในการดูดซึมกลูโคสโดยขึ้นอยู่กับขนาดของความเข้มข้นในช่วงความเข้มข้น 0.125 -

0.5 มิลลิกรัม/ มิลลิลิตร และขึ้นกับระยะเวลาในการทดสอบการกระตุ้นเพื่อใช้กลูโคสในเซลล์กล้ามเนื้อชนิด C2C12 ผลลัพธ์เหล่านี้แสดงให้เห็นถึงกิจกรรมทางชีวภาพของสารสกัดเอทานอลแบบหยาบจากกล้วยน้ำว้ามะลิอ่อง ซึ่งในอนาคตมีความจำเป็นที่จะต้องทำการศึกษาต่อไปเพื่อพัฒนาสารสกัดให้เป็นยาต้านเบาหวานได้

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Major Program Functional Food and Nutrition

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ABSTRACT

Banana inflorescence is known as banana flower, banana blossom, or banana heart that belongs to the Musaceae family. It is widely known to have a great potential for control of diabetes. However, the ability of an ethanolic extract from banana inflorescence to stimulate glucose uptake in skeletal muscle cell line has not been illustrated. Therefore, extraction and characterization of ethanolic extract from banana inflorescence was performed. Additionally, the antioxidant and antidiabetic activities of the extract were investigated. The inflorescence from 'Kluai Namwa Mali-Ong' was used in this study. These results showed that the banana inflorescence extracts contained carbohydrate, protein, phenolic, flavonoid, and fatty acids. The extract also exhibited antioxidant activities in 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging (DPPH), ferric ion reducing antioxidant power (FRAP), and 2,2'-azinobis (3- ethylbenzothiazoline- 6- sulphonic acid) free radical scavenging activity (ABTS) assays. The inhibitory concentration of the extract to scavenge 50% (IC_{50}) values in the DPPH and ABTS assays were 27.89 $\pm$ 0.54 and 21.33 $\pm$ 0.87 mg/mL, respectively. In addition, the antioxidant activity in the FRAP assay was 752.21 $\pm$ 17.09 mM TE/g extract. The biochemical profiling of the extract was characterized using the ultra-high-performance liquid chromatography-electrospray ionization quadrupole time-of-flight

mass spectrometry (UHLC-ESI-QTOF/MS) technique in both positive and negative electrospray ionization modes. A total of ten and eleven major compounds in the positive and negative-ion modes were identified. Moreover, the extract showed a dose-dependent effect in a concentration range from 0.125 – 0.5 mg/mL and time-dependent effect on a stimulation of glucose uptake in C2C12 myotubes without cytotoxicity to the cells. These results suggest the biological activities of the crude ethanolic extract from 'Kluai Namwa Mali-Ong'. Future experimentations will be required to develop the extract into an antidiabetic drug.

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Piyapat Aiemcharoen

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LIST OF ABBREVIATIONS AND SYMBOLS

%	=	Percent
°C	=	Degree Celsius
ABTS	=	2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate)
BPC	=	Base peak chromatogram
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
et al.	=	et ali (Latin) and colleagues
ESI	=	Electrospray ionization
FAME	=	Fatty acid methyl ester
FID	=	Flame ionization detector
FRAP	=	Ferric reducing antioxidant power
g	=	Gram
GAE	=	Gallic acid equivalent
GDM	=	Gestational diabetes mellitus
GC	=	Gas chromatography
IC_{50}	=	The half maximal inhibitory concentration
L	=	Liter
LC	=	Liquid chromatography
MS	=	Mass spectrometry
m	=	Meter
M	=	Molar
mg	=	Milligram
min	=	Minute

mL	=	Milliliter
mm	=	Millimeter
mM	=	Millimolar
MTT	=	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
m/z	=	Mass to charge ratio
µg	=	Microgram
µL	=	Microliter
µm	=	Micrometer
µM	=	Micromolar
MUFA	=	Monounsaturated fatty acid
nm	=	Nanometer
nM	=	Nanomolar
pH	=	Potential of hydrogen ion
PUFA	=	Polyunsaturated fatty acids
QE	=	Quercetin equivalent
rpm	=	Revolution per minute
ROS	=	Reactive oxygen species
RT	=	Room temperature
T1DM	=	Type 1 diabetes mellitus
T2DM	=	Type 2 diabetes mellitus
TE	=	Trolox equivalent
SFA	=	Saturated fatty acids
UHPLC	=	Ultra high performance liquid chromatograph

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURES

Banana (*Musa* spp.) tree belongs to the Musaceae family. Banana is one of the top ten exported fresh fruits production in Thailand and is also classified as one of the most important economical fruit crops in the world (Rattanapan & Ounsaneha, 2020). In Thailand, banana trees can be found everywhere, and there are more than fifty varieties of the plants. After cultivation, other parts of the banana are used for different purposes. Banana inflorescence, a dark purple-red heart shaped structure, is a by-product of banana cultivation. It is also known as the "banana flower", "banana blossom", or "banana heart".

Banana inflorescence of Kluai Namwa Mali-Ong (ABB genome type) cultivar, an ancient Thai banana, can be found in Thailand and Southeast Asia. This cultivar is a hybrid variety of two wild types of banana: *Musa acuminate* (AA) and *Musa balbisiana* (BB). Many studies reported a good source of nutritional benefits from banana inflorescence as it commonly contains carbohydrate, protein, lipid, vitamin, mineral, fibers, and natural phytonutrients (Sidhu and Zafar 2018; Lau et al., 2020). Also, it possesses several biological activities such as antimicrobial, anti-inflammatory, cardiovascular protective, antioxidant, antidiabetic activities, antiulcer, and anti-HIV activities (Pushpaveni et al., 2019; Lau et al., 2020).

Banana inflorescence could be a major source of antioxidants that can scavenge free radicals and confer protection from oxidative stress. In addition, antidiabetic activity can be found in banana inflorescence (Jawla et al., 2012; Sheng et

al., 2014; Ramu et al., 2015). Some compounds, especially polyphenols with an antidiabetic effect have been identified from different cultivars of inflorescences (Sheng et al., 2011; Ramu et al., 2014; Vilhena et al., 2018). Nowadays, several investigators have demonstrated an antidiabetic activity of extracts from banana flowers or inflorescence in both *in vitro* and *in vivo* studies by stimulating glucose uptake in muscle cells or inducing diabetes in rats (Jawla et al., 2012; Ramu et al., 2014; Krishnan and Sinija 2016; Arun et al., 2017). Together, those findings illustrate the health benefit of banana flowers and inflorescence. Although many investigations have shown the antioxidant and antidiabetic activities of banana inflorescence extract, the information on the composition of active compounds of *Musa* ABB cv. Kluai Namwa with antidiabetic effect is limited. Therefore, this study used an ethanolic extract of the banana inflorescence from 'Kluai Namwa Mali-Ong' variety to analyze its phytochemical composition, antioxidant activities and the antidiabetic effect on C2C12 myotubes.

Review of Literature

1. Banana (*Musa* spp.)

Banana plant, a herbaceous flowering plant, has an underground stem and a pseudostem and grows well in a humid tropical climate with high temperature and humidity conditions in the regions of Central and South America, Africa, and Southeast Asia. This plant is widely consumed around the world. The Banana Market Review (2020) estimated that in 2020, the highest shipments were approximately 16.5 million tonnes from Latin America and the Caribbean with lower exports from Asia (4.4 million tonnes) and Africa (0.6 million tonnes). In addition, the imports of bananas remained relatively stable at around 19.8 million tonnes (Food and Agriculture Organization of the United Nations, 2021).

1.1 Taxonomy of banana

The scientific classification of the cultivated varieties is separated from the general taxonomy of bananas. There are ten levels of banana classification these different levels are shown in Figure 1-1.

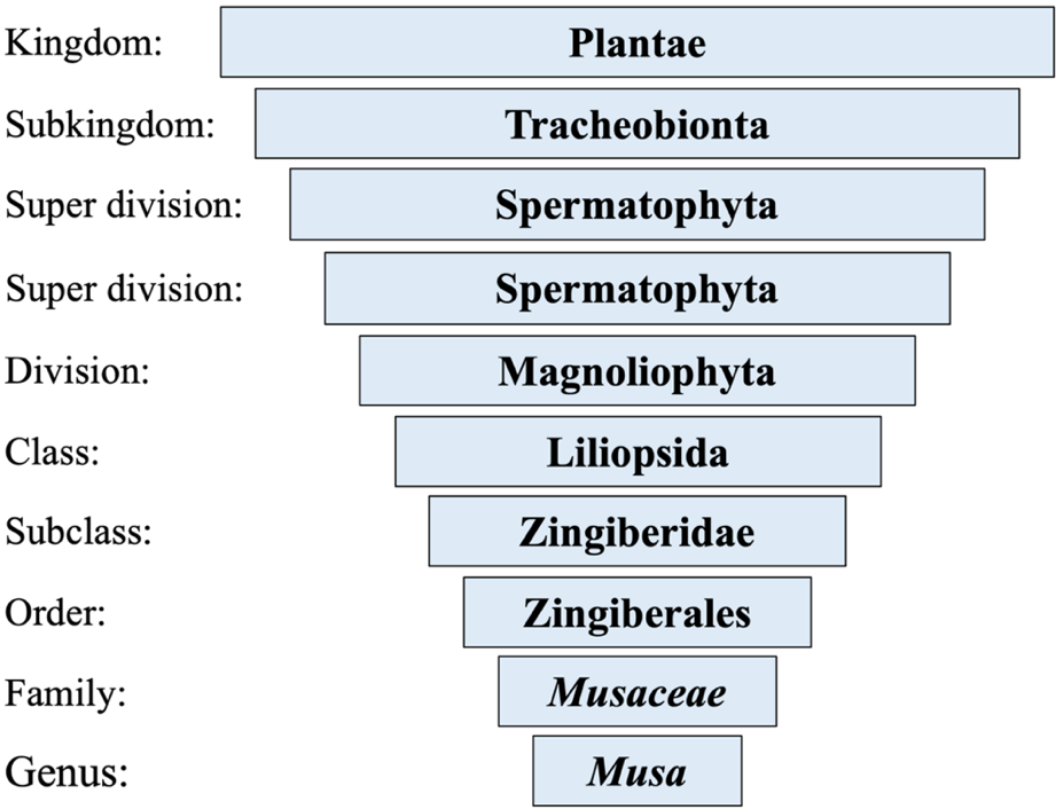


Figure 1-1. The scientific taxonomy of banana plant (Qamar and Shaikh, 2018)

1.2 Genomic classifications of banana

Banana belongs to *Musa* species can be divided into different groups according to the chromosome numbers and ploidy as well as the characteristics of the two wild types: *Musa acuminata* (genome type A, AA) and *Musa balbisiana* (genome type B, BB) (Martin et al., 2017). These two varieties are primitive edible bananas and are endemic to Southeast Asia and Western Pacific region. These are important species used for developing banana cultivars that give rise to the various major genomic groups.

There might be more than 100 varieties of banana plants in Thailand. Different cultivars of banana are classified for different purposes. For example, banana can be used as a dessert or consumed as a fresh fruit or a cooked food. A dessert banana

is one of the edible bananas that are eaten fresh upon ripening. The genomic classification of bananas is shown in Figure 1-2. which are divided into the wild and hybrid type. Two genetic types of wild type are including the diploid of AA and BB and the three types of hybrid (*Musa* x *paradisiaca*) groups are AAB, ABB, and AABB (Valmayor et al., 2000).

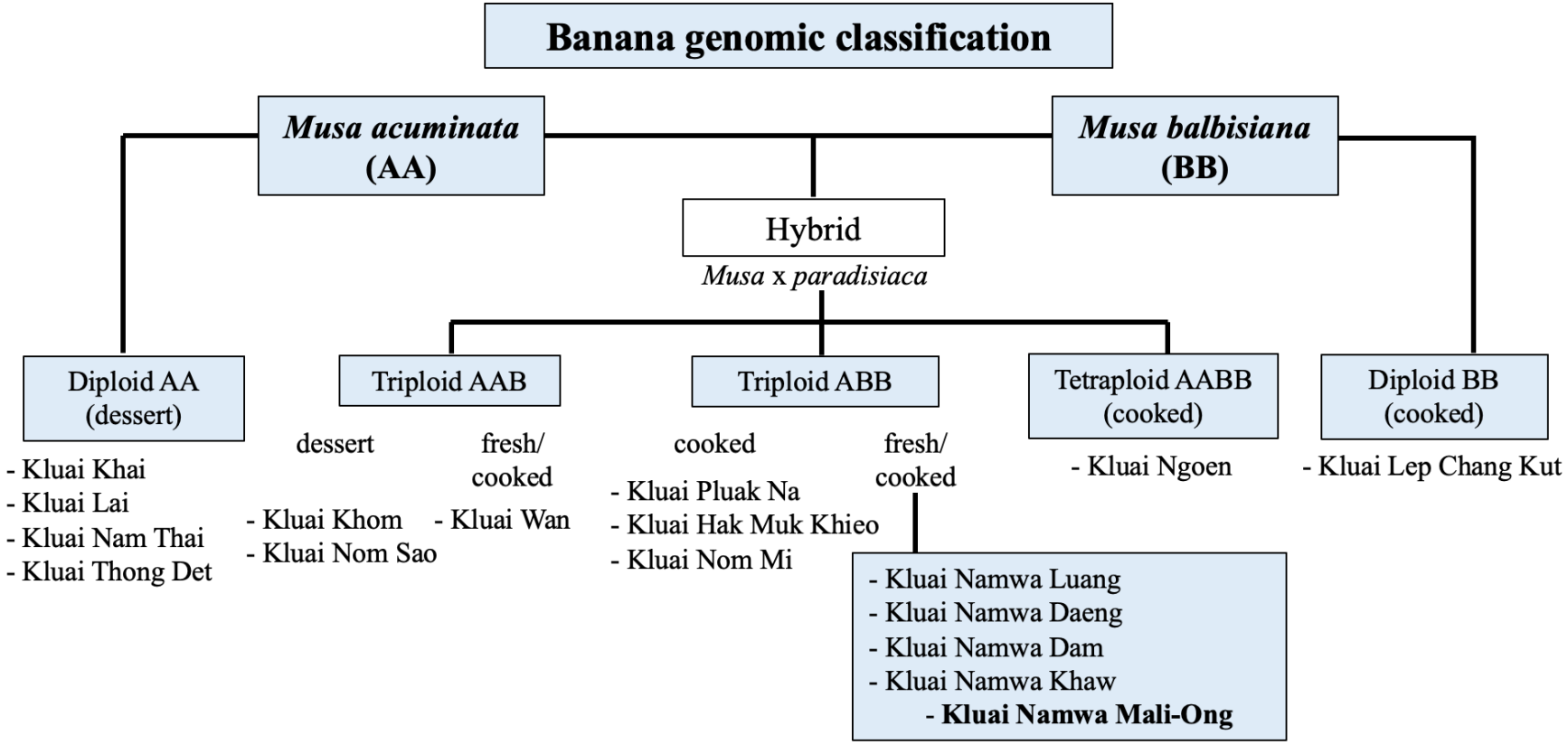


Figure 1-2. Banana genomic and cultivars classification in Thailand (Adapted from (Valmayor et al., 2000)

1.3 Banana inflorescence

Each banana plant develops only one inflorescence. The structure of an inflorescence is shown in Figure 1-3. It consists of a stalk and many bracts between rows of flowers that are arranged in a spiral row (Fahn, 1953). The inflorescence produces terminally at the end of the plant as an extension from the pseudostem. In an inflorescence, male and female flowers are found. The male flowers have normal stamens which form normal pollen for a reproduction function, while the female flowers have an ovary which develops into banana fruits.

Kluai Namwa (ABB genome type) is well-known and widely consumed throughout Thailand. All parts of the tree can be utilized. People consumed banana inflorescence or used it as a vegetable or an ingredient in salad, curries, and soup for a long time (Padam et al., 2014; Pushpaveni et al., 2019).

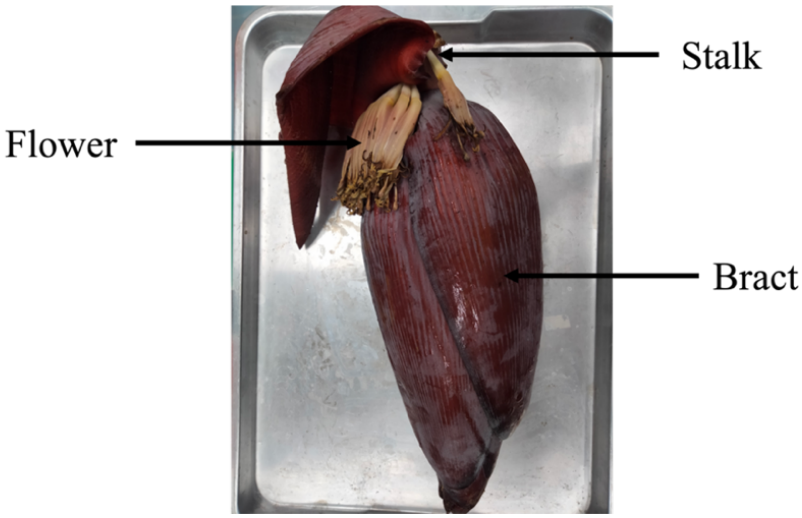


Figure 1-3. The structure of the banana inflorescence

1.4 Nutritional value of banana inflorescence

Banana inflorescence contains several nutrient constituents such as carbohydrate, protein, fat, vitamins, and minerals (such as phosphorous, calcium, potassium, copper, magnesium, and iron). It is rich in dietary fiber, which can be found in both the outer and inner bracts (Begum and Deka, 2019). Many studies used banana flowers and inflorescence to determine the proximate composition of the extract (Jayamurthy et al., 2011; Swe, 2012; Schmidt et al., 2015; Krishnan and Sinija, 2016; Ramu et al., 2017; Basumatary and Nath, 2018). The proximate compositions of the banana inflorescence from different cultivars and plant materials are shown in Table 1-1. Overall, the nutritional values of the banana inflorescence revealed various compositions containing macronutrients and micronutrients. In addition, the banana inflorescence seems to have high moisture contents. Accurate moisture content is an important and widely used measurement in the quality control of the extract.

Table 1-1. Proximate composition of banana inflorescences

Parameter	*Musa acuminata*	*Musa cavendishii*	*Musa* spp. (Poovan)	*Musa* spp. (Monthan)	*Musa* spp. (Nanjangud Rasa Bale)	*Musa balbisiana*	*Musa* spp. (Baxijiao)	*Musa* spp. (Paradisical)	*Musa sapientum*	*Musa paradisiaca* (Phee kyan)	*Musa chiliocarpa* (Thee hmwe)
Origin	Brazil	Brazil	India	India	India	India	China	China	India	Myanmar	Myanmar
Carbohydrate (g/100 g)	ND	ND	95.23	95.61	53.78	10.18	91.39	90.80	69.45	ND	ND
Crude protein (g/100 g)	1.79	1.46	1.99	1.43	19.60	1.79	2.07	1.62	6.23	13.28	15.82
Crude fat (g/100 g)	0.43	0.39	0.43	0.54	5.79	0.54	0.40	0.60	6.23	0.60	0.40
Ash (g/100 g)	1.56	1.09	3.21	2.42	6.51	1.71	1.19	1.24	11.80	6.37	7.20
Moisture (g/100 g)	91.00	90.73	90.10	90.23	8.33	85.44	90.58	89.42	ND	9.37	7.36
Crude fiber (g/100 g)	ND	4.83	12.82	12.42	70.07	7.68	4.96	5.74	6.21	0.08	1.15
Reference	Fingolo et al., 2012	Schmidt et al., 2015	Krishnan and Sinija, 2016	Krishnan and Sinija, 2016	Ramu et al., 2017	Basumatary and Nath, 2018	Sheng et al., 2011	Sheng et al., 2011	Jayamurthy et al., 2011	Swe, 2012	Swe, 2012

ND; not determine

2. Maceration method of banana inflorescence

Maceration extraction is a very simple and popular extraction method based on a solid-liquid extraction. The whole plant needed to be ground into small pieces and soaked in an organic solvent and frequently stirred (Abubakar and Haque, 2020). The mixture then is filtered to isolate the extract from residues.

Solvent extraction is the most frequently used to extract bioactive compounds from medicinal plants. The solvent for extraction should be chosen based on the chemical compounds contained within the plants (Qamar and Shaikh, 2018). Generally, alcohol is widely used to extract plant substances. Ethanol extraction is one of the techniques used in plant extraction because ethanol can be consumed. Ethanol can extract both polar and non-polar plant constituents, including hydrophilic or lipophilic molecules. Examples of the chemical compounds found in the ethanolic extract of flowers or inflorescence from different banana cultivars are shown in Table 1-2.

Table 1-2. Summary of the ethanolic extract of banana studies (Adapted from Lau *et al*., 2020)

Banana cultivars	Classification	Part of banana	Chemical components	Biological activities	References
Musa spp. Baxijiao and Paradisiaca	AAA/AAB	Flower	Total phenolics, flavonoids, saponins, vitamin E	Antioxidant activities	Sheng et al., 2011
Musa paradisiaca	ND	Flower	ND	Antimicrobial and antihyperglycemic activities	Jawla et al., 2012
Musa spp. var. Nanganjud rasa bale	ND	Flower	Umbelliferone and lupeol	Antidiabetic activities	Ramu et al., 2014
Musa spp. Baxijiao	AAA	Flower	Vanillic acid, ferulic acid, β-sitosterol, daucosterol, 9-(4′-hydroxylphenyl)-2-methoxyphenalen-1-one	Antidiabetic activities	Sheng et al., 2014
Musa spp. var. Nanjangud rasa bale	ND	Flower	Polyphenols, steroids	Antimicrobal and cardiovascular protective	Ramu et al., 2015
Musa cavendishii	AAA	Inflorescence (male flower and bract)	Total phenolics and flavonoids	Antioxidant activities	Schmidt et al., 2015
Musa spp. Poovan and Monthan	ABB	Flower	Total phenolics and flavonoids, vitamin E	Antioxidant activities	Krishnan and Sinija, 2016
Musa balbisiana	BB	Inflorescence	Total phenolics and flavonoids	Antioxidant activities	Revadigar et al., 2017
Musa spp.	ND	Flower	β-sitosterol, 31-norcyclolaudenone, and (24R)-4α, 14α 4-trimethyl-5α-cholesta-8, 25(27)-dien-3β-ol	Antidiabetic activities	Sheng et al., 2017

ND; not determine

3. Phytochemical compounds in banana inflorescence extract

A traditional medical plant containing beneficial phytochemicals may be converted to natural plant-derived natural products. The banana plant is well-known for several health benefits. Many various parts of the banana plants, including banana inflorescence, synthesize secondary metabolites such as phenolic acids, polyphenols, and flavonoids which act as antioxidants (Pandey and Rizvi, 2009).

Several *in vitro* activities of banana inflorescence extract using polar and non-polar organic solvents have been studied. Polyphenols are a class of natural small molecules that are widely occurring in the extract of banana flowers and inflorescence (Joseph et al., 2014; Krishnan and Sinija, 2016; Arun et al., 2017). Polyphenols are secondary substances that can broadly be classified in three classes; phenolic acid, flavonoids, and non-flavonoids (Figure 1-4). In previous studies, the phytochemical compounds of banana inflorescence presented a wide range of polyphenols and the polar solvents extracts showed higher polyphenol contents than the non-polar solvent extracts (Do et al., 2014; Thouri et al., 2017). Thus, the most efficient extraction depended on the solvent system.

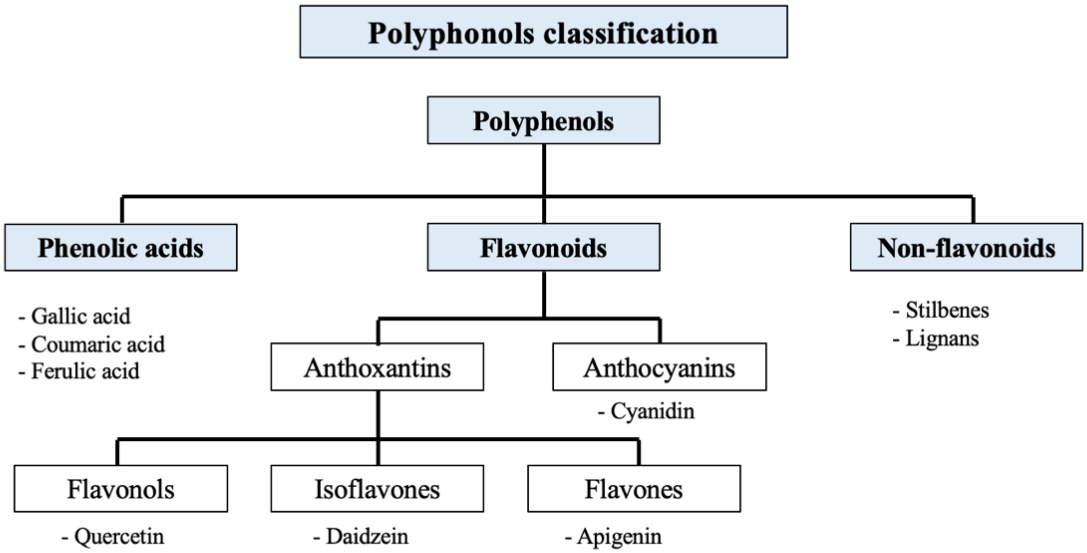


Figure 1-4. The classification of polyphenol (Adapted from Beconcini et al., 2020)

4. Biological activities of banana inflorescence

There are many the biological activities of banana inflorescence, including antioxidant and antidiabetic activities. Herein, the antioxidant and antidiabetic activities will be discussed.

4.1 Antioxidant activities

Antioxidants are usually small molecules or coenzymes that trap or neutralize free radicals and reactive oxygen species (ROS), which can potentially cause oxidative damage to cells or tissues. Antioxidants can be classified into two major types: endogenous antioxidants and exogenous antioxidants based on their activities (Figure 1-5). The endogenous antioxidants, which are products from the metabolism of body, are categorized into primary and secondary antioxidants. The system of endogenous antioxidants directly or indirectly contributes to defending against ROS. Examples of the primary antioxidant enzymes are superoxide dismutase (SOD),

catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). The secondary antioxidants are known to be scavengers of ROS, including glutathione (GSH), coenzyme Q10 (CoQ10), thioredoxin (TRX), ubiquinone, uric acid, lipoic acid, and bilirubin (Simioni et al., 2018). The other class of antioxidant, exogenous antioxidants, can be found in foods or supplements. There are many natural antioxidants in vegetables and fruits that have an important role of nutrition in human health (Vona et al., 2021). For example, vitamins, polyphenol, and fatty acids are antioxidants in vegetables.

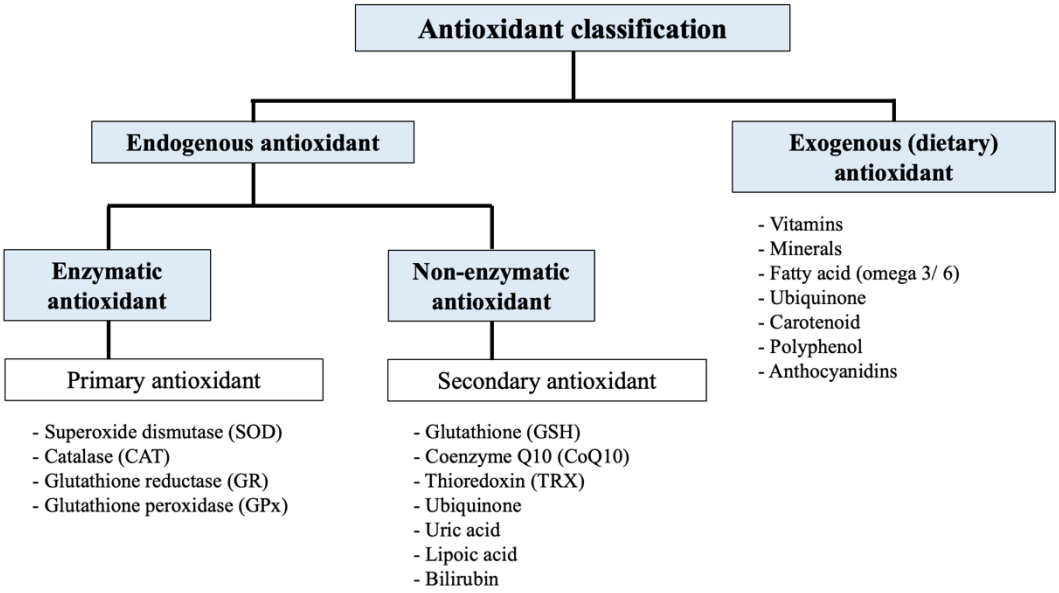


Figure 1-5. Scheme of antioxidant classification

Banana inflorescence contained several polyphenol compounds including β-sitosterol, flavonoids, saponin, catechin, and isoquercetin (Amornlerdpison et al., 2020). These compounds are known to have antioxidant activities. Epidemiological studies have shown that the intake of dietary antioxidants significantly

reduces the risk of diseases often caused by oxidative stress, such as diabetes, cancer, and cardiovascular disease (Nisha and Mini, 2014; Dal and Sigrist, 2016). Therefore, banana inflorescence is a good source of antioxidants and it may be used as a functional food to prevent oxidative diseases.

4.2 Antidiabetic activities

Diabetes mellitus (DM) is a chronic metabolic disease caused by uncontrollable elevated blood glucose levels. The prevalence of DM is currently one of the worldwide health problems that tend to increase over the long-term duration. Diabetes is characterized by hyperglycemia as one of the main symptoms of diagnosis (Ramu et al., 2015). There are three main types of diabetes: type 1, type 2 , and gestational diabetes. Type 1 diabetes mellitus (T1DM), also known as chronic autoimmune diabetes, is an endocrine disorder. It is caused by the destruction of the pancreatic β cells, creating an insulin deficiency condition of T1DM, resulting in an increase of blood glucose (hyperglycemia) (Katsarou et al., 2017; DiMeglio et al., 2018). Type 2 diabetes mellitus (T2DM), is the most common type of diabetes which is often developed from insulin resistance. Insulin resistance is characterized by a reduced response of the body to insulin stimulation of target tissues such as the liver, muscle, and adipose tissue (Freeman and Pennings, 2020). The last main type, gestational diabetes mellitus (GDM), occurs when a body cannot produce enough insulin during pregnancy. The risk of GDM can increase the development of T2DM after giving birth. However, this type usually disappears if the condition is detected early and well managed (Egan et al., 2020).

Medicinal plants have great potentials to confer health improvement and prevent disease for a long time. The possible antidiabetic activities of the ethanolic extract of banana inflorescence have also received a lot of records of its use for diabetes treatment. Several studies have utilized both *in vitro* and *in vivo* approaches. For example, Arun and colleagues reported the phytochemicals-rich extract of *M. paradisiaca* banana inflorescence promoted glucose uptake in L6 myotubes in a dose-dependent manner (Arun et al., 2017). The methanolic extract of the banana inflorescence from *Musa paradisiaca* is used to study the antidiabetic effect for stimulation and insulin production in the mice models (Ojewole and Adewunmi, 2003). Ramu and colleagues demonstrated that the crude extracts of banana inflorescences from *Musa* sp. var. Nanjangud rasa bale and isolated umbelliferone and lupeol compounds show varying degrees of inhibitory effect toward α-glucosidase and α-amylase (Ramu et al., 2014). Moreover, previous *in vivo* studies, either using alloxan or streptozotocin which is widely used to induce experimental diabetes in animals model, showed that the banana inflorescence extracts from several cultivars are useful in lowering blood glucose and preventing diabetic complications (Pari and Umamaheswari, 2000; Bhaskar et al., 2011; Nisha and Mini, 2014). Therefore, banana inflorescence could potentially be used as an antidiabetic functional food.

Research objectives

1. To perform ethanol extraction with banana inflorescences.
2. To identify the chemical composition of the banana inflorescence using techniques in phytochemistry.
3. To determine the antioxidant activities of the banana inflorescence extract.
4. To evaluate the toxicity of the banana inflorescence extract on C2C12 myotubes.
5. To investigate the effect of the banana inflorescence extract on glucose uptake activity of C2C12 myotubes.

CHAPTER 2

MATERIALS AND METHODS

1. Materials and Equipment

1.1 Instruments

Instruments	Model	Company
Autoclave	HA-300M	Hirayama/ TOMY Digital Biology
Biological Safety Cabinet (BSC)	Safemate 1.2 (Class II)	EuroClone®
CO_2 incubator	SafeGrow 188	EuroClone®
Desiccator		WEIFO®
Duran bottle		SCHOTT Duran®
Electronic balance (2 digits)	PG5002-S	Mettler Toledo®
Electronic balance (4 digits)	AL-204	Mettler Toledo®
Electronic pipette		Corning®
Erlenmeyer flask		PYREX®
Filter paper No.1		Whatman
Filter units and bottle top filters	Nalgene™	Thermo Scientific™
Glass tube		PYREX®
Hemocytometer		BOECO
High-speed refrigerated centrifuge	Allegra 64R	Beckman Coulter™
High-speed refrigerated centrifuge	MX307	TOMY Digital Biology
Hot air oven		Binder
Hot plate stirrer	C-MAG HS7	IKA®
Incubator		Memmert
Magnetic stirrer with stir bar		Clifton Laboratory
Micropipette		Corning®

Microplate reader	Synergy HT	BioTek®
Microplate reader	SPECTROstar Nano	BMG LABTECH
Microscope	CKX53	Olympus
Pasteur pipette		Nest biotechnology
Pipette tip		Corning®
pH meter	713	Metrohm
Reservoirs, multiwell 8 channels		Socorex
Rotary evaporator	Hei-VAP	Heidolph
Serological pipettes		Nunc™
Syringe		Nipro
UV-Visible spectrophotometer	G20	Thermo Scientific™
Vortex mixer	G-560E	Scientific industries
Water bath		Grant Instruments™

1.2 Materials

Chemicals	Company
1,1-diphenyl-2-picrylhydrazyl (DPPH)	Sigma-Aldrich
2,2'-azinobis-(3-ethylbenzothaizoline-6-sulfonic acid)	Sigma-Aldrich
2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)	Sigma-Aldrich
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)	Sigma-Aldrich
Absolute ethanol	Labscan
Acetic acid	Qrec
Ascorbic acid	Sigma-Aldrich
Bovine Serum Albumin (BSA)	Sigma-Aldrich
C2C12 myoblast cell line	ATCC® CRL-1772™
Coomassie Brilliant Blue G-250	Sigma-Aldrich
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich
Dulbecco's Modified Eagle's Medium (DMEM)	Gibco™
Fetal Bovine Serum (FBS)	Invitrogen
Ferric chloride hexahydrate	Sigma-Aldrich
Folin-Ciocalteu's phenol reagent	Sigma-Aldrich
Glucose	Sigma-Aldrich
Gallic acid	Sigma-Aldrich
Glucose (GO) assay kit	Sigma-Aldrich
Horse serum (HS)	Gibco™
Hydrochloric acid (HCl)	Sigma-Aldrich
Methanol	Labscan
Penicillin/ streptomycin antibiotic	Gibco™
Phenol detached crystals	Loba Chemie
Phosphate-Buffered Saline (PBS), 10X	Thermo Fisher Scientific™
Phosphoric acid	J.T. Baker®

Potassium persulfate	Sigma-Aldrich
Quercetin	Sigma-Aldrich
Sodium bicarbonate ($NaHCO_3$)	Sigma-Aldrich
Sodium carbonate (Na_2CO_3)	Carlo ERBA
Sulfuric acid (H_2SO_4)	J.T. Baker®
Trypsin-EDTA solution	Gibco™
Trolox	Sigma-Aldrich

2. Methods

In this study, the laboratory experiments were encompassed with the ethanolic extraction, determination of bioactive compounds, and biological activity. The following brief laboratory experiment diagram as shown in Figure 2-1.

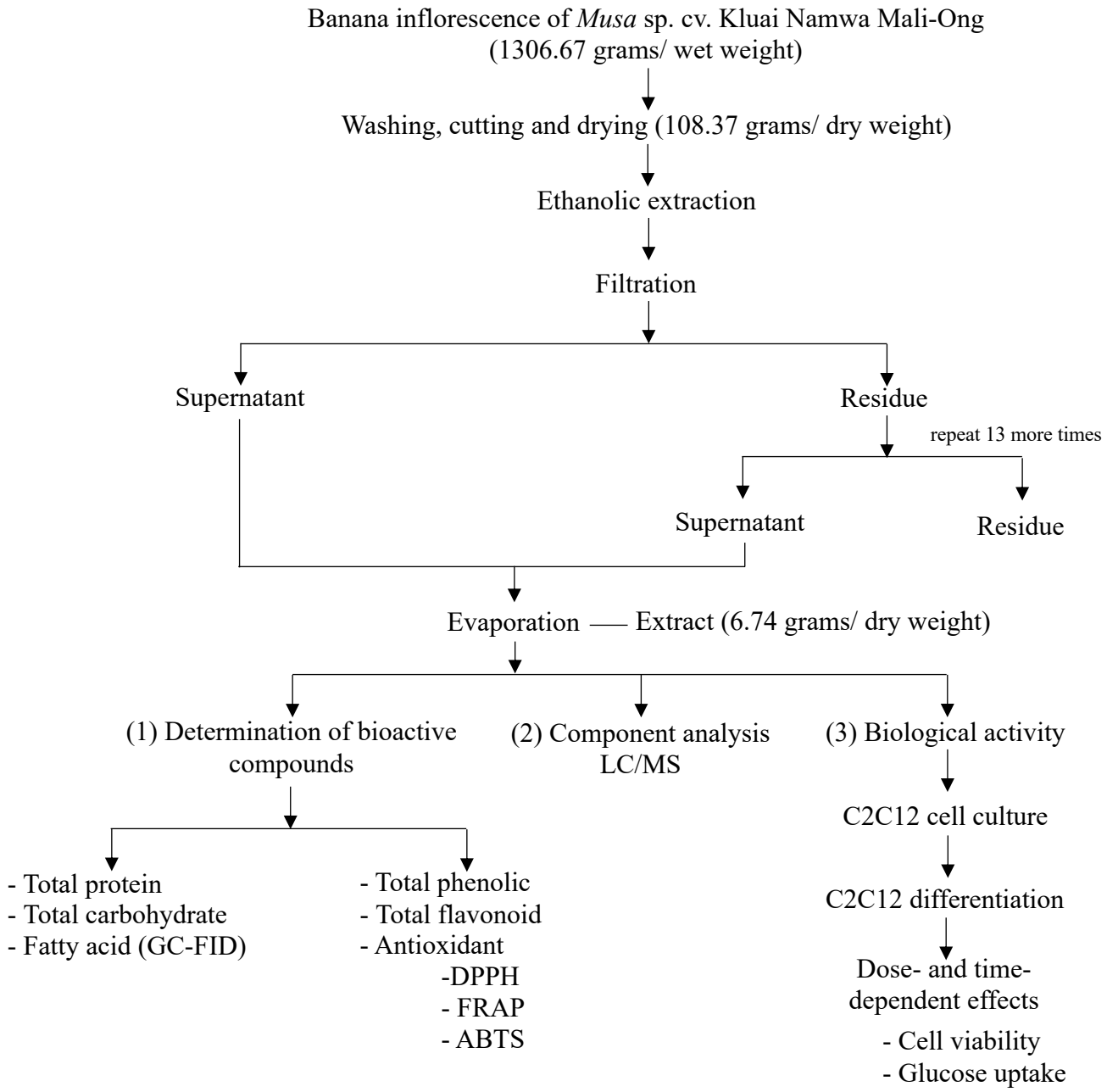


Figure 2-1. Schematic diagram of laboratory experiment

Part 1. Characterize bioactive compounds from banana inflorescence

1.1 Banana inflorescences preparation

Banana inflorescences used in this study were ‘Mali-Ong’ Kluai Namwa from Chuenkamon Garden in Phatthalung, Thailand in 2021. The whole banana inflorescences (including the bracts and flowers) were washed and chopped into small pieces and dried at 70°C in a hot air oven for 3 days. The dried inflorescences approximately 100-110 g were ground into a powder using a dry blender then kept in a air-tight plastic bag and stored in a desiccator before extraction.

1.2 Extraction of banana inflorescence

Dried banana inflorescences from 1.1 were placed in a 1 L Duran bottle and extracted using a maceration process. In this study, the dried banana inflorescences were extracted with absolute ethanol at room temperature. Two hundred milliliters of absolute ethanol were added to the Duran bottle and the residue from process was re-extracted for 13 extraction times. Extraction was performed the condition by stirring a mixture for 12-16 hours. A total of 1.65 liters of ethanol was used for extraction. The extracts were further filtered through a Whatman No. 1 filter paper. The obtained extraction were stored in a cold room then further evaporate under reduced pressure, whereas the residue after extraction was dried and kept in a desiccator for future use.

1.3 Rotary evaporation

The extraction of banana inflorescences was concentrated using a rotary evaporator to remove the solvent. The temperature was kept at 50°C and the vacuum

was set at 100 mbar. The evaporation was performed until the volume of the extract was not changed. The recovery percentage was calculated using the equation 1 (Fatiha et al., 2012). The extracts were collected in a vial and kept in a -20°C freezer until needed.

Calculation

$$\text{Yield of extract (\%).} = \frac{\text{weight of extract (g)}}{\text{weight of dried banana inflorescence used (g)}} \times 100 \quad (1)$$

1.4 Total protein determination

Total protein concentration was determined using the Bradford's assay (Bradford, 1976). In brief, bovine serum albumin (BSA) was used as a standard protein. The standard protein was prepared at a concentration range from 100-1600 µg/mL. The standard BSA was kept on ice at all times. The 5X Bradford solution was prepared by dissolving 100 mg Coomassie Brilliant Blue G-250 in 47 mL absolute methanol, followed by an addition of 100 mL 85% (w/v) phosphoric acid. Then, the final volume was adjusted to 200 mL with distilled water. The 5X Bradford solution was diluted to 1X solution and was mixed well with each sample and standard (2.5 mL Bradford reagent: 50 µL sample or standard BSA) in a glass tube. Twenty minutes after the incubation at room temperature, the mixture was transferred to a plastic cuvette. Each sample was measured the absorbance at 595 nm using a UV-Visible spectrophotometer. The estimation of total protein content of the extract was calculated based on the standard curve of BSA protein.

1.5 Total carbohydrate determination

Method for total carbohydrate determination was modified from Dubois and colleagues (Dubois et al., 1951). Briefly, five percent phenol (200 µL) was added to 200 µL sample or standard glucose solution (3.125-100 µg/mL) in a glass tube. After vortexing, 1 mL of concentrated sulfuric acid (H_2SO_4) was added and the mixture was incubated at room temperature for 30 minutes. Then, the absorbance at 470 nm for each sample was measured. The concentration of total carbohydrates in the sample can be calculated from the standard curve of glucose.

1.6 Total phenolic determination

The method for measuring total phenolic compounds was modified from a previous publication (Abdelhady et al., 2011). The ethanolic extract of banana inflorescence was dissolved in absolute ethanol to 10-40 mg/mL concentrations. Then, 40 µL of 10% Folin-Ciocalteau reagent was added to 20 µL of the extracts. After incubating for 6 minutes, 40 µL of 7.5% sodium carbonate solution (Na_2CO_3) and 100 µL of distilled water were added to the reaction mixture. Then, the incubation was continued in the dark for 1 hour at room temperature. After incubation, the absorbance at 765 nm was measured using a microplate reader. Gallic acid was used as a standard solution at the concentration range from 25-100 µM, and the results were reported as mg gallic acid equivalents (GAE)/ g of extract.

1.7 Total flavonoid determination

The method of quantifying total flavonoid content (TFC) in banana inflorescence extract was performed as previously described (Mahmood et al., 2011). Briefly, 20 µL of the ethanolic extract or a quercetin (standard solution at 25-300 µM) was mixed with 40 µL of 5% sodium nitrite ($NaNO_2$), 40 µL of 10% aluminium chloride ($AlCl_3$), and 100 µL of 1 M sodium hydroxide (NaOH). Absolute ethanol was used as a reagent blank. After vortexing and incubating at room temperature for 30 minutes, the absorbance at 510 nm was measured using a microplate reader. The results of total flavonoid contents were calculated as mg of quercetin equivalents (QE)/ g of extract.

1.8 Fatty acid analysis using gas chromatography with flame ionization detection (GC-FID)

The composition of volatile compounds of the extract was analyzed using Gas Chromatograph-Flame Ionization Detector (GC-FID) with Agilent Technologies (GC 7820A, USA). The fatty acids were converted to fatty acid methyl esters (FAMEs) by transesterification of fats with methanol. FAMEs was transferred to gas chromatography vials, and the condition of GC was equipped with the FID detector and the column. The dimensions of the column were 30 m x 0.32 mm x 0.25 µm. Total run time was 27 minutes with the following conditions: the initial oven temperature was 265°C, the detector temperature was 300°C, the injection volume was 1.0 µL with the flow rate 1.0 mL/min, and the split ratio was 50:1 for the injection. The identification of fatty acid constituents was identified by matching their mass GC-FID spectra with the known compounds in the library and database data.

1.9 Antioxidant determination

1.9.1 DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical molecule and has a dark-colored crystal. DPPH assay is a common antioxidant assay based on electron transfer (ET). In principle, antioxidant compounds convert DPPH radical to a more stable DPPH molecular product by a donating hydrogen atom, changing from a purple color compound into a yellow color compound (Figure 2-2), which can be measured by the absorbance at 517 nm (Sadeer et al., 2020; Xiao et al., 2020).

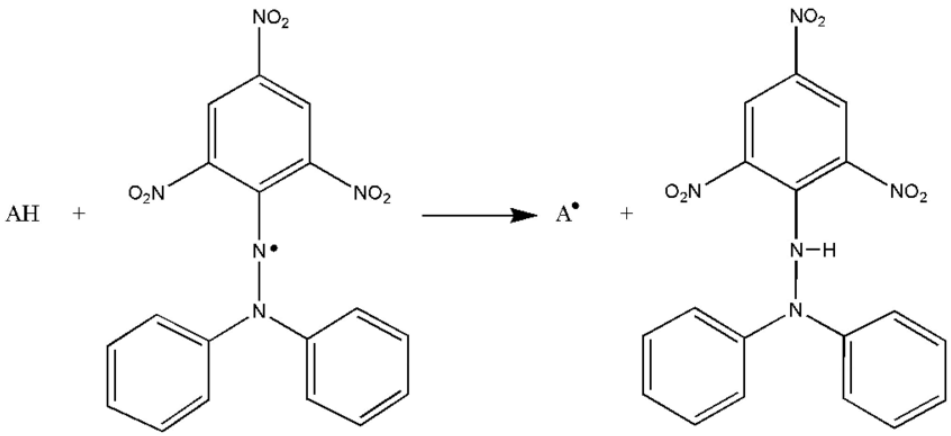


Figure 2-2. The reaction between DPPH radical and antioxidant to form DPPH (Sadeer et al., 2020)

The free radical scavenging activity of banana inflorescence extract was measured as described previously (Mahmood et al., 2011). Briefly, 20 µL of the banana inflorescence extract or Trolox (standard solution at 100-300 µM) was mixed with 180 µL of DPPH reagent in a 96-well plate. After incubating in the dark for 40 minutes at room temperature, the absorbance at 517 nm was measured. The antioxidant activity of the extract was reported as percent of inhibition (equation 2).

Calculation

% Inhibition = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$ (2)

Where, $Abs_{control}$ = absorbance of DPPH radical + methanol

Abs_{sample} = absorbance of DPPH radical + sample extract

The scavenging activity was plotted with the percent inhibition of DPPH scavenged versus concentration of standard curve of Trolox concentration. The IC_{50} value (the inhibitory concentration of the ethanolic extract provided to scavenge 50% of DPPH free radical scavenging activity) of the extract was also calculated.

1.9.2 FRAP assay

The FRAP assay is based on the ability of an antioxidant pH at 3.6 to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to a blue form color of ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) complex (Figure 2-3). The reaction of FRAP can be measured at 593 nm to test the potential of iron reduction and hydrogen atom transfer (Xiao et al., 2020).

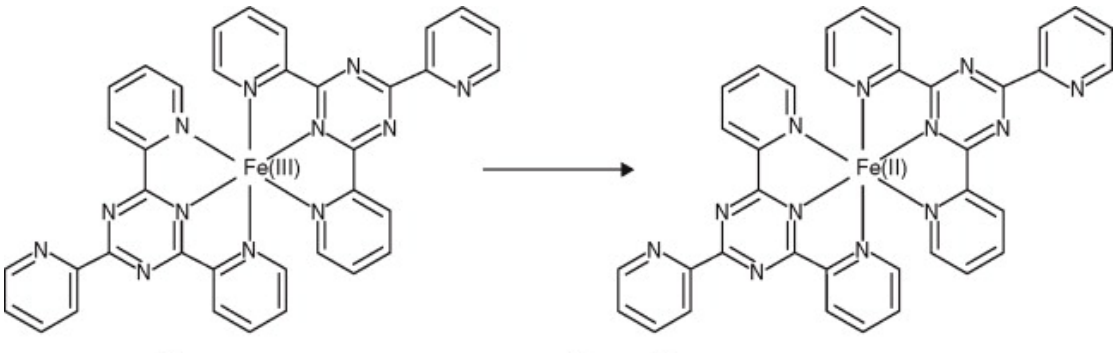


Figure 2-3. The reaction between Fe^{3+}-TPTZ complex and antioxidant to form Fe^{2+}-TPTZ complex (Sadeer et al., 2020)

Briefly, 20 μL of the banana inflorescence extracts (10-40 mg/mL), or a standard solution was added directly to the 96-well microplate, followed by 180 μL of working FRAP solution. The FRAP working solution was prepared by mixing 300 mM acetate buffer at acidic conditions (pH 3.6), 10 mL TPTZ in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1. The FRAP working solution was prepared daily and warmed at 37°C for 10 minutes before use. The mixtures were shaken and incubated for 30 minutes at room temperature in the dark condition. After incubation, the absorbance was measured at 593 nm with a microplate reader. Absolute methanol was used as a reagent blank. Trolox solution was used as a standard in a concentration range from 100-700 μM. The results were expressed as mg of Trolox equivalent (TE)/ g of extract. The antioxidant capacity of extracts was determined based on increasing in Fe (II)-TPTZ absorbance by calculating the percentage of antioxidant capacity. The FRAP values were calculated and expressed as mM Trolox equivalent (TE) per gram extract.

1.9.3 ABTS radical scavenging assay

The ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate)) assay is a colorimetric assay for analyzing the ability of anti-oxidation. This assay is based on the relative ability of antioxidants to scavenge the ABTS radical. The ABTS cation radical is created by the oxidation of ABTS by potassium persulfate, and the cation can absorb the light at 743 nm (Figure 2-4). The color changed from bluish-green color to pale blue color.

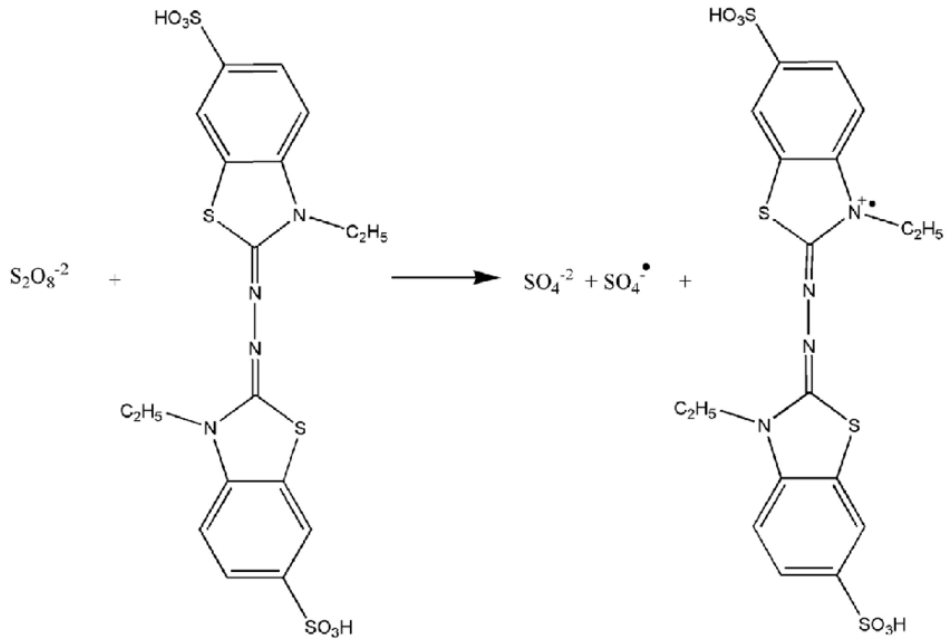


Figure 2-4. The reaction between ABTS radical and antioxidant to form ABTS (Güdr, 2016)

Briefly, the working solution was mixed in 1:1 ratio of ABTS and potassium sulfate overnight. Prior to use, the ABTS stock solution was diluted to generate an absorbance in the range 0.700 $\pm$ 0.025 at 734 nm using a microplate reader. The sample was prepared by adding 20 µL of the banana inflorescence extracts (10-40 mg/mL) or Trolox (standard solution at 100-300 µM) to 180 µL of $ABTS^{\cdot+}$ working reagent in a 96-well microplate. Absolute methanol was used as a reagent blank. All reagents were mixed using the vortex mixer and incubated in the dark at room temperature for 15 minutes, the absorbance at 734 nm was measured. The antioxidant activity of the extract was calculated as the percent of inhibition as shown in equation 3. The IC_{50} value was also determined.

Calculation

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad (3)$$

Where, $Abs_{control}$ = absorbance of ABTS radical + methanol

Abs_{sample} = absorbance of ABTS radical + sample extract

1.10 Liquid chromatography-mass spectrometry method (LC/MS)

LC/MS was used for the targeted analysis of bioactive compounds in the crude ethanolic extract of banana inflorescence. Liquid chromatography analysis was performed with the ultra-high-performance liquid chromatography and coupled to an electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF/MS) technique with the 1290 Infinity II LC-6545 Quadrupole-TOF (Agilent technologies) in both positive and negative ionization modes. An aliquot of the extract was injected into the C18 column (150 × 2.1 mm, particle size 1.8 μm, Agilent technologies). The detection was performed through heated electrospray ionization (ESI) source and subjected to the positive and negative charged ions. The solvents used in the mobile phase consisted of water and 0.1% formic acid (A) and acetonitrile (B). The gradient elution was carried out as followed: 95% of A for 0-4 min, 5% of B for 4 - 20 min, 55% of A for 20-30 min, 5% of A for 30-40 min, and 95% of A for 40-45 min, using a flow rate of 0.2 mL/min. The specific compounds of the extract were recorded in the positive and negative ionization modes by comparing the m/z ratio with that of the known compounds in the library and databases. The results were reported on the basis of the compounds identification, retention time, molecular formula, mass to charge ratio (m/z), and type of compounds.

Part 2. Biological activity of the banana inflorescence extract on C2C12 myotubes

2.1 Cell culture medium preparation

2.1.1 Complete DMEM supplemented with 10% fetal bovine serum (FBS)

FBS and 100X penicillin/ streptomycin antibiotics were prewarmed in a 37°C water bath. Low-glucose Dulbecco's Modified Eagle Medium (DMEM, Gibco™) supplemented with 1.5 g of sodium bicarbonate was completely dissolved in 850 mL of sterile distilled water. After adjusting the pH to 7.1-7.2, FBS and 100X penicillin/streptomycin was added to 10% and 1X final concentration, respectively. Then, the total volume was adjusted to 1000 mL with distilled water. The medium was filtered through a 0.2 μm bottle-top filter and aliquoted into 50 mL falcon tubes. The tubes were labeled and stored in a refrigerator or -20°C until future use.

2.1.2 Complete DMEM supplemented with 2% horse serum (HS)

To induce C2C12 myoblast fusion, the cells were allowed to reach 70-80% confluence before changing to the differentiation medium. This medium was prepared as described in the section 2.1.1, except that the 2% horse serum was substituted for the 10% FBS.

2.2 Thawing C2C12 cells

An aliquot of C2C12 cells in a cryogenic vial was removed from the liquid nitrogen tank and quickly thawed in a 37°C water bath. The cell suspension (approximately 1 mL) was gently transferred to a 9-mL warm DMEM medium

supplemented with 10% FBS in a 15 mL falcon tube. Centrifugation at 2,000 rpm was performed for 2 minutes, and the medium was removed using an aspirator. The cell sediment was gently resuspended with 1 mL complete DMEM supplemented with 10% FBS and plated onto a 10-cm culture dish. The cells were revived in a 37°C incubator with 5% CO_2. After a day of plating, the cells were visualized under a light microscope. If the cell density reached 80-90% confluency, subculturing would be performed. However, if the confluency was lower than 50% and with many floating cells, the DMEM supplemented with 10% FBS medium would be replenished. The incubation was continued for 1 more day and subculturing would perform if the confluency reached 70-80%.

2.3 Subculturing

Subculturing was performed every two days or when the confluency of the cell reaches approximately 70-80%. To start subculturing, the old DMEM medium was removed using an aspirator. After washing once with 10 mL 1X phosphate-buffered saline (PBS), the cells were detached from the plate by adding 2 mL of 1X trypsin (0.025%) and incubating at 37°C under 5% CO_2 for 5 minutes. To recover the cells, 8 mL of fresh complete DMEM medium supplemented with 10% FBS was added, then they were gently resuspended and subsequently transferred into a 15 mL falcon tube for centrifugation at 2000 rpm for 2 minutes. The supernatant was aspirated off, then the cells were gently resuspended in 1 mL complete DMEM. The total number of the cells were determined as described in the section 2.4. To subculture, 2.5×10^5, 5×10^3, 2.4×10^4 cells were plated into a 10-cm, 96-well, or a 24-well cell culture plate,

respectively, and the number of passages were counted for every successive subculturing.

2.4 Counting cells

C2C12 cells were counted before seeding using a hemacytometer (Figure 2-5). In brief, 10 µL of cell suspension was mixed with 30 µL of 0.4% trypan blue. Then, 10 µL of the mixture was transferred into a hemacytometer for counting. The total number of cells and cell density were also calculated using equations 4 and 5, respectively.

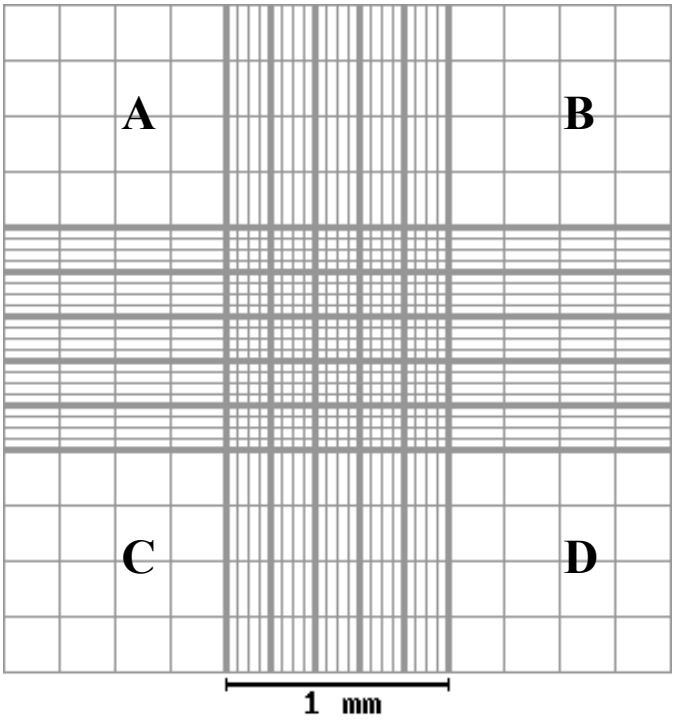


Figure 2-5. Counting cells with hemacytometer

Calculation

Total number of cells = Total number of viable cells in 4 quadrants x 10^4 cells/mL (4)

Where, Total number of viable cells = A+ B+ C+ D

Cell density = (Average of total number of viable cells) x 4 x 10^4 cells/mL (5)

Where, $\frac{\text{Total number of viable cells}}{4}$

2.5 C2C12 cell differentiation

C2C12 cells were seeded at a density of 5.0 x 10^3, 2.4 x 10^4 viable cells/well in 96-well or a 24-well cell culture plate, respectively. For differentiating the cells to myotube (Yaffe and Saxel, 1977), cells were grown to confluence and medium was changed to completed DMEM supplemented with 2% HS. The culture medium was replenished every 2 days. After 6 days of culturing, the myoblasts were differentiated into myotubes, which could be visualized under a light microscope.

2.6 Dose- and time-dependent effects

C2C12 myotubes were treated with an increasing concentration of the ethanolic extract from the banana inflorescence. A blank control (untreated cells) and positive controls (insulin, metformin) were included. The medium was collected for the glucose assay after 24 hours of treatment (see below), whereas the remaining cells on the plate were tested for their viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay or the cell viability assay.

The time-dependent effect was performed similarly. C2C12 myotubes in 24-well culture plate were treated with 0.125-0.5 mg/mL concentration of the ethanolic extract and the medium was collected at different time points. Glucose assay and cell viability assay were performed as described below.

2.7 Cell viability assay

MTT or (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay was used to determine the viability of the treated cells (Mosmann, 1983). In brief, after washing cells with phosphate-buffered saline (PBS), 0.5 mg/mL MTT in the complete DMEM supplemented with 2% horse serum was added to the cells. After incubating for 2 hours, cells were washed with PBS before dimethyl sulfoxide (DMSO) was added to dissolve formazan crystals. The absorbance at 570 nm was measured using a microplate reader spectrophotometer. The cell viability was determined as described in equation 6.

Calculation

$$\% \text{ cell viability} = (Abs_{sample} / Abs_{control}) \times 100 \quad (6)$$

Where, $Abs_{control}$ = absorbance of control

Abs_{sample} = absorbance of banana inflorescence extract

2.8 Glucose assay

The glucose contents in the medium from treated cells were determined using a glucose assay kit (Sigma-Aldrich). Differentiated C2C12 cells in 96-well culture plates were treated with the crude ethanolic extract at different concentrations. The dose-dependent effect of C2C12 myotubes was treated with the ethanolic extract in a concentration range from 0.125-0.5 mg/mL for 24 hours. Insulin and metformin were used as positive controls for the measurement of glucose uptake, while the untreated cells were used as a negative control. The stock solution of glucose was prepared using a 2-fold serial dilution method to generate glucose in a concentration

range from 6.25-100 µg/mL. Fifty µL of glucose reagent was added to 25 µL of the samples. Subsequently, the mixtures were incubated for 30 minutes at 37°C. The reaction was stopped by adding 50 µL of 12N sulfuric acid (H_2SO_4). The absorbance at 540 nm was measured using a microplate reader. The time-dependent experiment was performed similarly. Media from treated C2C12 myotubes at different time points were collected to measure the glucose uptake. The percentage of stimulation and the ratio of the fold changes against the untreated cell was also calculated using equation 7 and 8, respectively.

Calculation

$$\% \text{ stimulation} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100 \quad (7)$$

$$\text{Ratio of the fold changes} = Abs_{control}/Ab_{sample} \quad (8)$$

Where, $Abs_{control}$ = absorbance of control

Abs_{sample} = absorbance of banana inflorescence extract

3. Statistical analysis

At least three independent experiments were performed and each data set was obtained in triplicate. Statistical analysis was analyzed by using One-Way analysis of variance (ANOVA). The Duncan's multiple-range test was applied for mean comparison. Different letters (i.e., a, b, and c) were assigned to indicate the datasets that show a significant difference ($p < 0.05$).

CHAPTER 3

RESULTS AND DISCUSSION

1. Extraction yield of banana inflorescence

A total of five whole banana inflorescences of Kluai Namwa Mali-Ong cultivar were extracted with absolute ethanol and the solvent evaporated until dryness. The average yield of crude ethanolic extracts from five inflorescences was expressed as a percent of the concentrated extract (w/w). The weight and yield extraction of by-products from banana inflorescences were summarized in Table 3-1. The residue was dried in the oven at 70°C for approximately 3 days and stored for future experiments.

Table 3-1. The average amount of fresh and dried weights, percent dry weight of banana inflorescence, weight, total solid, and yield of the five banana inflorescences.

Categories	Average amount
Fresh banana inflorescence (g)	1306.67 ± 211.73
Dried banana inflorescence (g)	108.37 ± 17.29
Moisture content (%wet basis)	91.7 ± 0.38
Dry weight of inflorescence (% of fresh banana inflorescence)	8.30 ± 0.38
Weight of the extract (g)	7.30 ± 1.29
Total solid of the extract (% of the weight of the extract)	85.66 ± 1.90
Yield of the extract (% of dried weight of banana inflorescence)	6.74 ± 0.62

The values shown are the average ± standard deviation (n = 5).

2. Quantitative phytochemical screenings of ethanolic extracts of banana inflorescence

Proximate compositions of banana inflorescence extract were analyzed. The present study showed the quantitative phytochemical screening of various phytoconstituents such as carbohydrate, protein, phenolic acid, and flavonoid contents. The results for the estimation of proximate analysis in the extract are presented in Table 3-2.

Table 3-2. Proximate composition of crude ethanolic extract including total carbohydrate, total protein, total phenolic, and flavonoid contents.

Component analyze	Average amount
Total carbohydrate (% of the weight of the extract)	38.53 ± 3.00
Total protein (% of the weight of the 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

Data are presented as mean values ± standard deviation (n = 3).

Compare to a fresh banana inflorescence from a study by Basumatary and Nath, the carbohydrate concentration in the extract was higher than the value reported for *Musa balbisiana* Colla inflorescence (10.18%) (Basumatary and Nath, 2018). However, the carbohydrate contents of the extract was lower than that in the extract from *Musa balbisiana* of Poovan banana (95.23%) and Monthan cultivars in India (95.61%) (Krishnan and Sinija, 2016), Baxijiao banana (91.39%), and Paradisiaca banana (90.80%) in China (Sheng et al., 2011). In addition, the present of carbohydrate

content in the methanolic extract from *Musa sapientum* was 69.45% (Jayamurthy et al., 2011).

The protein concentration in the extract was lower than the protein content from studies reported of *Musa* sp. of Nanjangud Rasa Bale banana (19.60%), Thee hmwe banana (15.82%) and Phee kyan (13.28%) cultivars (Swe, 2012; Ramu et al., 2017). In contrast, other studies showed less protein contents in their inflorescence extract than that in this study. For example, a study by Basumatary and Nath reported 1.79% total protein content in the extract from *Musa balbisiana* (Basumatary and Nath, 2018). Other studies by Krishnan and Sinija showed that their inflorescence extracts from *Musa* spp. cultivar Poovan and Monthan contained 1.99% and 1.43% protein concentration, respectively (Krishnan and Sinija, 2016). These data reveal that total carbohydrate and protein contents from the crude ethanolic extract were inconsistent a varied among studies because different types of banana inflorescences may have different quantities source of macronutrients. In addition, the extraction conditions used such as temperature, duration, and the ratio of sample to extraction solvent may have some effects (Sulaiman et al., 2017; Andres et al., 2020).

The plant secondary metabolites, phenolic acid and flavonoid contents were evaluated in this extract. Total phenolic and flavonoid contents in the extract were 8.06 $\pm$ 0.70 mg GAE/ g extract and 4.79 $\pm$ 0.07 mg QE/ g extract, respectively. Results from a study done by Thaweesang showed total phenolic contents of the florets and bract extract from banana inflorescence of *Musa* ABB cv. Kluai Namwa was 1235.94 $\pm$ 217.48 and 741.79 $\pm$ 97.34µg GAE/ g extract, which is lower than the results in this study (Thaweesang, 2019). However, when compared to the total flavonoid contents

reported by Revadigar and colleagues, the ethanolic extract of *Musa balbisiana* Colla inflorescence contained the total flavonoid concentration at 4.85 ± 0.05 mg QE/ g extract (Revadigar et al., 2017), which is comparable to the flavonoid concentration found in the extract in this study.

In this observation, the screening of banana inflorescence extract using several techniques revealed the presence of various phytochemical compounds such as phenolics and flavonoids. Epidemiological studies reported that long-term consumption of medicinal plants rich in polyphenols, which possess antioxidant activities, may help decrease the risk of chronic inflammation of heart disease (Scalbert and Williamson, 2000; Pandey and Rizvi, 2009). Moreover, increased consumption of dietary polyphenol was shown to prevent cellular oxidative stress and many diseases including diabetes mellitus, cancers, neurodegenerative diseases, inflammatory disorders, and infectious diseases (Pandey and Rizvi, 2009). Therefore, the consumption of banana inflorescence may confer a protection to various major human diseases.

3. Ethanolic extract of banana inflorescence contains weak antioxidant activities of the *in vitro* study

The antioxidant assays of DPPH, FRAP and ABTS were classified as electron transfer-based assays (Xiao et al., 2020). The stable radical DPPH and ABTS have been widely used to estimate the free radical scavenging activities of the antioxidants in plant extracts. The inflorescence extract exhibited the inhibitory activity of the different concentrations of the extract in the range of 10-40 mg/mL that shown in Table 3-3.

Table 3-3. The percentage inhibition of the banana inflorescence extract using DPPH and ABTS assay.

Concentration (mg/mL)	**Antioxidant activities (%inhibition)**	
	DPPH assay	**ABTS assay**
10	18.70 ± 6.50	35.57 ± 0.80
20	37.48 ± 4.51	48.85 ± 1.86
40	70.50 ± 5.76	73.32 ± 1.45

The values shown are the average ± standard deviation (n = 3).

The results provided that the inhibitory activity of the extract in the ABTS assay was more efficient than that in the DPPH assay in all range of the extract concentrations. The percent inhibition of the extract on ABTS assay presented range from 35.57 to 73.32%, whereas DPPH assay range from 18.70 to 70.50%. Antioxidant activities were presented as IC_{50} values or the concentration of the extract at 50% inhibition of free radical scavenging. The IC_{50} values of the extract on both DPPH and ABTS were shown in Table 3-4.

Table 3-4. IC_{50} values of the banana inflorescence extract.

Extract	**Component analyze** IC_{50} **(mg/mL)**	
	DPPH assay	**ABTS assay**
Banana inflorescence	27.89 $\pm$ 0.54	21.33 $\pm$ 0.87

The values shown are the average $\pm$ standard deviation (n = 3).

From Table 3-4, it was noticed that the ABTS assay is more sensitive for identifying and detecting the antioxidant activities than the DPPH assay. These data suggest that the ABTS assay radical scavenging activities respond faster and have higher antioxidant capacity than DPPH assay (Lee *et al*., 2015).

The antioxidant activities that were expressed as millimolar Trolox equivalent per gram of extract were calculated. As shown in Table 3-5, the extract showed the highest reducing ability in the FRAP assay while DPPH and ABTS radical scavenging assays showed no significance ($p < 0.05$) in the extract.

Table 3-5. Antioxidant activities of the banana inflorescence extract.

Extract	Antioxidant activities (mM TE/g extract)		
	DPPH assay	**ABTS assay**	**FRAP assay**
Banana inflorescence	$415.35^{b} \pm 25.21$	$465.31^{b} \pm 14.56$	$752.21^{a} \pm 17.09$

The values shown are the average ± standard deviation (n = 3).

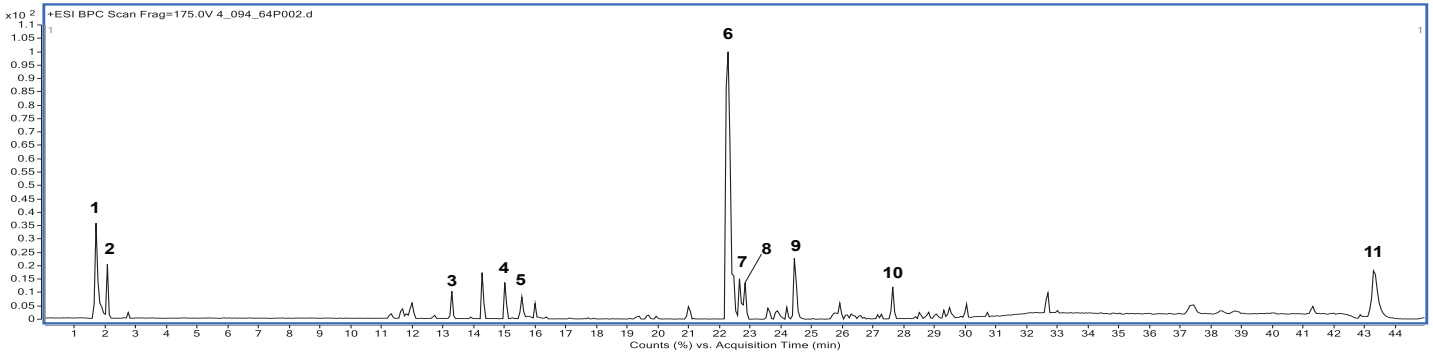
Antioxidant activities of ethanolic extract from banana flowers or inflorescences was previously determined. Previous findings from Sheng and colleagues reported the antioxidant activities of *Musa* sp. Baxijiao and Paradisiaca extracts in DPPH, ABTS, and FRAP assays. They showed that the IC_{50} values of the extracts were within 2.12 to 5.84 μg/mL concentrations (Sheng et al., 2011). In a study by Schmidt and colleagues, the IC_{50} value of the ethanol extract from *Musa cavendishii* banana inflorescence in the DPPH assay was 0.31 mg/mL. (Schmidt *et al*., 2015). Additionally, Thaweesang used a DPPH assay to show that the IC_{50} values of the ethanolic extract from the fresh and blanched Kluai Namwa inflorescence were 1.27 and 1.30 mg/mL, respectively (Thaweesang, 2019). Another study by Amornlerdpison and colleagues suggested that the IC_{50} values of the crude ethanolic extract of banana inflorescence using ABTS assay was 2.93 ± 0.01 (Amornlerdpison et al., 2020).

Therefore, other studies represented higher antioxidant activities of ethanolic extract of banana inflorescence. This study found that the ethanolic extract of banana inflorescence was a weak antioxidant. Because the inflorescence in this study was dried at 70°C, the high heat could possibly destroy the antioxidant activities. Consistently, many studies used room temperature or lower than 70°C under ventilation to dry banana inflorescence (Padam et al., 2014; Amornlerdpison et al., 2020; Chiang et al., 2021). To further verify the antioxidant compounds in the extract, the ultra-high-performance liquid chromatography and coupled to an electrospray ionization quadrupole time-of-flight mass spectrometry (UHPLC-ESI-QTOF/MS) analysis was conducted and the results are discussed in the next section.

4. The chemical composition in the extract using UHPLC-ESI-Q-TOF MS/MS

Base peak chromatograms in the positive and negative-ionization modes of the extract are shown in Figures 3-1. The peaks in the chromatogram represent the major compounds in the extract. Eleven compounds were identified in the positive-ionization mode and ten compounds in the negative-ionization mode. The analysis of phytochemical compounds, retention time, molecular formula, mass to charge ratio (m/z) values, and classification of compounds was summarized in Tables 3-6. and 3-7.

(A)



(B)

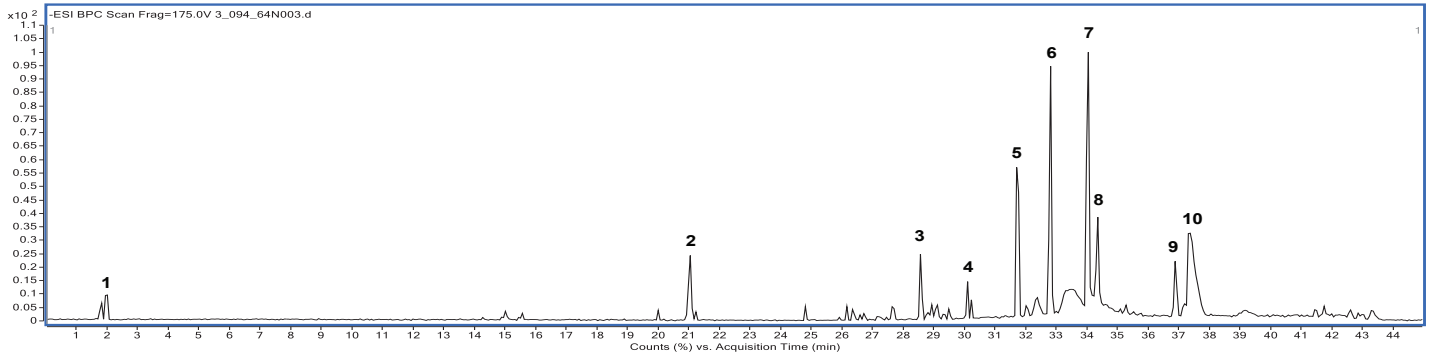


Figure 3-1. Base peak chromatogram (BPC) scan of the crude ethanolic extract from banana inflorescence by UHPLC-ESI-QTOF/MS analysis. **(A)** shows BPC scan from the positive electrospray ionization mode. **(B)** shows BPC scan from the negative electrospray ionization mode.

Table 3-6. Compounds identification, retention time, molecular formula, mass to charge ratio (m/z), and type of compounds in the mass spectrometry from the positive electrospray ionization mode UHPLC-ESI-QTOF/MS.

Peak	Compounds	Retention time (min)	Molecular formula	Fragments m/z	Classification
1.	2-Amino-3-methyl-1-butanol	1.734	$C_5H_{13}N\ O$	104.1073	amino alcohol
2.	Adenine	2.126	$C_5H_5N_5$	136.062	purine nucleobase
3.	Solanocapsine	13.342	$C_{27}H_{46}N_2O_2$	453.3438	steroid alkaloid
4.	L-4-Hydroxy-3-methoxy-a-methylphenylalanine	15.143	$C_{11}H_{15}NO_4$	248.0896	aromatic L-alpha-amino acid
5.	PI(O-16:0/16:0)	15.605	$C_{41}H_{81}O_{12}P$	814.5788	phosphatidylinositol
6.	Xestoaminol-C	22.497	$C_{14}H_{31}NO$	230.2487	1,2-aminoalcohols
7.	2-hydroxyhexadecanoic acid	22.713	$C_{16}H_{32}O_3$	290.27	hydroxy fatty acid
8.	3-Ethyltridecan-2-one	22.859	$C_{15}H_{30}O$	244.2644	long-chain fatty aldehyde
9.	Phytosphingosine	24.455	$C_{18}H_{39}NO_3$	318.3013	amino alcohol
10.	Kolanone	27.695	$C_{33}H_{42}O_4$	520.3412	monoterpenoid
11.	1-Palmitoyl-2-linoleoyl PE	43.009	$C_{39}H_{74}NO_8P$	716.523	phosphatidylethanolamine

Table 3-7. Compounds identification, retention time, molecular formula, mass to charge ratio (m/z), and type of the compounds in the mass spectrometry from the negative electrospray ionization mode UHPLC-ESI-QTOF/MS.

Peak	Compounds	Retention time (min)	Molecular formula	Fragments m/z	Classification
1.	R-(+)-2-Pyrrolidone-5-carboxylic acid	2.091	$C_5H_7NO_3$	128.0356	amino acid
2.	11,12,13-trihydroxy-9-octadecenoic acid	21.068	$C_{18}H_{34}O_5$	329.2338	long-chain fatty acid
3.	(±)12(13)-EpOME	28.537	$C_{18}H_{32}O_3$	295.2281	monounsaturated epoxy fatty acid
4.	13(S)-HODE	30.148	$C_{18}H_{32}O_3$	295.2283	long-chain fatty acid
5.	α-Linolenic Acid	31.825	$C_{18}H_{30}O_2$	277.2177	polyunsaturated fatty acid
6.	Chaulmoogric acid	32.898	$C_{18}H_{30}O_2$	279.2337	monounsaturated long-chain fatty acid
7.	Isopalmitic acid	34.122	$C_{16}H_{32}O_2$	255.2339	methyl-branched-chain fatty acid
8.	Vaccenic acid	34.393	$C_{18}H_{34}O_2$	281.2487	trans fatty acid
9.	Stearic acid	36.940	$C_{18}H_{36}O_2$	283.2643	saturated long-chain fatty acid
10.	3R-hydroxy-eicosanoic acid	37.396	$C_{20}H_{40}O_3$	327.2906	long-chain fatty acid

From the positive-ionization mode, the major compounds identified are amino alcohols, amino acids, steroid alkaloids, and fatty acids. The highest peak at the retention time of 22.497 minutes was identified as Xestoaminol-C (peak 6) compounds. The study for comparative the effect of antioxidants and antidiabetics by Sandikapura and colleagues also reported 2-amino-3-methyl-1-butanol, adenine, Xestoaminol-C, and phytosphingosine in their inflorescence extract (Sandikapura et al., 2018). These compounds may have antioxidant and antidiabetic activities. Moreover, solanocapsine, which is a steroid alkaloid component, showed many activities, including antibacterial activity and cytotoxicity against HeLa cell lines (Dongre et al., 2007; Almoulah et al., 2017). Kolanone, a natural polyisoprenyl benzophenone, was presented to exhibit significant antimicrobial activity in the extract (Hussain et al., 1982; Madubunyi, 1995).

In addition some bioactive compounds in the negative-ionization mode showed anti-diabetic activities, mainly belonging to the fatty acids. The highest peak on the chromatogram showed isopalmitic acid at the retention time of 34.122 minutes. The antioxidant activity of isopalmitic acid (peak 7) has been reported by Khoo and colleagues (Khoo et al., 2019). In addition, α-linolenic acid (peak 5) was shown to have both antioxidant and antidiabetic activities (Comlekcioglu, 2019; Kato et al., 2000; Khoo et al., 2019; Savych et al., 2020; Smorowska et al., 2021). The compounds at peaks 8, 9 and 10 were identified as vaccenic acid, stearic acid, and 3R-hydroxy-eicosanoic acid, respectively. A study by Jacome-Sosa and colleagues reported the suppression of intestinal inflammatory diseases and antioxidant properties of vaccenic acid (Jacome-Sosa et al., 2016). Moreover, long-chain fatty acids such as stearic acid and 3R-hydroxy-eicosanoic acid were shown the properties for preventing chronic diseases (Ahmad et al., 2012; Maheshwari et al., 2020; Smorowska et al., 2021). The

identification of bioactive compounds in the extract using the UHPLC-ESI-QTOF/MS -based approaches is an important analysis, which covered many groups of compounds. Thus, a detailed chemical profiling of the crude extract is required to determine the phytochemical for antioxidant and antidiabetic activities by the UHPLC-MS/MS. The results from UHPLC-MS/MS were consistent with the antioxidant analysis that presented weak antioxidant activities and the identification of compounds showed low levels of polyphenols. Most of the compounds found in the crude ethanolic extract were identified as other types of compounds such as amino acids, amino alcohols, steroids, and fatty acids. Among those compounds, some fatty acids were previously shown to possess an antidiabetic activity (Ramu et al., 2017; Bolaños et al., 2021). Therefore, it is hypothesized that the ethanolic extract from Kluai Namwa Mali-Ong may have an antidiabetic activity. To test this hypothesis, the ability of the extract to stimulate glucose uptake in a muscle cell line was investigated.

5. Cell viability of the ethanolic extract of banana inflorescence promotes C2C12 differentiation in a dose-dependent manner

Skeletal muscle cell lines have been used to evaluate the antidiabetic activity of extracts, including C2C12 muscle cells derived from mouse skeletal muscle (Tortorella and Pilch, 2002; DeFronzo and Tripathy, 2009; Honardoost et al., 2016). This study used the differentiated C2C12 myotubes to study the effect of ethanolic extract of banana inflorescence on glucose metabolism.

The cell viability of the banana inflorescence extract on C2C12 myotubes was analyzed using an MTT assay and the results were shown in Figure 3-2. The extract was used at concentrations from a range between 0.125-0.5 mg/mL. The results show that all treatments on C2C12 myotubes conferred cell viability greater than 80%, suggesting that the extracts are not toxic to the cells. Moreover, the positive controls, which are insulin and metformin also showed no toxicity effect on C2C12 cells.

From the results, it was observed that percent cell viability of the treated cells was increased proportionally to the extract concentration. These results were consistent with previous findings that show the viability of banana inflorescence extract treated in C2C12 mouse myotubes cells was significantly increased as compared with the untreated cells in a dose-dependent manner (Han et al., 2019; Safarzad et al., 2020). These findings may be associated with the increasing cell viability from the extract treatment with different concentrations stimulating on C2C12 myotubes. Therefore, concentrations of the ethanolic extract ranging from 0.125 to 0.5 mg/ml and positive controls (5.88×10^{-4} mg/mL insulin and 8.28×10^{-2} mg/mL metformin) were used for further experiments.

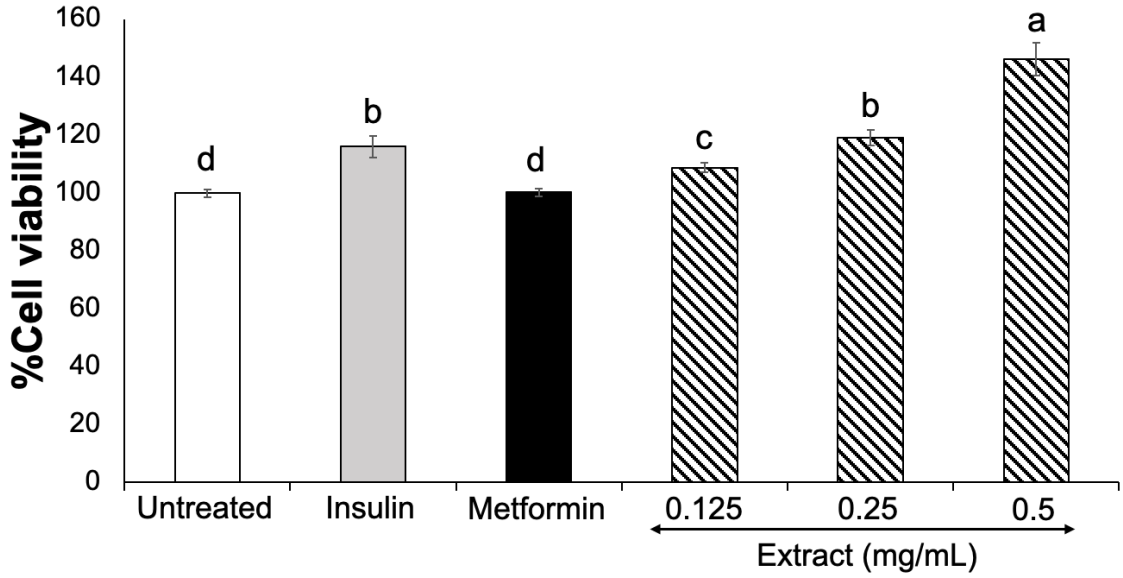
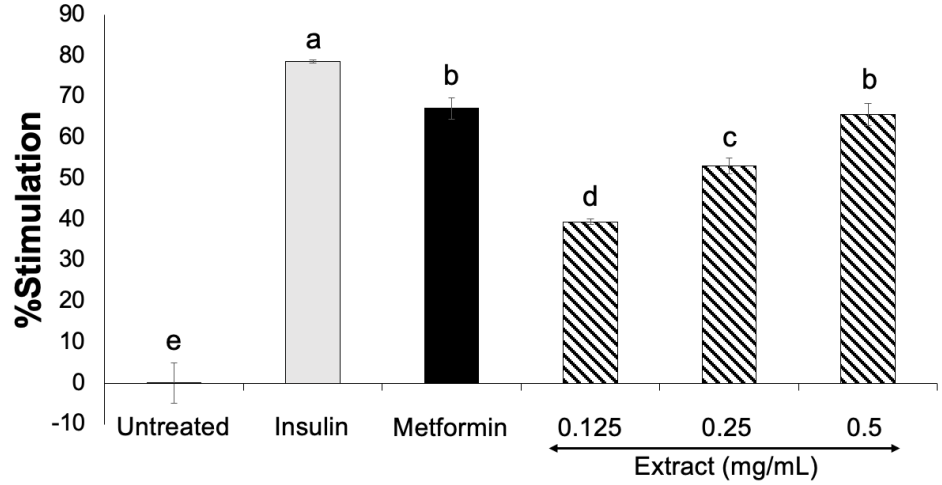


Figure 3-2. The effect of the extract on cell viability of C2C12 myotubes. The result was expressed as the percentage of the untreated cells. After incubating C2C12 cells with the banana inflorescence extract at different concentration (0.125-0.5 mg/mL), in comparison with positive controls (5.88×10^{-4} mg/mL insulin and 8.28×10^{-2} mg/mL metformin). Different letters (a - d) were assigned to indicate the datasets that show a significant difference ($p < 0.05$). Data are presented as mean values $\pm$ standard deviation ($n = 3$).

6. The ethanolic extract of banana inflorescence promotes glucose uptake stimulatory effect on differentiated C2C12 myotubes

The glucose uptake stimulatory effect of the extract on C2C12 myotubes was analyzed using an indirect glucose assay (measuring how much glucose is left in the medium rather than measuring how much glucose is uptaken into the cells). The dose- and time-dependent effect of the extract was investigated. Insulin (hormone) and metformin (an antidiabetic drug) was included in the experiment as positive controls that stimulate glucose uptake in the muscle cells. The results showed that the extract at the concentration of 0.125, 0.25, and 0.5 mg/mL conferred 21.41±2.17 %, 42.83±1.48 %, and 61.47±1.84% stimulation of glucose uptake, respectively (Figure 3-3A). In addition, glucose uptake of the extract was also significantly expressed in terms of the ratio to untreated of 1.32-, 1.78- and 2.67-fold (Figure 3-3B), compared with the untreated cells. The banana inflorescence extract at 0.5 mg/mL also showed no significance ($p < 0.05$) for glucose uptake in the C2C12 cells compared with the positive control metformin. Treatment of C2C12 cells with the ethanolic extract significantly increased glucose uptake in a dose-dependent manner ($p < 0.05$), with the highest concentration (0.5 mg/mL) increased glucose uptake similar to metformin (~61.70% stimulation of glucose uptake). These data suggest that the extract from banana inflorescence at all the concentrations tested promotes glucose uptake in a dose-dependent manner.

(A)



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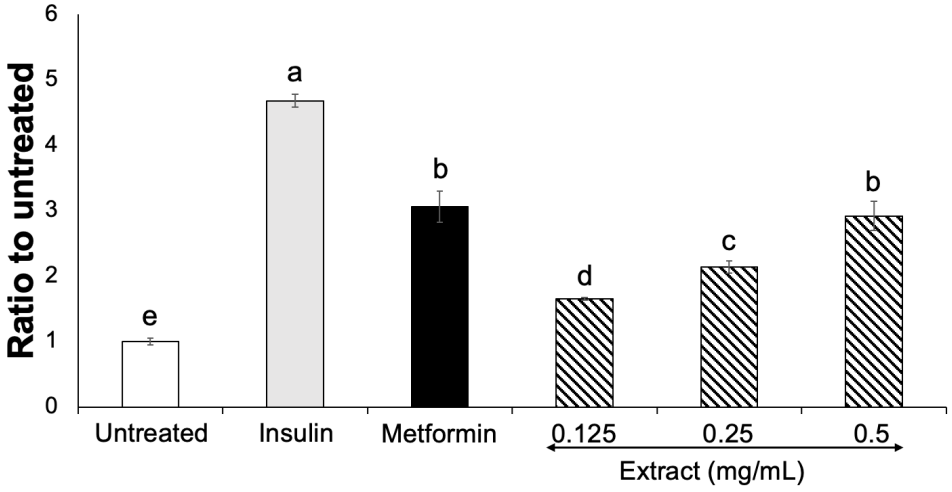

Figure 3-3. The extract stimulates glucose uptake in C2C12 myotubes in a dose-dependent manner. **(A)** The glucose uptake was expressed as % of the untreated. **(B)** The glucose uptake was expressed as a ratio to untreated cells after incubating C2C12 cells with the banana inflorescence extract (0.125-0.5 mg/mL), in comparison with positive controls (5.88×10^{-4} mg/mL insulin and 8.28×10^{-2} mg/mL metformin). Different letters (a - e) were assigned to indicate the datasets that show a significant difference ($p < 0.05$). Data are presented as mean values ± standard deviation ($n = 3$).

For the time-dependent manner, the media of cell culture were collected at different time points of 12, 15, 18, 21 and 24 hours. The results showed that the extract and positive controls promoted significant differences in glucose uptake from 18 hours to a maximum of 24 hours compared to the untreated cells (Figures 3-4 and 3-5).

At the present, the information about the stimulation of glucose uptake in muscle cells by ethanolic extract from banana inflorescence remains scarced. Several studies used the extract of banana inflorescences and the other parts of banana *in vitro* and *in vivo* assays. For example, a study *in vitro* assay by Bhaskar and colleagues analyzed the effects of banana flower and pseudostem extracts (*Musa* sp. cultivar elakki bale) on the Ehrlich ascites tumor cells (EAT). The results presented that methanol and aqueous solvent extractions could promote using 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) uptake in the EAT cells (Bhaskar et al., 2011). Additionally, Pari and Maheshwari reported that an oral intake of banana flower extracts for 30 days significantly reduced the blood sugar levels in rats (Pari and Umamaheswari, 2000). A study by Jamuna and Nandini suggested that feeding banana flower extracts to diabetic rats reduced diabetic nephropathy complications and controlled hyperglycemia by observing the components of glucose transporters at the molecular level through GLUTs/SGLTs in the kidney (Jamuna and Nandini, 2014). Other studies by Ramu and colleagues reported that the ethanol extract of banana flowers showed varying degrees of inhibitory effect on α-glucosidase and expressed the ability of glucose uptake stimulation by suppressing an antihyperglycemic effect in normal and alloxan-induced diabetic rats (Ramu et al., 2014). Moreover, Vilhena and colleague reported that using the extract from *Musa x paradisiaca* flowers and bracts

in streptozotocin (STZ)-induced diabetic rats showed the decrease in fasting glycemia as compared with the untreated diabetic group (Vilhena et al., 2018).

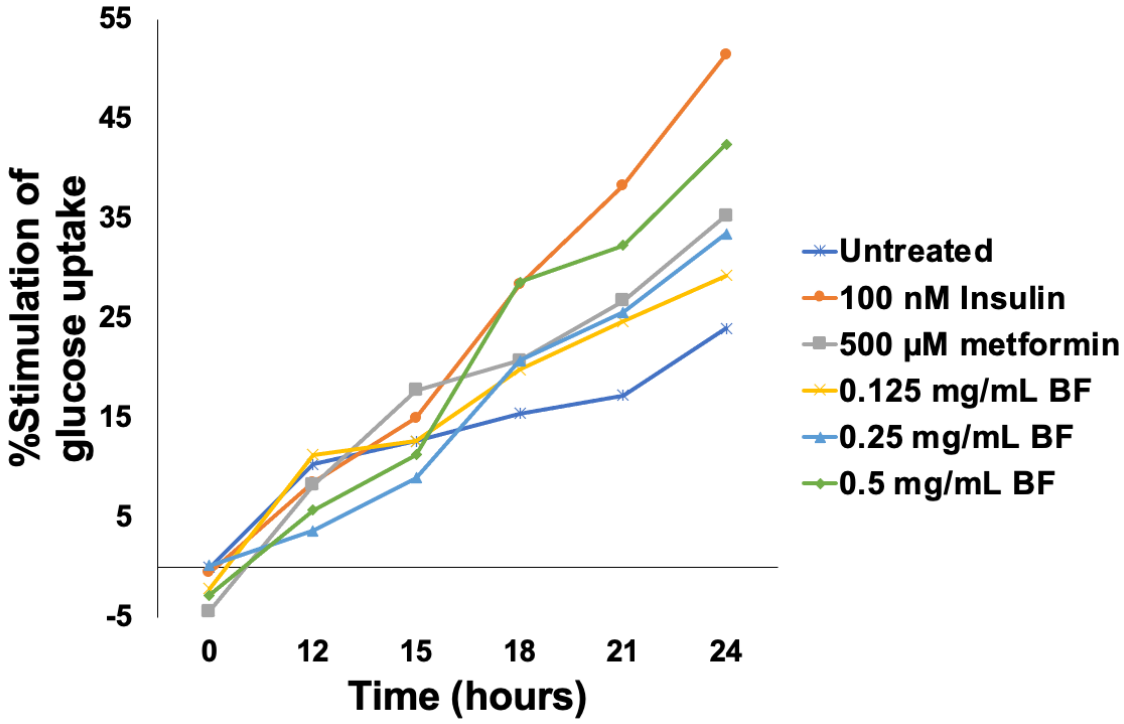

Figure 3-4. Time-dependent effect of the banana inflorescence extract on glucose stimulation on C2C12 myotubes. The glucose uptake (expressed as a %stimulation of the untreated cell) after incubating C2C12 cells with the banana inflorescence extract (0.125-0.5 mg/mL), in comparison with positive controls (5.88×10^{-4} mg/mL insulin and 8.28×10^{-2} mg/mL metformin). Data are presented as mean values ± standard deviation (n = 3).

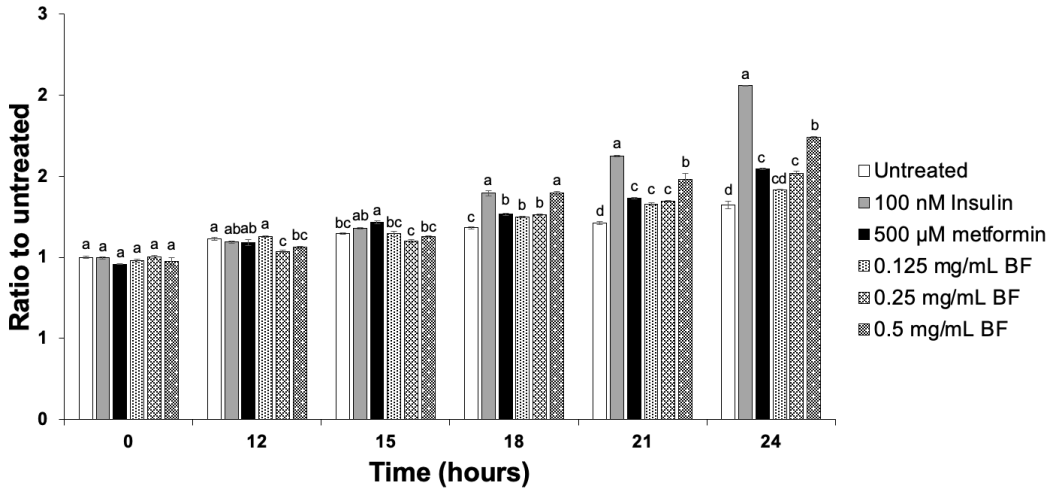

Figure 3-5. Time-dependent glucose uptake stimulation on C2C12 myotubes. The glucose uptake was expressed as a ratio to untreated cells after incubating C2C12 cells with the banana inflorescence extract (0.125-0.5 mg/mL), in comparison with positive controls (5.88×10^{-4} mg/mL insulin and 8.28×10^{-2} mg/mL metformin). Different letters (a - d) were assigned to indicate the datasets that show a significant difference ($p < 0.05$). Data are presented as mean values ± standard deviation ($n = 3$).

The skeletal muscle is the principal site for essential metabolism that plays a role in maintaining blood glucose homeostasis and glucose utilization (Evans et al., 2019; Merz and Thurmond, 2020). In this study, the extract from Kluai Namwa Mali-Ong could promote glucose uptake in dose-and time-dependent manners Therefore, the ethanolic extract of banana inflorescence could potentially be used in diets and used as an anti-diabetic food additive to control diabetic diseases.

The ability of the extract to stimulate glucose uptake in the cell line was consistent with the hypothesis that fatty acids identified in the UHPLC-ESI-QTOF/MS may possess the antidiabetic activity. Thus, further analysis on the fatty acids in the extract was conducted and the results are discussed next.

7. Fatty acid composition in the banana inflorescence extract revealed the biological activities

Generally, fat can be found in banana inflorescence. Several studies reported the fatty acid contents in different cultivars of bananas and also showed to have varying levels (Nadeeshani et al., 2021; Bolaños et al., 2021). However, the fatty acid contents in the banana inflorescence extract of 'Kluai Namwa Mali-Ong' has not been studied. Thus, gas chromatography with flame ionization detection (GC-FID) was used to analyze fatty acid profiles from the extract. The detailed fatty acids profile and the chromatogram of the extract are shown in Table 3-8. and Figure 3-6., respectively.

Table 3-8. Fatty acid profile of the crude ethanolic extract from banana inflorescence identified by GC-FID analysis

No.	Retention time (min)	Area (%)	Fatty acid
1.	10.232	0.16544	Myristic acid (C14:0)
2.	11.412	0.27123	Pentadecanoic acid (C15:0)
3.	12.555	22.02084	Palmitic acid (C16:0)
4.	12.870	0.31388	Palmitoleic acid (C16:1)
5.	15.217	1.92162	Stearic acid (C18:0)
6.	15.584	3.89340	Oleic acid (C18:1)
7.	16.463	24.85882	Linoleic acid (C18:2)
8.	17.629	6.36497	Linolenic acid (C18:3)
9.	18.461	0.98957	Arachidic acid (C20:0)
10.	18.717	0.48341	Eicosenoic acid (C20:1)
11.	20.692	1.36141	Behenic acid (C22:0)
12.	20.988	1.30437	Erucic acid (C22:1)
13.	23.448	1.28866	Lignoceric acid (C24:0)
14.	23.877	1.42122	Selacholeic acid (C24:1)

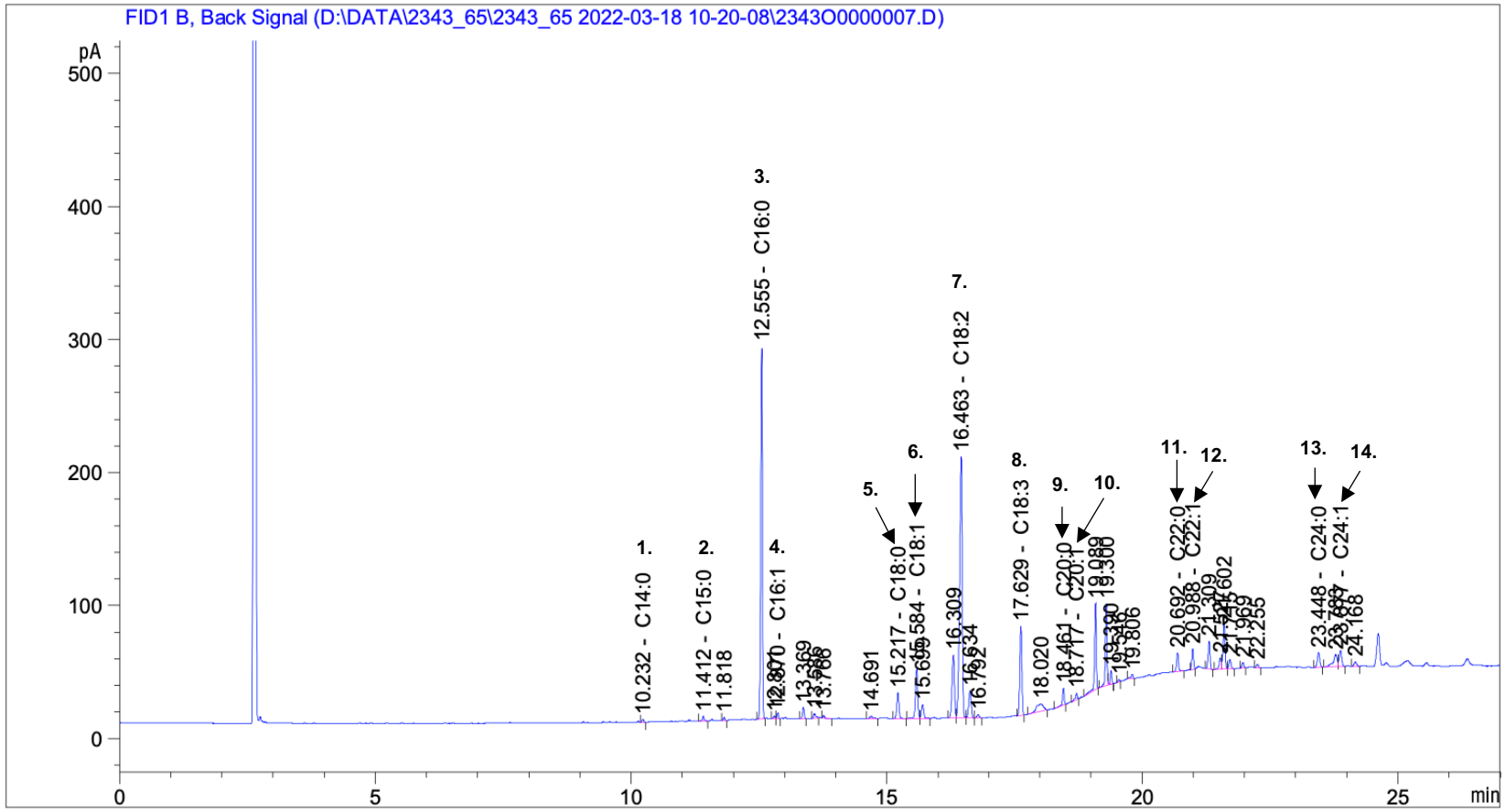

Figure 3-6. Chromatogram of the fatty acid profile from the crude ethanolic extract of the banana inflorescence extract using GC-FID analysis.

The crude ethanolic extract of banana inflorescence presented a total of fourteen major different volatile compounds. The characteristic of the volatile fatty acid in the extract was identified as the different proportion of fatty acids. From Table 3-8 there are seven saturated fatty acids (SFA), including myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0). Five compounds were monounsaturated fatty acids (MUFA), which are palmitoleic acid (C16:1), oleic acid (C18:1), eicosenoic acid (C20:1), erucic acid (C22:1), and selacholeic acid (C24:1). Additionally, two polyunsaturated fatty acids (PUFA): linoleic acid (C18:2, ω6) and linolenic acid (C18:3, ω3) were identified. Based on these findings, the main components of the fatty acids from banana inflorescence extract were linoleic acid (24.85882%), followed by palmitic acid (22.02084%), and linolenic acid (6.36497%).

It has been known that banana inflorescence contains many kinds of fatty acids. For example, a study by Sheng and colleagues showed the major compositions of MUFA and PUFAs groups such as oleic acid, linoleic acid and α-linolenic acids in their inflorescence extract (Sheng et al., 2011). In addition, the study by Ramu and colleagues reported that the dominant content from total fatty acids in banana inflorescence was linoleic acid (Ramu et al., 2017). The other study by Bolanos and colleagues reported the detailed composition of fatty acid profiles in dried banana flower and pseudostem. The results showed the high contents of PUFAs, MUFA and SFA, respectively (Bolaños et al., 2021). These findings are in agreement with the results from this study.

Saturated fatty acids are linked to increase the risk of heart diseases and stroke (Siri-Tarino et al., 2010). Palmitic acid is one of the most common SFA groups found in natural plants. It is considered that a higher intake of palmitic acid is associated with an increased relative risk of developing cardiovascular diseases (Ebbesson et al., 2015; Shramko et al., 2020). However, PUFAs, such as linoleic acid and linolenic acid, are the most frequently found fatty acids in plants. They contain hydrocarbon chains with two or more double bonds and are considered essential fatty acids (Minihane and Lovegrove, 2006). Many investigations have focused on the effects of long-chain PUFAs in lowering the risk of heart disease, especially sudden heart failure. (Abdelhamid et al., 2018; Shramko et al., 2020; Zheng et al., 2022).

At the present, the dietary guidelines recommend that increasing a high proportion of PUFAs and reducing the SFA intake have been shown to prevent the major cardiovascular diseases (Kang and Wang, 2005; Astrup et al., 2011). These suggest that the extract from banana inflorescences contains a good source of essential fatty acids, and which have a good health benefits. Possible that the fatty acids in the extract confer the antidiabetic activities, by promoting glucose uptake in the muscle cells.

It is important to note that the fatty acids identified in both UHPLC-ESI-Q-TOF/MS and GC-FID techniques might not be the *bona-fide* antidiabetic compounds. It is also possible that other types of compounds in the extract may also contribute to the antidiabetic activity. Future experimentation will be required to identify the active antidiabetic components in the inflorescence extract from Kluai Namwa Mali-Ong.

CHAPTER 4

CONCLUSION AND SUGGESTION

In this study, the crude ethanolic extracts of five banana inflorescences (*Musa* ABB cv. Kluai Namwa 'Mali Ong') were extracted and analyzed. The selected extract from one banana inflorescence is identified the proximate compositions such as total carbohydrate, total protein, total phenolic, and total flavonoid contents. The antioxidant activities of the extract were analyzed using the DPPH, FRAP, and ABTS assays. The results show that the ethanolic extract of banana inflorescence contains weak antioxidant activities. UHPLC-ESI-Q-TOF/MS was used to identify the chemical composition of the extract. The details of the chemical compositions of the extract are covered in many groups of compounds including alcohols, amino alcohols, alkaloids, and fatty acids. C2C12 skeletal muscle cells were used to investigate the effect on cell viability and glucose uptake activity. The highest concentration at 0.5 mg/mL considers safe for C2C12 myotubes with the cell viability more than 80%. Moreover, all tested concentrations of the extract showed the glucose uptake activity in a dose- and time-dependent effect on C2C12 myotubes. In addition, the identification of the fatty acid profiles presented the compounds that have a potential for antidiabetic activity. Therefore, the ethanolic extract of banana inflorescence could potentially be used in diets as an antidiabetic food additive to control diabetic diseases.

Future recommendation

The extract will be recommended to identify the active compounds and the antidiabetic mechanism of the extract.

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APPENDIX

Appendix A. Preparation of the ethanolic extracts of five banana inflorescences

Table 6-1. The amount of fresh and dried weights, percent dry weight of banana inflorescence, weight, total solid, and yield of the inflorescence extract of five banana inflorescences.

Sample	Wet weight (g)	Dry weight (g)	%Dry weight	Moisture content (%wet basis)	Weight after evaporation (g)	%Total solid	%Yield of extraction
1	1439.64	126.08	8.76	91.24	7.70	87.69	6.11
2	1067.10	92.62	8.68	91.32	5.59	85.38	6.04
3	1087.22	87.33	8.03	91.97	6.37	77.37	7.29
4	1431.45	115.73	8.08	91.92	8.13	80.93	7.02
5	1507.95	120.11	7.97	92.03	8.71	83.91	7.25

Appendix B. Antioxidant activities of ethanolic extract of banana inflorescence

1. DPPH assay

Table 6-2. The amount of standard Trolox concentration with DPPH assay.

Std.	Replicate	O.D.	O.D.std-O.D.blank	Mean	S.D.
0	1	0.421	0.391	0.390	0.0012
	2	0.421	0.391		
	3	0.419	0.389		
100	1	0.318	0.276	0.275	0.0042
	2	0.320	0.278		
	3	0.312	0.27		
200	1	0.201	0.164	0.164	0.0015
	2	0.199	0.162		
	3	0.202	0.165		
300	1	0.109	0.063	0.061	0.0026
	2	0.108	0.062		
	3	0.104	0.058		

*Blank: contained standard Trolox and ethanol instead of DPPH solution
Control: contained ethanol instead of antioxidant solution

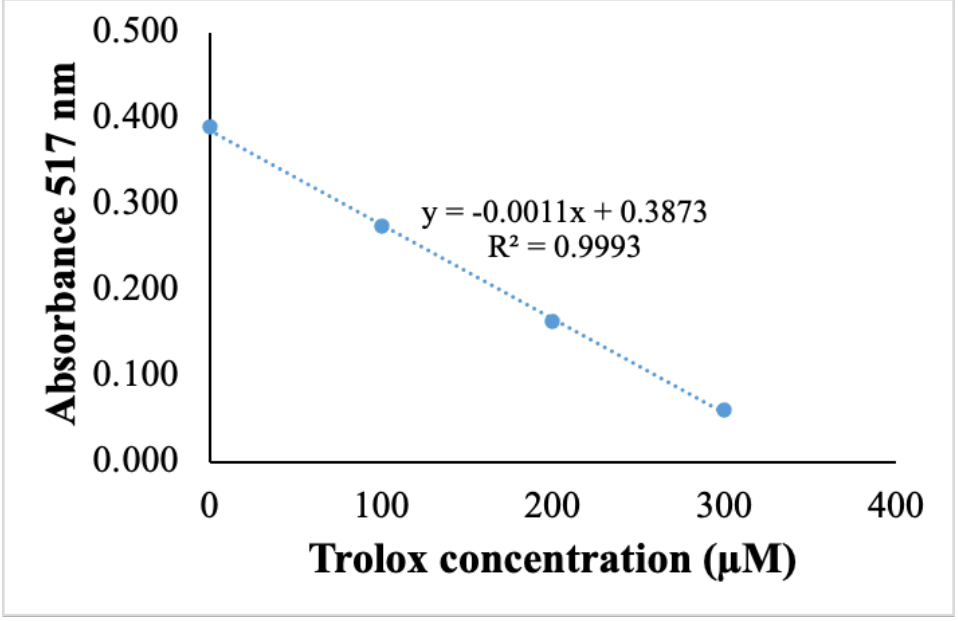

Figure 6-1. Trolox concentration vs absorbance of DPPH standard curve.

2. FRAP assay

Table 6-3. The amount of standard Trolox concentration with FRAP assay.

Std.	Replicate	O.D.	O.D.std-O.D.blank	Mean	S.D.
0	1	0.128	0.086	0.083	0.0046
	2	0.120	0.078		
	3	0.128	0.086		
100	1	0.294	0.252	0.260	0.0068
	2	0.304	0.262		
	3	0.307	0.265		
200	1	0.535	0.525	0.523	0.0072
	2	0.539	0.529		
	3	0.525	0.515		
300	1	0.648	0.606	0.637	0.0289
	2	0.685	0.643		
	3	0.705	0.663		
400	1	0.885	0.843	0.876	0.0302
	2	0.926	0.884		
	3	0.944	0.902		
500	1	1.148	1.106	1.122	0.0144
	2	1.170	1.128		
	3	1.175	1.133		
600	1	1.275	1.233	1.248	0.0160
	2	1.289	1.247		
	3	1.307	1.265		
700	1	1.450	1.407	1.470	0.0561
	2	1.533	1.49		
	3	1.557	1.514		

*Blank: contained standard Trolox and ethanol instead of FRAP solution
Control: contained ethanol instead of antioxidant solution

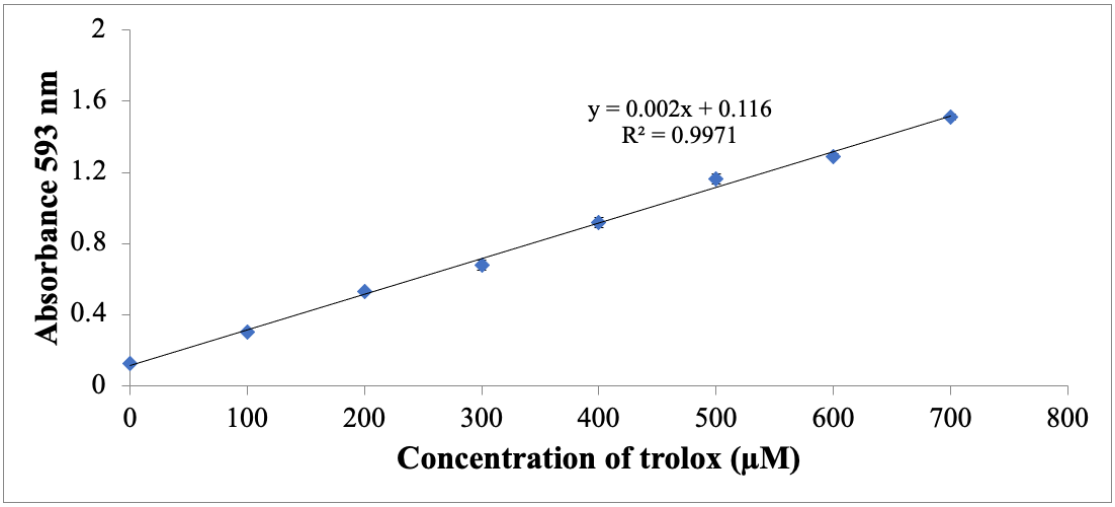

Figure 6-2. Trolox concentration vs absorbance of FRAP standard curve.

3. ABTS assay

Table 6-4. The amount of standard Trolox concentration with ABTS assay.

Std.	Replicate	O.D.	O.D.std-O.D.blank	Mean	S.D.
0	1	0.544	0.503	0.503	0.0035
	2	0.547	0.506		
	3	0.54	0.499		
100	1	0.417	0.375	0.369	0.0090
	2	0.401	0.359		
	3	0.416	0.374		
200	1	0.273	0.232	0.234	0.0049
	2	0.272	0.231		
	3	0.281	0.24		
300	1	0.126	0.084	0.079	0.0095
	2	0.127	0.085		
	3	0.110	0.068		

*Blank: contained standard Trolox and ethanol instead of ABTS solution
Control: contained ethanol instead of antioxidant solution

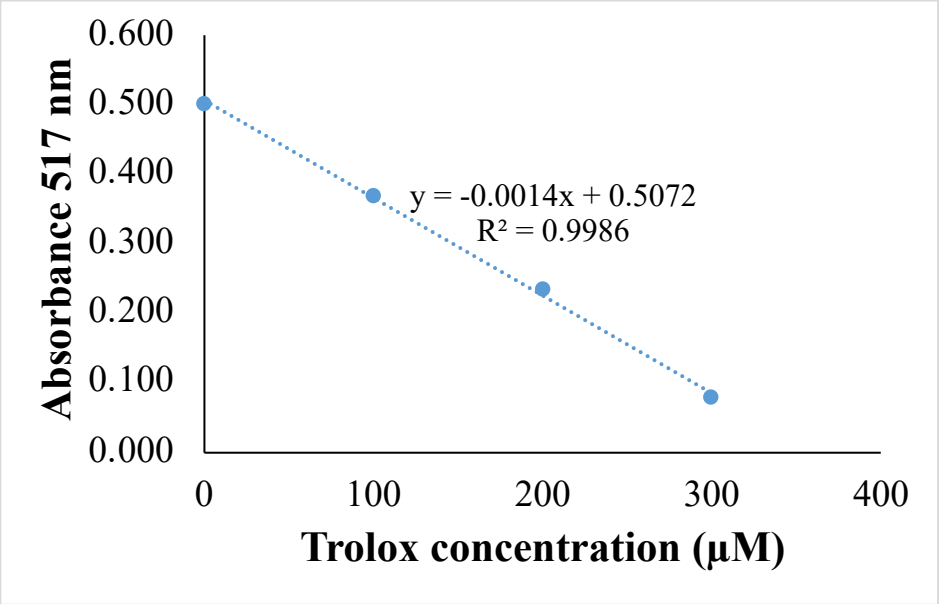

Figure 6-3. Trolox concentration vs absorbance of ABTS standard curve.

Appendix C. Effect of the ethanolic extract of banana inflorescence on C2C12 myotubes

1. MTT assay

Table 6-5. Cell viability using MTT assay.

Sample	Absorbance 570 nm	%cell viability	%cell viability average	S.D.
Untreated	1.882	99.05	100.0	1.35
	1.888	99.37		
	1.929	101.53		
100 nM insulin	2.127	111.95	116.1	3.72
	2.263	119.11		
	2.228	117.26		
500 μM metformin	1.900	100.00	100.2	1.30
	1.931	101.63		
	1.882	99.05		
0.125 mg/mL of extract	2.031	106.89	108.8	1.64
	2.084	109.68		
	2.086	109.79		
0.25 mg/mL of extract	2.311	121.63	119.2	2.66
	2.273	119.63		
	2.211	116.37		
0.5 mg/mL of extract	2.811	147.95	146.3	5.74
	2.869	151.00		
	2.658	139.89		

2. Glucose oxidase assay

2.1 Dose-dependent effect

Table 6-6. Percent stimulation of glucose uptake using glucose oxidase assay.

Sample	Absorbance 540 nm	%stimulation	%stimulation average	S.D.
Untreated	0.237	5.578	0.13	4.92
	0.261	-3.984		
	0.254	-1.195		
100 nM insulin	0.053	78.884	78.62	0.46
	0.053	78.884		
	0.055	78.088		
500 µM metformin	0.089	64.542	67.20	2.59
	0.076	69.721		
	0.082	67.331		
0.125 mg/mL extract	0.081	67.729	65.60	2.71
	0.084	66.534		
	0.094	62.550		
0.25 mg/mL extract	0.112	55.378	53.12	1.97
	0.121	51.793		
	0.120	52.191		
0.5 mg/mL extract	0.151	39.841	39.44	0.69
	0.151	39.841		
	0.154	38.645		

Table 6-7. Ratio to untreated cell of glucose uptake using glucose oxidase assay.

Sample	Absorbance 540 nm	Ratio to untreated	Ratio average	S.D.
Untreated	0.237	1.06	1.00	0.05
	0.261	0.96		
	0.254	0.99		
100 nM insulin	0.053	4.74	4.68	0.10
	0.053	4.74		
	0.055	4.56		
500 μM metformin	0.089	2.82	3.06	0.24
	0.076	3.30		
	0.082	3.06		
0.125 mg/mL extract	0.081	3.10	1.65	0.02
	0.084	2.99		
	0.094	2.67		
0.25 mg/mL extract	0.112	2.24	2.14	0.09
	0.121	2.07		
	0.120	2.09		
0.5 mg/mL extract	0.151	1.66	2.92	0.22
	0.151	1.66		
	0.154	1.63		

2.2 Time-dependent effect

Table 6-8. Time-dependent effect at absorbance 540 nm using glucose oxidase assay.

Time	Replicate	UT	100 nM Insulin	500 µM metformin	0.125 mg/mL	0.25 mg/mL	0.5 mg/mL
0	1	0.428	0.454	0.458	0.449	0.436	0.452
	2	0.442	0.446	0.456	0.436	0.434	0.420
	3	0.421	0.426	0.447	0.442	0.439	0.462
	Mean	0.430	0.442	0.454	0.442	0.436	0.445
	S.D.	0.011	0.014	0.006	0.007	0.003	0.022
12	1	0.372	0.389	0.395	0.379	0.403	0.412
	2	0.391	0.395	0.404	0.390	0.415	0.407
	3	0.397	0.404	0.406	0.390	0.413	0.405
	Mean	0.387	0.396	0.401	0.386	0.410	0.408
	S.D.	0.013	0.007	0.006	0.006	0.007	0.003
15	1	0.392	0.334	0.347	0.344	0.342	0.347
	2	0.373	0.350	0.285	0.375	0.337	0.366
	3	0.383	0.345	0.348	0.369	0.398	0.356
	Mean	0.382	0.343	0.327	0.363	0.359	0.356
	S.D.	0.010	0.008	0.036	0.016	0.034	0.010
18	1	0.383	0.294	0.310	0.327	0.333	0.306
	2	0.379	0.290	0.334	0.365	0.349	0.278
	3	0.377	0.297	0.327	0.375	0.287	0.306
	Mean	0.379	0.294	0.323	0.356	0.323	0.297
	S.D.	0.003	0.003	0.013	0.026	0.032	0.016
21	1	0.377	0.250	0.309	0.339	0.326	0.315
	2	0.360	0.266	0.298	0.349	0.268	0.301
	3	0.379	0.267	0.330	0.341	0.324	0.293
	Mean	0.372	0.261	0.312	0.343	0.306	0.303
	S.D.	0.010	0.010	0.016	0.005	0.033	0.011
24	1	0.338	0.199	0.296	0.329	0.248	0.264
	2	0.346	0.222	0.283	0.286	0.300	0.268
	3	0.326	0.213	0.280	0.300	0.315	0.299
	Mean	0.337	0.212	0.286	0.305	0.288	0.277
	S.D.	0.010	0.011	0.009	0.022	0.035	0.019

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