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Optimization Method for Phenolic Compounds Extraction from Plant (Albizia myriophylla)

Syazwan Aizat Bin Adnan

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DECLARATION

I declare that this thesis entitled "Optimization Method for Phenolic Compounds Extraction from Plant (Albizia myriophylla)" is the result of my own research except as cited in the references.

Signature :

Student's Name : Syazwan Aizat Bin Adnan

Date :

Verified By:

Signature :

Supervisor's Name : Dr. Siti Roshayu binti Hassan

Stamp :

Date :

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ABSTRACT

Albizia Myriophylla, or Tebu Gajah generally a plant or herb as an alternative to antidiabetic medicine widely used in ancient times because of its benefits and functions in treating diseases such as reducing glucose in the blood. However, the scientific substance that responds was unknown. To overcome this problem research was carried out using GCMS testing to identify the respective bioactive compounds that were actually responsible for anti-diabetic properties. This research aims to investigate the effect of different process parameters on the yield of extracted phenolic compounds of Tebu Gajah using the method of Soxhlet. For this purpose, the experiment was run using different parameters and the best for extraction of Tebu Gajah was methanol as solvent, at 5 hours,. The best parameter for enhancing the yield of the phenolic compound was the extraction solvent.

Keywords: Tebu Gajah, Albizia Myriophylla, Phenolic compound, Extraction.

ABSTRAK

Albizia Myriophylla, atau Tebu Gajah umumnya merupakan tumbuhan atau herba sebagai alternatif kepada ubat antidiabetik yang digunakan secara meluas pada zaman dahulu kerana khasiat dan fungsinya dalam merawat penyakit seperti mengurangkan glukosa dalam darah. Walau bagaimanapun, bahan saintifik yang bertindak balas tidak diketahui. Untuk mengatasi masalah ini kajian telah dijalankan menggunakan ujian GCMS untuk mengenal pasti sebatian bioaktif masing-masing yang sebenarnya bertanggungjawab terhadap sifat anti-diabetes. Penyelidikan ini bertujuan untuk mengkaji kesan parameter proses yang berbeza terhadap hasil ekstrak sebatian fenolik Tebu Gajah menggunakan kaedah Soxhlet. Untuk tujuan ini, eksperimen dijalankan menggunakan parameter yang berbeza dan yang terbaik untuk pengekstrakan Tebu Gajah ialah metanol sebagai pelarut, pada 5 jam. Parameter terbaik untuk meningkatkan hasil sebatian fenolik ialah pelarut pengekstrakan..

Kata kunci: Tebu Gajah, Albizia Myriophylla, sebatian fenolik, ekstrak

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1.1 Background study

In both traditional and modern medicine, medicinal plants are utilised with the goal of promoting health, providing treatment for a specific ailment, or both. In 2002, the Food and Agriculture Organisation calculated that more than 50,000 medicinal plants were utilised globally. Out of the roughly 30,000 plants for which a usage of any type is known, The Royal Botanic Gardens, Kew more conservatively estimated in 2016 that 17,810 plant species have a therapeutic application. The three primary types of benefits that medicinal plants may offer are: health benefits to those who use them as medicines; financial benefits to those who collect, process, and sell them; and societal benefits, such as job possibilities, tax revenue, and a more productive workforce. However, the development of plants or extracts with potential medical applications is hampered by a lack of strong scientific support, poor drug development procedures, and inadequate funding.

Albizia Myriophylla, also known as Tebu Gajah in Malaysia, was an example of an herbal crop that was still relatively unknown to the general population. It was a member of the Mimosoideae subfamily and genus Albizia. However, this herbal plant was more well-known in India, Sri Lanka, Burma, Thailand, Laos, Cambodia, and Vietnam, where the locals had used it since ancient times. For example, in Vietnam, they used stems because of their sweet taste to replace licorice in their system of traditional medicine. The many portions of this medicinal plant had long been utilized in conventional medicine on diabetes treatment among South-East Asians and other human ailments. Observed that traditional medicinal plant knowledge had always guided the hunt for new remedies. Traditional medical techniques were now considered alternative medicine. Considering the fact that scientific evidence did not support their efficacy or their mechanisms of action. The extracts from this plant had been shown to have analgesic, antibacterial, anti-diabetic, anti-allergic, anti-fungal, anti-cancer, anti-inflammatory, anti-leukaemia antimicrobial, and antioxidant properties.

This study was to investigate the optimization method of phenolic extraction from plants in Tebu Gajah making use of the Response Surface Methodology (RSM) technique. The goal of the study is to use RSM to increase the level of gallic acid in Tebu Gajah.

1.2 Problem Statement

Phenolic or phenolcarboxylic acids, a type of phytochemical known as a polyphenol, are one of the primary forms of phenolic compounds found in plants. They can be found in a variety of meals made from plants, although their concentrations are highest in leaves, seeds, and the skins of fruits and vegetables. Albizia myriophylla were being choose as Albizia myriophylla has high amount of phenolic. One of the most prevalent phenolic acids in the plant world is gallic acid, also known as 3,4,5-trihydroxybenzoic acid, with the CAS number 149-91-7. It is a colourless chemical that is somewhat yellow and crystalline, widely used in the food and pharmaceutical industries. So, this experiment was taken to determine total gallic acid in Albizia myriophylla as gallic acid ability to scavenge free radicals and act as antioxidants, can prevent the oxidation and rancidity of oils and fats. The phenolic being chosen as phenolic has many benefits and advantages. Phenolic benefits to humans are Phenolic compounds have been utilised to treat a variety of common human illnesses, such as hypertension, metabolic issues, incendiary infections, and neurodegenerative disease. Phenolic compounds have the ability to block enzymes linked to the development of human diseases. There are already research being done about phenolic compounds extraction but it if focus on its parameters meanwhile what I am focusing on is optimization of phenolic extraction and want to measure level of gallic acid in Albizia myriophylla. I want to optimize using response surface methodology (RSM). Response surface methodology (RSM) is a set of optimisation techniques for factorial variable sets that cause the response to attain a specified maximum or minimum value. Gallic acid has many benefits to human. Gallic acid can reduce DNA damage and contains potent anti-aging actives. So, by measuring gallic acid levels we can use Albizia Myriophylla for skincare purpose.

1.3 Expected Outcome

By the end of this experiment, I can identify total yield of gallic acid in Tebu Gajah using Soxhlet Extraction. By doing this, I can identify which solvent can extract better in Soxhlet extraction. Besides through RSM, I can identify total phenolic compound that being extracted.

1.4 Objective

- To optimize the yield of gallic acid recovery from Albizia Myriophylla.
- To study effect of parameter on the yield from Albizia Myriophylla.
- To characterize the phenolic compounds from Albizia Myriophylla.

1.5 Scope Study

Gallic acid level in Tebu Gajah was measured in this study through extraction using RSM. Tebu Gajah will first be extracted, and then been extracted using Soxhlet extraction and its optimisation and level of gallic acid will be checked by RSM.

1.6 Significances of study

This study showed Tebu Gajah, as one of the medicinal plants with variety of benefits to human health. This study conducted to consider the potential of different process parameters on the yield of extracted phenolic compounds in Tebu Gajah. The different process parameters on the yield were expected to improve the total phenolic compound in Tebu Gajah for various applications. Thus, this study was essential to fill commercial herbs to treat various ailments.

Chapter 2

Literature Review

2.1 Herbal Plants

A wide range of plants are referred to as "medicinal plants" when discussing herbal therapy. While the term "herb" comes from a combination of the Latin word "herba" and the old French word "herbe." Since ancient times, people have found and utilised in conventional medical practises, therapeutic plants—often referred to as medicinal herbs are used. Plants serve a range of functions, such as defence and protection against pests, maladies, and herbivorous mammals that synthesise hundreds of chemical compounds. Herbal medicine has been used for countless years and is ingrained in many different cultures. Traditional medical practises including Ayurveda, Traditional Chinese Medicine (TCM), and Native American medicine have long understood the therapeutic effects of plants.

The bioactive substances found in herbal plants, such as alkaloids, flavonoids, phenols, terpenes, and essential oils, are what give them their therapeutic powers. The body may respond to these substances in a variety of ways, including through their antioxidant, analgesic, and anti-inflammatory properties. Various parts of herbal plants, such as their fresh or dried leaves, flowers, stems, roots, seeds, or extracts, can be employed. Depending on the plant and desired medicinal effect, they are made as teas, infusions, decoctions, tinctures, powders, capsules, and ointments.

Plant meals contain the vast majority of naturally occurring bioactive substances, including antioxidants and secondary metabolites. The most prevalent class of secondary metabolite discovered in plants were phenolic chemicals. They are crucial for the plant's pigmentation, development, and reproduction as well as its defence against infections and predators. The powerful phytoalexin and astringency of the plants were mostly responsible for this. They had been shown to have anti-allergic, anti-inflammatory, antioxidant, hepatoprotective, antiviral, and anticarcinogenic properties. On the other hand, the most research on their antioxidant activity has probably focused on their capacity to prevent the formation of free radicals and scavenge those that already present in living things.

A variety of fresh or dried herbs, tablets, capsules, powders, teas, and extracts are used to make herbal medicines. They were a particular class of easily accessible dietary supplement. Herbal medications were primarily used as alternative or complementary therapies, as a supplement to avoid sickness, and to maintain or improve one's health. An estimated 80% of people worldwide used traditional herbal medicine as their primary form of healthcare. e. According to University Technology Malaysia (UTM), by creating a soft gel capsule product from the Sky fruit seed oil, also known as Swietenia Macrophylla, the essence of the Sky fruit seed oil was successfully extracted. Herbs were grown 17 increasingly abundant and accessible, and they were also being widely produced and cared for. It's a plant with value in terms of medicinal, nutritional, and aromatic properties. We can observe that each form of the herbal plant had its unique characteristics, including various nutrients and purposes. (Camille Keisha Mahendra, 2021). Tebu Gajah is one illustration of a herbal plant that is frequently used. Tebu Gajah is a type of plant found in the forests of Malaysia as well as in other ASEAN countries. This plant is found on mountain slopes, riverbeds, and among other herbs.

2.1.1 Tebu Gajah (Albizia Myriophylla)

The genus Albizia Myriophylla, also referred to as Tebu Gajah, contains more than 160 species of tropical and subtropical trees. The generic name "Albizia" honours Italian aristocrat Filippo Degli Albizzi, who introduced the plant to Europe for the first time in the middle of the eighteenth century. Common names for the plants included yet, because the generic name's outdated spelling—with a double "z"—was still in use, there were also silk trees, plants, and sirises, they were also referred to as albizzias. A common term for certain species was mimosa. However, it relates to species of Mimosa plants. This plant, which was also known as "Cha Em Thai" in Thailand, was widely distributed throughout Southeast Asian countries and was also called "Cay Song Ran" in Vietnam. According to traditional medicine, it had a variety of pharmacological effects, including blood pressure, cholera,and laxatives. The following conditions had frequently treated the lips, cough, urticaria, chills, and boils in children. Licorice was Albizia Myriophylla Benth's botanical name.

Tebu Gajah is well known traditionally as being a useful source to its advantages and for being a useful source of alternative anti-diabetic medications that are safer and more effective. Additionally, these medicinal plants must be introduced to society so that it can recognise them through further study and formally recognise their ability to treat a variety of maladies. Therefore, this study demonstrated that Tebu Gajah was rich in anticancer, antidiabetic, antibacterial, and other health-beneficial compounds. Its -glucosidase inhibitory effect was shown in one in vitro investigation, but its hypoglycaemic effect had never been proven scientifically in vivo. Various traditional medicinal plants have recently been tested on animals to see if they have any anti-diabetic properties. The current experiment examines the possibility of hypoglycemia in the stem of Tebu Gajah.

Figure1: Albizia Myriophylla

2.1.2 Taxonomy,morphology and ecology of Tebu Gajah

A subfamily of Mimosoideae known as Tebu Gajah, or Albizia Myriophylla, contains quickly expanding subtropical and tropical trees and shrubs. It can be found all over Southeast Asia, is common in lowland woodlands in tropical Asia, and is easily farmed in tropical climes. Below is a taxonomy of Tebu Gajah.

This plant has a short lifespan and a variety of behaviours. They were mostly little trees, ranging in size from a scrambling shrub to a robust climber. The stems, which grow to a height of 2-4 metres, start out green while young and turn brown as they get older. The branches were spherical, and the downward-curving hook-like prickles at the base of the leaf scars were up to 5 mm long. The small flowers were bundled and contained stamens that were considerably longer than the petals, with many more than ten filaments, whereas the leaves had a hairy texture, were made up of many small leaflets, and had two glands on the petiole that were 10-15 cm long. The flower head has 10–12 white, sessile blossoms that are up to 1 mm long. The clusters are 10–16 cm long, club-shaped at the tip, and extensively branched. The flowers on the borders have funnel- or bell-shaped petals, funnel-shaped corollas, stamens with corolla tubes and smooth bulbs, petals that are one millimetre long, corollas that are four millimetres long, and pale yellow feathers on the corollas.

2.1.3 Application of Tebu Gajah

Tebu Gajah's wood, leaves, and roots were frequently used for food and medicine. The aqueous bark extract was used in other herbal supplements and as a tonic in Peninsular Malaysia. It was used as an alternative to traditional diabetic treatment. The aqueous bark extract significantly reduced blood sugar levels in streptozotocin nicotinamide-induced diabetic rats, according to this study. Because diabetes and obesity have a similar ethology, Tebu Gajah bark extract may have an anti-obesity impact, either directly or indirectly, through antioxidant or free radical scavenging effects.Tebu gajah bark was used to prevent obesity in a patent in the United States but not as an anti-obesity medication because the bitter flavour of the saponin discourages overeating.

Tebu Gajah was beneficial not just for medical purposes but also for animal husbandry. Elephants love to eat the tree trunk when it is still wet or fresh because it has a sweet taste and is therefore more appetising to them. In actuality, it was also appropriate for use in jewellery, particularly in the form of rosaries made from its core or ring stones. In addition, the core can be pounded into a fine point similar to a spear and inserted into the cigarette to provide flavour for the smoker. Because smoking the cigarette will make it tasteless, this was necessary.

It was also used to treat cases of fever and coughing. It requires 10 to 20 grammes of Tebu Gajah and was washed. After boiling the combination over low heat until the solution's colour changes in the pot, turn off the fire. Use the boiled water within a day after drinking it and leaving a residue. It is necessary to utilise this medication consistently in order to receive obvious therapeutic results. Additionally, it reduces bleeding from tiny incisions made from chewed or crushed leaves as well as several skin disorders. For mouthwash, they used Tebu Gajah stems, which worked better in preventing plaque. There aren't any clinical trials or other pieces of research particularly examining Albizia myriophylla's capacity to lessen bleeding. The majority of the literature on Albizia species focuses on the phytochemical makeup and putative biological functions of these plants. According to these studies, Albizia species, particularly Albizia myriophylla, may have anti-inflammatory, wound-healing, and antioxidant qualities that could indirectly help to lessen bleeding due to chemical compound that inside Albizia Myriophylla contain Albizia myriophylla contains gallic acid, which may directly encourage the expression of antioxidant genes, making it a potential agent for wound healing.

2.2 Phenolic Compound

Phenolic substances are second-generation metabolites that are produced when pentose phosphate and plant shikimic acid break down phenylpropanoid. They can be simple phenolic compounds or sophisticated polymerized materials, but they will always have benzene rings having a single or many hydroxyl groups. The distribution of phenolics, which are the most noticeable secondary metabolites in plants, may be seen throughout the entire metabolic process. The compounds that make up these polyphenols, also known as phenolic chemicals, include different types of molecules, including simple flavonoids, phenolic acids, complicated flavonoids, and coloured anthocyanins. These phenolic chemicals are typically connected to plant defence mechanisms. However, phenolic metabolites also play a significant role in other processes, such as accelerating pollination, providing colour for concealment and defence against herbivores, and having antibacterial and antifungal properties.

2.2.1 Extraction Phenolic Compound

Extraction was crucial in the process of obtaining phenolic substances from plant tissue. It could be traditional or non-conventional. Traditional methods have been used, including hydro distillation, Soxhlet, boiling, soaking, and maceration. However, the Soxhlet extraction method was the one that was most frequently used to extract phenolic compounds because it had lower processing costs, was simple to use, was appropriate initially and in bulk extraction, was good for the overall recovery of extracts, and compared to other traditional approaches, it was quicker and more efficient like maceration or percolation. However, those with greater yields can be obtained using a vastly reduced amount of solvent compared to other unconventional techniques. The ultrasound-assisted extraction method (UAE) is one illustration of this.

A more advanced procedure than the traditional one was ultrasound-assisted extraction (UAE). In another study plan, Temperature, output amplitude, duty cycle, sample size, and total extraction time were some of the variables that Palma et al. investigated for the extraction of phenolic compounds from grapes and compared the best conditions with customary extraction techniques. The outcomes demonstrated that ultrasound-assisted extraction could extract phenolic chemicals with a greater yield and in a lot less time—six minutes as opposed to sixty—than conventional extraction methods. In the majority of cases, the UAE was able to extract phenolic chemicals more quickly, more effectively, and with better outcomes than usual. In order to increase the extraction yield of phenolic chemical, this work combined the methods of UAE and Soxhlet. Both the UAE and the Soxhlet were alternatives to the more conventional techniques, and they offered the potential to increase extraction effectiveness while simultaneously promoting environmental preservation by using fewer organic solvents. According to

Zhang et al.'s (2018) study, "Optimisation of Ultrasound-Assisted Extraction of Bioactive Compounds from Plant Materials Using Response Surface Methodology: RSM was employed in this work to optimise the extraction of bioactive compounds from plant materials utilising ultrasound technology. The researchers looked at the effects of extraction time, temperature, and the solvent-to-material ratio on the extraction yield. RSM was used to develop a prediction model and pinpoint the best extraction conditions for the greatest yield.

2.2.2 Phenolic Compound Properties

Phenol, a chemical substance that was "poly," which suggests it was made up of more than one compound, was the name of its structure. Phenol is also known as polyphenol or phenolic compound. Metabolites having a single or several phenolic residues, as well as different quantities of oxy-groups and substituents made up plant phenolic compounds. Both conjugated and free phenolic chemicals are present in plants. The complex of phenolic compounds can contain the phenol carboxylic acids and their derivatives, simple phenols and quinones, flavones, flavanols, catechins, and leukoanthocyans, even within a single plant species. They produce orto- and semi-quinone radicals, which interact with proteins and combine with metal ions to create complexes. The distribution of substitutions on the aromatic rings, the quantity and location of hydroxyl groups, and other factors all affect how antioxidant-active phenolic compounds are. These qualities, together with a wide range of structural options, make polyphenols very important to plant life. Important biological processes like photosynthesis, respiration, the manufacture of cell- and tissue-supporting substances, the development of defence mechanisms, and the regulation of auxin transport are covered.

It has long been understood that polyphenols are good for your health. When they get into the body, they can work as antioxidants and shield bodily cells from damage caused by free radicals Consuming a variety of fruits and vegetables was essential since eating enough polyphenols can shield the body's cells from damage. Amylase absorption can be restricted while treating conditions involving glucose absorption, such as diabetes, using plant-derived chemicals and phenolic compounds. Contrarily, the risk of metabolic syndrome can be decreased by phenolic acids and flavonoids. The structural differences of polyphenols also impact how they separate and dissolve. The structure of any compound, for instance, affected the degree of polarity, conjugation, and contact with the sample matrix phenolics with a high molecular weight were commonly insoluble due to their structural makeup.

The persistence of phenolic substances also varies because of their uneven dispersion in plants; certain phenolic chemicals, for instance were stable while others were oxidation-prone, thermolabile, or volatile.

The structural differences of polyphenols also influence their solubility and properties of separation. The structure of any compound, for instance, affected the polarity level, conjugation, and interaction with the sample matrix. Due to the high molecular weight phenolics' structural composition, they were typically insoluble.Furthermore, the stability of phenolic compounds varies as a result of their uneven distribution throughout plants; for instance, some phenolic compounds were volatile, thermolabile, or susceptible to oxidation.

According to Keerati Thamapan et al., sweetness compound concentrations of A. Myriophylla, such as saponin, sugar, and flavonoid compounds, were effectively performed with RSM optimization study, which revealed that the extract Albizia Myriophylla by Ultrasound assisted extraction was 178 times sweeter than sucrose. According to Goh Yong Meng et al., ABZ bark extracts may had the capacity to manage chronic metabolic illnesses influenced by oxidative variables, such as diabetes mellitus and obesity. These was because in the ABTS, DPPH, and FRAP tests, the yield of extraction had demonstrated varied quantities of phenolics, flavonoids, and saponin with varying degrees of antioxidant activity. (Keerati Thamapan, 2019)

According to Azmah saat, the effectiveness of using Tebu Gajah bark and virgin coconut oil to lower blood sugar levels in diabetics was evaluated by looking at its antidiabetic effects in rats. After being given orally to rats with and without diabetes induced by streptozotocin, the aqueous bark extract of Tebu Gajah and virgin coconut oil were tested for their hypoglycaemic activity. Rats were separated into 8 groups as part of their research on lowering blood glucose levels. Four groups of rats were healthy, and another four groups had diabetes that was produced. Diabetes was induced by intraperitoneal injection of 60 mg/kg body weight, and measurements of body weight and fasting blood glucose levels were taken on days 0, 15, 30, and 45. This research revealed Tebu Gajah's aqueous bark extract and virgin coconut oil have anti-diabetic properties since they reduce the serum glucose levels of diabetic rats. The diabetic rats' body weight also rises as a result of the medication. (Azmah Saat, 2013)

2.2.3 Total Yield of Phenolic Compound in Tebu Gajah

Antioxidants have recently gained widespread recognition as a crucial topic that experts from all around the world are currently delving deeply into. About ten years ago, this trend started. Antioxidants had a well-deserved reputation for having positive effects, especially when it came to human health. It has developed into an essential element for maintaining human health. The main cause of the antioxidant's beneficial benefits is its capacity to stop oxidative damage by protecting cells from the damage caused by oxidative stress, we can reduce the risk of developing chronic diseases including cancer, heart conditions, as well as illnesses associated with chronic inflammation and degeneration. All phenolic compounds absorb UV light, and some of them have 280 nm absorption. The metal oxide reduction products had a blue tint and a broad light absorption spectrum with a maximum of 765 nm.

The total phenol concentration of Tebu Gajah bark extracts was determined using the Folin-Ciocalteau assay, which requires mixing with 2.5 mL of 10-fold diluted Folin-Ciocalteau reagent and 2.0 mL of 7.5% sodium carbonate, 300 mg of each extract was combined. Each test sample was made into three copies, and the mixture was incubated at 40 degrees Celsius for 30 minutes. Then, using a spectrophotometer, the absorbance of the reaction mixtures is calculated in milligrammes of gallic acid equivalents. The investigation's findings indicate that the extract made from ethyl acetate (EAE), which has 0.77 mg of gallic acid equivalent (GAE) per milligramme of extract, contains the most phenolic acid.

2.4 Gas Chromatography Mass Spectrometry (GCMS)

Gas Chromatography Mass Spectrometry (GCMS) is an advanced analytical technique employed across diverse scientific to unravel the complexities of chemical mixtures. This methodology shows the strengths of two distinct but different methods: gas chromatography and mass spectrometry. Gas serves as the first stage in the process, separating the sample based on their differential affinity for an early phase within a chromatographic column. The sample is vaporized during this procedure and then put into a stream of carrier gas that moves it through the column. Due to their interactions with the stationary phase, the components of the sample are separated according to their volatile, polar, and size constituents.

The individual components are sent into the mass spectrometer for additional examination after being separated by GC. By ionizing the separated molecules and subsequently determining their mass-to-charge ratios and fragmentation patterns, mass spectrometry plays a crucial role in this step. The sample molecules undergo ionization, which produces ions. These ions are then separated and identified in the mass analyzer according to their mass-to-charge ratios. Mass spectrometry offers precise information about the molecular structure and content of the analytes by detecting the amount and distribution of these ions.

The combination of mass spectrometry with gas chromatography in gas-chromatographic mass spectrometry (GCMS) enables accurate target component identification and quantification in complicated mixtures. In domains like environmental science, where GCMS is used to identify and measure pollutants, pesticides, and other contaminants in air, water, and soil samples, this feature is especially helpful. In the field of forensic science, GCMS helps with trace evidence, explosives, and drug analysis, supporting law enforcement organizations in their criminal investigations. Similar to this, GCMS plays a key role in pharmaceutical research and development by helping to characterize the active components, contaminants, and degradation products present in medication formulations. Moreover, GCMS finds use in quality assurance and food safety, facilitating the identification of pollutants, adulterants, and residues in food items.

The development of portable and compact systems, along with the incorporation of hyphenated techniques like GC-MS/MS and GCxGC-MS, which improve sensitivity, selectivity, and analytical throughput, are the results of the ongoing advancements in GCMS technology. Additionally, the capabilities of GCMS have been further enhanced by advancements in data analysis algorithms and sample preparation techniques, allowing researchers to tackle ever-more complicated analytical challenges. In the future, the continued development of GCMS has the potential to significantly contribute to global innovation and advancements in industrial applications, scientific research, and regulatory compliance.

Chapter 3

Materials And Method

3.0 Materials and Method

3.1 Materials

Tebu Gajah or Albizia Myriophylla was collected as much as 600 grams in village Ayer Lanas Jeli, Kelantan.

3.2 Chemical

In this study the chemical were use methanol, and ethyl acetate for extraction. The chemical solvent was provided from laboratory UMK Jeli.

3.3 Methods

3.3.1 Raw Material Preparation

Albizia Myriophylla will be collected nearby places in Jeli. Tebu Gajah will be collected for 1 kilograms . Then, Tebu Gajah was cut in small sizes used machete equipment. Then dried in the oven at 103°C for 24 hours or until dried. After drying, tebu gajah been grind in powder form using grinding machine that being provided at UMK warehouse and the sample kept in an airtight container.after that, the sample being blend using heavy blender to produce a thin and smaller sizes . then, the sample also being sieved to collect only the powder form to being used in extraction. The preparation being repeated until achieved 600 grams of fine blend powder. 10 grams of the material were used for each experiment. The sample was placed in a beaker, with solvent added at a constant ratio of 1:11 (w/v) for 44 minutes, then fixed using ultrasound-assisted extraction (UAE). The sample was then put in a thimble and transferred to the Soxhlet extraction. Three times were spent doing this. As a sample was being tested, the extract was being rotary evaporated. By dividing the amount of dry mass recovered after extraction by the dry weight of the powder before use, the yield for each extract was determined as a percentage.

Figure 2 : Albizia Myriophylla or Tebu Gajah that has been blended. .

3.4 Process Study

3.4.1 Response Surface Methodology

A statistical test design known as the response surface methodology combines various levels of components into a single experiment. It is applied for evaluating complex situations with several concurrently impacting factors, including interactions. For this, test arrays are utilised. A RSM is a DOE that employs specifically designed arrays for computing interactions and quadratic responses. Three parameters are also being used in the test. First is extraction time. In a variety of scientific and industrial applications, the extraction time—that is, the length of the extraction process—has a substantial impact on the effectiveness and results of extraction. Longer extraction periods, which are crucial to yield because they provide the extraction solvent more time to permeate and dissolve more target chemicals from the source material, typically lead to higher yields. Second is temperature. Temperature that being use is crucial in playing part at using RSM Software as a parameter. Temperature is a significant variable in extraction processes, exerting a dramatic influence on the efficiency and selectivity of extraction. Temperature increases typically speed up the mass transfer of solutes from the source material to the solvent, increasing the extraction rate. Higher solubility and diffusion rates at elevated temperatures can result in increased target compound yields. Temperature has a complicated impact on extraction, though, since very high temperatures can also cause thermally sensitive chemicals to degrade. Lastly, I am using solvent volume as the third parameter. One crucial factor that can have a big impact on the effectiveness and results of an extraction process is the volume of solvent utilized. The solubility of target chemicals and their migration from the source material to the solvent are directly influenced by the solvent volume. Since larger solvent volumes have a greater capacity to dissolve and take away the target molecules, they frequently produce higher extraction yields. On the other hand, decreased yields and partial extraction can result from reduced solvent volumes.

Independent variables	Units	Coded value	
		-1	1
Solvent Volume	ml	300	400
Temperature	^{o}C	10	70
Extraction time	H	4	6

Table 1 : Parameter for Soxhlet Extraction

Std	Run	Space Type	Factor 1 A:Solvent Volume mL	Factor 2 B:temperature C	Factor 3 C:Time Hours	Response 1 Total Yield grams
11	13	Axial	350	9.77311	5	20.97
4	19	Factorial	400	50	4	20.19
7	20	Factorial	300	50	6	21.58
16	17	Center	350	35	5	21.41
10	15	Axial	434.09	35	5	21.92
8	16	Factorial	400	50	6	21.29
17	18	Center	350	35	5	21.11
2	14	Factorial	400	20	4	20.63
15	3	Center	350	35	5	20.06
13	8	Axial	350	35	4	20.88
19	9	Center	350	35	5	19.19
20	10	Center	350	35	5	19.27
14	11	Axial	350	35	6.68179	18.9
9	12	Axial	265.91	35	5	18.38
5	2	Factorial	300	20	6	18.56
6	1	Factorial	400	20	6	18.91
3	6	Factorial	300	50	4	19.54
18	5	Center	350	35	5	18.37
1	4	Factorial	300	20	4	19.52
12	7	Axial	350	60.2269	5	18.91

Table 2 : Number of sample

3.4.2 Soxhlet Extraction

The plant extract (10g) from Tebu Gajah is placed inside the extraction device using a Soxhlet extractor thimble. Using the feed-to-solvent ratios of 1:25, 128 and 1:31 w/v, the 33 solvents are measured in a conical flask. The mixture is refluxed using a heat mantle for varying extraction times of 2 hours, 4 hours, and 6 hours. The extract solution is given time to cool to room temperature after the extraction period. The liquid is then dried with the use of a rotary evaporator after being filtered through a cone of filter paper and weighed. Utilising rotating evaporators, the solvent is then separated from the extract. The extract's TPC extraction yield was then assessed. The experimental method was repeated three times, and the mean and standard deviation were noted. TPC extraction yield can be calculated by the number of bacteria on a petri dish x 1 / dilution factor. After that amount is multiplied by the dilution obtained.

For this work, I use three different parameters to determine the total phenolic compound for this extraction. First is the solvent volume. Then the second parameter is temperature. The temperature must be watch carefully because the solvent may be drain and can cause the round conical flask to break. So the temperature must be watched also the solvent so it do not drain when the extraction run. Lastly, the third parameter is extraction hours. Extraction hours being set for 4 hours, 5 hours and 6 hours. A different time being used to test whether it affect the extraction or not.

Then, I also use different solvent which is ethyl acetate and methanol. The reason I use different solvent is to know which chemical is better for albizia myriophyla. Because certain chemicals do not shows the exact result. So different sample must use a different chemicals.

Ethyl acetate plays a pivotal role in extraction processes, serving as a versatile and effective solvent for isolating compounds from natural sources. Ethyl acetate, with the chemical formula CH3COOC2H5, is an ester formed through the reaction between acetic acid (CH3COOH) and ethanol (C2H5OH). The chemical structure of ethyl acetate involves a carbonyl group (C=O) from the acetic acid portion and an alkoxy group (-OC2H5) from the ethanol portion. Also, ethyl acetate is relatively easy to derive through a straightforward chemical reaction. It is commonly produced through the esterification reaction between acetic acid (CH3COOH) and ethanol (C2H5OH).

Meanwhile for methanol, the chemical formula CH3OH, is the simplest alcohol and a colorless, volatile liquid. The chemical structure of methanol consists of a single carbon (C) atom bonded to three hydrogen (H) atoms, forming the methyl group (CH3), and one oxygen (O) atom, forming the hydroxyl group (-OH). The molecule has a tetrahedral geometry around the carbon atom, with the oxygen atom and three hydrogen atoms arranged around it. Yes, methanol is relatively straightforward to derive through various industrial processes. One common method for methanol production is the catalytic hydrogenation of carbon monoxide (CO) in the presence of hydrogen gas (H2).

Also , through the time I run the extraction, I can conclude that methanol not easy to drain even when use a high temperature. The solvent volume do not drain as many as ethyl acetate. Ethyl acetate mostly will be drain and the solvent will turn dark due to albizia myriophylla. Meanwhile, methanol do not easily to change colours. It only shows a little yellowish.

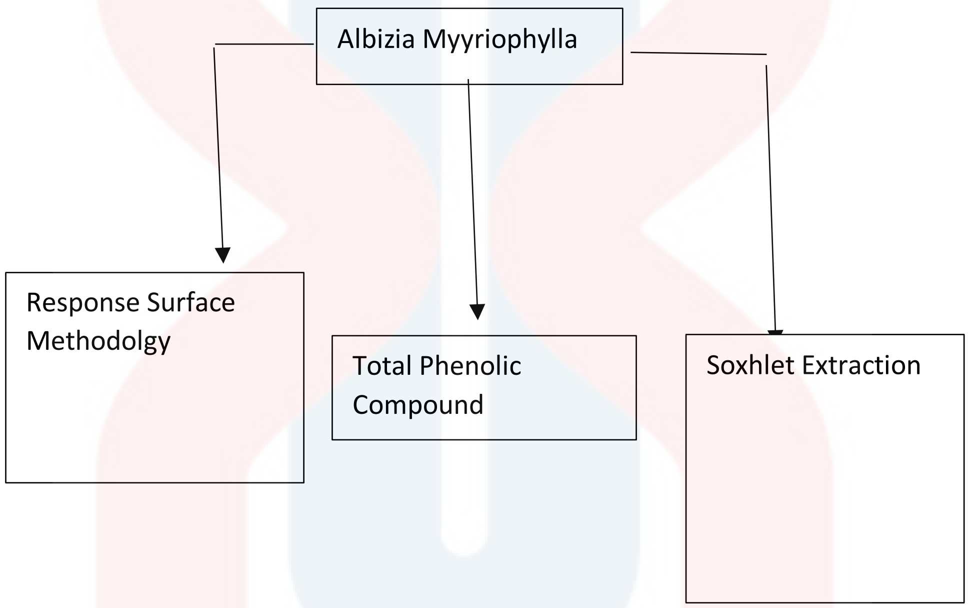
$$yield\ of\ extracts\ \% = \frac{weight\ of\ extracts\ from\ plant\ sample (w)}{weight\ of\ dried\ plant\ sample\ (w)} \text{ x100}$$

Figure 3 : Soxhlet Extraction of Tebu Gajah

3.4.2.1 Subsection Analysis

The extracted compound of gallic acid were being verified by comparing the physical properties such as color. The color were much brighter when using methanol as the solvent for the extraction instead of using ethyl acetate which is shown darker . it is shows that ethyl acetate can extract better as a solvent than using methanol.

3.4.3 Research Flow Chart



CHAPTER 4

RESULT AND DISCUSSION

4.1 Analytical Analysis

4.1.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The purpose of this work is to determine component that contain inside Albizia Myriophylla and mainly focused on gallic acid. Gallic acid, a polyphenolic molecule, has received interest due to its antioxidant characteristics and possible medicinal applications. We aim to detect the existence of gallic acid as well as other bioactive compounds that promote antidiabetic features by applying GC-MS to water extracts and solid raw of bark A. myriophylla. This analytical method separates and identifies particular chemical ingredients in a complex combination using mass spectra and retention times.

The GC-MS shows that a solid rarw bark of Albizia Myriophylla can shows a total of 18 peaks corresponding to the bioactive components that relating their peak retention time, peak area (%), and mass spectral fragmentation patterns to those of the known compounds described by the National Institute of Standards and Technology (NIST) library.). The GC-MS study of *A.myriophylla* solid raw revealed that several key bioactive in Pyridine (1.58%), Hexadecanoic acid, methyl ester (5.99%), 9-12-Octadecadienoic acid, methyl ester (1.80%), 9-Octadecadienoic acid, methyl ester (1.80%), Chondrillasterol (10.54%), and Lupeol (4.01%).

Comparatively, the findings showed that the plant's bark has a substantially larger total phenolic content in its aqueous extract than in its solid raw form. Although the liquid sample contains 68 chemicals in total, only 37 of those compounds can be recognized. The results showed that several significant bioactive compounds were present in the liquid form of the water extract of A. myriophylla's bark, including phenolic found in solid raw, octanoic acid (1.58%), cyclopentadecane (0.13%), hexadecane (0.07%), 9-octadecadienoic acid, methyl ester (2.82%), tetracosanoic acid, methyl ester (0.12%), and squalene (0.24%). These compounds have also been reported in the literature to exhibit microbial inhibitory activity, antioxidants, anti-inflammatory properties, and antidiabetic effect.

4.1.2 Response Surface Methodology

"Total yield" in chemical processes describes the total amount of a certain product that is produced at the conclusion of a reaction or extraction. In chemical synthesis, manufacturing, and extraction operations, obtaining a high total yield is frequently the main goal because it has a direct impact on the productivity and profitability of the process. Response Surface Methodology (RSM) comes into play when trying to maximize the factors that affect a compound's overall yield.

The purpose of this method is to determine the total yield that can being used for medicinal purpose. The RSM method shows that the parameter which solvent volume, extraction time and temperature plays a huge role in Soxhlet extraction. Although the data that achieved are mostly identical to eachother but we can verify that the total phenolic compound that contain in Albizia Myriophylla are many , so that Albizia myriophylla are a wise choices for medicinal purpose especially for inflammatory .

As per the figure, we can see that the scatter plot shows a linear graph that shows an increasing linear line.

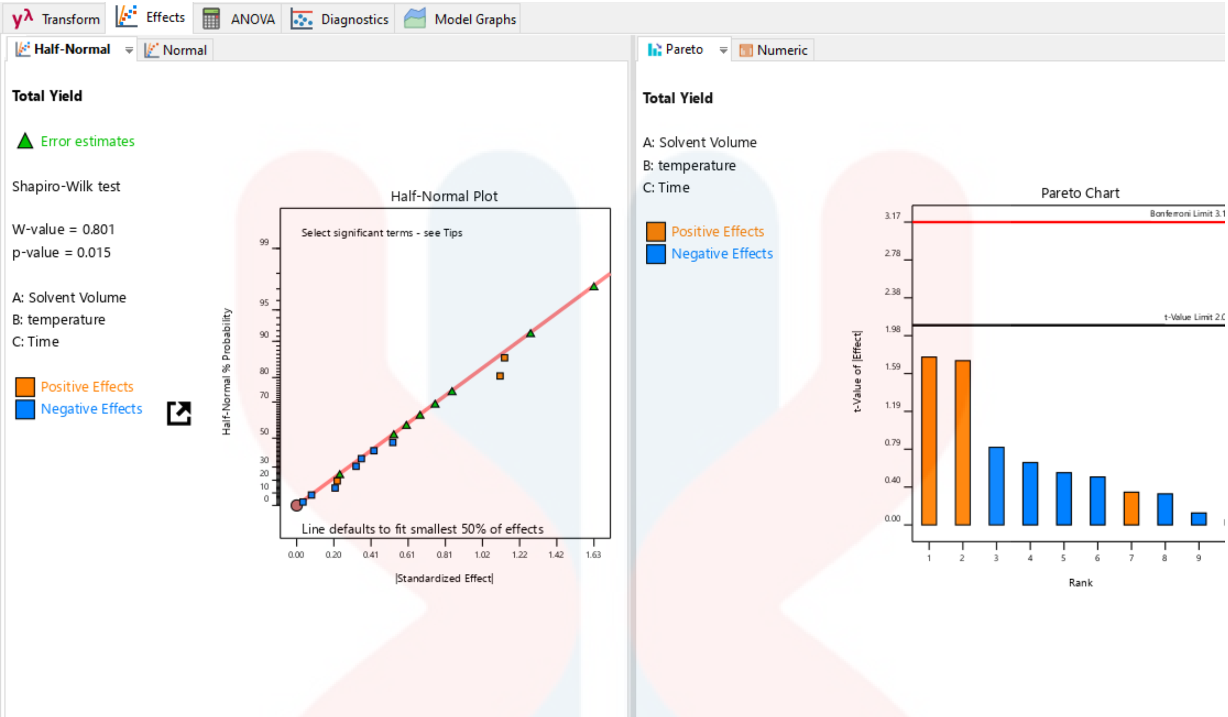


Figure 4 : The result of RSM

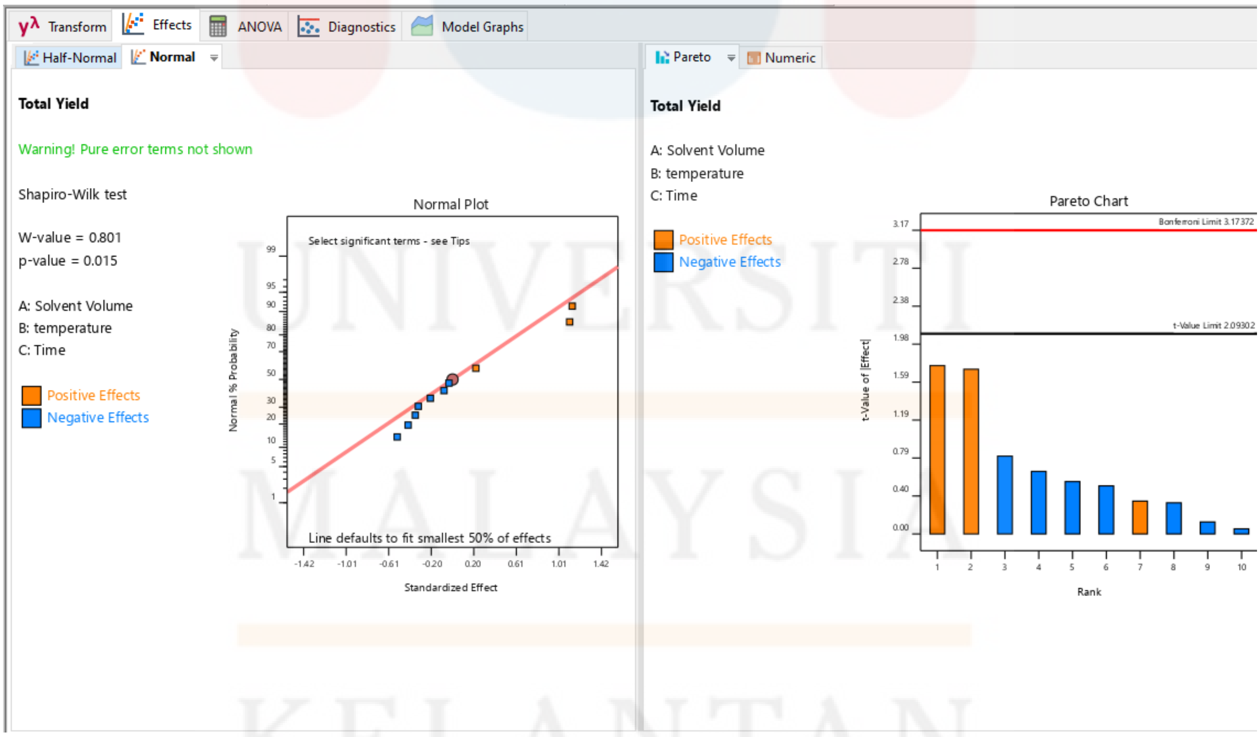


Figure 5: The result of RSM

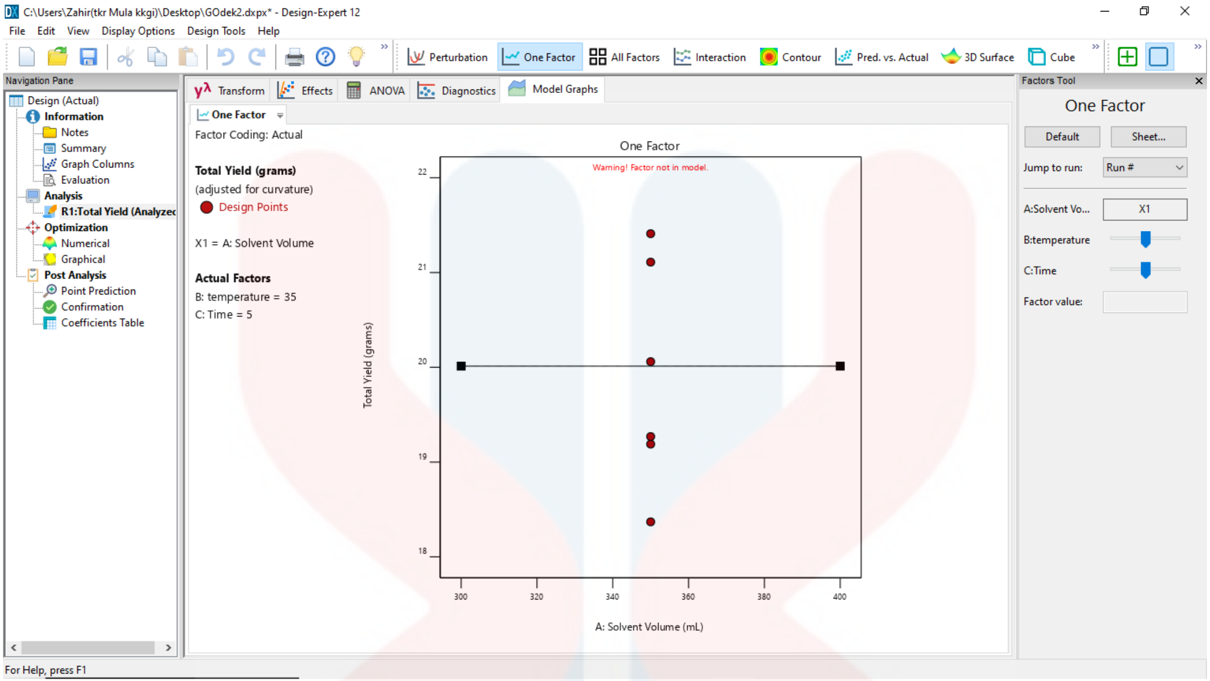


Figure 6 : The result of RSM

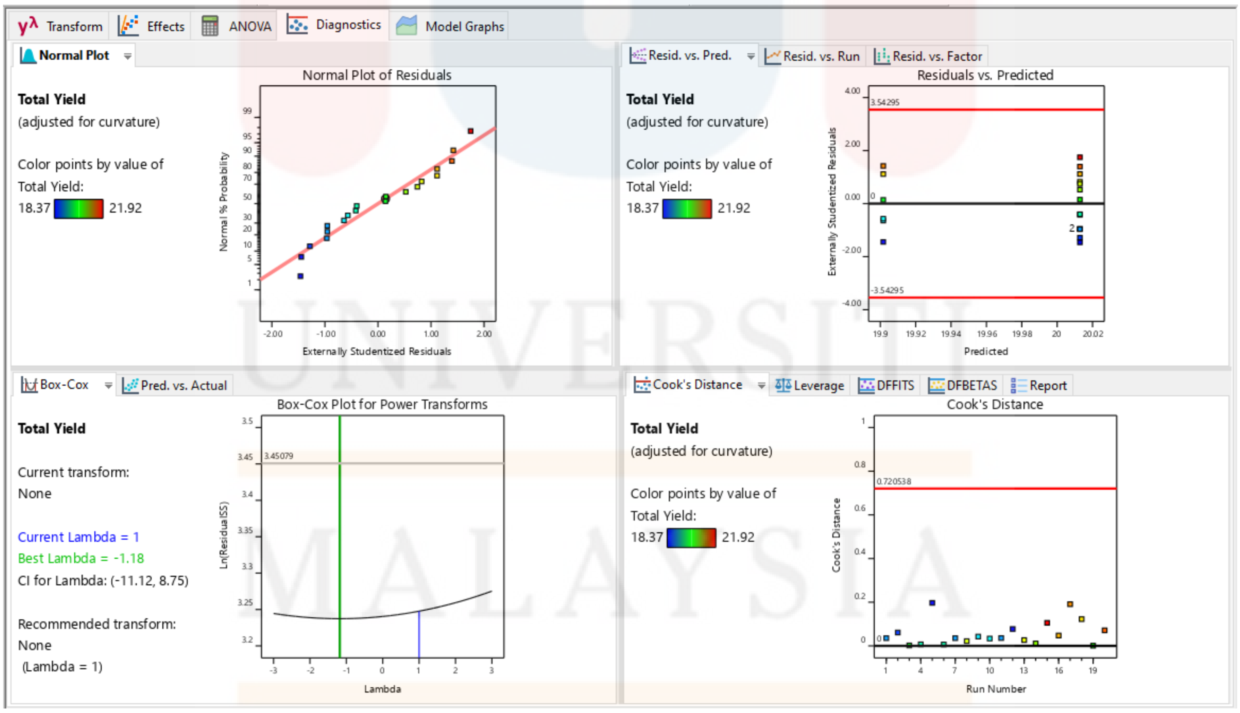


Figure 7 : The result of RSM

Conclusion

To optimise tebu gajah extraction process, a Soxhlet extraction has been applied in current work. Some variables including Temperature, Solvent Volume and extraction time were investigated . GCMS were strongly impacted by all the investigated parameters. By balancing the rate of total phenolic compound and GCMS , the ideal extraction conditions were determined to be ethyl acetate for 5 hours . this study findings the best parameters were extraction time . 5 hours of extraction time were better than 4 and 6 hours. This was because the 5 hours were the time where it not too high and too low. So it can extracted nicely.

Recommendation

Through this study, I recommend other to further finding another parameter that works best with gallic aicd. There are maybe another parameter that can significantly find anything inside tebu gajah. Also, gallic acid has many more benefits especially in medicinal purposes.

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