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Phytochemical analysis and antioxidant activity of Musa Balbisiana bract extract

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Independent University, Bangladesh (IUB)

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*Phytochemical Analysis and Antioxidant Activity of Musa Balbisiana
Bract Extract*



A dissertation submitted to the Department of Pharmacy, Independent University, Bangladesh (IUB) in partial fulfillment of the degree of Bachelor of Pharmacy

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I want to express my gratitude to everyone who made this academic project memorable and significant, whether directly or indirectly.

Declaration

I thus sincerely affirm that this dissertation is the product of my own original research and was finished entirely by me. This dissertation's content, data, analysis, and conclusions are derived from independent study and experimentation that I conducted as part of my undergraduate research program.

My distinguished supervisor, Dr. Nasrin Akhter of the School of Pharmacy and Public Health at Independent University, Bangladesh, provided direct supervision and direction for all experimental procedures, data collecting, and analysis. Throughout the whole study, the research was conducted in compliance with appropriate scientific principles and ethical standards thanks to her unwavering support, insightful advice, and academic guidance.

I further declare that no other institution has received this dissertation, in whole or in part, for the purpose of awarding a professional or academic degree. This work exhibits my commitment to the subject of pharmaceutical and health sciences and shows how hard I worked to finish this research assignment.

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Table of Contents

Acknowledgement	2
Declaration	3
Abstract	10
Chapter 1 - Introduction.....	11
1.1 Background of the study	12
• Importance of natural antioxidants	12
• Oxidative stress and free radicals.....	13
• Increasing interest in plant-based bioactive compounds	14
1.2 Medicinal values of Banana Blossom.....	15
• Definition and Classification.....	15
• Structure of Phenolic Compounds	16
• Structure of Flavonoid Compounds	16
• Role in Plant Defense.....	17
• Health Benefits.....	17
1.3 Antioxidant Activity.....	18
• Free Radicals and Reactive Oxygen Species (ROS).....	18
• Mechanism of Oxidative Damage.....	18
• Importance of Antioxidant Assays	19
• Importance of Antioxidant Assays	20
1.4 DPPH Radical Scavenging Assay.....	20
• Mechanism of Radical Scavenging.....	21
• Color Change Reaction	21
• Advantages and Limitations.....	21
1.5 Total Phenolic Content (TPC) Assay	22
• Mechanism of Redox Reaction	22
• Gallic Acid Standard Curve	23

• Expression as mg GAE/g Extract.....	23
• Expression as mg GAE/g Extract.....	24
1.6 Mechanism of Antioxidant Action of Phenolics	25
• Structural Features Influencing Antioxidant Activity	26
1.7 Factors Affecting Phenolic Content	26
• Solvent Polarity	26
• Extraction Time	27
• Temperature.....	27
• Plant Maturity.....	27
• Plant Maturity.....	27
1.8 Total Flavonoid Content (TFC) Assay	27
• Principle of Total Flavonoid Content Method	27
• Mechanism of Color Formation.....	28
• Quercetin Standard Curve	29
Expression as mg GAE/g Extract	29
1.9 Aim of the Study	30
1.10 Significance of the Study	30
1.11 Correlation Between Phenolic Content and Antioxidant Activity	31
1.12 Medicinal values of <i>Musa balbisiana</i> bract.....	33
• Botanical Classification	34
• Morphology	35
• Global Production and Economic Importance.....	36
1.13 <i>Musa balbisiana</i> Bract as an Underutilized Plant By-Product.....	37
• Composition of <i>Musa balbisiana</i> Bract	37
• Reported Phytochemicals.....	37
• Potential for Value-Added Products.....	39
1.14 Phytochemical Composition of <i>Musa balbisiana</i> Bract	39

• Phenolics	40
• Flavonoids	40
Alkaloids.....	40
• Tannins.....	40
1.15 Previous Studies on <i>Musa balbisiana</i> Bract	40
• Phytochemical Studies	40
• Phenolic and Flavonoid Content	41
• Antioxidant Activity	41
• Research Gap and Significance.....	41
1.16 Correlation Between Phenolic Content and Antioxidant Activity	41
• Evidence from Previous Studies.....	42
• Evidence from Previous Studies.....	42
Chapter 2 - MATERIALS AND METHODS	44
2.1 List of the Chemicals	45
2.2 List of the Apparatus	46
2.3 Sample Collection	46
2.4 Sample Preparation	47
• Drying Method	47
• Grinding and Sieving	48
• Storage Conditions	48
2.5 Extraction Procedure.....	48
• Solvent Selection.....	48
• Method of Extraction	49
• Filtration.....	49
Yield Calculation.....	49
Determination of Extract Yield	50
2.6 Chemicals and Reagents	50

• Solvents	51
2.7 Determination of Antioxidant Activity (DPPH Assay).....	52
• DPPH Radical Scavenging Assay	52
• Reagents and Apparatus:	52
Ascorbic Acid 400 μ g/ml (standard solution)	53
Serial Dilution (standard preparation).....	53
Extract solution preparation	54
Blank Preparation.....	55
2.8 Determination of Total Phenolic Content.....	55
• Reagents and apparatus.....	56
• Preparation of Standard Stock Solution.....	56
Preparation of Extract stock Solution	56
Preparation of 7.5% Sodium Carbonate solution	57
Preparation of Folin- Ciocalteu Reagent.....	58
Preparation of Standard and Sample.....	58
Folin–Ciocalteu Reagent:.....	59
2.9 Determination of Total Flavonoid Content.....	59
Preparation of Standarded Querectin Solution	60
Preparation of Extract Stock Solution	60
Preparation of 10% Aluminum chloride solution	61
Preparation of 1M Potassium acetate solution.....	61
Preparation of Standard and Sample.....	62
Aluminum chloride.....	62
Potassium acetate	63
Chapter 3 - RESULTS.....	64
3.1 Calculation of Percentage % inhibition (DPPH).....	65
3.2 Graph (concentration vs inhibition) - DPP	66
3.3 IC ₅₀ value (DPPH).....	67
Calc. of IC 50 value of Sample	68

3.4 Calculation of (Total Phenolic Content)	69
3.5 Graph of Total Phenolic Content	70
3.6 TPC Result	71
3.7 Calculation of (Total Flavonoid Content)	73
3.8 Graph of Total Flavonoid Content	74
3.9 TFC Result.....	75
3.10 Correlation Between TPC, TFC and DPPH Activityt.....	77
Chapter 4 - Discussion	78
4.1 Interpretation of DPPH Results	79
4.2 Interpretation of TPC Results	79
Values of Absorbance.....	79
Value of TPC.....	80
4.3 Interpretation of TFC Results.....	80
Values of Absorbance.....	80
Value of TFC.....	81
4.4 Comparison with Previous Studies.....	81
4.5 Comparison with Other Fruit Species.....	82
4.6 Relationship Between Phenolic Content and Antioxidant Activity.....	82
4.7 Possible Mechanisms of Action.....	83
4.8 Limitations of the Study.....	83
Chapter 5 - CONCLUSION.....	86
5.1 Conclusion	87
5.2 Recommendations.....	88
Significance of Advanced Characterization.....	89
Isolation of Active Compounds.....	90
Rationale for Isolation.....	90
Common Isolation Techniques.....	90
Application to <i>Musa balbisiana</i>	91

Benefits of Isolation.....	91
Application in Food and Pharmaceutical Industries.....	92
Potential Pharmaceutical Applications.....	92
Pharmaceutical Industry Applications.....	93
Significance and Industry Relevance.....	94
Chapter 6 - REFERENCES.....	95
References.....	96
References.....	98

Abstract

Musa balbisiana bracts contain various naturally occurring phytochemicals that may contribute to antioxidant activity. The present investigation was conducted to assess the antioxidant potential of methanolic extract prepared from *Musa balbisiana* bracts by evaluating its total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity. Fresh bracts were collected and thoroughly washed to remove impurities. The materials were then shade-dried and pulverized into a fine powder, followed by extraction with methanol. Different concentrations of the prepared extract were tested against DPPH radicals to determine its free radical scavenging ability. The findings indicated that the antioxidant activity increased with increasing extract concentration. The IC₅₀ value was calculated as **99.59 µg/mL**, reflecting the ability of the extract to neutralize free radicals when compared with the selected standard.

TPC was estimated by applying the Folin-Ciocalteu colorimetric method and the result was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The extract exhibited a TPC of **10.91 mg GAE/g extract**, suggesting the presence of phenolic constituents that may be involved in its antioxidant effect. In addition, TFC was determined and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract). The obtained TFC value was **19.60 mg QE/g extract**, indicating the occurrence of flavonoid compounds within the extract. The observed DPPH scavenging activity, together with the detected phenolic and flavonoid contents, suggests that these phytochemical constituents may play an important role in the antioxidant properties of *Musa balbisiana* bract extract.

Overall, the study suggests that *Musa balbisiana* bracts are a promising source of natural antioxidant compounds. Their utilization may provide valuable bioactive substances from an underused agricultural by-product, with potential applications in functional foods, nutraceuticals, food preservation, and pharmaceutical research. Further studies on phytochemical characterization, extraction optimization, active compounds, and in vivo evaluation are needed to confirm their functional and therapeutic potential.

Keywords: *Musa balbisiana* bract, Methanolic extract, Antioxidant activity, DPPH radical scavenging, Total phenolic content, Total flavonoid content, Gallic acid equivalents, Quercetin equivalents, IC₅₀, Bioactive compounds, Phytochemicals, Nutraceuticals

Chapter 1 - Introduction

1.1 Background of the Study

Importance of Natural Antioxidants

Natural antioxidants are biologically active compounds that help protect the body against oxidative damage caused by reactive free radicals and other harmful molecules generated through normal metabolic activities as well as environmental exposure. When the production of these reactive substances exceeds the body's protective capacity, oxidative stress can occur, leading to damage of important cellular components such as lipids, proteins, and DNA. Prolonged oxidative stress has been associated with the aging process and the development of several chronic health conditions Betteridge, 2000; Lobo *et al.*, 2010; Valko *et al.*, 2007.

Antioxidants can reduce oxidative damage by donating electrons or hydrogen atoms to unstable free radicals, thereby converting them into more stable forms and limiting harmful chain reactions within biological tissues. Regular exposure to antioxidant-rich foods may therefore contribute to maintaining cellular integrity and overall physiological balance. Natural antioxidants have been studied for their possible protective roles against various disorders, including cardiovascular diseases, diabetes, cancer, and neurodegenerative conditions.

Plant-based foods are considered important dietary sources of antioxidant compounds. Fruits, vegetables, whole grains, nuts, seeds, and various plant-derived beverages contain diverse phytochemicals, including phenolic compounds and flavonoids, which may contribute to their antioxidant properties. In addition to protecting cells from oxidative damage, these compounds may support normal immune function and help maintain healthy physiological processes. Therefore, the identification and evaluation of antioxidant-rich plant materials have become important areas of nutritional, pharmaceutical, and natural product research. In this context, the investigation of underutilized plant materials such as *Musa balbisiana* bracts may provide an opportunity to identify valuable natural sources of bioactive compounds with potential health and functional applications Betteridge, 2000; Lobo *et al.*, 2010; Valko *et al.*, 2007.

Oxidative Stress and Free Radicals

Oxidative stress occurs when the formation of reactive molecules in the body exceeds the ability of its antioxidant defense mechanisms to neutralize them effectively. Free radicals are highly reactive chemical species that contain one or more unpaired electrons, making them unstable and capable of reacting with nearby cellular components. In biological systems, many of these reactive molecules are generated from oxygen and are collectively associated with reactive oxygen species (ROS). Important examples include superoxide anion, hydroxyl radical, and hydrogen peroxide (Betteridge, 2000).

Reactive oxygen species are naturally produced as part of normal physiological processes. Mitochondrial energy production is one of the major intracellular sources of ROS, while additional amounts may be generated during inflammatory responses and immune-system activity. Under normal conditions, the body maintains a balance between the production and removal of these reactive species through its antioxidant defense system. However, exposure to environmental factors such as cigarette smoke, air pollution, radiation, and certain toxic chemicals can increase ROS formation and disturb this balance Valko *et al.*, 2007;.

The body's antioxidant protection involves both enzymatic and non-enzymatic components. Important antioxidant enzymes include superoxide dismutase, catalase, and glutathione peroxidase, while vitamins such as vitamin C and vitamin E contribute to the non-enzymatic defense system. When ROS production becomes excessive, these protective mechanisms may become insufficient, resulting in oxidative damage to essential cellular molecules. Lipids can undergo peroxidation, proteins may experience structural and functional alterations, and oxidative damage to DNA can contribute to genetic mutations and impaired cellular function.

Continuous oxidative stress has been associated with the development and progression of several chronic diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative diseases such as Parkinson's and Alzheimer's disease. The connection between free radical-induced damage and aging was notably proposed by Denham Harman through his free radical theory of aging in 1956. Nevertheless, free radicals and ROS are not exclusively harmful, as controlled amounts also participate in normal cellular signaling and immune defense. Therefore, maintaining an appropriate balance between free radical generation and antioxidant protection is essential for preserving cellular function and reducing oxidative damage. This balance also highlights the importance of investigating

natural antioxidant sources, including plant-derived materials rich in phenolic and flavonoid compounds Halliwell and Gutteridge, 2015.

Increasing Interest in Plant-Based Bioactive Compounds

In recent years, plant-derived bioactive compounds have received considerable attention in scientific and medical research because of their potential role in maintaining health and reducing the risk of various diseases. Plant bioactive compounds are naturally occurring substances that can produce biological effects in the body beyond their basic nutritional contribution. A wide range of these compounds is found in fruits, vegetables, cereals, legumes, herbs, and medicinal plants. (Cory *et al.*, 2018; Pandey and Rizvi, 2009)

Major groups of plant-based bioactive substances include polyphenols, flavonoids, carotenoids, alkaloids, terpenoids, and phytosterols. Numerous studies have suggested that these compounds may possess a variety of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, and neuroprotective effects. Their beneficial actions are associated with several biological mechanisms, such as reducing oxidative stress, influencing cellular signaling pathways, regulating gene expression, and supporting normal metabolic functions. (Heim *et al.*, 2002; Rice-Evans *et al.*, 1997)

Among these compounds, polyphenols and flavonoids are particularly important because of their ability to interact with reactive free radicals and contribute to the body's antioxidant defense system. Carotenoids are also recognized for their potential protective effects against cellular damage and their contribution to normal immune function. The combined presence of different phytochemicals in plant materials may therefore provide multiple biological benefits and make plants valuable sources of naturally occurring functional compounds. (Pandey and Rizvi, 2009; Shahidi and Ambigaipalan, 2015)

The increasing prevalence of chronic diseases, together with growing consumer interest in natural and health-promoting products, has encouraged further investigation of plant-derived bioactive substances. Research in this field has expanded toward their possible incorporation into nutraceuticals, functional foods, dietary products, and pharmaceutical preparations. As a result, plant-based bioactive compounds are increasingly being considered important components of preventive nutrition and complementary approaches to healthcare. The exploration of less-utilized plant materials, including *Musa balbisiana* bracts, may further contribute to

the discovery of natural sources of phenolic and flavonoid compounds with potential biological applications. (Shahidi and Yeo, 2016)

1.2 Phenolic Compounds in Plants

Definition and Classification

Phenolic compounds are an important group of plant secondary metabolites characterized by the presence of one or more hydroxyl groups attached to an aromatic ring. They play significant roles in plant growth, development, reproduction, and defense against environmental stresses. These compounds are also widely studied because of their potential antioxidant and other biological activities. (Dai and Mumper, 2010)

Based on their chemical structure, phenolic compounds can be classified into several major groups, including simple phenols, phenolic acids, flavonoids, tannins, lignans, and stilbenes. Flavonoids can be further divided into subclasses such as flavonols, flavones, flavanones, isoflavones, anthocyanins, and catechins. The structural diversity of these compounds contributes to their wide range of biological and functional properties in plants. (Heim *et al.*, 2002; Rice-Evans *et al.*, 1997)

Structure of Phenolic Compounds

An aromatic benzene ring (C₆) with one or more hydroxyl (–OH) groups makes up the fundamental structure of phenolic substances. For instance, flavonoids have two aromatic rings (A and B) joined by a three-carbon bridge to form a heterocyclic C ring, while phenolic acids have a simple aromatic ring with a carboxylic acid group. Tannins are larger polyphenolic polymers, and stilbenes consist of two phenyl groups linked by a two-carbon ethylene bridge. Phenolics' broad spectrum of chemical and biological activities is supported by their structural diversity. (Heim *et al.*, 2002)

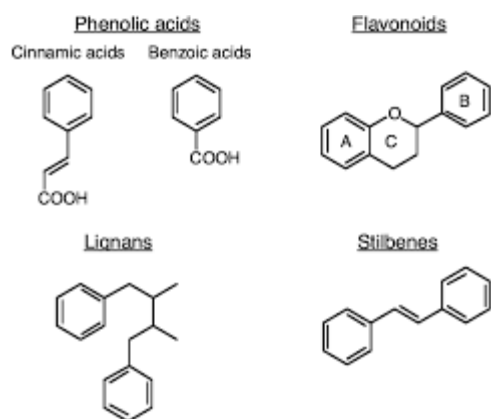


Figure: Basic chemical structures of major phenolic compounds (e.g., phenolic acid, flavonoid, tannin). (Cory *et al.*, 2018)

Structure of Flavonoid Compounds

Flavonoids are a major group of naturally occurring polyphenolic compounds found widely in plants. Their basic structure consists of **15 carbon atoms ($\text{C}_6-\text{C}_3-\text{C}_6$)** arranged into two aromatic rings, known as the A and B rings, connected through a three-carbon heterocyclic C ring. The A and C rings form the central flavonoid skeleton, while the B ring is generally attached to the C ring. (Heim *et al.*, 2002; Rice-Evans *et al.*, 1997)

Variations in the number and position of hydroxyl, methoxy, and other functional groups produce different types of flavonoids. Major subclasses include flavonols, flavones, flavanones, isoflavones, anthocyanins, and flavanols. These structural differences influence their chemical properties and biological activities, particularly their ability to act as antioxidants.

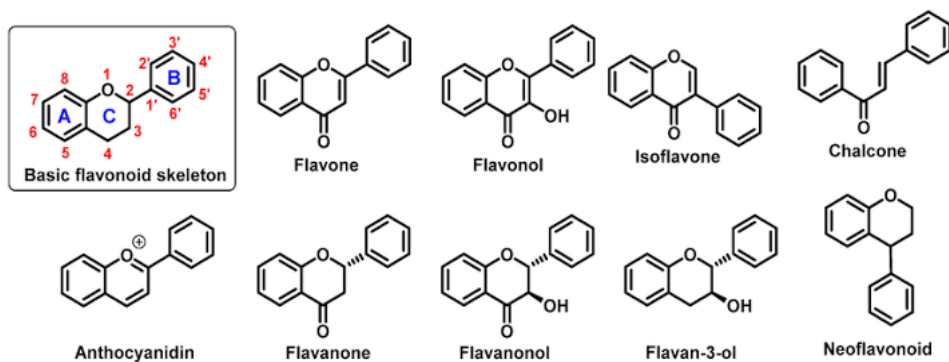


Figure: Basic chemical structure of a flavonoid compound showing the A, B, and C rings (Heim *et al.*, 2002).

Role in Plant Defense

Phenolic compounds help plants protect themselves from biological and environmental stresses. They can inhibit harmful bacteria and fungi and may also discourage herbivores due to their bitter taste or biological effects. Phenolics also contribute to plant tissue strength through their role in lignin formation. (Shahidi and Ambigaipalan, 2015)

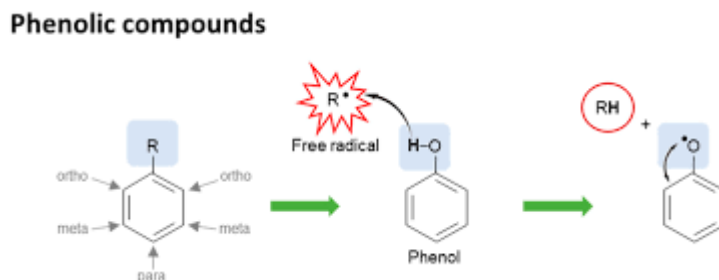
Moreover, phenolic compounds help protect plant cells from UV radiation and oxidative stress by scavenging reactive oxygen species (ROS). Their production may increase during pathogen attack, physical damage, or unfavorable environmental conditions. Thus, phenolics are an important part of the plant's natural defense system. (Heim *et al.*, 2002)

Health Benefits

Phenolic compounds are widely studied for their antioxidant and anti-inflammatory properties. They help protect cells from oxidative damage by neutralizing free radicals, reducing reactive oxygen species (ROS), and binding metal ions. These activities may help protect DNA, proteins, and lipids from cellular damage.

Phenolic acids and flavonoids have been associated with potential protective effects against chronic conditions such as cancer, diabetes, cardiovascular diseases, and neurological disorders. Their anti-inflammatory activity may involve reducing pro-

inflammatory enzymes and signaling pathways. Therefore, phenolics are considered valuable natural antioxidants for functional foods and nutraceutical applications.



Suggested Image 2: Mechanism of phenolic compounds scavenging free radicals and reducing oxidative stress(Betteridge, 2000).

1.3 Antioxidant Activity

Free Radicals and Reactive Oxygen Species (ROS)

Free radicals are highly reactive molecules or atoms that contain one or more unpaired electrons, making them capable of causing chain reactions in biological systems. Reactive oxygen species (ROS) include superoxide anion ($\text{O}_2^{\bullet-}$), hydroxyl radical ($\bullet\text{OH}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2$).

ROS are naturally produced during normal cellular metabolism, particularly in mitochondria, and also during immune responses. At normal levels, ROS can act as signaling molecules involved in cell growth, differentiation, and defense. However, excessive ROS production can disturb the cellular antioxidant balance and lead to oxidative stress and cellular damage (Betteridge, 2000;

Mechanism of Oxidative Damage

When ROS levels surpass the antioxidant defenses of the cell, oxidative damage takes place. Three primary routes are involved in the mechanism:

- **Lipid Peroxidation:** When ROS attack polyunsaturated fatty acids in cell membranes, they produce reactive aldehydes like malondialdehyde (MDA) and lipid radicals, which compromise the integrity of the membrane.
- **Protein Oxidation:** ROS cause structural changes and enzyme deactivation by oxidizing amino acid side chains and rupturing peptide bonds.

- DNA Damage: ROS contribute to mutagenesis and genomic instability by causing base alterations, strand breakage, and cross-linking.

Aging and the pathophysiology of several diseases, including as cancer, diabetes, neurodegeneration, and cardiovascular disorders, are linked to this oxidative damage.

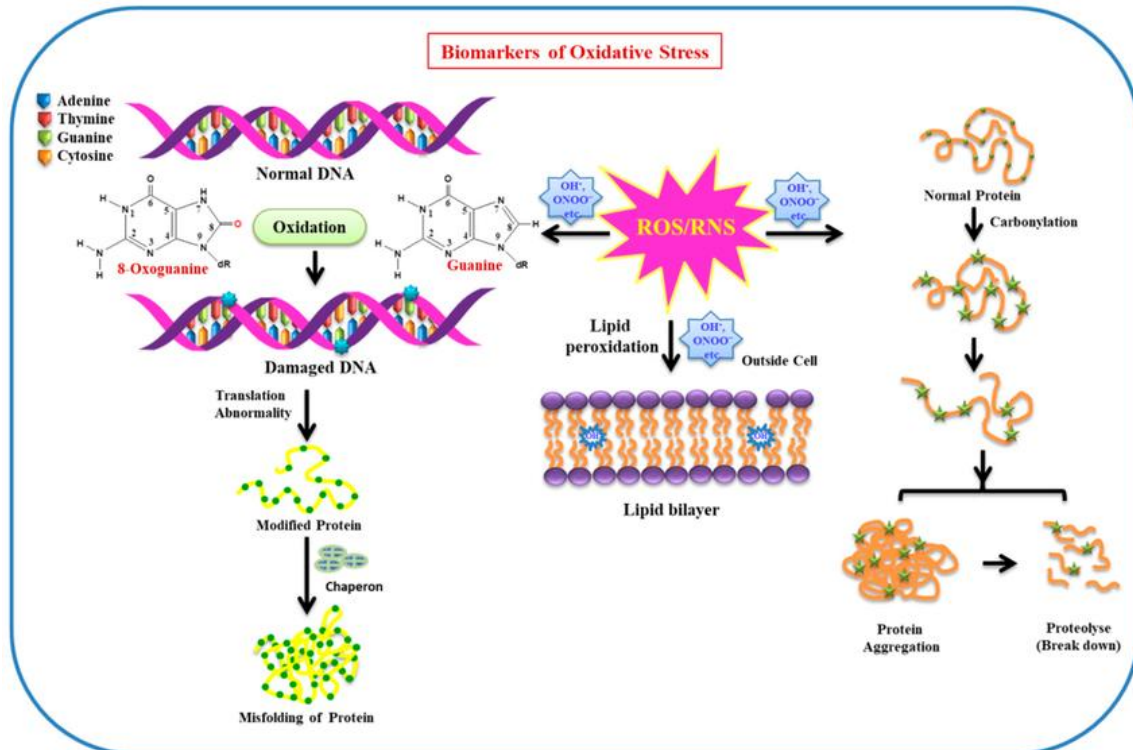


Figure: Diagram showing ROS formation and oxidative damage to lipids, proteins, and DNA(Betteridge, 2000;.

Importance of Antioxidant Assays

Antioxidant assays are experimental techniques used to assess a compound's or plant extract's capacity to scavenge free radicals. These tests are necessary for:

- Measure the antioxidant capacity of foods, plants, and bioactive substances. Identify possible defense mechanisms against oxidative stress.
- Evaluate the effectiveness of synthetic and natural antioxidants.

DPPH radical scavenging, ABTS assay, FRAP (Ferric Reducing Antioxidant Power), and ORAC (Oxygen Radical Absorbance Capacity) are examples of

common antioxidant assays. These tests assist researchers in identifying bioactive substances produced from plants, such as flavonoids and polyphenols, that can reduce cellular damage caused by ROS and promote health.

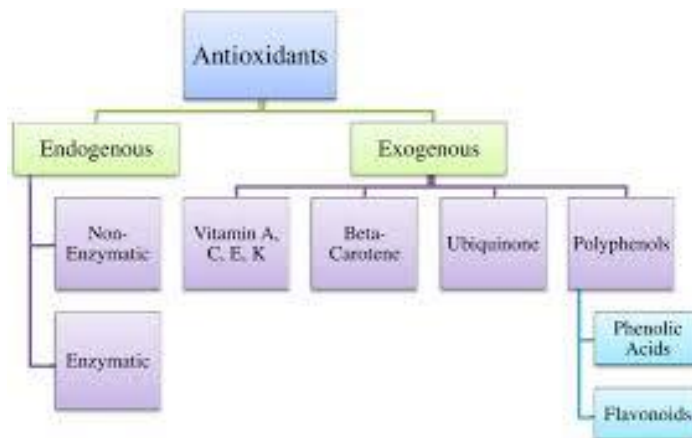


Figure: Flowchart of antioxidant assay types and their mechanisms(Betteridge, 2000;).

1.4 DPPH Radical Scavenging Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

Principle of DPPH Assay

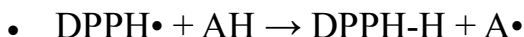
The ability of a substance or plant extract to scavenge free radicals is evaluated experimentally using antioxidant tests. These examinations are required for: • Assess the antioxidant potential of foods, plants, and bioactive compounds.

- Determine potential oxidative stress defense mechanisms.
- Assess the efficacy of both natural and artificial antioxidants.

Common antioxidant tests include ORAC (Oxygen Radical Absorbance Capacity), FRAP (Ferric Reducing Antioxidant Power), ABTS assay, and DPPH radical scavenging. These assays help researchers find plant-based bioactive compounds, such flavonoids and polyphenols, that can lessen ROS-induced cellular damage and improve health (Brand-Williams *et al.*, 1995; Blois, 1958).

Mechanism of Radical Scavenging

The mechanism involves **electron or hydrogen atom transfer** from the antioxidant to the DPPH radical:



Here, AH represents the antioxidant molecule, which donates a hydrogen atom to the DPPH radical (DPPH•), neutralizing it and forming a non-radical DPPH-H molecule. This reaction stops the chain reaction of free radicals and reflects the antioxidant potential of the compound.

Color Change Reaction (Purple → Yellow)

Because of its delocalized electron, the DPPH radical has a rich violet/purple color in solution. Depending on the amount of antioxidants available, the unpaired electron is quenched upon reduction by an antioxidant, turning the solution yellow or colorless. Spectrophotometric measurement is made possible by this observable color shift, which is a crucial aspect of the test.

Measurement at 517 nm

A UV-visible spectrophotometer is usually used to track the reaction at 517 nm, which is the wavelength at which DPPH exhibits its greatest absorbance. The antioxidant's capacity to scavenge radicals is directly correlated with a gradual decrease in absorbance at 517 nm. The antioxidant concentration needed to eliminate 50% of DPPH radicals is known as IC₅₀, and it is frequently used to express the results.

Advantages and Limitations

Advantages:

- Simple, rapid, and reproducible.
- Requires small sample amounts.
- Widely used for screening antioxidant activity of plant extracts.

Limitations:

- DPPH reacts only with compounds soluble in organic solvents (ethanol, methanol).

- Not suitable for aqueous extracts.
- Reaction kinetics vary with different antioxidants, which may affect accuracy.
- Does not mimic biological conditions fully, as it is a chemical assay rather than a cellular model.

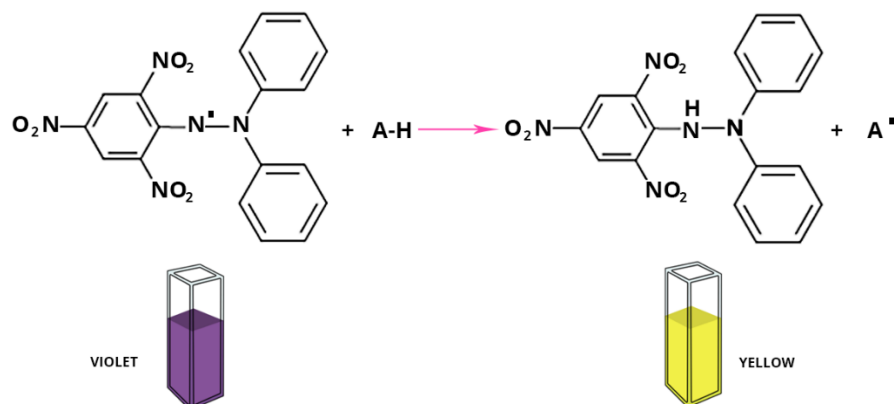


Figure: Diagram showing DPPH radical (purple) being reduced by an antioxidant to DPPH-H (yellow) and measurement at 517 nm (Brand-Williams *et al.*, 1995;

1.5 Total Phenolic Content (TPC) Assay

Principle of Folin–Ciocalteu Method

A popular colorimetric test for determining the total phenolic content (TPC) of plant extracts, meals, and drinks is the Folin–Ciocalteu (F–C) method. The Folin–Ciocalteu reagent, a combination of phosphomolybdate and phosphotungstate, reacts with phenolic chemicals to form the basis of the procedure. Under alkaline conditions, phenolic chemicals function as reducing agents and transfer electrons to the F–C reagent, forming a blue complex. Spectrophotometry can be used to quantify the concentration of phenolics in the sample, which is correlated with the color's intensity.

Mechanism of Redox Reaction

The Folin-Ciocalteu assay's mechanism is basically a redox reaction:

- Quinones are produced by oxidizing phenolic compounds (Ar–OH).
- A blue-

colored Mo(V)/W(V) combination is produced by reducing the Folin–Ciocalteu reagent (Mo(VI)/W(VI)).

In simplified terms:



This electron transfer reaction forms the basis of the spectrophotometric measurement of total phenolics.

Gallic Acid Standard Curve

The process of quantification involves comparing the sample's absorbance to a standard calibration curve made with gallic acid, a reference phenolic substance. Under the same circumstances as the samples, serial dilutions of gallic acid are reacted with the F-C reagent, and absorbance is recorded (usually at 765 nm). The phenolic content of the sample can be calculated using a linear regression equation derived from the standard curve.

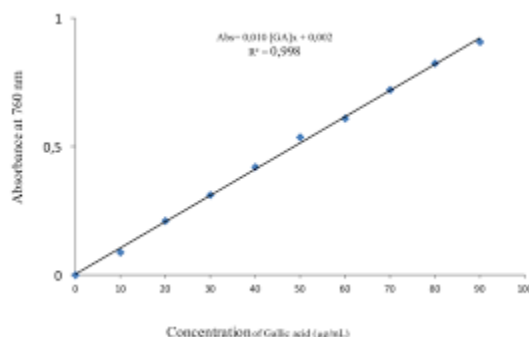


Figure: Example of a gallic acid standard curve (absorbance vs. concentration).

Expression as mg GAE/g Extract

Milligrams of gallic acid equivalents per gram of extract (mg GAE/g) is a typical way to express a sample's total phenolic content. Comparing various samples and investigations is made possible by this standardization.

For example:

$$\text{TPC (\%)} = \frac{\text{GAE}}{C_{\text{initial}}} \times 100$$

This method provides a simple and reproducible estimate of total phenolics in plant-derived materials and is frequently applied in antioxidant studies.

The total Phenolic content of the *Musa balbisiana* bract extract is expressed as milligrams of quercetin equivalents per gram of extract (mg GAE/g). The flavonoid content is calculated from the quercetin calibration curve using the following equation:

$$\text{TPC (mg GAE/g)} = (C \times V \times \text{DF}) / W$$

Where:

- **C** = Concentration of flavonoids obtained from the quercetin calibration curve (mg/mL)
- **V** = Volume of the extract solution (mL)
- **DF** = Dilution factor
- **W** = Weight of the plant extract used (g)
- **QE** = Quercetin equivalents

Therefore, the final result is reported as **mg GAE/g extract**. A higher value indicates a greater amount of Phenolic compounds in the *Musa balbisiana* bract extract.

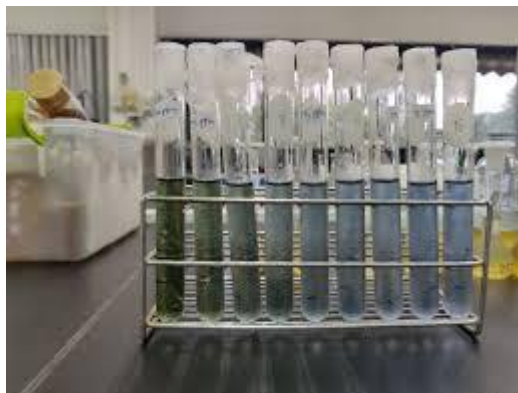


Figure: Color change of the F–C reaction in a test tube, blue intensity corresponding to phenolic concentration (Valko *et al.*, 2007)

1.6 Mechanism of Antioxidant Action of Phenolics

Phenolic compounds are important plant secondary metabolites that possess significant antioxidant properties. Their antioxidant activity mainly depends on their ability to donate hydrogen atoms or electrons to unstable free radicals and stabilize the resulting molecules. Through these mechanisms, phenolics can reduce oxidative damage to important biomolecules such as lipids, proteins, and DNA.

Key Mechanisms

Free Radical Scavenging

Phenolic compounds can neutralize free radicals and reactive oxygen species (ROS) by donating hydrogen atoms or electrons. The resulting phenoxyl radical is relatively stable because its unpaired electron can be delocalized through the aromatic structure. This helps reduce the continuation of oxidative chain reactions. (Heim *et al.*, 2002)

Metal Chelation

Some phenolic compounds can bind transition metal ions such as Fe^{2+} and Cu^{2+} . These metals can promote the formation of highly reactive hydroxyl radicals through reactions such as the Fenton reaction. Metal chelation therefore helps reduce the generation of reactive radicals and limits oxidative damage. (Heim *et al.*, 2002)

Inhibition of Oxidative Enzymes

Certain phenolic compounds may inhibit enzymes involved in oxidative processes, including lipoxygenase, cyclooxygenase, and xanthine oxidase. By reducing the activity of these enzymes, phenolics may decrease the formation of reactive oxygen species and associated oxidative stress. (Rice-Evans *et al.*, 1997)

Synergistic Interactions

Phenolics can interact with other antioxidant compounds, including vitamins and carotenoids. Such interactions may help maintain antioxidant activity and improve the overall ability of a biological system to control oxidative reactions. (Shahidi and Ambigaipalan, 2015)

Chain-Breaking Activity

Phenolic compounds can interrupt lipid peroxidation by donating hydrogen atoms to lipid peroxy radicals. This converts reactive radicals into more stable products and slows the propagation of oxidative reactions, thereby helping to protect lipids from further degradation.

Structural Features Influencing Antioxidant Activity

The antioxidant effectiveness of phenolic compounds depends on their chemical structure. Important structural factors include: (Heim *et al.*, 2002)

- **Number and Position of Hydroxyl Groups:** The number and arrangement of hydroxyl groups can influence hydrogen or electron donation and radical-scavenging ability. (Rice-Evans *et al.*, 1997)
- **Conjugation and Resonance:** Delocalization of electrons within the aromatic structure helps stabilize the phenoxyl radical formed after hydrogen donation.
- **Substituents:** Groups such as methoxy and glycosyl substituents can modify the antioxidant properties and chemical behavior of phenolic compounds.

Overall, phenolic compounds can act through several complementary mechanisms to reduce oxidative stress. Their antioxidant properties make them important bioactive constituents of plants and support their potential applications in food preservation, nutraceuticals, and pharmaceutical research.

1.7 Factors Affecting Phenolic Content

The phenolic content of plant extracts can be influenced by several factors related to the plant material and the extraction process. Important factors include solvent polarity, extraction time, temperature, and the maturity or developmental stage of the plant. Understanding these factors is important for obtaining maximum phenolic recovery while maintaining the stability of bioactive compounds.

Solvent Polarity

The polarity of the extraction solvent has a major influence on the solubility and recovery of phenolic compounds. Polar solvents such as methanol, ethanol, and

water are generally effective for extracting many hydrophilic phenolics, while less polar solvents may be more suitable for relatively lipophilic compounds. A mixture of solvents can sometimes improve extraction efficiency by dissolving phenolics with different chemical properties. The choice of solvent may also influence the antioxidant activity of the resulting extract.

Extraction Time

Extraction time affects the amount of phenolic compounds transferred from the plant material into the solvent. A short extraction period may result in incomplete recovery, whereas excessively long extraction can promote oxidation or degradation of some sensitive phenolic compounds. Therefore, an appropriate extraction time should be selected to obtain a good yield while maintaining compound stability.

Temperature

Temperature can affect the solubility, diffusion, and extraction efficiency of phenolic compounds. Moderate temperatures may improve solvent penetration and increase the release of phenolics from plant tissues. However, excessive heat may degrade heat-sensitive compounds and reduce their antioxidant activity. Thus, suitable temperature control is important for balancing extraction efficiency and phenolic stability.

Plant Maturity

The phenolic composition of plant materials can vary according to their developmental or maturity stage. Young and mature tissues may contain different types and amounts of phenolic compounds. Environmental conditions and plant stress can also influence phenolic production and accumulation. Selecting an appropriate maturity stage of *Musa balbisiana* bracts can therefore help obtain consistent phytochemical content and antioxidant activity.

1.8 Total Flavonoid Content (TFC) Assay

Principle of Total Flavonoid Content Method

The Total Flavonoid Content (TFC) assay is a commonly used colorimetric method for estimating the amount of flavonoid compounds present in plant extracts. Flavonoids are an important group of polyphenolic compounds that contribute to the

antioxidant properties of many plant materials. In this study, the TFC assay is used to estimate the flavonoid content of *Musa balbisiana* bract extract.

The method is commonly based on the reaction between flavonoid compounds and aluminum chloride (AlCl_3). Flavonoids form a colored complex with aluminum ions under suitable reaction conditions. The intensity of the color produced depends on the concentration of flavonoids present in the sample. The developed color is measured using a UV-Visible spectrophotometer, and the absorbance value is used to estimate the flavonoid concentration.

Mechanism of Color Formation

The aluminum chloride method involves the formation of a complex between aluminum ions and the hydroxyl and carbonyl groups present in flavonoid molecules. When the flavonoid compounds react with AlCl_3 , a stable colored complex is formed. This color development allows the flavonoid concentration to be estimated through spectrophotometric measurement.

In simplified form:



The intensity of the developed color is generally proportional to the amount of flavonoid compounds present in the extract. Therefore, measurement of absorbance provides an indirect estimation of the total flavonoid content.

Quercetin Standard Curve (Chang *et al.*, 2002; Zhishen *et al.*, 1999)

Quantification of total flavonoids is commonly performed by comparing the absorbance of the sample with a calibration curve prepared using quercetin as the reference standard. Different concentrations of quercetin are prepared and treated with the reagents under the same experimental conditions as the plant extract. The absorbance of the standard solutions is then measured using a UV-Visible spectrophotometer, commonly at around **420 nm**, depending on the specific procedure used. (Chang *et al.*, 2002)

A calibration curve is prepared by plotting the absorbance values against the corresponding concentrations of quercetin. A linear regression equation obtained

from this standard curve is then used to calculate the flavonoid content of the *Musa balbisiana* bract extract.

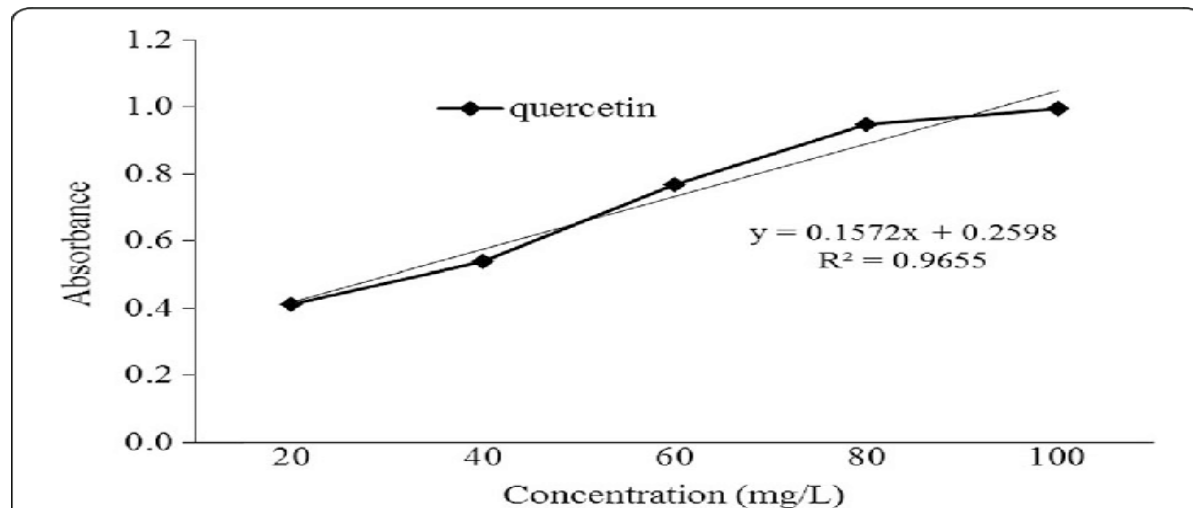


Figure: Quercetin standard calibration curve showing the relationship between absorbance and concentration (Zhishen *et al.*, 1999).

Expression as mg QE/g Extract

The total flavonoid content of the *Musa balbisiana* bract extract is expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). The flavonoid content is calculated from the quercetin calibration curve using the following equation: (Sadebo *et al.*, 2026; Food Chemistry, 2025)

$$\text{TFC (mg QE/g)} = (\text{C} \times \text{V} \times \text{DF}) / \text{W} \text{ (Shahidi and Yeo, 2016)}$$

Where:

- **C** = Concentration of flavonoids obtained from the quercetin calibration curve (mg/mL) (Altemimi *et al.*, 2017)
- **V** = Volume of the extract solution (mL) (Sadebo *et al.*, 2026)
- **DF** = Dilution factor (Altemimi *et al.*, 2017)
- **W** = Weight of the plant extract used (g)
- **QE** = Quercetin equivalents

Therefore, the final result is reported as **mg QE/g extract**. A higher value indicates a greater amount of flavonoid compounds in the *Musa balbisiana* bract extract.

1.9 Aim of the Study

The aim of this study is to investigate the phytochemical composition and antioxidant activity of *Musa balbisiana* bract extract, an underutilized plant material with potential bioactive properties. The study focuses on determining the total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging activity using the DPPH assay.

The findings are expected to provide information about the antioxidant potential of *Musa balbisiana* bracts and their possible health-related benefits. The study may also support the sustainable utilization of this plant material for potential applications in functional foods, nutraceuticals, and other value-added products.

1.10 Significance of the Study

Musa balbisiana bracts are often considered an underutilized plant material and are generally discarded after use. However, these bracts may contain various biologically active compounds, particularly phenolic compounds and flavonoids, which are known for their antioxidant properties. Therefore, investigating the phytochemical composition and antioxidant activity of *Musa balbisiana* bract extract can provide useful information about its potential as a natural source of bioactive compounds.

The determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) can help estimate the amount of important phenolic and flavonoid compounds present in the extract. In addition, the DPPH radical scavenging assay can be used to evaluate its ability to neutralize free radicals. The results of these analyses may help establish a relationship between the presence of phenolic and flavonoid compounds and the antioxidant activity of the bract extract.

From an environmental and economic perspective, the utilization of *Musa balbisiana* bracts may contribute to reducing plant-based waste and promoting sustainable use of agricultural resources. Instead of treating the bracts as discarded material, their possible recovery as a source of valuable phytochemicals could

support waste valorization and the development of more sustainable approaches to plant resource management.

The findings may also have potential applications in the food, nutraceutical, pharmaceutical, and functional food industries. If the extract demonstrates significant antioxidant activity, it could be further investigated for use as a natural antioxidant source, dietary supplement ingredient, or other value-added product. Such utilization could increase the economic importance of an otherwise underused plant material. Finally, this study will provide baseline information on the phytochemical characteristics and antioxidant potential of *Musa balbisiana* bract extract. The results may serve as a useful reference for future research investigating its individual bioactive compounds, biological activities, safety, and possible applications. Overall, the study contributes to the growing interest in plant-derived antioxidants and the sustainable utilization of underexplored botanical resources.

1.11 Correlation Between Phenolic Content and Antioxidant Activity

Phenolic compounds are considered important contributors to the antioxidant activity of many plant extracts. Their ability to donate hydrogen atoms or electrons, neutralize free radicals, and chelate metal ions can help reduce oxidative reactions. Therefore, a higher Total Phenolic Content (TPC) is often associated with stronger antioxidant activity. However, antioxidant activity is not determined by phenolics alone, as other compounds such as flavonoids, vitamins, and other phytochemicals may also contribute.

Evidence from Studies

Radical Scavenging Assays

Plant extracts containing higher levels of phenolic compounds often show stronger free radical scavenging activity in assays such as DPPH, ABTS, and FRAP. Phenolic hydroxyl groups can donate hydrogen atoms or electrons to unstable radicals, helping to stabilize them and prevent further oxidative reactions.

Metal Chelation and Reducing Power

Phenolic compounds can bind transition metal ions and reduce oxidized substances, thereby limiting the formation of highly reactive radicals. Extracts with higher

phenolic content may therefore show greater reducing power and antioxidant capacity in appropriate in-vitro assays.

Quantitative Correlation

Several studies have reported a positive relationship between TPC and antioxidant activity in different plant extracts. However, the strength of this relationship can vary depending on the plant species, extraction method, phenolic composition, and antioxidant assay used. Therefore, TPC can provide useful information about antioxidant potential but should not be considered the only determining factor.

Influence of Phenolic Structure

The antioxidant effectiveness of individual phenolic compounds depends not only on their concentration but also on their chemical structure. Factors such as the number and position of hydroxyl groups, conjugation, and molecular arrangement can influence their radical-scavenging ability. Thus, both the quantity and structural characteristics of phenolic compounds contribute to the overall antioxidant activity.

Overall, the relationship between TPC and antioxidant activity is useful for evaluating the antioxidant potential of plant extracts. In the present study, TPC results can be compared with DPPH radical scavenging activity to better understand the contribution of phenolic compounds to the antioxidant properties of *Musa balbisiana* bract extract.

1.12 Medicinal values of *Musa balbisiana* bract



Figure: Field of *Musa balbisiana* bract (Dai and Mumper, 2010)

Musa balbisiana is a species of banana belonging to the family Musaceae and is widely distributed throughout tropical and subtropical regions. It is an important banana species and is recognized for its relatively hardy nature and ability to grow under different environmental conditions. The plant produces large bracts that surround and protect the developing inflorescence. Although the fruit and other parts of the banana plant have received considerable nutritional and scientific attention, the bracts are comparatively less explored as a potential source of bioactive compounds. (Heim *et al.*, 2002; Rice-Evans *et al.*, 1997)

Plant tissues of *Musa* species contain a diverse range of phytochemicals, including phenolic compounds, flavonoids, tannins, alkaloids, and other secondary metabolites. These constituents may contribute to various biological activities, particularly antioxidant activity. Phenolic and flavonoid compounds are of special interest because they can interact with reactive oxygen species and free radicals, thereby helping to reduce oxidative damage. The presence and concentration of these phytochemicals can vary depending on the plant variety, geographical conditions, maturity, and extraction method. (Cory *et al.*, 2018)

The bract of *Musa balbisiana* represents an underutilized plant material that may have potential for the recovery of valuable natural bioactive compounds. Investigating its phytochemical composition and antioxidant properties can provide useful information regarding its possible nutritional, pharmaceutical, and functional

applications. In particular, assessment of total phenolic content, total flavonoid content, and DPPH radical scavenging activity can provide an initial indication of its antioxidant potential. (Shahidi and Ambigaipalan, 2015)

With increasing interest in naturally derived antioxidants and sustainable utilization of plant resources, *Musa balbisiana* bracts may offer an alternative source of phytochemicals with potential health-related applications. Further investigation of their bioactive constituents may support their future use in nutraceuticals, functional foods, natural antioxidant formulations, and other pharmaceutical or industrial applications. Therefore, systematic evaluation of *Musa balbisiana* bract extract is relevant for exploring the biological value of this relatively underutilized part of the plant. (Shahidi and Yeo, 2016)

Botanical Classification

Musa balbisiana is a species of flowering plant belonging to the Musaceae family within the order Zingiberales. It is a herbaceous monocotyledonous plant that is widely distributed in tropical and subtropical regions. The botanical classification of *Musa balbisiana* is as follows: (Swargiary *et al.*, 2021; Bartholomew *et al.*, 2003)

- **Kingdom:** Plantae – multicellular, photosynthetic plants (Bartholomew *et al.*, 2003; Swargiary *et al.*, 2021)
- **Phylum:** Tracheophyta – vascular plants (Ali *et al.*, 2020)
- **Class:** Liliopsida – monocotyledonous flowering plants (Swargiary *et al.*, 2021)
- **Order:** Zingiberales – an order of tropical monocotyledonous flowering plants (Al-Daihan and Bhat, 2012; Swargiary *et al.*, 2021)
- **Family:** Musaceae – the banana family (Swargiary *et al.*, 2021)
- **Genus:** *Musa* – a genus comprising bananas and plantains (Al-Daihan and Bhat, 2012)
- **Species:** *Musa balbisiana* (Swargiary *et al.*, 2021)

Musa balbisiana is a perennial herbaceous monocot characterized by a pseudostem formed from tightly overlapping leaf sheaths and large, broad leaves. The plant produces an inflorescence enclosed by prominent bracts, which gradually open as the flowers and developing fruits emerge. The bracts are an important structural part of the inflorescence and may contain various naturally occurring phytochemicals. Its classification within the genus *Musa* and family Musaceae reflects its close botanical relationship with other cultivated and wild banana species. (Sadebo *et al.*, 2026; Food Chemistry, 2025)

The species is also considered an important genetic resource in banana diversity and has contributed to the development of several cultivated banana varieties. Because different parts of the plant contain biologically active compounds, *Musa balbisiana* has attracted interest in phytochemical and pharmacological research. In particular, investigation of its bracts may provide useful information about their phenolic, flavonoid, and antioxidant properties. (Sadebo *et al.*, 2026)

Morphology

Musa balbisiana is a perennial, herbaceous, monocotyledonous plant characterized by a robust pseudostem and large, broad leaves. The pseudostem is formed by the overlapping leaf sheaths and provides structural support to the plant. Its leaves are generally elongated, broad, and green, with a prominent midrib and distinct parallel venation. The plant develops a large inflorescence that emerges from the central region of the pseudostem and is composed of numerous flowers arranged along a central axis. (Ali *et al.*, 2020)

The inflorescence is protected by large, conspicuous bracts that are initially tightly arranged around the developing flowers. These bracts are typically elongated to ovate in shape and may show variations in color ranging from green to reddish or purplish shades depending on their maturity and variety. As the inflorescence develops, the bracts gradually open or bend backward, exposing successive groups of flowers. Their thick and relatively firm structure provides protection to the developing floral parts during the early stages of growth. (Swargiary *et al.*, 2021)

The flowers of *Musa balbisiana* are arranged in clusters beneath the bracts, with female flowers generally occurring toward the basal portion of the inflorescence and male flowers toward the distal region. Following pollination and fruit development, the plant produces fruits that are generally smaller and contain numerous hard seeds compared with many cultivated seedless bananas. The fruit shape, see development, leaf characteristics, and inflorescence structure are important morphological features for identifying the species. (Sadebo *et al.*, 2026; Food Chemistry, 2025)

The bracts of *Musa balbisiana* are particularly relevant to the present study because they represent an underutilized part of the plant and may contain various phytochemicals. Their biological importance may be associated with the presence of phenolic compounds, flavonoids, and other secondary metabolites. Therefore, examination of the morphological characteristics of the bract provides an important basis for its identification and for further investigation of its phytochemical

composition and antioxidant properties. (Swargiary *et al.*, 2021)



Figure: Banana Blossom (Swargiary *et al.*, 2021)

Global Production and Economic Importance

Bananas and plantains are among the most important tropical fruit crops and are cultivated extensively across Asia, Africa, Latin America, and other tropical and subtropical regions. *Musa balbisiana* is an important wild and cultivated banana species that occurs widely throughout South and Southeast Asia. Although it is not generally produced on the same commercial scale as major dessert banana cultivars, the species has considerable agricultural and genetic importance because of its adaptability, resistance to environmental stresses, and contribution to the genetic diversity of cultivated bananas. (Sadebo *et al.*, 2026; Food Chemistry, 2025)

Musa balbisiana is particularly valuable as a genetic resource in banana improvement programs. Its genetic characteristics have contributed to the development of several cultivated banana varieties, especially those belonging to the AAB and ABB genomic groups. The species is also utilized locally for food and other traditional purposes in different regions. Banana cultivation as a whole supports the livelihoods of farmers and contributes to agricultural economies through the production, marketing, processing, and distribution of fruits and related products. (Vuong *et al.*, 2013; Vu *et al.*, 2018)

In addition to its agricultural value, different parts of the *Musa* plant have attracted increasing attention as potential sources of useful bioactive compounds. Large

quantities of plant residues, including leaves, pseudostems, peels, and bracts, are generated during banana cultivation and harvesting. Rather than treating these materials solely as agricultural waste, their phytochemical content can be investigated for potential nutritional, pharmaceutical, and industrial applications. In particular, *Musa balbisiana* bracts may contain phenolic compounds, flavonoids, and other secondary metabolites that could contribute to antioxidant activity. (Pereira and Maraschin, 2015)

The growing interest in sustainable agriculture and the development of value-added products from agricultural by-products has increased the importance of exploring underutilized plant materials. Utilization of *Musa balbisiana* bracts as a potential source of natural antioxidants may provide an additional approach for reducing agricultural waste while recovering biologically valuable compounds. Therefore, investigation of the phytochemical composition and antioxidant properties of *Musa balbisiana* bracts may have both scientific and economic significance and could support future applications in nutraceutical, functional food, pharmaceutical, and natural antioxidant products. (Sadebo *et al.*, 2026; Food Chemistry, 2025)

1.13 *Musa balbisiana* Bract as an Underutilized Plant By-Product

Composition of *Musa balbisiana* Bract

The bract of *Musa balbisiana* is an important structural component of the banana inflorescence and represents a relatively underutilized part of the plant. These bracts consist of plant tissues containing structural components such as cellulose, hemicellulose, and lignin, which contribute to their firm and fibrous nature. In addition to these structural materials, the bracts may contain proteins, minerals, carbohydrates, and various secondary metabolites. (Swargiary *et al.*, 2021)

Phytochemical constituents such as phenolic compounds, flavonoids, tannins, and other naturally occurring compounds have been reported or investigated in different parts of *Musa* species. Phenolic and flavonoid compounds are particularly important because of their potential antioxidant properties and their ability to interact with reactive free radicals. The concentration and composition of these bioactive constituents may vary depending on species, maturity, geographical origin, and extraction conditions. (Al-Daihan and Bhat, 2012)

Although banana fruits are widely utilized as food, several non-fruit parts of the plant are often discarded or used only for limited traditional purposes. The generation of these plant residues creates an opportunity for recovering useful compounds and

developing value-added products. In this context, *Musa balbisiana* bracts may serve as a potential source of naturally occurring phytochemicals with functional and biological significance. (Dai and Mumper, 2010)

The investigation of *Musa balbisiana* bracts is therefore relevant from both scientific and environmental perspectives. Determination of their total phenolic content, total flavonoid content, and antioxidant activity can provide valuable information about their bioactive potential. Effective utilization of these underexplored plant materials may contribute to reducing agricultural waste and promoting the sustainable use of banana plant resources. Furthermore, the recovery of antioxidant compounds from bracts could support future applications in nutraceuticals, functional foods, natural preservation systems, and pharmaceutical research. (Sultana *et al.*, 2009)

Reported Phytochemicals

Musa balbisiana and other members of the *Musa* genus are recognized as sources of a variety of naturally occurring phytochemicals that may contribute to their nutritional and biological properties. Different parts of the banana plant have been investigated for the presence of secondary metabolites, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, terpenoids, and other related constituents. The occurrence and concentration of these compounds may differ according to the plant part, geographical origin, maturity, and extraction method. (Sadebo *et al.*, 2026)

Phenolic compounds and flavonoids are of particular interest because of their potential contribution to antioxidant activity. These compounds can interact with reactive oxygen species and free radicals and may help reduce oxidative damage. Flavonoids such as quercetin and related phenolic constituents have been investigated in *Musa* species because of their antioxidant and other potential biological activities. Tannins and other secondary metabolites may also contribute to the overall biological characteristics of banana plant extracts. (Sadebo *et al.*, 2026; Food Chemistry, 2025)

The non-edible parts of banana plants, including peels, leaves, pseudostems, and bracts, may contain appreciable amounts of phytochemicals and therefore represent potential sources of value-added bioactive compounds. In comparison with the commonly consumed fruit, these plant parts are generally less utilized and are frequently discarded after agricultural activities. Their phytochemical investigation may therefore provide an opportunity to identify useful compounds from an otherwise underexploited plant resource. (Shahidi and Yeo, 2016)

In the case of *Musa balbisiana* bracts, the presence of phenolic and flavonoid compounds is particularly relevant to antioxidant research. Evaluation of total phenolic content and total flavonoid content, together with DPPH radical scavenging activity, can provide an initial assessment of the relationship between these phytochemicals and the antioxidant potential of the extract. Further phytochemical profiling and compound identification would be necessary to determine the individual constituents responsible for the observed biological activity and to establish their possible applications in nutraceutical, functional food, and pharmaceutical research. (Sadebo *et al.*, 2026)

Potential for Value-Added Products

Musa balbisiana bracts are an underutilized plant material that may offer potential for the development of value-added products. Instead of being discarded as agricultural waste, these bracts can be investigated as a source of useful phytochemicals, particularly phenolic and flavonoid compounds with potential antioxidant properties. (Shahidi and Yeo, 2016)

The bioactive compounds obtained from bract extracts may have future applications in nutraceuticals, functional foods, pharmaceuticals, and natural antioxidant formulations. In addition, utilizing banana bracts can help reduce agricultural waste and promote the sustainable use of plant resources. Therefore, further research on their phytochemical composition and biological properties may support the development of useful and economically valuable products. (Altemimi *et al.*, 2017)

1.14 Phytochemical Composition of *Musa balbisiana* Bract

Phenolics

Phenolic compounds are important plant secondary metabolites characterized by one or more aromatic rings containing hydroxyl groups. They are known for their antioxidant properties and can help neutralize free radicals and bind metal ions. In *Musa balbisiana* bracts, phenolic compounds may contribute significantly to the antioxidant activity of the extract. Their production can also increase in response to environmental stresses such as UV radiation, pathogen attack, and physical injury. The presence of phenolic compounds in *Musa* plant materials has been reported through various phytochemical investigations.

Flavonoids

Flavonoids are a major group of naturally occurring polyphenolic compounds found in many plants. They are associated with several biological activities, particularly antioxidant, anti-inflammatory, and antimicrobial effects. The presence of flavonoids in *Musa balbisiana* bracts may contribute to the biological and antioxidant properties of the extract. Therefore, determination of the Total Flavonoid Content (TFC) is useful for evaluating the potential antioxidant capacity of the bract extract.

Tannins

Tannins are high-molecular-weight polyphenolic compounds that can interact with proteins and other biological molecules. They possess antioxidant and astringent properties and may also contribute to plant defense. The presence of tannins in *Musa* plant materials suggests that they may be part of the phytochemical composition of *Musa balbisiana* bracts and could contribute to their overall biological activity (Swargiary *et al.*, 2021).

Alkaloids

Alkaloids are nitrogen-containing secondary metabolites that occur naturally in many plants. They are associated with various biological activities, including antimicrobial and protective effects. Phytochemical screening of *Musa* plant materials has indicated the presence of alkaloid compounds. In *Musa balbisiana* bracts, these compounds may contribute to the plant's natural defense mechanisms and overall phytochemical profile.

1.15 Previous Studies on *Musa balbisiana* Bract

Phytochemical Studies

Previous phytochemical investigations on *Musa* species have reported the presence of several bioactive compounds, including phenolics, flavonoids, tannins, alkaloids, and other secondary metabolites. These compounds are considered important because of their potential antioxidant and other biological activities. The phytochemical composition may vary depending on the plant species, plant part, geographical origin, maturity, and extraction method.

Phenolic and Flavonoid Content

Studies on different parts of *Musa* plants have indicated that phenolic and flavonoid compounds can contribute significantly to their antioxidant properties. These compounds are capable of donating electrons or hydrogen atoms to neutralize free radicals. Therefore, the determination of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) provides useful information about the concentration of these important phytochemicals in *Musa balbisiana* bract extract.

Antioxidant Activity

Several studies investigating *Musa* plant materials have reported antioxidant activity using different in-vitro assays, including DPPH radical scavenging, ABTS, and reducing power assays. The antioxidant effects observed in these studies are generally associated with the presence of phenolic and flavonoid compounds. The DPPH assay is particularly useful for evaluating the free-radical scavenging ability of plant extracts and is therefore suitable for assessing the antioxidant potential of *Musa balbisiana* bract extract.

Research Gap and Significance

Although different parts of *Musa* species have been investigated for their phytochemical and antioxidant properties, comparatively limited attention has been given to the bracts of *Musa balbisiana* as a potential source of bioactive compounds. Further investigation of this underutilized plant material may provide valuable information regarding its phenolic and flavonoid content and free-radical scavenging activity. Therefore, the present study focuses on TPC, TFC, and DPPH radical scavenging activity to evaluate the phytochemical and antioxidant potential of *Musa balbisiana* bract extract.

1.16 Correlation Between Phenolic Content and Antioxidant Activity

Phenolic and flavonoid compounds are important secondary metabolites that may contribute significantly to the antioxidant activity of plant extracts. Their antioxidant properties are mainly associated with their ability to donate hydrogen atoms or electrons, neutralize free radicals, and, in some cases, chelate metal ions. Therefore, extracts containing higher levels of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) may exhibit stronger antioxidant activity. However, antioxidant activity is not determined by phenolic and flavonoid compounds alone,

as other phytochemicals and naturally occurring antioxidants may also contribute to the overall antioxidant capacity of a plant extract (Sulaiman *et al.*, 2011; Alothman *et al.*, 2009).

Evidence from Previous Studies

1. Radical Scavenging Activity

Plant extracts rich in phenolic and flavonoid compounds often demonstrate stronger free-radical scavenging activity in antioxidant assays such as DPPH, ABTS, and FRAP. Phenolic and flavonoid molecules contain hydroxyl groups that can donate hydrogen atoms or electrons to unstable free radicals, thereby stabilizing the radicals and reducing oxidative reactions. Consequently, higher TPC and TFC values may be associated with greater radical-scavenging potential.

2. Metal Chelation and Reducing Power

Phenolic and flavonoid compounds may also contribute to antioxidant activity through metal-chelating and reducing mechanisms. They can interact with transition metal ions and may reduce oxidized compounds, thereby limiting the formation of highly reactive free radicals. As a result, plant extracts containing considerable amounts of phenolics and flavonoids may exhibit enhanced reducing power and antioxidant capacity in suitable in-vitro assays.

3. Quantitative Relationship Between TPC, TFC and Antioxidant Activity

Several studies have reported a positive association between TPC, TFC, and antioxidant activity in different plant extracts. In general, an increase in phenolic and flavonoid content may be accompanied by improved radical-scavenging activity. However, the strength of this relationship can vary depending on the plant species, extraction solvent, extraction conditions, composition of individual phenolic and flavonoid compounds, and the antioxidant assay employed. Therefore, TPC and TFC are useful indicators of antioxidant potential but should not be considered the sole determinants of antioxidant activity.

4. Influence of Phenolic and Flavonoid Structure

The antioxidant effectiveness of individual phenolic and flavonoid compounds depends not only on their concentration but also on their chemical structure. The number and position of hydroxyl groups, degree of conjugation, and molecular

arrangement can influence their ability to scavenge free radicals and interact with reactive species. Thus, both the quantity and structural characteristics of phenolic and flavonoid compounds can affect the overall antioxidant activity of a plant extract.

5. Relationship with DPPH Radical Scavenging Activity

The DPPH radical scavenging assay is commonly used to evaluate the free-radical-scavenging ability of plant extracts. A higher scavenging activity indicates a greater ability of the extract to neutralize DPPH radicals. Since phenolic and flavonoid compounds can act as hydrogen or electron donors, their presence may contribute to the observed DPPH scavenging activity. Therefore, comparing TPC and TFC values with DPPH results can provide a better understanding of the possible contribution of these compounds to the antioxidant properties of the extract.

Chapter 2 - MATERIALS AND METHODS

2.1 List of the Chemicals

Preparation of DPPH Solution (0.004% w/v)

- Weighed 2 mg of DPPH.
- Dissolved in 50 mL methanol using a volumetric flask.
- Transferred into an amber bottle to protect from light.
- Stored in dark conditions to prevent degradation.

Preparation of Ascorbic Acid Standard (400 µg/mL)

- Weighed 40 mg ascorbic acid.
- Dissolved in 100 mL methanol.
- Mixed thoroughly to obtain a uniform standard solution.

Preparation of Sodium Carbonate (7.5% w/v)

- Weighed 3.75 g Na₂CO₃.
- Dissolved in distilled water and made up to 50 mL.

Preparation of Folin–Ciocalteu Reagent

- Took 10 mL Folin–Ciocalteu reagent.
- Diluted with 90 mL distilled water.
- Stored in an amber bottle.

Preparation of 10% Aluminum chloride Reagent

- Took 10g aluminum chloride
- Diluted with 100 mL distilled water.

Preparation of 1M Potassium Acetate Reagent

- Took 9.82g Potassium acetate
- Diluted with 100 mL distilled water.

2.2 List of the Apparatus

- UV-Visible Spectrophotometer
- Analytical Balance
- Volumetric Flasks
- Rotary Evaporator
- Test Tubes
- Fumehood
- Shaker
- Test Tube Stand
- Amber Glass Bottle

2.3 Sample Collection

Selection of *Musa balbisiana* Bracts

The selection of suitable plant material is an important step for obtaining reliable and consistent experimental results. Fresh and healthy *Musa balbisiana* bracts were collected from a local source. The samples were selected based on their maturity, physical condition, and uniformity.

The selection criteria included:

- **Maturity:** Fully developed and healthy bracts were selected to obtain representative phytochemical content.
- **Physical Integrity:** Bracts showing fungal infection, discoloration, bruising, or mechanical damage were excluded.
- **Uniformity:** Bracts with relatively similar size and maturity were selected to minimize variation during extraction.

Careful selection of healthy and relatively uniform bracts helps reduce variation in phytochemical composition and extraction efficiency, thereby improving the reliability of the experimental results.

Cleaning and Preparation

After collection, the *Musa balbisiana* bracts were cleaned and prepared for extraction following standard laboratory procedures:

The collected bracts were thoroughly washed with clean running water to remove soil, dust, and other surface impurities. After washing, the bracts were further cleaned following standard laboratory procedures to minimize potential microbial and surface contamination. Unwanted portions and damaged parts were carefully removed before further processing. The cleaned bracts were then cut into small and relatively uniform pieces to increase the surface area and facilitate the efficient extraction of bioactive compounds. Finally, the prepared samples were either processed immediately or stored in suitable airtight containers under appropriate conditions until further extraction.

Proper cleaning and preparation of the *Musa balbisiana* bracts are important for removing unwanted materials and obtaining a suitable sample for the extraction and subsequent analysis of **TPC, TFC, and DPPH radical scavenging activity**.

2.4 Sample Preparation

Drying Method

After collection and cleaning, the *Musa balbisiana* bracts were dried to reduce moisture content and help preserve their bioactive compounds. Two commonly used drying methods are air-drying and oven-drying.

- **Air-Drying:** The bracts were arranged in a single layer on clean trays and dried at room temperature (approximately 25–30°C) in a well-ventilated area away from direct sunlight. This method helps minimize heat-induced degradation of sensitive phytochemicals.
- **Oven-Drying:** Alternatively, the bracts may be dried in a hot-air oven at approximately 40–50°C until a constant weight is achieved. Controlled oven drying can provide faster and more uniform moisture removal, although excessive temperature may cause degradation of heat-sensitive compounds.

The drying method can influence the recovery and stability of phenolic and flavonoid compounds and may consequently affect the antioxidant activity of the extract.

Grinding and Sieving

After drying, the *Musa balbisiana* bracts were ground into a fine powder using a suitable laboratory grinder or blender. The powdered material was then passed through an appropriate sieve, preferably around **40–60 mesh**, to obtain relatively uniform particle size.

Uniform particle size increases the surface area available for contact with the extraction solvent and can improve extraction efficiency. It also helps maintain consistency and reproducibility between samples.

Storage Conditions

The dried and powdered bract material was stored in clean, airtight, moisture-resistant containers to protect it from moisture, contamination, and degradation of bioactive compounds. For short-term storage, the sample may be kept at room temperature under dry conditions, while refrigerated storage at approximately **4°C** may be used for longer periods when appropriate.

Proper drying, grinding, and storage are important steps for maintaining the quality and stability of the phytochemicals before extraction and subsequent determination of TPC, TFC, and DPPH radical scavenging activity.

2.5 Extraction Procedure

Solvent Selection

The selection of an appropriate solvent is an important factor in the extraction of phenolic and flavonoid compounds from *Musa balbisiana* bracts. Commonly used solvents include methanol, ethanol, water, and their mixtures. Polar solvents are generally effective for extracting many phenolic and flavonoid compounds because of their ability to dissolve relatively polar phytochemicals. Alcohol–water mixtures may also improve the recovery of a wider range of bioactive compounds.

Method of Extraction

The dried and powdered *Musa balbisiana* bract material was extracted using a suitable solvent. Depending on the laboratory procedure, extraction may be carried out by maceration or Soxhlet extraction.

- **Maceration:** The powdered plant material is soaked in the selected solvent for a specific period, usually 24–48 hours, at room temperature with occasional shaking. This method is simple and suitable for compounds that may be sensitive to heat.
- **Soxhlet Extraction:** In this method, the powdered sample is continuously extracted with the selected solvent through repeated cycles of heating and condensation. It allows efficient contact between the solvent and plant material and can provide good recovery of extractable phytochemicals.

The extraction technique should be selected based on factors such as extraction time, solvent compatibility, and the stability of heat-sensitive bioactive compounds.

Filtration

After completion of the extraction, the mixture was filtered using Whatman No. 1 filter paper to separate the solid plant residue from the liquid extract. Filtration removes suspended particles and provides a clearer filtrate suitable for subsequent concentration.

Concentration Using Rotary Evaporator

The filtered extract was concentrated using a rotary evaporator under reduced pressure to remove the extraction solvent while minimizing excessive exposure to heat. A controlled temperature, commonly around 40–50°C, may be used depending on the solvent and laboratory procedure. The concentrated crude extract was collected and stored in a properly sealed container at approximately 4°C until further analysis.

Yield Calculation

The percentage extraction yield was determined by comparing the weight of the dried extract with the initial weight of the plant material used for extraction.

Extraction Yield (%) = (Weight of Dried Extract / Weight of Initial Sample) × 100

The extraction yield provides an estimate of the amount of extract obtained from the original plant material and can be useful for evaluating extraction efficiency.

Determination of Extract Yield

If these are your actual measured values:

Weight of empty Petri dish = 70.328 g

Weight of Petri dish + dried extract = 72.178 g

Weight of Extract = (72.178 – 70.328)

= 1.850 g

Therefore, the total amount of dried *Musa balbisiana* bract extract obtained was **1.850 g**.

2.6 Chemicals and Reagents

DPPH Solution (0.004% w/v)

- 2 mg of DPPH was weighed and dissolved in 50 ml of methanol in a 50 ml volumetric flask.
- The solution was transferred into an amber reagent bottle and stored in the dark to prevent photodegradation.

Ascorbic Acid Standard Solution (400 µg/ml)

- 40 mg of ascorbic acid was weighed and dissolved in 100 ml of methanol in a 100 ml volumetric flask.
- This solution served as the reference standard for antioxidant activity assays.

Sodium Carbonate Solution (7.5% w/v)

- 3.75 g of Na₂CO₃ was weighed and dissolved in distilled water in a 50 ml volumetric flask.
- The solution was made up to the mark with distilled water.

Folin–Ciocalteu Reagent (Diluted)

- 10 ml of Folin–Ciocalteu reagent was added to a 100 ml volumetric flask.
- The mixture was diluted with 90 ml of distilled water up to the mark.
- The diluted reagent was transferred into an amber glass bottle and stored appropriately.

Gallic acid

- Weigh 25 mg gallic acid and transfer to a 5 mL volumetric flask or vial.
- Add methanol, dissolve completely, and make up the volume to 5 ml.
- Mix well and label (5 mg/mL, methanol).

Querectin standard solution preparation (5000 µg /ml)

- 25mg of querectin was weighed and added to 5ml of methanol in a testube.

Aluminium Chloride solution (10% w/v)

-10g AlCl₃ is weighed and add 100ml distill water in a 100ml volumetric flask.

1M Potassium Acetate solution (mw=98.15g/ml)

-9.82g sodium acetate was weighed and and 100ml distill water was added in a 100ml volumetric flask

Solvents

- Methanol or ethanol was used as the extraction and assay solvent due to its ability to dissolve phenolic compounds and DPPH.
- Distilled water was used for preparing sodium carbonate solution and diluting Folin–Ciocalteu reagent to ensure purity and avoid contamination.

2.7 Determination of Antioxidant Activity (DPPH Assay)

DPPH Radical Scavenging Assay

We evaluated antioxidant capacity using the DPPH assay. DPPH (1,1-diphenyl-2-picryl-hydrazyl) is a stable free radical that changes color when it's reduced by an antioxidant. By measuring this color change with UV-Vis spectroscopy, we can quantify how much DPPH has been scavenged. Testing a range of antioxidant concentrations lets us calculate the IC₅₀-the concentration required to quench 50% of the DPPH signal. In this context, a lower IC₅₀ means stronger antioxidant activity, while a higher IC₅₀ Indicates weaker activity.

Reagents and Apparatus:

1. DPPH (1,1 Diphenyl 2- Picryl Hydrazyl) (2mg)
2. Methanol 300ml
3. Ascorbic acid/butylated hydroxytoluene as standard (40mg)
4. Banana blossom extract (40mg),
5. UV spectrophotometer

Preparation of Reagents

DPPH 0.004% w/v

- Accurately weighed 2 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) using an analytical balance. The weighed DPPH was carefully transferred into a clean, dry 50 mL volumetric flask.
- A small volume of methanol was added initially to dissolve the solid, and the flask was gently swirled until the DPPH was completely dissolved, forming a deep violet solution.
- Additional methanol was then added gradually until the volume reached the 50 mL calibration mark. The flask was stoppered and inverted several times to ensure proper mixing and homogeneity of the solution.

- The prepared DPPH solution was transferred into an amber reagent bottle and stored in the dark at room temperature to prevent photodegradation until further use.

Ascorbic Acid 400 μ g/ml (standard solution

- Accurately weighed 40 mg of ascorbic acid using an analytical balance. The weighed ascorbic acid was transferred into a clean and dry 100 mL volumetric flask.
- A small volume of methanol was added to dissolve the solid completely, and the flask was gently swirled until a clear solution was obtained.
- After complete dissolution, additional methanol was added gradually until the solution reached the 100 mL calibration mark. The flask was then stoppered and inverted several times to ensure uniform mixing.
- The prepared standard solution of ascorbic acid was stored in a properly labeled container for further experimental use.

Serial Dilution (standard preparation)

Stock = 400 μ g/ml [40mg in 100ml]

- From the standard stock solution (400 μ g/ml) 5ml was taken in a test tube, which produced a concentration of (400 μ g/ml).
- From the previously prepared concentration (400 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (200 μ g/ml).
- From the previously prepared concentration (200 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (100 μ g/ml).
- From the previously prepared concentration (100 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (50 μ g/ml).
- From the previously prepared concentration (50 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (25 μ g/ml).
- From the previously prepared concentration (25 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (12.5 μ g/ml).

Extract solution preparation

40mg extract was weighed in a balance and taken in a volumetric flask (100ml) and was filled with 100ml methanol up to the mark.

Serial Dilution (extract preparation)

Stock = 400 μ g/ml [40mg in 100ml]

- From the Extract stock solution (400 μ g/ml) 5ml was taken in a test tube, which produced a concentration of (400 μ g/ml).
- From the previously prepared concentration (400 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (200 μ g/ml).
- From the previously prepared concentration (200 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (100 μ g/ml).
- From the previously prepared concentration (100 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (50 μ g/ml).
- From the previously prepared concentration (50 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (25 μ g/ml).
- From the previously prepared concentration (25 μ g/ml) 2.5ml was taken in a test tube and 2.5ml methanol was added, which produced a concentration of (12.5 μ g/ml).

Standard and Extract

Take 6 new test tubes for both standard and extract, take 2ml of each concentration and add 2ml prepared DPPH solution into each concentration of standard and extract. Finally wrap each test tube with aluminum foil (to prevent light from entering).

Control Preparation

Using a clean pipette, 2.5 mL of the previously prepared DPPH solution was transferred into a clean, dry test tube. To this, 2.5 mL of methanol was added.

The contents of the test tube were gently mixed to ensure uniform distribution of the solution.

The test tube was then completely wrapped with aluminum foil to prevent exposure to light, as DPPH is light-sensitive and may undergo photodegradation. The wrapped test tube was kept aside under dark conditions until further analysis.

Blank Preparation

Using a clean measuring pipette, 5 mL of methanol was transferred into a clean, dry test tube.

The mouth of the test tube was immediately covered with aluminum foil to minimize evaporation, as methanol is a volatile solvent.

The prepared methanol blank was kept aside under appropriate laboratory conditions for subsequent use in the assay.

INCUBATE all the test tubes of (**standard, extract, control & blank**) for
(30 MINUTES)

Measurement at 517 nm

When DPPH is mixed with an antioxidant, the radical is converted to 1,1-diphenyl-2-picryl-hydrazine (DPPH-H). As DPPH is reduced, Its absorbance at 517 nm falls, so the degree of discoloration from purple to yellow directly reflects the hydrogen-donating (scavenging) capacity of the test compound or extract.

2.8 Determination of Total Phenolic Content

Gallic acid equivalents (GAE), which are commonly used to express TPC analysis, provide consistent comparisons between various samples and investigations. The approach is preferred for both normal laboratory analysis and research applications due to its affordability, simplicity, and reproducibility. In food science, pharmacognosy, phytochemistry, and nutraceutical development, where phenolic chemicals are strongly linked to antioxidant capacity and overall bioactivity, determining the total phenolic content is essential. All things considered, TPC evaluation is a significant predictor of the possible health benefits and qualitative characteristics of plant-based compounds.

Reagents and Apparatus:

1. Folin Ciocalteu Reagent – 5ml
2. Sodium Carbonate – 3.75gm
3. Gallic Acid – 25mg
4. Methanol – 250ml
5. UV spectrophotometer

Preparation of Standard Stock Solution

25mg of Gallic Acid was precisely weighed using an analytical balance. Then Transfer it into a dry, clean 10mL Test Tube then add 5mL Methanol by using a Pipette. Lastly lock the test tube with its stopper after that shake the test tube thoroughly until the solution dissolves completely, which produced a concentration of 5000 µg/mL stock solution .

Serial dilution (standard)

Then took 1 mL from this stock in a clean ,dry 10mL test tube and add 4mL Methanol in it (1000 µg/mL) then lock the test tube with its stopper and The test tube was repeatedly inverted to ensure that the solution was evenly mixed.

- From the stock (1000 µg/mL) took 1mL stock solution and add 4mL Methanol
- Took 0.5ml from the stock (1000 µg/ml) and add 4.5ml methanol that produced 5ml (100 µg/ml)
- Took 2.5ml from previous (100 µg/ml) and add 2.5ml methanol in it that produced 5ml (50 µg/ml)
- Took 2.5ml from previous (50 µg/ml) and add 2.5ml methanol in it that produced 5ml (25 µg/ml)
- Took 2.5ml from previous (25 µg/ml) and add 2.5ml methanol in it that produced 5ml (12.5 µg/ml)
- Took 2.5ml from previous (12.5µg/ml) and add 2.5ml methanol in it that produced 5ml (6.25 µg/ml)

Preparation of Extract stock Solution

25mg of extract was precisely weighed using an analytical balance. Then Transfer it into a dry, clean 10mL Test Tube then add 5mL Methanol by using a Pipette. Lastly lock the test tube with its stopper after that Shake the test tube thoroughly until the

solution dissolves completely, which produced a concentration of 5000 µg/mL stock solution .

Serial dilution (extract)

Then took 1 mL from this stock in a clean ,dry 10mL test tube and add 4mL Methanol in it (1000 µg/mL) then lock the test tube with its stopper and The test tube was repeatedly inverted to ensure that the solution was evenly mixed.

- From the stock (1000 µg/mL) took 1mL stock solution and add 4mL Methanol
- Took 0.5ml from the stock (1000 µg/ml) and add 4.5ml methanol that produced 5ml (100 µg/ml)
- Took 2.5ml from previous (100 µg/ml) and add 2.5ml methanol in it that produced 5ml (50 µg/ml)
- Took 2.5ml from previous (50 µg/ml) and add 2.5ml methanol in it that produced 5ml (25 µg/ml)
- Took 2.5ml from previous (25 µg/ml) and add 2.5ml methanol in it that produced 5ml (12.5 µg/ml)
- Took 2.5ml from previous (12.5µg/ml) and add 2.5ml methanol in it that produced 5ml (6.25 µg/ml)

Preparation of Reagents

Preparation of 7.5% Sodium Carbonate solution

3.75 g of sodium carbonate (Na_2CO_3) was precisely weighed using an analytical balance. To prevent material loss, the weighed sample was carefully transferred using a funnel into a dry, clean 50 mL volumetric flask. To fully dissolve the substance, a tiny amount of distilled water was added. A clear solution was produced by slowly swirling the flask until the sodium carbonate was completely dissolved. Following full dissolve, more distilled water was progressively added until the meniscus's bottom just touched the 50 mL volumetric flask's calibration mark. After that, the flask was repeatedly inverted and stoppered to guarantee that the solution was evenly mixed.

Preparation of Folin- Ciocalteu Reagent

Using a graduated pipette, measure out 5 mL of Folin-Ciocalteu reagent, then pour it into a sterile 50 mL volumetric flask. The reagent was dissolved and diluted by adding distilled water. Using pure water, the volume was gradually increased to the 50 mL calibration mark (around 45 mL added). To guarantee complete mixing and consistent dilution, the flask was stoppered and inverted multiple times.

After being prepared, the diluted Folin-Ciocalteu reagent was moved into an amber-colored glass bottle to shield it from light and kept in the proper storage conditions until needed again.

Preparation of Standard and Sample

Took 5 new clean, dry test tube for each standard and sample. Take 0.5ml of each concentration and add 2.5ml Folin – ciocalteu reagent and also add 2.5ml of sodium carbonate . Then wrap the test tube with aluminum foil (to prevent light from entering).

Control Preparation

Took a clean ,dry new test tube and add both previously prepared 2.5ml folin-ciocalteu reagent and sodium carbonate of 2.5ml also add 0.5ml methanol by using clean pipette .wrap the test tube aluminum foil (to prevent the light from entering).

The contents of the test tube were gently mixed to ensure uniform distribution of the solution.

Blank Preparation

Using a clean measuring pipette, 5 mL of methanol was transferred into a clean, dry test tube.

The mouth of the test tube was immediately covered with aluminum foil to minimize evaporation, as methanol is a volatile solvent.

The prepared methanol blank was kept aside under appropriate laboratory conditions for subsequent use in the assay.

INCUBATE all the test tubes of (**standard, extract, control & blank**) at 25°C for **(20 MINUTES)**

Measurement at 760 nm

Folin–Ciocalteu Reagent:

When Folin–Ciocalteu reagent reacts with phenolic compounds, a blue-colored complex is formed under alkaline conditions. The intensity of the blue color is proportional to the amount of phenolic compounds present in the extract. The absorbance of the developed color is measured at 760 nm, and a higher absorbance indicates a higher total phenolic content.

Sodium Carbonate:

Sodium carbonate provides the alkaline medium required for the reaction between phenolic compounds and the Folin–Ciocalteu reagent. It facilitates the formation of the blue-colored complex, whose absorbance is measured at 760 nm. The intensity of the color reflects the concentration of phenolic compounds present in the sample

2.9 Determination of Total Flavonoid Content

The Total Flavonoid Content (TFC) of *Musa balbisiana* bract extract was determined using the aluminum chloride (AlCl_3) colorimetric method, with quercetin as the standard. The absorbance was measured using a UV-Visible spectrophotometer at approximately 415 nm. A quercetin calibration curve was prepared, and the flavonoid content of the extract was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g). The TFC value was used to evaluate the flavonoid concentration and antioxidant potential of the *Musa balbisiana* bract extract.

Reagents and Apparatus:

1. Quercetin - 25mg
2. Aluminium Chloride - 10mg
3. Potassium acetate - 9.82gm
3. Banana blossom extract- 25mg
4. UV spectrophotometer

Preparation of Reagents

Preparation of Standardized Quercetin Solution

25mg of Quercetin was precisely weighed using an analytical balance. To prevent material loss, then transfer it into a dry, clean 10mL Test Tube then add 5mL Methanol by using a Pipette. Lastly lock the test tube with its stopper after that Shake the test tube thoroughly until the solution dissolves completely, which produced a concentration of 5000 $\mu\text{g/mL}$ stock solution

Serial dilution(standard)

Then took 1 mL from this stock in a clean ,dry 10mL test tube and add 4mL Methanol in it (1000 $\mu\text{g/mL}$) then lock the test tube with its stopper and The test tube was repeatedly inverted to ensure that the solution was evenly mixed.

- From the stock (1000 $\mu\text{g/mL}$) took 1mL stock solution and add 4mL Methanol
- Took 0.5ml from the stock (1000 $\mu\text{g/ml}$) and add 4.5ml methanol that produced 5ml (100 $\mu\text{g/ml}$)
- Took 2.5ml from previous (100 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (50 $\mu\text{g/ml}$)
- Took 2.5ml from previous (50 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (25 $\mu\text{g/ml}$)
- Took 2.5ml from previous (25 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (12.5 $\mu\text{g/ml}$)
- Took 2.5ml from previous (12.5 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (6.25 $\mu\text{g/ml}$)

Preparation of Extract Stock Solution

25mg of extract was precisely weighed using an analytical balance. Then Transfer it into a dry, clean 10mL Test Tube then add 5mL Methanol by using a Pipette. Lastly lock the test tube with its stropper after that Shake the test tube thoroughly until the solution dissolves completely. which produced a concentration of 5000 $\mu\text{g/mL}$ stock solution .

Serial dilution (extract)

Then took 1 mL from this stock in a clean , dry 10mL test tube and add 4mL Methanol in it (1000 $\mu\text{g/mL}$) then lock the test tube with its stopper and The test tube was repeatedly inverted to ensure that the solution was evenly mixed.

- From the stock (1000 $\mu\text{g/mL}$) took 1mL stock solution and add 4mL Methanol
- Took 0.5ml from the stock (1000 $\mu\text{g/ml}$) and add 4.5ml methanol that produced 5ml (100 $\mu\text{g/ml}$)
- Took 2.5ml from previous (100 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (50 $\mu\text{g/ml}$)
- Took 2.5ml from previous (50 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (25 $\mu\text{g/ml}$)
- Took 2.5ml from previous (25 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (12.5 $\mu\text{g/ml}$)
- Took 2.5ml from previous (12.5 $\mu\text{g/ml}$) and add 2.5ml methanol in it that produced 5ml (6.25 $\mu\text{g/ml}$)

Preparation of 10% Aluminum chloride solution

10g of Aluminum chloride (AlCl_3) was precisely weighed using an analytical balance. To prevent material loss, the weighed sample was carefully transferred using a funnel into a dry, clean 100 mL volumetric flask. To fully dissolve the substance, a tiny amount of distilled water was added. A clear solution was produced by slowly swirling the flask until the sodium carbonate was completely dissolved.

Following full dissolve, more distilled water was progressively added until the meniscus's bottom just touched the 100 mL volumetric flask's calibration mark. After that, the flask was repeatedly inverted and stoppered to guarantee that the solution was evenly mixed.

Preparation of 1M Potassium acetate solution

9.82g of Potassium acetate (CH_3COOK) was precisely weighed using an analytical balance. To prevent material loss, the weighed sample was carefully transferred using a funnel into a dry, clean 100mL volumetric flask. To fully dissolve the substance, a tiny amount of distilled water was added. A clear solution was produced

by slowly swirling the flask until the sodium carbonate was completely dissolved. Following full dissolve, more distilled water was progressively added until the meniscus's bottom just touched the 100 mL volumetric flask's calibration mark. After that, the flask was repeatedly inverted and stoppered to guarantee that the solution was evenly mixed

Preparation of Standard and Sample

Took 5 new clean, dry test tube for each standard and extract. Take 1ml of each concentration and add 3ml methanol, add 200 AlCl₃, 200 potassium acetate, add 5.6ml distill water in each test tube.

Control Preparation

Took a clean ,dry new test tube and add both previously prepared 200μL and 200 μL of potassium acetate also add 1ml methanol by using clean pipette .wrap the test tube aluminum foil.

The contents of the test tube were gently mixed to ensure uniform distribution of the solution.

Blank Preparation

Using a clean measuring pipette, 5 mL of methanol was transferred into a clean, dry test tube.

The mouth of the test tube was immediately covered with aluminum foil to minimize evaporation, as methanol is a volatile solvent.

The prepared methanol blank was kept aside under appropriate laboratory conditions for subsequent use in the assay.

INCUBATE all the test tubes of (**standard, extract, control & blank**)at 25°C for **(20 MINUTES)**

Measurement at 420 nm

Aluminum chloride

When aluminum chloride reacts with flavonoid compounds, a yellow-colored complex is formed. The intensity of the color depends on the amount of flavonoids present in the extract. As the complex is formed, its absorbance is measured at **420**

nm, so the degree of color development directly reflects the flavonoid content of the test compound or extract.

Potassium acetate

When potassium acetate is added to the reaction mixture, it helps maintain the required reaction conditions for the formation of the aluminum–flavonoid complex. The resulting color intensity is measured at **420 nm**, and the absorbance directly reflects the concentration of flavonoid compounds present in the test compound or extract

Chapter 3 - RESULTS

3.1 Calculation of Percentage % inhibition (DPPH)

Percentage inhibition (%)

$$= \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100\%$$

Conc. (µg/mL)	% Inhibition		Abs (Sam)	Abs (Ctrl)	Ctrl- Sam	Ctrl- Sam/Ctrl	% Inhibition
400.00	91.56		0.0385	0.456	0.4175	0.9156	91.561
200.00	64.05		0.1639		0.2921	0.6405	64.050
100.00	50.16		0.2273		0.2287	0.5016	50.162
50.00	33.70		0.3023		0.1537	0.3370	33.700
25.00	24.02		0.3465		0.1095	0.2402	24.022
12.50	22.24		0.3546		0.1014	0.2224	22.240

Table 01: Calculation of % inhibition (DPPH) for each concentration of sample

Percentage inhibition (%)

$$= \frac{\text{Absorbance (control)} - \text{Absorbance (std)}}{\text{Absorbance (control)}} \times 100\%$$

Conc. (µg/mL)	% Inhibition	Abs (Std)	Abs (Ctrl)	Ctrl- Std	Ctrl- Std/Ctrl	% Inhibition
400.00	86.80	0.0602	0.456	0.3958	0.8680	86.800
200.00	70.28	0.1355		0.3205	0.7028	70.281
100.00	65.88	0.1554		0.3006	0.6588	65.880
50.00	40.21	0.2726		0.1834	0.4021	40.211
25.00	33.36	0.3039		0.1521	0.3336	33.362
12.50	30.54	0.3167		0.1393	0.3054	30.540

Table 02: Calculation of % inhibition (DPPH) for each concentration of Standard

3.2 Graph (concentration vs inhibition) – DPP

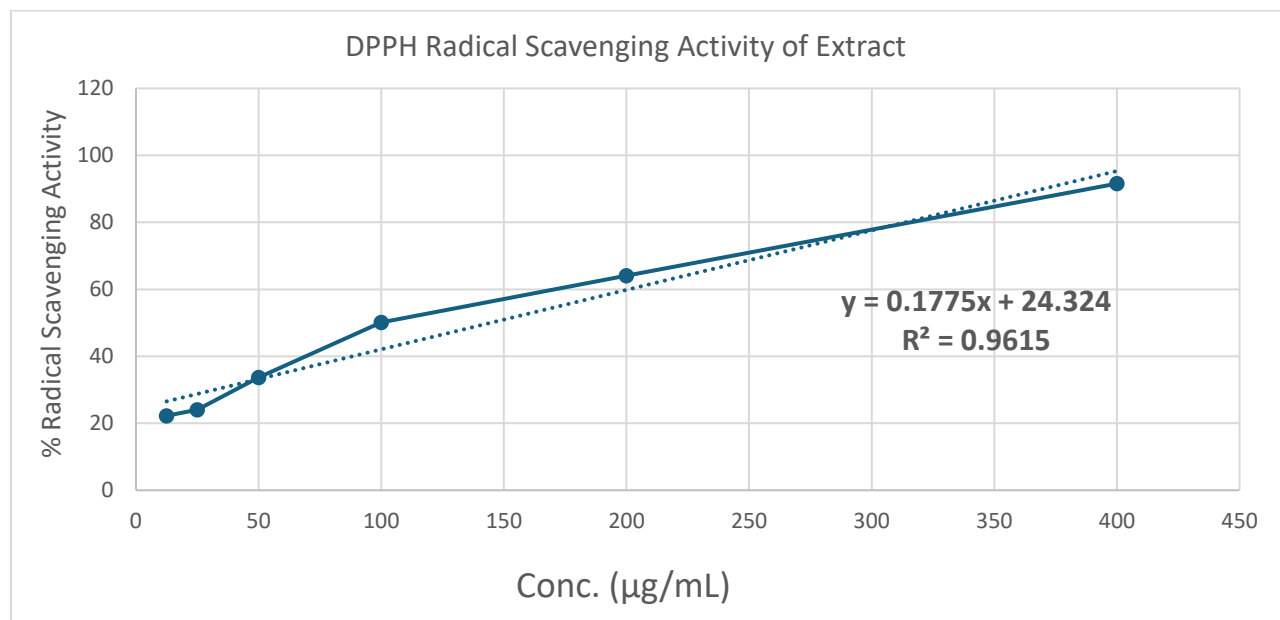


Figure 01: DPPH Radical Scavenging Activity of Extract

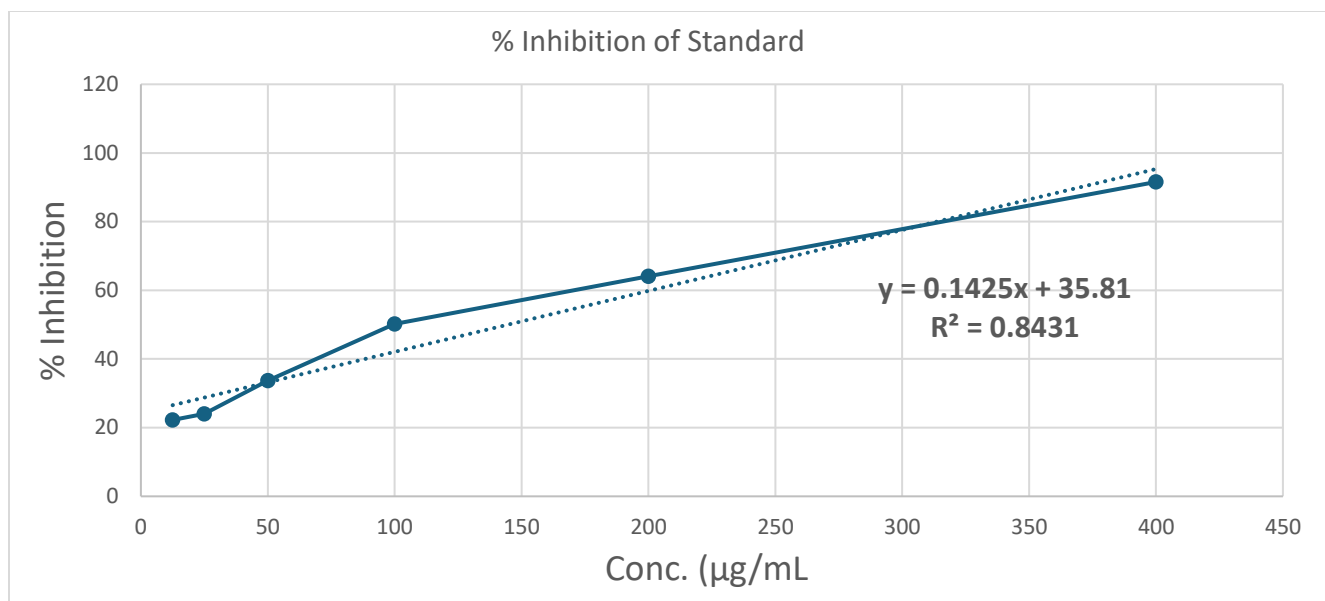


Figure 01: DPPH Radical Scavenging Activity of Standard

3.3 IC₅₀ value (DPPH)

- IC₅₀ = concentration of drug that produces **50% inhibition** of response.
- Perform experiment with **multiple drug concentrations**.
- Measure biological response (e.g., enzyme activity, cell viability).
- Calculate % inhibition:

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100\%$$

- Plot:
 - X-axis → **concentration**
 - Y-axis → **% inhibition**
- Fit a sigmoidal dose-response curve (using software like GraphPad Prism or Microsoft Excel).
- The concentration at **50% inhibition** on the curve = **IC₅₀ value**.

Calc. of IC ₅₀ value of Sample	
Y value	50%
Slope, m	0.1775
Y-Intercept, C	24.324
X value (IC ₅₀)	Y-C/m
Y-C	25.676
IC ₅₀	144.65

Table 03: Calculation of IC₅₀ value of Sample

Calc. of IC ₅₀ value of Standard	
Y value	50%
Slope, m	01425
Y-Intercept, C	35.810
X value (IC ₅₀)	Y-C/m
Y-C	14.19
IC ₅₀	99.59

Table 04: Calculation of IC₅₀ value of Standard

3.4 Calculation of (Total Phenolic Content)

$$y = 0.001865x + 0.00125$$

Step 1: The Regression Equation

For your thesis, define the relationship between concentration (x) and absorbance (y) as:

- **Equation:** $y = 0.001865x + 0.00125$
- **Slope (m):** 0.001865
- **Intercept (c):** 0.00125

Step 2: Calculate Concentration (C)

To find the concentration of your sample (x) in $\mu\text{g/mL}$, rearrange the equation to solve for x:

$$x = \frac{A_{\text{sample}} - 0.00125}{0.001865}$$

Step 3: Total Phenolic Content (TPC) Calculation

Insert values into the TPC formula:

$$TPC = \frac{C \times V \times DF}{W}$$

Specific parameters:

- **V (Volume):** 5 ml
- **DF (Dilution Factor):** 5
- **W (Weight):** 25 mg

$$TPC = \frac{C \times 5 \times 5}{25}$$

3.5 Graph of Total Phenolic Content

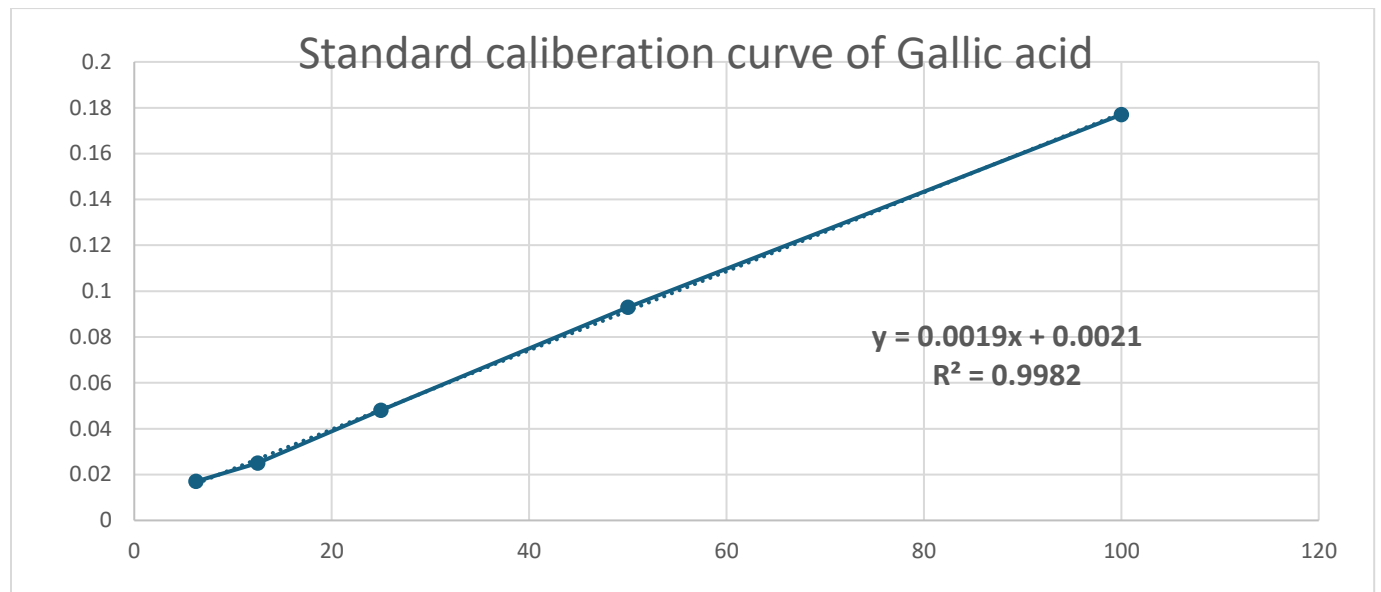


Figure 03: Standard calibration curve of Gallic acid

3.6 TPC Result

Calculate Concentration (C):

Here, calculate concentration using this formula

$$\text{formula } x = \frac{A_{\text{sample}} - 0.00125}{0.001865}$$

calculate the concentration for each sample absorbance readings:

Sample Abs(y)	Calculation: (y-0.00125)/0.00187	Concentration C (µg/mL)
0.011	(0.011 - 0.00125) / 0.00187	6.30
0.017	(0.017 - 0.00125) / 0.00187	8.45
0.021	(0.021 - 0.00125) / 0.00187	10.59
0.026	(0.026 - 0.00125) / 0.00187	13.27
0.031	(0.031 - 0.00125) / 0.00187	15.95

Table 05: Calculation of concentration for each absorbance of sample

From this table got concentrations ,

C1 = 6.30 µg/mL, C2 = 8.45 µg/mL , C3 = 10.59 µg/mL, C4 = 13.27 µg/mL,

C5 = 15.95 µg/mL

Total Phenolic Content (TPC) Calculation

Insert values into the TPC formula:

$$\begin{aligned} TPC &= \frac{C \times 5 \times 5}{25} \\ &= \frac{C}{1} \end{aligned}$$

Concentration C (µg/mL)	Concentration C (µg/mL)	TPC Calculation (C/1)	TPC (mg GAE/g)
C1	6.300	6.300/ 1	6.300
C2	8.445	8.445/ 1	8.445
C3	10.590	10.590/ 1	10.590
C4	13.271	13.271/ 1	13.271
C5	15.592	15.592/ 1	15.592

Table 06: Calculation of TPC (mg GAE/g) for each concentration

$$\begin{aligned}
 \text{Average TPC} &= \frac{C1+C2+C3+C4+C5}{5} \\
 &= \frac{6.30+8.45+10.59+13.27+15.59}{5} \\
 &= \frac{54.70}{5} \\
 &= 10.91
 \end{aligned}$$

The Total Phenolic Content of the analyzed samples ranged from 6.300 to 15.592 mg GAE/g and average TPC value is 10.91 mg GAE/g, ± 5 mg GAE/g. These values represent the phenolic concentration relative to the dry weight of the sample, calculated based on the Gallic Acid standard curve.

3.7 Calculation of (Total Flavond Content)

$$y = 0.001724x + 0.005208$$

Step 1: The Regression Equation

For your thesis, define the relationship between concentration (x) and absorbance (y) as:

- **Equation:** $y = 0.001724x + 0.005208$
- **Slope (m):** 0.001724
- **Intercept (c):** 0.005208

Step 2: Calculate Concentration (C)

To find the concentration of your sample (x) in $\mu\text{g/mL}$, rearrange the equation to solve for x:

$$x = \frac{A \text{ sample} - 0.005208}{0.001724}$$

Step 3: Total Flavonoid Content (TFC) Calculation

Insert values into the TFC formula:

$$TFC = \frac{C \times V \times DF}{W}$$

Specific parameters:

- **V (Volume):** 5 ml
- **DF (Dilution Factor):** 5
- **W (Weight):** 25 mg

$$TFC = \frac{C \times 5 \times 5}{25}$$

3.8 Graph of Total Flavonoid Content

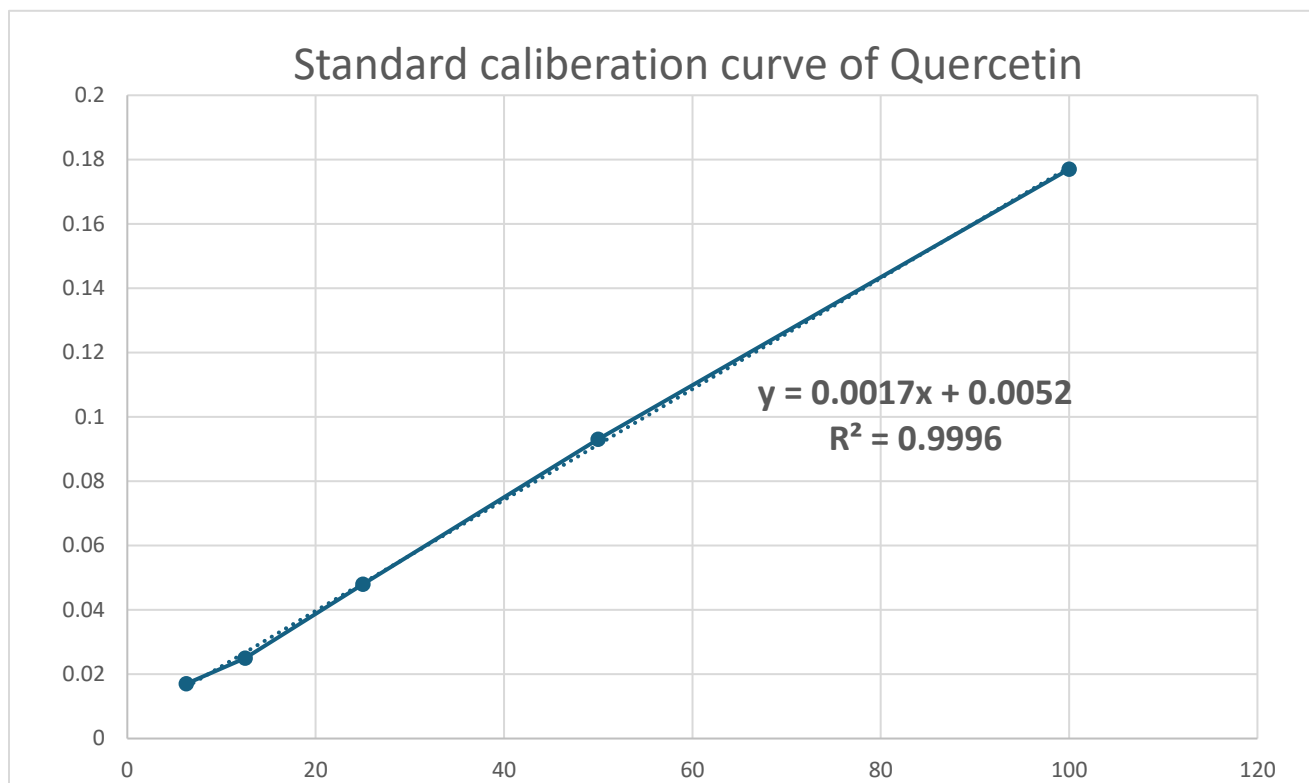


Figure 04: Standard calibration curve of Quercetin

3.9 TFC Result

Calculate Concentration (C)

Here, calculate concentration using this formula

$$\text{formula } x = \frac{A \text{ sample} - 0.005208}{0.001724}$$

calculate the concentration for each sample absorbance readings.

Sample Abs(y)	Calculation: (y- 0.005208)/ 0.001724	Concentration C (µg/mL)
0.029	(0.029-0.005208)/ 0.001724	13.80
0.033	(0.033-0.005208)/ 0.001724	16.12
0.039	(0.039-0.005208)/ 0.001724	19.60
0.044	(0.044-0.005208)/ 0.001724	22.51
0.049	(0.049-0.005208)/ 0.001724	25.41

Table 07: Calculation of concentration for each absorbance of sample

From this table got concentrations ,

C1 = 13.80 µg/mL, C2 = 16.12 µg/mL , C3 = 19.60 µg/mL, C4 = 22.51 µg/mL,

C5 = 25.41 µg/mL

Total Flavonoid Content (TFC) Calculation

Insert values into the TFC formula:

$$TFC = \frac{C \times 5 \times 5}{25}$$
$$= \frac{C}{1}$$

Concentration C (µg/mL)	Concentration C (µg/mL)	TFC Calculation (C/1)	TFC (mg QE/g)
C1	13.80	13.80/1	13.80
C2	16.12	16.12/1	16.12
C3	19.60	19.60/1	19.60
C4	22.51	22.51/1	22.51
C5	25.41	25.41/1	25.41

Table 08: Calculation of TFC (mg QE/g) for each concentration

$$\begin{aligned}\text{Average TFC} &= \frac{C1+C2+C3+C4+C5}{5} \\ &= \frac{13.80+16.12+19.60+22.51+25.41}{5} \\ &= \frac{97.93}{5} \\ &= 19.60\end{aligned}$$

The Total Flavonoid Content of the analyzed samples ranged from 13.80 to 25.41 mg QE/g and average TFC value is 19.60mg QE/g, ± 5 mg QE/g. These values represent the Flavonoid concentration relative to the dry weight of the sample, calculated based on the Quercetin standard curve.

3.10 Correlation Between TPC, TFC and DPPH Activity

To determine whether the phenolic compounds present in *Musa balbisiana* bract extract contribute significantly to its antioxidant activity, the relationship between Total Phenolic Content (TPC) and DPPH radical scavenging activity was evaluated.

The IC₅₀ value of the *Musa balbisiana* bract extract in the DPPH assay was found to be 144.65 µg/mL, whereas ascorbic acid, used as the standard antioxidant, showed a lower IC₅₀ value of 99.59 µg/mL. Since a lower IC₅₀ value indicates a greater free-radical scavenging capacity, ascorbic acid exhibited stronger antioxidant activity than the plant extract. The comparatively higher IC₅₀ value of the extract indicates a moderate level of antioxidant activity.

The Total Phenolic Content (TPC) of the *Musa balbisiana* bract extract was determined to be 10.91 mg gallic acid equivalent (GAE)/g of extract, indicating the presence of phenolic compounds in the sample. The findings suggest a positive association between phenolic content and antioxidant activity. Phenolic compounds can contribute to DPPH radical scavenging through their ability to donate hydrogen atoms or electrons and thereby neutralize free radicals. Therefore, the measured phenolic content may partly explain the antioxidant activity observed in the *Musa balbisiana* bract extract, although its activity was lower than that of the standard ascorbic acid.

The moderate antioxidant activity of the extract may be influenced by the amount, composition, and structural diversity of its phenolic compounds. Different phenolic compounds may exhibit different antioxidant capacities depending on their molecular structures and functional groups

Chapter 4 - Discussion

4.1 Interpretation of DPPH Results

Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, the sample's antioxidant activity was assessed and compared to a reference antioxidant at doses of 400, 200, 100, 50, 25, and 12.5 µg/mL.

Values of Absorbance

At doses of 400, 200, 100, 50, 25, and 12.5 µg/mL, the sample's absorbance values were 0.0385, 0.1639, 0.2273, 0.3023, 0.3465 and 0.3546, respectively. As concentration grew, absorbance gradually decreased, suggesting that at greater concentrations, free radical scavenging activity increased. At the corresponding concentrations, the absorbance values for the standard were 0.0602, 0.1355, 0.1554, 0.2726, 0.3039 and 0.3167. The standard showed a concentration-dependent drop in absorbance, which was more noticeable at higher concentrations, much like the sample.

IC₅₀ Values

It was found that the sample's IC₅₀ value was 144.65 µg/mL, while the standard's was 99.59 µg/mL. In comparison to the sample, the standard showed more capability for scavenging radicals because a lower IC₅₀ value denotes stronger antioxidant activity. The sample's significantly low antioxidant efficacy is indicated by the higher dose needed to produce 50% suppression of DPPH radicals. The findings show that the sample has quantifiable antioxidant activity that varies with concentration. However, the sample's higher IC₅₀ value indicates that its antioxidant capacity is lower than the normal. The sample's overall free radical scavenging activity is moderate.

4.2 Interpretation of TPC Results

Using a spectrophotometric technique, the sample's total phenolic content was calculated and compared to a standard reference chemical at concentrations of 100, 50, 25, and 12.5, 6.25 µg/mL.

Values of Absorbance

The total phenolic content of the *Musa balbisiana* bract extract was determined using a spectrophotometric method and compared with the standard calibration curve. The standard was prepared at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The corresponding absorbance values of the sample were 0.011, 0.017, 0.021, 0.026, and 0.031, respectively. The results showed a gradual increase in absorbance with increasing concentration, indicating a positive relationship between concentration and absorbance. Similarly, the standard showed absorbance values of 0.014, 0.026, 0.051, 0.090, and 0.190 at the respective concentrations. The standard therefore demonstrated a clear increasing trend in absorbance with increasing concentration, which is consistent with the expected response of a spectrophotometric assay. These absorbance values were subsequently used to determine the phenolic concentration of the sample from the standard calibration curve.

Value of TPC

The Total Phenolic Content (TPC) of the *Musa balbisiana* bract extract was found to be 10.91 mg GAE/g extract. This result indicates the presence of phenolic compounds in the extract, which may contribute to its antioxidant activity.

The TPC value of the crude extract is lower than that of pure gallic acid because the extract contains a mixture of different compounds. The presence of phenolic constituents may contribute to the reducing and free-radical-scavenging properties of the extract.

The observed variation in absorbance suggests that the experiment may be repeated to improve the linearity of the calibration curve and obtain more reliable TPC results.

4.3 Interpretation of TFC Results

Using a spectrophotometric technique, the sample's total phenolic content was calculated and compared to a standard reference chemical at concentrations of 6.25, 12.5, 25, and 50, 100 µg/mL.

Values of Absorbance

At concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL, the absorbance values of the sample were 0.029, 0.033, 0.039, 0.044, and 0.049, respectively. The absorbance

gradually increased with increasing concentration, showing a positive relationship between concentration and absorbance.

For the standard gallic acid, the corresponding absorbance values were 0.017, 0.025, 0.048, 0.093, and 0.177, respectively. The standard showed a clearer increase in absorbance with concentration, indicating a stronger concentration-dependent response.

Value of TFC

The Total Flavonoid Content (TFC) of the *Musa balbisiana* bract extract was found to be 19.60 mg quercetin equivalent (QE)/g extract. This result indicates the presence of flavonoid compounds in the extract, which may contribute to its antioxidant activity.

The flavonoid content of the crude extract is expressed as quercetin equivalents because the extract contains a mixture of different compounds rather than pure quercetin. The findings suggest that flavonoid constituents may contribute to the antioxidant properties of the extract.

The observed variation in absorbance suggests that the experiment may be repeated to improve the linearity of the calibration curve and obtain more reliable TFC results.

4.4 Comparison with Previous Studies

The Total Flavonoid Content (TFC) and antioxidant activity of the *Musa balbisiana* bract extract can be interpreted in relation to previous studies on *Musa* species. Several studies have reported that phenolic and flavonoid compounds contribute significantly to the antioxidant properties of banana plant tissues. A higher concentration of these bioactive compounds is often associated with stronger free-radical-scavenging activity in assays such as DPPH (Swargiary *et al.*, 2021; Sadebo *et al.*, 2026).

The present study showed a TFC of 19.60 mg QE/g extract, indicating the presence of flavonoid compounds in the *Musa balbisiana* bract extract. These compounds may contribute to the antioxidant activity observed in the extract. However, the antioxidant capacity of a crude plant extract can vary depending on the plant part, extraction solvent, concentration, and composition of bioactive compounds.

Previous research on different *Musa* species and plant parts has also demonstrated variation in total phenolic and flavonoid contents and antioxidant activity. Such differences may result from genetic variation, maturity, environmental conditions, and extraction methods. Overall, the present findings support the potential of *Musa balbisiana* bracts as a source of flavonoid compounds with possible antioxidant properties (Swargiary *et al.*, 2021; Sadebo *et al.*, 2026).

4.5 Comparison with Other Fruit Species

The relationship between Total Phenolic Content (TPC) and antioxidant activity has been investigated in various fruit species using the Folin–Ciocalteu and DPPH methods. In general, extracts with higher phenolic and flavonoid contents often show stronger free-radical-scavenging activity, which is reflected by lower IC₅₀ values.

Previous studies on different fruit extracts have reported variations in TPC, TFC, and DPPH activity depending on the species, plant part, extraction solvent, and experimental conditions. Methanolic extracts often show considerable antioxidant activity because methanol can effectively extract many phenolic and flavonoid compounds.

The present *Musa balbisiana* bract extract showed moderate antioxidant activity with an IC₅₀ value of 144.65 µg/mL, compared with the standard, which showed an IC₅₀ value of 99.59 µg/mL. The comparatively higher IC₅₀ value indicates lower radical-scavenging potency than the standard. Differences in antioxidant activity among plant extracts may be attributed to variations in phytochemical composition and extraction efficiency.

Overall, the present findings are consistent with previous research showing that phenolic and flavonoid compounds can contribute significantly to the antioxidant activity of plant-derived extracts.

4.6 Relationship Between Phenolic Content and Antioxidant Activity

The relationship between Total Phenolic Content (TPC) and antioxidant activity was evaluated by comparing the TPC results with the DPPH radical scavenging activity of the *Musa balbisiana* bract extract. Phenolic compounds can donate hydrogen atoms or electrons and stabilize free radicals, thereby contributing to antioxidant activity.

In the present study, the extract showed a DPPH radical scavenging activity with an IC_{50} value of 144.65 $\mu\text{g/mL}$, indicating moderate antioxidant activity. The detectable phenolic content of the extract may contribute to this activity.

However, antioxidant activity is influenced not only by the total amount of phenolics but also by their chemical structure, concentration, and interaction with other phytochemicals such as flavonoids. Differences in extraction efficiency and assay conditions may also affect the results.

Overall, the findings suggest that phenolic and flavonoid compounds may contribute to the antioxidant activity of *Musa balbisiana* bract extract. Further phytochemical analysis and correlation studies would help establish the relationship between phenolic content and antioxidant activity more precisely.

4.7 Possible Mechanisms of Action

The antioxidant activity of *Musa balbisiana* bract extract may result from interactions between its phytochemicals, particularly phenolic and flavonoid compounds, and free radicals. These compounds can donate hydrogen atoms or electrons to stabilize reactive radicals and reduce oxidative damage. (Rice-Evans *et al.*, 1997)

Phenolic compounds may also chelate transition metal ions such as iron and copper, thereby reducing the formation of reactive oxygen species through Fenton-type reactions. In addition, phenolics and flavonoids may act together with other phytochemicals to enhance the overall antioxidant effect. (Heim *et al.*, 2002)

Furthermore, these compounds can help inhibit lipid peroxidation and protect cellular components from oxidative damage. Overall, the antioxidant activity of the extract may involve free-radical scavenging, metal chelation, and synergistic interactions among its phytochemicals. (Shahidi and Ambigaipalan, 2015)

4.8 Limitations of the Study

Although this study provides useful information on the phytochemical composition and antioxidant activity of *Musa balbisiana* bract extract, several limitations should be considered when interpreting the findings. These limitations may affect the generalization and broader application of the results.

Method of Extraction and Choice of Solvent:

The extraction method and solvent used can significantly influence the yield and composition of bioactive compounds. In this study, only one solvent system was used, which may not have extracted all phenolic, flavonoid, and other antioxidant compounds present in the *Musa balbisiana* bract. Therefore, the actual antioxidant potential of the plant material may be higher than the observed results.

Limited Characterization of Phytochemicals:

The study focused mainly on the determination of total phenolic content, total flavonoid content, and DPPH radical-scavenging activity. Specific phenolic compounds, flavonoids, carotenoids, vitamins, and other individual bioactive constituents were not identified or quantified. Therefore, it was not possible to determine which specific compounds were mainly responsible for the observed antioxidant activity.

Limitations of Concentration and Range

The sample and standard were evaluated within a predefined concentration range. This limited range may affect the accuracy of the IC_{50} determination, particularly if the actual IC_{50} value falls outside the tested concentrations. Variations in absorbance at some concentrations may also have influenced the overall results.

In Vitro Nature of the Study

The antioxidant activity was evaluated only under in vitro experimental conditions. Factors such as absorption, metabolism, bioavailability, and distribution of phytochemicals within the body were not investigated. Therefore, the observed antioxidant activity cannot be directly considered equivalent to the physiological effects in humans or animals.

Variability of the Sample:

The phytochemical content and antioxidant activity of *Musa balbisiana* bracts may vary depending on geographical location, maturity stage, environmental conditions, cultivation practices, and storage conditions. Since the study was conducted using a

limited sample source, the findings may not fully represent all *Musa balbisiana* bracts.

Possible Experimental Errors:

Small variations during sample preparation, pipetting, reagent preparation, incubation time, and spectrophotometric measurement may have affected the absorbance values. Such experimental variations can influence the calculated TPC, TFC, and antioxidant activity.

Effect of Solvent Polarity

The use of a single solvent limits the extraction of compounds with different polarities. Phenolic and flavonoid compounds may differ in their solubility depending on solvent polarity. Using solvents with different polarities or solvent mixtures in future studies may improve the recovery of a wider range of bioactive compounds and provide a more comprehensive assessment of the antioxidant potential of *Musa balbisiana* bract extract

Chapter 5 - CONCLUSION

5.1 Conclusion

This study highlights the potential of *Musa balbisiana* bract extract as a natural source of bioactive compounds by evaluating its phytochemical composition and antioxidant activity. The extract showed detectable phenolic and flavonoid contents along with free-radical-scavenging activity under in vitro conditions. The Total Phenolic Content (TPC) of the extract was found to be 10.91 mg gallic acid equivalent (GAE)/g extract, while the Total Flavonoid Content (TFC) was 19.60 mg quercetin equivalent (QE)/g extract. These findings indicate the presence of appreciable amounts of phenolic and flavonoid compounds in the bract extract.

The antioxidant activity of the extract was evaluated using the DPPH radical scavenging assay. The extract showed an IC₅₀ value of 144.65 µg/mL, whereas the standard showed an IC₅₀ value of 99.59 µg/mL. The comparatively higher IC₅₀ value of the extract indicates lower radical-scavenging potency than the standard, but it still demonstrates measurable antioxidant activity. The observed activity may be associated with the phenolic and flavonoid compounds present in the extract, although other phytochemicals and their possible synergistic interactions may also contribute to the overall antioxidant effect (Sadebo *et al.*, 2026; Shahidi and Yeo, 2016).

The antioxidant activity of *Musa balbisiana* bract extract may involve several mechanisms, including free-radical scavenging, hydrogen atom or electron donation, metal-ion chelation, and inhibition of oxidative chain reactions. These mechanisms can help stabilize reactive species and reduce oxidative damage. The presence of phenolic and flavonoid compounds may therefore play an important role in the antioxidant properties observed in the present study.

The findings also suggest that the antioxidant activity of the extract cannot be attributed solely to the total amount of phenolic or flavonoid compounds. Factors such as the chemical structure of individual compounds, their concentration, extraction efficiency, and interactions among different phytochemicals may influence the final antioxidant activity. Therefore, further investigation is necessary to establish a more precise relationship between phytochemical composition and antioxidant potential (Sadebo *et al.*, 2026; Shahidi and Yeo, 2016).

Despite these findings, the study has certain limitations. The use of a single extraction solvent and limited phytochemical characterization may have restricted the recovery and identification of some bioactive compounds. In addition, the antioxidant activity was evaluated only by an in vitro DPPH assay, which cannot

directly represent the physiological effects, bioavailability, or metabolism of these compounds in vivo. Variations in sample preparation, reagent handling, and spectrophotometric measurements may also influence the results.

Nevertheless, the present findings demonstrate that *Musa balbisiana* bract has potential as an underutilized source of natural antioxidant compounds. Its detectable phenolic and flavonoid contents and measurable DPPH radical-scavenging activity suggest possible applications in functional foods, nutraceuticals, natural antioxidant formulations, and other value-added products. Further studies should focus on optimizing extraction conditions, identifying individual bioactive compounds, evaluating additional antioxidant assays, and conducting in vivo studies to confirm its biological potential (Sadebo *et al.*, 2026; Shahidi and Yeo, 2016).

In summary, the study provides preliminary evidence that *Musa balbisiana* bract extract contains phenolic and flavonoid compounds and possesses measurable antioxidant activity. The findings support the potential utilization of *Musa balbisiana* bracts as a valuable plant-based source of bioactive compounds and provide a basis for further research into their nutritional, functional, and possible health-related applications (Shahidi and Yeo, 2016).

5.2 Recommendations

High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique that can be used to separate, identify, and quantify individual phenolic compounds present in plant extracts. In future studies, HPLC analysis of *Musa balbisiana* bract extract could be performed to identify specific phenolic acids and flavonoids, such as caffeic acid, ferulic acid, p-coumaric acid, and other relevant bioactive compounds.

Detailed HPLC profiling would provide a better understanding of the individual phytochemicals responsible for the antioxidant activity of the extract. It could also help establish a relationship between specific compounds and the observed DPPH radical-scavenging activity. Furthermore, HPLC analysis may support the standardization and quality control of *Musa balbisiana* bract extract by determining the consistency of its major bioactive constituents across different batches. This information could be useful for future development of functional foods, nutraceuticals, and other value-added products. (Sadebo *et al.*, 2026)

Gas Chromatography–Mass Spectrometry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) combines the separation capability of gas chromatography with the identification capability of mass spectrometry. In future studies, GC-MS analysis could be used to investigate the volatile and semi-volatile phytochemicals present in *Musa balbisiana* bract extract, including terpenoids, aromatic compounds, fatty acid derivatives, and other suitable secondary metabolites. (Shahidi and Yeo, 2016)

GC-MS analysis can provide valuable information about the molecular characteristics, retention behavior, and relative abundance of detectable compounds. This may help identify bioactive constituents that could contribute to the antioxidant or other biological activities of the extract. It can also complement spectrophotometric assays such as TPC, TFC, and DPPH by providing information about individual chemical constituents rather than only measuring total compound groups.

Furthermore, GC-MS may help detect minor or previously unidentified volatile and semi-volatile constituents that are not characterized by conventional spectrophotometric methods. Therefore, combining GC-MS with HPLC and other phytochemical techniques could provide a more comprehensive understanding of the chemical composition and potential bioactivity of *Musa balbisiana* bract extract. (Altemimi *et al.*, 2017)

Significance of Advanced Characterization

Further phytochemical characterization using HPLC and GC-MS would provide more detailed information about the chemical composition of *Musa balbisiana* bract extract. Such advanced analytical techniques could:

- **Identify specific bioactive compounds:** Determine the individual phenolic, flavonoid, and other detectable phytochemicals that may contribute to the antioxidant activity of the extract.
- **Support quantitative correlation:** Provide quantitative information that can be correlated with DPPH radical-scavenging activity and IC₅₀ values, helping to better understand the contribution of individual compounds to antioxidant activity.
- **Evaluate variation in samples and extraction methods:** Allow comparison of phytochemical composition among samples collected from different locations, maturity stages, and extraction conditions.

- **Support standardization and product development:** Help establish a consistent phytochemical profile and support the development of standardized, bioactive-rich *Musa balbisiana* bract extracts for potential use in functional foods, nutraceuticals, and other value-added applications.

Isolation of Active Compounds

Identifying the specific compounds responsible for the biological activities of *Musa balbisiana* bract extract is important for understanding its antioxidant potential. Isolation of active compounds allows individual phenolics, flavonoids, and other secondary metabolites to be separated from the crude extract for detailed structural and biological investigation.

Rationale for Isolation

Spectrophotometric assays such as DPPH, TPC, and TFC provide information about the overall antioxidant activity and the total amount of particular groups of compounds, but they do not identify individual molecules. Isolation of active compounds can help researchers:

- Determine which specific phytochemicals contribute most to the antioxidant activity.
- Evaluate the dose-response relationship of purified compounds.
- Investigate structure-activity relationships and understand how functional groups influence radical scavenging and metal-chelating activities.
- Provide purified reference compounds for further analytical techniques such as HPLC and LC-MS.

Common Isolation Techniques

Several chromatographic and preparative techniques can be used for the isolation of bioactive compounds from *Musa balbisiana* bract extract:

- **Column Chromatography:** A commonly used technique for separating compounds according to their polarity and interaction with the stationary phase. Silica gel and other suitable materials can be used for fractionation and purification.
- **Fractionation:** The crude extract can be separated into different fractions using solvents of increasing polarity, such as hexane, ethyl acetate, and

methanol. Each fraction can then be evaluated for antioxidant activity to identify the most active fraction.

- **Preparative Thin-Layer Chromatography (Prep-TLC):** This technique can be used to separate relatively small quantities of individual compounds based on their movement over a stationary phase. Separated compounds can be recovered for further analysis.
- **Preparative HPLC:** Preparative HPLC can be used to obtain relatively high-purity compounds from selected fractions, which can subsequently be subjected to structural and biological characterization.

Application to *Musa balbisiana*

Phenolic acids, flavonoids, and other potentially bioactive compounds present in *Musa balbisiana* bract extract can be separated and studied individually. The antioxidant activity of isolated compounds can then be evaluated and compared with that of the crude extract. Such investigations may also help determine whether the compounds act independently or show synergistic or antagonistic interactions when present together.

Challenges in Isolation

- **Complex Matrix:** Plant extracts contain numerous compounds with similar chemical properties, which can make separation and purification difficult.
- **Low Abundance:** Some bioactive compounds may occur in very small amounts and therefore require sensitive analytical methods and sufficient quantities of starting material.
- **Compound Stability:** Certain phenolic and other bioactive compounds may be sensitive to heat, light, oxygen, or prolonged processing, which can affect their stability and biological activity.

Benefits of Isolation

Isolation of active compounds from *Musa balbisiana* bract extract may support:

- **Pharmacological studies:** Purified compounds can be evaluated through in vitro and, where appropriate, in vivo biological studies.
- **Nutraceutical development:** Identified bioactive compounds may support the development of products with defined phytochemical profiles.
- **Standardization:** Purified marker compounds can be useful for quality control and standardization of *Musa balbisiana* bract-based products.

Application in Food and Pharmaceutical Industries

The findings of this study indicate that *Musa balbisiana* bract extract may have potential applications in the food, nutraceutical, and pharmaceutical sectors because of its detectable phenolic and flavonoid contents and measurable antioxidant activity. Further research and safety evaluation would be necessary before practical or commercial applications.

Applications in the Food Industry

1. Use of Natural Antioxidants for Food Preservation (Shahidi and Yeo, 2016)

Oxidative deterioration can reduce food quality by causing rancidity, color changes, and loss of nutritional value. The antioxidant compounds present in *Musa balbisiana* bract extract may have potential as natural antioxidant ingredients to help reduce oxidative processes in food systems. This could be particularly relevant to the growing interest in plant-based and naturally derived food ingredients.

2. Nutraceuticals and Functional Foods (Shahidi and Yeo, 2016)

The presence of phenolic and flavonoid compounds, together with the observed DPPH radical-scavenging activity, suggests that *Musa balbisiana* bract extract may have potential for future development as a nutraceutical or functional food ingredient. However, further studies on toxicity, bioavailability, stability, and biological activity are required before such applications can be established.

3. Food Fortification (Shahidi and Yeo, 2016)

Following appropriate safety and stability evaluations, antioxidant-rich fractions or standardized extracts from *Musa balbisiana* bracts could potentially be investigated for incorporation into selected food products such as bakery products, cereals, beverages, or other formulations. Such applications may increase the functional value of foods by providing additional plant-derived bioactive compounds.

Potential Pharmaceutical Applications

The phenolic and flavonoid constituents of *Musa balbisiana* bract extract may also be of interest for pharmaceutical and health-related research because of their potential antioxidant properties. Further investigation could focus on isolated compounds, mechanisms of action, cellular studies, toxicity evaluation, and in vivo

models to determine their possible therapeutic relevance. However, the current in vitro findings alone are not sufficient to establish pharmaceutical efficacy.

Pharmaceutical Industry Applications

1. Phytopharmaceutical Development

The phenolic and flavonoid compounds present in *Musa balbisiana* bract extract may have potential for future development of plant-based pharmaceutical or nutraceutical products. The observed antioxidant activity suggests that the extract may be investigated for applications related to oxidative stress. However, further studies involving compound isolation, toxicity evaluation, pharmacological testing, and standardization are required before any therapeutic application can be considered.

2. Supplementary Applications for Oxidative Stress (Shahidi and Ambigaipalan, 2015)

Oxidative stress is associated with various chronic health conditions, including cardiovascular disorders, diabetes, inflammation, and age-related diseases. The antioxidant compounds present in *Musa balbisiana* bract may potentially support endogenous antioxidant defense mechanisms. Further in vitro, cellular, and in vivo studies are necessary to determine whether the extract or its isolated compounds could have beneficial effects when used as a supplementary treatment.

3. Cosmeceutical Applications

Plant-derived antioxidants are increasingly investigated for their potential role in protecting skin from oxidative damage and premature aging. The phenolic and flavonoid constituents of *Musa balbisiana* bract extract may therefore have potential applications in cosmetic and cosmeceutical formulations, such as antioxidant creams, serums, and other skin-care products. Further studies are required to evaluate skin compatibility, stability, safety, and actual protective effects.

Significance and Industry Relevance

The present study demonstrates that *Musa balbisiana* bract contains detectable phenolic and flavonoid compounds and possesses measurable antioxidant activity, with a DPPH IC₅₀ value of 144.65 µg/mL. These findings indicate that the bract, which is often underutilized, may have potential beyond its traditional or agricultural use.

The utilization of *Musa balbisiana* bract as a source of natural bioactive compounds could contribute to the development of functional foods, nutraceuticals, cosmeceuticals, and other value-added products. However, practical industrial application would require further optimization of extraction methods, detailed phytochemical characterization, standardization of active constituents, stability studies, and comprehensive safety and toxicological evaluations. Further research could therefore help determine the feasibility of utilizing *Musa balbisiana* bract as a sustainable source of natural antioxidant compounds.

Chapter 6 - REFERENCES

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