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Antioxidant Evaluation of *Etlingera elatior* extracts using Ultrasonic Assisted Extraction (UAE) in different solvent

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DECLARATION

I declare that this thesis entitled **Antioxidant Evaluation of *Etlingera elatior* extracts using Ultrasonic Assisted Extraction (UAE) in different solvent** was the results of my own research except as cited in the references.

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Antioxidant Study and Extraction of *Etlingera elatior* using Ultrasonic Assisted Extraction (UAE) in Ethyl Acetate, Methanol and Hexane.

Abstract

Etlingera elatior, also known as Kantan, was a popular traditional drug and food enhancer in Malaysia. In this study, methanol, hexane and ethyl acetate were used as a solvent and an Ultrasonic Assisted Extraction (UAE) extractor was applied in the extraction process. The yield of methanol extracted by ultrasonic method was 9.56% in hexane, 9.55% in ethyl acetate, and 9.31% in hexane. The 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay was used to measure the antioxidant activity of the Etlingera elatior extraction. The concentration of antioxidant activity was calculated using the IC_{50} extraction of *Etlingera elatior*. Based on the study, *Etlingera elatior* concentration potential for antioxidant activity against DPPH free radicals was 39.10 µg/ml in methanol, 37.03 µg/mL in hexane, and 25.00 µg/mL in ethyl acetate. The primary component are observed with the highest phytochemical analysis score was oleic acid, caryophyllene, hexadecenoic acid, stigmasterol and dodecanoic acid.

Keywords: DPPH, Antioxidant, GC-MS

Abstrak

Etlingera elatior, juga dikenali sebagai Kantan, merupakan ubat tradisional dan penambah makanan yang popular di Malaysia. Dalam kajian ini, metanol, heksana dan etil asetat digunakan sebagai pelarut dan pengekstrak. Ultrasonik Assisted Extraction (UAE) digunakan dalam proses pengekstrakan. Hasil metanol yang diekstrak melalui kaedah ultrasonik ialah 9.56% dalam heksana, 9.55% dalam etil asetat, dan 9.31% dalam heksana. Ujian penghapusan radikal bebas 2,2-diphenyl-1-picryhydrazyl (DPPH) digunakan untuk mengukur aktiviti antioksidan pengekstrakan elatior Etlingera. Kepekatan aktiviti antioksidan dikira menggunakan pengekstrakan IC_{50} Etlingera elatior. Berdasarkan kajian, potensi kepekatan Etlingera elatior untuk aktiviti antioksidan terhadap radikal bebas DPPH ialah 39.10 µg/ml dalam metanol, 37.03 µg/mL dalam heksana, dan 25.00 µg/mL dalam etil asetat. Komponen utama diperhatikan dengan skor analisis fitokimia tertinggi ialah asid oleik, caryophyllene, asid heksadesenoik, stigmasterol dan asid dodekanoik.

Kata kunci: DPPH, Antioksidan, GC-MS

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DPPH	2,2-diphenyl-1-picryhydrazyl	1
IC_{50}	Half-maximal inhibitory concentration	1
ml	Mililitre	2
µg	Microgram	3
mg	Miligram	3
kHz	Kilohertz	10
UV	Ultraviolet	11

CHAPTER 1

INTRODUCTION

1.1 Background of study

Etlingera elatior, commonly known as *Etlingera elatior*torch ginger, was a captivating floral gem native to Southeast Asia. Beyond its stunning crimson inflorescence and fragrant notes, Zingiberaceae member harbours a treasure trove of bioactive compounds with promising medicinal properties. Among these, antioxidant activity reigns supreme, offering potential protection against chronic diseases and oxidative stress. However, unlocking the full spectrum of these beneficial compounds necessitates efficient extraction methods that maximize their yield and potency. This chapter delves into the potential of ultrasonic-assisted extraction (UAE) as a powerful tool for unlocking the remarkable antioxidant potential of *Etlingera elatior*.

Etlingera elatior boasts a diverse array of antioxidant-rich phytochemicals, including phenolic acids, flavonoids, and terpenoids (Chan et al., 2011). These potent molecules neutralize free radicals, those destructive byproducts of cellular metabolism, before they can wreak havoc on our cells and tissues. Studies have demonstrated *Etlingera elatior*'s potent antioxidant activity in scavenging free radicals like DPPH and ABTS, surpassing even established antioxidants like ascorbic acid (Vimala et al., 2013). This impressive antioxidant capacity positions *Etlingera elatior* as a promising candidate for combating oxidative stress-related ailments like cancer, cardiovascular diseases, and neurodegenerative disorders.

Traditional extraction methods often employ harsh solvents and high temperatures, potentially degrading these delicate bioactive compounds. UAE emerges as a gentler and more efficient alternative. By harnessing the power of ultrasound waves, UAE disrupts cell walls and enhances mass transfer, facilitating the release of desired compounds into the solvent (Chemat & Khan, 2011). This translates to faster extraction times, higher yields, and superior preservation of bioactive properties compared to conventional methods (Gong et al., 2019).

Currently popular was one such technique called ultrasonication. Ultrasonication was the process of introducing powerful ultrasonic waves into liquids and slurries. High shear forces and turbulences, along with notable pressure and temperature differentials, are all the result of intense sonication-induced acoustic cavitation. By stirring up particles, shattering droplets, and upsetting cells, these forces have the effects of homogenization, dispersion, emulsification, and extraction (Alice Muellemiestre 2017). Methanol was a potential substitute for conventional organic solvents in extraction processes due to its many benefits. High yields of excellent extracts can be obtained by combining methanol solvents with ultrasonic extraction (Qing Wen Zhang, 2018).

The unique attributes of both “bunga kantan” and UAE converge to create a potent synergy. The rich phytochemical profile of Etlingera elatior offers a plethora of antioxidant compounds for extraction, while UAE's gentle and efficient approach ensures their optimal recovery. Studies have shown that UAE significantly enhances the extraction yield and antioxidant activity of Etlingera elatiorextracts compared to conventional methods (Gong et al., 2019). This makes UAE a powerful tool for unlocking the full antioxidant potential of this remarkable plant.

The extracted *Etlingera elatior* antioxidants hold immense potential for diverse applications. They can be incorporated into functional foods and beverages to bolster their health benefits. Their antibacterial and anti-inflammatory properties make them promising candidates for natural preservatives and cosmetic ingredients. Furthermore, ongoing research explores their potential role in disease prevention and therapeutic interventions. *Etlingera elatior*, with its vibrant beauty and potent antioxidant arsenal, stands as a testament to nature's ingenuity. By harnessing the power of UAE, we can unlock its full potential, paving the way for novel nutraceuticals, pharmaceuticals, and cosmeceuticals. Further research continues to unravel the multifaceted applications of *Etlingera elatior*'s antioxidant riches, promising a future where this fragrant gem from Southeast Asia flourishes not only in gardens but also in the realm of human health and well-being.

1.2 Problem statement

The problem statement in this study was to investigate types of phytochemical compound exist in *Etlingera elatior* after extraction using ultrasonication assisted extraction in hexane, ethyl acetate and methanol. Limited study has been conducted to understand the potential of *Etlingera elatior* either in medical fields or product development. Therefore, the focus in this research study will be the antioxidant study using 2,2-diphenyl-1- picryhydrazyl (DPPH) and IC_{50} reading to understand the results better. The study also aims to give new insight and knowledge about the good and benefits of *Etlingera elatior*.

1.3 Objective

1. To extract Etlingera elatior using Ultrasonic Assisted Extraction (UAE) in hexane, ethyl acetate and methanol.
2. To investigate the antioxidant study using 2,2-diphenyl-1- picryhydrazyl (DPPH) free radical scavenging assay.
3. To investigate the types of phytochemical compound, exist was in Etlingera elatior using GC-MS.

1.4 Scope of study

This study assessed the phytochemical analysis and antioxidant activity of *Etlingera elatior* ultrasonicated in hexane, ethyl acetate and methanol extract. This research was conducted in University Malaysia Kelantan (UMK) Campus Jeli. The result was carried out after 2 months. As the result, conclude that the presence phytochemical compound exists in the sample extracted such as oleic acid. The higher antioxidant potential was recorded against DPPH free radicals.

1.5 Significance of study

According to this study, *Etlingera elatior* flower extract has a high antioxidant action against oxidative stress caused by lead and may be used as a therapy for lead poisoning. *Etlingera elatior* antioxidants were extracted by ultrasonography with a solvent mixture of methanol, hexane, and ethyl acetate. The most recommended method for separating phytochemical compounds from *Etlingera elatior* was ultrasonic extraction. Superior extract yields can be obtained in a relatively short amount of time thanks to sonication, which completes the extraction process. To identify the particular active antioxidants contained in this plant extract, more investigation was necessary.

CHAPTER 2

LITERATURE REVIEW

2.1 Etlingera elatior

Etlingera elatior flour washes in shady understories, reaching heights of up to 4 meters (Chan et al., 2011). Its striking appearance stems from its distinctive inflorescence, a dense cone-shaped cluster of bright pink bracts surrounding delicate white or yellow flowers (NParks, 2012). These bracts, which persist for several weeks, add a touch of exotic flair to gardens and floral arrangements. Beneath the showy exterior lies a network of fleshy rhizomes, the source of the plant's culinary and medicinal properties (Vimala, 2013). *Etlingera elatior* (torch ginger), a flowering plant native to Southeast Asia, has gained significant attention in recent years due to its diverse medicinal properties and potent antioxidant activity. Its flowers, leaves, and rhizomes are traditionally used in local communities for treating various ailments such as cough, fever, and rheumatism (Ng et al., 2013). Scientific research has increasingly validated these traditional uses, revealing a wealth of bioactive compounds like flavonoids, phenolics, and terpenoids in *Etlingera elatior*, contributing to its impressive antioxidant capacity (Ibrahim et al., 2019).

This chapter delves into the existing literature on the antioxidant activity of *Etlingera elatior* and explores the effectiveness of ultrasonic assisted extraction (UAE) as a method for maximizing the extraction yield and bioactivity of its antioxidant compounds. The immature flower buds of *Etlingera elatior* are the culinary star of the show. Their texture and fragrant, citrusy notes with a hint of ginger add a unique dimension to Southeast Asian cuisine. Finely chopped buds enhance salads like rojak and lend depth to fragrant curries like laksa (NParks, 2012). Beyond Southeast Asia, *Etlingera elatior* was gaining recognition among adventurous chefs, finding its way into innovative dishes and cocktails.

Etlingera elatior holds a deep-rooted connection with traditional medicine in Southeast Asia. Indigenous communities have long utilized its various parts for treating ailments like earaches, wounds, and even postpartum wastes (Vimala, 2013). Recent scientific research has shed light on the plant's potential pharmacological properties, including antibacterial, antifungal, and cytotoxic activities (Chan et al., 2011). These findings pave the way for further exploration of *Etlingera elatior*'s therapeutic potential.

Etlingera elatior's vibrant beauty and fragrant presence transcend the realm of culinary and medicinal uses. In cultures across Southeast Asia, it was woven into the fabric of tradition and folklore. Its striking inflorescence adorns ceremonial offerings, symbolizing prosperity, and good fortune. The delicate scent of *Etlingera elatior* fills the air during festive occasions, evoking a sense of cultural identity and belonging, with its alluring beauty, culinary versatility, and rich cultural significance stands as a testament to the wonders of the natural world. As we delve deeper into its botanical secrets and therapeutic potential, we can appreciate the multifaceted value of this captivating plant. By understanding and celebrating *Etlingera elatior*, we not only enrich our culinary repertoire and explore its medicinal possibilities, but also connect with the vibrant cultural tapestry of Southeast Asia.

2.2 Antioxidant study of Etlingera elatior

In recent years, there has been an increasing amount of literature in antioxidants and phytochemistry. *Etlingera elatior* inflorescence was known to have high antioxidant properties. Most of the studies on the antioxidant activities of inflorescence of E. elatior were limited to rhizomes and leaves (Maimulyanti et. al, 2015). Several studies also have demonstrated the strong antioxidant activity of *Etlingera elatior* extracts. Sulaiman et al. (2011) evaluated the antioxidant potential of different parts of the plant using various assays, including DPPH, ABTS, and FRAP. Their results revealed significant antioxidant activity in all parts, with the highest observed in the rhizomes, followed by the leaves and flowers. This finding suggests that *Etlingera elatior* can be a valuable source of natural antioxidants.

However, in this research the methodology chosen using DPPH to determine the antioxidant activity of *Etlingera elatior*. By using DPPH stock solutions, the antioxidant level of plants that had been extracted in Ethyl acetate, Hexane and Methanol can be studied based

on the results obtained through standard curve plot. A spectrophotometer was used to get the reading of abs of each extraction that has been diluted with DPPH stock solution but with different concentrations. It was important to understand the concept of how antioxidant activity works to get the research to achieve the objective. By understanding the formula, the correct reading for antioxidant study can be calculated accurately. The scavenging activity can be measured using Equation 2.1 below:

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

Equation 2.1 shows equation of how to calculate DPPH radical scavenging activity.

Numerous studies have revealed that oxidative stress, which can lead to the generation of free radicals, may have a major role in the development of several degenerative illnesses, asthenia diseases, pulmonary diseases, and inflammatory diseases. Free radicals can act as reductants or oxidants, giving or receiving an electron from biological molecules. These consist of nitric oxide radicals, hydroxyl radicals, superoxide, hydrogen peroxide, and oxygen singlet (Jadid et. al, 2017). The human body has evolved a special defence mechanisms to combat the presence of these free radicals through the synthesis of endogenous antioxidants (Jadid et al. 2017). Further research has identified the specific compounds responsible for this activity. Ibrahim et al. (2019) isolated and characterized flavonoids from *Etlingera elatior* flowers and demonstrated their potent free radical scavenging capacity. Likewise, Ting et al. (2020) attributed the antioxidant activity of *Etlingera elatior* leaves to the presence of phenolic acids and terpenoids. These studies highlight the complex interplay of various bioactive compounds contributing to the overall antioxidant capacity of *Etlingera elatior*.

2.2.1 Hexane, Ethyl Acetate and Methanol as solvent

The most widely used and documented solvents for oil extraction are hexane, petroleum ether, diethyl ether, ethanol, n-heptane, isopropanol, acetone, chloroform, methanol, and 1-butanol (Shivani et al., 2011). Plants, oil extraction efficiency, impact on the environment, and renewability of various extraction solvents are all subject to variation. Hexane, Ethyl Acetate and Methanol has been used as the extraction solvent in this study. Antioxidants can

be extracted from plants using a variety of methods, including maceration, Soxhlet extraction, subcritical water extraction, supercritical fluid extraction, and ultrasound aided extraction. However, the solvent employed for extraction has an impact on antioxidant activity and extraction yield in addition to the extraction technique. Different antioxidant chemicals may or may not be soluble in each solvent depending on their molecular properties and polarity (Nihal et. al, 2006). Polyphenols are commonly extracted from plant matrices using polar solvents. Aqueous combinations of methanol, ethanol acetone, and ethyl acetate, respectively, are the best solvents. It is well established that ethanol is a safe solvent to use in polyphenol extraction and that it is safe for ingestion by humans. Higher molecular mass flavanols can be effectively extracted using aqueous acetone, but lower molecular weight polyphenols are often more effectively extracted using methanol (Do et. al, 2014).

2.2.2 Ultrasonicated of plant extraction

Ultrasonic assisted extraction (UAE) has emerged as a promising alternative to conventional extraction methods for *Etlingera elatior* due to its numerous advantages. UAE utilizes high-frequency ultrasonic waves to create cavitation bubbles in the solvent, promoting mass transfer and enhancing the release of bioactive compounds from the plant matrix (Chew et al., 2015). This leads to several benefits, including reduction of extraction time. UAE significantly reduces the extraction time compared to conventional methods, often by up to 50% or more (Kadam et al., 2016). This not only improves efficiency but also minimizes the potential degradation of heat-sensitive antioxidants. Next, Increased extraction yield**.** The cavitation effect of ultrasound disrupts cell walls and facilitates enhanced penetration of the solvent into the plant material, resulting in higher yields of extracted antioxidants (Chew et al.,2015). Thus, improved selectivity. By adjusting the ultrasonic parameters, UAE can be tailored to selectively extract specific antioxidant compounds from *Etlingera elatior*, offering greater control over the composition of the extract. Reduced solvent consumption. UAE often requires lower solvent volumes compared to conventional methods, making it a more environmentally friendly and sustainable approach (Kadam et al., 2016).

2.2.3 Factors affecting antioxidant study of *Etlingera elatior*

Several factors influence the antioxidant activity of *Etlingera elatior*, including plant part. As mentioned earlier, different parts of the plant exhibit varying levels of antioxidant activity. Studies have shown that rhizomes typically possess the highest concentration of antioxidants, followed by leaves and flowers (Sulaiman et al., 2011). Next, the extraction method**.** The extraction method significantly impacts the yield and bioactivity of antioxidant compounds. Conventional methods like maceration and reflux extraction often suffer from limitations such as prolonged extraction times, high solvent consumption, and potential degradation of heat-sensitive compounds (Kadam et al., 2016). Moreover, another factor could be solvent selection. The choice of solvent plays a crucial role in selectively extracting different antioxidant compounds from *Etlingera elatior*. Polar solvents like methanol and ethanol are effective for extracting phenolics and flavonoids, while non-polar solvents like hexane are more suitable for terpenoids (Ting et al., 2020). Finally, extraction parameters. Factors like extraction temperature, time, and solvent-to-material ratio influence the efficiency and selectivity of antioxidant extraction. Optimizing these parameters was crucial for maximizing the yield and bioactivity of desired compounds (Chew et al., 2015).

2.3 Effectiveness of UAE for Antioxidant Extraction from Etlingera elatior

Several studies have demonstrated the effectiveness of UAE for extracting antioxidants from E. elatior. Mohamed et al. (2013) compared UAE with maceration and reported a 2.5-fold increase in total phenolic content and a 4-fold increase in antioxidant activity using UAE. Similarly, Chew et al. (2019) observed a 30% higher total phenolic yield and a 20% higher antioxidant capacity with UAE compared to maceration. These findings highlight the superior efficiency of UAE in extracting bioactive antioxidants from E. elatior.

The effectiveness of UAE was strongly influenced by its operating parameters, including solvent type, ultrasonic power, extraction time, and temperature (Zakaria et al., 2019). Optimization of these parameters was crucial for maximizing antioxidant yield and activity. For instance, studies have shown that ethanol-water mixtures are generally more effective solvents than pure water for extracting phenolic compounds from E. elatior (Mohamed et al., 2013). Additionally, moderate ultrasonic power levels and extraction times often provide

optimal results, while excessively high temperatures can degrade antioxidant activity (Zakaria et al., 2019).

The identification of bioactive compounds and pharmaceutical applications also has been determined. UAE extracts of *Etlingera elatior* have been shown to contain a diverse range of bioactive compounds with potential pharmaceutical applications. Phenolic acids such as gallic acid, ellagic acid, and p-coumaric acid exhibit potent antioxidant, anti-inflammatory, and anticancer properties (Zakaria et al., 2017). Flavonoids like quercetin and kaempferol possess similar bioactivities and additionally demonstrate neuroprotective and antidiabetic effects (Mohamed et al., 2013). Gingerols, the characteristic pungent compounds of E. elatior, exhibit anti-inflammatory, analgesic, and antitumor activities (Zakaria et al., 2019).

These identified bioactive compounds hold promising potential for various pharmaceutical applications. The antioxidant properties of E. elatior extracts could be harnessed in nutraceuticals and functional foods to combat oxidative stress-related diseases (Chew et al., 2019). The anti-inflammatory and analgesic activities of gingerols could be explored in the development of natural pain relievers and anti-inflammatory agents (Zakaria et al., 2019). Furthermore, the anticancer potential of phenolic acids and flavonoids warrants further investigation for the development of novel cancer therapies (Mohamed et al., 2013).

2.4 GC-MS Analysis

In this research, methodology used to identify the potential phenolic compound was using Gas Chromatographic Mass Spectrophotometer (GC-MS). As an example, in a study by Mohamed et al. (2013), UAE with ethanol-water mixtures was employed to extract phenolics from *Etlingera elatior*. GC-MS analysis revealed the presence of various phenolic acids and flavonoids, contributing to the potent antioxidant activity observed in the extract. Another study also shows that from Zakaria et al. (2017) used GC-MS to identify and quantify phenolic compounds in different parts of Orthosiphon stamina's. The findings demonstrated significant variations in phenolic profiles between leaves, stems, and flowers, highlighting the importance of tissue selection for maximizing phenolic extraction.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Etlingera elatior was bought from the morning market, dried at temperatures of 60ºC for 24 hours. The dried *Etlingera elatior* was then blended using a heavy-duty blender and crushed using ball milling in order to get the powdery effect of *Etlingera elatior*.

3.2 Extraction

Etlingera elatior was cleaned and washed with clean water. The material was cut into small pieces and been blend until become a dust and ready for extraction, 10 grams of fresh *Etlingera elatior* flower buds were mixed in 150 ml of hexane, ethyl acetate and methanol.

3.3 Ultrasonication extraction method

The powered flower buds of *Etlingera elatior* were then macerated with methanol, ethyl acetate and hexane solvent. Then it was sonicated using sonicator Fisher Scientific (FB 15063). The extract was filtered with Whatman filter paper o. 1 in a 28 Bunchner funnel were concentrated using vacuum with a rotary evaporator. Parameter set at 30ºC for the

temperature, frequency of ultrasonicating at 20kHz and time set for 45 minutes (3 Times stop). All samples were stored in the dark at 4 °C until further analysis. After the extraction, min of yield calculate in percentage as the reading of yield and crude has been recorded and repeated three times.

3.4 Antioxidant Activity

The antioxidant activity was evaluated by the free radical scavenging activity (DPPH) method. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was free radical but stable. DPPH solution was initially violet in color which fades when antioxidants are donated hydrogen. The change in color was monitored by spectrophotometer and DPPH free radical scavenging activity was calculated. Stock solutions of 0.1 mM DPPH in methanol were made. Test samples of extract were made at 200, 100, 50, and 25 µg/mL in methanol, hexane and ethyl acetate. The absorbance was measured at 514 nm by spectrophotometer pharmaspec UV-1700 (Shimadzu). After 30 minutes and % scavenging was calculated by the equation where, Ao = Absorbance of DPPH solution and AT = Absorbance of test or reference sample. The % scavenging was then plotted against concentration and regression line were obtained in the graph to calculate IC_{50}. IC_{50} was defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%.

3.5 Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS was performed using a Hewlett Packard 6890 Gas Chromatograph with a 5973N Mass Selective Detector. The HP-5 column (30m x 0.25 mm x 0.25 µm film thickness) was made of fused silica capillary (Agilent Technologies, USA). Helium was used as the carrier gas, flowing at a rate of 1.0 ml/min. The oven temperature was controlled to raise at a rate of 20°C/min from 50°C (held for two minutes) to 280°C (held for ten minutes). The temperatures of the injection and interface were established at 250°C and 280°C, correspondingly. Spitless mode was used to inject a 1-ml sample, which was then analysed in MS full scan mode (m/z 40-650). At 70 eV, the electron ionization was fixed. The ChemStation programme was used to acquire the data.

3.5.1 Identification of Phytochemical Compounds

The mass spectrum of the GC-MS was interpreted based on the database of the National Institute of Standards and Technology (NWAST02) and Wiley275 libraries with matches of ≥80 % to identify the phytochemical compounds.

CHAPTER 4

RESULT AND DISSCUSSION

The chapter presents the results of the phytochemical analysis and antioxidant activity of *Etlingera elatior* using pure hexane, ethyl acetate and methanol solvent gathered from the research. Data presented and analysed include their discussion.

4.1 Extraction of *Etlingera Elatior* in hexane, ethyl acetate and methanol solvent

In this study, the extraction method and the result extraction of *Etlingera elatio*r with presented include discussion.

Table 4.1 shows yield percentage for extraction of *Etlingera elatior* in hexane, ethyl acetate and methanol solvent.

Extraction of *Etlingera elatior* in Hexane Solvent

	Repeat 1	Repeat 2	Repeat 3
Dried Ee	10.00g	10.00g	10.00g
Crude extraction of Ee	0.911g	0.956g	0.998g
Yield (%)	9.11	9.56	9.98
Min of Yield (%)		9.55	

Extraction of *Etlingera elatior* in Ethyl Acetate Solvent

	Repeat 1	Repeat 2	Repeat 3
Dried Ee	10.00g	10.00g	10.00g
Crude extraction of Ee	0.951g	0.943g	0.901g
Yield (%)	9.51	9.43	9.01
Min of Yield (%)		9.31	

Extraction of *Etlingera elatior* in Methanol Solvent

	Repeat 1	Repeat 2	Repeat 3
Dried *Etlingera elatior*	10.00g	10.00g	10.00g
Crude extraction	0.958g	0.901g	1.011g
Yield (%)	9.58	9.01	10.11
Min of Yield (%)		9.56	

Based on the table above, to extract phytochemicals from plants, several processes must be completed, including homogenization, extraction, grinding, and milling. The primary procedure for extracting phytochemicals from plant substances is extraction, out of all these procedures. The chemical makeup of phytochemicals, the extraction technique, sample particle size, the solvent utilised, and the existence of interfering compounds all have an impact on extraction efficiency (Do et al, 2014). The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the sample. Under the same extraction time and temperature, solvent and composition of sample are known as the most important parameters. In this study, *Etlingera elatior* extracts were obtained by using water and different concentrations of hexane, ethyl acetate and methanol. Extraction yields ranged from 9.58% for hexane extract to 10.11% for *Etlingera elatior* extract in hexane solvent (Table 4.1.1).

The min of yield percentage in pure hexane will be 9.56%. Meanwhile in pure ethyl acetate, the extraction yields ranged from 9.11% for hexane extract to 9.98%. The min of yield percentage in pure ethyl acetate will be 9.55%. Thus, yield of *Etlingera elatior* extract in methanol range from 9.51% to 9.01%. The min of yield percentage in pure methanol will be 9.31%. The min of yield percentage shows consistency in documentation of the yield percentage. These finding shows that it is possible that compounds other than phenolics were removed, which increases yield. This could be explained by the fact that proteins and carbohydrates dissolve more readily in water and methanol than in ethanol and acetone (Do et al, 2014).

4.2 Antioxidant activity for *Etlingera elatior* extract in hexane, ethyl acetate and methanol solvent.

In this section showed the result of antioxidant activity of *Etlingera elatior* by using 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay.

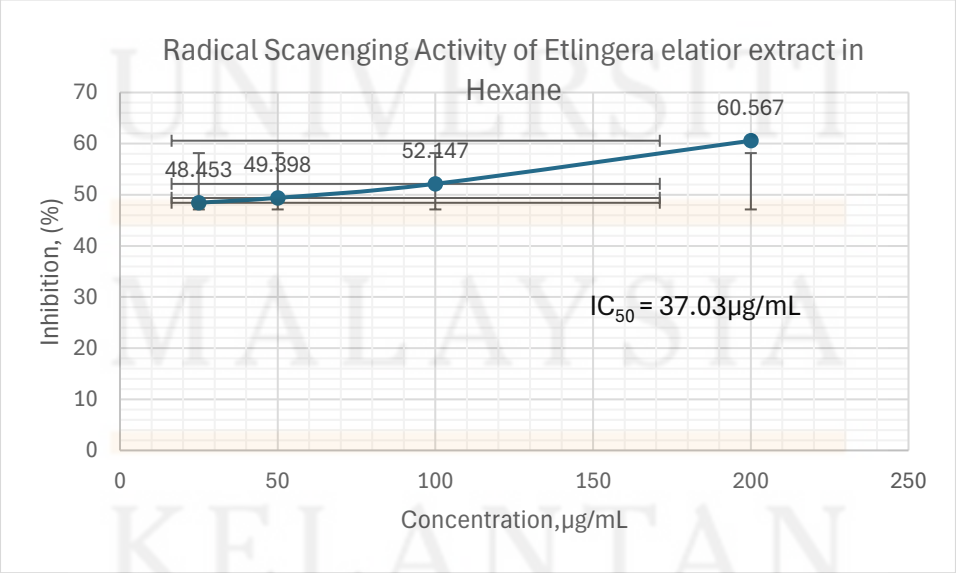
4.2.1 Antioxidant activity of *Etlingera elatior* extract in hexane solvent.

Table 4.2.1 RSA of *Etlingera elatior* extract against DPPH in hexane solvent.

Calculation of Radical Scavenging Activity (RSA) and IC_{50} from DPPH Assay				
Absorbance				
Concentration	**Control**	**Sample**	**% RSA**	**IC_{50}**
25	1.164	0.600	48.4	37.03
50	1.164	0.589	49.3	
100	1.164	0.557	52.1	
200	1.164	0.459	60.5	

*%RSA = Percentage of Radical Scavenging Activity

Figure 4.2.1 *Etlingera elatior* percentage of scavenging DPPH at different concentration in hexane extract.



In this study, showed that The DPPH radical scavenging activity in hexane extract from *Etlingera elatior* were recorded in terms of percentage inhibition or percentage scavenging of DPPH as shown in table 4.2.1 and Figure 4.2.1. The result showed that the absorbance decreases because of a colour change from purple to yellow, as the radical was scavenged by anti-radicals, through donated of hydrogen to give reduced from DPPH. The linear regression found in Figure 4.2.1. IC_{50} value was determine through the graph that has been plotted. The ability of *Etlingera elatior* in different extract to donated proton to DPPH free radical was accessed in this assay. Concentration of extract scavenging 50% of DPPH radical shown in Table 4.2.1 and Figure 4.2.1. The concentration antioxidant activity of *Etlingera elatior* was (IC_{50} = 37.03µg/mL). Increased free radical activity was shown by the reaction mixture's lower absorbance. The following formula was used to determine the % DPPH scavenging effect % inhibition or DPPH scavenging action = $A_0 – A_1 / A_0 \times 100$ where A_1 represented the absorbance while the test or standard sample was present, and A_0 represented the absorbance of the control response (Leaves and Leaves 2014).

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

However, based on the IC_{50} result of 37.03µg/mL indicate that antioxidant activity does exists in the plant extraction of *Etlingera elatior* in hexane solvent. A compound's IC_{50}, which was achieved based on the graph, expresses the quantity of antioxidant needed to reduce the DPPH concentration by 50%. It was inversely correlated with a compound's antioxidant capability. A compound's increased antioxidant activity was indicated by a lower IC_{50}. The extracts' IC_{50} values for the DPPH radical scavenging activity experiment are displayed based on the research. The highest DPPH radical activity was discovered in the 100% ethanol extract (IC_{50}= 70.06 mg/mL) (Do, Angkawijaya et al. 2014). Based on the IC_{50} result of plant extract in hexane solvents, it can be concluded that the radical scavenging activity of antioxidant activity ability at good category to scavenge free radical matter.

To gain a broader perspective on Etlingera elatior's antioxidant potential, let's consider a study by (Zhou et al, 2018), which employed the Ultrasound-Assisted Extraction (UAE) method instead of hexane extraction. This study revealed an IC_{50} value of 22.5 µg/mL for the Etlingera elatior extract obtained using the UAE method. The IC_{50} value of 22.5 µg/mL achieved through the UAE method is lower than the 37.3 µg/mL obtained with

hexane extraction. This suggests that the UAE method might be more efficient in extracting potent antioxidant compounds from Etlingera elatior. This observation highlights the potential influence of extraction methods on the yield and bioactivity of the extracted compounds. The choice of solvent plays a crucial role in the extraction process. Hexane is a nonpolar solvent, primarily extracting nonpolar compounds like fats and oils. In contrast, the UAE method often utilizes polar solvents like water or ethanol, which are more effective in extracting polar compounds like phenolic antioxidants. This difference in solvent polarity likely explains the observed variation in IC_{50} values between the two studies.

An IC_{50} value of 37.3 µg/mL in hexane solvent classifies the sample as a strong antioxidant. However, it's essential to remember that this is just one indicator of its potential health benefits. Further research, including cell-based and in vivo studies, is necessary for a comprehensive evaluation. Additionally, exploring different extraction methods, as demonstrated by the study using the UAE method, can provide valuable insights into optimizing the extraction of bioactive compounds from natural sources. Choosing the appropriate extraction method based on the target compounds and their desired properties is crucial for maximizing the potential benefits of natural products like *Etlingera elatior*.

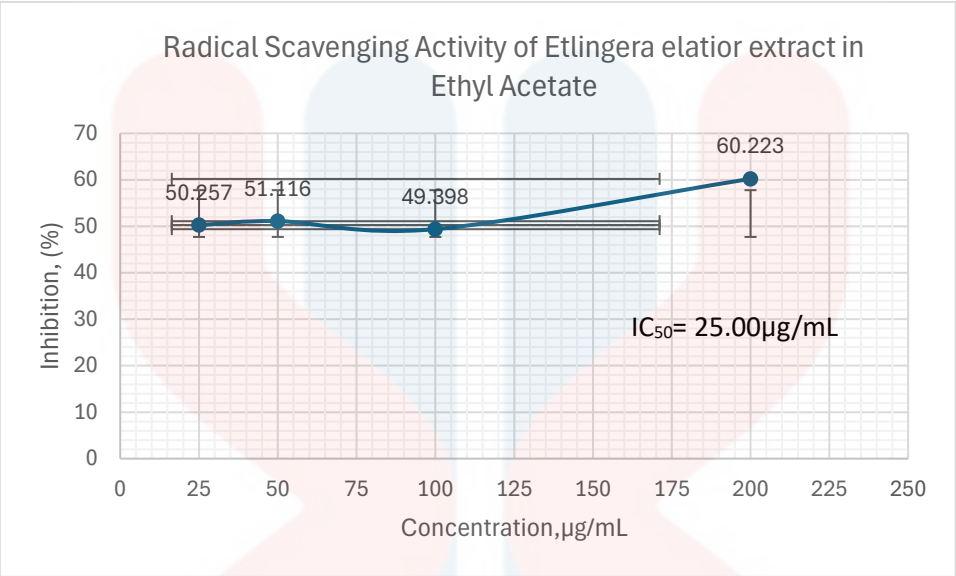
4.2.2 Antioxidant activity of *Etlingera elatior* extract in ethyl acetate solvent.

Table 4.2.2 RSA of *Etlingera elatior* extract against DPPH in ethyl acetate solvent.

Calculation of Radical Scavenging Activity and IC_{50} from DPPH Assay				
Absorbance				
Concentration	**Control**	**Sample**	**% RSA**	**IC_{50}**
25	1.164	0.579	50.2	25.00
50	1.164	0.569	51.1	
100	1.164	0.589	49.3	
200	1.164	0.463	60.2	

*%RSA = Percentage of Radical Scavenging Activity

Figure 4.2.2 *Etlingera elatior* percentage of scavenging DPPH at different concentration in ethyl acetate extract.



In this study, showed that The DPPH radical scavenging activity in methanol extract from *Etlingera elatior* were recorded in terms of percentage inhibition or percentage scavenging of DPPH as shown in Table 4.2.2 and Figure 4.2.2. The result showed that the absorbance decreases because of a colour change from purple to yellow, as the radical was scavenged by anti-radicals, through donated of hydrogen to give reduced from DPPH. The linear regression found in Figure 4.2.2. IC_{50} value was determine through the graph that has been plotted. The ability of *Etlingera elatior* in different extract to donated proton to DPPH free radical was accessed in this assay. Concentration of extract scavenging 50% of DPPH radical shown in Table 4.2.2 and Figure 4.2.2. The concentration antioxidant activity of *Etlingera elatior* was (IC_{50} = 25.00µg/mL). Increased free radical activity was shown by the reaction mixture's lower absorbance. The following formula was used to determine the percentage DPPH scavenging effect of percentage inhibition or DPPH scavenging action = $A_0 – A_1 / A_0 \times 100$ where A_1 represented the absorbance while the test or standard sample was present, and A_0 represented the absorbance of the control response (Leaves and Leaves 2014).

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

Based on the classification, an IC_{50} value of 25.00 µg/mL in ethyl acetate solvent can be categorized as a strong antioxidant. This indicates a notable capacity to scavenge free radicals, potentially offering promising implications for its use as a therapeutic agent or nutraceutical ingredient. Based on the classification, an IC_{50} value of 25.00 µg/mL in ethyl acetate solvent can be categorized as a strong antioxidant. This indicates a notable capacity to scavenge free radicals, potentially offering promising implications for its use as a therapeutic agent or nutraceutical ingredient.

To gain a broader perspective on *Etlingera elatior*'s antioxidant potential, let's consider a study by (Zhou et al, 2018), which employed the Ultrasound-Assisted Extraction (UAE) method instead of ethyl acetate extraction. This study revealed an IC_{50} value of 22.5 µg/mL for the *Etlingera elatior* extract obtained using the UAE method. The IC_{50} values of both studies, 25.00 µg/mL for ethyl acetate and 22.5 µg/mL for UAE, fall within the category of strong antioxidants. However, the slightly lower IC_{50} value obtained with the UAE method suggests that it might be more efficient in extracting potent antioxidant compounds from *Etlingera elatior*.

The choice of solvent plays a crucial role in the extraction process. *Ethyl acetate* is a moderately polar solvent, capable of extracting a wider range of compounds compared to nonpolar solvents like hexane. However, compared to highly polar solvents like water or ethanol, it might have lower efficiency in extracting certain polar antioxidants like phenolic compounds. This difference in solvent polarity likely contributes to the observed variations in IC_{50} values between the two studies. An IC_{50} value of 25.00 µg/mL in ethyl acetate solvent classifies the sample as a strong antioxidant, indicating significant free radical scavenging potential. However, it is essential to remember that this is just one indicator, and further research, including cell-based and in vivo studies, is necessary for a comprehensive evaluation. Additionally, exploring different extraction methods, as demonstrated by the study using the UAE method, can provide valuable insights into optimizing the extraction of bioactive compounds from natural sources. Choosing the appropriate extraction method based on the target compounds and their desired properties is crucial for maximizing the potential benefits of natural products like *Etlingera elatior*.

4.2.3 Antioxidant activity of *Etlingera elatior* extract in methanol solvent.

Table 4.2.3 RSA of *Etlingera elatior* extract against DPPH in methanol solvent.

Calculation of Radical Scavenging Activity (RSA) and IC_{50} from DPPH Assay				
Absorbance				
Concentration	**Control**	**Sample**	**% RSA**	**IC_{50}**
25	1.164	0.618	46.9	39.10 µg/mL
50	1.164	0.553	52.5	
100	1.164	0.463	60.2	
200	1.164	0.448	61.5	

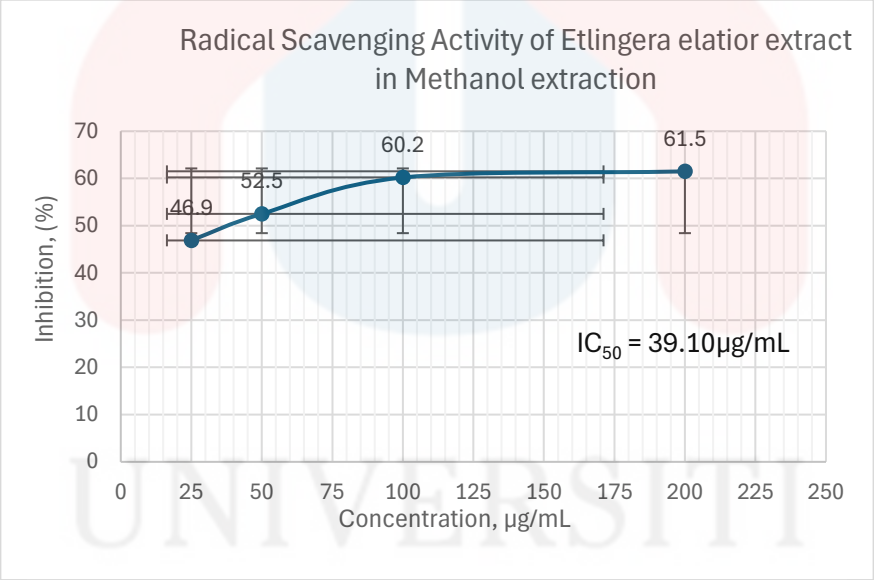


Figure 4.2.3 *Etlingera elatior* of % scavenging of DPPH at different concentration in methanol extract.

In this study, showed that The DPPH radical scavenging activity in methanol extract from *Etlingera elatior* were recorded in terms of percentage inhibition or percentage scavenging of DPPH as shown in Table 4.2.3 and Figure 4.2.3. The result showed that the absorbance decreases because of a colour change from purple to yellow, as the radical was scavenged by anti-radicals, through donated of hydrogen to give reduced from DPPH. The linear regression found in Figure 4.2.3. IC_{50} value was determine through the graph that has been plotted. The ability of *Etlingera elatior* in different extract to donated proton to DPPH free radical was accessed in this assay. Concentration of extract scavenging 50% of DPPH

radical shown in Table 4.2.3 and Figure 4.2.3. The concentration antioxidant activity of *Etlingera elatior* was (IC_{50} = 39.10µg/mL). Increased free radical activity was shown by the reaction mixture's lower absorbance. The following formula was used to determine the percentage DPPH scavenging effect of percentage inhibition or DPPH scavenging action = $A_0 – A_1 / A_0 \times 100$ where A_1 represented the absorbance while the test or standard sample was present, and A_0 represented the absorbance of the control response (Leaves and Leaves 2014).

$$\text{DPPH radical scavenging activity} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100\%.$$

However, based on the IC_{50} result of 39.10µg/mL indicate that antioxidant activity does exists in the plant extraction of *Etlingera elatior* in methanol. A compound's IC_{50}, which was achieved based on the graph, expresses the quantity of antioxidant needed to reduce the DPPH concentration by 50%. It was inversely correlated with a compound's antioxidant capability. A compound's increased antioxidant activity was indicated by a lower IC_{50}. The extracts' IC_{50} values for the DPPH radical scavenging activity experiment are displayed based on the research. In the realm of antioxidant research, the IC_{50} value serves as a crucial gauge for assessing a substance's efficacy in neutralizing free radicals. It signifies the concentration required to inhibit 50% of the activity, often measured in the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. While a definitive threshold universally defining a "good" IC_{50} does not exist, general classifications provide valuable insights. Typically exhibit IC_{50} values below 50 µg/mL. Examples include well-known antioxidants like vitamin C and certain plant-derived polyphenol. Moderate antioxidant of IC_{50} values generally fall within the range of 50 to 100 µg/mL Meanwhile weak antioxidant characterized by IC_{50} values exceeding 100 µg/mL. Based on this classification, the sample under investigation, with an IC_{50} of 39.1 µg/mL in methanol solvent, can be categorized as a strong antioxidant. This indicates a notable capacity to scavenge free radicals, potentially offering promising implications for its use as a therapeutic agent or nutraceutical ingredient.

It is crucial to acknowledge that the IC_{50} value derived from the DPPH assay provides only a preliminary assessment. Other factors influencing a substance's overall antioxidant potential including bioavailability. Even if a compound demonstrates a low IC_{50} in vitro, its effectiveness in the human body depends on how well it is absorbed, distributed,

and metabolized. Next, specificity. Different free radicals possess varying reactivities, and an antioxidant might exhibit selectivity towards certain types of free radicals over others. Thus, synergy. The human body possesses a complex network of antioxidants working synergistically to combat free radicals. While the DPPH assay evaluates a single compound in isolation, the true antioxidant potential might be greater when combined with other antioxidants.

To gain a broader perspective on *Etlingera elatior*'s antioxidant potential, let's consider a study by (Mao et al., 2018) that employed the Ultrasound-Assisted Extraction (UAE) method instead of methanol extraction. This study revealed an IC_{50} value of 12.9 µg/mL for the *Etlingera elatior* extract obtained using the UAE method. The IC_{50} value of 12.9 µg/mL, achieved through the UAE method, is notably lower than the 39.1µg/mL obtained with methanol extraction. This suggests that the UAE method might be more efficient in extracting potent antioxidant compounds from *Etlingera elatior*. This observation highlights the potential influence of extraction methods on the yield and bioactivity of the extracted compound. Although the sample is classified as a powerful antioxidant with the IC_{50} value of 39.1 µg/mL in methanol solvent, it is important to keep in mind that this is only one piece of the evidence. Additional investigation, encompassing both in vitro and in vivo investigations, is needed to thoroughly assess the possible health advantages of this specimen. Furthermore, as the study employing the UAE method shows, experimenting with various extraction techniques might yield important insights for maximising the extraction of chemicals that are bioactive from natural sources.

4.3 IC_{50} data analysis of Etlingera elatior extraction in methanol, hexane and ethyl acetate.

Table 4.3 shows IC50 reading average for sample extracted in hexane, ethyl acetate and methanol that had been repeated in three times.

IC_{50} Reading Average	
Solvent	**Average**
Hexane	35.94
Ethyl Acetate	36.64
Methanol	26.12

To ensure the accuracy and reliability of IC_{50} reading of each alcoholic extracted of *Etlingera elatior*, a DPPH radical Scavenging activity has been repeated three times to measure the sustainability of IC_{50} reading for hexane extraction, methanol extraction and ethyl acetate extraction. By measuring the reading three times at four different concentration which are 25, 50, 100 and 200 with 0.1mM of DPPH diluted in methanol solvent. A graph has been plotted to shows the reading of IC_{50} in three different days where the reading was measured in methanol, hexane and ethyl acetate at 517nm abs.

Based on the table above, for plant extracted in hexane solvent average reading of IC_{50} was 39.54µg/mL. At this rate, the most efficient reading of IC_{50} for plants extracted in hexane can be concluded that *Etlingera elatior* that has been extracted in hexane solvent does have scavenging activity in fighting free radical. Meanwhile for plant extracted in ethyl acetate solvent, the average reading of IC_{50} was 36.64µg/mL. At this rate, the most efficient reading of IC_{50} for plant extracted in ethyl acetate solvent can be concluded that *Etlingera elatior* that has been extracted in hexane solvent does have scavenging activity in fighting free radical.

Thus, it can be concluded that IC_{50} average reading for plant extraction in methanol was 26.12µg/mL. The most efficient reading chosen which was 26.12µg/mL as it can be indicate that the lower the reading the better the radical scavenging ability in this antioxidant study.

This signifies that the methanol extract possessed the highest antioxidant activity, requiring the least amount of extract to scavenge 50% of the DPPH free radicals compared to the other solvents. Conversely, the hexane extracts consistently demonstrated the highest IC_{50} values (36.64µg/mL), indicating the weakest antioxidant activity among the tested solvents. The ethyl acetate extract displayed intermediate antioxidant activity with IC_{50} values ranging AT 25.00µg/mL. These trends remained consistent across all three reading days, suggesting good reproducibility of the results.

Several factors could contribute to the observed differences in antioxidant activity between the extracts could be solvent polarity. Methanol was the most polar solvent among the three, followed by ethyl acetate and then hexane. Polarity plays a crucial role in extracting different classes of bioactive compounds from plant materials. More polar solvents can extract a wider range of polar compounds, including phenolic compounds and flavonoids, known for their antioxidant properties (Dai & Mumper, 2010). This aligns with the observed trend of higher antioxidant activity in the more polar methanol extract compared to the less polar hexane extract.

Next, compound solubility. Different bioactive compounds have varying solubilities in different solvents. The methanol extract might have effectively solubilized compounds with significant antioxidant potential, while the hexane extract may have primarily extracted compounds with lesser antioxidant activity. Moreover, synergistic effects. The antioxidant activity of plant extracts often erases from the combined action of multiple bioactive compounds, rather than solely due to individual ones (Synymon & Kalogeropoulos, 2017). The methanol extract might have extracted a combination of compounds that synergistically enhance its antioxidant activity compared to the other extracts.

4.4 GC-MS Analysis of *Etlingera elatior* extract in methanol, hexane, and ethyl acetate solvent.

Based on the graph and table belows, chemical compound along with its function of chemical has been defined in order to identify the potential of *Etlingera elatior*'s in pharmacology field.

4.4.1 GC-MS analysis of *Etlingera elatior*'s extract in hexane solvent.

Figure 4.41 shows GC-MS analysis for *Etlingera elatior*'s extract in hexane graph composition.

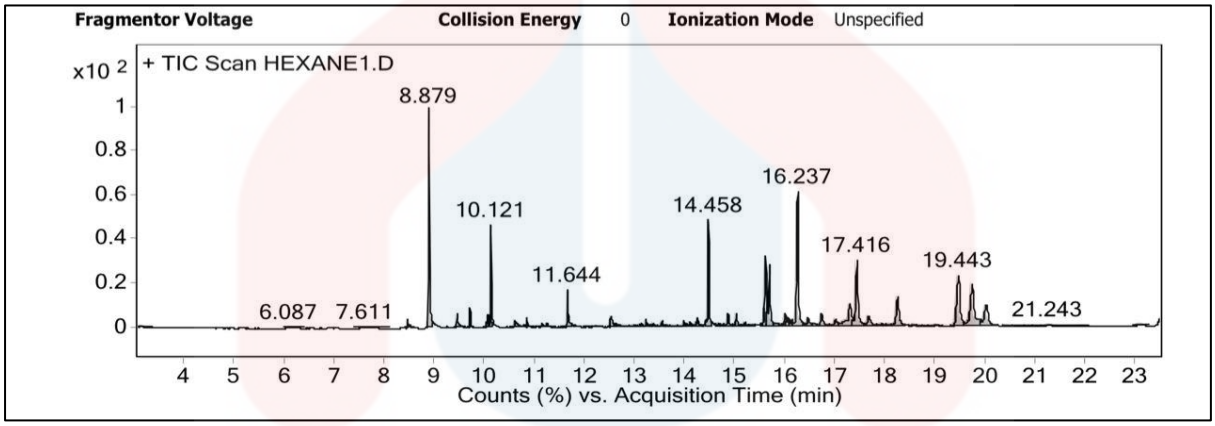


Table 4.4.1 Major compound in *Etlingera elatior* extract in hexane solvent and classifications.

No.	Compound Name	Molecular Formula	Rt (Min)	Percentage Area	Peak Score	Biological Activity
1	Dodecanal	$C_{12}H_{24}O$	8.459	0.32	94	possible toxic activity against tropical mosquitoes transmitting dangerous illnesses

						(Sharopov, Valiev et al. 2017)
2	Caryophyllene	$C_{15}H_{24}$	8.645	0.02	95	anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016)
3	Bicyclo[7.2.0]undec-4-ene, 4,11,11 -trimethyl-8-methylene-,[1R-(1R*,4 Z,9S*)]-	$C_{15}H_{24}$	8.645	0.02	87	antimicrobial properties (Rahman, Ahmad et al. 2014)
4	Dodecanoic acid	$C_{12}H_{24}O_2$	9.445	1.01	99	strong antimicrobial activity against many Gram-positive bacteria (Nakatsuji, Kao et al. 2009)
5	Vaccenic acid	$C_{18}H_{34}O_2$	9.964	0.06	86	enhances anti-tumor immunity (Fan, Xia et al. 2023)
6	Acetic acid, chloro-, octadecyl ester	CH_3COOH	10.438	0.16	96	No study found
7	Chloroacetic acid,	$C_{17}H_{33}ClO_2$	10.438	0.16	93	No study found

	pentadecyl este					
8	Tetradecanoic acid	$C_{14}H_{28}O_2$	10.596	0.87	98	antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014)
9	Trichloroacetic acid, hexadecyl es	$C_{18}H_{33}Cl_3O_2$	10.734	0.09	90	No study found
10	1-Octadecene	$C_{18}H_{36}$	10.734	0.09	86	No study found
11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	11.465	0.08	95	antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, hemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015)
12	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	12.748	0.09	90	No study found
13	Decanoic acid	$C_{10}H_{20}O_2$	13.670	0.11	91	strong antimicrobial activity against many Gram-positive bacteria (Nakatsuji, Kao et al. 2009)
14	Eicosane	$C_{20}H_{42}$	13.637	0.26	95	No study found

There are many chemical compounds and their biological activity defined in extraction of *Etlingera elatior*'s in hexane solvent. To understand the better scorer compound in this sample, major compound of samples has been listed and identified the biological activity. Thus, this way analysis toward the potential impact of *Etlingera elatior*'s can be seen. The highest score of chemical compounds with score of 99% in the table of major compound found with score of >80% will be the presence of dodecanoic acid. Dodecanoic acid also known as strong antimicrobial activity against many Gram-positive bacteria (Nakatsuji, Kao et al. 2009). Moreover, tetradecanoic also found in this sample which function as a antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014). Next, hexadecenoic acid, methyl ester are also compound that has major score in the sample with 95% which function as antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, hemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015). Caryophyllene also found in this sample with score of 85%, function as anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016). To achieve the objective of the experiment, based on the major compound with highest score above 80% in the sample found are more focusing to the compound that function as antioxidant activity.

However, there are also chemical compounds with major score that the biological activity has no study found yet about the function of the chemical compound. It has come to the limitations of research where there is no further study about these compounds function. The chemical compound such as 9,12-Octadecadienoic acid at 90% score and eicosane at 95% score. Based on the findings, it can be concluded that the *Etlingera elatior*'s extract in hexane solvent has many chemical compounds found with various number of biological activities indicate it can fight radical particle through scavenging of antioxidant activity.

4.4.2 GC-MS analysis of *Etlingera elatior*'s extract in Ethyl Acetate solvent.

Figure 4.4.2 shows GC-MS analysis for *Etlingera elatior*'s extract in ethyl acetate solvent graph composition.

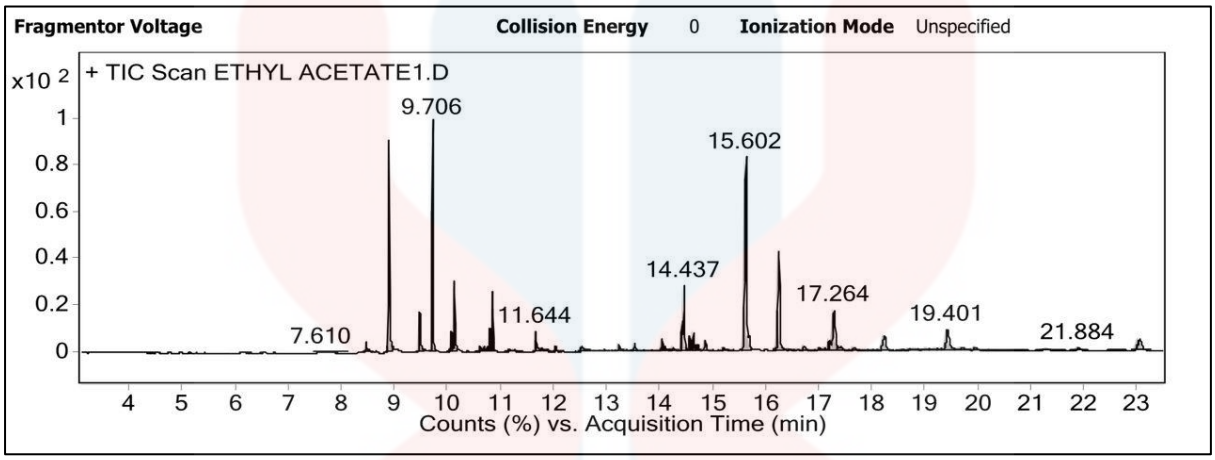


Table 4.4.2 GC-MS Analysis of Etlingera elatior of ethyl acetate1 in ethanol and classifications.

No. Peak	Compound Name	Molecular Formula	Rt (Min)	Percentage Area	Score	Biological Activity
1	Undecane	$C_{11}H_{24}$	6.086	0.04	86	No study found
2	Caryophyllene	$C_{15}H_{24}$	8.644	0.08	99	anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016)
3	Pentafluoropropionic acid, dodecyl ester	$C_3HF_5O_2$	9.024	0.73	94	No study found

4	Chloroacetic acid, tridecyl ester	$C_2H_3ClO_2$	9.024	0.73	91	No study found
5	Cyclododecane	$C_{12}H_{24}$	9.279	0.88	98	No study found
6	cwas-9-Tetradecen-1-ol	$C_{14}H_{28}O$	10.065	1.03	91	No study found
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	10.603	0.56	98	antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014)
8	Cyclohexadecane		11.244	0.19	94	No study found
9	cwas-13-Octadecenoic acid		11.465	0.04	80	No study found
10	n-Hexadecanoic acid	$C_{16}H_{34}$	11.611	1.33	99	antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, hemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015)
11	Z-14-Nonacosane	$C_{29}H_{58}$	19.788	0.55	95	No study found
12	Stigmasterol	$C_{29}H_{48}O$	21.884	0.59	95	rebuilding mechanisms related to estrogen effects , acting as an

						intermediate in the biosynthesis of androgens, estrogens, and corticoids (Ikegawa, 1978)

There are many chemical compounds and their biological activity defined in extraction of *Etlingera elatior*'s in ethyl acetate solvent. To understand the better scorer compound in this sample, major compound of samples has been listed and identified the biological activity. Thus, this way analysis toward the potential impact of *Etlingera elatior*'s can be seen. The highest score of chemical compounds with score of 99% in the table of major compound found with score of >80% will be the presence of hexadecenoic acid, methyl ester. This compound also has major score in the sample with 95% which function as antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, haemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015). Caryophyllene also found in this sample with score of 99%, function as anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016). Tetradecanoic acid also found in *Etlingera elatior* extract in ethyl acetate with score 98%. It is function as antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014). To achieve the objective of the experiment, based on the major compound with highest score above 80% in the sample found are more focusing to the compound that function as antioxidant activity. Moreover, similar compound may be found in the extracts in hexane solvent however with different score. This study indicates that antioxidative compound found in ethyl acetate solvent has higher score.

However, there are also chemical compounds with major score that the biological activity has no study found yet about the function of the chemical compound. It has come to the limitations of research where there is no further study about these compounds function. The chemical compound such as Undecane at 86% score, Pentafluoropropionic acid, dodecyl ester at 95% score, Chloroacetic acid, tridecyl ester at 91% score, Cyclododecane at 94% score, cwas-13-Octadecenoic acid at 80% score and lastly Z-14-Nonacosane at 95% score. Based on the findings, it can be concluded that the *Etlingera elatior*'s extract in ethyl acetate solvent has many chemical compounds found with various number of biological activities indicate it can fight radical particle through scavenging of antioxidant activity.

4.4.3 GC-MS analysis of *Etlingera elatior*'s extract in methanol solvent.

Figure 4.4.3 shows GC-MS analysis for *Etlingera elatior* extract in methanol solvent graph composition.

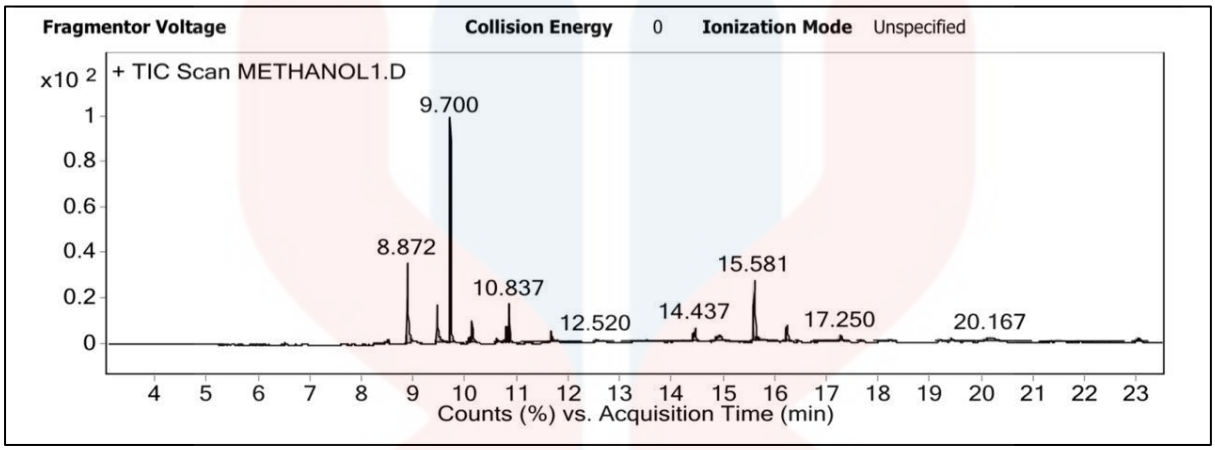


Table 4.4.3 Major compound in *Etlingera elatior* extract in methanol solvent and classifications.

No.	Compound Name	Molecular Formula	Rt (Min)	Percentage Area (%)	Score	Biological Activity
1	Caryophyllene	$C_{15}H_{24}$	8.645	0.07	91	anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016)
2	Dodecanoic acid or lauric acid	$C_{12}H_{24}O_2$	9.444	3.80	99	strong antimicrobial activity against many Gram-positive bacteria (Nakatsuji, Kao et al. 2009)
3	2-Butenedioic	$C_{16}H_{28}O_4$	9.706	10.57	90	Anti-bacteria,

	acid (Z)-, monododecyl ester					antioxidant (Thelma and Balasubramanian 2021)
4	Tetradecanoic acid or myrwastic acid	$C_{14}H_{28}O_2$	10.603	1.18	98	antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014)
5	Oleic acid	$C_{18}H_{34}O_2$	11.030	0.20	94	apoptotic activity leading to the selective death of tumor cells (Fontana, Spolaore et al. 2013)
6	Hexadecanoic acid	$C_{16}H_{32}O_2$	11.465	0.21	95	antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, hemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015)
7	Stigmast-4-en-3-one	$C_{29}H_{48}O$	14.912	2.75	90	antimicrobial activity against some bacteria including Streptococcus gordonii and Streptococcus sanguinwas. (Udobre, Etim et al.)

Based on the table above, there are many chemical compounds and their biological activity defined in extraction of *Etlingera elatior*'s in methanol solvent. To understand the better scorer compound in this sample, major compound of samples has been listed and identified the biological activity. Thus, this way analysis toward the potential impact of *Etlingera elatior*'s can be seen. The highest score of chemical compounds with score of 95% in the table of major compound found with score of >80% will be the presence of hexadecenoic acid. This compound also has major score in the sample which function as antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, haemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015). Caryophyllene also found in this sample with score of 91%, function as anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016). Tetradecanoic acid also found in *Etlingera elatior* extract in methanol with score 98%. It is function as antiurease, antielastase and antioxidant activities (Sokmen, Hasdemir et al. 2014). 2-Butenedioic acid (Z)-, monododecyl ester also found in the extracts of methanol solvent with score 90% with function such as Anti-bacteria, antioxidant (Thelma and Balasubramanian 2021). To achieve the objective of the experiment, based on the major compound with highest score above 80% in the sample found are more focusing to the compound that function as antioxidant activity. Moreover, similar compound may be found in the extracts in hexane and ethyl acetate solvent however with different score. This study indicates that antioxidative compound found in ethyl acetate solvent has higher score.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The radical scavenging activity of extracted plant in hexane, ethyl acetate and methanol show that the percentage of antioxidant activity in scavenging free radical has activity. The activity measures based in the percentage of radical scavenging activity using DPPH assay. The result than recorded in four different concentrations which was 25, 50, 100 and 200. For extraction of *Etlingera elatior* in hexane, ethyl acetate and methanol graph of inhibition percentage and concentration (25, 50, 100 and 200) has been plotted to get the IC_{50} values of hexane, ethyl acetate and methanol extraction. The IC_{50} value of plant extracted in methanol was 39.10µg/mL, for plant extracted in hexane will be 37.03µg/mL and for plant extracted in ethyl acetate will be 25.00 µg/mL. The IC_{50} value of plant extracted in ethyl acetate was 25.00µg/mL. To test the reliability data, the reading of percentage radical scavenging activity has been repeated in three different days to get the reading of IC_{50}. For *Etlingera elatior* in hexane will be 35.94 µg/mL, in ethyl acetate solvent will be 36.64µg/mL and in methanol solvent will be 26.12µg/mL. The GC-MS analysis of *Etlingera elatior* in three types of solvent also has been done. Analysis of all three sample has indicate finding of chemical compound such as caryophyllene as anti-inflammatory, anticarcinogenic, antimicrobial, antioxidative and analgesic activities (Fidyt, Fiedorowicz et al. 2016), hexadecenoic acid as antioxidant, 5-alpha-reductase inhibitor, antifibrinolytic, haemolytic and antimicrobial activities (Gomathi, Kalawaselvi et al. 2015).

The chemical compound found in all three samples indicate that *Etlingera elatior* indeed has many benefits specially to function as an oxidative compound and fight free radical scavenging. Based on the study that has been conducted, it can be conclude *Etlingera elatior* has many benefits which can be as an anti-aging product for human healthcare generally.

Thus, it also can be transformed into bioproduct such as body scrub, supplement and also essential oil.

5.2 Recommendation

The finding suggest that methanol was the most effective solvent for extracting antioxidants from *Etlingera elatior*. This information was valuable for future research aiming to isolate and identify the specific bioactive compounds responsible for the observed antioxidant activity in the methanol extract. Furthermore, investigating the individual and combined effects of these compounds could provide insights into their potential applications in food preservation, medicine, and cosmetics. It was important to acknowledge that the current data only provides a preliminary understanding of the antioxidant activity in *Etlingera elatior* extracts. Further studies are warranted to:

a) **Isolate and identify the specific bioactive compounds** responsible for the antioxidant activity in the methanol extract. Techniques like chromatography and mass spectrometry can be employed for this purpose.

b) **Evaluate the synergistic effects** of different bioactive compounds isolated from the extracts. This could involve testing combinations of compounds and assessing their combined antioxidant activity compared to individual ones.

c) **Compare the antioxidant activity** of *Etlingera elatior* extracts with other commonly used natural antioxidants. This would provide a context for the potential applications of these extracts.

d) **Investigate the in vivo effects** of *Etlingera elatior* extracts, particularly focusing on their potential health benefits and safety profiles.

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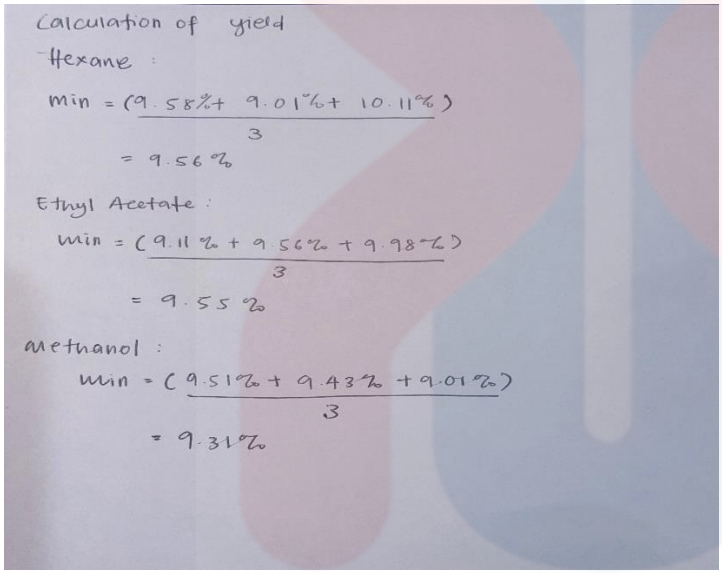
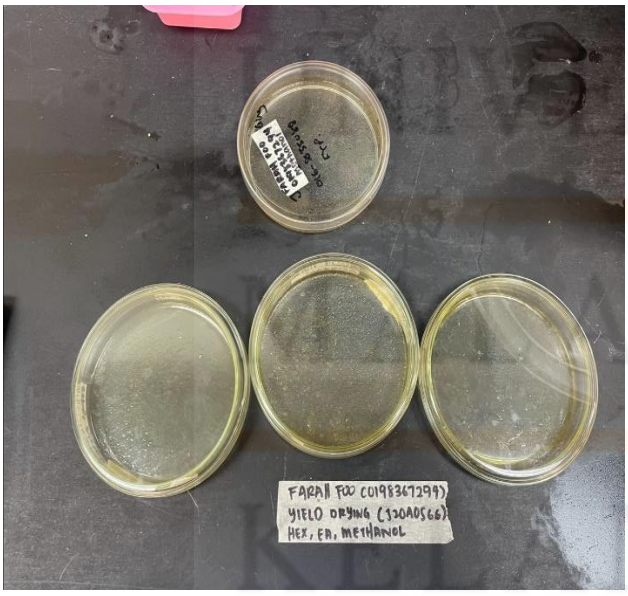
APPENDIX

The table shows IC_{50} reading.

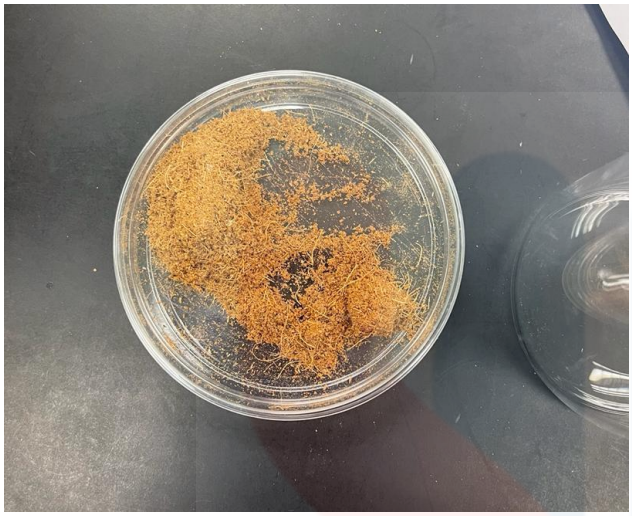
IC_{50} Reading of E.elatior extract in Hexane, Ethyl Acetate and Methanol			
Solvent	**Repeated 1**	**Repeated 2**	**Repeated 3**
Methanol	35.00 µg/mL	38.81 µg/mL	34.02 µg/mL
Hexane	37.03 µg/mL	38.89 µg/mL	37.47 µg/mL
Ethyl Acetate	25.00 µg/mL	25.67 µg/mL	27.70 µg/mL



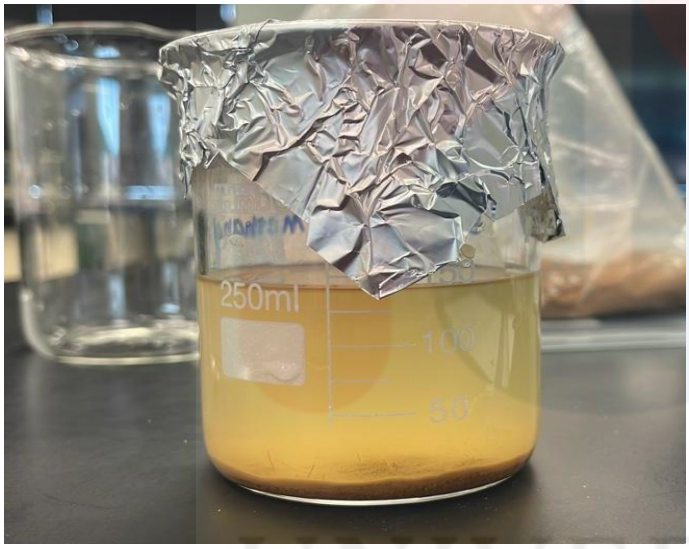
Calculation of yield percentage



Dried crued of extracts in hexane, ethyl acetate and methanol solvent



Dried *Etlingera elatior*



Extracted sample after going in UAE water bath