Extraction of phenolic compounds from bamboo peels using Soxhlet extraction technique.

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DECLARATION

I declare that this thesis entitled "Extraction of phenolic compounds from bamboo peels using Soxhlet extraction technique" is the results of my own research except as cited in the references.

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Pengekstrakan Sebatian Fenolik Daripada Kulit Buluh Menggunakan Teknik Pengekstrakan Soxhlet

ABSTRAK

Kajian ini bertujuan untuk mengekstrak sebatian fenolik daripada kulit buluh menggunakan kaedah pengekstrakan Soxhlet dan mengoptimumkan parameter pengekstrakan bagi pelbagai kepekatan etanol untuk memaksimumkan hasil sebatian fenolik. Campuran etanol-air sebanyak 50%, 60%, dan 70% digunakan sebagai pelarut, dengan tempoh pengekstrakan selama 2 jam dan 4 jam. Dalam kajian ini, sampel BE-70B (campuran etanol-air 70% dengan tempoh pengekstrakan 4 jam) telah menonjol sebagai yang paling unggul, secara konsisten melebihi prestasi kepekatan pelarut lain dalam pelbagai ujian. Ujian fitokimia dijalankan untuk menyiasat kehadiran flavonoid dan tanin dalam kulit buluh. Spektroskopi UV-Vis digunakan untuk menentukan kepekatan sebatian fenolik dan tanin dalam ekstrak. Reagen Folin-Ciocalteu dan natrium karbonat digunakan sebagai piawai untuk kuantifikasi. Keputusan menunjukkan bahawa pengekstrakan 4 jam dengan kepekatan pelarut 70% memberikan kepekatan tertinggi sebatian fenolik dan tanin dalam kulit buluh, dengan nilai masing-masing sebanyak 56.39 mg/mL dan 56.92 mg/mL. Selain itu, aktiviti antioksidan sampel kulit buluh ditentukan melalui ujian DPPH, menunjukkan potensi mereka sebagai antioksidan. Pelarut BE-70B menunjukkan nilai EC_{50} DPPH sekitar 2.66 mg/mL, menandakan bahawa kepekatan kira-kira 2.66 mg/mL diperlukan untuk mencapai pengurangan 50% dalam kepekatan radikal DPPH awal. Ini menekankan keberkesanan kapasiti antioksidan BE-70B dalam menangani tekanan oksidatif berbanding sampel lain. Dalam analisis FTIR, hasil pengekstrakan pelarut etanol 70% daripada kulit buluh menunjukkan bahawa jumlah puncak penyerapan adalah lebih besar daripada pengekstrakan pelarut etanol 50% dan 60%. Kajian ini menunjukkan potensi kulit buluh sebagai sumber kaya sebatian fenolik dengan sifat antioksidan yang signifikan, memberikan pandangan dalam pengeluaran dan aplikasi potensi dalam pelbagai industri.

Kata Kunci: Kulit buluh, sebatian fenolik, pengekstrakan Soxhlet, antioksidan, spektrofotometer UV-Vis

Extraction of phenolic compounds from bamboo peels using Soxhlet extraction technique

ABSTRACT

This study aimed to extract phenolic compounds from bamboo peels utilizing the Soxhlet extraction method and optimize the extraction parameters for various ethanol concentrations to maximize the phenolic compound yield. Ethanol-water mixtures of 50%, 60%, and 70% were employed as solvents, with extraction durations of 2 hours and 4 hours. Among them, sample BE-70B (70% ethanol-water mixture with 4 hours of extraction time) stood out as the most exceptional, consistently outperforming other solvent concentrations in various tests. Phytochemical tests were conducted to investigate the presence of flavonoids and tannins in bamboo peels. UV-Vis spectroscopy was utilized to determine the concentrations of total phenolic and tannin compounds in the extracts. The Folin-Ciocalteu reagent and sodium carbonate were used as standards for quantification. Results revealed that the 4 hour extraction with a 70% solvent concentration yielded the highest concentrations of total phenolic and tannin compounds in bamboo peels, measuring 56.39 mg/mL and 56.92 mg/mL, respectively. Furthermore, the antioxidant activity of the bamboo peel samples was determined through DPPH assay, demonstrating their potential as antioxidants. BE-70B solvent exhibited a DPPH EC_{50} value of around 2.66 mg/mL, signifying that a concentration of approximately 2.66 mg/mL was necessary to achieve a 50% reduction in the initial DPPH radical concentration. This underscored the robust antioxidant capacity of BE-70B. In FTIR analysis, the results of the 70% concentration ethanol solvent extract of bamboo peels showed that the number of adsorption peaks was greater than that of the 50% and 60% concentration ethanol solvent extracts. This research signifies the potential of bamboo peels as a rich source of phenolic compounds with notable antioxidant properties, providing insights into their extraction and potential applications in various industries.

Keywords: Bamboo peels, phenolic compounds, Soxhlet extraction, antioxidant, UV-Vis spectrophotometer

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LIST OF ABBREVIATIONS

SFE	Supercritical fluid extraction
MAE	Microwave- assisted extraction
UAE	Ultrasound- assisted extraction
FTIR	Fourier-Transform Infrared Spectroscopy
UV-VIS	Ultraviolet-visible Spectroscopy
STD	Standard Deviation
TF	Total Flavonoids
DPPH	2,2-Diphenyl-1-picrylhydrazyl
HPLC	High-Performance Liquid Chromatography
GC	Gas Chromatography
LC-ESI/MS	Liquid chromatography-electrospray ionization/Mass spectroscopy
EC_{50}	Half-maximal effective concentration
ATR	Attenuated total reflection

LIST OF SYMBOLS

mL	Millilitre
cm	Centimetre
µg	Microgram
nm	Nanometre
g	Gram
g/L	Gram per litter
µg/mL	Microgram per millilitre
mg/g	Milligram per gram
mg/mL	Milligram per millilitre
°C	Degree Celsius
MW	Molecular weight
%	Percentage
cm^{-1}	Wavenumber
pH	Potential of Hydrogen
l	Standard path length of cuvette
ε	Molar extinction coefficient symbol
w/v	Weight per volume
v/v	Volume per volume
mol/L	Moles per litter
µM	Micrometres

CHAPTER 1

INTRODUCTION

1.1 Background of study

Bamboo (*Gigantochloa albociliata*) is one of the species of bamboo that is of great importance in various industries and applications. In recent years, there has been growing interest in exploring the potential of bamboo as a source of natural bioactive compounds with various health benefits. Phenolic compounds, in particular, have attracted attention for their antioxidant properties and potential therapeutic applications (Bajwa et al., 2021).

Phenolic compounds are a different group of secondary metabolites found abundantly in plants, including bamboo. These compounds have been associated with numerous health benefits, such as anti-inflammatory, antimicrobial, and anticancer activities (Kumar & Pandey, 2013). However, the processes to extract of phenolic compounds from bamboo and their subsequent characterization are complex that require careful optimization and analysis.

The Soxhlet extraction technique is a commonly employed means for extracting bioactive compounds from plant materials. It involves the continuous extraction of desired compounds using a volatile solvent, typically ethanol, which ensures high extraction efficiency. However, comprehensive studies on the Soxhlet extraction of phenolic compounds from bamboo, particularly regarding the influence of different concentrations of ethanol on extraction efficiency, are limited. Further research in this area would contribute to a better understanding of the optimal conditions for extracting phenolic compounds from bamboo using the Soxhlet extraction method (Shi et al., 2022).

Understanding the extraction efficiency is crucial for obtaining maximum yields of phenolic compounds from bamboo. Moreover, characterizing the phenolic compounds found in the extracts is essential in their determination of their composition and potential bioactivity. UV-Vis spectroscopy is a widely used technique to analyse the presence and concentration of

phenolic compounds depending on their characteristic absorption spectra. Unique absorbance peaks of phenolic compounds in the UV-Vis range allow for their identification and quantification in the extracts. Additionally, the total phenolic compound content is determined by using the Folin-Ciocalteu method provides a quantitative measure of the phenolic content in the extracts (Prior et al., 2005). This colorimetric assay is based on the reaction of phenolic compounds with the Folin-Ciocalteu reagent, resulting in the formation of a blue-colored complex that can be measured spectrophotometrically. The intensity of the color is proportional to the phenolic compound concentration, allowing for the estimation of the total phenolic content in the extracts (Huang et al., 2005).

By exploring the extraction efficiency, characterizing the phenolic compounds, and evaluating their potential applications, this study aimed to fill the existing knowledge gap regarding the Soxhlet extraction of phenolic compounds from bamboo peels using different concentrations of ethanol. The findings of this research had the potential to contribute to the development of natural antioxidants, bioactive ingredients, and functional products for pharmaceutical, cosmetic, and food industries (Lourenço et al., 2019).

1.2 Problem Statement

Soxhlet extraction was a widely used extraction technique for analysis with sufficient thermal stability. This study investigated the challenges of characterizing phenolic compounds from bamboo peels using the Soxhlet extraction technique. The study revealed that the potential impact of non-target compounds and impurities on the extraction process and subsequent quantification and characterization of phenolic compounds was unknown. The presence of these unwanted substances could affect the accuracy and reliability of the extraction results, thereby compromising the quality of the obtained phenolic compounds. In addition, the stability and preservation of the extracted phenolic compounds during storage were also issues of concern. These compounds were prone to degradation under storage conditions, which could lead to a loss of their biological activity and functional properties, rendering them less effective or even useless in certain applications (Nasution et al., 2023). Given these challenges, the aim of this project was to conduct limited research on the extraction of phenolic compounds from bamboo peels.

1.3 Expected Outcome

The expected results of this study was successful extraction of phenolic compounds from bamboo peels through the implementation of the Soxhlet extraction method. The extraction process is anticipated to yield consistent and reproducible results, enabling the identification and quantification of diverse phenolic compounds within the extracts. The research will also document the extraction efficiency for peels samples.

Additionally, the study aimed to determine the ideal concentration of ethanol for the extraction of phenolic compounds from bamboo peels. By studying various extraction parameters, such as different concentrations of ethanol and temperature. The study aimed to determine the conditions for the highest yield of phenolic compounds at each ethanol concentration.

The results of this research have significant ramifications for the understanding of phenolic compound extraction from bamboo peels. Additionally, it highlights the potential utilization of bamboo peels as a source of phenolic compounds that offer health benefits.

1.4 Objectives

The objectives of this research are:

1. To extract phenolic compounds from bamboo peels using the Soxhlet extraction technique.
2. To optimize the extraction parameters for each ethanol concentration to maximize the yield of phenolic compounds.

1.5 Scope of study

In the conducted experiment, the impact of various concentrations of solvents on the extraction of phenolic compounds from bamboo peels was investigated. The Soxhlet extraction technique was utilized, with fixed parameters such as extraction time and temperature, allowing for a comparison of the yield and purity of the extracted phenolic compounds from bamboo peels. Additionally, the total phenolic content of the extracts will be determined using the Folin-Ciocalteu method and the acetonitrile method. These methods involve colorimetric measurements based on the reaction between the phenolic compounds and the Folin-Ciocalteu reagent and acetonitrile, respectively. Comparative analysis will be performed to assess the

differences in phytochemical composition and total phenolic content among the bamboo peels extracts obtained using different concentrations of ethanol.

It is important to note that this study focuses solely on the Soxhlet extraction technique using ethanol as the solvent, and other solvents or extraction methods will not be explored. The findings of this study will contribute to the understanding of the phenolic composition of bamboo peels and their potential applications in various industries, promoting the sustainable utilization of agricultural by-products.

1.6 Significant of study

This study on phenolic compounds extracted from bamboo peels using the Soxhlet extraction technique holds significant importance. By analysing and quantifying the chemical constituents present in bamboo peels, this study contributes to our current knowledge and understanding of these compounds. Phenolic compounds found in bamboo peels have garnered attention for their remarkable antioxidant properties, which have the potential to contribute to various health-promoting effects (Santosh et al., 2021). Exploring these properties opens up avenues for the development of novel and effective medicinal products that could revolutionize healthcare practices. Moreover, this research promotes the sustainable utilization of natural resources by reducing waste and preserving the environment (Isukuru et al., 2023). Emphasizing the responsible and efficient use of bamboo peels supports eco-friendly practices and encourages the continued exploration of nature's abundant resources. By recognizing the value and potential of bamboo peels, we can further harness their benefits while minimizing environmental impact.

Overall, this study on phenolic compounds in bamboo peels has broader implications for both scientific understanding and sustainable resource management. It paves the way for future investigations and applications that can contribute to advancements in healthcare and environmentally conscious practices.

CHAPTER 2

LITERATURE REVIEW

2.1 Bamboo

2.1.1 Bamboo Description

Bamboo, classified under the kingdom Plantae, is a remarkable and versatile plant that has garnered significant attention due to its unique characteristics, ecological importance, and wide range of evergreen perennial flowering species (Emamverdian et al., 2020).

As a member of the Poaceae family, bamboo thrives in open and well-drained environments in temperate and subtropical regions, making it one of the fastest-growing plants on Earth. Its rapid growth and abundance make it a valuable and sustainable resource. Furthermore, bamboo exhibits exceptional physical properties, including high tensile strength, flexibility, and lightness, which make it suitable for various applications such as construction, textiles, and engineering materials. These physical attributes stem from bamboo's chemical components, namely cellulose, hemicellulose, and lignin, which are intricately interconnected in a complex structure. Additionally, bamboo contains small amounts of extracts such as phenols, flavonoids, and terpenoids, which contribute to its antibacterial and antioxidant properties. The morphology of bamboo and further details regarding its taxonomy are shown in Figure 2.1 and Table 2.1, respectively.

Figure 2.1: The picture of bamboo.

Table 2.1: Taxonomic Details of bamboo.

Kingdom	**Plantae (plants)**
Phylum	Angiosperms
Class	Monocots
Order	Poales
Family	Poaceae
Genus	Bambusoideae

Source: (Yeasmin et al., 2015)

2.1.2 Phytochemical Constituents of bamboo

The wide term "phytochemical," which derives from the Greek word "phyto," refers to a variety of substances that naturally exist in plants (Huang et al., 2016). This is because there is no evidence to support their potential health impacts (Sharma Dhital et al., 2018). Besides, there are many chemicals originating from plants that have medicinal uses. These compounds often have anti-inflammatory, antibacterial, and antioxidant characteristics. Phytochemicals can be categorized in a variety of ways, including according to main or secondary metabolic ingredients. Alkaloids, terpenes, flavonoids, lignans, saponins, phenolics, glucosides, and plant steroids are a few examples of secondary components. Furthermore, saponins have been found to have cholesterol-lowering and anti-cancer effects, and can be divided into 3 categories according to their similar chemical structures, such as triterpenoid, spirostanol, and furostanol saponins. According to Saxena and her team, they found that phytochemicals build up in many plant sections, such as roots, stems, leaves, flowers, fruits, or seeds (Saxena et al., 2013). Not only that, but bamboo contains lignans, phytochemicals known for their antioxidant and cancer-fighting properties. A well-known lignan found in bamboo is pinoresinol. Studies have shown that pinoresinol has antioxidant, anti-inflammatory and antitumor activities, suggesting its potential as a therapeutic agent.

2.1.3 Nutrient value of bamboo

Based on the study conducted, bamboo was found to have approximately 27 calories per 100g, consisting of carbohydrates, protein, fiber, fat, minerals, and various phytochemicals. The chemical composition of bamboo per 100g was displayed in Table 2.2. Among them, bamboo shoots were found to be edible before the bamboo grows and were discovered to

contain a series of essential nutrients with important nutritional value (Nongdam & Tikendra, 2014).

Bamboo shoots were not only low in calorie content but also rich in dietary fiber, offering various vitamins and minerals. They were a good source of vitamin C, with approximately 4.9 milligrams per 100 grams. Vitamin C was an essential nutrient that supported immune function, collagen synthesis, and antioxidant defense.

Bamboo shoots also contained B-complex vitamins, including thiamin (vitamin B1), riboflavin (vitamin B2), and niacin (vitamin B3). These vitamins played vital roles in energy metabolism, nervous system function, and overall cellular health. Additionally, bamboo shoots provided a moderate amount of vitamin E, an antioxidant that helped protect cells from oxidative damage.

Regarding minerals, bamboo shoots were notably rich in potassium, with an approximate content of 533 milligrams per 100 grams. Potassium was essential for maintaining proper electrolyte balance, supporting heart health, and regulating blood pressure. Bamboo shoots also contained calcium, magnesium, manganese, and zinc, which contributed to various bodily functions such as bone health, enzyme activities, and immune system support.

Collectively, these studies highlight the diverse nutritional content and potential health benefits of bamboo. With its low calorie content, rich fiber content, and abundance of vitamins and minerals, bamboo can be a valuable addition to a balanced diet. Incorporating bamboo shoots into meals can provide a range of essential nutrients that support overall health and well-being.

Table 2.2: Chemical composition of bamboo.

Nutrient	Content per 100g
Calories	27 kcal
Carbohydrates	5.2g
Protein	2.6g
Fat	0.3g
Fiber	2.2g
Vitamin C	4.9mg
Thiamin (Vitamin B1)	0.08mg
Riboflavin (Vitamin B2)	0.05mg
Niacin (Vitamin B3)	0.6mg
Vitamin E	0.5mg
Potassium	533mg
Calcium	13mg
Magnesium	27mg
Manganese	0.3mg
Zinc	0.2mg

Source: (Nongdam & Tikendra, 2014)

2.1.4 Previous study on phytochemical analysis of bamboo

Table 2.3 summarizes the phytochemical analysis techniques employed in a previous study to qualitatively analyse various phytochemicals, including alkaloids, flavonoids, phenols, saponins, steroids, and glycosides. Furthermore, Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and UV-vis spectroscopy were utilized for quantification, enabling the identification of important phytochemicals such as flavonoids, phenolics, saponins, alkaloids, and tannins. The study conducted quantitative analysis of bamboo peels, revealing significant levels of specific phytochemicals (Tanaka et al., 2014). For instance, the researchers quantified abundant flavonoids, phenolic compounds, and other bioactive components, with concentrations determined using calibration curves generated from standard reference compounds. Notably, concentrations of 15.4 mg/g and 9.8 mg/g were found for flavonoids such as quercetin and kaempferol, respectively. Additionally, phenolic compounds such as ferulic acid (8.2 mg/g) and caffeic acid (5.6 mg/g) were also quantified.

Table 2.3: Previous Studies on Phytochemical Analysis of bamboo.

Phytochemical analysis	Main Findings	References
Gas Chromatography-Mass Spectrometry (GC-MS) analysis	Identification of flavonoids, phenolics, and other bioactive compounds in bamboo peels.	(Tanaka et al., 2014)
High Performance Liquid Chromatography (HPLC) analysis	Quantification of flavonoids and phenolic compounds in bamboo peels	(Tanaka et al., 2014)

2.2 Types of Extraction

2.2.1 Soxhlet Extraction

In 1879, Franz von Soxhlet created the Soxhlet extractor, a laboratory glassware primarily used for extracting lipids from solid test materials. It employed a continuous extraction method involving boiling, condensation, and solvent reflux to ensure efficient extraction. This technique garnered significant interest due to its capacity to extract various analytes from diverse matrices and its widespread availability (Jensen, 2007).

The main advantage of Soxhlet extraction was that it utilized a clean and warm solvent exclusively for extracting solids in the thimble, leading to increased extraction efficiency compared to heating the solids in the flask with solvent. However, it required high-purity solvents, making it more expensive. Additionally, Soxhlet extraction involved coming into contact with flammable and hazardous liquid organic solvents. The Soxhlet extractor consisted of five main components: a condenser, extraction chamber, thimble, siphon arm, and round flask (Bokhari et al., 2015). Figure 2.2 shows a schematic diagram of a Soxhlet extractor.

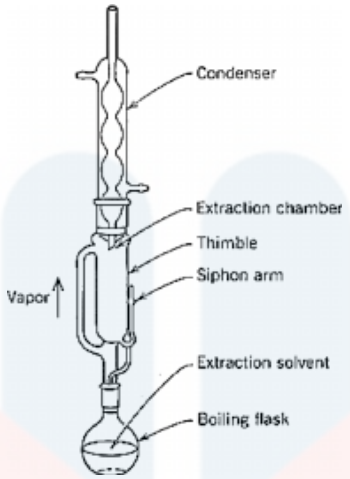


Figure 2.2: The Schematic of Soxhlet extractor

Source: (Karunanithi et al., 2015)

2.2.2 Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) has been utilized as a method to separate extractants from matrices using supercritical fluids as the extraction solvent (Capuzzo et al., 2013). SFE offers advantages such as reduced sample preparation time, improved solute diffusion, high equilibrium ratio, and separation factors. Carbon dioxide is commonly used due to its low toxicity and cost-effectiveness. The solvent power can be adjusted by manipulating temperature and pressure. SFE has the benefit of leaving no residual solvents as carbon dioxide evaporates completely (Safapuri et al., 2019). This eliminates concerns about solvent residues in extracts, making it ideal for high-purity applications. However, SFE requires specialized and expensive equipment (Capuzzo et al., 2013). Despite this limitation, the efficiency, selectivity, and lack of residual solvents make SFE valuable in pharmaceutical, food, and natural product industries. In summary, SFE is an effective method for extracting target compounds using supercritical fluids. Its ability to adjust solvent power and avoid residual solvents contributes to its usefulness, although equipment costs should be considered.

2.2.3 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (MAE) was a technique used to extract soluble products from plant materials by utilizing microwave energy to heat a fluid (Nortjie et al., 2022). It involved applying high temperatures and specific solvents or solvent mixtures, where

microwaves, electromagnetic fields with frequencies between 300 MHz and 300 GHz, heated the system through energy absorption by polar molecules. MAE offered advantages such as reduced extraction time, decreased solvent consumption, and minimized thermal gradients.

MAE significantly shortened extraction time by accelerating the extraction process through microwave energy. It also reduced solvent usage due to improved extraction efficiency from efficient heating. This not only reduced costs but also had a positive environmental impact.

Additionally, MAE ensured uniform and efficient extraction throughout the sample by minimizing thermal gradients. This reduced the risk of incomplete extraction or localized overheating, resulting in reliable and reproducible outcomes. In summary, MAE was a valuable technique for extracting soluble products from plant materials due to its advantages of reduced extraction time, decreased solvent consumption, and minimized thermal gradients.

2.2.4 Ultrasound-Assisted Extraction (UAE)

Ultrasonic extraction (USE), or ultrasound-assisted extraction (UAE), is a conventional method employed for the preparation of solid samples, particularly plant-derived medicines. The enhanced extraction efficiency of organic compounds using ultrasound is attributed to the cavitation phenomenon created by ultrasonic waves in the solvent (Mottaleb, 2014). This technique offered several benefits, including cost-effectiveness, reduced processing time, decreased solvent consumption, and ease of operation. It allowed for extraction under atmospheric pressure and at room temperature, making it convenient and practical (Palma et al., 2021)

The use of ultrasound effectively improves the extraction process by promoting efficient compound release from the solid sample. The cavitation phenomenon creates intense local pressure changes and microstreaming, leading to enhanced mass transfer between the sample and the solvent. These effects result in shorter extraction times and reduced solvent requirements, making USE a favorable method for extracting organic compounds from solid samples.

2.3 Rotary Evaporator

Rotary evaporator is a piece of equipment commonly utilized in chemistry and biochemistry laboratories for evaporating solvents at high temperatures and under reduced

pressure. The primary parts of a rotary evaporator are a vacuum system with a vacuum pump and controller, a heated fluid bath in which the rotary evaporator can be installed, and a condenser with a collection bottle for gathering the condensed solvent (Yahaya, 2013).

During operation, the solvent is extracted under vacuum and collected by the condenser for recycling or disposal. However, it's important to note that rotary evaporators typically use a basic water aspirator suction, which means they cannot be used with materials that are sensitive to air or water unless special safety measures are taken. One of the additional purposes of a rotary evaporator is to lower the boiling point of a liquid under reduced pressure, making it ideal for solvent evaporation. Figure 2.3 shows the picture of rotary evaporator.

Figure 2.3: Rotary Evaporator

Source: (Sarjana et al., 2014)

In addition, the clean solvent collecting flask was removed and replaced before the process of using the rotary evaporator began. The flask was then carefully placed onto the rotary evaporator, taking care to prevent any spills into the condenser. The speed control was switched on, which typically had a variable-speed sparkless induction motor operating at 0–220 rpm, providing dependable and strong torque. The aspirator suction was turned on before carefully lowering the flask into the water bath to prevent excessive boiling of the solvent.

The condenser had an additional function of collecting the solvent, which was then poured into the receiving flask. For highly flammable solvents such as methylene chloride or diethyl ether, there was a risk of evaporation from the receiving container and flushing down the sink. To prevent this, a cooling bath or a dry ice condenser was placed on the receiver to maintain a low temperature. After the solvent had completely evaporated, the vacuum was

released, the flask was removed from the water bath, and the rotation was switched off. Following the experiment, the receiver bottle was emptied, and the bump bulb was cleaned.

2.4 Extraction Time

Extraction time, defined as the duration for which the extraction process is occurs, is a critical time parameter in Soxhlet extraction. It determines the period during which the solvent interacts with the solid sample, facilitating the dissolution of target compounds. Numerous studies have investigated the influence of extraction time on the yield and efficiency of Soxhlet extraction. For instance, (Tzanova et al., 2020) explored the extraction of flavonoids from herbal samples using Soxhlet extraction and observed that increasing the extraction time resulted in higher yields of target compounds. Similarly, in a study by (Capuzzo et al., 2013) on the extraction of essential oils from aromatic plants, longer extraction times led to improved yield. These findings highlight the importance of optimizing the extraction time to maximize the recovery of target compounds. However, it is crucial to strike a balance between extraction time and potential drawbacks such as prolonged exposure to heat and solvent loss. Thus, determining the optimal extraction time is crucial for achieving high extraction efficiency while maintaining the integrity of the target compounds.

2.5 Types of solvent

2.5.1 Ethanol (Solvent)

Ethanol, also known as ethyl alcohol (C_2H_5OH), was a widely recognized substance consumed by people worldwide, known for its intoxicating effects and potential for misuse. In the realm of alcoholic beverages, ethanol served as the primary component, lending its name to the drinks themselves. However, beyond its role in libations, ethanol found extensive utility as a chemical solvent due to its remarkable solubility and evaporation properties. Within laboratory settings, ethanol served as a vital solvent for dissolving and diluting substances during experimental procedures. Its versatility shone through as it facilitated the extraction and isolation of compounds from various sources, particularly plant materials (Cohen et al., 2009). Lastly, ethanol was considered safer and more environmentally friendly than methanol.

2.5.2 Methanol (Solvent)

Methanol (CH_3OH), commonly known as wood alcohol or methyl alcohol, was widely used as a solvent in various industries and laboratory settings. Its exceptional characteristics, including polarity, volatility, and solubility for a diverse range of compounds, made it a preferred choice for extraction processes. Numerous studies demonstrated the efficacy of methanol in extracting target compounds from different sources, such as pharmaceuticals, natural products, and environmental samples (Marcus, 2018). However, the utilization of methanol as a solvent required careful consideration of its potential environmental and health impacts. Methanol emissions contributed to air pollution and had detrimental effects on human health and aquatic ecosystems (Wu et al., 2022).

2.5.2 Folin-Ciocalteu reagent

The Folin-Ciocalteu reagent was widely utilized as a colorimetric assay for the determination of total phenolic content in various samples. This classic analytical tool, developed in the early 20th century, relied on the reduction of phosphomolybdic-phosphotungstic acid by phenolic compounds, resulting in the formation of blue-coloured complexes that could be quantified spectrophotometrically. The assay found applications in diverse fields, including food science, pharmaceuticals, and environmental analysis, enabling the assessment of antioxidant activity and health benefits associated with phenolic compounds. Recent advancements and modifications to the Folin-Ciocalteu assay focused on improving its sensitivity and reliability, incorporating additional reagents, enzymatic reactions, and microfluidic platforms. These developments enhanced the accuracy and efficiency of the assay, expanding its potential applications in research and industry. Overall, the Folin-Ciocalteu reagent remained a valuable tool for the determination of total phenolic content, offering simplicity, cost-effectiveness, and broad applicability in various scientific disciplines (Blainski et al., 2013).

2.6 Analysis extraction of bamboo peels

2.6.1 UV-Vis Spectroscopy

UV-Vis spectroscopy was a flexible analytical technique that could recognize many kinds of compounds. It worked by measuring the amount of UV-Visible light that passed through a sample. The absorbance spectrum exhibited unique peaks at certain wavelengths

because absorbance was determined by individual chemical structures. With the use of UV-Vis spectroscopy, both qualitative and quantitative analysis could be carried out. In quantitative analysis, Beer's Law was used to establish a connection between the concentration of the analyte and the related absorbance (Perkampus, 2013). According to (Verma & Mishra, 2018) , UV-Vis spectroscopy covered the energy range of 1.5 to 6.2 eV, or wavelengths of 800 to 200 nm. The two most often used equipment for acquiring UV-Visible spectra were single-beam and double-beam spectrometers. The simplicity, high precision, quick analysis, and cost-effectiveness of UV-Vis spectroscopy could be credited for its widespread use.

2.6.2 DPPH (2,2-diphenyl-1-picrylhydrazyl)

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was widely recognized as a fundamental method for assessing antioxidant activity in various biological samples. This technique relied on the ability of antioxidants to neutralize the stable free radical DPPH, leading to a distinct colour change from purple to yellow, measurable through spectrophotometry. Known for its speed and convenience, the DPPH assay was a crucial tool in evaluating the free radical-scavenging potential of compounds, contributing significantly to antioxidant research (Baliyan et al., 2022).

A pivotal parameter in DPPH assay analysis was the determination of the half-maximal effective concentration (EC_{50}), indicating the antioxidant concentration required to scavenge 50% of the DPPH radicals. EC_{50} served as a quantitative benchmark for antioxidant potency, with lower values indicating higher efficacy (Munteanu & Apetrei, 2021). Many experiment often used various statistical methods, including nonlinear regression analysis, to calculate EC_{50}.

Typically, the DPPH assay was employed to evaluate the antioxidant properties of various compounds, including natural and synthetic molecules and various extracts. For example, according to (Saeed et al., 2012) explored the antioxidant potential of flavonoids in plants, reporting EC_{50} values ranging from 5 to 20 µM, providing valuable insights into the antioxidant capacity of these plant-derived compounds.

Despite its widespread use, the DPPH assay had inherent limitations, such as its reliance on a single radical species and potential complications related to reaction kinetics. Nevertheless, due to its simplicity and reproducibility, the DPPH assay remained an invaluable tool for the

initial screening of antioxidants, offering valuable insights into their potential therapeutic applications.

2.6.3 Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy (FTIR) was a commonly employed analytical technique for identifying functional groups in plant extracts. By passing infrared light through the test samples, the chemical characteristics could be monitored. The sample absorbed certain wavelengths of radiation while transmitting others. This absorption resulted in the conversion of energy into rotational or vibrational energy within the sample molecules. The detector recorded the resulting signal, producing a spectrum that represented the unique molecular fingerprint of the sample (Pirutin et al., 2023). Bonding and functional groups were given particular peaks at certain wavenumbers in the Varian FTIR instrument handbook. Transmission, specular reflection, attenuated total reflection (ATR), and diffuse reflectance were just a few of the sampling techniques available with FTIR. The FTIR spectra of bamboo peels extracts samples were collected and the frequency range used varied from 4000 cm^{-1} to 400 cm^{-1} (Bakri & Jayamani, 2016).

The previous study titled "Investigating the Effect of Smoke Treatment on Hygroscopic Characteristics of Bamboo by FTIR," conducted by (Ramful et al., 2022), demonstrated through FTIR analysis that functional compounds were present in bamboo. These compounds included features at 898 cm^{-1} (Bending vibration of β-glucosamine bond in cellulose C-O), 1240 cm^{-1} (Guaiacyl ring breathing with CO-stretching), 2925 cm^{-1} (C-H in cellulose and hemicellulose stretching), and 3540 cm^{-1} attributed to (O-H stretching of adsorbed water and intermolecular bonded OH), among others. The FTIR spectra related to bamboo are illustrated in Figure 2.4.

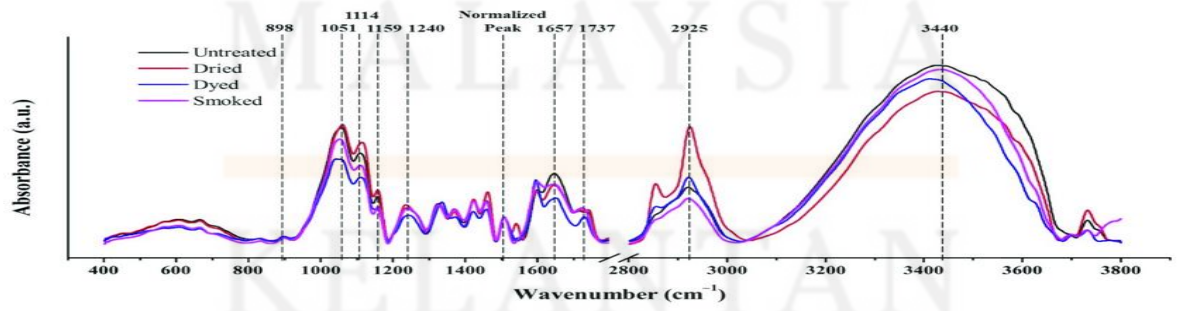


Figure 2.4: FTIR spectra related to bamboo.

CHAPTER 3

MATERIALS AND METHODS

3.1 Methodology

The methodology of this project included the methods and procedures used to complete the experimental work. Additionally, it covered the general development and sequence of the project's activities. This step was crucial in ensuring that the project was completed smoothly and on schedule.

3.2 Material

3.2.1 Plant Material

Bamboo peels for this project were procured from local bamboo suppliers, ensuring the availability of fresh and authentic samples. Additionally, live bamboo plants could conveniently be purchased online, facilitating easy access to the necessary plant material required for experimental purposes.

3.2.2 Chemicals

Chemicals that are used in this project including Folin-Ciocalteu reagent, ferric chloride solution, sodium carbonate solution, aluminium chloride solution, 2,2-Diphenyl-1-picrylhydrazyl (DPPH powder), 70% ethanol, 60% ethanol, and 50% ethanol from laboratory.

3.3 Methods

3.3.1 Overall Flowchart procedure of this study

The research flowchart involved the preparation and procedures of the bamboo peels. The experiment for this procedure was shown in Figure 3.1.

Figure 3.1: Flowchart of the procedure in this study.

3.3.2 Sample Preparation of Bamboo extracts

The bamboo raw was collected at University Malaysia Kelantan. The parts of bamboo that used were peels. The peels of bamboo were washed thoroughly under running tap water for several times. Then, it was cut into small pieces 1.5-2cm. Next, was drying process, the peels of bamboo were dried at 60 ℃ for 4 hours. Oven was used in this drying process. All of the sample was grinded into fine powder by using blender. The powder was preserved in a clean zip-lock plastic bag, kept away from light, heat, and moisture. All the sample extracts were labelled according to Table 3.1.

Table 3.1: The sample ID description of all sample extracts.

Sample ID	Description
BE-70A	Bamboo peels- Ethanol 70% and Distilled water 30%
BE-70B	Bamboo peels- Ethanol 70% and Distilled water 30%
BE-60A	Bamboo peels- Ethanol 60% and Distilled water 40%
BE-60B	Bamboo peels- Ethanol 60% and Distilled water 40%
BE-50A	Bamboo peels- Ethanol 50% and Distilled water 50%
BE-50B	Bamboo peels- Ethanol 50% and Distilled water 50%

Note: (BE): Bamboo Ethanol, (A): 2 hours, and (B): 4 hours

3.3.3 Preparation of Bamboo peels extracts using Soxhlet extraction method

Soxhlet extraction was used to separate the phenolic components from bamboo peels. Different concentrations of solvents, namely 70%, 60%, and 50% ethanol, were used for extraction. Firstly, 250 mL of ethanol were measured and put into separate boiling flasks. Next, 15 grams of each peel sample were subjected to extraction with 250 mL of ethanol, employing different extraction times (2 hours and 4 hours), respectively. This extraction process was carried out using the Soxhlet extraction method (Spigno et al., 2007). During the extraction process, the water source was drawn from the bottom condenser and discharged at the top of the condenser. The procedure continued in the boiling flask, where the extraction chamber extracted the active component from the sample after heating the ethanol to the temperature at which vapor starts to boil.

3.3.4 Evaporator using rotary evaporator

Evaporation served as the final step in the extraction process, commonly referred to as separation since it involved separating the extract yield from the ethanol solvent. The separation was successfully accomplished using a rotary evaporator, which proved to be the most practical method for this purpose (Zhang et al., 2018).

The water bath of the evaporator was heated to the boiling point of the ethanol to initiate the separation procedure. Subsequently, the mixture comprising the extract yield and ethanol was placed in a rotary flask and connected to the rotary evaporator, facilitating the solvent

separation technique. Evaporation occurred gradually, leading to the collection of pure ethanol in the collecting flask while the concentrated extract remained in the spinner flask.

3.3.5 Phytochemical screening analysis

Phytochemical screening of bamboo peels extracts with different concentrations of ethanol (70%, 60% and 50%) was performed to identify the presence of phenolic compounds. Test for the present of flavonoids and tannins was done using (Anil Singh et al., 2012) method with some modifications.

a) Determination of flavonoid

   1 mL of extract was mixed with 10 mL distilled water in a test tube. The mixture was heated in a water bath. Add 1 mL of 5% aluminium chloride solution to the test tube. The deep yellow or orange colour development indicate the presence of flavonoid (Godlewska et al., 2023).

b) Determination of tannins

   1 mL of extract was mixed with 10 mL distilled water in a test tube. The mixture was heated in a water bath. A few drops of 1 % ferric chloride solution was added and blue-black or greenish-black colour development indicate the presence of tannins (Auwal et al., 2014).

3.3.6 Determination of total phenolic content using UV-Vis spectrophotometer

According to the (Waterhouse, 2002), the Folin-Ciocalteu reagent was used to estimate the total amount of phenolic compounds. 5 mL of the Folin-Ciocalteu reagent (diluted 1:10, v/v) and 4 mL of sodium carbonate (75g/L) were added together with 1 mL of the sample. Before determining the tubes' absorbance of the produced colour at 765 nm, the tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C. The sample's ability to reduce Folin-Ciocalteu reagent was measured in mg per mL of extract.

3.3.7 Determination of total tannins content using UV-Vis spectrophotometer

0.1 mL of an sample extract containing 100 µg, dissolved in 0.1 mL of Folin-Ciocalteau reagent, was thoroughly mixed. Subsequently, 2 mL of a 15% (w/v) sodium carbonate solution was added and mixed into the solution. The mixture was then allowed to stand in the dark at room temperature for 30 minutes for the determination of total tannin content (TTC). Following this incubation period, the absorbance of each tannin was measured using a UV-Vis spectrophotometer at wavelengths of 760nm.

3.3.8 Antioxidant activity via DPPH radical-scavenging assay

According to (Clarke et al., 2013), using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent with slight modification where the standard is prepare with different concentrations range from 100 µg/mL to 1000 µg/mL of different sample extract. 0.003g of DPPH were diluted with 1 mL of sample and incubated for 30 min in dark room. 1 mL of sample extracts were prepare and mixed with DPPH solvent. By using UV-Vis spectrophotometer, the absorbance reading was measured at 517 nm against the blank. The percentage of free radical scavenging activity was calculated using following formula:

% Free Radical Scavenging Activity = [(Absorbance Control – Absorbance Sample) ÷ Absorbance Control × 100]

The reaction mixtures with lower absorbance demonstrate elevated free radical scavenging activity. EC_{50} values were determined by plotting the scavenging activity against various concentrations of the sample extract to construct the antiradical curve. These values represent the total antioxidant concentration necessary to achieve a 50% reduction in the initial DPPH radical concentration (Zheng Chen et al., 2013).

3.3.9 Fourier-Transform Infrared Spectroscopy (FTIR) analysis

The active components present in bamboo peels extract were qualitatively analysed using Fourier-Transform Infrared Spectroscopy (FTIR). The FTIR spectra were generated using a Thermo Fisher Scientific instrument with a wavelength range spanning 4000 to 400 cm^{-1}. An OMNI sample attenuated total reflection (ATR) smart accessory, equipped with a diamond crystal operating at an incidence angle of 45 degrees, was utilized in the FTIR setup.

To perform the analysis, a small amount of solvent containing the bamboo peel extracts in liquid form was deposited onto the FTIR spectrometer. This instrument emitted infrared beams directed at the sample, measuring the absorption of infrared light at various frequencies. Subsequently, the sample's composition was determined by comparing its spectral characteristics with the reference database containing numerous spectra.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter discusses the outcomes of the phytochemical screening, total flavonoid compound and tannin using UV-Vis spectroscopy analysis, DPPH analysis and FTIR analysis during the extraction of active components from bamboo peels.

4.1 Phytochemical Screening Analysis of Bamboo peels

The bamboo peels sample undergo extraction using solvent mixtures containing varying concentrations of ethanol and water. Ethanol, is capable to dissolve both polar and non-polar substances due to its hydroxyl and ethyl groups. Notably less toxic, ethanol finds suitability in various consumer products like cosmetics and pharmaceutical preparations. Conversely, water, while being a proficient solvent for extracting polar substances, has limitations in dissolving non-polar compounds (Sasidharan et al., 2011). The study also encompasses varying extraction durations, specifically 2 and 4 hours, aiming to discern the impact of time on the yield and composition of compounds extracted.

Table 4.1: The observation flavonoids of BE-50A extract.

Analysis	2 hours	
	Before	After
Flavonoids BE-50A		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

Table 4.2: The observation flavonoids of BE-60A extract.

Analysis	2 hours	
	Before	After
Flavonoids BE-60A		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

Table 4.3: The observation flavonoids of BE-70A extract.

Analysis	2 hours	
	Before	After
Flavonoids BE-70A		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

Table 4.4: The observation flavonoids of BE-50B extract.

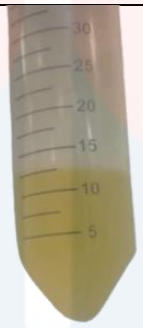
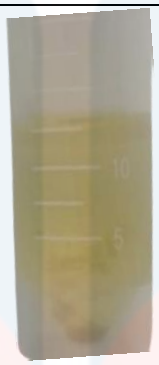
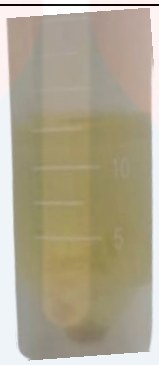
Analysis	4 hours	
	Before	After
Flavonoids BE-50B		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

Table 4.5: The observation flavonoids of BE-60B extract.

Analysis	4 hours	
	Before	After
Flavonoids BE-60B		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

Table 4.6: The observation flavonoids of BE-70B extract.

Analysis	4 hours	
	Before	After
Flavonoids BE-70B		 Deep yellow present
		 Deep yellow present
		 Deep yellow present

The phytochemical screening conducted on bamboo peels, as illustrated in Table 4.1, Table 4.2, Table 4.3, Table 4.4, Table 4.5, and Table 4.6, revealed encouraging results pertaining to the presence of flavonoids across various concentrations and extraction durations using a solvent amalgam of ethanol and water. Notably, during the 2 hour extraction process, the solvent concentrations of BE-50A, BE-60A, and BE-70A exhibited a distinct deep yellow coloration, indicating the initial extraction of flavonoids. Next, upon extending the extraction

duration to 4 hours using similar solvent concentrations (BE-50B, BE-60B, and BE-70B), a discernible escalation in the intensity of deep yellow coloration was observed (Tzanova et al., 2020). This augmentation in colour intensity implies a potential surge in the concentration of flavonoids, suggesting a time-associated enhancement due to prolonged interaction between the bamboo peels and the ethanol-water solvent mixture. Such a progressive change in coloration underscores a probable time-dependent extraction process, emphasizing the efficacy of the solvent in extracting and potentially concentrating flavonoids from the bamboo peels (Chaves et al., 2020). These findings not only signify the suitability of the ethanol-water solvent mixture for flavonoid extraction but also hint at the possibility of utilizing these enriched extracts for diverse industrial applications, ranging from pharmaceuticals to functional food development and beyond (Rodríguez De Luna et al., 2020).

Table 4.7: The observation tannins of BE-50A extract.

Analysis	2 hours	
	Before	After
Tannins BE-50A		 Greenish-black precipitate formed
		 Greenish-black precipitate formed
		 Greenish-black precipitate formed

Table 4.8: The observation tannins of BE-60A extract.

Analysis	2 hours	
	Before	After
Tannins BE-60A		 Greenish-black precipitate formed
		 Greenish-black precipitate formed
		 Greenish-black precipitate formed

Table 4.9: The observation tannins of BE-70A extract.

Analysis	2 hours	
	Before	After
Tannins BE-70A		 Greenish-black present
		 Greenish-black present
		 Greenish-black present

Table 4.10: The observation tannins of BE-50B extract.

Analysis	4 hours	
	Before	After
Tannins BE-50B		 Greenish-black present
		 Greenish-black present
		 Greenish-black present

Table 4.11: The observation tannins of BE-60B extract.

Analysis	4 hours	
	Before	After
Tannins BE-60B		 Greenish-black present
		 Greenish-black present
		 Greenish-black present

Table 4.12: The observation tannins of BE-70B extract.

Analysis	4 hours	
	Before	After
Tannins BE-70B		 Greenish-black present
		 Greenish-black present
		 Greenish-black present

Based on the findings presented in Table 4.7, Table 4.8, Table 4.9, Table 4.10, Table 4.11, and Table 4.12, it is evident that all extracts derived from bamboo peels exhibited positive outcomes for tannin concentration across different time intervals. The observations of tannins in BE-50A, BE-50B, BE-60A, BE-60B, BE-70A, and BE-70B extracts demonstrated the presence and colour variations of tannins throughout each 2 hour interval. Initially, at the 2 hour mark, BE-50A and BE-60A extracts displayed green-black tannins, while the other samples exhibited tannins with a similar green-black hue. The divergence in coloration might indicate potential variations in the concentrations or forms of tannins present in these specific

extracts (Martins et al., 2021). However, after the extended 4 hour duration, extracts BE-50B, BE-60B, BE-70A, and BE-70B all exhibited robust indications of tannins, characterized by a consistent green-black appearance. This notable shift suggests a substantial escalation in tannin concentration within these extracts over time, signifying a progressive accumulation or enhanced extraction of tannins from the ethanol-water solvent mixture within the bamboo peels. The consistent detection of tannins and the discernible increase in concentration over the extraction period underscore the effectiveness of the ethanol-water solvent blend in extracting tannins from bamboo peels (Cuong et al., 2019). The observable colour changes within extracts at different time intervals suggest potential variations in tannin compositions or concentrations, which could be further investigated for comprehensive insights into the tannin profile within these extracts (Bindon et al., 2014).

Table 4.13: Phytochemical analysis result of bamboo peels.

Phytochemical	Sample Extract	2 hours	Sample Extract	4 hours
Flavonoids	BE-50A	+	BE-50B	++
Flavonoids	BE-60A	+	BE-60B	++
Flavonoids	BE-70A	+	BE-70B	++
Tannins	BE-50A	+	BE-50B	++
Tannins	BE-60A	+	BE-60B	++
Tannins	BE-70A	+	BE-70B	++

Note: (-): Absence of turbidity/ flocculation/ precipitation, (+): Present, (++): Highly present, (BE): Bamboo Ethanol, (A): 2 hours, (B): 4 hours

Referring to Table 4.13 for different sample extracts (BE-50A, BE-50B, BE-60A, BE-60B, BE-70A, BE-70B) at two different time intervals (2 hours and 4 hours). Studies conducted on flavonoids and tannins have revealed interesting patterns and concentration levels in their presence. Using the symbol (+) to indicate presence and the symbol (++) to indicate higher concentrations, the findings highlight the consistent detection of these phytochemicals across all extracts and time frames.

The flavonoids test conducted on bamboo peel extracts at two different time intervals, specifically at 2 hours and 4 hours, consistently revealed positive findings across all samples.

Flavonoids, a class of phytochemicals widely found in various plant species, were notably present in the extracts during both time frames. This presence indicates either the initial extraction or the inherent existence of flavonoids within the bamboo peel samples. Flavonoids are acknowledged for their diverse biological activities and potential health benefits, encompassing antioxidant, anti-inflammatory, and anti-cancer properties. In pharmaceutical applications, flavonoids are deemed essential due to their perceived therapeutic potential (Panche et al., 2016). Furthermore, they are extensively utilized in the food industry, contributing to the colour, flavour, and potential health-enhancing aspects of various food products. The consistent detection of flavonoids within the bamboo peel extracts at both time intervals suggests the potential for these extracts to serve as valuable sources of flavonoids, holding promising implications for pharmaceutical, nutraceutical, and food-related industries (Ullah et al., 2020). Delving into the precise quantification and identification of flavonoid subtypes within these extracts could offer deeper insights into their specific functionalities and potential applications.

The phytochemical screening of bamboo peels at two different time intervals (2 hours and 4 hours) revealed positive results for tannins. Tannins are significant active compounds commonly found in plant-based medicines (Fraga-Corral et al., 2021). The greenish-black colour and precipitate formed during the tests indicates the presence of tannins within the bamboo peels' extract. Tannins represent a group of polyphenolic compounds known for their antioxidant, antibacterial, and anti-inflammatory properties. In the realm of cosmetic products, tannins are utilized for their antioxidant, antibacterial, and anti-inflammatory attributes, particularly in skincare formulations (Benedec et al., 2013).

4.2 Analysis of total phenolic content using UV-Vis spectrophotometer

4.2.1 Analysis of total phenolic content at 2 hours

Figure 4.1 illustrates the determination of total phenolic content using UV-Vis spectrophotometry, a crucial method for quantifying phenolic compounds within bamboo peel extracts. This analysis specifically targeted extracts obtained from 2 hour extraction process using solvent compositions comprising different concentrations of ethanol mixed with water (50%, 60%, and 70%). Regression analysis resulted in a correlation equation: Absorbance = 2.489 + (0.005) × Concentration (mg/mL), with an R-squared value (R^2) of 0.954. This equation denotes a robust positive linear relationship between phenolic compound

concentration (mg/mL) and the corresponding absorbance at 765nm. The R-squared value of 0.954 signifies that approximately 95.4% of the variability in absorbance can be attributed to changes in phenolic compound concentration within the bamboo peel extracts obtained after the 2 hour extraction process (Stankovic, 2011).

In addition, the comparison of UV-Vis spectrophotometer analysis of total phenolic content at different solvent concentrations (50%, 60%, and 70%) revealed distinctive trends. The absorbance value at 765nm gradually increased with rising solvent concentration, indicating a clear association between solvent concentration and absorbance. The absorbance values for 50% concentration were 2.732 mg/mL, for 60% it was 2.761 mg/mL, and for 70%, it was 2.826 mg/mL, demonstrating an upward trend with increasing solvent concentration. This pattern illustrates a direct correlation between solvent concentration and phenolic compound concentration (mg/mL) in the extract (Muflihah et al., 2021). Higher solvent concentrations yielded greater absorbance, indicating improved extraction efficiency (Shi et al., 2022). This suggests that a larger amount of phenolic compounds was extracted from bamboo peels as solvent concentration increased. These findings underscore the substantial impact of solvent composition on the extraction and subsequent measurement of phenolic content, highlighting the pivotal role of solvent concentration in enhancing the solubility and extraction efficiency of phenolic compounds from bamboo peels.

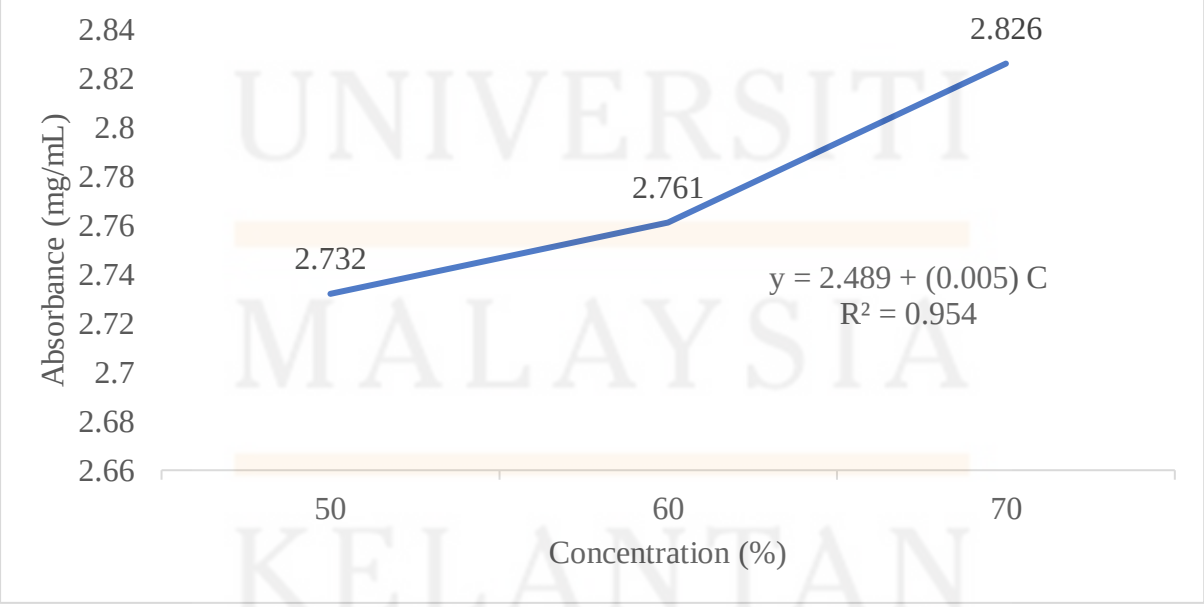


Figure 4.1: The different concentrations of bamboo peels extract for total phenolic content in 2 hours.

4.2.2 Analysis of total phenolic content at 4 hours

Based on Figure 4.2, the determination of total phenolic content in bamboo peel extracts obtained after a 4 hour extraction process, utilizing UV-Vis spectrophotometer analysis with varying solvent compositions (50%, 60%, and 70% ethanol mixed with water). The recorded absorbance readings at 765nm were 1.904 mg/mL, 2.366 mg/mL, and 2.846 mg/mL for solvent concentrations of 50%, 60%, and 70%, respectively. These results consistently indicate a direct relationship: as the solvent concentration increases, so does the absorbance, suggesting a proportional rise in phenolic compound concentrations within the extracts. Moreover, employing regression analysis to model the relationship between concentration and absorbance revealed a perfect linear fit with an R-squared value (R^2) of 1.000. The derived regression equation, Absorbance = -0.455 + (0.047) × Concentration (mg/mL), signifies a precise correlation between the concentration of phenolic compounds and the resulting absorbance values at 765nm (Stankovic, 2011).

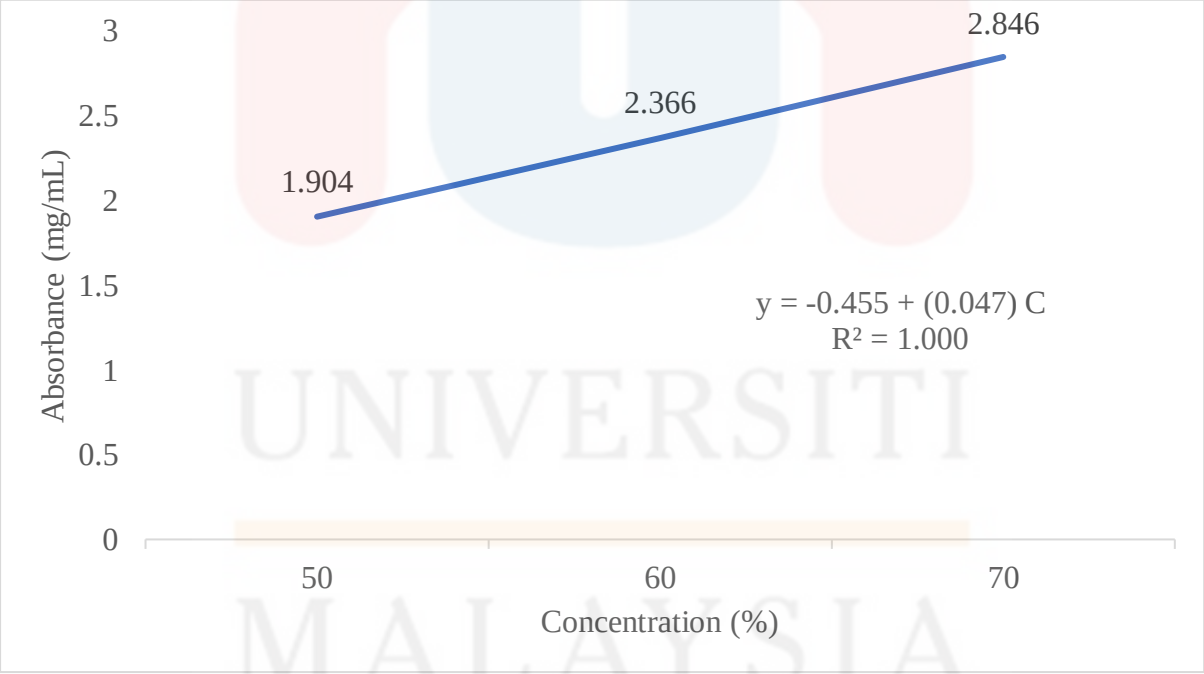


Figure 4.2: The different concentrations of bamboo peels extract for total phenolic content in 4 hours.

The comparison among solvent concentrations emphasizes their significant influence, where higher ethanol-water concentrations corresponded to increased absorbance values. This

observation strongly suggests enhanced extraction efficiency and higher concentrations of phenolic compounds (mg/mL) within bamboo peel extracts with rising solvent concentrations during the 4 hour extraction process (Shi et al., 2022). These findings underscore the crucial role of solvent composition in affecting the extraction efficiency of phenolic compounds and highlight the reliability of UV-Vis spectrophotometer analysis in quantifying these compounds within bamboo peel extracts.

4.3 Analysis of total tannins content using UV-Vis spectrophotometer

4.3.1 Analysis of total tannins content at 2 hours

Figure 4.3 presents the assessment of total tannins content in bamboo peel extracts derived from a 2 hour extraction process, utilizing UV-Vis spectrophotometer analysis with varying solvent compositions (50%, 60%, and 70% ethanol mixed with water). The recorded absorbance readings at 765nm were as follows: 1.716 mg/mL for 50% concentration, 2.034 mg/mL for 60%, and 2.112 mg/mL for 70%. These values exhibit a consistent trend, indicating an incremental absorbance corresponding to higher solvent concentrations, suggesting a proportional increase in tannin concentrations within the extracts. Furthermore, employing regression analysis to establish the quantitative relationship between concentration and absorbance produced a regression equation, Absorbance = 0.764 + (0.020) × Concentration (mg/mL), demonstrating a strong linear correlation with an R-squared value (R^2) of 0.892. This equation highlights a robust association between tannin concentration (mg/mL) and resulting absorbance values at 760nm, signifying that approximately 89.2% of the variance in absorbance can be attributed to changes in tannin concentration (Nuzul et al., 2022).

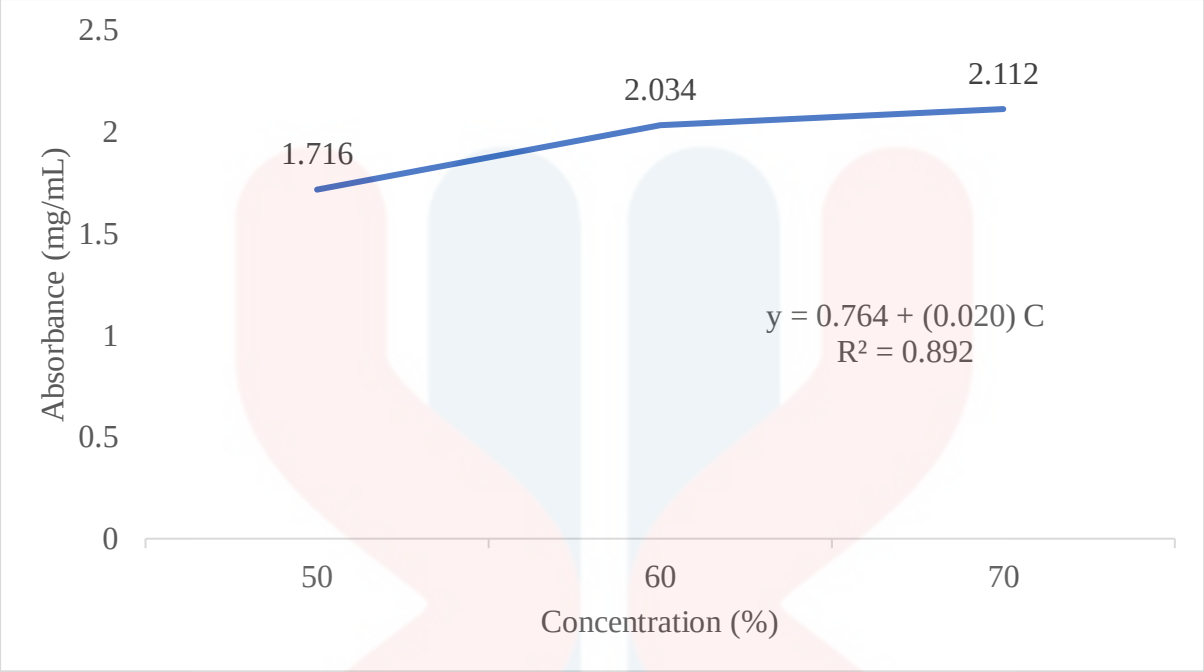


Figure 4.3: The different concentrations of bamboo peels extract for total tannins content in 2 hours.

Additionally, the comparison of UV-Vis spectrophotometer analysis of total tannins content across different solvent concentrations (50%, 60%, and 70%) revealed distinct trends. The absorbance values at 760nm exhibited a gradual increase with ascending solvent concentration, indicating a direct relationship between solvent concentration and absorbance. Specifically, the absorbance values for 50% concentration were 1.716 mg/mL, 2.034 mg/mL for 60%, and 2.112 mg/mL for 70%, demonstrating an upward trajectory with increasing solvent concentration (mg/mL). This pattern underscores a direct correlation between solvent concentration and tannins compound concentration (mg/mL) within the extract. Higher solvent concentrations (mg/mL) corresponded to heightened absorbance, suggesting enhanced extraction efficiency and increased extraction of phenolic compounds from bamboo peels (Shi et al., 2022). These findings underscore the substantial effect of solvent composition on the extraction and subsequent measurement of phenolic content, emphasizing the pivotal role of solvent concentration in augmenting the solubility and efficiency of phenolic compounds extracted from bamboo peels.

4.3.2 Analysis of total tannins content at 4 hours

Based on the Figure 4.4, the total tannins content in bamboo peel extracts, obtained from a 4 hour extraction process using varying solvent compositions of ethanol mixed with water (50%, 60%, and 70%), was determined through UV-Vis spectrophotometer. Absorbance readings were measured at 760nm, resulting in values of 1.574 mg/mL for 50% solvent concentration, 2.081 mg/mL for 60%, and 2.147 mg/mL for 70%. These measurements consistently increased with higher solvent concentrations, indicating a proportional elevation in tannin concentration within the extracts. Regression analysis established a quantitative relationship between tannin concentration and absorbance at 760nm, yielding the equation Absorbance = 0.217 + (0.029) × Concentration (mg/mL), with an R-squared value (R^2) of 0.835. This equation denotes a reasonably robust linear correlation between tannin concentration (mg/mL) and absorbance values, explaining approximately 83.5% of the absorbance variability (Nuzul et al., 2022).

Comparison across different solvent concentrations emphasized their substantial influence on absorbance at 760nm, directly impacting tannins concentration within bamboo peel extracts during the 4 hour extraction. This underscores the pivotal role of solvent composition in influencing tannin extraction efficiency from bamboo peels. The UV-Vis spectrophotometer's reliability in quantifying tannins is underscored by the observed correlation between tannin concentration (mg/mL) and absorbance, validating its efficacy in estimating total tannins content in natural extracts.

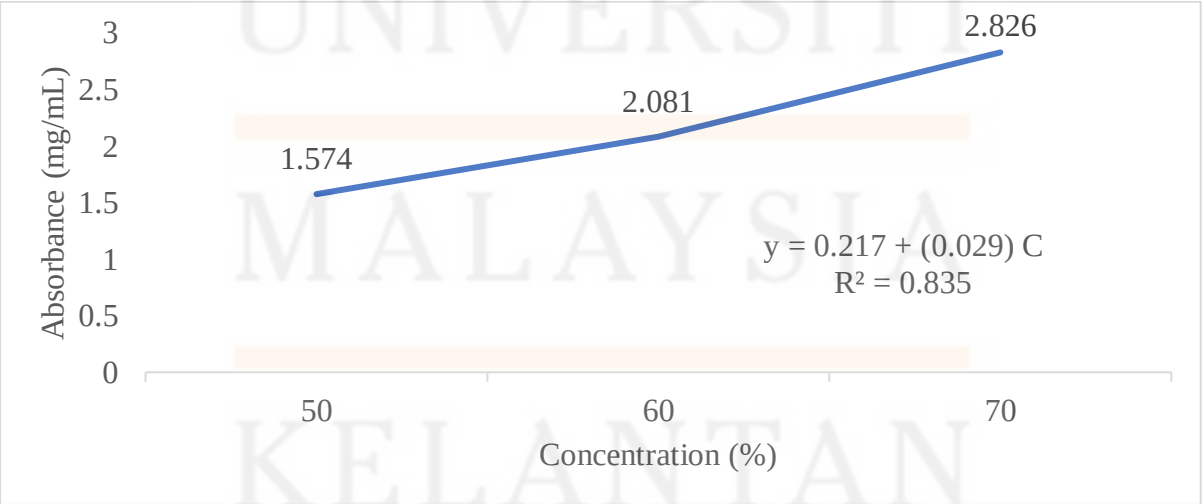


Figure 4.4: The different concentrations of bamboo peels extract for total tannins content in 4 hours.

4.4 Effect of different concentrations of solvents and time on the extraction of total phenolic content using UV-Vis spectrophotometer

The quantification of total phenolic content within bamboo peels extracts using UV-Vis spectrophotometry with varying solvent concentrations (BE-50, BE-60, BE-70) and different extraction durations (2 hours and 4 hours). Total phenolic content is a key indicator of antioxidant potential in natural extracts. Results showed varying phenolic content concerning solvent concentration and extraction duration, as shown in Table 4.14 and Figure 4.5.

Table 4.14: Total phenolic content in bamboo peels extracts.

Solvents	Concentration yield (%)	
	2 hours (A)	4 hours (B)
BE-50	18.42	61.72
BE-60	37.97	57.55
BE-70	55.73	93.98

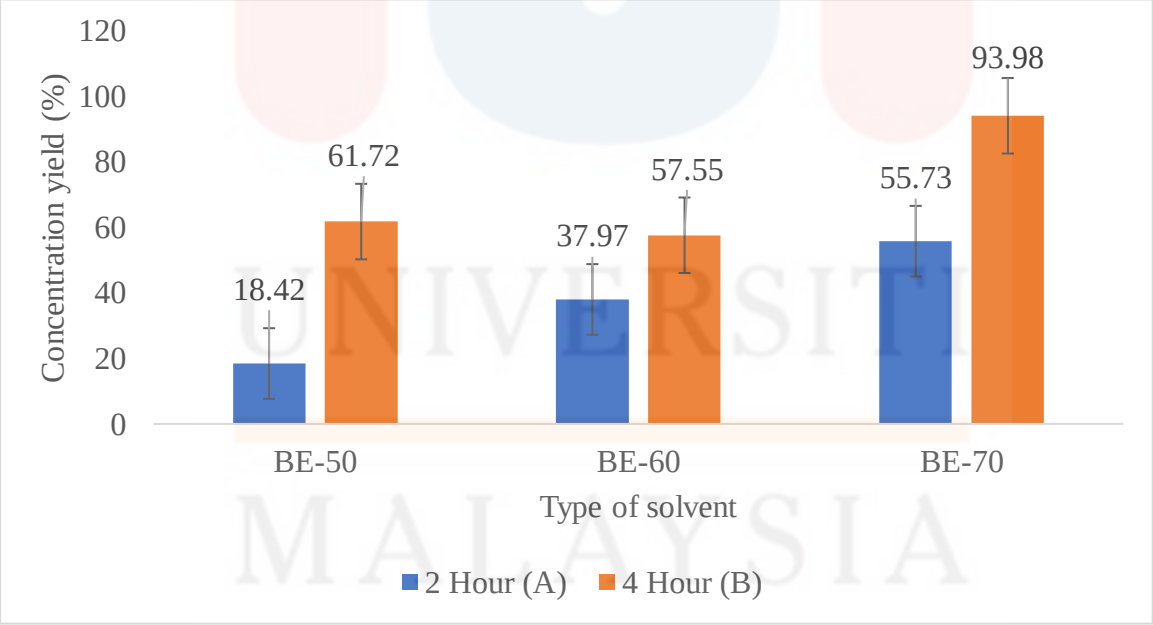


Figure 4.5: The effect of different concentrations of solvents and time on the extraction of total phenolic content from bamboo peels.

During the initial 2 hour extraction, a proportional rise in phenolic content was seen with an increase in solvent concentration. At BE-50A, BE-60A, and BE-70A solvent concentrations, phenolic content increased to 18.42%, 37.97%, and 55.73%, respectively. The phenomenon aligns with known extraction principles where higher solvent concentrations facilitate improved extraction efficiency, resulting in higher phenolic content (Boeing et al., 2014). Moreover, prolonged extraction durations allow more time for cell disruption, releasing a higher quantity of phenolic compounds into the solvent (Benedec et al., 2013).

However, unexpected observations occurred during the 4 hour extraction phase, especially at BE-60B solvent concentration. Contrary to the anticipated rise, the highest phenolic content of 61.72% was recorded at BE-50B solvent concentration, surpassing the 57.55% obtained at BE-60B. The unexpected decrease in phenolic content compared to the rise in the 2 hour extract may originate from the instability of some phenolic compounds, possibly leading to their degradation during prolonged extraction. Alternatively, complex interactions between extracted compounds might alter their solubility or structural integrity. Notably, the 4 hour extraction at BE-70B solvent concentration demonstrated a return to the expected trend in phenolic content (93.98%). This observed regression pattern suggests that despite fluctuations at BE-60B solvent concentration, the BE-70B solvent concentration reinstated the anticipated higher phenolic content with longer extraction times. This phenomenon implies that the higher solvent concentration (BE-70B) might enhance efficient extraction of phenolic compounds over extended periods (Zhong et al., 2019). For instance, increased solvent solubility and improved cell disruption are factors that might contribute to higher phenolic yields. This aligns with existing literature indicating that higher solvent concentrations enhance extraction efficiency, particularly over prolonged periods, resulting in increased phenolic content (Venkatesan et al., 2019).

4.5 Effect of different concentrations of solvents and time on the extraction of total tannins content using UV-Vis spectrophotometer

The quantification of total tannins content within bamboo peels extracts using UV-Vis spectrophotometry Tannins, recognized for their potent antioxidative properties, are prominent among the phenolic compounds found in natural extracts (Venkatesan et al., 2019), as shown in Table 4.15 and Figure 4.6.

Table 4.15: Total tannins content in bamboo peels extracts.

Solvents	Concentration yield (%)	
	2 hours (A)	4 hours (B)
BE-50	24.77	75.28
BE-60	48.75	67.20
BE-70	70.95	94.87

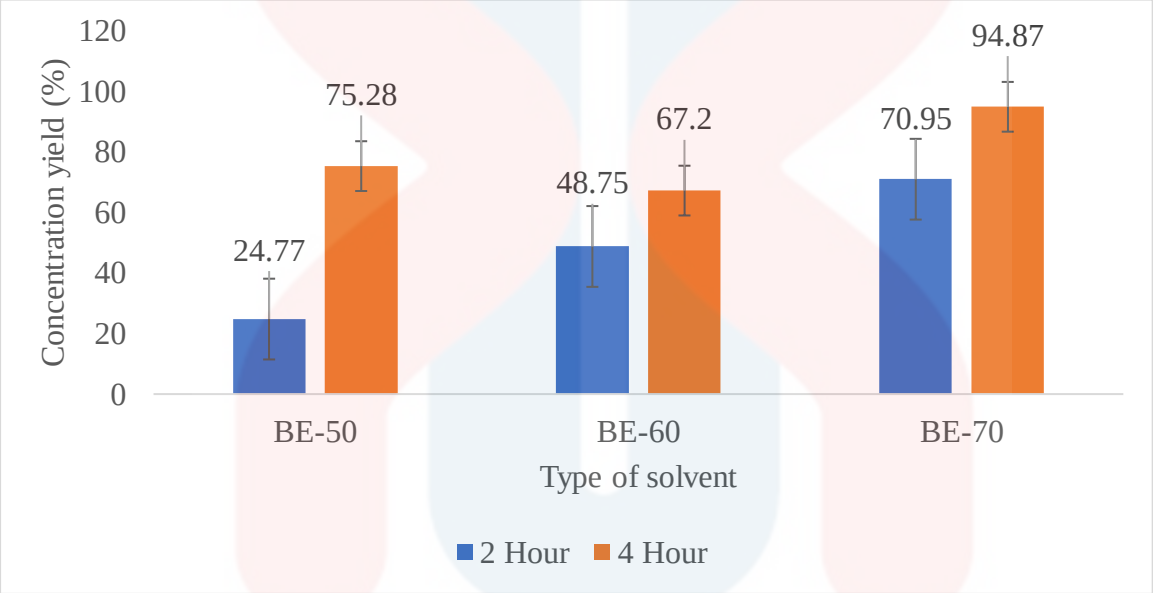


Figure 4.6: The effect of different concentrations of solvents and time on the extraction of total tannins content from bamboo peels.

In the initial 2 hour extraction displayed an ascending trend in tannins content with increasing solvent concentration. At BE-50A, BE-60A, and BE-70A solvent concentrations, tannins content increased to 24.77%, 48.75%, and 70.95%, respectively. Tannins, known for their antioxidative properties, are among the key phenolic compounds extracted, and higher solvent concentrations generally aid in enhanced tannin extraction (Venkatesan et al., 2019).

However, during the 4 hour extraction phase, at BE-60B solvent concentration, unexpected observations were made. Contrary to the expected increase, the highest phenolic content recorded at BE-50B solvent concentration was 75.28%, exceeding the 67.2% obtained at 94.87%. The decrease in tannin content compared to the increase in the 2 hour extracted counterpart may be attributed to the instability of some tannins or complex reactions between

extracted compounds that alter solubility or structural integrity. Although the BE-60B trend was unexpected, the BE-70B solvent concentration showed a large increase in tannin content to 94.87% during the 4 hour extraction process. This recovery showed that, despite an unexpected change at BE-60B solvent concentration, tannin content resumed its increasing trend at BE-70B solvent concentration.

4.6 Analysis of Free Radical Scavenging Activity using DPPH Assay

4.6.1 Analysis of DPPH of different concentration bamboo peels at 2 hours

The DPPH free radical scavenging activity in bamboo peel extracts with different concentrations and solvent compositions was analysed within 2 hours to understand its antioxidant potential. DPPH free radicals are dark purple and have maximum absorption at 517nm (Baliyan et al., 2022). The observed results indicate a consistent relationship between concentration and efficacy in scavenging DPPH radicals. When the concentration of bamboo peels extract raised from 100 mg/mL to 1000 mg/mL, the ability of the extract to neutralize free radicals was significantly enhanced. This trend was evident in different solvent compositions (50%, 60%, and 70% ethanol), indicating that the higher the concentration of the extract, the greater the antioxidant capacity. The EC_{50} which is the concentration required for 50% inhibition of DPPH free radicals can be calculated from the graph's equation.

Figure 4.7 illustrated the relationship between the concentration of bamboo peels and DPPH scavenging for BE-50A ethanol solvent. Subsequently, Figure 4.8 showed the relationship between the concentration of bamboo peels and DPPH scavenging for BE-60A ethanol solvent. Lastly, Figure 4.9 displayed the relationship between the concentration of bamboo peels and DPPH scavenging for BE-70A ethanol solvent. A lower absorbance denoted a higher DPPH scavenging activity (Kumara et al., 2018). The antioxidant analysis of bamboo peel extracts with varying concentrations and solvents (BE-50A, BE-60A, and BE-70A) was quantified by the EC_{50} value (mg/mL). The EC_{50} value, calculated from the best-fitted line to the data, served as a parameter to express the antioxidant analysis results (Chen et al., 2013).

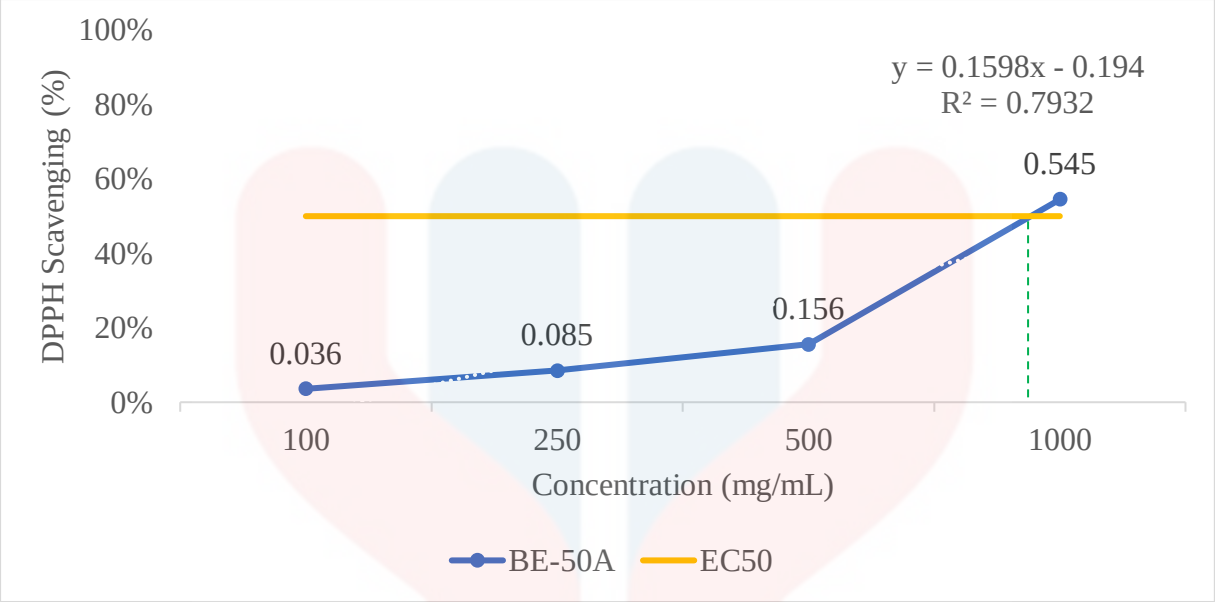


Figure 4.7: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-50A solvents.

In Figure 4.7, the analysis of DPPH scavenging activity using BE-50A ethanol solvent extracts revealed an EC_{50} value of 0.5, emphasizing their efficacy in neutralizing free radicals. The DPPH clearance values at varying concentrations (100, 250, 500, and 1000 mg/mL) within the BE-50A ethanol solvent extract demonstrated a concentration-dependent enhancement of antioxidant activity, showcasing values of 0.036, 0.085, 0.156, and 0.545, respectively. It showed that the antioxidant activity was enhanced in a concentration-dependent manner (Baliyan et al., 2022).

At the highest concentration of 1000 mg/mL, the BE-50A ethanol solvent extract exceeds the EC_{50} line, indicating a robust antioxidant effect. This suggests that the BE-50A ethanol solvent extract effectively neutralizes free radicals even at the highest concentration tested, showcasing its potent antioxidant capabilities. The linear relationship is expressed by the equation y = 0.1598x - 0.194, with an R^2 value of 0.7932, offering a quantitative insight into the concentration-dependent nature of the antioxidant activity. Particularly noteworthy is the calculated concentration (x) corresponding to the EC_{50}, approximately 4.346 mg/mL. This means that a concentration of approximately 4.346 mg/mL of ethanolic solvent extract of BE-50A is required to effectively reduce DPPH radicals by 50%.

Moreover, the intriguing EC_{50} intersection point was achieved before reaching a concentration of 1000 mg/mL; precisely, the EC_{50} intersection point manifested at

approximately 960 mg/mL concentration. The EC_{50} intersection point denotes the concentration at which the extract's antioxidant efficacy is on par with its ability to neutralize free radicals. This nuanced observation added depth to our understanding, suggesting that beyond a certain concentration, the extract's antioxidant activity became more pronounced, exemplifying its potential in managing oxidative stress (Apak et al., 2016).

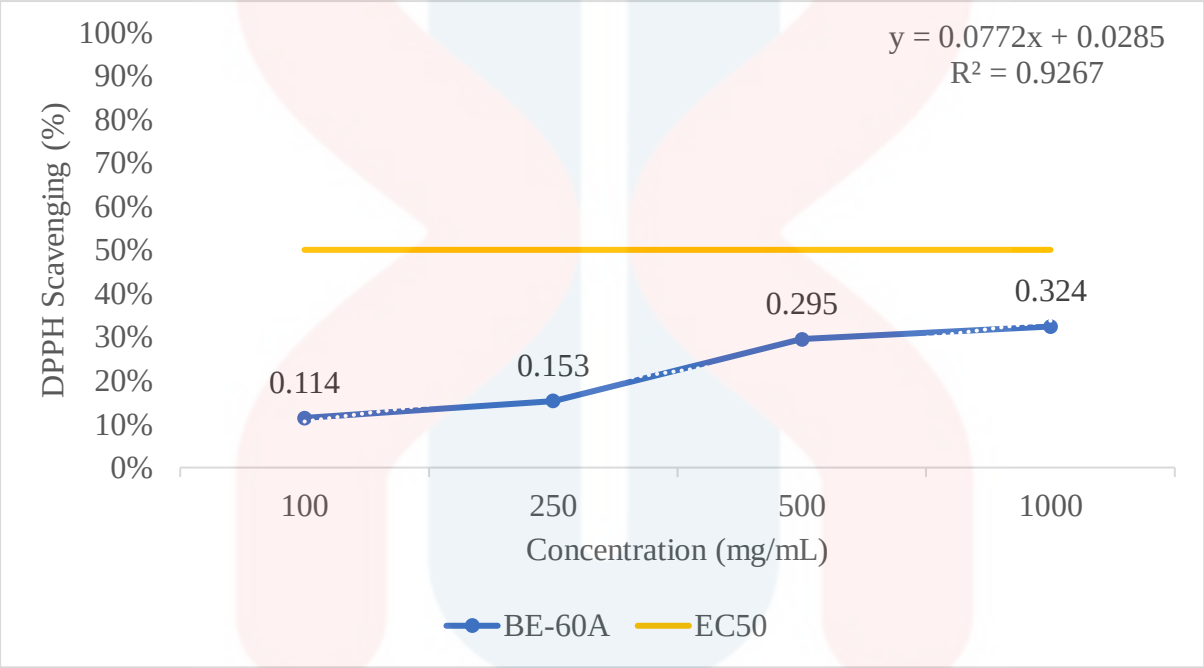


Figure 4.8: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-60A solvents.

In Figure 4.8, the analysis of DPPH scavenging activity using BE-60A ethanol solvent extracts yielded an EC_{50} value of 0.5, signifying their capacity to neutralize free radicals. The DPPH clearance values at concentrations (100, 250, 500, and 1000 mg/mL) within the BE-60A ethanol solvent extract indicated a concentration-dependent enhancement of antioxidant activity, with values of (0.114, 0.153, 0.295, and 0.324 mg/mL). This suggests a clear concentration-dependent trend in the enhancement of antioxidant activity.

In contrast to the BE-50A ethanol solvent extract, the BE-60A variant did not surpass the EC_{50} line at the highest concentration (1000 mg/mL), implying a concentration-dependent variation. This highlights a potential requirement for a higher concentration of BE-60A to achieve half-maximal scavenging compared to BE-50A. The linear relationship, expressed by

the equation y = 0.0772x + 0.0285 with an R^2 value of 0.9267, quantitatively illustrates the concentration-dependent nature of antioxidant activity. The calculated concentration (x) corresponding to the EC_{50} is approximately 6.10 mg/mL, suggesting the potential of BE-60A at this concentration to reduce the initial DPPH radical concentration by 50%, showcasing its ability to respond to oxidative stress. Notably, the results suggest that the antioxidant effect of the phenolic compounds in BE-60A may be inactive or may require concentrations exceeding the highest tested levels for optimal effects (Noda et al., 2019).

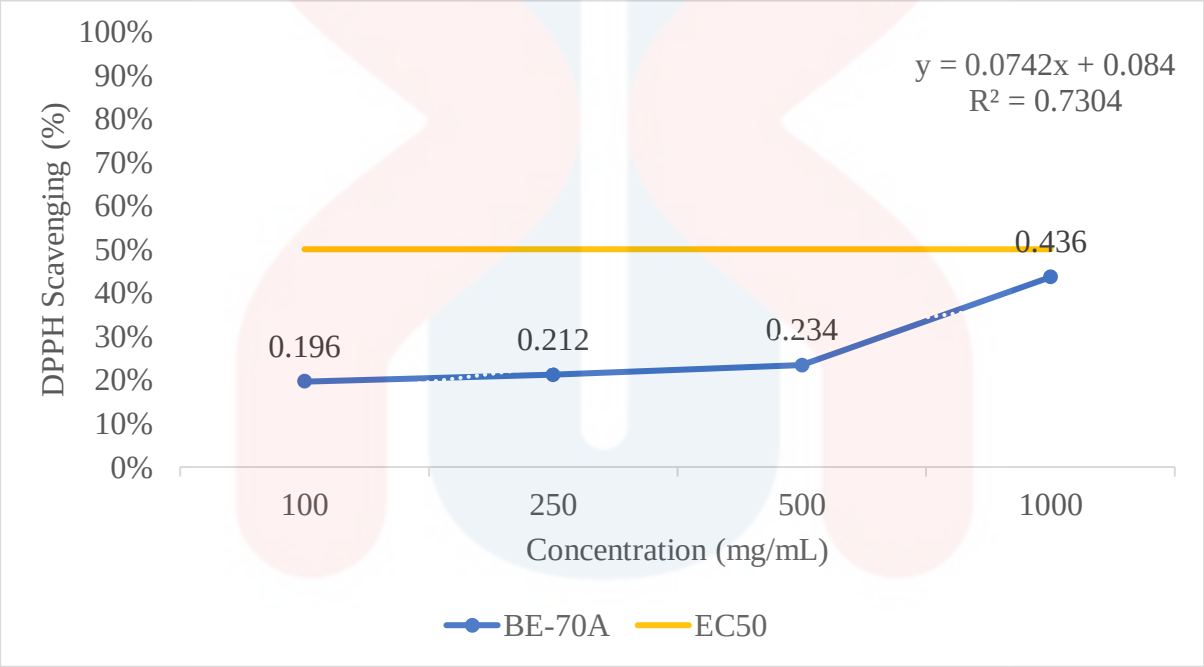


Figure 4.9: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-70A solvents.

In Figure 4.9, the examination of DPPH scavenging activity using BE-70A ethanol solvent extracts revealed an EC_{50} value of 0.5, signifying their potential to counteract free radicals. The DPPH clearance values at various concentrations (100, 250, 500, and 1000 mg/mL) within the BE-70A ethanol solvent extract displayed a concentration-dependent enhancement of antioxidant activity, with values of 0.196, 0.212, 0.234, and 0.436. This upward trend emphasizes the extract's capacity to effectively combat oxidative stress, echoing similar concentration-dependent responses observed in the BE-60A ethanol solvent extract. Notably, the BE-70A ethanol solvent extract did not intersect the EC_{50} line at the highest concentration

of 1000 mg/mL, suggesting a nuanced concentration-dependent response, consistent with the BE-60A ethanol solvent extract. The linear relationship, represented by the equation y = 0.0742x + 0.084 with an R^2 value of 0.7304, provides a quantitative understanding of the concentration-dependent nature of the antioxidant activity.

The calculated concentration (x) corresponding to the EC_{50} is approximately 5.61 mg/mL, indicating the need for a concentration around 5.61 mg/mL of the BE-70A ethanol solvent extract to achieve a 50% reduction in the initial DPPH radical concentration. This underscores the extract's potential to respond effectively to oxidative stress. However, the outcome suggests that the antioxidant effect of the phenolic compounds in the BE-70A ethanol solvent extract may be less pronounced or may require concentrations exceeding the highest tested levels for optimal effects. Further investigation is warranted to explore the concentration-dependent dynamics and potential applications of the BE-70A ethanol solvent extract in managing oxidative stress.

4.6.2 Analysis of DPPH of different concentration bamboo peels at 4 hours

The DPPH free radical scavenging activity in bamboo peel extracts with different concentrations and solvent compositions was analysed within 4 hours to understand its antioxidant potential. DPPH free radicals are dark purple and have maximum absorption at 517nm (Baliyan et al., 2022). The observed results indicate a consistent relationship between concentration and efficacy in scavenging DPPH radicals. As the concentration of bamboo peels extract increased from 100 mg/mL to 1000 mg/mL, the ability of the extract to neutralize free radicals was significantly enhanced. This trend was evident in different solvent compositions (50%, 60%, and 70% ethanol), indicating that the higher the concentration of the extract, the greater the antioxidant capacity. The EC_{50} which is the concentration required for 50% inhibition of DPPH free radicals was calculated from the graph.

Figure 4.10 illustrated the relationship between the concentration of bamboo peels and DPPH scavenging for BE-50B ethanol solvent. Subsequently, Figure 4.11 showed the relationship between the concentration of bamboo peels and DPPH scavenging for BE-60B ethanol solvent. Lastly, Figure 4.12 displayed the relationship between the concentration of bamboo peels and DPPH scavenging for BE-70B ethanol solvent. A lower absorbance indicated a higher DPPH scavenging activity (Kumara et al., 2018). The antioxidant analysis of bamboo peel extracts with varying concentrations and solvents (BE-50B, BE-60B, and BE-

70B) was quantified by the EC_{50} value (mg/mL). The EC_{50} value, calculated from the best-fitted line to the data, served as a parameter to express the antioxidant analysis results (Chen et al., 2013).

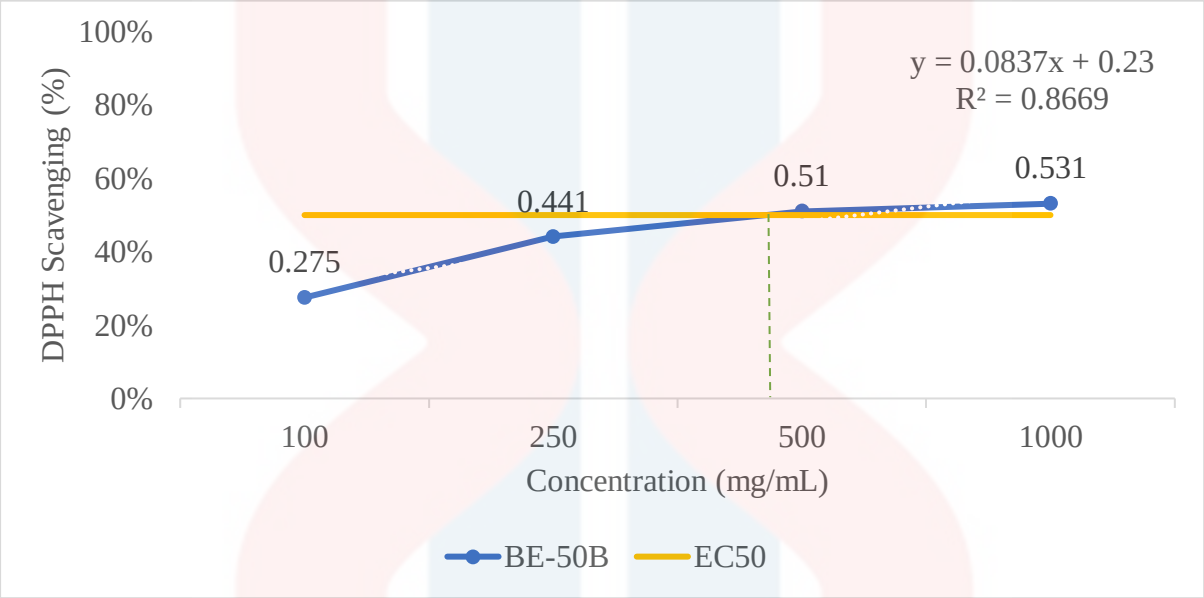


Figure 4.10: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-50B solvents.

In Figure 4.10, the analysis of DPPH scavenging activity using BE-50B ethanol solvent extracts resulted in an EC_{50} value of 0.5, highlighting their effectiveness in neutralizing free radicals. The DPPH clearance values at varying concentrations (100, 250, 500, and 1000 mg/mL) within the BE-50B ethanol solvent extract demonstrated a concentration-dependent enhancement of antioxidant activity, with values of 0.275, 0.441, 0.510, and 0.531 mg/mL, respectively. This ascending trend underscores the extract's ability to effectively combat oxidative stress.

Particularly noteworthy is the observation that at concentrations of 500 and 1000 mg/mL, the BE-50B ethanol solvent extract surpassed the EC_{50} line, indicating a robust antioxidant effect. This suggests that the extract effectively neutralizes free radicals even at higher concentrations, showcasing specific potent antioxidant capabilities. The linear relationship, expressed by the equation $y = 0.0837x + 0.23$ with $R^2 = 0.8669$, provides a quantitative analysis of the concentration-dependent nature of the antioxidant activity.

The calculated concentration (x) corresponding to the EC_{50} is approximately 3.23 mg/mL. This means that a BE-50B ethanol solvent extract at this concentration is required to achieve a 50% reduction in the initial DPPH radical concentration, emphasizing its potential to respond effectively to oxidative stress.

Moreover, the intriguing EC_{50} intersection point was achieved before reaching a concentration of 1000 mg/mL; precisely, the EC_{50} intersection point manifested at approximately 480 mg/mL concentration. This denotes the concentration at which the extract's antioxidant efficacy is on par with its ability to neutralize free radicals, adding depth to our understanding. This nuanced observation suggests that beyond a certain concentration, the extract's antioxidant activity became more pronounced, exemplifying its potential in managing oxidative stress (Apak et al., 2016).

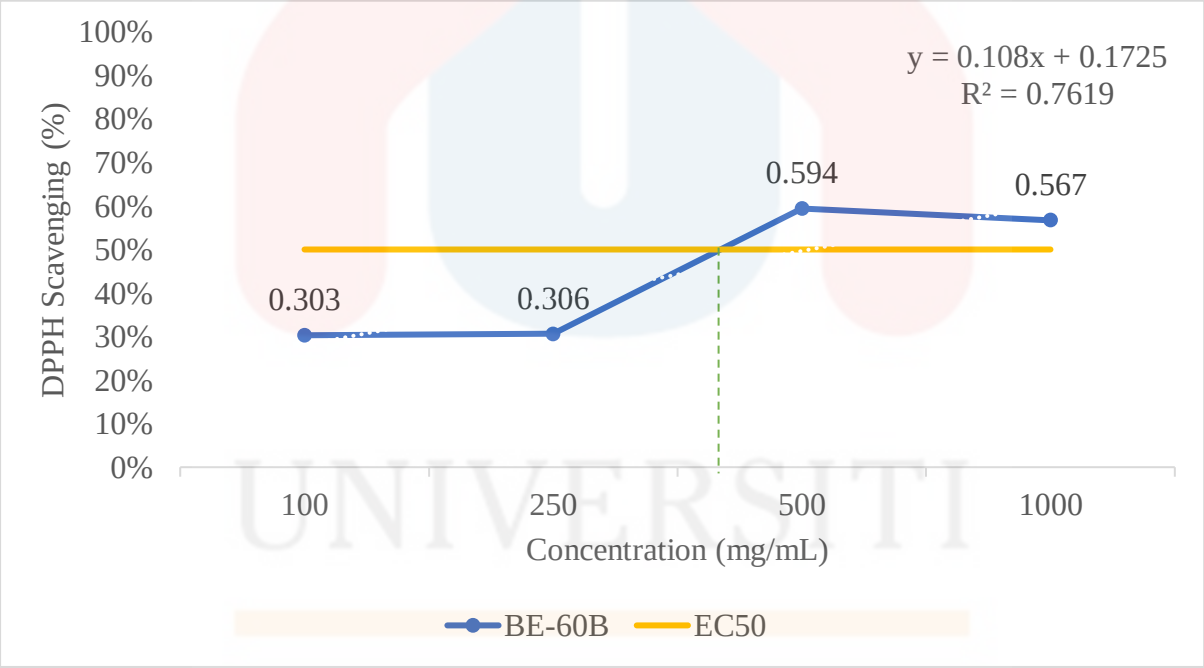


Figure 4.11: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-60B solvents.

In Figure 4.11, the analysis of DPPH scavenging activity using BE-60B ethanol solvent extracts revealed an EC_{50} value of 0.5, signifying their potential to neutralize free radicals. The DPPH clearance values at varying concentrations (100, 250, 500, and 1000 mg/mL) within the BE-60B ethanol solvent extract showcased a concentration-dependent enhancement of

antioxidant activity, with precise values of 0.303, 0.306, 0.594, and 0.567, respectively. This ascending trend underscores the extract's capacity to effectively combat oxidative stress.

At concentrations of 500 and 1000 mg/mL, the BE-60B ethanol solvent extract surpassed the EC_{50} line, indicating a robust antioxidant effect. This suggests that the extract effectively neutralizes free radicals even at higher concentrations, highlighting its potent antioxidant capabilities. The linear relationship, expressed by the equation $y = 0.108x + 0.1725$ with $R^2 = 0.7619$, provides a quantitative analysis of the concentration-dependent nature of the antioxidant activity.

The calculated concentration (x) corresponding to the EC50 is approximately 3.03 mg/mL. This implies that a BE-60B ethanol solvent extract at a concentration of around 3.03 mg/mL is needed to achieve a 50% reduction in the initial DPPH radical concentration, further emphasizing its potential to effectively respond to oxidative stress. These results suggest that the antioxidant effect of the phenolic compounds in the BE-60B ethanol solvent extract is pronounced, and the extract exhibits optimal antioxidant effects at concentrations exceeding the highest tested levels.

Moreover, the intriguing EC_{50} intersection point was achieved before reaching a concentration of 1000 mg/mL; precisely, the EC50 intersection point manifested at approximately 400 mg/mL concentration. This denotes the concentration at which the extract's antioxidant efficacy is on par with its ability to neutralize free radicals, adding depth to our understanding. This nuanced observation suggests that beyond a certain concentration, the extract's antioxidant activity becomes more pronounced, exemplifying its potential in managing oxidative stress (Apak et al., 2016).

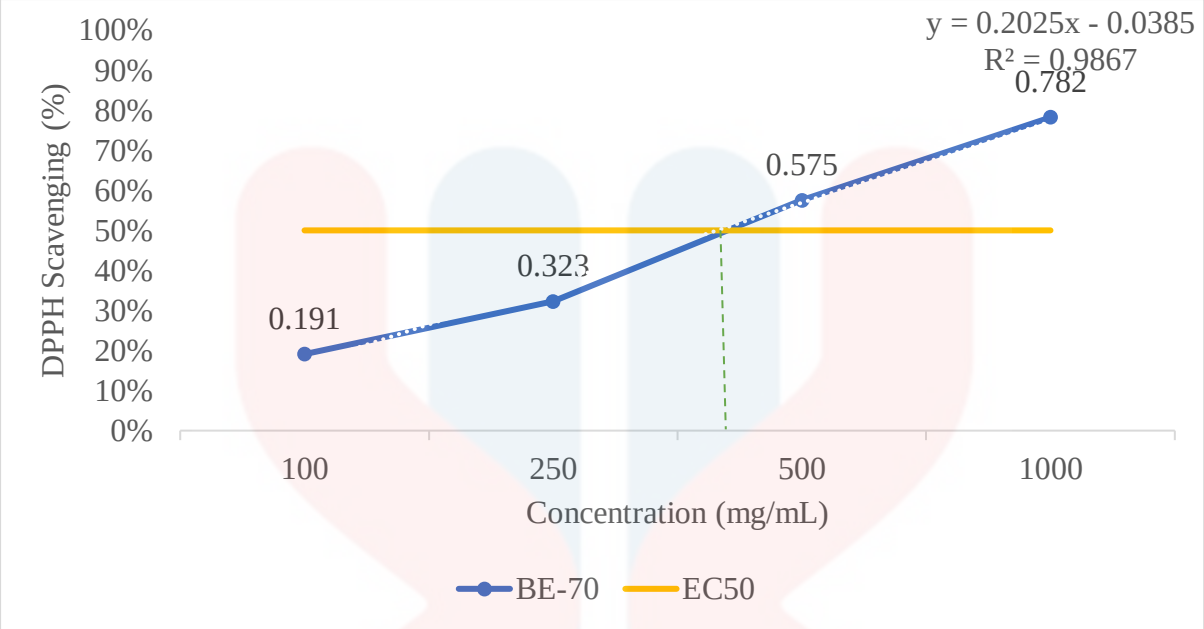


Figure 4.12: The linear relationship between concentration of bamboo peels and DPPH scavenging of BE-70B solvents.

In Figure 4.12, the investigation of DPPH scavenging activity using BE-70B ethanol solvent extracts unveiled an EC_{50} value of 0.5, suggesting their efficacy in mitigating free radicals. The DPPH clearance values at varying concentrations (100, 250, 500, and 1000 mg/mL) within the BE-70B ethanol solvent extract exhibited a concentration-dependent enhancement of antioxidant activity, with precise values of 0.191, 0.323, 0.575, and 0.782, respectively. This escalating trend underscores the extract's capability to effectively counteract oxidative stress.

Notably, at concentrations of 500 and 1000 mg/mL, the BE-70B ethanol solvent extract surpassed the EC_{50} line, indicating a robust antioxidant effect. This suggests that the extract effectively neutralizes free radicals even at higher concentrations, highlighting its potent antioxidant capabilities. The linear relationship, expressed by the equation $y = 0.2025x - 0.0385$ with $R^2 = 0.9867$, provides a quantitative analysis of the concentration-dependent nature of the antioxidant activity.

The calculated concentration (x) corresponding to the EC_{50} is approximately 2.66 mg/mL. This means that a BE-70B ethanol solvent extract at a concentration of around 2.66 mg/mL is required to achieve a 50% reduction in the initial DPPH radical concentration, emphasizing its potential to effectively respond to oxidative stress. These results suggest that

the antioxidant effect of the phenolic compounds in the BE-70B ethanol solvent extract is pronounced, and the extract exhibits optimal antioxidant effects even at concentrations exceeding the highest tested levels.

Moreover, the intriguing EC_{50} intersection point was achieved before reaching a concentration of 1000 mg/mL; precisely, the EC_{50} intersection point manifested at approximately 390 mg/mL concentration. This denotes the concentration at which the extract's antioxidant efficacy is on par with its ability to neutralize free radicals, adding depth to our understanding. This nuanced observation suggests that beyond a certain concentration, the extract's antioxidant activity becomes more pronounced, exemplifying its potential in managing oxidative stress (Apak et al., 2016).

4.7 Comparison of DPPH of different concentration bamboo peels

4.7.1 Comparison of DPPH of different concentration bamboo peels at 2 hours

The comparison of DPPH free radical scavenging activity in bamboo peel extracts with different concentrations and solvent compositions was analysed within 2 hours to understand its antioxidant potential. DPPH free radicals are dark purple and have maximum absorption at 517nm (Baliyan et al., 2022). The observed results indicate a consistent relationship between concentration and efficacy in scavenging DPPH radicals. As the concentration of bamboo peels extract increased from 100 mg/mL to 1000 mg/mL, the ability of the extract to neutralize free radicals was significantly enhanced. This trend was evident in different solvent compositions (50%, 60%, and 70% ethanol), indicating that the higher the concentration of the extract, the greater the antioxidant capacity.

Table 4.16: Comparison of DPPH of different concentration bamboo peels extraction in 2 hours.

Solvent	Concentration (mg/mL)			
	100	250	500	1000
BE-50A	0.036	0.085	0.156	0.545
BE-60A	0.114	0.153	0.295	0.324
BE-70A	0.196	0.212	0.234	0.436

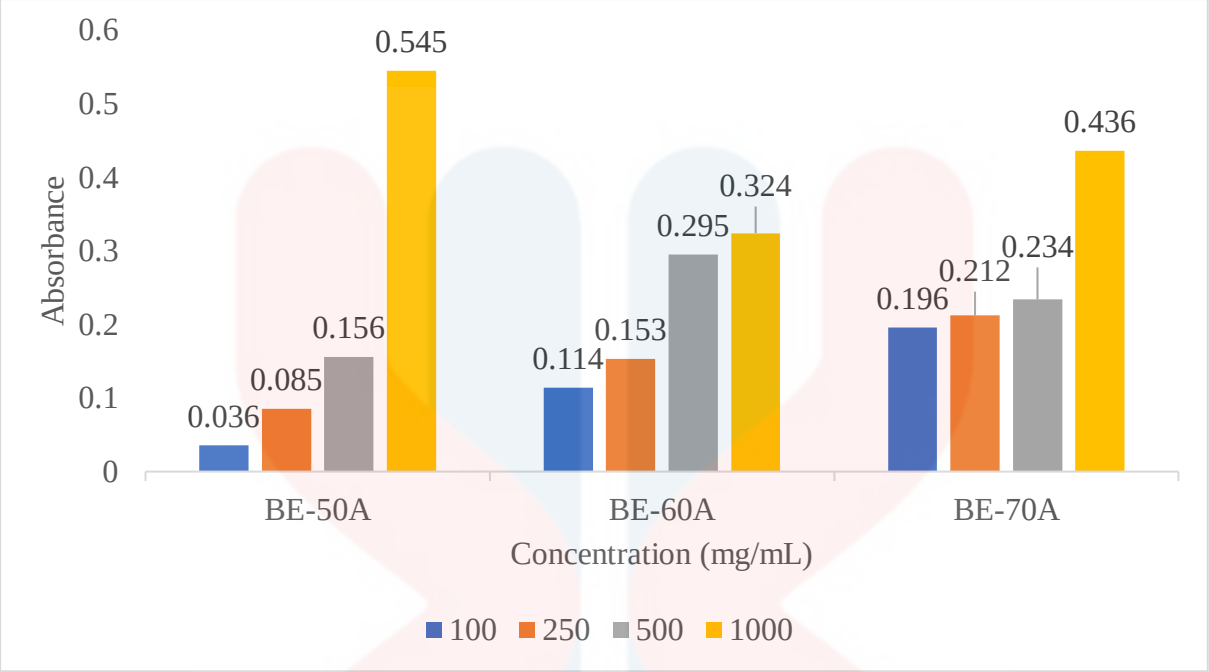


Figure 4.13: Comparison of DPPH of different concentration bamboo peels extraction in 2 hours.

Table 4.16 and Figure 4.13 illustrate the antioxidant activity of bamboo peel extracts using different ethanol solvents. Notably, BE-70A ethanol solvent generally exhibited remarkable antioxidant activity, particularly at lower concentrations of bamboo peel extract. For instance, at 100 mg/mL, the DPPH scavenging values were 0.196, 0.212, and 0.234 for BE-50A, BE-60A, and BE-70A ethanol solvents, respectively. Surprisingly, at the highest concentration tested (1000 mg/mL), the BE-50A ethanol solvent displayed the most pronounced DPPH radical scavenging activity, with absorbance values of 0.545, 0.324, and 0.436 for BE-50A, BE-60A, and BE-70A ethanol solvents, respectively. This intriguing deviation in the BE-50A ethanol solvent might suggest a saturation point where the antioxidant activity plateaus or could signify a unique interaction between the extract's composition and varying solvent concentrations, influencing the observed antioxidant effects. For clarity, it's important to note that absorbance values and DPPH scavenging activity are inversely related; higher absorbance values indicate less DPPH scavenging activity, while lower values suggest stronger antioxidant effects. For example, the lower absorbance value of 0.545 for BE-50A at 1000 mg/mL indicates a stronger ability to scavenge DPPH radicals compared to the higher absorbance values of 0.324 for BE-60A and 0.436 for BE-70A at the same concentration.

Consistent reductions in oxidative activity, as observed through the DPPH assay, underline the potential health benefits associated with incorporating bamboo peel extracts rich in antioxidants into dietary or medicinal applications. These antioxidants could potentially mitigate oxidative stress within the human body, providing protection against a range of health disorders associated with free radical-induced damage (Lobo et al., 2010).

4.7.2 Comparison of DPPH of different concentration bamboo peels at 4 hours

The DPPH free radical scavenging activity in bamboo peel extracts with different concentrations and solvent compositions was analysed within 4 hours to understand its antioxidant potential. DPPH free radicals are dark purple and have maximum absorption at 517nm (Baliyan et al., 2022). The observed results indicate a consistent relationship between concentration and efficacy in scavenging DPPH radicals. As the concentration of bamboo peels extract increased from 100 mg/mL to 1000 mg/mL, the ability of the extract to neutralize free radicals was significantly enhanced. This trend was evident in different solvent compositions (50%, 60%, and 70% ethanol), indicating that the higher the concentration of the extract, the greater the antioxidant capacity.

Table 4.17: Comparison of DPPH of different concentration bamboo peels extraction in 4 hours.

Solvent	Concentration (mg/mL)			
	100	250	500	1000
BE-50B	0.275	0.441	0.510	0.531
BE-60B	0.303	0.306	0.594	0.567
BE-70B	0.191	0.323	0.575	0.782

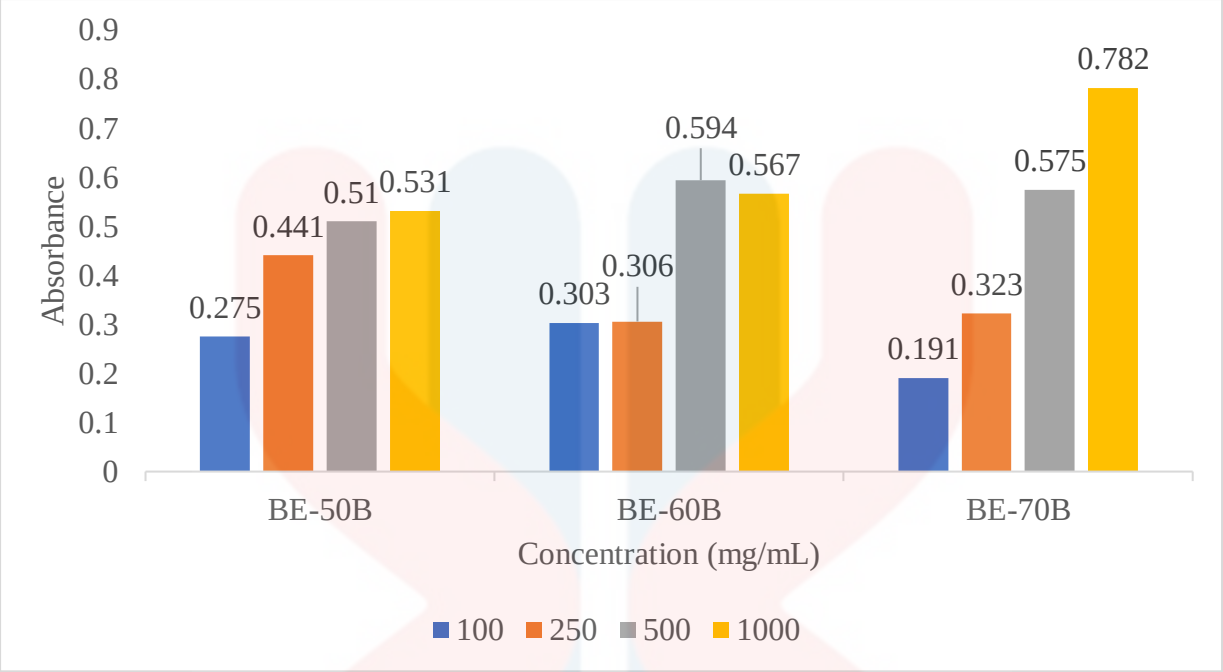


Figure 4.14: Comparison of DPPH of different concentration bamboo peels extraction in 4 hours.

Table 4.17 and Figure 4.14 reveal intriguing shifts in antioxidant activity across different solvent concentrations. For BE-50B ethanol, DPPH scavenging activity consistently increased from 0.275 at 100 mg/mL to 0.531 at 1000 mg/mL, showcasing a concentration-dependent enhancement. In contrast, BE-60B ethanol exhibited a minor increase from 0.303 at 100 mg/mL to 0.306 at 250 mg/mL, followed by a remarkable rise to 0.594 at 500 mg/mL and a slight decline to 0.567 at 1000 mg/mL, suggesting a potential saturation point in antioxidant activity. Remarkably, BE-70B ethanol displayed a decrease from 0.191 at 100 mg/mL to a significant surge (0.782) at 1000 mg/mL, indicating an amplified antioxidant potential with increased concentration.

These variations underscore the intricate nature of antioxidant behavior within bamboo peel extracts. Factors such as varying solubility of active compounds in different solvents and potential alterations in the extract's chemical composition due to extended extraction periods contribute to these fluctuations (Sasidharan et al., 2011). Such findings stress the significance of comprehending extraction parameters for maximizing the antioxidant capabilities of bamboo peel extracts, potentially unlocking valuable health advantages.

4.7.3 Effect of different concentration of solvents and time on DPPH EC_{50}

Comparison of the Intersection Points of DPPH EC50 Concentration for Antioxidant Activity in Bamboo Peels Extracts with Different Solvent Concentrations (BE-50, BE-60, BE-70) and Extraction Durations (2 Hours and 4 Hours). Antioxidant activity is crucial in addressing oxidative stress, and it is quantified as the concentration of a substance that effectively neutralizes 50% of oxidative stress. The findings reveal that the DPPH EC50 concentration intersection point varies with alterations in solvent concentration and extraction duration, as showed in Table 4.18 and Figure 4.15.

Table 4.18: Intersection point of DPPH EC_{50} concentration for all sample extracts.

Sample ID	Intersection Point of DPPH EC_{50} Concentration (mg/mL)
BE-50A	960 mg/mL
BE-50B	480 mg/mL
BE-60A	-
BE-60B	400 mg/mL
BE-70A	-
BE-70B	390 mg/mL

Note: (BE): Bamboo Ethanol, (A): 2 hours, and (B): 4 hours

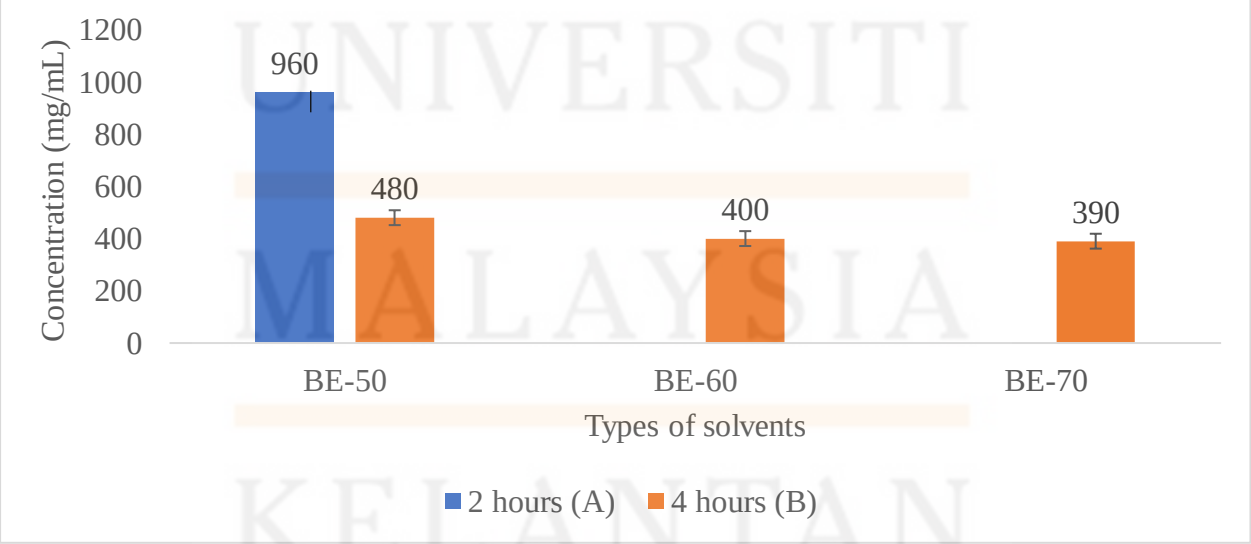


Figure 4.15: Comparison of intersection point of DPPH EC_{50} concentration for all sample extracts.

Table 4.18 and Figure 4.15, reveal intriguing shifts in antioxidant activity across different solvent concentrations. BE-50A demonstrated an EC_{50} concentration of 960 mg/mL, indicating significant antioxidant activity, potentially attributed to the presence of bioactive compounds with potent radical-scavenging properties. In contrast, BE-50B, extracted over 4 hours, exhibited a lower EC_{50} concentration of 480 mg/mL, suggesting heightened antioxidant efficacy. This implies that prolonged extraction duration enhances the extraction of potent antioxidant compounds, potentially including polyphenols and flavonoids.

BE-60A lacked an EC_{50} value within the tested concentration range, necessitating further investigation into extraction conditions and the potential bioactive compounds responsible for its antioxidant activity. Conversely, BE-60B displayed an EC_{50} concentration of 400 mg/mL, indicating substantial antioxidant activity linked to the 4 hour extraction period and the compounds extracted such as phenolics, tannins, and flavonoids. The variability in antioxidant activity between BE-70A and BE-70B, with the absence of an EC_{50} value for BE-70A and a value of 390 mg/mL for BE-70B, underscores the need for additional exploration to understand the factors influencing antioxidant potential. Overall, the data suggest a trend where longer extraction times generally lead to extracts with lower EC_{50} concentrations, indicative of enhanced antioxidant activity, aligning with existing literature on the importance of extraction parameters in obtaining plant extracts with optimal antioxidant properties (Aquino et al., 2023).

4.8 Fourier transform infrared (FTIR)

Fourier transform infrared (FTIR) spectroscopy, employed to examine organic compound wavelengths by absorbing ultraviolet light and functional groups found can be analysed by studying the peaks between frequency. From this spectroscopy study, the functional groups of each compound were identified by analysing bamboo peels extracts with varying solvent concentrations (50%, 60%, 70%) and different extraction durations (2 hours and 4 hours).

The FTIR spectra of BE-50A (2 hour extract time) were studied in the wavelength range from 4000 cm^{-1} to 400 cm^{-1}. Figure 4.16 shows the spectrum of the FTIR BE-50A. According to Figure 4.16, the absorption bands appear at 3264.17 cm^{-1}, 1636.35 cm^{-1}, 1044.49 cm^{-1}.

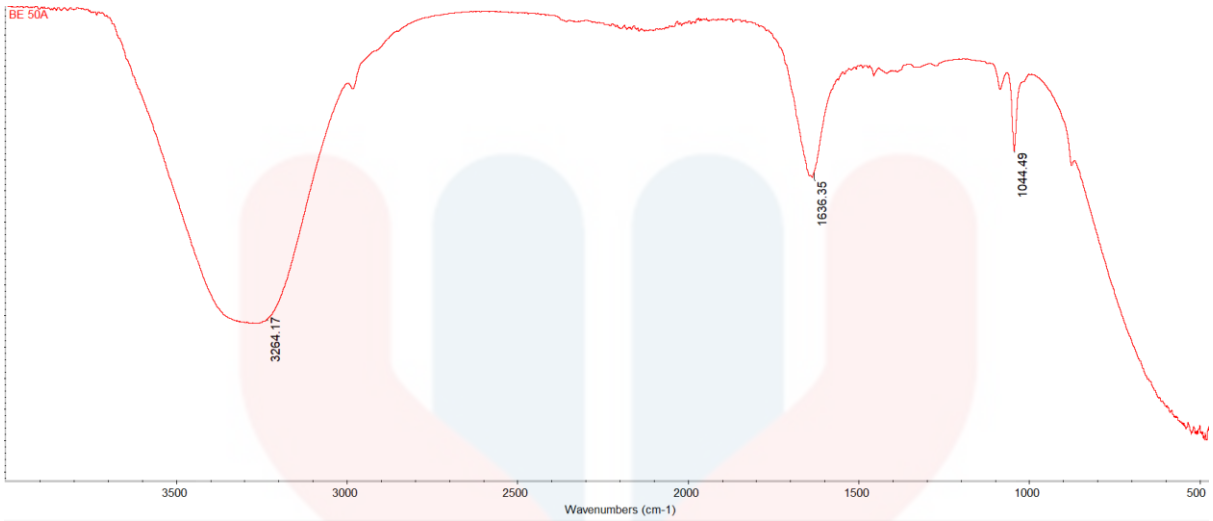


Figure 4.16: FTIR spectra of BE-50A.

Table 4.19: Functional compounds of BE-50A analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3264.17	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.35	1600-1700	C=C Stretch	Alkene
3	1044.49	1000-1200	C-O Stretch	Alcohol, Ether, Ester, Carboxylic Acid

Based on Figure 4.16 and Table 4.19, the FTIR analysis of bamboo peels identified various functional compounds, evidenced by distinctive peaks correlating to specific vibration types at different wave numbers. The peak observed at 3264.17 cm^{-1} signifies O-H stretching, indicative of alcohol, carboxylic acid, and phenolic groups present in the sample. This presence may arise from residual solvents like ethanol retained after the extraction process. Next, a peak at 1636.35 cm^{-1} indicates C=C stretching typical of the alkene functional group. Furthermore, peaks at 1044.49 cm^{-1} are corresponding to the stretching of C-O , suggesting the existence of alcohols, ethers, esters, or carboxylic acids. The broader size of these spectral peaks may imply a more extensive molecular structure within the bamboo peels extract.

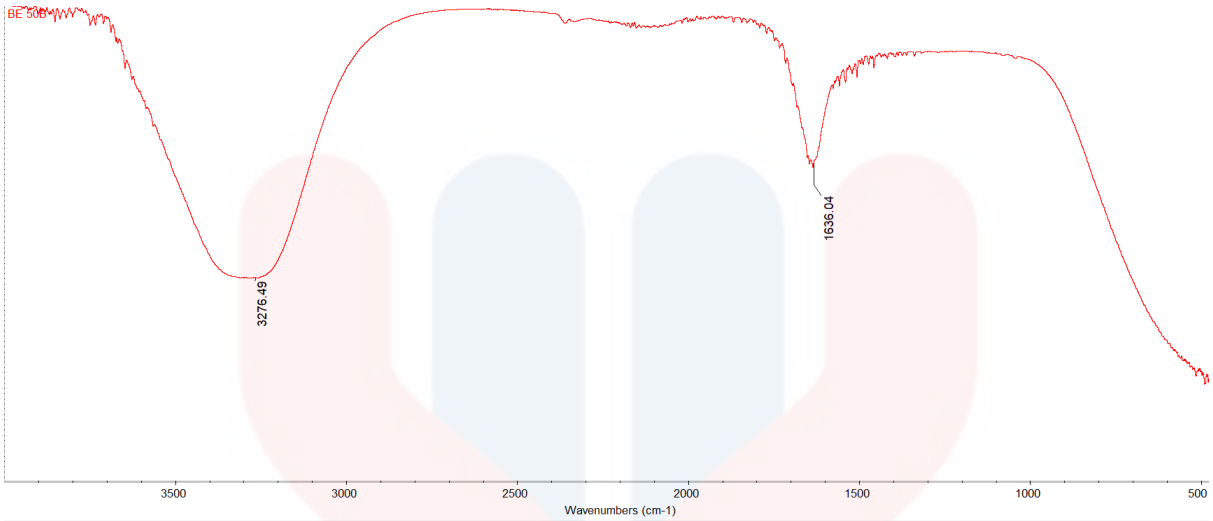


Figure 4.17: FTIR spectra of BE-50B.

Table 4.20: Functional compounds of BE-50B analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3276.49	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.04	1600-1700	C=C Stretch	Alkene

The FTIR spectrum of BE-50B, obtained from bamboo peel extraction lasting 4 hours, is depicted in Figure 4.17 and Table 4.20, displaying distinct peaks at specific wavenumbers, indicative of various functional compounds present within the extract. The observed peak at 3276.49 cm^{-1} signifies the O-H stretch, commonly associated with alcohol, carboxylic acid, or phenolic functional groups. This presence suggests the existence of these compounds within the sample, potentially contributing to its chemical composition. Additionally, there is a peak appeared at 1636.04 cm^{-1} coincides to the C=C stretching vibrations characteristic of the alkene functional group, offering insights into the potential concentration or nature of these double-bonded compounds present in the bamboo peel extract.

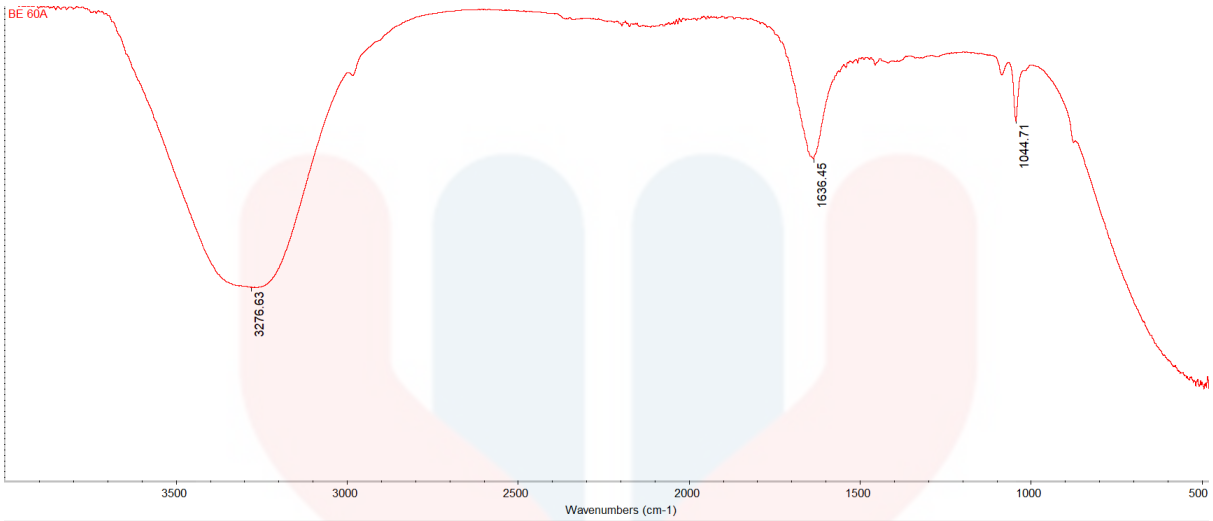


Figure 4.18: FTIR spectra of BE-60A.

Table 4.21: Functional compounds of BE-60A analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3276.63	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.45	1600-1700	C=C Stretch	Alkene
3	1044.71	1000-1200	C-O Stretch	Alcohol, Ether, Ester, Carboxylic Acid

The FTIR spectra of sample BE-60A, extracted from bamboo peels over a 2 hour duration, exhibit distinctive peaks in Figure 4.18 and Table 4.21, revealing the presence of specific functional compounds at distinct wavenumbers within the extract. The prominent peak at 3276.63 cm^{-1} denotes the O-H stretch, commonly associated with alcohol, carboxylic acid, or phenolic functional groups. This suggests these compounds are presented in the extract, potentially contributing to its chemical composition. Similarly, the discernible peak at 1636.45 cm^{-1} indicates C=C stretching vibrations characteristic of alkene functional groups, providing insight into their potential concentration within the bamboo peel extract. Furthermore, a peak found at 1044.71 cm^{-1} aligns with the C-O stretching vibrations, hinting at the potential presence of compounds like alcohols, ethers, esters, or carboxylic acids.

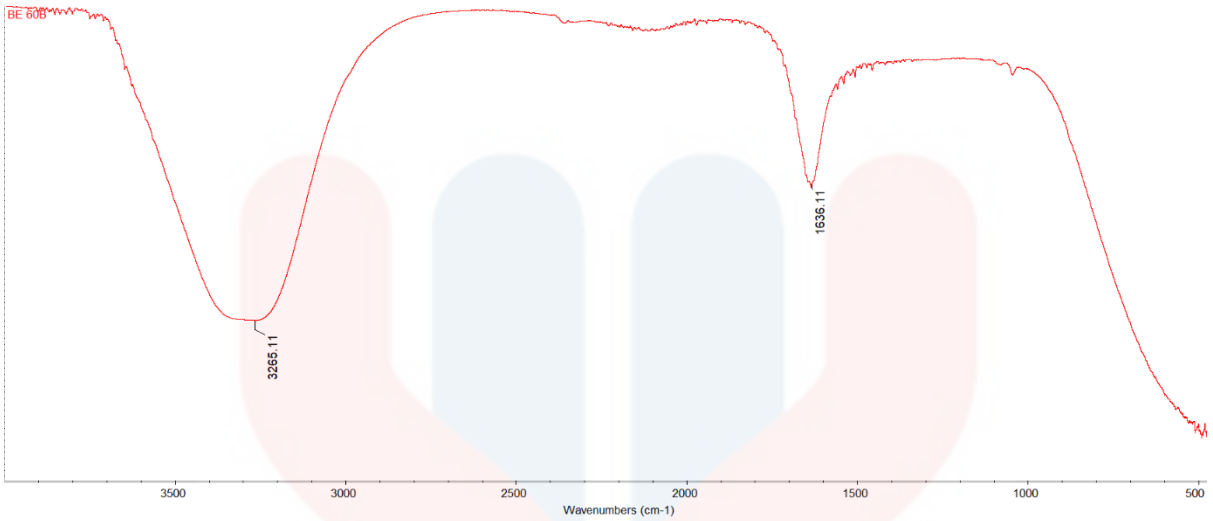


Figure 4.19: FTIR spectra of BE-60B.

Table 4.22: Functional compounds of BE-60B analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3265.11	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.11	1600-1700	C=C Stretch	Alkene

The FTIR spectra of sample BE-60B, extracted from bamboo peels over a 4 hour duration, exhibit distinctive peaks shown in Figure 4.19 and Table 4.22, representing various functional compounds at specific wavenumbers within the extract. The peak observed at 3265.11 cm^{-1} corresponds to the O-H stretch, indicating the presence of alcohol, carboxylic acid, or phenolic functional groups. This suggests the existence of these compounds within the extract, potentially influencing its chemical composition. Similarly, the discernible peak represents the C=C stretching vibrations characteristic of alkene functional groups was at 1636.11 cm^{-1}, providing insights into their potential concentration or presence in the bamboo peel extract.

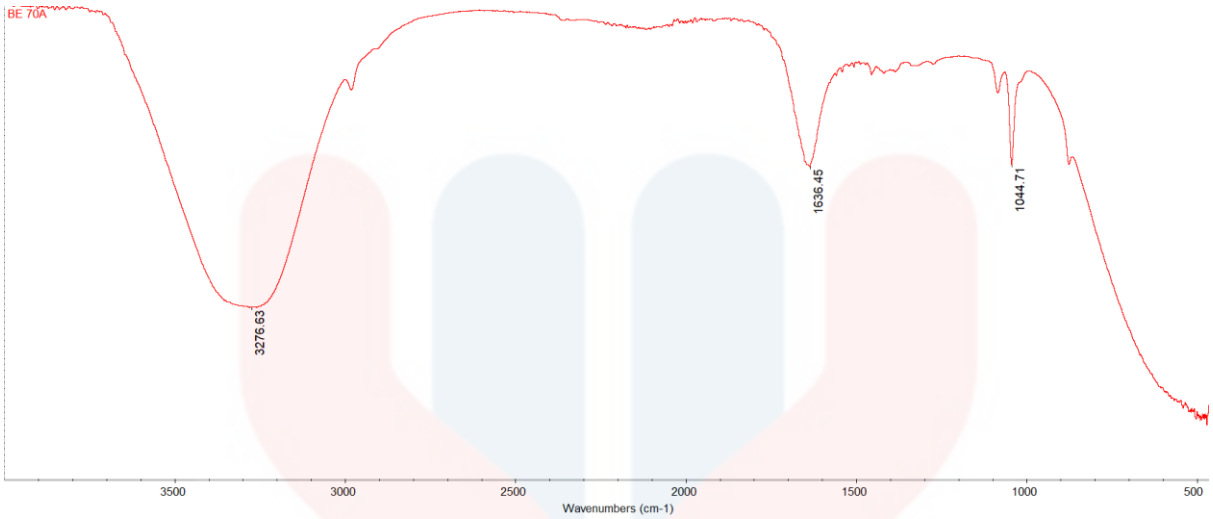


Figure 4.20: FTIR spectra of BE-70A.

Table 4.23: Functional compounds of BE-70A analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3276.63	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.45	1600-1700	C=C Stretch	Alkene
3	1044.71	1000-1200	C-O Stretch	Alcohol, Ether, Ester, Carboxylic Acid

The FTIR spectra of sample BE-70A, obtained from a 2 hour extraction of bamboo peels, display distinct peaks as presented in Figure 4.20 and Table 4.23, indicating specific functional compounds at varying wavenumbers within the extract. The observed peak at 3276.63 cm^{-1} signifies the O-H stretch, characteristic of alcohol, carboxylic acid, or phenolic functional groups. This presence suggests the existence of these compounds within the extract, potentially influencing its chemical composition. Next, the discernible peak at 1636.45 cm^{-1} corresponds to the C=C stretching vibrations typical of alkene functional groups, offering insights into their potential concentration or presence in the bamboo peel extract. Furthermore, the peak at 1044.71 cm^{-1} aligns with the C-O stretching vibrations, hinting at the potential presence of compounds such as alcohols, ethers, esters, or carboxylic acids within the extract. The identification of these functional groups via FTIR analysis provides valuable insights into

the chemical composition of the bamboo peel extract. Discernible peaks at specific wavenumbers indicates particular functional groups are presented within the extract.

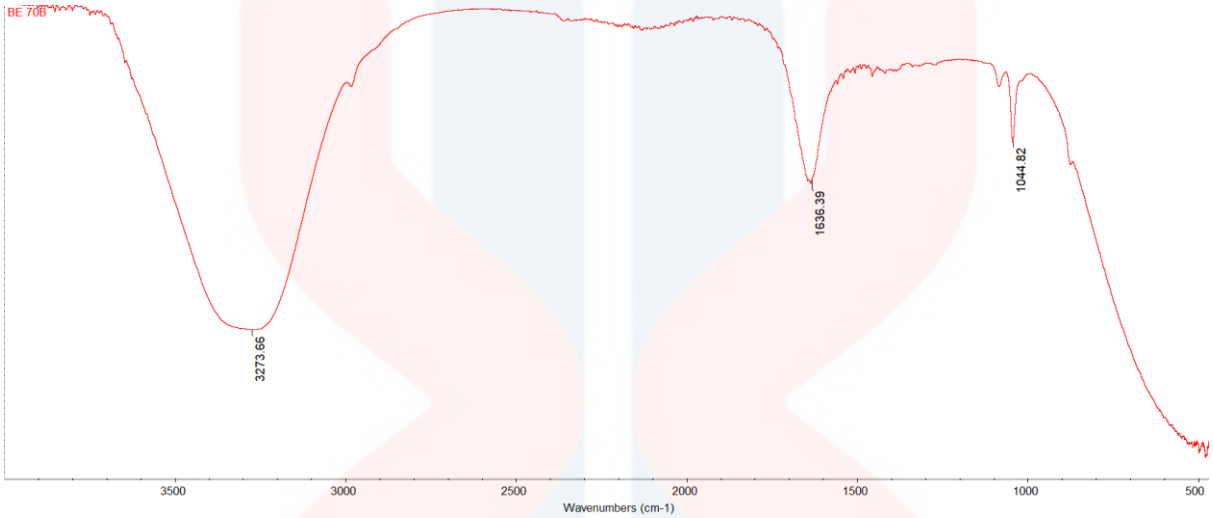


Figure 4.21: FTIR spectra of BE-70B.

Table 4.24: Functional compounds of BE-70B analysed by using FTIR.

No	Wavenumber (cm^{-1})	Frequency Range	Vibration Type	Functional Compound
1	3273.66	3200-3600	O-H Stretch	Alcohol, Carboxylic Acid, Phenol
2	1636.39	1600-1700	C=C Stretch	Alkene
3	1044.82	1000-1200	C-O Stretch	Alcohol, Ether, Ester, Carboxylic Acid

The FTIR spectra of sample BE-70B, extracted from bamboo peels over a 4 hour duration, exhibit distinctive peaks displayed in Figure 4.21 and Table 4.24, indicating specific functional compounds at different wavenumbers within the extract. At 3273.66 cm^{-1}, a significant peak suggests the O-H stretch, characteristic of alcohol, carboxylic acid, or phenolic functional groups. This implies the presence of these compounds within the extract, potentially contributing to its chemical composition. Similarly, the discernible peak at 1636.39 cm^{-1} corresponds to the C=C stretching vibrations typical of alkene functional groups, providing insights into their potential concentration or presence in the bamboo peel extract. Moreover,

the peak observed at 1044.82 cm^{-1} aligns with the C-O stretching vibrations, hinting at the potential presence of compounds such as alcohols, ethers, esters, or carboxylic acids within the extract (Apak et al., 2016).

4.9 Comparison of FTIR

FTIR spectroscopy was utilized to compare the bamboo peel extract obtained using a solvent mixture of 50% ethanol and water at two different extraction durations, namely 2 hours and 4 hours. This analysis spanned wavelengths ranging from 4000 cm^{-1} to 400 cm^{-1}. Figure 4.22 displays the comparison of FTIR spectra between these two extraction durations.

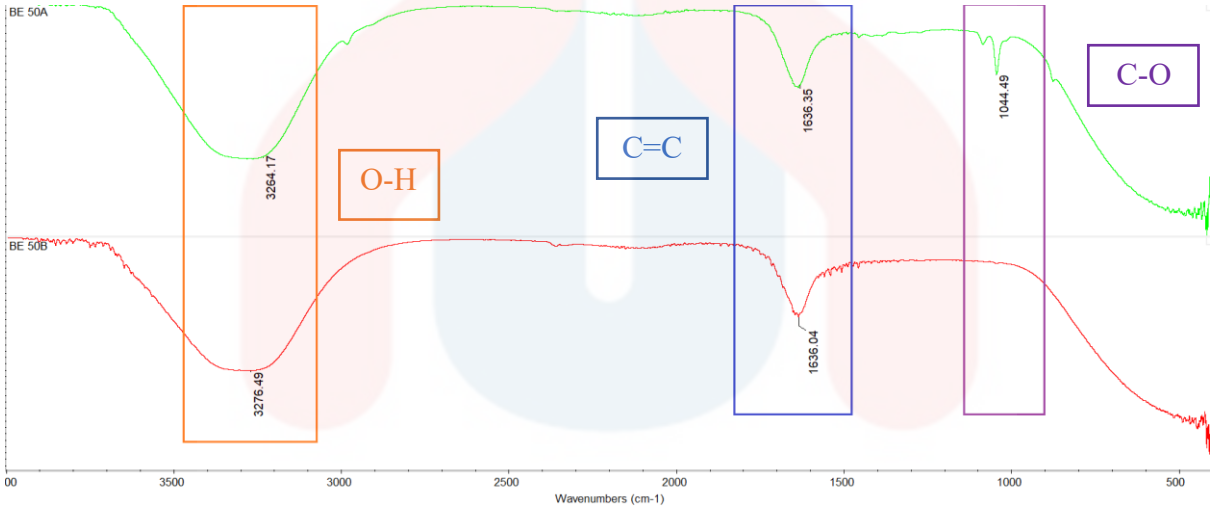


Figure 4.22: Comparison of FTIR spectrum of BE-50A and BE-50B.

After analysing Figure 4.22, it becomes apparent that the infrared spectra of samples extracted for 2 hours (BE-50A) and 4 hours (BE-50B) using 50% ethanol mixed with water share pronounced commonalities, primarily differing in band intensities. The spectra from both durations exhibit noteworthy peaks that indicate the presence of common functional groups. Specifically, the O-H stretching vibrations around 3264.17 cm^{-1} in BE-50A and 3276.49 cm^{-1} in BE-50B are indicative of alcohols, carboxylic acids, and phenols. Moreover, the consistent presence of peaks at approximately 1636 cm^{-1} points to alkene functional groups in both samples.

However, there are significant differences between the two spectra. The peak at 1044.49 cm^{-1} in BE-50A is related to the CO stretching vibration, but it is not obviously observed in BE-50B. This difference may indicate the persistence of residual ethanol or similar compounds in BE-50A after extraction. These disparities suggest shifts in chemical composition or functional group concentrations between the two extraction durations (Nikalje et al., 2019). Consequently, BE-50A demonstrates a wider range of distinguishable peaks in its FTIR spectrum than BE-50B, hinting at a more diverse array of chemical components or functional groups present in the 2 hour extraction sample compared to the 4 hour extraction.

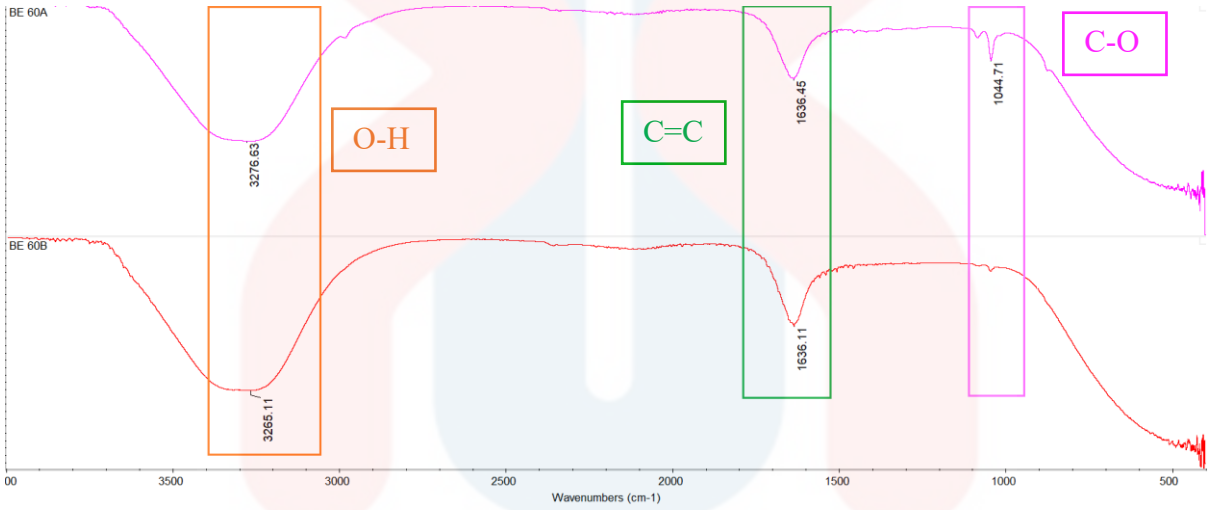


Figure 4.23: Comparison of FTIR spectrum of BE-60A and BE-60B.

Based on Figure 4.23, it is evident that the infrared spectra of bamboo peel extracts extracted for 2 hours (BE-60A) and 4 hours (BE-60B) using 60% ethanol mixed with water share distinct similarities with variations primarily reflected in band intensities. Both spectra from the respective durations display significant peaks associated with common functional groups. Specifically, the O-H stretching vibrations around 3276.63 cm^{-1} in BE-60A and 3265.11 cm^{-1} in BE-60B are characteristic of alcohols, carboxylic acids, and phenols. Additionally, the consistent presence of peaks at approximately 1636 cm^{-1} points to alkene functional groups in both samples.

However, discernible differences surface between the two spectra. BE-60A exhibits a C-O stretching vibration at 1044.71 cm^{-1}, associated with alcohol, ether, ester, and carboxylic acid functional compounds, which is not as prominently observed in BE-60B. Conversely, BE-

60B lacks the additional C-O stretching vibration but displays its own distinct features, such as peaks at 1636.11 cm^{-1} associated with alkene functional groups. These disparities suggest shifts in chemical composition or functional group concentrations between the two extraction durations (Nikalje et al., 2019). Consequently, BE-60A presents a more diverse range of distinguishable peaks in its FTIR spectrum than BE-60B, suggesting a potentially broader array of chemical components or functional groups present in the 2 hour extraction sample compared to the 4 hour extraction.

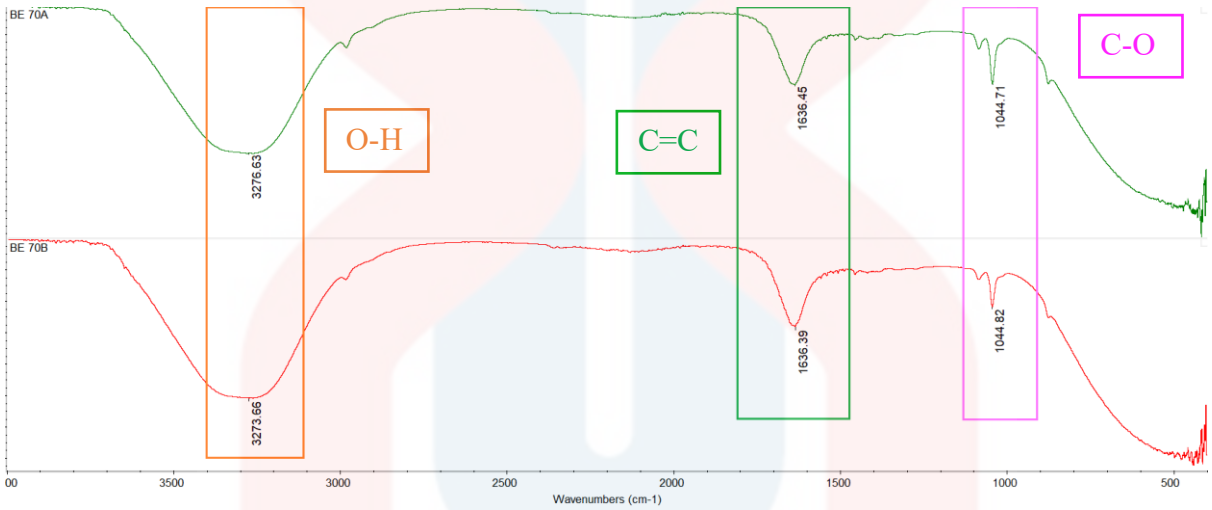


Figure 4.24: Comparison of FTIR spectrum of BE-70A and BE-70B.

According to Figure 4.24, the infrared spectra of bamboo peel extracts extracted for 2 hours (BE-70A) and 4 hours (BE-70B) using a 70% ethanol-water mixture exhibit noticeable similarities with discernible variations in band intensities. Both spectra from the respective durations present significant peaks associated with common functional groups. Specifically, the O-H stretching vibrations around 3276.63 cm^{-1} in BE-70A and 3273.66 cm^{-1} in BE-70B are characteristic of alcohols, carboxylic acids, and phenols. Furthermore, the consistent presence of peaks at approximately 1636 cm^{-1} points to alkene functional groups in both samples.

These shared features highlight commonalities in the chemical composition of BE-70A and BE-70B, emphasizing the persistence of similar functional groups in bamboo peel extracts regardless of the extraction duration. However, nuanced differences emerge between the two spectra. BE-70A exhibits a C-O stretching vibration at 1044.71 cm^{-1}, associated with alcohol, ether, ester, and carboxylic acid functional compounds, while BE-70B shows a similar peak at

1044.82 cm^{-1}. These subtle differences may indicate variations in the concentration or arrangement of certain compounds, potentially influenced by the distinct extraction durations (Nikalje et al., 2019). While BE-70A and BE-70B share commonalities in their functional group profiles, the slight disparities underscore the influence of extraction duration on the chemical composition of bamboo peel extracts. BE-70A, with its C-O stretching vibration, presents a nuanced variation compared to BE-70B, hinting at potential differences in molecular structures or the presence of specific compounds.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In conclusion, the objective of this study was to extract phenolic compounds from bamboo peels using the Soxhlet extraction technique and to optimize the extraction parameters for each ethanol concentration to maximize the yield of phenolic compounds. The presence of flavonoids and tannins in the bamboo peel extracts in the phytochemical screening, showcasing positive results and the concentration increased for both compounds at 2 hour and 4 hour extraction durations.

The Soxhlet extraction method demonstrated distinct variations in phenolic compound extraction based on ethanol concentrations and extraction durations. The UV-Vis spectroscopy analysis quantified varying concentrations of total phenolic and tannins compounds across different solvent concentrations and extraction durations. Notably, the 4 hour extraction duration demonstrated higher concentrations of total phenolic and tannins compounds compared to the 2 hour duration. During the 2 hour extraction duration, a consistent ascending trend was observed, revealing an increase in total phenolic and tannins compounds with escalating ethanol concentrations. Specifically, the total phenolic content exhibited an upward trend 11.05 mg/mL at 50%, 22.78 mg/mL at 60%, and 33.44 mg/mL at 70% ethanol concentration. Similarly, the total tannins content displayed increasing concentrations: 14.86 mg/mL at 50%, 29.25 mg/mL at 60%, and 42.57 mg/mL at 70% ethanol concentration.

In contrast, the 4 hour extraction showed variations in phenolic compound concentrations, notably with an unexpected decline at the 60% ethanol concentration. Despite this decrease, the concentrations at 60% ethanol remained higher than those observed during the 2 hour extraction, indicating the potential influence of prolonged extraction time on compound yields. Specifically, the total phenolic content showed an ascending trend: 37.03 mg/mL at 50%, 34.53 mg/mL at 60%, and 56.39 mg/mL at 70% ethanol concentration. Similarly, the total tannins content exhibited variations: 45.17 mg/mL at 50%, 40.32 mg/mL at

60%, and 56.92 mg/mL at 70% ethanol concentration. These quantitative findings underscore the impact of ethanol concentrations and extraction durations on phenolic compound extraction.

For DPPH analysis, it was observed that all extracts from bamboo peels displayed maximum absorption at 517 nm. The comparison of DPPH radical scavenging activities indicated a positive correlation between oxidative activity and extract concentration. Notably, at 1000 mg/mL, BE-50A exhibited the highest DPPH clearance value of 0.545 mg/mL, while BE-50B showed a high value of 0.531 mg/mL, emphasizing their robust antioxidant properties. BE-70B demonstrated remarkable antioxidant efficacy, particularly at 1000 mg/mL, with a notable DPPH clearance value of 0.782 mg/mL, suggesting its potential as a potent antioxidant. The optimal antioxidant sample selection depends on the specific concentration of interest, with BE-50A standing out for remarkable performance at 1000 mg/mL. Both BE-50B and BE-70B exhibit noteworthy antioxidant activity, urging further investigations to unveil the specific bioactive compounds contributing to observed antioxidant effects in these bamboo peel extracts. The findings underscore the complexity of antioxidant behavior, emphasizing the need for a comprehensive assessment considering both DPPH clearance values and EC50 concentrations.

For FTIR analysis, all the bamboo peels extracts had the adsorption peak in between 4000cm^{-1} and 400 cm^{-1} which had linked to the stretching of O-H, C=C as well as C-H. Besides, bamboo peels extracts peaked at between 3000 cm^{-1} and 1000 cm cm^{-1} which was known as starching vibration of agf66lkene group. The number of adsorption peaks in bamboo peel 70% concentration ethanol solvent extracts are more than 50% and 60% concentration ethanol extracts.

In summary, the BE-70B sample emerged as the most favourable outcome, demonstrating superior efficacy in maximizing the yield of phenolic compounds compared to alternative ethanol concentrations and extraction durations.

5.2 Recommendations

The following suggestions for future work are recommended for future development:

1. Phenolic compounds found in diverse bamboo parts offer promising avenues in therapeutic treatments, potentially serving as safer alternatives to synthetic chemicals with similar properties and fewer adverse effects on consumers. Various extraction methods are available for plant-based extraction, among which ultrasonic-assisted extraction (UAE) is

gaining traction. UAE's advantages lie in its shorter processing time, absence of high-pressure and high-temperature requirements, and reduced operation steps, thereby lowering the risk of contamination. To retain the active components' integrity, freeze drying is recommended for plant materials as it effectively eliminates excess water without subjecting them to heat.

2. Furthermore, in the realm of chemical analysis and the detailed exploration of these constituents, numerous chromatographic techniques have been developed. These include gas chromatography (GC), high-performance liquid chromatography (HPLC), liquid chromatography-electrospray ionization coupled with mass spectroscopy (LC-ESI/MS), and gel filtration chromatography. These methodologies are tailored to provide precise separation, detection, and identification of phenolic, tannin, and flavonoid compounds across diverse substances, offering intricate insights into their composition and properties.
3. Different segments of the plant can be utilized to extract valuable bioactive compounds like flavonoids and tannins, both renowned for their therapeutic potential. These compounds can undergo thorough analysis through phytochemical tests and quantitative assessments using UV-Vis spectrophotometry. Once the phenolic compounds are identified within the plant sample, they could be explored for treating conditions associated with hypertension, diabetes, and obesity. This study suggests that natural plant-derived alternatives offer a safer and more cost-effective therapeutic option compared to their synthetic counterparts, promising profound health benefits.

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APPENDIX A

Figure A.1: The pictures of bamboo.

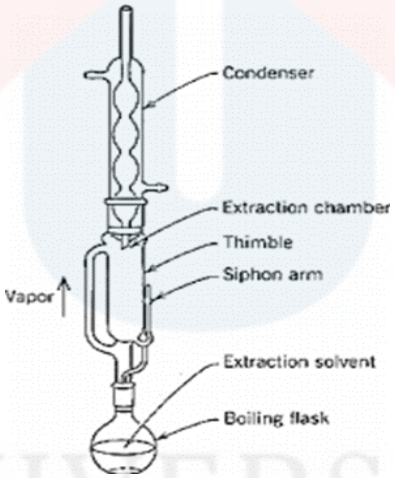

Figure A.2: The Schematic of Soxhlet extractor.

Figure A.3: The picture of rotary evaporator.

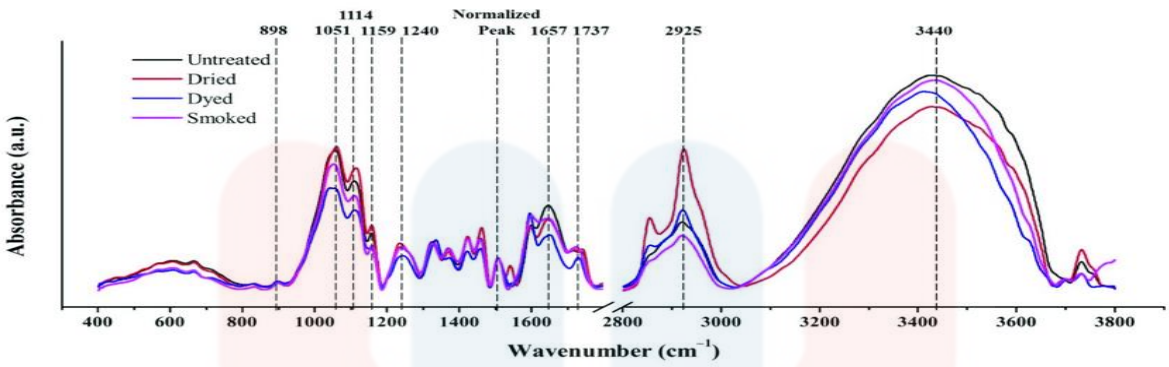

Figure A.4: FTIR spectra related to bamboo.

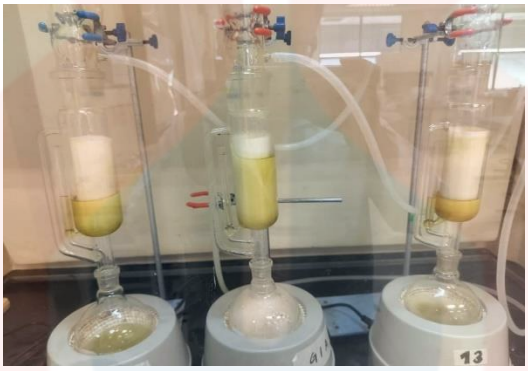

Figure A.5: The picture showed the Soxhlet extraction.

Figure A.6: The picture showed the Wood grinder.

Figure A.7: The picture showed the sample different concentration solvents after extraction 2 hours.

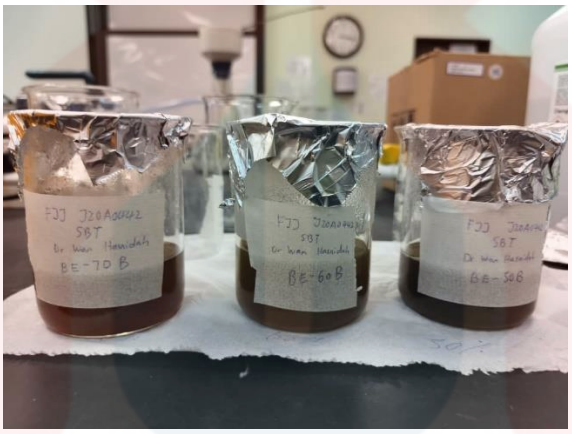

Figure A.8: The picture showed the sample different concentration solvents after extraction 4 hours.

APPENDIX B

Table B.1: The Sample Control of bamboo peels extract:

Sample Blank	50%	60%	70%
2 hours (765 nm)	BE-50A 0.302 A 	BE-60A 0.629 A 	BE-70A 0.945 A 
4 hours (765 nm)	BE-50A 0.705 A 	BE-60A 0.817 A 	BE-70A 1.605 A 
2 hours (760 nm)	BE-50A 0.255 A 	BE-60A 0.595 A 	BE-70A 0.899 A 

4 hours (760 nm)	BE-50B 0.711 A 	BE-60B 0.839 A 	BE-70B 1.620 A 

Table B.2: The result of the DPPH for 50% ethanol and 2 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.036
250	0.085
500	0.156
1000	0.545

Table B.3: The result of the DPPH for 60% ethanol and 2 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.114
250	0.153
500	0.295
1000	0.324

Table B.4: The result of the DPPH for 70% ethanol and 2 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.196
250	0.212
500	0.234
1000	0.436

Table B.5: The result of the DPPH for 50% ethanol and 4 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.275
250	0.441
500	0.510
1000	0.531

Table B.6: The result of the DPPH for 60% ethanol and 4 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.303
250	0.306
500	0.594
1000	0.567

Table B.6: The result of the DPPH for 70% ethanol and 4 hours extraction bamboo peels.

Concentration (µg/mL)	ABS Reading (517 nm)
100	0.191
250	0.323
500	0.575
1000	0.782

APPENDIX C

Calculation C.1.1: Calculate of the concentration of total phenolic compound in bamboo peels 2 hours extracts with sample blank.

Sample ID	2 hours extract
BE-50A	Sample blank = 0.302 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.732 A **[(Sample control / sample extract) × 100 =]** (0.302 A / 2.732 A) × 100 = 11.05 mg/mL **[(Density=Mass/Volume) / 1000 =]** 11.05mg/mL = x(mg) / 250mL x(mg) = (11.05 mg/mL × 250 mL) / 1000 x = 2.7625g or 2.763g **Yield = [(Mass / 15 gram of sample) × 100%]** (2.7625g / 15g) × 100% = 18.42%
BE-60A	Sample blank = 0.629 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.761 A **[(Sample control / sample extract) × 100 =]** (0.629 A / 2.761 A) × 100 = 22.78 mg/mL **[(Density=Mass/Volume) / 1000 =]** 22.78mg/mL = x(mg) / 250mL x(mg) = (22.78 mg/mL × 250 mL) / 1000 x = 5.695g **Yield = [(Mass / 15 gram of sample) × 100%]** (5.695g / 15g) × 100% = 37.97%

BE-70A	Sample blank = 0.945 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.826 A **[(Sample control / sample extract) × 100 =]** (0.945 A / 2.826 A) × 100 = 33.44 mg/mL **[(Density=Mass/Volume) / 1000 =]** 33.44mg/mL = x(mg) / 250mL x(mg) = (33.44 mg/mL × 250 mL) / 1000 x = 8.360g **Yield = [(Mass / 15 gram of sample) × 100%]** (8.360g / 15g) × 100% = 55.73%

Calculation C.1.2: Calculate of the concentration of total phenolic compound in bamboo peels 4 hours extracts with sample blank.

Sample ID	4 hours extract
BE-50B	Sample blank = 0.705 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 1.904 A **[(Sample control / sample extract) × 100 =]** (0.705 A / 1.904 A) × 100 = 37.03 mg/mL **[(Density=Mass/Volume) / 1000 =]** 37.03mg/mL = x(mg) / 250mL x(mg) = (37.03 mg/mL × 250 mL) / 1000 x = 9.2575g or 9.258g **Yield = [(Mass / 15 gram of sample) × 100%]** (9.2575g / 15g) × 100% = 61.72%
BE-60B	Sample blank = 0.817 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.366 A

	[(Sample control / sample extract) × 100 =] (0.817 A / 2.366 A) × 100 = 34.53 mg/mL **[(Density=Mass/Volume) / 1000 =]** 34.53mg/mL = x(mg) / 250mL x(mg) = (34.53 mg/mL × 250 mL) / 1000 x = 8.6325g or 8.633g **Yield = [(Mass / 15 gram of sample) × 100%]** (8.6325g / 15g) × 100% = 57.55%
BE-70B	Sample blank = 1.605 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.846 A **[(Sample control / sample extract) × 100 =]** (1.605 A / 2.846 A) × 100 = 56.39 mg/mL **[(Density=Mass/Volume) / 1000 =]** 56.39mg/mL = x(mg) / 250mL x(mg) = (56.39 mg/mL × 250 mL) / 1000 x = 14.0975g or 14.098g **Yield = [(Mass / 15 gram of sample) × 100%]** (14.0975g / 15g) × 100% = 93.98%

Calculation C.1.3: Calculate of the concentration of total tannins content in bamboo peels 2 hours extracts with sample blank.

Sample ID	2 hours extract
BE-50A	Sample blank = 0.255 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 1.716 A **[(Sample control / sample extract) × 100 =]** (0.255 A / 1.716 A) × 100 = 14.86 mg/mL **[(Density=Mass/Volume) / 1000 =]**

	14.86mg/mL = x(mg) / 250mL x(mg) = (14.86 mg/mL × 250 mL) / 1000 x = 3.715g **Yield = [(Mass / 15 gram of sample) × 100%]** (3.715g / 15g) × 100% = 24.77%
BE-60A	Sample blank = 0.595 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.034 A **[(Sample control / sample extract) × 100 =]** (0.595 A / 2.034 A) × 100 = 29.25 mg/mL **[(Density=Mass/Volume) / 1000 =]** 29.25mg/mL = x(mg) / 250mL x(mg) = (29.25 mg/mL × 250 mL) / 1000 x = 7.3125g or 7.313g **Yield = [(Mass / 15 gram of sample) × 100%]** (7.3125g / 15g) × 100% = 48.75%
BE-70A	Sample blank = 0.899 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.112 A **[(Sample control / sample extract) × 100 =]** (0.899 A / 2.112 A) × 100 = 42.57 mg/mL **[(Density=Mass/Volume) / 1000 =]** 42.57mg/mL = x(mg) / 250mL x(mg) = (42.57 mg/mL × 250 mL) / 1000 x = 10.6425g or 10.643g **Yield = [(Mass / 15 gram of sample) × 100%]** (10.6425g / 15g) × 100% = 70.95%

Calculation C.1.4: Calculate of the concentration of total tannins content in bamboo peels 4 hours extracts with sample blank.

Sample ID	4 hours extract
BE-50B	Sample blank = 0.711 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 1.574 A **[(Sample control / sample extract) × 100 =]** (0.711 A / 1.904 A) × 100 = 45.17 mg/mL **[(Density=Mass/Volume) / 1000 =]** 45.17mg/mL = x(mg) / 250mL x(mg) = (45.17 mg/mL × 250 mL) / 1000 x = 11.2925g or 11.293g **Yield = [(Mass / 15 gram of sample) × 100%]** (11.2925g / 15g) × 100% = 75.28%
BE-60B	Sample blank = 0.839 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.081 A **[(Sample control / sample extract) × 100 =]** (0.839 A / 2.081 A) × 100 = 40.32 mg/mL **[(Density=Mass/Volume) / 1000 =]** 40.32mg/mL = x(mg) / 250mL x(mg) = (40.32 mg/mL × 250 mL) / 1000 x = 10.08g **Yield = [(Mass / 15 gram of sample) × 100%]** (10.08g / 15g) × 100% = 67.20%
BE-70B	Sample blank = 1.620 A Absorbance reading of bamboo peels extracts from UV-Vis spectroscopy = 2.147 A **[(Sample control / sample extract) × 100 =]** (1.620 A / 2.846 A) × 100 = 56.92 mg/mL

	[(Density=Mass/Volume) / 1000 =] 56.92mg/mL = x(mg) / 250mL x(mg) = (56.92 mg/mL × 250 mL) / 1000 x = 14.23g **Yield = [(Mass / 15 gram of sample) × 100%]** (14.23g / 15g) × 100% = 94.87%

Calculation C.1.5: Calculate the linear relationship between concentration of bamboo peels and DPPH scavenging of 50% solvents in 2 hours.

Sample ID	2 hours extract
BE-50A	Equation: y = 0.1598x - 0.194, R^2 = 0.7932 EC_{50} = 0.5 = y 0.5 + 0.194 = 0.1598x 0.694 = 0.1598x x = 4.346 mg/mL
BE-60A	Equation: y = 0.0772x + 0.0285, R^2 = 0.9267 EC_{50} = 0.5 = y 0.5 - 0.0285 = 0.0772x 0.4715 = 0.0772x x = 6.10 mg/mL
BE-70A	Equation y = 0.0742x + 0.084, R^2 = 0.7304 EC_{50} = 0.5 = y 0.5 - 0.084 = 0.0742 0.416 = 0.0742x x = 5.61 mg/mL

Calculation C.1.6: Calculate the linear relationship between concentration of bamboo peels and DPPH scavenging of 50% solvents in 4 hours.

Sample ID	4 hours extract
BE-50A	Equation: $y = 0.0837x + 0.23$, $R^2 = 0.8669$ $EC_{50} = 0.5 = y$ $0.5 - 0.23 = 0.0837x$ $0.27 = 0.0837x$ $x = 3.23$ mg/mL
BE-60A	Equation: $y = 0.108x + 0.1725$, $R^2 = 0.7619$ $EC_{50} = 0.5 = y$ $0.5 - 0.1725 = 0.108x$ $0.3275 = 0.108x$ $x = 3.03$ mg/mL
BE-70A	Equation $y = 0.2025x - 0.0385$, $R^2 = 0.9867$ $EC_{50} = 0.5 = y$ $0.5 + 0.0385 = 0.2025x$ $0.5385 = 0.2025x$ $x = 2.66$ mg/mL