Development of Nanoemulgel formulation containing extract of *Etlingera elatior*

Heilen Binti Ansayal
J20A0451

A reported submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Forest Resources Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UMK

2024

DECLARATION

I declare that this thesis entitled "title of the thesis" is the results of my own research except as cited in the references.

Signature : ____________________

Student's Name : HEILEN BINTI ANSAYAL____________________

Date : 20/1/24____________________

Verified by:

Signature : ____________________

Supervisor's Name : Dr.Nurul Akmar Binti Che Zaudin____________________

Stamp : ____________________

Date : ____________________

ACKNOWLEDGEMENT

It is a great pleasure to address people who helped me throughout this project to enhance my knowledge and practical skills especially in research area. First and foremost, I express my deepest gratitude to Dr. NuruL Akmar Binti Che Zaudin, my project supervisor, for their unwavering guidance, invaluable insights, and continuous support throughout the entirety of this research endeavor. Their mentorship has been a beacon, illuminating the path to academic and professional growth. Their commitment to excellence and dedication to fostering a conducive learning environment have been instrumental in shaping the trajectory of this project. Also for their expert advice, constructive critiques, and the generosity with which they shared their wealth of knowledge. Their inputs have been instrumental in refining the conceptual framework and methodology, elevating the quality of this research.

My appreciation further extends to the Universiti Malaysia Kelantan for providing the necessary resources, research facilities, and a stimulating academic environment that has been crucial to the success of this project. The support from the Universiti Malaysia Kelantan has been a cornerstone in facilitating a conducive atmosphere for intellectual exploration and scholarly pursuits.

Heartfelt thanks are also due to my fellow undergraduate students who have been steadfast companions on this academic journey. Their camaraderie, shared insights, and collaborative spirit have added depth and richness to the collective learning experience. The exchange of ideas and the collective pursuit of knowledge have been a source of inspiration and motivation.

In closing, I extend my deepest appreciation to my family and friends for their unwavering support, encouragement, and understanding throughout this academic pursuit. Their patience, encouragement, and belief in my abilities have been a constant source of strength, propelling me forward even in the face of challenges.

Finally, as this project draws to a close, I reflect on the collective efforts and collaborative spirit that have made this endeavor a reality. This acknowledgment is a testament to the collaborative ethos that underlies the academic community, where shared knowledge and support foster an environment of growth and exploration.

Development of nanoemulgel formulation containing extract of *Etlingera elatior*

ABSTRACT

This research aims to develop nanoemulgel formulation containing the extract of *Etlingera elatior* and to study the characteristic of nanoemulgel containing the extract of Etlingera *elatior*. The nanoemulgel characterization involved assessing its ph measurements, spreadibility test, viscosity test and physical appearance. Initial steps included obtaining *Etlingera elatior* extract through Soxhlet extraction and rotary evaporator processes and water as a solvent. Before making nanoemulgel, the nanoemulsion formulation was prepared using the low energy emulsification method. Nanoemulsion formulations were obtained from the ternary phase diagram involving distilled water, soybean oil and surfactant. The nanoemulsions consists of 60% Tween 80 and glycerol, 30% water and 10% soybean oil. Nanoemulgel was obtained by combining nanoemulsion containing extraction *Etlingera elatior* with Carbopol 940 as a gelling agent. The result revealed that the nanoemulgels containing extraction *Etlingera elatior* ph measurements indicated that the solution was acidic and not suitable for the skin. The viscosity measurements enabled researchers to comprehend the relative thickness of each nanoemulgel formulation in the result comparison. The spreadibility resulted that the nanoemulgel formulation significantly influenced the spreadibility of the importance of formulation in product development. The potential application of nanoemulgel in the field of medicine is very useful in the production of cream products.

Keywords: *Etlingera elatior*, nanoemulsion,nanoemulgel,ternary phase diagrams,features,

Pembangunan formulasi nanoemulgel yang mengandungi ekstrak daripada *Etlingera elatior*

ABSTRAK

Kajian ini bertujuan untuk membangunkan formulasi nanoemulgel yang mengandungi ekstrak *Etlingera elatior* dan mengkaji ciri-ciri nanoemulgel yang mengandungi ekstrak *Etlingera elatior*. Pencirian nanoemulgel melibatkan penilaian pengukuran pH, ujian kecerapan, ujian kepekatan, dan penampilan fizikalnya. Langkah permulaan termasuk mendapatkan ekstrak *Etlingera elatior* melalui proses penyulingan Soxhlet dan pemutaran vakum dan air sebagai pelarut. Sebelum membuat nanoemulgel, formulasi nanoemulsi disediakan menggunakan kaedah emulsifikasi tenaga rendah. Formulasi nanoemulsi diperoleh daripada gambarajah fasa ternari yang melibatkan air suling, minyak soya, dan surfaktan. Nanoemulsi terdiri daripada 60% Tween 80 dan gliserol, 30% air, dan 10% minyak soya. Nanoemulgel diperoleh dengan menggabungkan nanoemulsi yang mengandungi ekstrak *Etlingera elatior* dengan Carbopol 940 sebagai agen pengemulsi. Hasil kajian menunjukkan bahawa nanoemulgel yang mengandungi ekstrak *Etlingera elatior* mempunyai pengukuran pH yang menunjukkan bahawa larutan tersebut bersifat asidik dan tidak sesuai untuk kulit. Pengukuran kepekatan membolehkan penyelidik memahami ketebalan relatif setiap formulasi nanoemulgel dalam perbandingan hasil. Ujian kecerapan menunjukkan bahawa formulasi nanoemulgel secara signifikan mempengaruhi kecerapan pentingnya formulasi dalam pembangunan produk. Potensi penggunaan nanoemulgel dalam bidang perubatan sangat berguna dalam penghasilan produk krim.

Kata kunci: Bunga Kantan (Jack) R.M, nanoemulsi,nanoemulgel,gambar rajah fasa ternary,ciri-ciri

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS (optional)

g	gram
pa.s	pascal seconds
cm	centimeter
ml	mililiter
nm	nanometer
NE	Nanoemulsion
Ee	*Etlingera elatior*
FG	Formula Gel

LIST OF SYMBOLS (optional)

%	Percentage
°C	degree Celcius
*	asterism

CHAPTER 1

INTRODUCTION

1.1 Background Of Study

There are Zingiberaceae species in every tropical and subtropical climate, although the majority of them are found in Asia. *Etlingera elatior* (Jack) R.M. Smith is a species of plant native to Sumatra, Indonesia, and widely distributed throughout Southeast Asia. It is referred to as "Kantan" in Malaysia and "kecombrang" in Indonesia. Botanically, the *Etlingera elatior* species are quite appealing because of their variegated red and pink sun hues on their bracts and blossoms. (Yeat, 2016)

Etlingera elatior, often known as torch ginger, is a well-known plant in Southeast Asia. It's inflorescences are traditionally utilised for both culinary and medicinal purposes. The inflorescence's smell and perfume are incredibly distinctive. It has been used for a very long time as a traditional flavour and medicine. Women who have used post-parfum bathe in water infused with *Etlingera elatior* leaves and other aromatic herbs to eliminate body smell (Eric Wei Chiang Chan, 2011)

Etlingera elatior's inflorescence is renowned for its exceptional antioxidant properties. Rhizomes and leaves have been the primary research sites for the antioxidant properties of *Etlingera elatior* inflorescence (Yau Yan Lim, 2011)

In traditional medicine systems, *Etlingera elatior* has been used for centuries to treat various ailments. It is believed to possess anti-inflammatory, antioxidant, antimicrobial, and digestive properties. The plant is also utilized in cultural ceremonies, rituals, and traditional practices in Southeast Asian cultures. The interest in *Etlingera elatior* stems from its rich phytochemical composition. The plant contains various bioactive compounds, including flavonoids, phenolic acids, terpenoids, and essential oils. These compounds are responsible for the plant's characteristic aroma, flavor, and potential therapeutic effects. (Liza Noordin, 2022)

Numerous scientific studies have focused on exploring the pharmacological and biological activities of *Etlingera elatior*. Researchers have investigated its antioxidant potential, anti-inflammatory effects, antimicrobial activity, anti-cancer properties, and other therapeutic potentials. Studies have also examined its use in skincare and cosmetic applications due to its reported anti-aging, moisturizing, and soothing effects on the skin. (Liza Noordin, 2022) Additionally, there is growing interest in developing innovative formulations of *Etlingera elatior* extracts, such as nanoemulsions and nanoemulgels. These formulations aim to enhance the stability, bioavailability, and efficacy of the bioactive compounds for various applications, including topical delivery.

The background of study on *Etlingera elatior* encompasses a multidisciplinary approach, including botany, phytochemistry, pharmacology, biotechnology, and formulation science. Researchers seek to unravel the plant's chemical composition, elucidate its biological activities, and explore its potential applications in medicine, cosmetics, and food industries. The diverse array of studies on *Etlingera elatior* highlights its significance as a valuable natural resource with potential benefits in various fields. Continued research on this plant can contribute to a deeper understanding of its therapeutic properties, formulation development, and sustainable utilization while respecting its cultural and traditional significance.

Nanoemulsions is the distribution of droplets of one liquid throughout another liquid using an emulsifying agent and processing techniques like high-pressure homogenization is known as a nanoemulsion. A nanoemulsion's droplet size, which is typically between 20 and 200 nanometers, gives it unique properties like increased stability, absorption, and bioavailability. The usage of nanoemulsions is widespread, especially in the culinary, pharmaceutical, cosmetic, and agricultural industries. (Preeti, 2023)

Nanoemulgel is a type of gel that utilizes nanotechnology to enhance its properties. Nanotechnology refers to the manipulation and use of materials at the nanometer scale, which is incredibly small (one nanometer is one billionth of a meter). In the case of nanoemulgel, nanotechnology is used to create tiny droplets of active ingredients that are dispersed evenly throughout the gel. These small droplets provide a larger surface area for the active ingredients to interact with the skin, leading to better absorption and more effective treatment. Moreover, the nanoemulgel also uses nano-sized particles to improve the texture and stability of the gel.The smaller particles allow for a smoother and more easily spreadable gel, and they also improve the shelf life of the product. Overall, the use of nanotechnology in nanoemulgel helps to improve the efficacy and usability of the gel, making it a popular choice in many skincare and pharmaceutical products. (Mahipal Reddy Donthi, 2023)

1.2 Problem Statement

Etlingera elator is widely grown for its ornamental value and has many medicinal properties. In addition, *Etlingera elatior* are also traditionally used to treat wounds on the skin. The trick is to put *Etlingera elatior* on the injured areas to treat the wounds. Its anti-microbial content is also able to prevent infection in the wound area. *Etlingera elatior* was investigated for its potential medicinal benefits, particularly in the healing of wounds. The cost and accessibility of wound creams, particularly for individuals with limited financial resources or in regions where healthcare infrastructure is lacking have created a pressing issue. Ensuring affordability and availability of effective wound care products is essential for promoting optimal wound healing outcomes for all individuals. Nanoemulgel serving as a natural moisturizer incorporated into wound cream formulation. This research to aimed wound cream in gel form utilizing *Etlingera elatior*, with a unique focus on employing a nanoemulgel formulation, which had not been previously explored in research. The methodology involved extracting *Etlingera elatior*, followed by incorporating it into a gel after undergoing the nanoemulsion process. The resultant gel was then formulated into a cream specifically intended for wound healing purposes. Notably, this *Etlingera elatior* wound cream exhibited favorable characteristics such as easy adherence to the skin and suitability for the surrounding skin environment. Moreover, the study confirmed its safety, as it was found to be non-toxic and non-irritating.

1.3 Objectives

The objectives of this research are,

1. To develop nanoemulgel formulation containing the extract of *Etlingera elatior*.
2. To study the characteristic of nanoemulgel containing the extract of Etlingera *elatior.*

1.4 Scope of Study

The research was conducted at the University Malaysia Kelantan (UMK*). Etlingera elatior* was extracted using a solvent, such as water. Subsequently, the extracted *Etlingera elatior* underwent the nanoemulsion formulation process using a low-energy emulsification method. The resulting nanoemulsion served as a key ingredient in developing a nanoemulgel by incorporating gelling agents. The formulation adopted the nanoemulgel approach.To analyze the characteristics of the nanoemulgel containing *Etlingera elatior*, various parameters were studied, including viscosity test, spreadibility test, pH measurements and physical appearance.

1.5 Significance of Study

By studying *Etlingera elatior,* researchers can uncover its potential benefits in medicine. The benefits of this wound cream in medical field are can increase knowledge in studying medicine. It is also can increase the economy in medicine. Besides, it is also making easier for patients who have small wounds to get *Etlingera elatior* wound cream at a pharmacy or clinic without having to go to a big hospital. In addition, the study can also be a reference for future researchers.

CHAPTER 2

LITERATURE REVIEW

2.1 *Etlingera elatior*

2.1.1 Background of *Etlingera elatior*

Figure 2.1*: Etlingera elatior* ((Jack) R.M. Smith (Yeat, 2016)

The Zingiberaceae family includes torch ginger (*Etlingera elatior*), commonly known as torch lily, wild ginger, or Philippine wax flower. It comes from Malaysia and Indonesia.Torch ginger is a clumping herbaceous plant that may be grown both sexually (through seeds) and asexually (through cuttings) (rhizomes). The inflorescence of torch ginger comes in three colours: red, pink, and white. Pink torch ginger is common in rural areas and jungle borders, but white and red torch ginger are uncommon. Torch ginger, particularly the flower bud, is popular in Southeast Asia for culinary purposes. Many Malay, Nyonya, Indonesian, and Thai recipes use it as a flavouring component. Other components of the torch ginger plant, such asthe rhizome, leaf, and flower, have long been used as a condiment and medicinal. (Yau Yan Lim, 2011)

According to (Yau Yan Lim, 2011), *Etlingera. elatior's* leaves, flowers, stems, and rhizome contained high levels7 of total phenolic and flavonoids. Flavonoids found in *Etlingera elatior* include quercetin, apigenin, kaempferol, luteolin, and myricetin. The compounds kaempferol 3-glucuronide, quercetin 3-rhamnoside, quercetin 3-glucoside, and quercetin 3-glucuronide were found in the leaves. We found quercetin, kaempferol, and kaempferol-3-O-glucoside in the flowers and stems.

2.1.2 Plant extraction

Extraction is the first step to separate the desired natural product from the raw material. Extraction methods include solvent extraction, distillation methods, pressing and polishing according to the principle of extraction. Solvent extraction is the most widely used method. The extraction of natural products proceeds through the following stages which is stage one the solvent penetrates into the solid matrix. Stage two, the solute dissolves in the solvent. Stage three the solute seeps out of the solid matrix and stage four the extracted solutionis collected. Any factor that increases the absorption and solubility in the above steps will facilitate extraction. The properties of the extracting solvent, the blood size of the raw material,the solvent-to-preparation ratio, the extracting temperature and the extracting period will affect the efficiency of the extraction. (Qing Wen Zhang, 2018).

2.1.3 Water as a solvent

Hexane, petroleum ether, diethyl ether, ethanol, nheptane, isopropanol, acetone, choloroform, methanol, and 1-buttanol are the most common and reported oil extraction solvents. (Shivani MPatel, 2011)

In this study, water has been choose as a solvent in extraction. Since it is the most polar solvent, a variety of polar substances can be extracted using it. Water is useful for extracting polar chemicals since it is a polar solvent. Many components of plants, including certain flavonoids, phenolics, and vitamins that are soluble in water, dissolve easily in it. It is inexpensive, nontoxic, nonflammable, and extremely polar; it dissolves a broad variety of compounds. Generally speaking, water is regarded as harmless. In industries where safety and the lack of hazardous residues are critical, such as food, pharmaceutical, and herbal extractions, it is commonly utilized. There are disadvantages using this solvent because the extract becomes concentrated with a lot of heat and encourages the growth of mold and germs. It may also cause hydrolysis. (J Pharm Bioallied, 2020)

2.1.4 Soxhlet as an extractor

In general, extraction procedures include Soxhlet extraction, maceration, decoctiona, infusion, percolation, superficial extraction, digestion,ultrasound-assisted extraction and microwave assisted extractions. (J Pharm Bioallied, 2020). In this studied, Soxhlet extraction has been choosen as an extraction method.

Soxhlet extraction was known as a continuous hot extraction. The apparatus is called Soxhlet extractor made up of glass. It is consists of a round bottom of flask, extraction chamber, siphon tube and condenser at the top. Plant material that has been dried, ground, and finely powdered is put inside a porous bag (thimble) that is made of sturdy filter paper or clean fabric and fastened shut. The thimble is placed inside the extraction chamber once the extraction solvent has been added to the bottom flask. After that, the solvent is heated from the bottom flask, evaporates, and flows through the condenser before condensing and flowing down to the extraction chamber, where it comes into contact with the medication to extract it.

Consequently, the solvent and the plant material that has been extracted flow back into the flask when the amount of solvent in the extraction chamber reaches the top of the siphon. The procedure is continued until the medication is fully extracted, which is achieved when no residue is left behind by the extraction chamber's solvent flowing out. This technique works well with plant materials that have insoluble contaminants and those that are only partially soluble in the selected solvent. On the other hand, thermolabile plant materials cannot be processed using this technology. Reduced solvent usage can yield greater drug extraction yields. Plant materials that withstand heat can also be used with it. High levels of heat could be delivered, and no filtration is necessary. (J Pharm Bioallied, 2020)

2.1.5 Rotary evaporator for solvent removal

In this study, Rotary evaporator has been used for solvent removal to completed the extraction. An alternative to heating a flask to the solvent's boiling point at one atmosphere is to use a rotating evaporator to remove a solvent from it. This method offers the advantages of being speedier and less likely to result in the sample's heat degradation. Consist of an induction motor that doesn't ignite spinning spinning an evaporation flask submerged in a water bath set to heat.

With the use of a speed controller, solvent vacuum distillation at both high and low temperatures can be accomplished rapidly and effectively. It stops the vacuum from foaming and the liquid from bumping. Slides and powders can also be dried in a vacuum.Heat-sensitive chemicals are extracted using low temperature vacuum distillation. Continuous liquid input into the evaporating flask and distillation under a controlled environment are made possible by the introduction. (C.Lemaire)

2.2 Nanotechnology

The word "nanotechnology" was first introduced in 1974 by the late Norio Taniguchi1 (University of Tokyo) to describe the ability to accurately construct materials at the scale of nanometres. In fact, this is its present definition; the term "engineer materials" is now used to refer to more than just materials alone; it is also used to describe the design, characterization, manufacture, and application of materials. The creation of materials, devices, and systems with precise control at nanoscale dimensions is the definition of nanotechnology. (Ramsden, 2016)

Nanotechnology has continued to be utilised in all domains where minuscule size plays a critical role in determining essential qualities, thanks to advancements in the fields of materials science, chemistry, and engineering during the past few decades. From biology and medicine to physics, engineering, and chemistry, they are utilised. The precise tagging of biological molecules is done using cadmium telluride nanoparticles. Sunscreens mostly consist of titanium dioxide nanoparticles, which are highly effective UV radiation blockers.

In particular, the cosmetics, pharmaceuticals, and food industries have increasingly used nanotechnology, one of the rapidly expanding technological uses. Products using nanotechnology have a business opportunity due to its exceptional qualities, such as large interfacial area, enhanced distribution of the active substances, and superior solubilization capacity in small droplet size. (Ting, 2020)

2.2.1 Nanoemulsion

Due to its versatility in delivering both hydrophilic and lipophilic medicines, nanoemulsions are currently attracting a lot of interest in the biopharmaceutical and cosmetics industries. They can serve as a medication delivery system that can be used in a variety of systemic ways, including topical, oral, and other methods. Products containing nanoemulsions might be semisolids like creams and balms or fluids like lotions and liniments. The droplet sizes of nanoemulsion which range from 20 nm to 500 nm, are colloidal dispersions made up of oil, water, and an emulsifier. Because it reduces the interfacial tension between the water and oil phases of the nanoemulsion, the emulsifier is essential for producing small-sized droplets. Consumers dislike the greasy feel and high oil content of water in oil (W/O) nanoemulsion despite their great emollient characteristics. However, oil in water nanoemulsion (O/W) is preferred because it speeds up absorption and since the formulation contains less oil, making it easier to wipe off the skin. (Ting, T. C, (2020)).

2.2.1 (a) Surfactant

Surfactants are amphiphilic compounds that decrease interfacial tension and stop droplet aggregation, giving stability to the nanoemulsions. Surfactants provide electrostatic, steric, or dual electro-steric stability at the oil and water interface because they are easily adsorbed there. (Abeeda Mushtaq, 2023) Surfactants possess amphiphilic properties, meaning that they consist of a polar portion and a non-polar portion. Because of this structure, surfactants can lower the interfacial tension between immiscible liquid phases, enabling the creation of systems that are nanoemulsified. The ratio of the hydrophilic and lipophilic groups of surfactant molecules, both in terms of size and strength, determines the HLB value. The activity of surfactants is to facilitate the production of the nanoemulsion; the kind of nanoemulsion creation and the stability of the system are determined by the value of HLB. There are three types of surfactants: cationic, non-ionic, and anionic. In detergents, shampoos, and soaps, anionics are the most widely used class. The polar area is even more polarized due to the oxygen atoms and negative charge. This kind of surfactant has great water solubility due to its high polarity. Quaternary nitrogen is the source of cationic surfactants. Their polar regions are positively charged, while the nitrogen atoms exhibit high electronegativity, drawing in the bond's electrons to partially offset the positive charge and reduce polarity. (Fernanda Almeida, 2022)

2.2.1 (b) Co-surfactant

Co-surfactant a chemical substance that is used in addition to a surfactant to improve its performance. Co-surfactant penetrates the surfactant layer and builds up significantly at the interface layer, improving the interfacial film's fluidity. Co-surfactants provide the interfacial film with the necessary flexibility to accept various curvatures needed to create a microemulsion throughout a broad composition range. The lipophilic chains of the surfactant should be suitably short or contain fluidizing groups if a single surfactant film is needed. A co-surfactant's functions include enhancing the interface's fluidity, destroying any liquid crystalline or gel structure that might impede the development of a microemulsion, and modifying the surfactant partitioning characteristic to modify the interface's HLB value and spontaneous curvature. (Muhammad Asri Abd Sisak, 2016)

2.2.1 (c) Oil Phase

There are two key factors that must be taken into account before choosing the right oil phase, making it the most significant excipient in the formulation since it determines the choice of the other ingredients for the microemulsion. First, the oil's capacity to dissolve the active component in medication. Additionally, the selection must yield a substantial microemulsion existence region. Comparatively speaking, oils with shorter hydrocarbon chains are easier to create a microemulsion than those with longer chains. The oil's capacity to dissolve lipophilic groups, on the other hand, is directly correlated with the length of its chain. (Muhammad Asri Abd Sisak, 2016)

2.2.2 Nanoemulgel

Due to its dual characteristics as a nanoemulsion and gel base, nanoemulgel is one of the most suitable choices for drug administration to the skin. Nanoemulgel has attained high patient acceptability due to the advantages of both nanoemulsion and gel. The use of nanoemulgel in the pharmaceutical industry is widespread. For the treatment of various local and systemic conditions, the formulations and development of nanoemulgel for the various delivery techniques have been the subject of several studies and investigations. Including transdermal, vaginal, ophthalmic, oral, and nasal to brain. The creation of natural, environmentally friendly goods with a variety of positive bioactivities is currently gaining popularity. For researchers looking to optimise the formulation of the application to meet market demands, plant-based oils and nanoemulgel may work wonderfully together. (Ting, 2020)

2.2.2 (a) Method to produce nanoemulgel

A produced nanoemulsion is coupled with an appropriate gel base in a multistep process called nanoemulgel development.

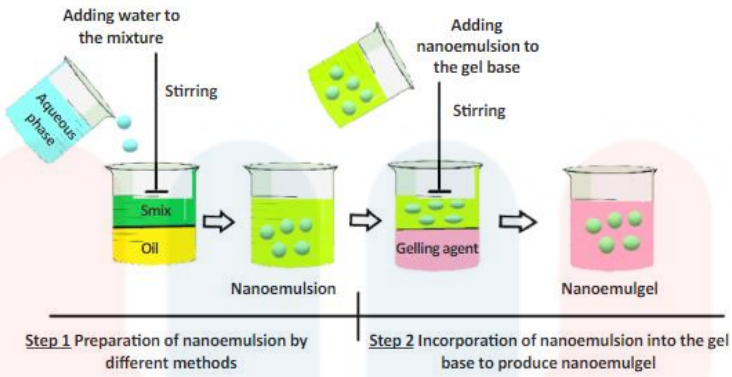


Figure 2.2: A procedure that combines a prepared nanoemulsion with a suitable gel base (Alaa R. Azeez, 2022)

2.2.2 (b) Application of nanoemulgel

A. Morsy et al. created an atorvastatin-loaded nanoemulgel in 2019 (50) for the purpose of promoting wound healing. A variety of formulations were developed, including nanoemulgel, atorvastatin-loaded gel, and emulgel. The in-vitro drug release profiles for atorvastatin from all manufactured formulations were 65% from gel, 55% from nanoemulgel, and 44% from emulgel after 6 hours. The development of atorvastatin as a nanoemulgel greatly enhanced its capacity to enter the skin. The nanoemulgel with atorvastatin loading displayed the highest percentage of wound contraction in the in vivo wound healing trials. After receiving atorvastatin-loaded nanoemulgel therapy for 21 days, a histopathological evaluation of the skin revealed that the histological structure of the skin had significantly improved. (Alaa R. Azeez, 2022)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Raw materials

The *Etlingera elatior* was taken in the UMK Jeli area and around the area that has a lot of *Etlingera elatior* in Kelantan. The fresh *Etlingera elatior* was washed with water to remove all the dirt. The *Etlingera elatior* was dry at a temperature of 24 to 25 °C, and stored overnight.

3.1.2 Chemicals and apparatus

The chemicals that have been used when doing this research were soy bean oil as oil phase, Tween 80 as a surfactant, glycerol as a co-surfactant, distilled water, gelling agent which is Carbopol 940. Meanwhile the apparatus that has been used is beaker, cylinder measurement, boiling flask, coil condenser, Soxhlet extractor, thimble, heater strirrer, homogenizer, strirrer, analytical weighing, knife, test tube, oven, viscometer, laser, Rotary Evaporator

3.2 Methods

3.2.1 Extraction of *Etlingera elatior*

The preparation of the plant material *Etlingera elatior* involved harvesting and cleaning to eliminate impurities. Subsequently, the extraction process was initiated. Typically, the plant material underwent extraction using a suitable solvent, namely water, to extract the bioactive compounds. Soxhlet extraction was employed for this purpose. The *Etlingera elatior* was dried at a temperature of 24 to 25 °C and stored overnight. The material was then cut into small pieces, blended until it turned into a powder, and made ready for extraction. The Soxhlet extraction method was utilized to proceed with the extraction process.

3.2.2 Formulation of nanoemulsion of *Etlingera elatior*

The research employed the low-energy emulsification technique, consisting of three steps. Initially, oil was gradually added to a water-surfactant mixture. Subsequently, water was incrementally added to an oil-surfactant solution. The final step involved combining all the ingredients to form the ultimate composition. Nano-emulsions with average droplet sizes of 50 nm and commendable kinetic stability were only achieved at specific oil weight fractions. Low-energy emulsification processes demand less energy compared to high-energy methods, utilizing the system's inherent chemical energy and requiring modest agitation. Approaches such as phase inversion and spontaneous emulsification exemplify low-energy methods. Spontaneous emulsification, a practical method for producing nanoemulsions, involves two liquid components: an aqueous phase and an organic phase. The aqueous phase accommodates water-soluble solvents, surfactants, and co-surfactants transferred from the organic phase. Initially, an organic phase comprising oil and surfactant is introduced into an aqueous phase consisting of water and co-surfactant. Rapid migration of water-miscible components into the aqueous phase, expanding the oil-water interfacial area, induces significant turbulence at the phase interface, resulting in the spontaneous formation of small oil droplets. (Alaa R. Azeez, 2022)

Table 3.1 Quantity of Extraction of *Ee*, Quantity of soy bean oil, Quantity of water, Quantity of surfactant & co-surfactant of F1 to F14

Formula	Quantity of Extraction *Ee*	Quantity of Soy bean oil (g)	Quantity of water(g)	Quantity of surfactant & co-surfactant (g)
F1	0.2	0.8	3	6
F2	0.3	0.7	3	6
F3	0.4	0.6	3	6
F4	0.5	0.5	3	6
F5	0.8	0.2	3	6
F6	0.7	0.3	3	6
F7	0.6	0.4	3	6
F8	0.6	1	2.4	6
F9	0.7	1	2.1	6
F10	1.2	1	1.8	6
F11	1.5	1	1.5	6
F12	2.4	1	0.6	6
F13	2.1	1	0.7	6

F14	1.8	1	1.2	6

* *Ee* represent *Etlingera elatior*

3.2.3 Formulation of nanoemulgel of *Etlingera elatior*

The enhanced *Etlingera elatior* nanoemulsion was combined with Carbopol gel to create the nanoemulgel. In the distilled water, the hydrogel was created by dispersing the gelling agent Carbopol 940. The dispersion was neutralised with triethanolamine, and the mixture was then agitated until a sticky gel base formed. The nanoemulgel was created by gradually adding the *Etlingera elatior* nanoemulsion to the gel while stirring continuously. (Ting, 2020) The amount of Carbopol 940 added to the nanoemulsion formulation was varied into 2%, 4%, 6%, 8% and 10%. The nanoemulsion formulation was formed as a nanoemulgel. The nanoemulsion formulation designated as F2 was utilized for blending with Carbopol 940 to produce the nanoemulgel

3.2.4 Characterization of nanoemulgel containing of *Etlingera elatior*

3.2.4 (a) Ph. Measurements

The pH of the nanoemulgel was measured by using digital pH meter. pH of the samples should be about 6-7 to match the skin condition. (Ting, 2020)

3.2.4 (b) Viscosity Test

When measuring the flow of any fluid, including liquids, semi-solids, gases, and even solids, viscosity is a crucial factor to consider. Measurements of viscosity are taken alongside the effectiveness and quality of the product. Everybody working in flow characterization, R&D, quality assurance, or fluid transfer has occasionally dealt with viscosity measurement of one kind or another. Viscosity is a measure of the resistance of a fluid to deform under shear stress and measure of fluid friction. (M.Maheshwar, 2018)

3.2.4 (c) Spreadability Test

The bioavailability effectiveness of gel formulations is determined by their spread ability, which demonstrates the area to which the formulation could easily spread when applied to skin or the affected part. The spreadability of various nanoemulgel formulations were determined by measuring the spreading diameter of 0.5 g of the sample between two horizontal glass plates after one minute. 5g weight was then applied to the upper plate. Each formulation was determined three times for the accuracy and consistency of the results. (Teo Chai Ting, 2020)

3.2.4 (d) Physical appearance

The visual properties of both nanoemulsion and nanoemulgel were evaluated and compared with the physical appearance of macroemulsion, nanoemulsion, and microemulsion, which were plotted on the ternary phase diagram for comparison. The appearance assessment included visually examining and analyzing the formulations to evaluate their macroscopic features, such as color, clarity, and consistency.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results

This section provides an overview of the findings obtained from the examination of nanoemulsion formulations and the development of nanoemulgel using water as a solvent for *Etlingera elatior,* as gathered from the research. The data collected is presented and thoroughly discussed in the subsequent sections.

4.1.1 Extraction of *Etlingera elatior* water solvent

In this study, showed the extraction method and the result extraction of *Etlingera elatior.*

Table 4.1: Extraction of *Etlingera elatior* water as solvent

	1	2	3
Weight of dried *Etlingera elatior*	10 g	10g	10g
Weight of crude extraction *Etlingera elatior*	0.954g	0.905gram	1.021g
Yield (%)	9.54	9.05	10.21
Min of Yield (%)		9.6	

The preparation of the plant material, Etlingera elatior, involved harvesting and thorough cleaning to eliminate impurities. Subsequently, the extraction process was initiated as the primary step in this research. To conduct the extraction, 10 grams of dried Etlingera elatior were precisely measured using analytical weighing. A beaker was filled with 300 ml of water, and this water was transferred to the boiling flask for the Soxhlet extraction process. Once completed, the extracted liquid was placed in the rotary with a boiling temperature of 64.7°C. The formula for calculating yields is provided below. The extraction of Etlingera elatior was performed three times, each time using the same quantity of dried Etlingera elatior (10 grams) to ensure ample yields. The average percentage yield for the extraction was determined to be 9.6%, representing the total yields in this study.

$$\% \, yields = \frac{\text{crude extraction}}{\text{dried extraction}} \, x \, 100$$

4.1.2 Formulation of nanoemulsion of extraction *Etlingera elatior*

Nanoemulsions, alternatively termed submicron emulsions, ultrafine emulsions, and miniemulsions, are colloidal particulate systems of submicron size. These systems are regarded as thermodynamically and kinetically stable isotropic dispersions, comprising two immiscible liquids—typically water and oil. Stabilization is achieved through an interfacial film composed of an appropriate surfactant and co-surfactant, resulting in the formation of a single-phase dispersion. (K.Gurpeet)

Table 4.2 : Nanoemulsion without containing active substance

Formula	Quantity of soy bean oil(g)	Quantity of water(g)	Quantity of Surfactant (g)	Quantity of co-surfactant (g)
F0	1	3	3	3

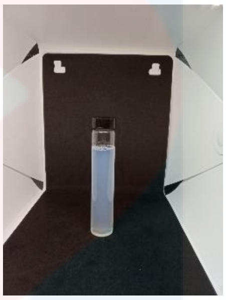


Figure 4.1: Nanoemulsion without containing active substance

Based on the results presented in Table 4.1, the formulation of nanoemulsion without the active substance (*Etlingera elatior* extraction) was demonstrated. This formulation included Tween 80 as the surfactant, with 3 grams of Tween 80 measured using analytical weighing and placed in a small beaker. Additionally, 3 grams of glycerol, serving as a co-surfactant, was mixed with Tween 80 after being measured. The oil phase, consisting of soybean oil, was also blended with Tween 80 and glycerol. The beaker was then placed on a magnetic stirrer for thorough mixing. Once well-blended, 3 grams of water was added to the beaker to mix with the surfactant, co-surfactant, and oil phase. The total volume of the combined oil phase, surfactant, co-surfactant, and water was 10 ml.

Subsequently, 50 ml of water was taken to stir on the magnetic stirrer. Using a disposable syringe, 1.5 ml from the 10 ml mixture containing co-surfactant, surfactant, oil phase, and water was taken and mixed with the 50 ml water. After thorough mixing, the resulting solution was placed into a test tube for a clear observation of the nanoemulsion, as depicted in Figure 4.1. This formulation was repeated three times to assess the stability of the nanoemulsion. After three repetitions, the results consistently indicated the formation of nanoemulsion.

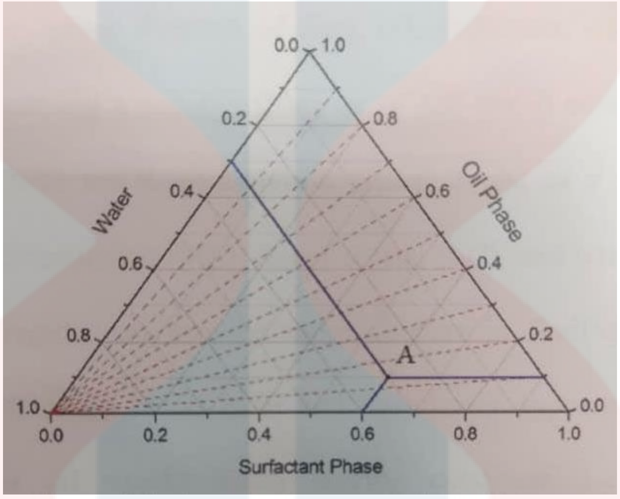


Figure 4.2 The ternary phase diagram for formula F0

The nanoemulsion fprmulation obtained in this research was based on the ternary phase diagram constructed by Wang (2009). The ternary phase diagram is utilized in nanoemulsion formulation to visualize and refine the composition of the system. Its purpose is to comprehend the phase interactions and behaviors among the components of the nanoemulsion, typically comprising oil, water, and surfactant/co-surfactant.

This diagram offers insight into the regions where various types of emulsions (e.g., macroemulsions, nanoemulsions, or microemulsions) are present, as well as the demarcations between these regions. By mapping the compositions of different formulations onto the ternary phase diagram researcher can pinpoint the ideal proportions of oil, water, and surfactant/co-surfactant for achieving stable nanoemulsions possessing desired attributes such as small droplet size, uniform droplet size distribution, and prolonged stability.

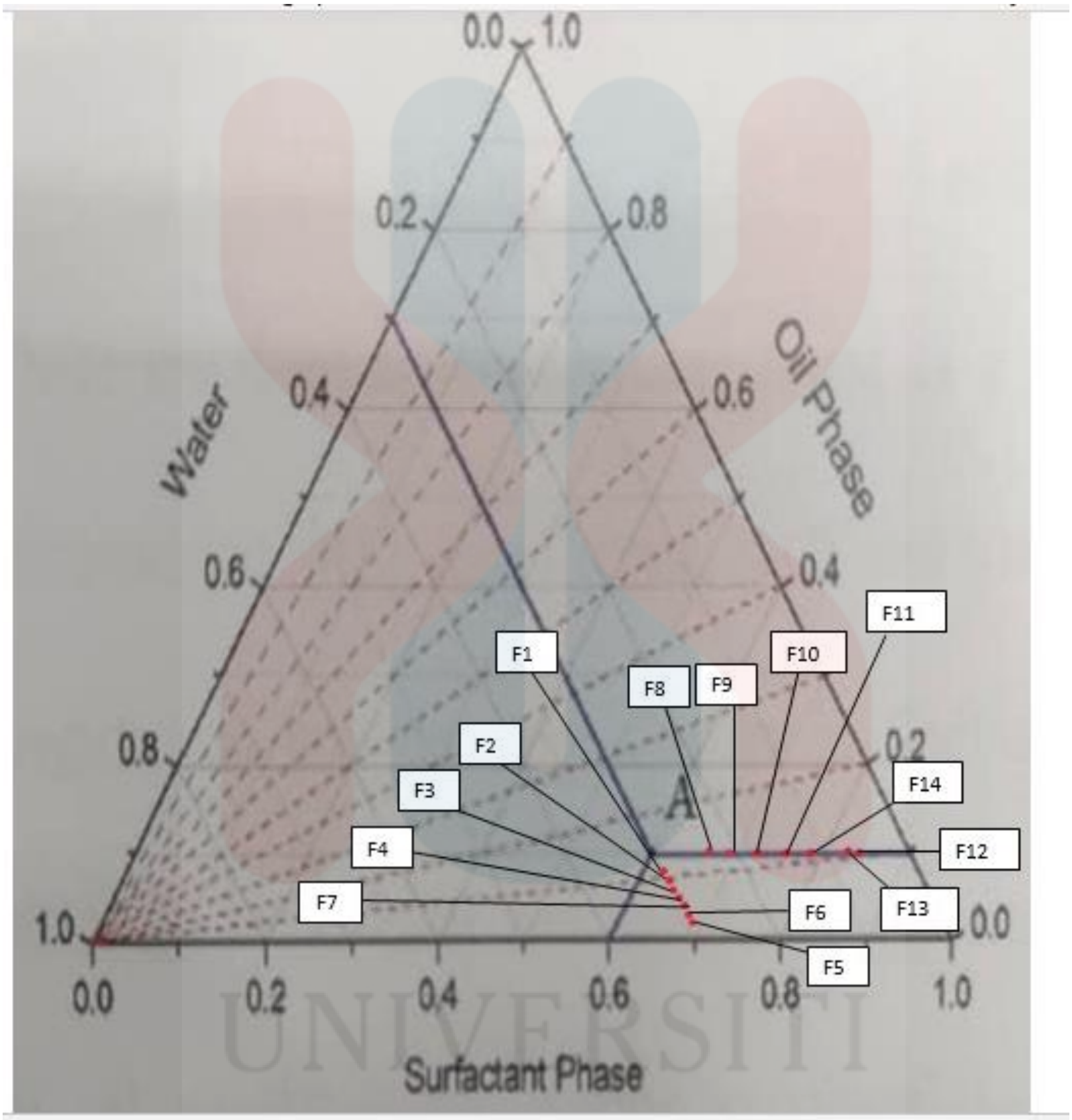
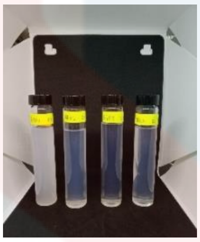
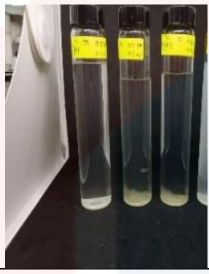
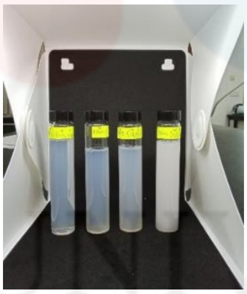
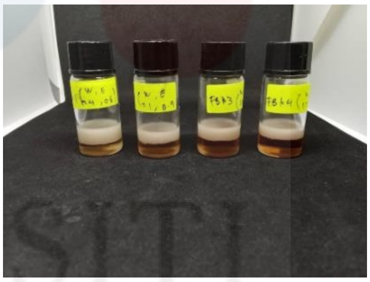
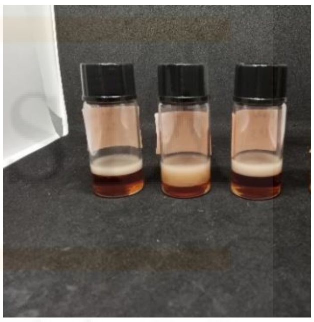


Figure 4.3 The ternary phase diagram to find formula that produced nanoemulsion. Point F1 until F14 was plotted on triangular graph

Table 4.3 Picture of nanoemulsion containing *Etlingera elatior* and nanoemulsions concentrated each formula

FORMULA	Nanoemulsion containing *Etlingera elatior*	Nanoemulsions Concentrated each formula
F1, F2, F3, F4		
F5, F6, F7		
F8, F9, F10, F11		
F12, F13, F14		

Based on the Table 3.1, there are 14 formula of nanoemulsions that had been recorded. The quantity of *Etlingera elatior* extraction, quantity of soybean oil, quantity of water and quantity of surfactant and co-surfactant were the quantity that need to added in each formula. Each value of this quantity influence every result.

Based on the information presented in Table 4.4, the results indicate that the color of F1 was white, whereas F2, F3, and F4 appeared bluish in comparison to F1. The formulation for F1 included 0.2 g of *Etlingera elatior* extraction, 0.8 g of soybean oil, 3 g of water, and 6 g each of surfactant and co-surfactant. In contrast, the quantities of *Etlingera elatior* extraction in F2, F3, and F4 were 0.3 g, 0.4 g, and 0.5 g, respectively. The quantities of soybean oil in F2, F3, and F4 were 0.7 g, 0.6 g, and 0.5 g, respectively. The quantities of water, surfactant, and co-surfactant remained consistent across all formulations, maintaining the same values as in F1. The values of *Etlingera elatior* extraction and soybean oil in F1, F2, F3, and F4 had a noticeable impact on the results, as evidenced in Table 4.5, where F1 exhibited a white color compared to the bluish appearance of F2, F3, and F4.

The formulations for F8, F9, and F10 exhibited a cloudy color, while F11, F12, F13, and F14 resulted in a white opaque color. In F8, F9, and F10, 0.6g, 0.7g, and 1.2g of *Etlingera elatior* extraction were added, along with 1g of soybean oil and 2.4g, 2.1g, and 1.8g of water, respectively. The quantities of surfactant and co-surfactant remained constant at 6g.

For F11, F12, F13, and F14, the quantities of *Etlingera elatior* extraction added were 1.5g, 2.4g, 2.1g, and 1.8g, respectively, with each formula incorporating 1g of soybean oil. The water quantities varied among these formulations, with F11, F12, F13, and F14 receiving 1.5g, 0.6g, 0.7g, and 1.2g of water, respectively. It can be inferred that the quantities of *Etlingera elatior* extraction, soybean oil, and water added in formulations F8 to F14 influenced the resulting color in each formulation.

4.1.3 Formulation of nanoemulgel of *Etlingera elatior*

The nanoemulgel was produced by mixing the nanoemulsion extraction of *Etlingera elatior* with Carbopol 940 and distilled water. In this study, gelling agent Carbopol 940 was applied at concentrations of 2%, 4%, 6%, 8%, and 10%. Carbopol 940 is known for its superior properties in thick formulations, excellent clarity, and the ability to produce low-viscosity gel. (Teo Chai Ting, 2020) When nanoemulgel adheres to the skin's surface, it releases oil droplets and penetrates the skin, allowing the medication to be delivered to the targeted area. Furthermore, because of its dual nature a nanoemulsion and a gel foundation combined into a single formulation with a small droplet size, making it capable of effectively delivering both hydrophilic and lipophilic drugs nanoemulgel is seen as a promising concept. (Barkat Ali Khan, 2023)

Table 4.4 Nanoemulgel from Nanoemulsion formulation F2

Carbopol 940	Nanoemulgel F2
2%	NG1
4%	NG2
6%	NG3
8%	NG4
10%	NG5

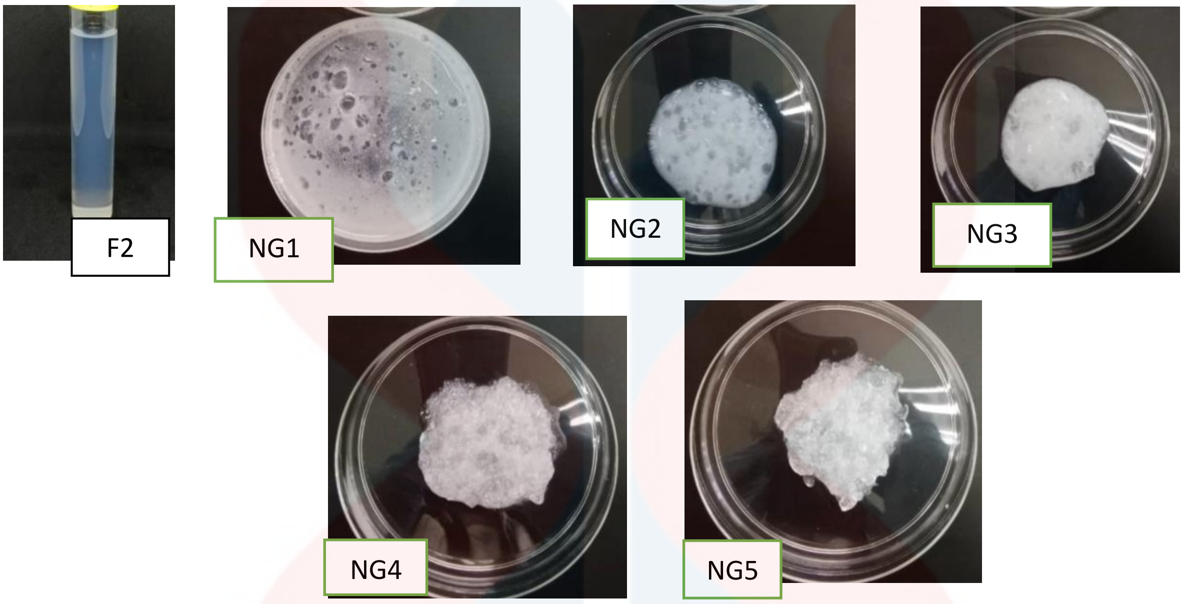


Figure 4.4 Nanoemulsion F2 and formulation nanoemulgel NG1 to NG5

The formula nanoemulsion that have been used in making nanoemulgel was F2. This formula had choosen because this formula succeeded produced a nanoemulsion. Varying concentrations was 2%, 4%, 6%, 8% and 10%.

4.2 Analysis of characterization of Nanoemulsion and Nanoemulgel containing extraction *Etlingera elatior*

4.2.1 Characterization of Nanoemulsion of extraction *Etlingera elatior* based on physical appearance, Tyndall effect and stability

4.2.1 (a) Physical appearance test for water, microemulsion, macroemulsion and nanoemulsion

Table 4.5 Physical appearance of microemulsion,macroemulsion,nanoemulsion and water

Solution	Water	Microemulsion	Macroemulsion	Nanoemulsion
Colour	Clear	Clear	White	Cloudy/Bluish
Transparency	Scattering	Transparent	Opaque	Transparent

Table 4.6 Physical appearance differences

Formula	Solution	Colour	Transparency
F0	Nanoemulsion	Cloudy	Transparent
F1	Macroemulsion	White	Transparent
F2	Nanoemulsion	Bluish	Transparent
F3	Nanoemulsion	Bluish	Transparent
F4	Nanoemulsion	Bluish	Transparent
F5	Microemulsion	Clear	Transparent
F6	Microemulsion	Clear	Transparent
F7	Microemulsion	Clear	Transparent
F8	Nanoemulsion	Cloudy	Transparent
F9	Nanoemulsion	Cloudy	Transparent
F10	Nanomemulsion	Cloudy	Transparent
F11	Macroemulsion	White	Opaque
F12	Macroemulsion	White	Opaque
F13	Macroemulsion	White	Opaque

F14	Macroemulsion	White	Opaque

The formulation of nanoemulsion of *Etlingera elatior* was showing that almost formula is nanoemulsion. Nanoemulsions are transparent due to their small size. Table 4.4 show some formula turn into nanoemulsions, macroemulsion and microemulsion. The formulation of nanoemulsion without containing active substance was 3g of co-surfactant which is the glycerol, 3g of surfactant which is the Tween 80, 3g water and 1g soybean oil as oil phase. This formula show the colour was cloudy. Bluish colour is categorized as a nanoemulsion. (Nurul Akmar Che Zaudin) Compared to formula F2, F3, F4, F8, F9 and F10 all this formula show a bluish and cloudy colour and transparent. Meanwhile formula F5, F6 and F7 show the colour was clear. The solution of this formula was microemulsion. (Alia A.Badawi, 2009) Meanwhile the colour of F11, F12, F13 and F14 was white and opaque. This solution of this formula was macroemulsion. (Nurul Akmar Che Zaudin) The nanoemulsions of droplet size was 5-200 nm. Meanwhile microemulsion was 5100 nm and for macroemulsion it was 0.1-100 μm (Kale, 2021) The appearance for this solution was opaque or turbid for macroemulsion and clear and transparent for microemulsion. Meanwhile the appearance for nanoemulsion it was cloudy or bluish and transparent. There are 3 test validity of the nanoemulsions samples which is the physical appearance differences, Tyndall effect and stability test. (Nurul Akmar Che Zaudin)

There are several factors that determine whether a formulation becomes a nanoemulsion, microemulsion, or macroemulsion. This is attributed to the type of emulsion formed, such as oil-in-water nanoemulsion, where oil droplets are dispersed in the continuous aqueous phase, or water-in-oil nanoemulsion, where water droplets are dispersed in the continuous oil phase. (R.Joshi, 2012). The changes in the the stability and physical properties of nanoemulsions can be affected by modifications in the composition of the water and oil phases. Particle size and stability can be impacted by changes in the ratios of surfactant or co-surfactant. The oil phase, which contains the distributed droplets, and the water phase, which acts as the continuous medium, are the two primary phases of nanoemulsions. These phases' composition has a major impact on the stability of the nanoemulsion. Physical and chemical features of the nanoemulsion are dependent on the kind of oil used and its composition. A formulation's overall stability might be impacted by the solubilities and interactions of certain oils with surfactants. The characteristics of the nanoemulsion can be affected by the water phase's composition, including the kind and purity of the water. The stability and shelf life of the formulation may be affected by the presence of ions or contaminants. Through their ability to lower the interfacial tension between water and oil, surfactants and co-surfactants are essential for stabilizing nanoemulsion. The emulsification process and the final droplet size can be impacted by variations in the surfactant to co-surfactant ratio. But however the value of co-surfactant and surfactant that vave been added always same and did not change which is 3g of co-surfactant and 3g of surfactant.

4.2.1 (b) Tyndall Effect

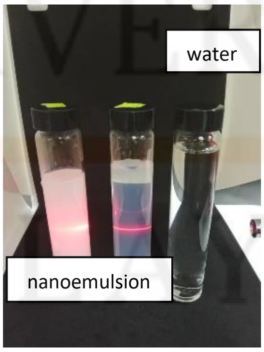


Figure 4. 5: Tyndall effect test

Nanoemulsions are nanoscale droplets containing a colloidal particle system. Light can be scattered by the colloidal particles as it travels through the particle-containing solution. Three distinct samples, one of which was a nanoemulsion made of distilled water and the other of which included extraction from *Etlingera elatior*, were exposed to light. The fact that a light beam could be clearly seen in the bottles holding the nanoemulsion sample demonstrated the stability of both formulations and the presence of nano-sized droplets that highlight the light path and make it visible to the human eye. The density of the particles in the solution and the frequency of the light both affect how much scattering occurs. (Nurul Akmar Che Zaudin)

4.2.1 (c) Stability Test

All the nanoemulsion sample were left at room temperature for 1 month to test its shelf life. But however, based on data that have been recorded on the visual observation the first day theres is no separation happen but sample did lose their stability after 2 weeks. Some samples were stable and no separation happening. Nanoemulsion was kinetically stable but after 2 weeks, separation phase is happening. This happened because of the microbial contamination. Nanoemulsions, especially those with a water component, may be susceptible to microbial contamination over time. This can lead to changes in the composition and stability of the nanoemulsion. Extreme temperature variations can impact the stability of nanoemulsions. Freezing or high temperatures can affect the structure and composition of the emulsion, leading to instability. Ostwald ripening is a phenomenon where larger droplets grow at the expense of smaller ones. If the formulation is not optimized, Ostwald ripening may occur, leading to changes in droplet size distribution and potential instability. (Juan Manuel Montes de Oca-Avalos, 2017)

4.2.2 Characterization of nanoemulgel *Etlingera elatior* ph measurement, viscosity test, spreadibility test and physical appearance

The characterization of nanoemulgel was characterized with some test which is stability test, ph measurement, viscosity test, spreadibility test and physical appearance.

4.2.2 (a) Ph measurement

Table 4.7 Ph of nanoemulgel

Sample	Carbopol 940	ph
NG0		7.07
NG1	2	3.5
NG2	4	3.1
NG3	6	3.2
NG4	8	3.3
NG5	10	3.3

NG0 represent Hydrogel

The pH of nanoemulgel were determined by using a calibrated pH meter. The ph for NG0 was 7.07. Meanwhile NG1, NG2, NG3, NG4 and NG5 was 3.5, 3.1, 3.2, 3.3,3.3. The readings were obtained for average of three times. The pH achieved was acidic which in the range of 3.1 to 3.5. It shows that the nanoemulgel formulations were not safe to be used on the skin and will causes any irritation when applied to the skin. The pH value of the nanoemulgel was gradually decreasing with the addition of nanoemulsions of extraction *Etlingera elatior* and more acidic compare to NG0 which is the blank hydrogel. The addition of the nanoemulsion extraction *Etlingera elatior* slightly influences the acidic condition of the formulation. Table 4.8 represents the average pH of the different nanoemulgel formulations. (Teo Chai Ting, 2020)

4.2.2 (b) Viscosity test

Table 4.8 Viscosity of nanoemulgel containing extraction *Etlingera elatior*

Sample	Carbopol 940	Viscosity (Pa.s)
NG1	2%	L
NG2	4%	1.28
NG3	6%	3.47
NG4	8%	4.32
NG5	10	6.35

Pa.s is unit of viscosity pascal seconds

Table 4.9 show the viscosity of nanoemulgel containing extraction *Etlingera elatior* of NG1, NG2, NG3, NG4, and NG5 which show different viscosity. The viscosity of NG1 is L which mean it is low. The viscosity for NG2 and NG3 was 1.28 and 3.47. Viscosity for NG4 and NG5 was 4.32 and 6,35. NG5 had high value compared to NG1. The larger value of the viscosity the high is the viscosity. The high viscosity was recorded in F9 which is 6.35.

Viscosity test was measure with the viscometer. Each of this formula bring a different value ot the viscosity. For the NG1 the viscosity is L which mean its low. The medium viscosity was in NG2, NG3 and NG4 which is 1.28, 3.47 and 4.32. Meanwhile NG5 had a highest viscosity. The viscosity of NG5 was enhanced due to the addition of gelling agent (Carbopol 940). The addition of gelling agent enhances viscosity of any preparation. (Barkat Ali Khan N. A., 2024) By structural characteristics, creams are opaque, viscous, ranged from non-greasy to mildly greasy texture and tend to evaporate or be absorbed when rubbed onto the skin. In comparison with ointments, creams are significantly less greasy, less viscous, less hydrating and more spreadable being used for their moistening and emollient properties. (Diana Stan, 2021) However, these days a lot of manufacturers see viscometers as an essential component of their R&D and process control initiatives. They are aware that viscosity measurements can frequently be used to examine some of the most crucial elements influencing a product's performance in the quickest, most accurate, and most trustworthy manner. The high viscosity also can useful in making certain food products, such as sauces, dressings, and condiments, benefit from high viscosity for a desirable texture and consistency. Also, high viscosity suitable for detergents and cleaning products such as gels and thickened detergents for better adherence to surface. (Nazneen Sultana, 2022)

4.2.2 (c) Spreadibility test

Table 4.9 Spreadibility of nanoemulgel containing extraction *Etlingera elatior*

Sample	Carbopol 940	Spreadibility (cm)
NG1	2%	9.2
NG2	4%	5
NG3	6%	4.1
NG4	8%	3.9
NG5	10%	3.4

The spreadibility test for nanoemulgel containing extraction *Etlingera elatior* for NG1 was 9.2cm. The spreadbility for NG2, NG3, NG4 and NG5 was 5cm, 4.1cm, 3.9cm and 3.4cm. The highest spreadibility was the NG1 and the low spreadibility was NG5. The spreadability of the nanoemulgel is one of the essential criteria for the selection of a topical delivery system.The spreadability of different nanoemulgel formulations was determined, and the result was tabulated in Table 4.10. The values obtained were found to be satisfactory and comply with the standard range. Larger value shows better spreadability that indicates the contentment of the formulation when applied to the skin, spreads easily, and displays the maximum slip and drag. From the result, NG1 recorded the largest value of spreadability. (Teo Chai Ting, 2020) Spreadability is the ability of a dosage form to spread out when applied to the skin. The therapeutic efficacy of topical formulation depends upon the spreadability. The spreadability coefficient of the topical product can change due to various factors, such as fluctuations in temperature. Lower temperatures, for example, can lead to an increase in the formulation's viscosity, thereby reducing its spreadability. Spreadibility and viscosity have the inverse relationship with each other. (Barkat Ali Khan N. A., 2023)

There are 3 significance of spreadibility test which is consumer the experience. The spreadibility is crucial for consumer acceptance. A product that spreads easily is more likely to be preferred by users. Secondly the uniform application which is good spreadibility ensures that the formulation is applied uniformly over the target area, which is crucial for consistent dosing and efficacy. Thirdly was ease of use. Products with good spreadibility are easier to handle and apply, making them more user-friendly. (Teo Chai Ting, 2020)

4.2.2 (d) Physical appearance

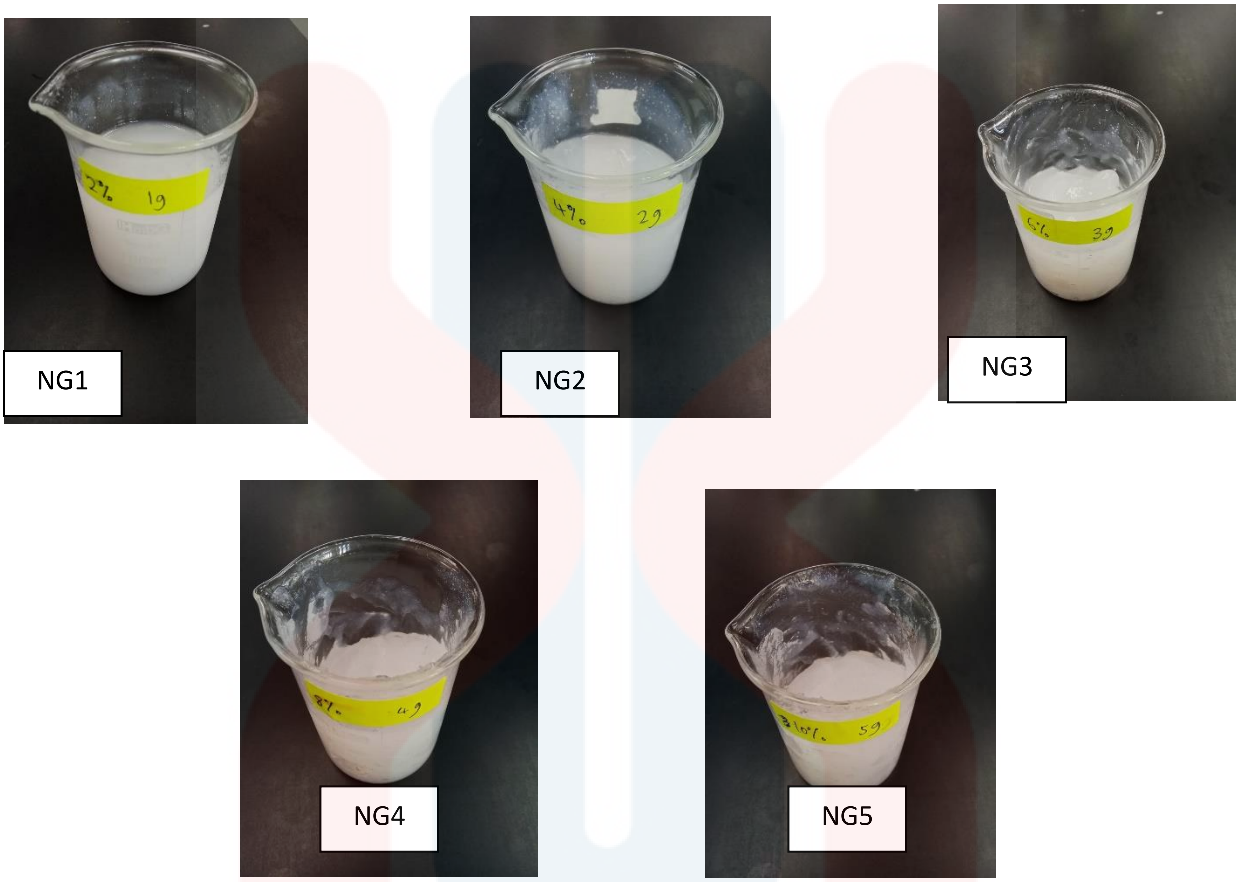


Figure 4.6 The nanoemulgel of NG1, NG2, NG3, NG4 and NG5 containing nanoemulsion extraction *Etlingera elatior*

Physical appearance of the nanoemulgel with different ratio shows similar result as nanoemulsions but they were all represented cloudier due to the addition of the gelling agent. The nanoemulgel produced usually was white viscous creamy accompanied by a smooth and homogeneous appearance that leads to the nanoemulgel being more easily spreadable with tolerable bioadhesion and fair mechanical characteristics. NG1 show too liquid compared to NG2. Meanwhile NG3 and NG4 is not too much liquid but not too much sticky. NG5 is a bit sticky.

The physical appearance for the nanoemulgel NG1, NG2, NG3, NG4 and NG5 was the colour of this 5 nanoemulgel was white cloudier. F2 more liquid or semi fluid compare to F9. F9 was thick and sticky The nanoemulgel produced usually was white viscous creamy accompanied by a smooth and homogeneous appearance that leads to the nanoemulgel being more easily spreadable with tolerable bioadhesion and fair mechanical characteristics. (Ting, 2020)

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

To conclude, this research has successfully developed formulations of nanoemulsion and nanoemulgel containing the extraction of *Etlingera elatior*, and characterized the nanoemulgel formulations. The extraction process involved the use of Soxhlet extraction with water as a solvent, followed by solvent removal using a rotary evaporator. The *Etlingera elatior* extraction was then incorporated into a nanoemulsion formulation consisting of oil phase, water, co-surfactant, and surfactant. Fourteen different formulations were tested to determine the number of nanoemulsions produced, with some exhibiting characteristics of macroemulsions, microemulsions, and nanoemulsions. Characterization tests including the Tyndall effect, visual appearance, and stability assessment were conducted on the nanoemulsion formulations, revealing the presence of nanoemulsions, microemulsions, and macroemulsions within each formulation. Subsequently, five different nanoemulgel formulations were developed and characterized using pH measurement, visual appearance, viscosity evaluation, and spreadability assessment. The analysis revealed that the pH levels of the nanoemulgels containing *Etlingera elatior* extraction fell within the range of 3.1 to 3.5, indicating an acidic nature. However, this pH range is not conducive to skin application, as the skin's optimal pH is around 7, which is neutral. This finding suggests that the nanoemulgel formulations may not be suitable for direct application to the skin due to their acidic pH levels. Further optimization of the formulations is necessary to adjust the pH to a more skin-friendly level, ensuring compatibility and safety for topical use. This highlights the importance of considering pH levels in formulation development, particularly for products intended for skin application, to ensure efficacy and minimize potential adverse effects.The observed viscosity trends, where Carbopol 940 concentration increases viscosity, follow rheological principles that control gel compositions.

Overall, this study highlights the importance of considering various factors, such as pH levels, in the development of formulations containing natural extracts for medicinal and cosmetic purposes. Further research and optimization efforts are needed to enhance the efficacy and suitability of these formulations for practical applications.

5.2 Recommendations

As a recommendation for further research, it would be beneficial to compare the extraction of Etlingera elatior using different solvents such as ethanol or methanol alongside water, to evaluate their effectiveness in the nanoemulsion formulation. This comparative study would provide valuable insights into the optimal solvent for extracting bioactive compounds from Etlingera elatior.Additionally, adjusting the pH of the nanoemulgel of Etlingera elatior could be explored by incorporating sodium hydroxide (NaOH) or Triethanolamine (TEA). NaOH is a potent base commonly used for pH adjustment, but its strong alkalinity requires careful handling. Conversely, TEA is a milder base, which may offer more controlled pH adjustment in the formulation process. Moreover, for a comprehensive understanding of the nanoemulsion formulation, further research could investigate the use of different solvents such as hexane, ethyl acetate, and methanol. Comparing these solvents in the extraction process would contribute to optimizing the formulation for enhanced efficacy and relevance in wound healing **applications.**

REFERENCES

Abeeda Mushtaq, S. M. (2023). Recent insights into Nanoemulsions: Their preparation, properties and applications. *Food Chemistry : X*.

Alaa R. Azeez, M. A. (2022). NANOEMULGEL AS A RECENT DRUG DELIVERY SYSTEM . *Military Medical*, 128-139.

Ali Ghasemzadeh, H. Z. (2015). Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of Etlingera elatior (Jack) R.M.Sm grown in different locations of Malaysia. *PubMed Central*.

Alia A.Badawi, S. A.-M. (2009). Preparation and Evaluation of Microemulsion Systems Containing Salicylic Acid. *National Library of Medicine*, 1081-1084.

Barkat Ali Khan, N. A. (2023). Formulation Development of Pharmaceutical Nanoemulgel for Transdermal Delivery of Feboxostat: Physical Characterization and In Vivo Evaluation. *European Journal Of Pharmaceutical Sciences*.

Barkat Ali Khan, N. A. (2024). Formulation development of pharmaceutical nanoemulgel for transdermal delivery of feboxostat: Physical characterization and in vivo evaluation. *European Journal Of Pharmaceutical Sciences*.

C.Lemaire, A. J. (n.d.). Solid Phase Extraction-An Alternative to the Use Of Rotary Evaporators for Solvent Removal in the Rapid Formulation of PET Radiopharmaceuticals. *Journal of Labelled Compounds and Radiopharmaceuticals*, 64-75.

Diana Stan, C. T.-B. (2021). Wound healing applications of creams and 'smart' hydrogels. *National Library Of Medicine*, 1218-1232.

Eric Wei Chiang Chan, Y. Y. (2011). Standarised herbal extract of chlorogenic acid from leaves of Etlingera elatior (Zingibereceae). *Pharmacognsy Research* , 84-178.

Fernanda Almeida, M. C. (2022). Influence of different surfactants on development of nanoemulsions containing fixed oil from an Amazon palm species. *Colloids and Surface A : Physicochemical and Engineering Aspects*.

J Pharm Bioallied, A. R. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy Bioallied Sciences*, 1-10.

Juan Manuel Montes de Oca-Avalos, R. J. (2017). Nanoemulsions : stability and physical properties. *Current Opinion in Food Science*, 1-6.

K.Gurpeet, S. (n.d.). Review of Nanoemulsion Formulation and Characterization Techniques. *Review Article*.

Kale, S. M. (2021). A Review On Nanoemulsion. *INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHS (IJCRT)*, 788-808.

Kamarza Mulia, R. M. (2018). Formulation and characterization of nanoemulgel mangosteen extract in virgin coconut oil for tropical formulation. *MATEC Web of Conferences*.

Liza Noordin, W. A. (2022). Etlingera elatior Flower Aqueous Extract Protects against Oxidatives Stress-Induced Nephropathy in a Rat Model of Type 2 Diabetes. *National Center for Biotechnology Information*.

M.Maheshwar. (2018). A REVIEW ARTICLE ON MEASUREMENT OF VISCOSITY. *INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY*, 69-77.

Mahipal Reddy Donthi, S. R. (2023). Nanoemulgel: A Novel Nano Carrier as a Tool for Topical Drug Delivery. *National Center for Biottechnology Information*.

Muhammad Asri Abd Sisak, R. D. (2016). STUDY ON THE EFFECT OF OIL PHASE AND COSURFACTANT ON MICROEMULSIONS SYSTEMS. *MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES*, 1409-1416.

Nazneen Sultana, J. A. (2022). *Drug Development Life Cycle.* CHAPTER METRICS OVERVIEW.

Nurul Akmar Che Zaudin, N. S. (n.d.). *Development and Characterization of Nanoemulsion Containing Essential Oil of Piper Betle as the Active Ingredient Via Low Energy Emulsification Method.*

Preeti, S. S. (2023). Nanoemulsion: An Emerging Novel Technology for Improving the Bioavailability of Drugs. *National Center for Biotechnology Information*.

Qing Wen Zhang, L. G. (2018). Techniques for extraction and isolation of natural products a comprehensive review. *Chinese Medicine*.

R.Joshi, R. P. (2012). AN OVERVIEW ON NANOEMULSION: A NOVEL APPROACH. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*, 4640-4650.

Ramsden, J. J. (2016). *What is nanotechnology?* Collegium Basilea (Institute Of Advanced Study), Basel, Switzerland: December 2016.

Shivani MPatel, P. K. (2011). Extraction and analysis of Jatropha curcas L.seed oil. *AFRICAN JOURNAL OF BIOTECHNOLOGY*, 18211-18213.

Teo Chai Ting, N. F. (2020). Development and Characterization of Nanoemulgel Containing Piper betle Essential Oil as Active Ingredient. *IINTERNATIONAL CONFERENCE ON SCIENCE AND TECHNOLOGY*, 1-7.

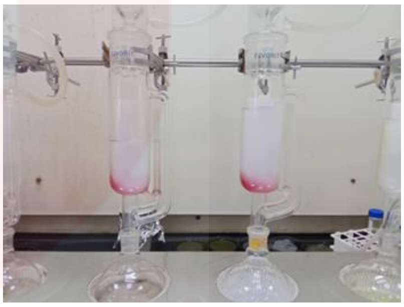
Ting, T. C. (2020). Development and Characterization of Nanoemulgel Containing Piper betle Essential Oil as Active Ingredient . *IOP Conference Series: Earth and Environmental Science*.

Yau Yan Lim, E. W. (2011). Standardised herbal extract of chologenic acid from leaves of Elingera elatior (Zingiberaceae). *Original Article*, 178-184.

Yeat, C. S. (2016). PHENOLOGICAL POSTHARVEST PHYSICOCHEMICAL AND ULTRASTRUCTYURAL CHARATERISTICS OF TORCH GINGER (Etlingera elatior (Jack) INFLORESCENCE AS A CUT FLOWER.

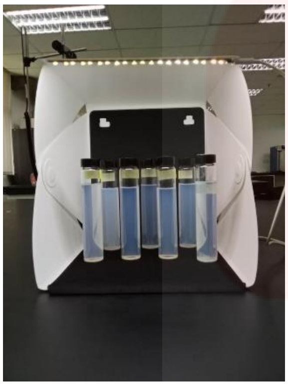
APPENDIX A

Extraction Process



APPENDIX B

Formulation Nanoemulsion



APPENDIX C

Formulation Nanoemulgel and The Characterization