# CHARACTERISATION OF JACKFRUIT (*Artocarpus heterophyllus*) LEAF EXTRACT: IMPACT OF DRYING AND ETHANOL/WATER RATIOS ON ANTIOXIDANT PROPERTIES AND DEVELOPMENT OF XANTHAN GUM-FISH GELATIN NANOEMULGEL

**By**

**HASWANI MAISARAH BINTI MUSTAFA**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**June 2024**

**FK 2024 67**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

# CHARACTERISATION OF JACKFRUIT (*Artocarpus heterophyllus*) LEAF EXTRACT: IMPACT OF DRYING AND ETHANOL/WATER RATIOS ON ANTIOXIDANT PROPERTIES AND DEVELOPMENT OF XANTHAN GUM-FISH GELATIN NANOEMULGEL

By

**HASWANI MAISARAH BINTI MUSTAFA**

**June 2024**

**Chairman : Nor Amaiza binti Mohd Amin, PhD**
**Faculty : Engineering**

The increasing demand for natural, sustainable, and effective delivery system for bioactive compounds has driven research into innovative formulations. Jackfruit leaves, known for this potent antioxidant properties, remain underutilised in pharmaceutical and cosmeceutical applications. However, the challenge lies in the optimising the drying processes to preserve their bioactive potential and integrating these extracts into stable and efficient drug delivery systems. Moreover, despite the growing interest in nanomeulgels as one of the promising drug delivery systems, many studies have yet to investigate the synergistic potential of natural stabilisers like xanthan gum and fish gelatin. This research aims to address these gaps by investigating the drying kinetics, selection of optimal solvent on antioxidant properties of jackfruit leaves (JL) from *Mastura* variety and developing a nanoemulgel formulation for topical applications. Initial phase study focused on the drying kinetics of JL using shade drying (SD), sun drying (SN), and oven drying at 40°C (OV). The Newton model was found to be the most suitable for describing moisture removal patterns of

JL. The moisture effective diffusivities were ranged from 2.920 x $10^{-10}$ to 6.814 x $10^{-10}$ $m^2/s$ over the temperature range studied and SD and OV were observed to preserve acceptable colour. All dried JL were ground into powder and macerated and extracted using various ethanol-water (E/W) extraction ratios (0%, 50%, 80% and 100%). Oven dried extracted using 80% E/W (OV80) able to preserve the highest antioxidant contents and antioxidant activities. The second phase involved the formulation of nanoemulgel stabilised with polymer of interest, single xanthan gum (Xan) and fish gelatin (Gel) and the polymer mixtures (MA – ME) incorporating the JL extract. Droplet size, zeta potential, polydispersity index (PDI), stability, morphology, and rheology of nanoemulgels integrated with JLE were determined. The sizes were all less than 250 nm, with PDI and zeta potential values in the range of 0.225 -0.319 and -47.34 -27.32 mV, respectively indicating colloidal stability. Rheological analysis demonstrated shear-thinning behaviour, while showing spherical, well dispersed droplet under microscope. Spreadability test revealed that all formulation has preferable spreadability feature (non-Newtonian flow) that required for the application of topical gel formulated. The selected nanoemulgel with desirable properties for topical application were observed in the *in vitro* release testing study for drug permeation. The result revealed that nanoemulgel with incorporation of 0.5% Xan and 2.0% Gel or labelled as MA showed significantly higher permeation rate (0.4294 ± 0.0062 $mg/cm^2$), than other formulations. MA was found to obey the Korsmeyer-Peppas equation, which may be interpreted through diffusion and erosion mechanisms. This discovery suggests that JL extract has the potential to be developed into sustainable and efficient bioactive delivery system.

**Keywords:** Fish Gelatin, Jackfruit Leaves, Nanoemulgel, Permeation, Xanthan Gum

**SDG:** GOAL 9: Industry, Innovation and Infrastructure, GOAL 12: Responsible Consumption and Production

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN EKSTRAK DAUN NANGKA (*Artocarpus heterophyllus*): IMPAK PENGERINGAN DAN NISBAH ETANOL/AIR TERHADAP SIFAT ANTIOKSIDAN DAN PEMBANGUNAN NANOEMULGEL GAM XANTAN-GELATIN IKAN**

Oleh

**HASWANI MAISARAH BINTI MUSTAFA**

**Jun 2024**

**Pengerusi : Nor Amaiza binti Mohd Amin, PhD**
**Fakulti : Kejuruteraan**

Permintaan terhadap sistem penhantaran bioaktif semulajadi, mampan dan berkesan semakin meningkat, memacu ke arah penyelidikan yang inovatif. Daun nangka yang kaya dengan sifat antioksidan, masih kurang dimanfaatkan dalam farmaseutikal dan kosmeseutikal. Walaubagaimanapun, cabaran utama adalah pada pengoptimuman proses pengeringan untuk mengekalkan potensi bioaktifnya, serta penghasilan sistem penghantaran ubat yang stabil dan cekap. Tambahan pula, walaupun minat terhadap nanoemulgel semakin meningkat sebagai salah satu sistem penghantaran ubat yang berpotensi, banyak kajian masih belum meneliti potensi terhadap sinergi penstabil semula jadi seperti gam xantan dan gelatin ikan. Kajian ini bertujuan untuk mengisi jurang penyelidikan tersebut dengan menyiasat kinetik pengeringan dan pemilihan pelarut yang optimum terhadap sifat antioksidan daun nangka dari varieti *Mastura* serta membangunkan formulasi nanoemulgel untuk aplikasi topikal. Fasa pertama kajian memberi tumpuan kepada kinetic pengeringan daun nangka menggunakan pengeringan secara redup (SD), pengeringan matahari (SN), dan pengeringan oven

pada 40°C (OV). Model Newton menunjukkan paling sesuai untuk menerangkan corak penyingkiran lembapan. Difusiviti efektif kelembapan adalah dalam julat 2.920 $x10^{-10}$ ke 6.814 x $10^{-10}$ $m^2/s$ untuk suhu yang dikaji dan SD dan OV didapati mengekalkan warna yang boleh diterima. Semua daun nangka kering dikisar menjadi serbuk dan diekstrak menggunakan pelbagai nisbah pengekstrakan etanol-air (E/W) (0%, 50%, 80% and 100%). Pengeringan oven yang diekstrak menggunakan 80% E/W (OV80) mampu untuk mengekalkan kandungan antioksidan dan aktiviti yang paling tinggi. Fasa kedua melibatkan nanoemulgel yang distabilkan dengan polimer pilihan, gam xantan (Xan) dan gelatin ikan (Gel) tunggal and campuran polimer (MA –ME) yang menggabungkan ekstrak daun nangka (JL). Nanoemulgel mengandungi ekstrak JL ditentukan berdasarkan ujian saiz titisan, potensi zeta, PDI, kestabilan, morfologi, dan reologi. Semua saiz menunjukkan kurang daripada 250 nm, dengan nilai PDI dan potensi zeta masing-masing di antara 0.225 -0.319 and -47.34 -27.32 mV menunjukan kestabilan koloid. Analisi reologi menunjukkan sifat aliran penipisan ricih, sambil menunjukkan bentuk sfera dan teragih dengan baik di bawah mikroskop.Ujian kebolehsebaran mendedahkan bahawa semua formula mempunyai ciri penyebaran (non-Newtonian flow) yang diperlukan untuk aplikasi gel topikal yang diformulasi. Nanomulgel yang terpilih dengan sifat yang diingini untuk aplikasi topikal ditentukan menggunakan kaedah pelepasan *in vitro* untuk resapan ubat. Hasil menunjukkan nanoemulgel yang digabungkan dengan 0.5% Xan and 2.0% Gel atau dilabel sebagai MA secara signifikan lebih tinggi daripada formula nanoemulgel lain. MA didapati persamaan Korsmeyer-Peppas, yang boleh ditafsirkan melalui mekanisma diffusi dan erosi. Penemuan ini mencadangkan ekstrak JL mempunyai potensi untuk dibangunkan menjadi sistem penghantaran bioaktif yang mampan dan efektif.

**Kata Kunci:** Gelatin Ikan, Daun Nangka, Nanoemulgel, Resapan, Gam Xanthan

**SDG:** MATLAMAT 9: Industri, Inovasi dan Infrastruktur, MATLAMAT 12: Penggunaan dan Pengeluaran yang Bertanggungjawab

## ACKNOWLEDGEMENTS

With the name of Allah, the Most Compassionate and Most Merciful

I would like to express my deepest gratitude to my mother and my siblings who have been my pillar of strength and for their unwavering support throughout my graduate journey. Their love and encouragement provided me strength and motivation to perservere through the challenge of this dissertation.

I want to acknowledge the invaluable guidance and assistance I received from my understanding supervisor, Dr. Nor Amaiza Mohd Amin and other supervisory committee whose mentorship and expertise were instrumental in this research. I am also immensely thankful to my Bioprocess Research group: Halimatun, Nadia, Ida, and Shaerah whose constant reassurance and assistance and understanding helped me maintain focus and determination.

I would like to extend my gratitude to all Process and Food Engineering's lab technicians for their assistance in providing support and guidance in this research as well. My deepest thank you to my funding bodies, Yayasan Bank Rakyat and UPM for the financial assistance and made this research possible.

Lastly, I want to thank me for perseverance, for believing in me, for doing all this hard work, never quitting and for just being me at all times. The completion of this dissertation would not been possible without collective support of these individuals and I am forever grateful for their presence throughout my PhD journey. Thank you so much.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

**Nor Amaiza binti Mohd Amin, PhD**
Senior Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

**Rabitah binti Zakaria, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

**Mohd Shamsul bin Anuar, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

**Azhari Samsu bin Baharuddin, PhD**
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

**ZALILAH MOHD SHARIFF, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 24 February 2025

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF APPENDICES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| °C | Degree celsius |
| ml | Millilitre |
| µm | Micrometer |
| nm | nanometer |
| h | Hour |
| cm | Centimetre |
| $cm^2$ | Centimetre square |
| $m^2$ | Meter square |
| s | Second |
| g | Gram |
| mg | Milligram |
| % | Percentage |
| $R^2$ | Determination coefficient |
| µM | Micro molar |
| mV | Milivolt |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Jackfruit or scientifically known as *Artocarpus heterophyllus* Lam. is also locally known as *Nangka* is one of the many tropical fruits that is popular and has found many uses in Malaysia. It is originated from India and also widely cultivated in other countries such as Thailand, Vietnam, Indonesia, Phillipines, Sri Lanka, China and Brazil (Zheng et al., 2014). Properties of its edible and non-edible parts such as the fruit, seed, skin, leaf, root, and twigs have been reported. The edible part such as the flesh and seeds are normally eaten as fresh and boiled or fried respectively and commercialised into various food products such as puree, juice, freeze dried light snack and as ice cream flavor. The non-edible part such as leaves was revealed to possess medicinal values such as antioxidant, anti-inflammatory, antimicrobial, anticancer, hypoglycaemic and hypolipidemic activities (Baliga et al., 2011; Jagtap and Bapat, 2010). Fresh leaves are highly perishable and prone to rapid degradation due to post harvest conditions such as enzymatic activity and microbial growth. These factors can cause to the loss of key bioactive compounds such as phenolics and flavonoids, which has the potential for pharmaceutical application. Furthermore, jackfruit leaves face challenges related to post-harvest storage and large-scale utilisation. Therefore, drying for future use could reduce this difficulty. Drying stabilises the leaves by reducing moisture content and able to retain the antioxidant, properties, making the leaves suitable for high-quality pharmaceutical applications (Kumar et al., 2022 and Inyang et al., 2018).

Drying is the most cost-effective way to preserve, enhance the shelf life, and improve the quality of jackfruit leaves. Process of drying helps to reduce the moisture content of the food. The main purpose of drying is to reduce the moisture content to a level where microbial spoilage and decomposition reactions are greatly reduced (Inyang et al., 2018). Higher drying temperatures reduce drying time but lead to poor product quality, surface heat damage and increased energy consumption. Mild, low-temperature drying conditions, on the other hand, can improve product quality but slow down drying, resulting in dry time was extended. There are many methods to achieve drying, some of the common methods include, sun drying, shade drying, oven drying, thin layer drying, thin layer drying, osmotic drying, spray drying and freeze drying.

When drying materials, effective empirical models of moisture diffusivity and drying kinetics are critical as they can be used to predict the drying time, temperature and energy required to dry the material. It enable researcher describe changes in physical and chemical and their rates quantitatively. This information can be used to optimise the drying process, thereby improving quality, yield, and profitability. Effective moisture diffusivity is a measure of how quickly moisture can move through a material. It is affected by the physical properties of the material, such as density, porosity, and curvature. Empirical models of drying kinetics can be used to predict how the drying rate will change over time as a function of the material's effective moisture diffusivity, drying temperature, and air velocity. By combining information from effective moisture diffusivity and an empirical model of drying kinetics, the drying time, temperature and energy required to dry a material can be predicted. By optimising drying process, it can improve quality, yield and profitability. Overall, an

effective empirical model of moisture diffusivity and drying kinetics is an important tool for optimising the drying process. Empirical modelling for drying kinetic can be developed based on Newton's law of cooling (Lewis, Page, and Modified Page) and Fick's second law of diffusion (Logarithmic, Henderson and Pabis, 2-term and 3-term exponential models and modified exponential models) can be used to describe the mechanisms of heat and mass transfer during the drying process (Kumar et al., 2022). By using these tools, the quality, yield, profitability and environmental impact of your drying process can be improved.

Jackfruit leaves was reported to have healing properties in treating many skins disease such as wounds and boils. Leaves extracts were mixed with other chemical compounds and used directly over the wounds during the study (Gupta, Jain, and Pathak, 2009). It was reported that jackfruit leaves contain the bioactive compound, artocarpin that have anti-inflammatory effect that act as wound healing activity (Chan et al., 2018). Jackfruit leaves extract was also reported to contain high antioxidant properties in the aqueous fraction (294.5 mg/g) and the ethyl acetate extract (361.2 mg/g). This was also reflected in the antioxidant activities where both extracts exhibited high scavenging activities (219.9 and 235.8 µg/mL, respectively) (Loizzo et al., 2010). The notable antioxidant properties of jackfruit leaves extract (JLE), makes it excellent candidate for incorporation into innovative drug delivery sytem. One such promising approach is nanoemulsion that stands out for their ability to improve absorption and effectiveness of bioactive compounds, enhancing their permeation through biological membranes.

Nanoemulsion is a drug delivery system that is able to enhance the permeation of drugs and bioactive compounds. Nanoemulsion (NE) is regarded as a system having two phases consisting of at least two liquids that are immiscible with each other with droplet size in nano range. Oil in water (O/W) and water in oil (W/O) emulsions are the example of emulsion systems that are normally used in a wide range of industries designed to encapsulate, and/or deliver active ingredient such as in pharmaceutical, cosmetics, food and agrochemical application (Komaiko and McClements, 2014). Nanoemulsion is a stable system that prevent against creaming, sedimentation, Ostwald ripening and aggregation. This is due to the stable steric repulsion between droplets that can repel each other with the presence of emulsifier surrounding the droplets. The emulsifier forms interfacial film to encapsulate the oil that is loaded with the drugs or the bioactive compounds. The droplet size range may vary depending on published reports, with some reported the nanoscale range between 10 – 100 nm, while others reported an upper limit of 500 nm or even 1000 nm. There is no agreement on the size range to define nanoemulsions due to variability in previous literature (Fan et al., 2020; Ren et al., 2019; Bitencourt et al., 2018; and Yukuyama et al., 2016).

There are several methods to produce an emulsion in nano size range, namely high energy (HE) methods and low energy (LE) methods.Nanoemulsion that is produced by HE requires specialised equipment that requires intense power and disruptive forces to breakup larger droplets forming small droplets in nanoscale range. Some examples of mechanical device that requires high energy are high pressure homogeniser (HPH), high shear homogeniser (HSH), microfluidise and ultrasonicator. The advantages of these methods are it can utilise a wide range of oil such as medium chain triglycerides (MCT) and long chain triglycerides (LCT), requires only small

amount of surfactants and is suitable for sample from medium to high viscosity materials. However, the drawbacks are it is not suitable for heat sensitive bioactive compounds, high maintenance and requires multipass homogenisation regime in order to obtain smaller size of droplets. Low energy methods rely on controlling the organic and aqueous phase interfacial properties in the presence of its surface-active molecules, such as their solubility and molecular geometry (Chang and Mcclements, 2014). A number of LE approaches have been developed including phase inversion temperature (PIT), phase inversion composition (PIC) and spontaneous emulsification (SE). These methods were preferred over HE because it utilises low operation cost, low energy equipment, require no special equipment and easy to administer effectively at producing fine droplets (Komaiko and Julian, 2015). It allows simple stirring mechanism over intensive preparation of HE method. However, the shortcomings of LE including a relatively high surfactant to oil ratio (SOR), limited type of surfactant and limited ranges of oil that work well as oil phase such as MCT. Larger droplets maybe formed as an outcome of choosing LCT for LE method. This may cause droplet growth, therefore cause instability of nanoemulsion (Gulotta et al., 2014).

Efficiency of drug delivery system for pharmaceutical application has been increasingly on research demand for its targeted site of action. Nevertheless, topically administered drugs suffer from drawbacks due to their systemic side effect such as hypersensitivity reactions and thinning of epidermis after prolonged use of drugs and the lack of effective concentration of the drug at the site of action, resulting in poor patient acceptance (Coondoo et al., 2014). There have been several reports on developments on topically administered nonsteroidal anti-inflammatory drugs (NSAIDs) having drawbacks on poor aqueous solubility and bioavailability (Anita et

al., 2021; and Elmataeeshy et al., 2018). Poor permeability of drugs may lead to expensive therapy cost and reduced in patient compliance in relation to the requirement of long term use of topical medications (Tan et al., 2012). The efficacy of a topical product should include a desirable and optimum consistency of formulation which helps to deliver and safeguard a suitable dose applied on the target application site. A disproportionate dose of a drug may cause undesirable side effect on the skin and wasted application. Unfortunately, the barrier function of human skin limits the permeability of the plentiful active agents in some topical formulation. One of the most important drawbacks of nanoemulsion designed for topical use is its low viscosity emerging from the tendency to slip from the skin surface (Froelich et al., 2017). Therefore, the need to develop an efficient drug delivery formulation is required. One of the most promising efficient topical applications is nanoemulsion based gel or also regarded as nanoemulgel. Nanoemulgel is a term used to define a nanoemulsion system that is incorporated with gelling system that incorporates polymer to increase viscosity of the formulation and to help enhance the drug permeability into skin, exerts dispersion onto skin and promote skin hydration in pharmaceutical products.

Previous researchers have reported on the use of natural and synthetic polymers to formulate nanoemulsion based gel system. Polysaccharide is a natural polymer used to tailor-made formulation for transdermal and topical drug delivery that works as an alternative to synthetic polymer (Barradas et al., 2018; Saidin et al., 2018). Djekic et al. (2016) reported the performance of thickening agent, xanthan gum towards the physical stability of emulsion studied. It was found out that optimised xanthan gum concentration (<0.75%) has better efficiency than reference hydrogel as a promising

carrier to enhance the drug delivery and showed no skin irritation. Apart from polysachharide, protein also serves as a gel matrix and the application has been reported to work similar to permeation enhancer. The research study by Wang and Zhang (2017) used dual proteins (zein and sodium caseinate) to study the characteristic and the physical stability of their nanoemulsion. Synthetic polymers serve similar purpose to increase the viscosity of nanoemulsion; i.e, Carbopol 940 was used to thickened nanoemulsion to enhance the permeation of drug for topical application for better drug loading capacity in comparison with other drug carriers (Ay Şenyiğit et al., 2021; and Ahmad et al., 2019). Biobased polymer or natural polymers have been used for a wide range of drug delivery application. They are eco-friendly, safe, biocompatible and serve as modifiers and stabiliser that are suitable for drug penetration into the skin (Alves et al., 2020). A deeper understanding and further investigations on the properties, the behavior, application and mechanisms of polysaccharide and protein blends of JLE nanoemulgel could be explored. This is essential especially if the utilisation of nanoemulgel in topical drug delivery or any other purposes are required.

## 1.2 Problem Statements

The increasing interest in natural sources of bioactive compound is driven by the demand for sustainable and health-promoting alternatives in food and pharmaceutical. Jackfruit leaf (*Artocarpus heterophyllus*), an agricultural byproduct are rich in bioactive compounds such as polyphenols and flavonoids, that exhibit strong antioxidant properties. However, effective utilisation of these bioactive compounds in functional applications, such as pharmaceuticals and cosmetics is hindered by several factors, including the lack of optimised processing methods that preserve their activity.

Traditional approaches to processing jackfruit leaves often fail to consider the impact of drying methods and solvent extraction conditions on the retention of antioxidant compounds. Drying, an essential pre-treatment step, significantly affects the bioactive content due to varying exposure to heat, light, and oxidative conditions. Similarly, the extraction solvent system plays a pivotal role in maximising bioactive recovery, with ethanol/water (E/W) ratios known to influence phenolic and flavonoids yields. Despite substantial research on plant-based antioxidants, limited studies systematically investigate the combined effects of drying conditions and E/W ratios on the antioxidant potential of jackfruit leaves. Addressing these gaps is critical for unlocking the full potential of jackfruit leaves as sustainable source of natural antioxidants.

Investigation of nanoemulsion based gel integrating polysaccharide and protein polymers complexes incorporated with JLE employing low energy method has yet been studied, where this could gather information on the understanding of the characteristics, rheological properties as well as its *in vitro* application. Up to date, there are no detailed kinetic models available that discuss specifically on the mechanism of the natural polymer blends in nano size range on jackfruit leaves extract loaded nanoemulgel. Hence, the investigation on the physical and chemical properties especially on rheological properties could predict the behaviour of nanoemulgel in order to contribute to the knowledge of preparing formulation and the process of synergistic effect by utilising these biopolymers.

Topical delivery of bioactive compounds is strongly limited by the poor skin permeability due to its poor solubility; bioavailability and the release was restricted to the stratum corneum. The formulation of the bioactive compounds into a biopolymer

based nanoemulsion has the potential to overcome this limitation. It is essential to obtain maximum drug delivery at targeted action site, which is safe, non-irritating and with high patient compliance. Due to their potential advantages over conventional gel, nanoemulgel can be used in the pharmaceutical industries to extend the shelf life of products by resisting product instability such as particle aggregation, gravitational separation, and flocculation. In depth research on the stability studies and characterisation of the rheological behavior of the developed formulation emphasising on the fundamental knowledge of its flow behavior as well as bioactive compound release kinetic studies is needed. The results of flow behavior properties are essential to predict the behaviour of the hydrogel thickened nanoemulsion. This properties may provide an insight to the decision of the extent of these biopolymers added in formulating towards creating desired properties of rheological behaviour for topical application. This could reduce the cost, energy, and time of the production for pharmaceutical application. This in turn, not only could help the local industries to generate income using underutilised resource but also could also be greatly useful to create numerous valuable products in the future such as in tissue engineering and biomedical application (Elmataeeshy et al., 2018).

## 1.3 Objectives of Research

This research builds on the challenges and gaps highlighted in the problem statement to address key issues related to maximising preservation and extraction of bioactive compounds. Additionally this study explores on formulating an innovative and effective drug delivery system. To address these issues effectively, the following objectives have been outlined to guide the research effectively.

1. To determine the drying behaviour and the effect of drying methods (shade drying, sun drying and oven drying) and various ethanol/water compositions on antioxidant the properties of *Artocarpus heterophyllus* var *Mastura* variety (J35) leaves extract.

2. To formulate and optimise nanoemulsion based gel loaded with jackfruit leaves extracts by varying biopolymer complexes concentration (xanthan gum and fish gelatin), oil and surfactant used.

3. To characterise the formulation developed in (2) based on the stability, rheological behaviour, spreadability, morphology behaviour and permeation of the drug/bioactive compound release into the skin membrane using *in vitro* Franz diffusion cell release testing.

## 1.4 Research Hypothesis

In this research, the hypothesis is as follows:

(a) The oven dried jackfruit leaves utilising 80 % ethanol water composition has higher antioxidant properties than sun dried and shade dried of other ethanol/water compositions.

(b) The best formulated gel has a good gel characteristics (particle size, ζ-potential polydispersity index, rheological and spreadability behaviour) are regarded as acceptable in drug delivery applications.

(c) The best formulated gel shows better drug release and permeation characteristics.

These hypotheses are based on the the idea that developing nanoemulgel reinforced with jackfruit leaves extract that has the potential of antioxidant properties which beneficial for the drug delivery application.

## 1.5 Significance of Study

The outcome of this study aims to develop and design an innovative drug delivery vehicle from underutilised jackfruit leaves extract containing bioactive compound (artocarpin). The significance of this study can be contributed in terms of (a) filling gaps in the literature and (b) knowledge in theory.

(a) Contribution to the literature: the study aims to fill a gap in the literature through focusing on developing and formulating drug delivery vehicle by blending two biopolymers (xanthan gum and fish gelatin). To date, the development of drug delivery mostly on single polymer, therefore this study will add to the literature on synergistic behaviour by adding more polymer for the enhancement of drug delivery.

(b) Knowledge in theory: the study aims to contribute to the understanding of the characterisics and behaviour of the developed nanoemulgel consist of biopolymer blends for the pharmaceutical application.

## 1.6 Scope of Research and Thesis Structure

This study is focused on designing the formulation of nanoemulgel from biopolymer complexes of xanthan gum and gelatine as a drug delivery vehicle for bioactive compound (artocarpin) from jackfruit leaves extract. In depth study has been performed to investigate the antioxidant properties of locally grown jackfruit leaves (Mastura variety) that has been dried using different drying techniques and different ethanol-water solutions. Extract from method that result in the highest antioxidant content were formulated into nanoemulsion and nanoemulgel. The stability studies, flow behaviour properties of the nanoemulsion and nanoemulgel were then investigated. The best formulation was tested in a permeability study to determine the maximum drug release of artocarpin into a synthetic membrane that mimic the stratum

corneum or human skin. The flow behaviour of the formulated nanoemulgel was then fitted to a rheological model that present the best fit of the desired properties of nanoemulgel formulation. Finally, the relationship between the structural and physicochemical behaviour of nanoemulgel were addressed and deliberated.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Overview of Jackfruit Industry in Malaysia

Jackfruit tree or locally known in Malaysia as 'nangka' is a tropical climacteric fruit. Scientifically known as *Artocarpus heterophyllus* Lam., jackfruit is a non-seasonal fruit and commonly consumed foods in Malaysia. Jackfruit is known to be originated from India, is widely cultivated in other countries such as Southeast Asia countries, i.e., Thailand, Vietnam, the Philippines and Indonesia and other countries such Sri Lanka, China, and Brazil. The plant is a low cost, abundance and readily available which provides a lot of nutritional content to rural communities in Southeast Asia, western part of Java and India (Daud et al., 2018).

Jackfruit is a tree-borne fruit producing up to 700 fruits per year, with a fruit that can reach up to 50 kg in weight with 60 – 90 cm in length. On average, per hectare of a land, 50 – 80 tonnes of fruits can be harvested. This tree categorised as monoecious which is a male and female producing flower. The stem is rough and straight whereas the plant bark is black or green, with up to 1.25 cm in thickness and discharge milky latex. The leaves are broad obovate, decurrent and elliptic in form (Ismail and Kaur, 2013; Saxena et al., 2011, and Prakash et al., 2009).

Previously, jackfruit has been planted in Kedah, and east coast of Malaysia (Terengganu and Kelantan). Over the years, a growing plantation cultivated in Johor, Perak, and Selangor because of good, well drained, and deep alluvial soil. The cultivation of jackfruit has rose to 4,675 ha which represented 2.5 percent of total fruit

area production. Production in 2020 was reported a total of 35.62 metric tons. The popular varieties are J32 (Mantin), J33 (Tekam Yellow) and J37 (Mastura). Jackfruit plantation industries have become one of the relevant industries in the EPP beside other industries such as papaya, pineapple, and banana (Meon, 2014). Figure 2.1 shows total area jackfruit plantation (%) being planted in Malaysia with Negeri Sembilan yielded the highest jackfruit production (11, 111 mt), followed by Pahang state at 8,399 mt and Johor with 4,572 mt. Jackfruit is also ranked at 16th place among 21 selected major tropical fruits in Malaysia (Dardak, 2019). Even though there are about 64 types of tropical fruits that can be cultivated in Malaysia, the Ministry of Agriculture and Agro-based Industry decided to have only 21 types of tropical fruits as commercial commodities that will be cultivated on commercial scale.

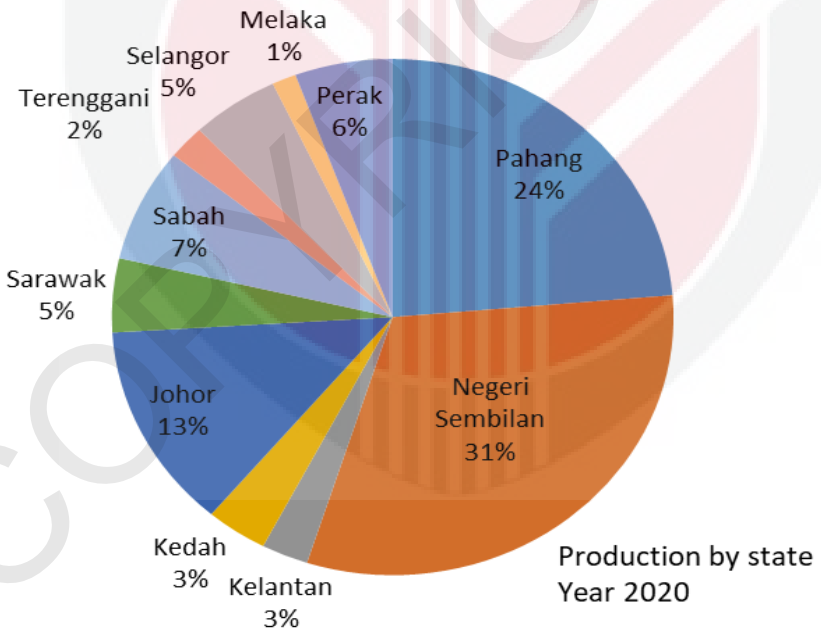



**Figure 2.1 : Total Jackfruit Plantation Area in Malaysia in 2020** (Biai, 2022)

More than 30 jackfruit varieties have been registered under the Department of Agriculture (DOA) and from all these there arethree jackfruit varieties that are

commercially grown in Malaysia known as Mantin (J32), Tekam Yellow (J33) and Mastura (J35). From these known jackfruits, Mastura (J35) variety has good characteristics such as the least latex production and highest average weight per fruit (can be more than 40 kg). Although these characteristics are good for fruit production, an important decision-making criterion for marketability of a plant is consumer acceptance. A survey was conducted on consumer acceptance towards jackfruit characteristics and the findings revealed that the four main factors attributed to the preference to jackfruit, are colour, texture, sweetness, and aroma. Overall, *Mastura* jackfruit has less crunchiness and less sweetness as opposed to consumers' preference. In particular, this provides information on decision of Mastura variety cultivation (Figure 2.2) in Malaysia.

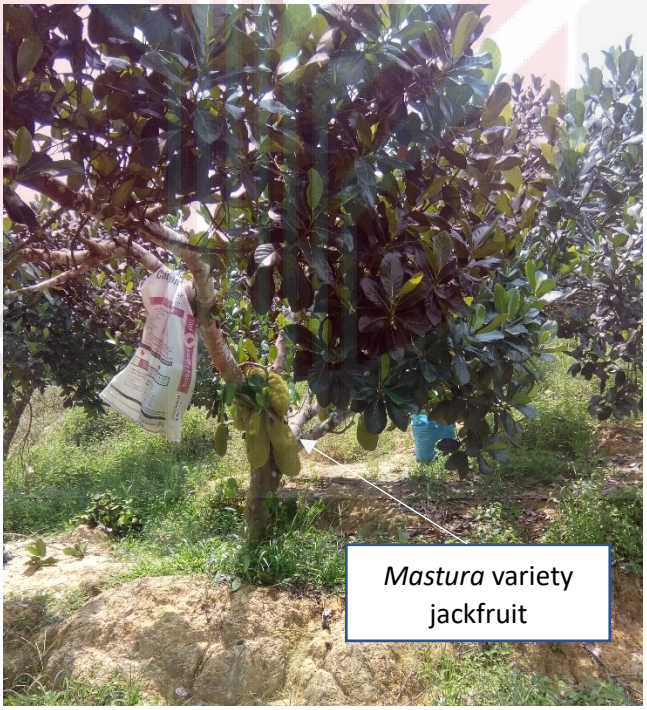



**Figure 2.2 : Mastura Jackfruit Tree Plantation Owned by Farmers' Organisation, Maran, Pahang Darul Makmur**

## 2.2 Economic Value and Major Uses of Jackfruit

Jackfruit contributed to the significant economic value and is given priority in commercialising cultivation in Malaysia. Exports of jackfruit made up only 0.03 % of total tropicals fruit produced in 2022 at 40,219.76 metric tons valued at RM 14.74 million. Most of the fruit were exported to China and other destinations including Singapore, Hong Kong, United States of America, Indonesia and other countries (Ministry of Agriculture and Food Security, 2022). However, exploitation on other form is yet to be explored as it is commonly sold as individual fresh bulb by local vendors (Abd Aziz et al., 2016). Other than used for fresh consumption, jackfruit is also processed into jackfruit 6 juice, ice cream, jackfruit chips and eaten as a vegetable. Among initiatives from the Department of Agriculture to develop the fruit is the Permanent Food Production Park which involved the leasing of government land to commercial entities to venture into food production activities. Forty two percent of 1,963 hectares of jackfruit are now grown in these food production parks involving 215 participants. In the 12th Malaysia long term crop development program, jackfruit has been selected among others to be given emphasis in the program to increase production and exports. Among ongoing initiatives is the use of Individual Quick Freezing (IQF) method to improve shelf life. Besides using jackfruit for fresh consumption and as a vegetable, there are also companies that use jackfruit as raw material to produce plant-based meat alternatives. A number of factors that are responsible for the low demand and lack of popularity are variation in fruit quality as stated earlier; including high juiciness (less crunchy) and low sweetness profile which may be undesirable to some consumers. The attractiveness of fruits is also based on the fruit size and colour.

Jackfruit is multi-purpose providing as food, furniture, varnishes, timber, livestock feed, industrial product, and medicinal uses. The edible part of a ripe jackfruit is normally eaten as fresh bulb and can be processed further into jam, snacks, jelly, or candy. The unripe part of edible jackfruit can be cooked and make into dishes eaten with chicken and eggs. Meanwhile, the inedible part such as straw, seed, leaves, latex, and pulp were reported to be used for extraction for medicinal purposes (Mustafa et al., 2020; Zhang et al., 2017), plastic food packaging film (Nazri et al., 2019) and varnishes (Ismail and Kaur, 2013).

As reported by the Pahang State Farmers Association (PASFA), a low demand on *Mastura* variety often results in surplus and waste, particularly the demand on *Tekam Yellow* variety peaks in the Malaysia. This reduced interest in *Mastura* jackfruit flesh has created backlog the plantations, further contributing to potential wastage that could lead into waste. This could be due to the demand of the fruit is less preferred by the consumers due to its low sweetness and high-water content properties (Johari et al., 2020). However, other parts of the plant, such as the leaves, offer promising opportunities for alternative applications as highlighted in previous studies, especially on nutritional content and antioxidant properties (Amadi et al., 2018 and Wang et al., 2017). The leaf of jackfruit (Figure 2.3) is physically dark green in colour and glossy, broad, and elliptic. The young shoots are often deeply lobed when juvenile. The structure of female heads is oblong ovoid receptacle while the male heads are sessile or on short peduncles receptacles (Ranasinghe et al., 2019).

**Figure 2.3 : Photograph of the *Mastura* Jackfruit Leaf**

## 2.3 Preservation of Jackfruit Leaves

Upon detachment from the twigs after harvesting, jackfruit leaves may deteriorate, thus reducing their shelf life. Therefore, there is a need for preservation to be able to prolong their shelf life. Drying is a critical pre-treatment step for jackfruit leaves before their utilisation in pharmaceutical application, primarily to preserve their antioxidant potential and ensure consistency and quality during subsequent processing. Fresh leaves are highly perishable, and their bioactive compounds such as phenolics and flavonoids, are prone to degradation due to enzymatic activity and microbial growth. Drying mitigates these risks by lowering moisture content, thereby limiting microbial activity and slowing enzymatic reactions that could degrade valuable antioxidant. However, during the drying process, deterioration and changes in properties lead to the undesirable impact on the quality of the product (Babu et al. 2018, and Reyes et al 2019).

Post-harvest handling of fresh jackfruit leaves poses challenges for obtaining large sample size for pharmaceutical use. The short helf life of fresh leaves exacerbate the difficulty maintaining consistent quality and supply. Drying enable the conversion of fresh leaves onto storable and transportable form, ensuring availability throughout the year and facilitating large scale extraction process. Properly dried leaves exhibit better stability in terms of bioactive compounds, making them more suitable for pharmaceutical application where reproducibility and efficacy are essential. (Babu et al., 2018, and Nahar and Sarker, 2019). Previous study by Yan et al. (2019) support the theory that fresh sample is susceptible to deterioration due to high moisture content, thereby causing loss of nutrient.

Additionally, the selection of drying method and conditions significantly influence the retention of antioxidant properties in plant leaves. For example, studies have shown that low temperature drying methods such as shade and oven drying at controlled temperature, can preserve higher level of phenolic and flavonoid compounds compared to high temperature or uncontrolled drying methods (Cheenkachom et al., 2022). This makes drying not only a logistical necessity but also quality-enhancing step in the preparation of jackfruit leaf-based pharmaceutical product.

Changes in colour, aroma, and degradation of bioactive compounds serve as an indicator of the oxidation process. Exposure to exhaustive long hours of drying time and high temperature could result in reduced antioxidant properties. Drying methods that are commonly used include convection drying, shade drying, and sun drying. Previous studies exposed jackfruit leaves to various drying methods, particularly to evaluate the effect of drying methods on the cellular constituent that can release the

phenolic compound from the plant material (Roshanak et al. 2016). Prakash et al. (2017) reported on the shade-dried jackfruit leaves for wound healing properties, focusing on the excision model and phytochemical screening for the presence of flavonoids. Ojwang et al. (2017) reported that the air-dried drying method of jackfruit leaves still contain an amount of antioxidant activities (65% to 78%).

The air-dried under the shade of jackfruit leaves collected from different districts in Uganda were also revealed to present an abundant amount of total phenolic content and total flavonoid content (ranging from 37.39 to 30.92 mg/g and 5.02 to 6.70 mg/g, respectively). For many years, different drying methods of leaves have been adopted to reach a compromise between quality and efficiency. Options included the commonly used method, the shade drying. Donkor et al. (2016) reported that natural drying may result in a slight decrease of flavonoid compound with the temperature increased. In contrast, a higher temperature (60 to 70 °C) leads to an increase in carvacrol content but causes darkening of the leaf colour (Rahimmalek and Goli 2013; Roshanak et al. 2016). However, the leaf colour may retain the colour quality demonstrated by lowering drying temperature to 40 °C (Chua et al. 2019).

During processing, the level of bioactive compounds can be significantly altered depending on the sample processing technique used. Many studies reported on the influence of postharvest processing factors (drying and extraction) on the pharmacological, phytochemical, and nutritional properties of samples. Conventional drying process always suffers from a long drying time with high energy consumption. This could result in reducing the quality content of samples. Drying technique often couple with extraction process for better process control without reducing the quality

of bioactive compounds, colour, and antioxidant properties. Thus, it could lead to better nutrient retention (Magangana et al., 2020).

Solvent extraction remains one of the most efficient methods for isolating bioactive compounbds (phenolics and flavonoids). The choice of solvent, extraction conditions, and techniques significantly influences the yield and antioxidant activity of the extracts. Solvents such as ethanol, methanol, acetone and water are commonly used, often in varying ratios, to optimise the extraction process. Based on previous finding by Hikmawanti et al. (2021), solvent extraction enable selective isolation target compounds by manipulating polarity, temperature and time. Their finding was in agreement with Lim et al. (2019) who stated that selection of optimal solvent may solubilise broader range of bioactive compounds at varying solvent ratio. Ethanol and methanol-based extraction have demonstrated high yield of phenolic and flavonoid compounds due to their intermediate polarity. A study by Hossain et al. (2018), reported that ethanol/water mixture provide a balance between safety and efficiency.

However, some limitation in solvent selection such as methanol, although this solvent is effective, it poses concerns on toxicity, limiting its application in food and pharmaceutical industries. On the other hand, utilising water as a solvent extraction is environmental friendly, it often yields lower concentrations of certain non-polar compounds (Claux et al., 2021). Ethanol is favoured for its safety profile, making it suitable for food and medicinal application, while methanol often provides slightly higher yields but is restricted to analytical purposes. Mixed solvents systems (e.g., ethanol/water) is increasingly preferred due to their ability to extract a broader spectrum of compounds. For instance, a study conducted by Lu et al. (2017) identified

that ethanol/water ratio at 8:2 concentrations was found to be optimal for extracting both polar and non-polar antioxidants from plant leaves.

Solvent extraction has proven to be an effective method for isolating antioxidant from jackfruit leaves, with ethanol/water systems emerging as the most practical and safe choice for food and pharmaceutical applications.

## 2.4 Extraction of Bioactive Compounds of Jackfruit Leaves

Jackfruit leaves are reported to contain bioactive compounds such as artocarpin, artocarpetin A, cycloheterophyllin, and artonins A and artonis B (Figure 2.4a) – Figure 2.4d) that has been investigated in many assays. The scavenging activity of cycloheterophyllin, and artonins A and artonis B are reported in DPPH, peroxyl and hydroxyl radicals. Together with these three compounds are also act as effective antioxidant against iron-induced lipid peroxidation in rat brain homogenate. Apart from that, a study conducted by Khan et al. (2003) shows that extract from leaves have antibacterial effects towards bacterial strain studied, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella thyphi* and *Serratia marcescens* just to name a few. Years later, Loizzo et al. (2010) also investigated the jackfruit leaves extract fraction against foodborne pathogens *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* by the agar diffusion and broth dilution method and observed that the plant sample has the possibility as antibacterial agents at concentration >1000 µg/ml. In a study on Malaysian jackfruit leaves by Sayuti et al. (2020), who reported that high total phenolic and flavonoid content using hot aqueous extraction method. It was also investigated that the jackfruit leaves revealed 4 compounds identified as chlorogenic acid, quercetin, rutin and kaempferol as their

bioactive compounds. Years later, a study reported by Noor et al. (2024) found that jackfruit leaves extracted using 50% ethanol/water found that it contain caffeic acid in their phenolic profiling with high free radical scavenging activities (86.48%). Caffeic acid in the jackfruit leaves offer many helth benefits including treatment for skin diseases and as anti-aging properties.

Another study by Prakash et al. (2013a) highlighted the bioactive compounds artocarpesin (5,7,2′,4′-tetrahydroxy-6-isoprenylflavone) (Figure 2.4e) and artocarpin(6-(3-methyl-L-butenyl)-5,2',4'-trihydroxy-3-isoprenyl-7-methoxyflavone in methanolic extract of leaves possess significant analgesic and immunomodulator activity in their study. Further investigation is suggested on the toxicity of leaves concentration normal human epithelial cell. Major health problem, diabetes is known for its seriousness that affects world population. In search for effective hypoglycemic agents, studies by Prakash et al. (2013b) found that methanol extracts of jackfruit leaf may be useful to have a potential anticonvulsant effect at 82.1% inhibition against the seizures induced by Maximal electroshock (MES) and Strychnine induced convulsions models on Swiss albino mice. Different study was conducted by using jackfruit leaves in ethanol extract and butanol extract (JFEE and JFBE, respectively) and revealed that both jackfruit extracts were also exerted hypoglycemic and hypolipidemic effect in STZ-diabetic rats conducted. The antioxidative activity of jackfruit extract resulted in significant lowering of serum protein level in STZ-diabetic, decreased in bad cholesterol level, LDL and showed inhibition activity in lipid peroxides. The findings could be attributed to the presence of quercitrin flavonoid that can attenuate the diabetic state by reducing the oxidative stress and thus have the ability to reduce the risk of cardiovascular disease (Omar et al., 2011).

An extended use of jackfruit species leaves also reported by Hafid et al. (2016) who found that dried leaves of *A. heterophyllus* Lam having good to moderate (>50%) free radical scavenging activities which were potent inhibitors of parasite growth both *in vitro* and *in vivo* tests activities. Thus, the leaves extract may offer another potential source for a new antimalarial drug. Recently, new phenolic compounds have been investigated by Wang et al. (2017) on leaves *A. heterophyllus* Lam. Study shows that one new 2-arylbenzofuran derivative, artocarstilbene B, one new benzaldehyde derivative, (E)-3,5- dihydroxy-4-(3-methylbut-1-enyl) benzaldehyde, as well as 18 known compounds were obtained from the study were tested on cancer cell lines using MTT method for its cytotoxicity studies. The study has demonstrated that many test compounds exhibited moderate to weak inhibitory activity against the proliferation of the PC-3, NCI-H460, and A549 cancer cell lines. Thus, it could add to another jackfruit leaves application to date.

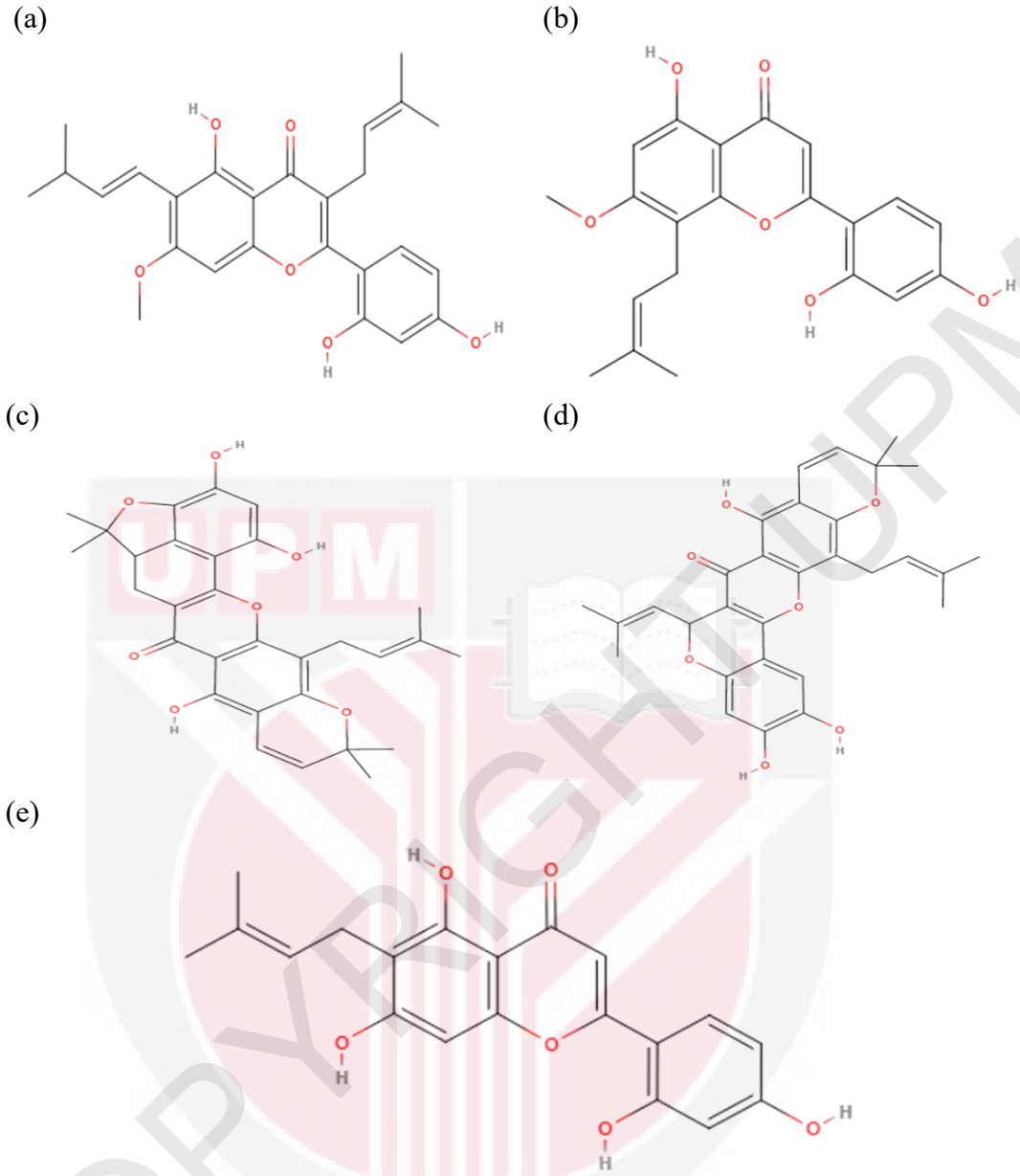



**Figure 2.4 : 2D Structure of Bioactive Compounds (a) Artocarpin; (b) Artocarpetin A; (c) Artonin A; (d) Cycloheterophyllin; (e) Artocarpesin Found in Jackfruit Leaves**

## 2.5 Application of Jackfruit Leaves in Pharmaceutical and Cosmeceutical Application

Nowadays, there is growing concern among consumers regarding the purchase of eco-friendly products to replace synthetic ingredients in topical pharmaceutical and cosmeceutical products. The inclusion of heavy chemicals or synthetic ingredients has

become a major concern for customers in their daily skincare routine. As a result, many pharmaceutical products have been developed and formulated using natural ingredients derived from plant extracts. Several studies have reported the advantages of plant extracts in treating various skin conditions. Ribeiro et al. (2015) discussed the use of plant extracts, such as tree barks, for the development and formulation of topical applications. Recent advances in cosmeceuticals, as reviewed by Draelos (2011), emphasise the use of functional cosmeceuticals, meaning that the ingredients used must come from raw materials that are generally recognized as safe (GRAS). These GRAS ingredients are primarily sourced from the plant kingdom. The search for plant sources, particularly non-edible parts such as leaves, flowers, twigs, barks, and roots, has garnered increasing interest among scientists today.

Jackfruit leaves contain valuable bioactive compounds that have been studied by many researchers for their anti-inflammatory and wound healing properties. The main bioactive compounds commonly found in jackfruit leaves are artocarpin, artocarpetin, and artocarpesin. In addition to their anti-inflammatory properties, these compounds also exhibit antioxidant, antimicrobial, and antiandrogen activities (Chan et al., 2018). Jackfruit leaves are not only limited to pharmaceutical and cosmeceutical applications but also find use as an adsorbent. Various industries, such as textile, plastic, and paper, discharge a wide range of inorganic and organic compounds. These compounds can cause toxicological and aesthetic problems that can affect aquatic life, soil, microflora, and fauna. Therefore, jackfruit leaf adsorbents have been found to be suitable due to their availability and reproducibility (Das and Mishra, 2019). Although jackfruit leaf possesed many health benefits such as antioxidant and antibacterial properties, the application in pharmaceutical area are still scarce. There are few reports explore on

the use of jackfruit leaves extract in the pharmaceutical application namely, ointment, film, and nanoemulsion. From the findings, all of the jackfruit leaves studied were able to deliver drug loaded with bioactive compounds to the action site. Table 2.1 illustrates the pharmaceutical and cosmeceutical applications of *Artocarpus heterophyllus* Lam. leaves by several researchers.

The economic potential of utilising waste resources can be realised through industrial processing to develop new topical antioxidants and skin lighteners. This maximises the use of underutilised and discarded resources and helps reduce waste generated from plantations, thus benefiting the ecosystem.

**Table 2.1 : Medicinal Application of Jackfruit Leaves**

| Application | Solvent extraction | Intended use | References |
|---|---|---|---|
| Ointment | Ethyl acetate | Wound healing | Periyanayagam and Karthikeyan (2013) |
| Film | Ethanol | Wound healing | Wijaya and Fitrya (2022) |
| Nanoemulsion | Alkalised water | Anticancer product | Calderon-chiu. (2021) |

## 2.6 Nanoemulsion

Drug delivery requires a system capable of carrying active ingredients and facilitating drug penetration into the skin layers. Consequently, various delivery systems have been developed to enhance the stability and solubility of drugs. Examples include liposomes (Kim, 2016), nanostructured lipid carriers (Ghate et al., 2016) and microemulsions (Patel et al., 2016). One intriguing approach to developing topical dosage forms involves the use of colloidal systems known as nanoemulsions (NE). Nanoemulsions consist of two immiscible liquids in which one liquid phase (dispersed phase) is suspended in the other liquid phase (continuous phase). Droplet sizes ranged

from 20 to 500 nm. Nanoemulsions are primarily categorised into two types: oil in water (O/W), where the oil phase is dispersed in the continuous phase (water); and water in oil (W/O), where the water phase is dispersed in the continuous phase (oil), as depicted in Figure 2.5 (Gupta et al., 2016). Depending on their composition, nanoemulsions can exhibit a visual appearance that ranges from transparent to turbid. They are kinetically stable systems that typically consist of mainly oil, water, and surfactants (sometimes with a co-surfactant) (Hong et al., 2017; Sonneville Auburn et al., 2018; McClements, 2012). In NE formulations, the oil phase plays a crucial role in the solubilisation of various lipophilic drugs for targeted disease treatment (Karami et al., 2019; Wang et al., 2017). The amount of oil used may vary (5% and 10% w/w depending on the administration site (Cardoso and Barradas, 2020). The choice of oil, whether of natural or synthetic origin, preferably comes from Food and Drug Administration (FDA)-approved and generally recognized as safe (GRAS) certified sources to ensure the safety, efficacy, and security of human use (Shao et al., 2018).

In addition to the oil, the emulsifier is another component of the oil phase in NE. The selection of emulsifier systems is based on their solubility and their emulsification ability (Donsi et al., 2012). Non-ionic surfactants are often preferred over other types of surfactants in many previous studies due to their HLB values, lower toxicity, and ability to solubilise in both oil and aqueous phases (Kaur and Mehta, 2017). Researchers have investigated the use of surfactant mixtures with a wide range of HLB values (Liu et al., 2019 and Liang et al., 2018). Studies have reported the usefulness of surfactant blends such as Span 80 and Tween 80 (Che Sulaiman et al., 2017; Salim, 2016) in improving the stability of nanoemulsions and enhancing skin permeability and solubility.

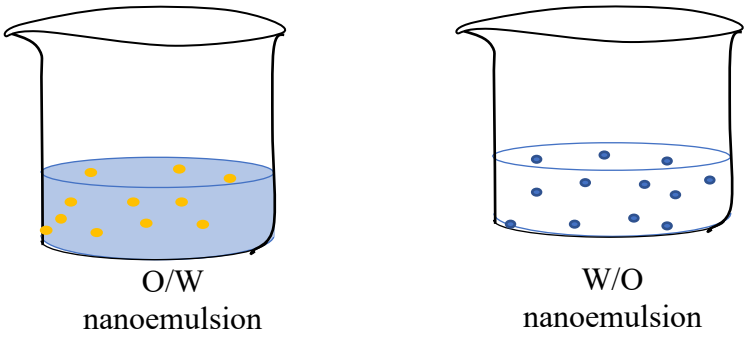



**Figure 2.5 : Oil in Water (O/W) and Water in Oil (W/O) Nanoemulsions**
(Adapted from McClements and Jafari, 2018a)

## 2.7 Properties and Advantages of Nanoemulsion

Nanoemulsions offer many advantages compared to conventional emulsions. These include high stability, improved interfacial contact for wider coverage, rapid absorption through enterocyte internalization, flexibility in selecting composition, ease of processing, enhanced drug solubility, and high bioavailability (Sonneville Auburn et al., 2018). A minimal amount of organic solvent is required during the development of nanoemulsions. The presence of emulsifiers exhibits excellent solubilisation capacity for both hydrophilic and hydrophobic actives at the oil-water interface. Surfactants effectively address kinetic instability issues such as sedimentation, flocculation, coalescence, and creaming. The smaller particle size in the nano range, surface charge, and reduction of surface tension by emulsifiers between the oil and water phases prevent the formation of instability phenomena such as sedimentation, flocculation, agglomeration, precipitation, and coalescence (Kale and Deore, 2017). Consequently, these factors help the nanoemulsion pass through biological membranes by temporarily altering cellular arrangement and improving interaction with cells after solubilising the lipid barrier or fusing with the cell wall's lipid bilayer interface.

Consequently, the NE can be used as a drug carrier for synthetic and natural origin efficiently at more specific target site of action and suitable in food, pharmaceutical and cosmetic industry. Drug delivery system can be categorised into emulsion, microemulsion and nanoemulsion. In general, these systems can be distinguished by method of preparation, solubility, stability and target of site application. The advantages and disadvantages of the emulsion, microemulsiom and nanoemulsion is summarised by numerous studies as presented in Table 2.2.

## 2.8 Production of Nanoemulsion

In the development of nanoemulsions, it is crucial to consider various factors that contribute to the production of stable formulations. The precise composition, emulsification method, and optimal preparation conditions are key elements for achieving high-quality, stable nanoemulsions. An excessive amount of emulsifier can lead to the formation of new micelles, which surround the droplets and prevent their instability (coalescence). Any remaining emulsifier in the system will dissociate and rapidly adsorb onto the surfaces of the newly formed droplets. Nanoemulsion can be synthesized via two different approaches: high energy (HE) methods and low energy (LE) methods as depicted in Figure 2.6 and Figure 2.7, respectively. Theoretically, high energy methods require high throughput energy mechanical devices such as high-pressure homogenisers, microfluidisers, and sonicators in order to create intense disruptive forces that allow the breakup the large oil droplets into nano-size droplets.

**Table 2.2 : Summary of the Advantages and Disadvantages of Nanoemulsion over Other Drug Delivery System**

| Types of drug delivery system | Advantages | Disadvantages | References |
|---|---|---|---|
| Emulsion | 1. To solubilise hydrophobic or oil soluble drugs<br>2. To enhance drug absorption through<br>3. To enhance topical absorption of drugs<br>4. To mask the disagree able taste and odour of drugs<br>5. To enhance palatability of nutrient oils | 1. Less stable as compared to other dosage forms<br>2. Possesses short shelf-life<br>3. Instability of emulsion such as creaming, cracking (breaking), flocculation during storage | Patel et al. (2010) |
| Microemulsion | 1. Easy preparation and simple scale up due to spontaneous formation ability<br>2. Enhance solubility of lipophilic drugs<br>3. It is thermodynamically more stable system as compared to conven-tional system and hence suitable for long term use | 1. Cost increased use to excess use of surfactant<br>2. Excess concentration of surfactants can lead to mucosal toxicity | Anton et al. (2018); Kale et al. (2017), Rai et al., (2018); Paliwal et al. (2019) |
| Nanoemulsion | 1. Able to enhance water solubility and bioavailability of lipophilic drugs<br>2. It is preferred dosage form to incorporate first pass metabolism mediated degradation prone drugs.<br>3. Instability like creaming, flocculation, coalescence, and sedimentation are rarely observed during storage | 1. Require correct formulation and fabrication of emulsion components. Ostwald ripening is the major issue in nanoemulsions. | Rai et al., (2018) |

In contrast, low energy methods require exploitation of low interfacial tension of a system to achieve reduction of droplet size such as spontaneous emulsification method, emulsion phase inversion (EPI) or also known as phase inversion composition (PIC) and phase inversion temperature (PIT) (Gupta et al., 2017; Hong et al., 2017).

### 2.8.1 High Energy Methods

The preparation of nanoemulsions (NE) via high-energy emulsification techniques (Figure 2.6) is thermokinetically stable. A higher degree of force is applied to break the intermolecular forces of attraction, including hydrogen bonding and Van der Waals forces, between liquids with very high surface tension and very low surface tension. When external energy in the form of shear, ultrasonic waves, and pressure is introduced, droplets are formed in the nano size range. However, a major drawback of using higher forces is the generation of extreme heat during these processes.

Nanoemulsions can be prepared using high-pressure homogenisers (HPH), which utilise higher degrees of force typically ranging from 6000 rpm to 10,000 rpm (Kaplan, 2019; and Sulaiman, 2016). Technically, the device consists of small gaps that are relatively small, forcing larger droplets to pass through these small gaps and break into smaller sizes (Yadav and Kale, 2020). The high energy consumption required to break the droplets into smaller ones exceeds the interfacial energy, while the remaining energy is dissipated as heat.

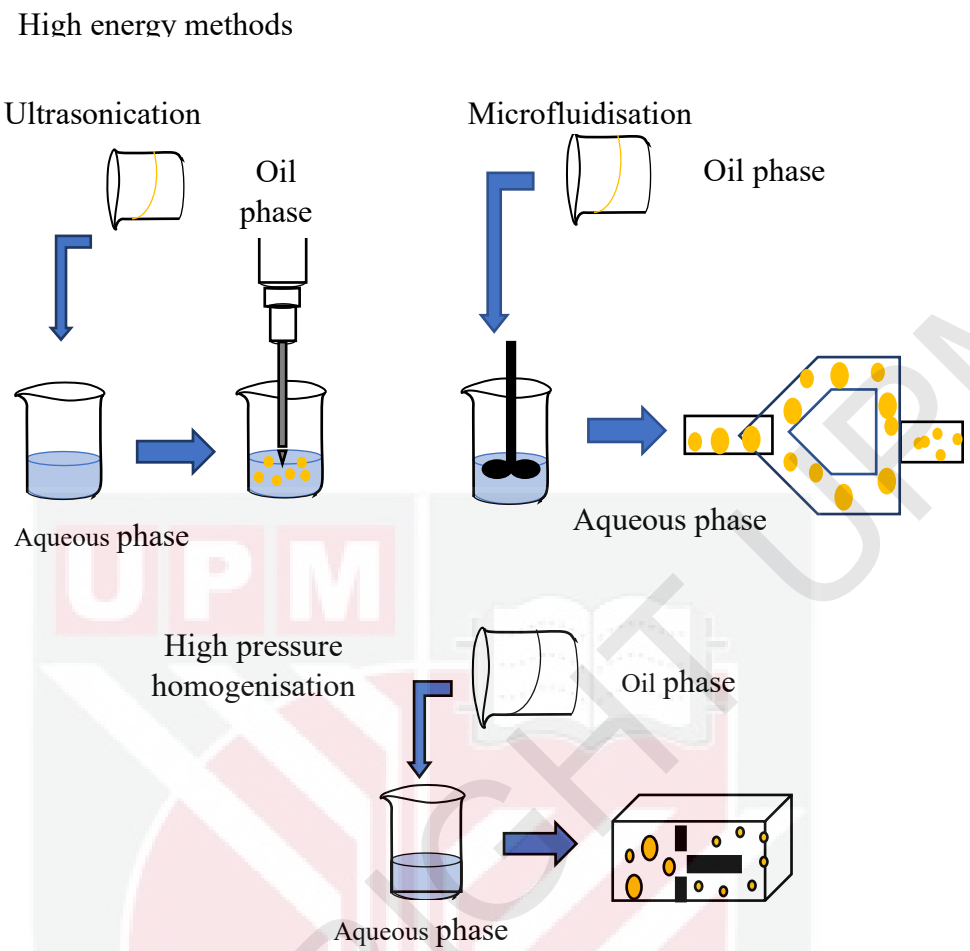



**Figure 2.6 : High Energy Methods Employed in Nanoemulsions Such as Ultrasonication, Microfluidication and High Pressure Homogenisation**

The concept behind utilising a microfluidiser is to develop intense disruptive forces capable of agitating and intermingling the oil and water phases, thereby creating tiny droplets as desired. The pressure used (ranging between 0 and 140 MPa), the number of treatments (ranging between 1 and 6), and the emulsifier concentration are important factors that affect the production of small droplets (Wang, 2020). The preparation of nanoemulsions using the microfluidisation method has also been reported for *Eucommia ulmoides* seed oil (Wang, 2020), thyme essential oil (Trujillo-Cayado et al., 2020), and cucurmin-MCT oil (Páez-Hernández et al., 2019). The preparation of nanoemulsions through an ultrasonicator has a similar effect to HPH.

This method offers benefits such as minimising the amount of surfactants used, requiring less time, and being a simple process. The speed used (ranging between 7,000 and 14,000 rpm), frequency (up to 20 kHz), and power settings are essential for the production of small droplets (Sarheed et al., 2020). Previous research has investigated the preparation of nanoemulsions incorporating various drugs, such as loratadine (Sarheed et al., 2020), folic acid (Ammar et al., 2020), and a mixture of olive oil and sesame oil (Saani et al., 2019).

### 2.8.2 Low energy methods

Low-energy methods are preferred over high-energy methods due to their ability to produce smaller droplets without the need for expensive equipment or excessive energy consumption. Researchers have taken a keen interest in low-energy methods because they enable the formation of small droplets by utilising the inherent energy within the emulsion system. Emulsification techniques involve altering the composition or temperature, which in turn affects the hydrophilic and lipophilic balance (HLB) of the encapsulated molecules. These approaches, employing simple stirring techniques, can yield comparable results to high-energy methods, such as fine droplets, low polydispersity index (PDI), and highly stable emulsion systems. Additionally, low-energy methods are energy-efficient and suitable for industrial-scale production.The production of nanoemulsion can be achieved via low energy methods such as spontaneous emulsification (SE), emulsion phase inversion (EPI) and phase inversion temperature (PIT) (Figure 2.7).

#### 2.8.2.1 Spontaneous Emulsification (SE)

Spontaneous emulsification involves gently mixing two liquid phases, resulting in an oil-in-water nanoemulsion system at a temperature where the surfactant has an affinity for the oil phase. The process is more effective at higher temperatures, and diluting the system with an aqueous phase adjusts the surfactant solubility, thereby promoting nanoemulsification. Once the surfactant mixture comes into contact with water, the thermodynamics of the system change, making it more soluble in the aqueous phase than the oil phase. This causes the aqueous phase to penetrate the oil/surfactant mixture, breaking up the oil and generating oil droplets in nanoform.Previous studies, such as those on hydrocarbon oil (Komaiko et al., 2014), β-carotene (Zhang et al., 2016), vitamin E acetate (Saberi et al., 2013), and *Phyllanthus urinaria* extract (Mahdi et al., 2011), have reported successful utilisation of the spontaneous emulsification method to produce stable nanoemulsions that remained stable during storage.

#### 2.8.2.2 Emulsion Phase Inversion (EPI)

Numerous studies have been conducted on the utilisation of EPI (Emulsion Phase Inversion) for producing nanoemulsions, specifically targeting smaller droplets in oil-in-water systems. This method involves using the minimum amount of water phase containing surfactants for the phase inversion system. The concept behind EPI is that a change in composition disrupts the hydrophilic-lipophilic balance (HLB) and leads to changes in the curvature of the surfactant monolayer. A wider range of surfactants with minimal dependence on temperature for their HLB, as proposed by Koroleva and Yurtov in 2012, can also be used. This method is also known as the emulsion inversion point or phase inversion composition. Previous studies have also investigated the

preparation of nanoemulsions using EPI with mixtures of edible docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Zhang et al., 2020), vitamin E (Hategekimana et al., 2015) and hydrocarbon oil (Komaiko and McClements, 2014).

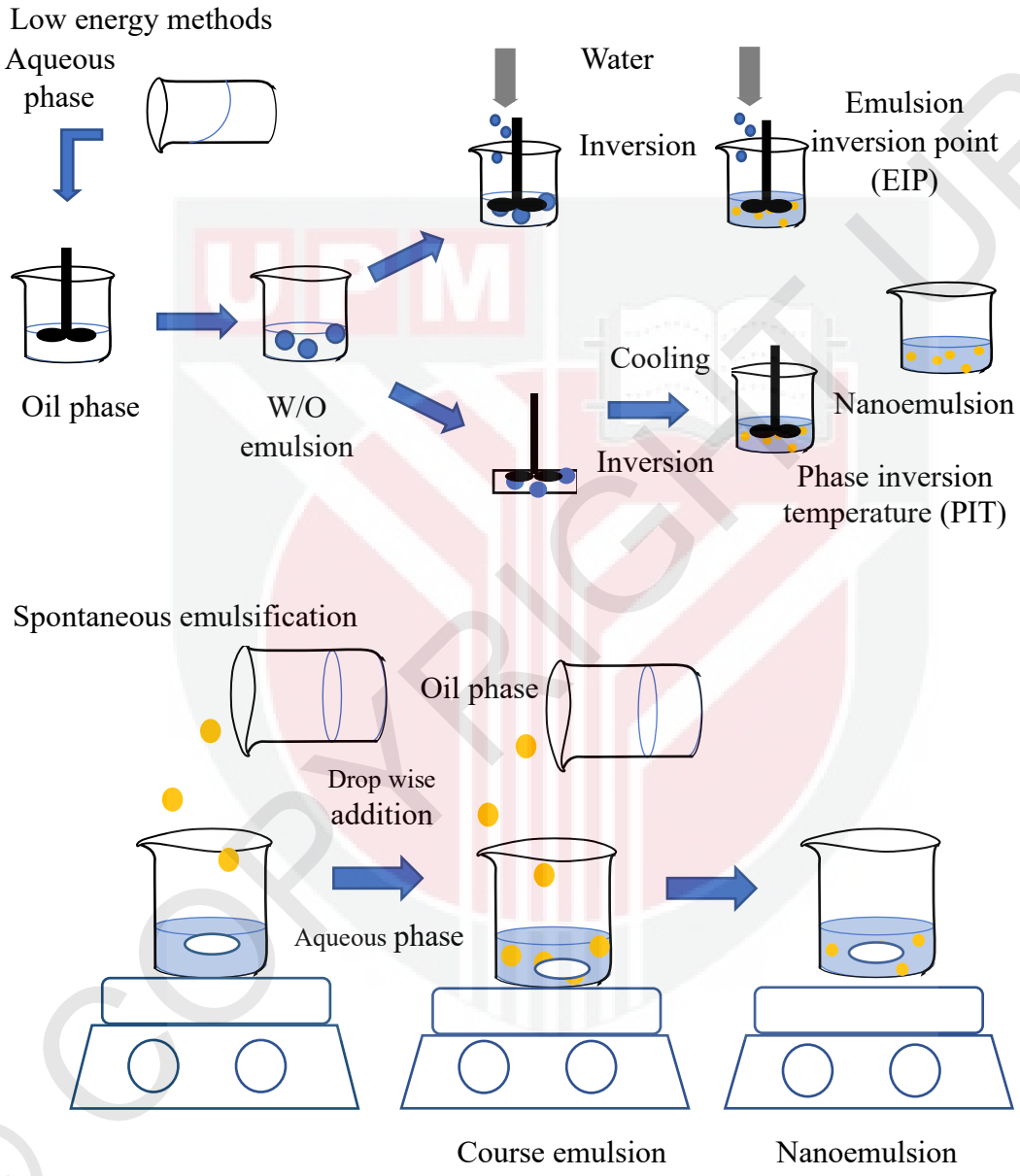



**Figure 2.7 : Low Energy Methods Employed in Nanoemulsions Such as Emulsion Inversion Point (EIP), Phase Inversion Temperature (PIT) and Spontaneous Emulsification**

#### 2.8.2.3 Phase Inversion Temperature (PIT)

The phase inversion temperature (PIT) method is a temperature-dependent emulsion system. This method relies on the temperature properties that allow for changes in distribution affinity toward water and oil based on temperature variations. Emulsifiers consist of hydrophilic (polar affinity) and hydrophobic (non-polar affinity) components. Below the transition temperature, the larger hydrated polar groups occupy the surface area of the emulsion system, resulting in an oil-in-water (O/W) emulsion. In contrast, above the transition temperature, a water-in-oil (W/O) emulsion system is formed as a result of the hydrocarbon chains (hydrophobic) occupying the system. By rapidly cooling both emulsion systems, stable nanoemulsions with low polydispersity index (PDI) and a narrow droplet size distribution can be produced. Studies on nanoemulsions using the PIT method have been reported by Chuesiang et al. (2018) for cinnamon oil and Ren et al. (2019) using n-tetradecane, both of which successfully produced stable nanoemulsions.

### 2.8.3 Advantages and Disadvantages of High Energy Methods and Low Energy Methods

The preparation of nanoemulsion basically can be divided into two methods; high energy (HE) and low energy (LE) methods. Method that was used to produce NE selected by the researchers may offer benefits for their research application as compared to another method and vice versa. One of the advantages of high energy methods in producing nanoemulsion is their ability to achieve small droplet sizes rapidly and efficiently. On the other hand, low energy methods offer the advantage of reduced equipment costs and lower risk of degradation or alteration of sensitive components within the emulsion. Although both methods can successfully produced

nano-size emulsion, there are still many challenges need to be addressed in the terms of extensive energy required and safety consideration. Therefore, to summarise, several researchers have highlighted the advantages and disadvantages of utilising HE and LE in their studies as presented in Table 2.3.

**Table 2.3 : Advantages and Disadvantages of High Energy Methods and Low Energy Methods**

| Nanoemulsion methods | Advantages | Disadvantages | References |
|---|---|---|---|
| High energy methods | Make use of mechanical devices to produce disruptive forces, use high energy input, cost inefficient | Limited to small batches | Yukuyama et al. (2016); Kim et al. (2018); |
| | Possibility to introduce various compounds into droplet in internal phase | High operational cost | |
| | Suitable for encapsulation of unlimited range of compounds and pharmaceuticals provided able to maintain stability at high pressure and shear rates | Require insulation for temperature and shear sensitive compounds | |
| Low energy method | Use internal chemical energy of system, use of simple stirring, energy efficient, non-destructive, no damage to the encapsulated molecule | Limited amount of oil phase;presence of solvent | Feng et al.(2020); Campani et al. (2016); Ševčíková et al. (2012); Solans and Sole (2012) |
| | Mainly depend on physicochemical properties of system especially surfactant properties | Limited to the non-ionic surfactant | |
| | Suitable for encapsulation of unstable substances that decompose in high energy devices such as protein, nucleic acid, peptides | Gradual addition of one phase into another; requires the presenceof liquid crystal (LC) ormid-range microemulsion(ME) phases | |
| | Low operational cost, easy for scale up | | |

### 2.8.4 Challenges in Nanoemulsion

The key challenges often encountered in the storage process of nanoemulsions include various forms of kinetic instability, such as Ostwald ripening, creaming, coalescence, sedimentation, and flocculation (Figure 2.8). Among these instabilities, Ostwald ripening poses the biggest problem. Ostwald ripening, also known as coarsening, is a process in which larger droplets grow at the expense of smaller droplets, resulting in a decrease in the total energy of the two-phase system as the size of the nanoemulsion increases (Abbasian Chaleshtari et al., 2022; Rai, 2018). Creaming and sedimentation are issues that typically occur under the influence of centrifugal and gravitational forces, respectively (Koroleva and Yurtov, 2012). Coalescence refers to the irreversible association of globules. If the repulsion forces between two globules are smaller than the attractive forces, they may collide and undergo coalescence, indicating irreversible association (Ali et al., 2013). In contrast, the collision of two globules may lead to flocculation when the intermolecular repulsive forces are strong enough to keep the globules separated at a distance.

The main problem often associated with nanoemulsions is the aggregation of droplets during the development process. Challenges such as creaming and sedimentation can be reduced by increasing the viscosity of the dispersion medium. Polysorbate 80, also known as Tween 80, is a commonly used nonionic surfactant that consists of poethers or polymers of ethylene oxide. It is frequently employed to stabilise aqueous formulations by maintaining distance between droplets through steric hindrance (Kaur and Mehta, 2017;

Li et al., 2016). The rate of Ostwald ripening can be decreased by reducing the solubility of the dispersed phase in the continuous phase, thereby slowing down the ripening process. One strategy involves adding gum to create a gel-like rheological behavior in an attempt to prevent Ostwald ripening (Trujillo-Cayado et al., 2020). To prevent destabilisation of nanoemulsions, such as flocculation, the adsorption of polyelectrolytes to the droplet surface can be employed to form a layer of droplet surface charge, preventing droplet-droplet collisions. However, the concentration of the polyelectrolyte must be carefully considered, as excessive concentration may promote flocculation. It is crucial to ensure that the repulsive attraction is sufficiently strong to prevent droplet aggregation, and additional agitation can be applied to disrupt the formed flocs (McClements and Jafari, 2018).

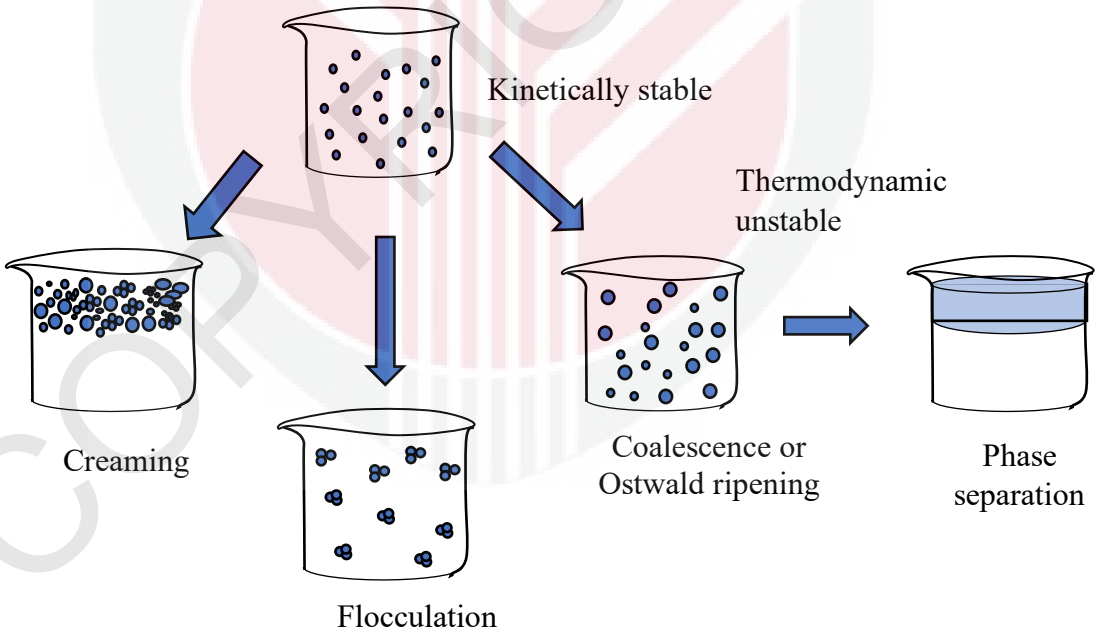



**Figure 2.8 : Nanoemulsion Instability**
(Adapted from Mcclements and Jafari, 2018)

### 2.8.5 Mechanism of Nano-Emulsification

The mechanisms of emulsification differ between nano-emulsification and conventional emulsions. Factors such as droplet size, droplet surface charge, and preparation methods contribute to the distinctions between the two types of emulsions. These factors favor greater stability during storage for nanoemulsions. The formation of smaller-sized globules in nanoemulsions results in less gravitational force and increased repulsive forces between the globules. Consequently, kinetic instabilities like creaming can cause the oil phase to separate and move upward in the container.

The emulsification process occurs when two immiscible liquids with low interfacial tension are combined in the presence of emulsifiers. This process involves two simultaneous steps: hydrodynamic breakdown and interfacial modification (Ravera et al., 2021). Hydrodynamic breakdown disperses the phase and suspends the dispersed phase into the medium of dispersion. Interfacial modification involves emulsifier adsorption at the interface. To achieve stable and smaller droplets, the dispersed phase requires significant stress, such as increasing temperature, applying an electric shock, inducing vibration, or using agitation. Emulsifiers are employed to reduce interfacial tension, minimising the need for excessive energy and stabilising droplet distribution. The selection of emulsifiers for nanoemulsion development depends on their solubility in water and oil phases, the type of nanoemulsion (oil-in-water or water-in-oil), and the desired hydrophilic-lipophilic balance (HLB) value (McClements and Jafari, 2018b). Oil-in-water nanoemulsions are stabilised by the presence of charge on the hydrophilic head of the surfactant surface and steric hindrance. On the other hand, water-in-oil nanoemulsions are stabilised by steric hindrance created by the hydrophobic tails of the emulsifier (Figure 2.9).

The stability of a nanoemulsion can be determined by measuring the zeta potential (ζ potential), which can have positive or negative values depending on the acidity or basicity of the nanoemulsion. If the nanoemulsion is acidic (pH 3-6), a negative charge will be observed, whereas a basic nanoemulsion (pH greater than 7) may exhibit a positive charge.

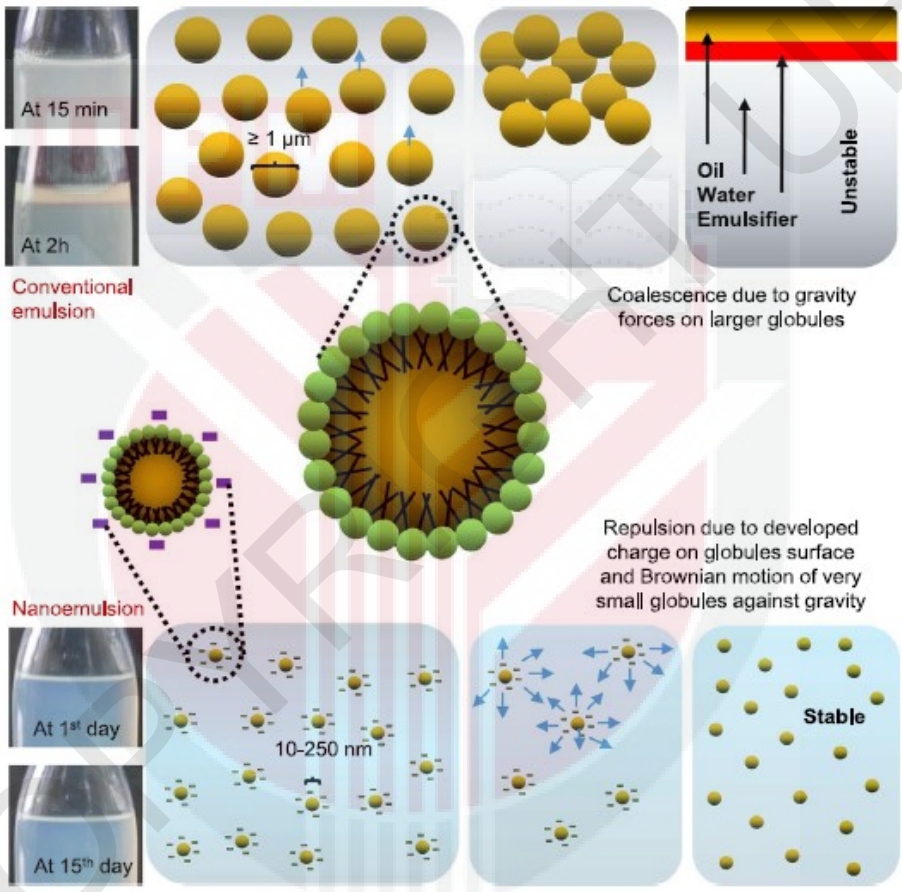



**Figure 2.9 : Comparison between Nanoemulsion and Conventional Emulsion in Terms of Droplet Size and Stability**
(Adopted from Rai et al. 2018)

### 2.8.6 Mechanism of Nanoemulsion Stabilisation

The stability of the NE formulation depends on several factors, including the composition of the emulsifier, droplet size, and surface charge. By combining

emulsifiers, it becomes possible to suspend the dispersed phase as small droplets in the dispersion medium, creating an elastic interface between the two immiscible liquids (McClements and Jafari, 2018b). This elastic interface surrounding the droplets allows them to withstand tension during deformation. Achieving a high level of nanoemulsion stability requires uniform droplet size distribution and the absence of gravitational forces as the droplets remain suspended in the dispersion medium.

When there is a higher degree of surface charge (electrostatic), the droplet molecules repel each other due to a strong intermolecular repulsive force. This phenomenon, known as Brownian motion, causes the charged nano-sized droplets to keep their distance from each other after an elastic collision (Erramreddy et al., 2019). The stability of nanoemulsion is influenced by the collective effects of gravitational force and electrostatic force. Gravitational force is typically associated with the size and weight of the droplets, while the repulsive force is attributed to the kinetic stability through Brownian motion or zeta potential (ζ potential) (Bhattacharjee, 2016). Emulsion systems with low molecular weight emulsifiers are considered stable when the ζ potential value exceeds ±30 mV, and they exhibit excellent stability when the ζ potential value approaches ±60 mV. A Zeta potential of ±20 mV or higher indicates short-term stability, while a range of -5 to +5 mV suggests rapid aggregation of the droplets (Jacobs et al., 2000; Mitri et al., 2011). On the other hand, in emulsions with high molecular weight surfactants, stability is maintained through a combination of steric hindrance and repulsive forces between similarly charged particles, which shifts the plane of shear to a greater distance, resulting in a lower observed ZP (Figure 2.10). Additionally, centrifugal force aids in maintaining the droplets in Brownian motion for a longer period, where the attractive force outweighs the repulsive force. The

characteristics of nanoemulsion, such as structure, charge, composition, and rheological behavior, can be adjusted by optimising the type or concentration of emulsifiers, the order of mixing ingredients, the mixing conditions, pH, and ionic strength (Rai et al., 2018).

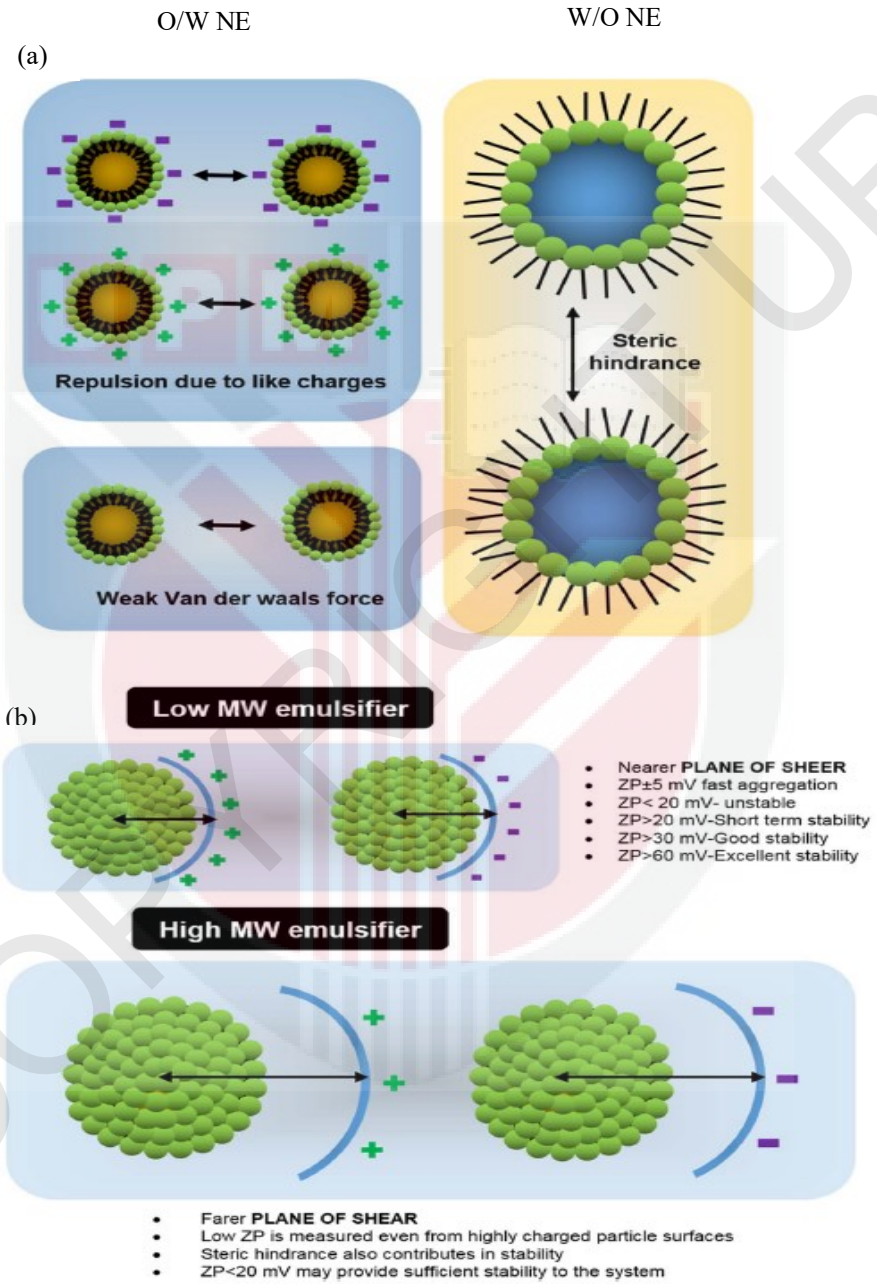



**Figure 2.10 : Nanoemulsion Stabilisation Mechanism. (a) Stability of O/W and W/O Nanoemulsion (Stabilised by Ionic or Non-Ionic Surfactants, (b) Stability of Nanoemulsion i.e., Stabilised Using Low and High Molecular Weight Surfactants** (Adopted from Rai et al. 2018)

### 2.8.7 Dispensing Nanoemulsion in the Final Dosage Form

The potential of nanoemulsion (NE) as a vehicle for controlled drug delivery and dispersion of active ingredients in skin layers has recently become increasingly important. Due to their small droplet size, they provide a high surface area that allows for effective transportation of active ingredients beneath the skin layers. NE, with its lipophilic interior, is more suitable for transporting hydrophobic compounds to the targeted site of action. The development of nanoemulsions is acceptable in pharmaceutical applications because kinetic instabilities such as creaming, flocculation, sedimentation, and coalescence can be overcome by incorporating surfactants into the system.

However, to enhance the adherence of the drug onto the skin, NE is usually thickened or incorporated with polymers to transform into a semisolid dosage form for easier administration through topical application. The addition of a polymer matrix to the nanoemulsion improves efficiency and spreadability, provides an emollient, non-greasy feeling, and increases the stability of the emulsion. The conversion from a gel to a sol state occurs when force is applied. The shear applied to the gel causes the polymer network to break, allowing for the diffusion and release of the drug content. The release of water from the polymeric network hydrates the skin and facilitates absorption into the stratum corneum. Wang et al. (2018) emphasised in their studies that gel incorporated with xanthan gum enables the release of vitamin C-loaded nanoemulgel at body temperature (37 °C) in an *in vitro* release study. Thickeners are used to increase the viscosity of the nanoemulsion and transform the solution into a semisolid dosage form in the case of O/W and W/O types of nanoemulsions. The selection of thickeners or gelling agents (stabilisers) varies, ranging from natural

polysaccharides such as xanthan gum and locust bean gum (LBG), protein polymers such as gelatin and zein, to synthetic agents such as carbopol and carbomer. However, when utilising a polymer in semisolid dosage forms, the composition needs to be optimised to prevent destabilisation of the nanoemulsion after the emulsification process.

Dispensing the formulation in a gel form is always easier and more advantageous than using a cream formulation, as semisolid cream formulations often encounter stability issues (Moulai Mostefa et al., 2006). It has also been observed that gel formulations containing proniosomes have a prolonged effect through membrane diffusion and a higher cumulative percentage release compared to marketed cream formulations (Sankar et al., 2009). Ahmad and colleagues (2017) reviewed that gel-based formulations provide better flux and prolonged release effects for drugs compared to other dosage forms. To illustrate the stabilisation mechanisms of W/O and O/W types of nanoemulsions and the stability of the polymeric network in semisolid dosage forms, Figure 2.11a and Figure 2.11b are provided.

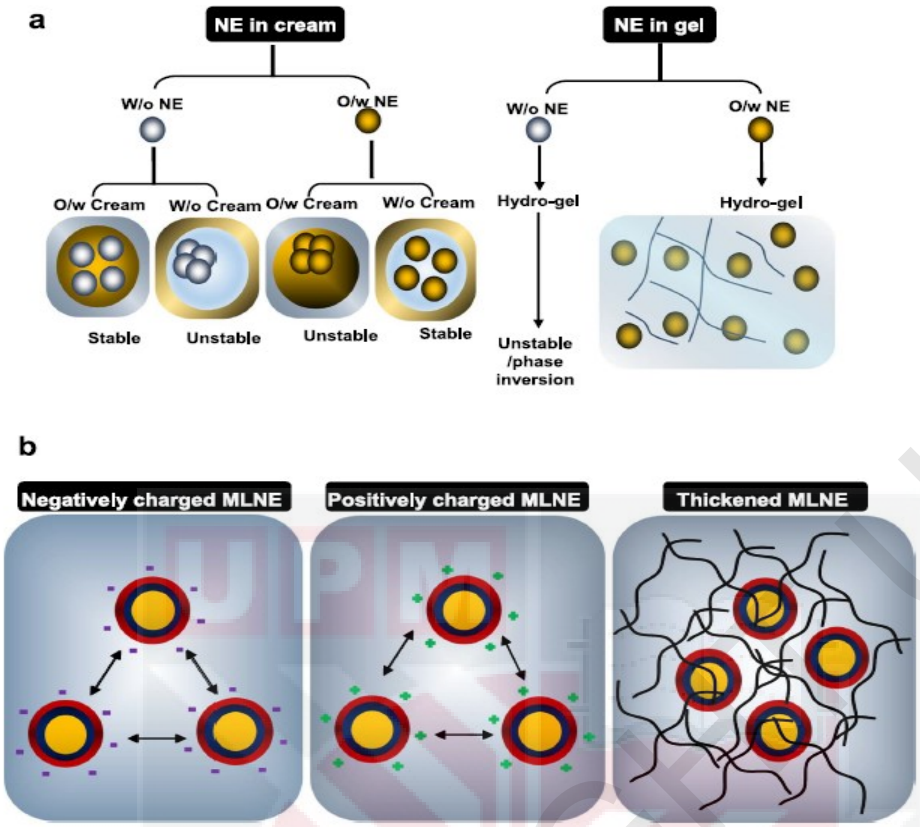
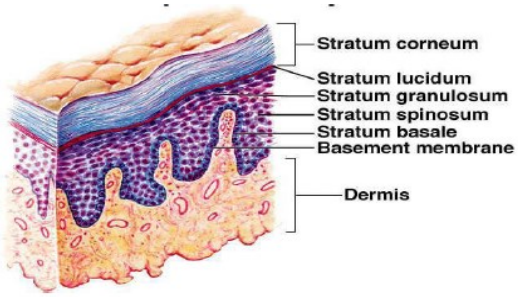



**Figure 2.11 : (a) Illustration of O/W and W/O Nanoemulsionstability of in Semi Solid Dosage Forms (Cream or Gel); (b) Illustration of Stability of Protein and Polysaccharide Stabilised Multilayered Nanoemulsion in Semi Solid Dosage Forms**
(Adopted from Rai et al. 2018)

## 2.9 Nanoemulsion Based Gel (Nanoemulgel)

There has been a growing interest in the past few decades regarding alternative drug carriers that can efficiently deliver substances into the skin, which is the largest organ in the human body. The human skin not only acts as a protective layer and barrier against harmful pathogens and toxins but also serves various normal functions such as thermoregulation, sensory detection, and fluid homeostasis. The protective barrier in human skin (Figure 2.12a) is mainly composed of the epidermis, particularly the stratum corneum. This structure consists of "bricks" (corneocytes) and "mortar" (intercellular lamellar lipid bilayers) (Figure 2.12b) (Zhang et al., 2013).

(a)




Epidermal layer

(b)

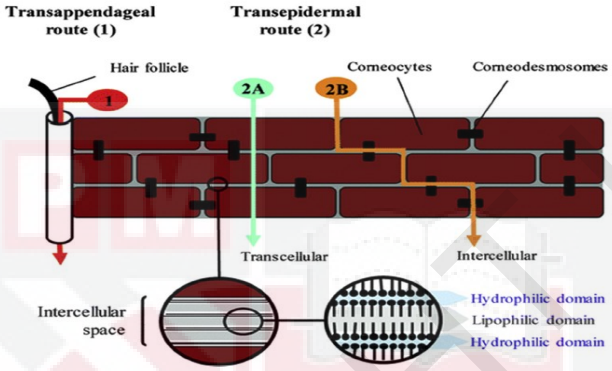



**Figure 2.12 : (a) A Diagram of Skin Layer (Epidermal Layer); (b) Structure of Brick and Mortar of Stratum Corneum**

Developing effective drug delivery approaches has been quite challenging, as drugs often fail to reach their intended site of action. Inadequate localization and penetration of drugs within the skin through percutaneous absorption are common problems faced by formulation scientists (Hussain et al., 2016). There are several delivery routes into the skin, with topical delivery (via the skin strata) and transdermal delivery (systemic exposure) being the most widely investigated. The penetration into the skin is hindered not only by the natural protection of human skin but also by factors such as low bioavailability and the composition and formulation of the drug delivery system, which can limit its effectiveness (Anand et al., 2019). Therefore, there is a necessity to design practical formulations that can enhance the transport of drugs across the skin membrane.

Nanoemulsion-based gel or nanoemulgel (NG) is a modified form of nanoemulsion where gel is added to the formulation to increase its viscosity (Ojha et al., 2022). The droplet size remains under 500 nm, ensuring better stability, enhanced solubility, and high drug loading capacity. Gelation is the process of transforming a free-flowing liquid emulsion into a non-flowing nanoemulsion (Mandal and Vishvakarma, 2023). The resulting non-flowing condition is typically semisolid with viscoelastic behavior. The choice of gelling agent, whether of natural or synthetic origin, and the mechanism that induces gelation determine whether the nanoemulgel formulation is physical (undergoing a sol-to-gel or gel-to-sol transition) or permanent. Table 2.4 provides a list of gelling agents that have been explored for the development of emulgels.

**Table 2.4 : List of Incorporation of Natural Polymer Using Polysaccharide, Proteins and Polysaccharide-Protein Copolymers Origin**

| Polymer | Modifications | Percentage amount added (%) | References |
|---|---|---|---|
| Polysaccharides | Chitosan | 2.0 | Barradas et al. (2018) |
| | Xanthan gum | 2.0; 0.25 – 1.0 | Wang et al. (2018); Djekic et al. (2016) |
| | Hydroxypropyl methylcellulose (hypromellose or HPMC) | 0.5-1.25 | Chandra et al. (2019) |
| | Carboxymethyl cellulose (CMC) | 1.0 | Morsy et al. (2019) |
| Proteins | Zein and sodium caseinate | 2.0 | Wang and Zhang (2017) |
| | Gelatin | 5.0 | Sabet et al. (2017) |
| | Keratin | 0.15 | Cheng et al. (2018) |
| Polysaccharides – proteins copolymer | Gelatin and xanthan gum | 0.4-4.0 : 0.2 | Wang et al. (2018) |
| | gelatin and κ-carrageenan | 1.0-2.0 : 0.05-1.0 | Derkach et al. (2015) |
| | Fish gelatin – gum Arabic | 1.0 : 1.0 | Anvari and Chung (2016) |
| Synthetic | Carbopol 940 | 1.0 | Ahmad et al. (2019); Elmateeshy et al. (2018); Dantas et al. (2016) Eid et al. (2014) |
| | Carbomer 934 | 0.5 | Dhawan et al. (2014) |

## 2.10 Properties of Nanoemulgel

The physicochemical properties of nanoemulgels, such as particle size, polydispersity index (PDI) distribution, zeta potential (ζ-potential), rheological behavior, and kinetic

release stability, have been found to affect the attributes of nanoemulgels (Barradas et al., 2018; Elmateeshy et al., 2018). Currently, there is growing interest in using gel-stabilised nanoemulsions to formulate stable topically applied drug delivery systems. To create a high-quality nanoemulgel, it is important to have small particle sizes, high zeta potential values, narrow PDI distributions, shear-thinning rheological properties, and enhanced skin permeation rates. Therefore, understanding the physicochemical properties of a nanoemulsion-based gel before formulating it as an excipient for drug delivery systems is vital. This knowledge helps in selecting the appropriate conditions and optimising the process during product development.

### 2.10.1 Particle Size and Polydispersity Index (PDI)

Measurement of particle size and polydispersity index (PDI) plays a significant role in determining the stability, appearance, rheological and optical properties, release profile, and bioavailability during the development of nanoemulgels. The droplet size of nanoemulgels typically ranges between 5 to 500 nm (Anand et al., 2019). Dynamic light scattering (DLS) technique is commonly used to measure droplet size. This technique involves observing the interaction between a laser beam and the dispersion to measure the transitional diffusion coefficient. The droplet size can be directly measured using an instrument like Nanosizer Malvern®, or it can be calculated using the Stokes-Einstein equation (Equation 2.1) when there is no interaction between droplets and the solution is diluted (Saifullah et al., 2016).

$$V = \frac{g}{18} D^2 \frac{\rho_d}{\eta} \qquad [2.1]$$

where:

V = rising velocity of the droplet (m/s)

g = gravitational constant (9.81m/s2)

D = droplet diameter (m)

d = density difference between heavy phase and

ligh phase(kg/m3)

η = dynamic viscosity

The dimension of a particle can be described by its particle size distribution (PSD), which represents the fractions of droplets within different size classes. The PSD is characterised using the polydispersity index (PDI), which reflects the size distribution of droplet diameters. The PDI can be measured by analyzing the intensity of scattered light as a function of the angle between the incident and scattered beams, following the light scattering theory (Saifullah et al., 2016).

### 2.10.2 Zeta Potential

The stability of nanoemulgel can be improved by incorporating polysaccharide/protein polymer into the system. The polysaccharide responsible for the surface charge changes and increase the thickness of the interfacial layers. Whilst protein, carry the opposite charges of the polysaccharide, and they are able to form electrostatic interaction that produce longer stability to nanoemulgel system (Yin et al., 2021). Typically, droplets entrapped in nanoemulgel possess an electric surface potential attributed to charged materials such as ionic emulsifiers, biopolymers, or mineral ions that adsorb onto the droplets (McClements, 2015). The electric surface potential plays a crucial role in determining the physicochemical properties of nanoemulgel, including aggregation stability, chemical stability, ingredient interactions, and surface adhesion. The electric properties of nanoemulgel droplets are commonly characterised

by their ζ potential, which typically falls within the range of -60 to +60 mV depending on the type of emulsifier and solution conditions (Kaplan et al., 2019; and McClements and Rao, 2011). Many polymers used in nanoemulsions have positive or negative charges on their surfaces. Charge neutralisation occurs when a charged polymer interacts with oppositely charged ions or molecules in a dispersion medium (such as water). This interaction neutralises the charge on the polymer, thereby reducing electrostatic repulsion between adjacent droplets. This allows the droplets to approach each other without repelling each other, contributing to the stability of the nanoemulgel. The neutralisation of charge is important when stabilising nanoemulsions as it helps prevent coalescence and aggregation.

The ζ potential of nanoemulsion droplets can be manipulated to be positive, neutral, or negative by selecting cationic, nonionic, or anionic emulsifiers. The magnitude (and sometimes the sign) of the ζ potential can be altered by adjusting the solution pH and ionic strength. For instance, the ζ potential of protein-coated droplets changes from highly positive at low pH to neutral at the isoelectric point and highly negative at high pH values (Zhang et al., 2020). Additionally, the electric characteristics of emulsifier-coated droplets can be modified through the adsorption of various surface-active agents such as ionic polysaccharides, proteins, or multivalent ions. The surface charge depends on the nature of the emulsifiers incorporated: the surface becomes negatively charged if an anionic surfactant is used and positively charged if a cationic surfactant is used (Hasenhuettl, 2019). Therefore, the stability of nanoemulsion which measured also by ζ potential highlighted in section 2.8.6 can be enhanced by reinforcing with polysaccharides/protein complex which have emulsifying ability to produce longer stability of the nanoemulgel.

### 2.10.3 Rheology

The gelation process converts a free-flowing liquid emulsion into a non-flowing formulation. Typically, such non-flowing formulations exhibit semisolid properties with viscoelastic behaviour, often demonstrating pseudoplastic (non-Newtonian) characteristics. This behaviour is characterised by a decrease in viscosity with increasing shear rate values. When shear stress is applied, it disrupts the arrangement of inorganic particles dispersed in the aqueous medium, leading to the collapse of weak interparticulate associations and indicating a tendency for flow. Similarly, for macromolecules, applied shear stress aligns the molecules in the direction of the stress, thereby weakening the resistance to flow (Rathod and Mehta, 2015).

Depending on the nature of the gelators and the mechanism of gelation induction, the emulgel can either be physical (exhibiting sol-to-gel and gel-to-sol transitions) or permanent (emulgel). Nanoemulgel, containing a liquid dispersion of xanthan gum, carboxymethyl cellulose (CMC), and other gelling agents, exhibits pseudoplastic properties. The viscosity of these gels decreases with an increasing rate of shear. The shearing action causes the disarrangement of molecules as shear stress increases, aligning their long axis and reducing resistance to flow, thus releasing the solvent from the gel matrix.

The rheograms demonstrating pseudoplastic behaviour have been observed in previous studies (Ahmad et al., 2019; Erramreddy et al., 2017). The small-amplitude oscillatory shear (SAOS) test is commonly used to describe dynamic viscoelastic behaviour. This test involves applying a small sinusoidal strain (or stress) and measuring the resulting stress (or strain). These small-amplitude oscillatory tests are

typically conducted in shear, hence the term SAOS, which stands for small-amplitude oscillatory shear, is commonly used to describe dynamic viscoelastic tests. The linear viscoelastic properties of a material are determined by its elastic modulus (G') and viscous modulus (G"). The measurement of energy accumulated and recovered per deformation cycle, represented by G', reflects the material's elastic behaviour. On the other hand, G" measures the dissipated energy and heat lost per deformation cycle, representing the material's viscous behaviour. When a material exhibits G' > G", it indicates a dominant elastic, solid-like behaviour. Conversely, when G" > G', the material exhibits viscous, liquid-like behaviour (Barradas et al., 2018).

In the strain sweep test, one can determine the rheological characteristics that relate to the structural patterns of formulations. The rheological behaviour of formulations is crucial for their application on the skin. The strain sweep test (Figure 2.13) reveals the linear viscoelastic region (LVR), where a sample can endure maximum deformation stress without undergoing structural changes. The material's structure remains unchanged within the LVR. However, beyond the LVR, the material's structure starts to break down and become disrupted, indicating the end of the linear viscoelastic regime of the formulations.

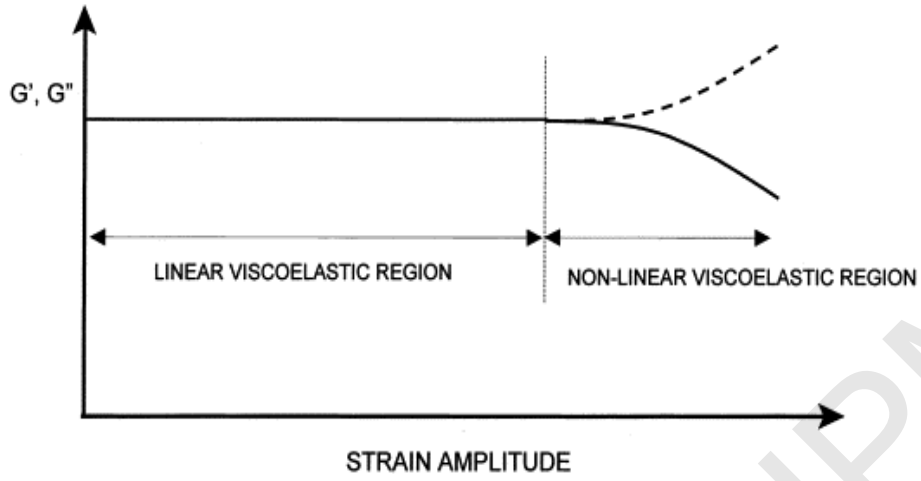



**Figure 2.13 : A diagram of the Strain Sweep Test Used in Determining the Linear Viscoelastic Region (LVR)**
(Adapted from Gunasekaran and Ak, 2000)

## 2.11 Natural Polymer for Drug Delivery System

Since 2000, the development of biopolymers as intelligent excipients has captivated formulation scientists, industrial researchers, and pharmacologists. This is primarily due to their favorable biological and chemical characteristics, such as low toxicity, a wide range of release profiles, high stability, and biocompatibility (Carter et al., 2019; Shariatinia, 2019). Biopolymers are complex biochemical entities that vary in their structural features and are derived from plants or living organisms. They can be broadly classified into three categories: carbohydrates, proteins, and nucleic acids (Krull et al., 2000). These compounds have the ability to modify, sustain, control, or even enhance the rate at which drugs enter the biological system.

### 2.11.1 Polysaccharide Based Polymer

Polysaccharides, which belong to the carbohydrate class, encompass a diverse range of polymeric oligosaccharides formed through glycosidic linkages between monosaccharide units. They are primarily sourced from natural origins such as plants

(e.g., pectin, cellulose, starch), animals (chitin, chitosan), microorganisms (xanthan gum, gellan gum, pullulan), and algae (carrageenan, alginate, and agar). Polysaccharides exhibit promising potential as biomaterials in nanomedicine applications due to their abundant availability in nature, biocompatibility, non-toxicity, affordability, water solubility, and bioactivity. In the structures of polysaccharides (Figure 2.14), there are various functional groups (hydroxyl, amino, carboxylic acid groups) present in their backbones, contributing to their diverse structural and functional properties (Dmour and Taha, 2018; and Debele et al., 2016).

To ensure the safety and protection of drugs from degradation, as well as to achieve sustained release at an optimal rate based on the formulated drug design, it is essential for the nanoemulgel to possess specific attributes. Numerous studies have investigated the utilisation of polysaccharides as gelling agents (Ali et al., 2023; Putro et al., 2023; and Barclay et al., 2019) in drug delivery systems. These studies explore the potential of polysaccharide-based nanoemulgels, which consist of hydrophilic outer shells surrounding hydrophobic drug cores, enabling the encapsulation of hydrophobic drugs. The cross-linking of the polymer matrix occurs when the drugs are added directly to the polymer solution. This interaction between the polymer and drugs relies on non-covalent interactions, such as hydrogen bonds and hydrophobic forces. The release of the encapsulated drug from the gel matrix primarily occurs through controlled diffusion, dependent on the mesh size of the nanoemulgels upon swelling (Figure 2.15).

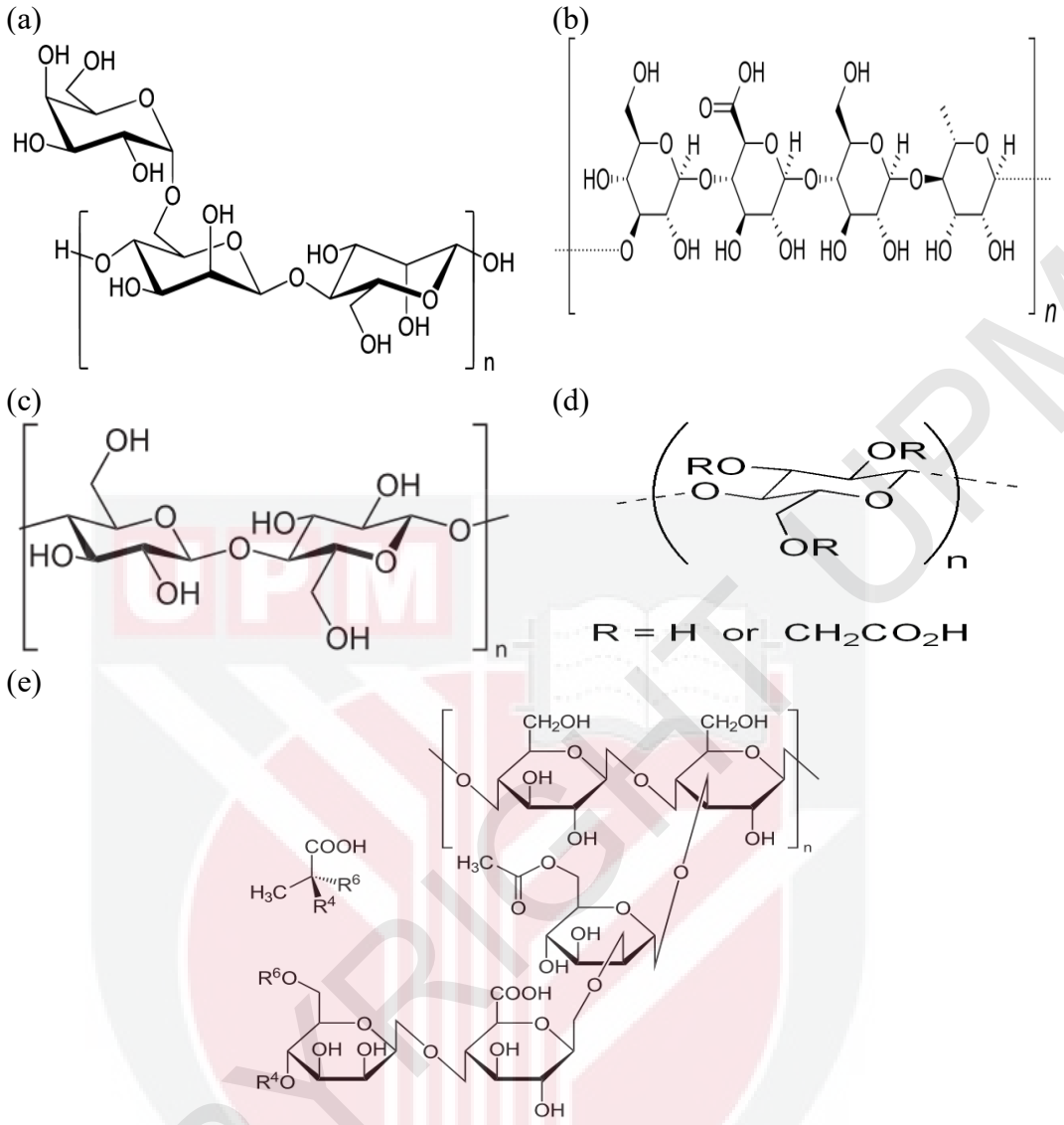



**Figure 2.14 : Different Sources of Polysaccharides Polymer (a) Guar Gum; (b) Gellan Gum; (c) Cellulose; (d) Carboxymethyl Cellulose; (e) Xanthan Gum**

Polysaccharides also contain a small amount of hydrophobic proteins, which contribute to their interfacial activity. A study conducted by Yin et al. (2012) on soy protein-soybean soluble polysaccharide complexes suggests that soy soluble polysaccharides can bind to soy proteins through electrostatic and hydrophobic interactions. Additionally, the neutral side chains of the polysaccharide can stabilise the protein/polysaccharide complexes in aqueous solutions, similar to the interactions

between hydrophobically modified ionic-neutral double hydrophilic block copolymers and proteins.

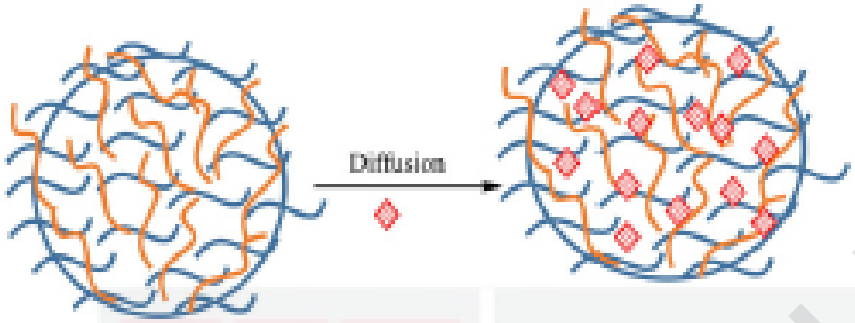



**Figure 2.15 : Drug Release of Encapsulated Drugs through Diffusion**
(Adapted from Debele et al., 2016)

Xanthan gum (Xan) is a commonly used polysaccharide polymer that functions as a gelling agent (Figure 2.14e). It is derived from the bacterium Xanthomonas campestris. XG consists of a primary unit composed of β-D-glucose linked at the 1 and 4 positions, which is identical to cellulose. It also has a trisaccharide branch side chain composed of a repeated unit of β-D-(1,2)-mannose attached to β-D-(1,4)-glucuronic acid, with a final β-D-mannose at the end (Saidin et al., 2018). Xanthan gum is a water-soluble compound that readily dissolves in both cold and hot water. It possesses multifunctional groups, such as polyhydroxyl and amino groups, which can facilitate hydrogen bonding. The linear molecular structure provides the chain with sufficient flexibility. This polysaccharide is generally recognized as safe (GRAS) and finds wide application as a stabiliser, gelling agent, and structural provider in the food, cosmetics, and pharmaceutical industries (Wang et al., 2018). Xanthan gum can form hydrogels when its aqueous solutions are annealed at high temperatures for a specific duration and then cooled (referred to as annealing and subsequent cooling-induced gelation). It exhibits superior gel characteristics at lower concentrations compared to

other polysaccharides (Freitas et al., 2014). In the pharmaceutical field, xanthan gum is utilised for various functions, including thickening agent, skin emollient, emulsion stabiliser, and emulsifier. This non-irritating substance demonstrates higher low-shear viscosity with notable shear thinning behavior compared to other hydrocolloids like locust bean gum, guar gum, and gellan gum (Kichou et al., 2022).

Due to the physicochemical and biological properties of polysaccharides, polysaccharide-based emulsions have a significant impact as drug carriers for various pharmaceutical agents. Most polysaccharides (or derivatised polysaccharides) have limited interaction with proteins (Debele et al., 2016). Therefore, the exploration of polysaccharide-based polymers as promising drug carriers for designing drug delivery systems (DDS) is an area of ongoing research.

### 2.11.2 Protein Based Polymer

In recent years, there has been an increasing interest among researchers in protein-stabilised emulsions for drug delivery. The use of protein-based biopolymers instead of synthetic polymers is favored due to their hydrophilic nature, biocompatibility, biodegradability, and safety. These protein biopolymers readily adsorb at the interface, reducing the interfacial tension and preventing disruption of droplets. As a result, they contribute to the production of more stable emulsions both thermodynamically and kinetically. Proteins consist of hydrophilic and hydrophobic residues distributed throughout their primary structure. In the tertiary conformation structure, proteins are folded, and the residues form segregated patches. The mechanism by which proteins stabilise emulsions involves a three-stage process: diffusion from the bulk to the interface vicinity, actual adsorption at the interface, and subsequent realignment of the

adsorbed protein. During adsorption, a portion of the hydrophobic groups becomes embedded in the oil phase, while the remaining groups remain in the aqueous phase. The protein then undergoes conformational changes to maximise interactions between groups and minimise unfavorable interactions. These conformational changes result in the formation of highly viscoelastic films, which prevent coalescence by promoting intermolecular interactions among the adsorbed proteins. The residues of the protein remaining in the aqueous phase provide steric stabilisation, preventing kinetic instabilities such as flocculation and coalescence.Previous studies (Zang et al., 2019; and Tamnak et al., 2016) have reported that protein concentrations ranging from 1% to 5% are typically added to develop stable emulsions. Protein biopolymers can be further enhanced through enzymatic and genetic modifications, not only to improve emulsion stability but also to enhance other properties. Mild processing conditions, such as controlled heating and shearing, can lead to partial denaturation and unfolding of proteins, allowing them to interact with more hydrophobic groups in the oil phase (Buoyer et al., 2012).

Protein biopolymers derived from natural sources, such as gelatin, collagen, silk, keratin, and elastin, have been extensively studied for their structural properties in various pharmaceutical applications. These protein biopolymers can serve as excipients in attractive drug delivery systems (DDS) for topical or transdermal routes, offering stable formulations due to their release behavior, degradation profile, and mucoadhesive nature (Jao et al., 2017). Additionally, plant-based proteins like zein from corn, soy protein, and gliadin have been investigated for their various functions in DDS, as reported in several previous studies (González et al., 2015; Patel et al., 2014; Xu et al., 2013).

### 2.11.3 Polysaccharide–Protein Based Polymer

Biopolymers derived from proteins and polysaccharides are excellent examples of stabilisers currently utilised in pharmaceutical industry applications. In the pharmaceutical industry, biopolymers serve various functions, such as capsule formation (gelatin), binding agents (arabic gum, chitosan), suspending agents (hypromellose), mucoadhesive agents (chitosan), and gel matrices for extended-release tablets (hypromellose). Protein biopolymers act as emulsifiers due to their ability to adsorb at the oil-water interface, thereby increasing emulsion stability. On the other hand, polysaccharide biopolymers function as emulsion stabilisers by forming a spider-web-like network in the continuous phase, thereby increasing the viscosity of the formulation, and transforming it into a gel-like state. These biopolymers can also be combined to form complexes through interactions such as covalent binding or electrostatic interactions. In the latter case, polysaccharide-protein complexes (PPCs) can be formed through the alternate layers of oppositely charged biopolymers, known as layer-by-layer (LBL) assembly. These multi-layer structures provide stability through electrostatic and steric interactions, thereby enhancing thermal stability and creating resistance to external treatments such as chilling, freezing, and thermal processing (Setiowati et al., 2020; Zang et al., 2019).

Ribeiro et al. (2017) reviewed PPCs from 2001 to 2015 and postulated that the undesirable properties of drug delivery carriers could be overcome by the synergistic effect of a planned biohybrid system, which optimises structures to facilitate interactions with the targets. Jeon et al. (2015) studied the quercetin-loaded multilayered liposome of chitosan-sodium hyaluronate and found that it improved

stability and skin permeability through electrostatic interactions, exhibiting sustainable behaviour as a drug delivery system.

Polymers function as emulsifiers or surfactants and play an important role in stabilising these nanoemulsions. They have hydrophilic (water-attracting) and hydrophobic (oil-attracting) regions that allow adsorption at the oil-water interface. If the concentration of these polymers is high enough, they can form cross-links between adjacent droplets, further increasing the stability of the nanoemulsion. Nanoemulgel system is stabilised by the polymer bridges that prevent droplets from growing together by physically connecting them. In the context of drug delivery, the combined effects of polymer crosslinking/bridging and charge neutralisation contribute to the formation and maintenance of stable nanoemulgel (Yin et al., 2021). This stability is critical for successful drug delivery. This ensures that the nanoemulgel remains intact during storage, transport, and administration, and that the drug is effectively released and absorbed into the body. Many studies on emulsion gel-based formulations stabilised by single polysaccharide biopolymers, single protein biopolymers, and their complexes are presented in Table 2.4. These biopolymers, whether used individually or in mixtures, are classified according to their main stabilising mechanisms and the nature of interactions in the mixtures of oppositely charged polysaccharides and proteins. These biopolymers find applications in the pharmaceutical, food, and beverage industries. In conclusion, polymers that enhance emulsions play a significant role in the field of targeted drug delivery as they enable the direct delivery of therapeutic agents to the intended site of action, thereby improving efficacy.

## 2.12 Kinetic Release of Drug Delivery

Human skin is affected by various biological variables, such as skin thickness, lipid content, and lipid composition. Therefore, there is a need to design a versatile delivery system to enhance drug absorption in specific areas of the skin. The pharmaceutical industry has made significant progress in designing drug delivery systems (DDS) with desired properties. It is essential to achieve a higher therapeutic effect of drugs or natural compounds through DDS, while minimising toxicological effects. Examples of these systems include gels, microspheres, liposomes, and others. Currently, there is a focus on enhancing formulations using polymers as delivery matrices. Controlled release of drugs through diffusion methods has been beneficial in achieving the desired sustained release profile for patient tolerance. Homs et al. (2018) emphasised the impact of polymer concentration on the release profile of dexamethasone (DXN). The study showed that DXN released at a much slower rate when using higher polymeric nanoemulsion, which is advantageous for controlled drug administration. A lower release of the drug indicates higher entrapment efficiency over time.

A delivery system was prepared using carbopol gel incorporating *Swietenia macrophylla* (SM) oil, manipulating various surfactant ratios to stabilise the gel formulation. It was observed that the formulation prepared with *Swietenia macrophylla* oil nanoemulgel significantly improved inflammation inhibition compared to the oil solution when applied in the form of nanoemulgels (Eid et al., 2014). Similar findings were reported by Jyothi and Koland (2016) regarding the use of single carbopol, single hydroxypropyl methyl cellulose K4M (HPMC K4M), and a mixture of carbopol-HPMC K4M as gelling agents in gel formulations. Interestingly, the combination of gelling agents exhibited better anti-inflammatory activity (57.78%)

in the paw volume of Albino Wister rats and showed a superior release kinetic profile (88.02%) compared to other formulations. This could be attributed to the optimum gelling properties, such as spreadability, viscosity, and the presence of a formed network, which contribute to the effectiveness of the gel formulation.

In an attempt to deliver 8-methoxsalen (8-MOP) drug encapsulated in a cationic polysaccharide (chitosan) thickened nanoemulsion, Barradas et al. (2018) found that the formulated DDS was more efficacious than the reference hydrogel. The kinetic release of 8-MOP-loaded hydrogel-thickened nanoemulsion (HTN) provided higher skin penetration and retention. The release kinetics followed the Higuchi model, which describes drug release as a diffusion-based process according to Fick's Law. Another study by Combrinck et al. (2014) reported that the type of polymer influenced the release and topical delivery of the active ingredient investigated. Whey protein isolate (WPI) in combination with other biopolymers was studied for its potential as a DDS. The researchers suggested that droplet charge affects stability and consequently, the release of the salicylic acid drug used. Among the various biopolymers tested, it was assumed that the positively charged amino group from chitosan interacted with the negatively charged skin surface, thereby enhancing drug penetration into the skin. Based on the results of previous kinetic release studies, it can be concluded that polymer-enhanced formulations hold promise as carriers for drug delivery systems.

Fine tuning rheology properties and other gelling properties for effective kinetic release are essential in drug delivery system. Ideal gelling system must show desired pharmacological activity in order to provide advantageous of DDS such as improve bioavailability, enhance the release of drugs, reduce toxicity and able achieve site-

specific action (Jacob et al., 2018). Therefore, in-depth understanding of kinetic release profile is required for fabricating biopolymer-enhanced formulation in future studies.

## 2.13 Kinetic Release Modelling

Kinetic modelling plays a crucial role in understanding and predicting the release behaviour of drug from pharmaceutical formulations. These factors include the physicochemical properties of the drug, the characteristics of the dosage form and the surrounding environment. The solubility and permeability of the drug and the drug's molecular weight and its partition coefficient all directly influence its release kinetics. Additionally, the type and composition of the dosage form, such as the presence of polymers or excipient and affect the drug release. Environmental factors such as pH, temperature and agitation, also impact drug release kinetics. These factors are essential for the development and optimisation of drug delivery systems, providing valuable insights for targeted and controlled release of pharmaceutical. Different kinetic models for drug release commonly used in understanding the release patterns of drugs from drug delivery systems are zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models (Ekenna et al., 2022). Zero order kinetic assumes constant release rate regardless of the drug concentration. First order kinetic on the other hand, considers a drug release rate that is proportional to the remaining drug concentration. The Higuchi model focuses on drug release from the matrix system, where the release rate depends on the square root of time. The Korsmeyer Peppas model is an employed model, for describing the way drugs are released from pharmaceutical dosage forms and was used to determine the mechanism of drug release and was graphically represented as log cumulative percent drug release versus log time. The value of the

release exponent "n" helps in understanding the drug release mechanism. For a value of ( n ) = 0.5, the drug release is considered to be Fickian diffusion (also called Case I transport), which is indicative of a typical drug release from a matrix system; 0.5 < ( n ) < 1.0, the drug release is considered to be anomalous, indicating a combined mechanism of diffusion and erosion of the polymeric matrix.; ( n ) = 1.0, the drug release is considered to be zero-order, which is often related to a purely erosion-driven mechanism and ( n ) > 1.0, the drug release is considered to be super case II transport. The Korsmeyer Peppas model is widely utilised due to its simplicity and its ability to effectively explain a range of drug release patterns. It finds application, in the development and assessment of pharmaceutical formulations aiding in understanding the release kinetics of drugs from diverse delivery systems (Jones et al., 2019). In the realm of kinetic modelling for drug release, the applications and limitations of this approach play a crucial role. The application of kinetic modelling facilitates the design of controlled-release profiles can be tailored for specific patients or disease. Kinetic modelling aids in the investigation of drug release from different dosage forms, such as topical gels, creams, oral tables, transdermal patches or implants (Parhi, 2022 and Tan et al., 2022). In conclusion, drug release kinetic modelling is an important area of research in the field of drug delivery systems. Mathematical models are used to determine the kinetics of drug release from drug delivery systems that can ultimately help to optimise the design of pharmaceutical formulation to yield information on the efficacy of various release models.

## 2.14 Mechanism of Drug Delivery

Although it is quite challenging to combine different properties of gelling agents into a single formulation with optimised properties, it is worth noting that complex

coacervation of biopolymers can overcome the limitations of emulsions, such as thermodynamic instability. The nature and properties of gelators have been reported to alter the drug release profile in a concentration-dependent manner when incorporated within the emulgel. In fact, an optimised concentration of gelators has been found to reduce the rate of drug release. The rate of drug release in the presence of a combination of gelators depends on the interaction between the gelator molecules and the gelator-drug molecule interaction. Typically, emulgel stability is significantly higher than that of emulsions due to increased viscosity of the external phase (Sharma et al., 2018).

The formulation of drug delivery systems enhanced by gels can improve interesting features such as mechanical properties and delivery kinetics, resulting in a sustained release profile of therapeutic molecules (Ribeiro et al., 2017). Sustained release over a longer period of time helps maintain therapeutic efficacy by minimising the frequency of dosage and improving patient compliance. Several researchers have reported on the drug release mechanism from the biopolymeric network of drug delivery systems (Jacob et al., 2018; Maphosa and Jideani, 2018 and Devi et al., 2017). The stability of nanoemulgel formulations depends on various parameters, including the distribution and size of oil droplets, the charge of droplets, pH, ionic strength, temperature of the preparation, cross-linker concentration, solubility of oil, drug loading, and encapsulation efficiency (Bouyer

et al., 2012; Barradas et al., 2018; Elmateeshy et al., 2018). Therefore, it is necessary to control these parameters when formulating a biopolymer-stabilised emulsion. The emulsifying process also plays a key role in influencing the microstructure of the

emulsion, particularly the droplet size. These tiny droplets serve as carriers for lipophilic drugs. By incorporating drugs into nanoemulgel, good stability can be achieved due to the drug's affinity to be solubilised in the oil phase. Fabrication of nanoemulgel formulations can accommodate a higher loading solubilisation capacity of the drug, improving the drug's affinity towards the skin lipid bilayer and allowing for better penetration through the skin. The development of gel-based nanoemulsion can help overcome the limitations of poorly soluble drugs.

Principally, drug release from a gel network involves the movement of drug molecules from the inner polymeric matrix towards the outer surface and then into the surrounding environment. The movement of the drug from the polymeric network can occur through a single mechanism or a synergistic action of mechanisms such as diffusion, dissolution, degradation, or swelling. In the case of an emulgel, drug release is primarily governed by the diffusion method, as illustrated in Figure 2.16.

Compared to both marketed and experimental gel formulations, nanoemulsion and nanoemulgel exhibit significantly higher *in vitro* release and permeation flux through the skin. In particular, the *in vitro* release and permeation flux of lornoxicam were found to be at their highest in the nanoemulsion formulation. The nanoemulgel, on the other hand, demonstrated a lower flux compared to the nanoemulsion, indicating a potential for prolonged drug release behavior (Choudhury et al., 2017). Typically, if drug diffusion across a polymer matrix is more pronounced than degradation, the mechanism of drug delivery is primarily driven by diffusion. In general, drug release follows first-order kinetics (via degradation of the matrix) rather than zero-order kinetics (via diffusion) (Lao et al., 2011). The particle size of the nanoemulgel plays a

crucial role in mediating drug release by influencing both diffusion and degradation mechanisms (Samala et al., 2016).

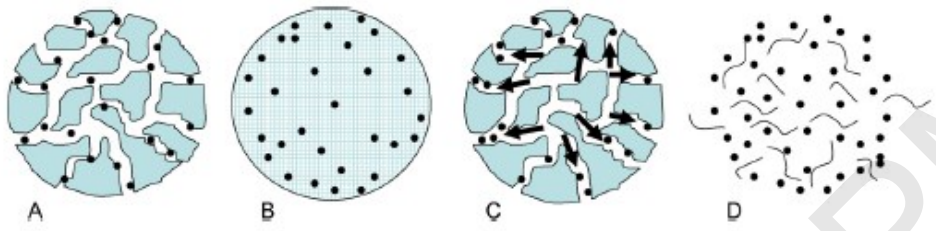



**Figure 2.16 : Drug Release Mechanisms from Polymeric Matrix: (A) Diffusion through Water Filled Pores, (B) Diffusion through the Polymer Matrix, (C) Osmotic Pumping, and (D) Erosion**
(Adopted from Fredenberg et al. 2011)

## 2.15 Advantages of Nanoemulgel

Nanoemulgel, a topical application, has the potential to improve patient compliance due to its enhanced release properties, non-greasiness, and non-irritating nature (Sengupta and Chatterjee, 2017). Numerous studies have been conducted to compare the bioavailability of nanoemulgel formulations with regular gels when applied to the skin (Mandal and Vishvakarma, 2023; Morsy et al., 2019; and Arora et al., 2015). Evaluation of drug permeation through the skin using Franz diffusion cells revealed that the nanoemulgel formulation exhibited significantly higher flux (0.154 mg/cm$^2$/h) compared to the commercially available gel formulation (0.132 mg/cm$^2$/h). In a comparative permeation study conducted by Arora et al. (2014), it was found that the optimised nanoemulgel formulation provided 1.2 times better permeation flux compared to the marketed gel formulation. Similar study was reported in experimental nanoemulgels, which improved the pharmacodynamic activity of the drug in the formulation (Choudhury et al., 2017). These findings suggest that the appropriate formulation can significantly enhance the thermodynamic activity of the drug,

facilitating its permeation and release into the skin. Additionally, the nanoemulgel matrix drug delivery system offers longer retention at the targeted active site and reduced side effects, further adding to its advantages.

Overall, nanoemulgel administered topically to the skin can serve as a viable alternative to conventional formulations. In addition to enhancing drug permeation, nanoemulgels also improve the pharmacokinetic profile, resulting in better therapeutic efficacy. The spreadability profile and reduced stickiness of the nanoemulgel formulation contribute to its better acceptance as a drug delivery system and increased patient compliance. In conclusion, nanoemulsion-based gel drug delivery systems have the potential to be effective, safe, and well-accepted for the topical delivery of lipophilic drugs. Despite a few limitations, nanoemulgel formulations have the possibility to replace conventional formulations in the future for the topical application of lipophilic drugs.

## 2.16 Concluding Remarks

The low demand for Mastura jackfruit has raised significant concerns regarding waste management, particularly regarding the non-edible part of the fruit, namely jackfruit leaves. Currently, the only known use for these leaves is as animal feed. However, there is a pressing need for alternative proposals to maximise the utilisation of jackfruit plant products. Regrettably, valuable compounds present in jackfruit leaves may have been overlooked by researchers due to limited research conducted in this area. Moreover, there has been a global increase in demand for the extraction of bioactive compounds from natural resources. This trend is driven by safety concerns and the desire for higher efficacy at a lower production cost. Consequently, the development

of nanoemulgel has emerged as a promising drug delivery system, opening up new possibilities for exploiting the potential of jackfruit leaves. By incorporating jackfruit leaves extract into a nanoemulgel using a low-energy method, it is possible to create an alternative application for this plant product. The developed nanoemulgel systems exhibit variations in terms of stability, physicochemical properties, rheology, and nanostructure, all of which play a crucial role in their suitability for drug delivery purposes.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Raw Material

Fresh jackfruit leaves (*Artocarpus heterophyllus* var. *Mastura*) were harvested from jackfruit plantation owned by Farmers' Organisation, Maran, Pahang Darul Makmur (Coordinate: 3.5829° N, 102.7748° E). Upon arrival, the leaves were washed with clean water to remove surface dirt and any debris. Then, it was packed in an average 500g polyethylene bag prior to drying. The leave was labeled as JL.

### 3.1.2 Chemicals and Reagents

Ethanol and methanol were purchased from J.Kollin, UK.Folin-Ciocalteureagent, sodium carbonate ($Na_2CO_3$), ferric chloride hexahydrate ($FeCl_3.6H_2O$), and starch powder were purchased from Merck, Germany. β-carotene, 2,4,6-tris-2,4,6-tripyridyl-*s*-triazine (TPTZ), sodium acetate trihydrate,1,1-diphenyl-2-picrylhydrazyl (DPPH), (+) catechin hydrate, gallic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid,97%) standards and fish gelatin were purchased from Sigma Chemical Co.(St Louis, MO, USA). Hexane was purchased from J.T.Baker. Palmester 3575 (caprylic/capric triglyceride) oil was kindly provided by KLK Oleo (Palm Oleo Sdn. Bhd). Coconut oil and palm kernel oil were purchased locally. Xanthan gum was purchased from Merck, Germany. Tween 80 (polyethylene glycol sorbitan monooleate), Tween 40 (polyoxyethylene sorbitan monopalmitate), Span 80 (sorbitan monooleate), and phosphate buffer were purchased from R and M Chemicals.

Artocarpin standard was purchased from Chemfaces (Wuhan, China). Squalene, and β-sitosterol standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol used for HPLC analyses were of HPLC grade purchased from Merck. All reagents used were of analytical grade unless otherwise stated.

## 3.2 Preparation of Jackfruit Leaves Extract (JLE)

Fresh jackfruit leaves (*Artocarpus heterophyllus* Lam. var. *Mastura*) were harvested from a jackfruit plantation owned by Farmers' Organization, Maran, Pahang Darul Makmur, Malaysia (3.5829° N, 102.7748° E). The leaves were labelled as JL. The moisture content of the sample was measured using a digital moisture analyser (MB45; Ohaus Corporation, Parsippany, NJ, USA) in triplicate. The leaves were weighed to (2.0 ± 0.01 g) and heated at 103 ± 0.1 °C until a constant weight was achieved. Jackfruit leaves collected were dried (shade-dried, sun-dried and oven dried at 40°C) until constant weight achieved as mentioned in the next section. Further processing is as detailed below and the processing flowchart is as shown in Figure 3.1.

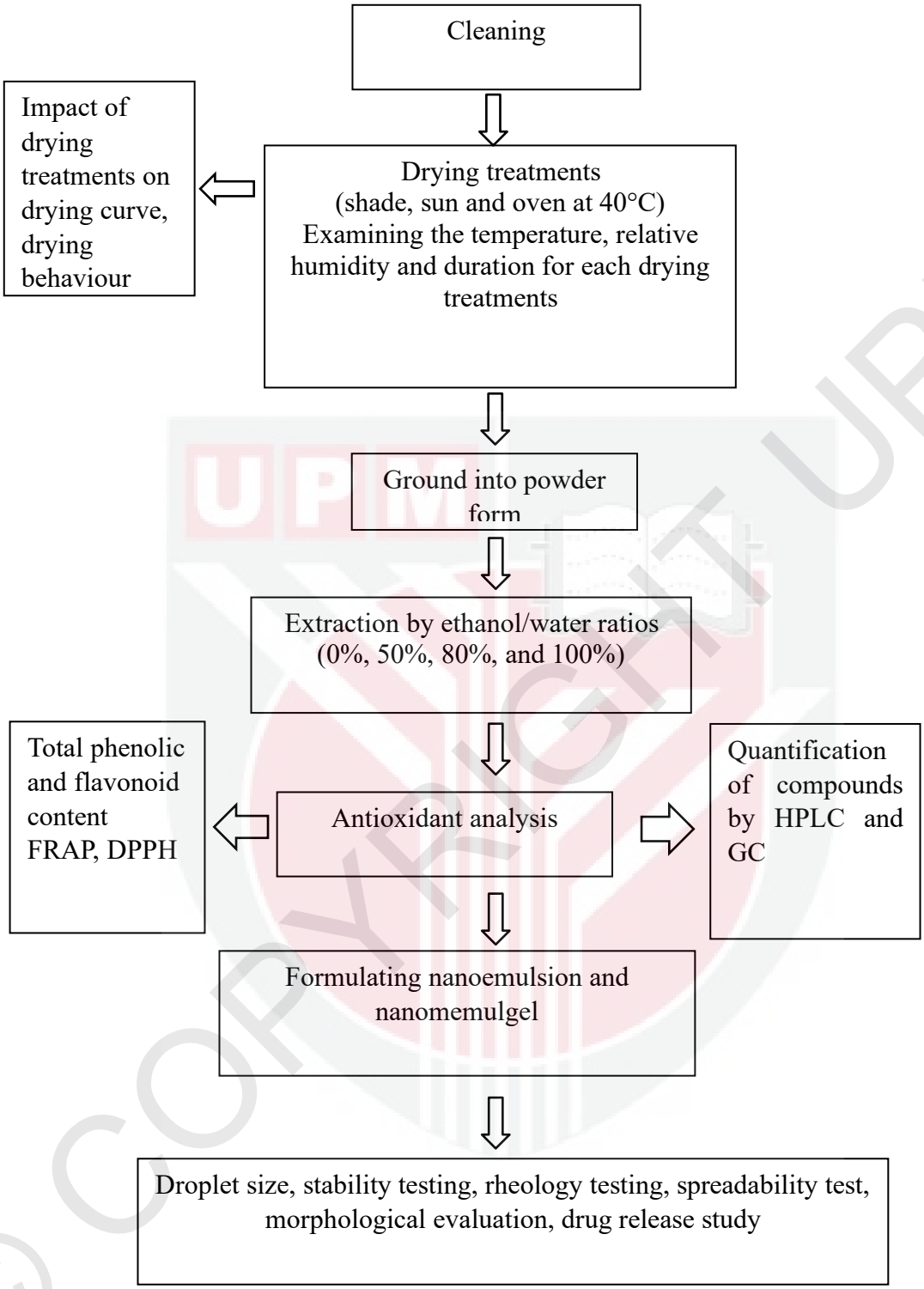



**Figure 3.1 : Flowchart for the Extraction and Formulation of JLE-Loaded Nanoemulgel**

### 3.2.1 Shade-Drying

Jackfruit leaves were shade-dried according to Ling et al. (2015) with modification. Samples were spread evenly as a thin layer on a net in a single layer under shade with average temperature and relative humidity ~25°C and ~58%, respectively. Samples were placed under shade and moisture content of jackfruit leaves were checked in an hourly interval for the first day, every 2 hours for the second and third day from 8:00 a.m to 10.00 p.m using digital moisture analyser (OHAUS, MB45, UK) in triplicates until constant weight achieved. Total hours of samples dried under the shade were recorded about 54 hours with all moisture contents in triplicate measurements were below 10% to reach constant weight).

### 3.2.2 Sun Drying

Jackfruit leaves were sun-dried according to Ling et al. (2015) with slight modification. Approximately 30 ± 0.5 g of fresh jackfruit leaves were evenly distributed and exposed to sunlight with average temperature readings and relative humidity approximately ~33°C and ~55%, respectively. All samples were allowed to dry from 8:00 h to 18:00 h and collected in the evening and moisture content reading were taken on hourly basis and measured using digital moisture analyser (OHAUS, MB45, UK) in triplicates until constant weight achieved. Total hours of samples dried under the sun were recorded about 10 hours with all moisture contents in triplicate measurements were below 10% to reach constant weight).

### 3.2.3 Oven Drying

Jackfruit leaves were oven-dried according to Ling et al. (2015). Approximately 30 ± 0.5 g of jackfruit leaves were dried at 40°C using an automatic electric oven (Memmert, German) in triplicates. Temperature at 40°C was selected to preserve sensitive bioactive compounds in the jackfruit leaf from any possible degradation. All leaves were evenly spread in oven to avoid stacking on each other to achieve maximum drying surface area. The moisture content of jackfruit leaves were checked in an hourly interval using digital moisture analyser (OHAUS, MB45, UK) until constant weight achieved. Total hours of samples dried in the oven at 40°C were recorded about 16 hours with all moisture contents in triplicate measurements were below 10%).

## 3.3 Empirical Modeling of Drying Kinetics

Table 3.1 shows the empirical models to describe the drying kinetics of jackfruit leaves for each method. The moisture ratios (MR) of JL were calculated using Equation 3.1 (Elhussein et al., 2018),

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad [3.1]$$

Where $MR$ is the moisture ratio, $M_t$ is the moisture (%) at time $t$, $M_0$ is the initial moisture (%), and $M_e$ is the moisture at equilibrium (%). Assuming the values of $M_e$ equal to zero are negligible, due to $M_e$ being relatively small compared to $M_t$ or $M_i$ (Doymaz and Kipcak, 2018).

Problem solver Microsoft Excel (Microsoft Corp., v. 2010, Redmond, WA, USA) was used to solve the seven nonlinear mathematical models (Table 3.1). Chi square ($\chi^2$) and root mean square error (RMSE) were used to evaluate the relationship of the experimental and predicted results in addition to the correlation coefficient ($R^2$). The following equations were employed to calculate $\chi^2$ (Equation 3.2) and RMSE (Equation 3.3),

$$\chi^2 = \frac{\sum_{i=1}^{n}(MR_i - \overline{MR})^2}{n-1} \quad [3.2]$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(MR_{i\,experimental} - MR_{i\,calculated}\right)^2}{n}} \quad [3.3]$$

Where $MR_{\text{i experimental}}$ is the concentration of experiment I, and $MR_{\text{i calculated}}$ is obtained from the model equation, and *n* represents the number of experiments.

**Table 3.1 : Empirical Model Applied for Drying Curves**

| Model name | Model equation | Reference |
|---|---|---|
| Newton | *MR* = exp(-k*t*) | Ayensu (1997) |
| Logarithmic | *MR* = a exp(-k*t*) + c | Yaldiz et al. (2001) |
| Verma | *MR* = a exp(-k*t*) + (1 - a) exp(-g*t*) | Verma et al. (1985) |
| Two term | *MR* = a exp(−$k_0$*t*) + b exp(−$k_1$*t*) | Togrul and Pehlivan (2004) |
| Midilli | *MR* = a exp(-k*t*^n) + bt | Midilli et al. (2002) |
| Page | *MR* = exp(-k*t*^n) | Hii et al. (2008) |
| Henderson and Pabis | *MR* = a exp(-k*t*) | Akpinar et al. (2003) |

## 3.4 Determination of Effective Diffusivity

Drying characteristics of biological products can be described by Fick's law diffusion model as represented by Equation 3.4,

$$\ln(MR) = \left(\frac{6}{\pi^2}\right) - \left(\frac{D_{eff}}{r^2}\right) x\, t \quad [3.4]$$

Where $D_{eff}$ is the effective diffusivity of moisture ($m^2/s$), $t$ is the time for drying (s), and $r$ is the mean thickness of the sample ($r = 7.8 \times 10^{-4}$ m). The form of Eq. 3.4 can be applied to particles with slab geometry assuming uniform initial moisture distribution. The diffusivity equation provides an approximate method to present a common quantitative comparison of various products in the aspect of the diffusion coefficient in the process of drying (Elhussein et al., 2018).

## 3.5 Colour Determination

The dried leaves were crushed and ground to a fine powder using a hammer mill (Perten Laboratory Mill 120; Perten Instruments, Hägersten, Sweden). Evaluations of colour were tested on a chromameter (Konica Minolta Colour Reader CR10; Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA). Measurement of colour was determined in $L^*$, $a^*$, and $\underline{b}^*$ coordinates, where $L$ is the lightness (0 = black, 100 = white). The $L^*$, $a^*$, and $b^*$ values are the averages of three readings. The chromaticity coordinate $a^*$ measures red (+) and green (-), and the chromaticity coordinate $b^*$ measures yellow (+) and blue (-). The $L^*$ and $a^*/\underline{b}^*$ values are commonly used as an index to report the colour quality (Mustafa et al., 2020).

## 3.6 Sample Extraction

All shade-dried (SD), oven-dried (OV) and sun-dried (SN) samples were ground by passing through a laboratory hammer mill (Perten Laboratory Mill 120; Perten Instruments, Hägersten, Sweden) with a 100-mesh sieve (0.149 mm aperture) to produce into final powdered form. All powdered samples were extracted using maceration method. The extraction was carried out by dissolving sample in ethanol/water composition (0%, 50%, 80% and 100%) with 5 g sample in 100 ml

solvent (in the ratio of 1:20) with modification (Safdar et al., 2017). Samples were left in the incubator shaker for 3 days with continuous stirring at 150 rpm. Next, the samples were filtered using Whatman No. 1 filter paper by vacuum filtration. The residue that was left on the filter paper was re-extracted three times optimum with fresh solvents and combined filtrates were pooled. Pooled filtrates were evaporated to dryness using rotary evaporator to afford thick green mass. Low temperature at 40°C for water bath was applied to minimise any possible degradation of the samples. Meanwhile, combined filtrates (0% ethanol) were pooled and lyophilised using freeze dryer to remove all the water excess. Table 3.2 summarises the ranges of variables conducted. All experiments in this study were performed in triplicate. Fresh leaves (FL) was used as control.

**Table 3.2 : Ranges of Experimental Variables**

| Drying Method | Ethanol Percentage (%) | Acronym |
|---|---|---|
| Shade (SD) | 100 | SD100 |
| | 80 | SD80 |
| | 50 | SD50 |
| | 0 (Water only) | SD-W |
| Sun (SN) | 100 | SN100 |
| | 80 | SN80 |
| | 50 | SN50 |
| | 0 (Water only) | SN-W |
| Oven at 40 °C (OV) | 100 | OV100 |
| | 80 | OV80 |
| | 50 | OV50 |
| | 0 (Water only) | OV-W |

## 3.7 Antioxidant Analysis

Antioxidant analysis that was conducted in this study were total phenolic content (TPC), total flavonoid content and antioxidant activities which were ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH).

### 3.7.1 Total Phenolic Content (TPC)

Total phenolic content assay of samples was determined using Folin-Ciocalteu assay as described by Mustafa et al. (2016). A 100µl of supernatant was mixed with 0.5ml Folin-Ciocalteu reagent (10 times dilution). The solution was added with 7ml of distilled water and allowed to stand at room temperature for 5 minutes. Then, 1.5ml sodium bicarbonate (60mg/ml) solution was added to the mixture and left at room temperature in dark place for 2 hours. Absorbance was read at 725nm against blank using UV-Visible spectrophotometer (Shimadzu UV1800; Shimadzu Scientific Instruments, Columbia, MD, USA). A calibration curve (Appendix A) was prepared, using a standard solution of gallic acid (0.0, 0.2, 0.4, 0.6, 0.8 and 1mg/ml). Results were expressed as gallic acid equivalents mg GAE/100g extract.

### 3.7.2 Total Flavonoid Content (TFC)

Total flavonoid content assay of samples was determined as described by Iqbal et al. (2007). One milliliter of sample was added to 4 ml distilled water. Then,0.3 ml sodium nitrite ($NaNO_2$) was added and left to stand in a dark place for 5 min. The solution was then added with 0.3 ml aluminium chloride ($AlCl_3$) left at dark place for 6 min. Two milliliters sodium hydroxide (NaOH) was added to the solution and made to a final volume of 10 ml. Absorbance was read at 510 nm against blank using UV–Visible

spectrophotometer (Shimadzu UV1800; Shimadzu Scientific Instruments, Columbia, MD, USA). A calibration curve (Appendix B) was prepared using a standard solution of artocarpin ranged from 0.0 to 1.0 mg/ml. Results were expressed as artocarpin equivalent mg AE/100 g extract.

### 3.7.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out according to method described by Liu et al. (2009). The mechanism of this method is based on the reduction of ferric 2,4,6-tripyridyl-s-triazine complex ($Fe^{3+}$-TPTZ) to its ferrous form ($Fe^{2+}$-TPTZ) in the presence of antioxidants. The FRAP reagent contains 20 mM $FeCl_3.6H_2O$, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 0.3 M acetate buffer, pH 3.6. It was freshly prepared and incubated at 37°C for 10 minutes in an incubator (Memmert GmbH + Co. KG, Schwabach, Germany). The FRAP reagent was mixed in the ratio of 1:1:10. Aliquot of 100 µl sample was mixed with 2.9 ml of FRAP reagent. The absorbance of the reaction mixture was measured using a spectrophotometer (Shimadzu UV1800; Shimadzu Scientific Instruments, Columbia, MD, USA) at 593 nm after incubation at room temperature for 1 hour. Trolox (1000 µM) was used for the calibration curve (Appendix C) and the results were expressed as µM of Trolox equivalents per mg extract weight (µM TE/mg EW).

### 3.7.4 1,1-diphenyl-2-picrylhydrazyl (DPPH) Assay

The antioxidant activity was carried out through evaluation of free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH). The determination was based on the method described by Yamaguchi et al. (1998) with some modifications. An aliquot (600µl) was added to 4.5 ml of 0.05 mM DPPH ethanolic solution. The mixture was

then thoroughly vortex and left for 20 minutes in the dark condition at room temperature. The absorbance was measured at 517nm against a blank of ethanol. Results were expressed as $IC_{50}$ and percentage inhibition of the DPPH radical was calculated according to the following equation:

$$\text{Scavenging effect (\%)} = 1 - \frac{\text{(Abs sample)}}{\text{(Abs control)}} \times 100 \qquad [3.5]$$

where Abs control is the absorbance of DPPH solution without sample.

$IC_{50}$ value was determined from the plotted graph of scavenging activity against the concentration of extracts and is defined as total antioxidant necessary to decrease the initial DPPH radical by 50%.

## 3.8 Quantification of Bioactive Compounds in Jackfruit Leaves Extracts by HPLC and GC Analyses

### 3.8.1 Preparation of Artocarpin Standard

Standard compound (artocarpin) was prepared in methanol and serial dilutions of standard concentration were diluted with distilled water. Various standard concentrations (0.00, 0.03, 0.06, 0.13, 0.25 and 0.5 mg/ml) were injected into HPLC system to establish standard calibration curve. Three replicate injections were performed for each concentration. The slope and $r^2$ were determined by the least-square linear regression analysis method.

### 3.8.2 Preparation of Jackfruit Leave Extracts

All samples were prepared by diluting 5 times of sample mentioned in section 3.6 with the solvent mixture. Samples were injected into the HPLC system under condition mentioned in section 3.8.3. Quantification of artocarpin in samples was compared with external standard method that matched the respective peak areas.

### 3.8.3 Quantification of Artocarpin

The sample with the highest amount of antioxidant properties from each treatment was further analysed for artocarpin content. Artocarpin in samples was analysed using a Shimadzu HPLC system (Shimadzu SPD-10AV, Shimadzu Scientific Instruments, Columbia, MD, USA), which was equipped with an ultraviolet/visible (UV/VIS) detector (SPD-10AV) (i.d. 4.6 × 250 mm, 5 µm column). The analysis was performed according to Septama and Panichayupakaranant (2016) with modification of the adjusted mobile phase. A mobile phase was prepared that consisted of methanol and water (85% methanol and 15% deionized water), at a flow rate of 1 mL/min. Detection of artocarpin using HPLC was coupled using a diode array detector (DAD) at 285 nm. All volume samples were injected at 20 µL. The identification of the compound was based on the comparison with external standard. The signals and area of each peak were processed using Class VP software (Shimadzu LabSolutions, v.6.14, Kyoto, Japan). The amount of sample was expressed as milligram per gram of extract weight (mg/g).

### 3.8.4 Quantification of Squalene and β-sitosterol by GC Analysis

The sample with the highest amount of antioxidant properties from each treatment was further analysed for volatile compounds. Samples were prepared according to by adapting the standard method Cd 11b-91 (Mustafa et al., 2020) using Shimadzu GC-2010 Plus equipped with split/split less injector and flame ionization detector (FID) (Shimadzu, Kyoto, Japan). Approximately, 0.02 g of the sample was weighed and added with 5-α-cholestane serving as an internal standard (ISTD), 0.3 mL pyridine, and 0.15 mL N-trimethylsilyl-N-methyl-trifluoroacetamide (MSTFA). The sample solution was mixed well and heated at 40 °C for silylation purposes. The GC analysis was performed using a capillary column, HT5 (12 m × 0.32 mm, i.d. 0.1 m, Scientific Glass Engineering Analytical Science (SGE), Melbourne, Australia). The temperatures of the flame ionization detector (FID) and injector were set at 370 °C and 360 °C, respectively. The oven temperature was programmed as follows: initial oven temperature at 80 °C, held for 1 minute, temperature ramping at 10°C $min^{-1}$ to 360°C$min^{-1}$andhold at final temperature for 15minutes.Hydrogen was used as a carrier gas at a flow rate of 30 mL/min. For the GC analysis, a split ratio of 1:10 was set, and a 1 µL of sample solution was injected into the GC system.

## 3.9 Preparation of Nanoemulsion

### 3.9.1 Screening of JLE for Solubility Study

Among sample quantified for their bioactive compounds in section 3.8.3 and 3.8.4, oven 80 (OV80) containing highest values of antioxidant properties and bioactive compounds was selected for the preparation of nanoemulsion. The solubility of JLE was determined according to Hussain et al. (2016) in various excipients; medium chain

triglyceride (MCT) oils (Palmester, palm kernel oil and coconut oil) and selected surfactants (Span 80, Tween 40 and Tween 80). An excess quantity of JLE was mixed with 1 mL solvent (oils and surfactants) in stoppered vials for 72 h at 37°C and in isothermal water bath shaker (Memmert, type 3047, Germany). The solvent-drug mixture was then centrifuged at 5000 rpm (Centrifuge-5804R, Eppendorf, Germany) for 15 min. The supernatant was filtered through 0.45 mm millipore membrane filters and diluted with ethanol. The amount of solubilised extract was quantified spectrophotometrically (Shimadzu UV1800; Shimadzu Scientific Instruments, Columbia, MD, USA) was read at 265 nm using ethanol as blank.

### 3.9.2 Surfactant to Oil Ratio (SOR)

The influence of surfactant concentration was investigated by varying the surfactant to oil ratio (SOR) as described by Komaiko and McClements (2014). The total oil phase (surfactant blends and oil) in the final system was held constant at 20% while the SOR was varied by altering the amounts of surfactant and water content in the final content as presented in Table 3.3.

**Table 3.3 : Surfactant to Oil Ratio of JLE Nanoemulsion System**

| SOR | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 |
|---|---|---|---|---|---|---|
| Surfactant mix (%) (T80:S80) (8 : 2) | 6.7 | 10 | 12 | 13.4 | 14.3 | 15 |
| Oil (%) | 13.3 | 10 | 8 | 6.6 | 5.7 | 5 |
| Water (%) | 80 | 80 | 80 | 80 | 80 | 80 |

The SOR was calculated in the following equations:

$$SOR = m_s/m_o \quad [3.6]$$

$$m_w = 100 - m_s/m_o \quad [3.7]$$

where $m_w$, $m_s$ and $m_o$ are the masses of water, surfactant and oil, respectively. SOR tested were between 0.5 – 3.0. Mixing speed was held at 700 rpm in 25 minutes and was conducted at room temperature.

### 3.9.3 Screening of Hydrophilic Lipophilic Balance (HLB) Composition

Hydrophilic lipophilic balance (HLB) is defined as numerical value which is specific for particular emulsifying agent expressing the strength of hydrophilic and lipophilic part of the molecule (emulsifying agent) that required to produce physically stable emulsion. The HLB of surfactant blends were calculated (Mahdi et al., 2011) in the Equation 3.8 according to the fractional of surfactant blends as follow:

$$HLB_{mix} = x(HLB_A) + y(HLB_B) \quad [3.8]$$

In order to develop nanoemulsion, concentration ratios of surfactant components were subjected to initial screening. Surfactant components, Tween 80 (T80) and Span 80 (S80) were mixed (smix) in different weight ratios ranging from 10:0 to 6:4 (Table 3.4). Different ratios surfactant component were titrated by adding oil phase (MCT oils and surfactants) in a dropwise manner into water phase until all components dissolved. All mixtures that formed transparent O/W system were checked for the particle size. The smallest particle size determined was used for the preparation of nanoemulsion. Visual observation of turbidity was observed for each formulation.

**Table 3.4 : Mass Ratio of Surfactant Blends Tween 80 and Span 80 (T80:S80)**

| **Surfactant** | | | **Mass ratio** | | |
|---|---|---|---|---|---|
| T80 | 6 | 7 | 8 | 9 | 10 |
| S80 | 4 | 3 | 2 | 1 | 0 |

### 3.9.4 Construction of Pseudoternary Phase Diagram

The phase behavior study for mixed surfactant system (T80:S80) was conducted by constructing pseudoternary phase diagram (PTD) as described by Mahdi et al. (2011). The pseudoternary phase diagram was plotted using Ternary Plot Chemix School version 8 software. Classification of phase state was made according to the physical appearance observed which was categorised into isotropic, homogeneous, and two or multiphase regions.

## 3.10 Development of Nanoemulsion Incorporated with JLE

Based on the solubility studies (section 3.9.1), Palmester 3575 was selected as oil phase, Tween 80 and Span 80 at a ratio 8:2 as surfactant mix (smix). Nanoemulsion was developed as described by Mehrnia et al. (2016) with modifications. An optimum 70 mg of JLE was added in the smix (T80 mix with S80) at the determined ratio and mixed continuously until fully dissolved using hotplate stirrer. Finally, the oil phase (JLE, smix and Palmester 3575 oil) was incorporated into the water phase by dropwise addition (ratio 2:8) at 50°C operating at 700 rpm speed to obtain homogenous JLE-nanoemulsion using spontaneous emulsification method (Figure 2.7). This nanoemulsion served as control and labeled as NE.

## 3.11 Development of Jackfruit Leaves Extract Nanoemulgel

Xanthan gum (Xan), fish gelatin (Gel) and xanthan gum – fish gelatin (Xan-Gel) mixtures were hydrated at a mass ratio (0.5 -2.5% w/v) in a sufficiently quantity distilled water overnight on a hotplate stirrer operating at 700 rpm at 50°C. The complexes were kept for 24 h to swell completely. The nanoemulgel formulations were

prepared by incorporating JLE nanoemulsion into Xan, Gel and Xan-Gel polymer gel matrix in a ratio 1:1 forming a nanoemulsion based gel (Elmataeeshy et al., 2018). The blends were labeled XA-XC for nanoemulsion added with xanthan gum, GA-GD for nanoemulsion incorporated with fish gelatin and MA-ME for nanoemulsion blends with Xan-Gel. Carbopol (plain nanoemulgel) was also prepared the same procedure as other nanoemulgels. Carbopol and nanoemulsion were used as positive control labeled as Carbopol and NE. Nanoemulgel compositions are presented in Table 3.5.

Jackfruit leaves extract (JLE) loaded nanoemulgels (NG) were examined for particle size, polydispersity index (PDI), zeta potential (ζ-potential), pH, thermodynamic stability studies, spreadability test, viscosity, morphology and permeation study (*in vitro* release testing).

**Table 3.5 : Composition of (a) Nanoemulsion; (b) Carbopol (c) Xanthan Gum Enhanced Nanoemulgel; (d) Fish Gelatin Enhanced Nanoemulgel; (e) Xan-Gel Enhanced Nanoemulgel**

**(a) Plain nanoemulsion composition**

| Composition | Weight (w/w)g |
|---|---|
| Water | 80 |
| Surfactant (T80:S80) (8:2) | 10 |
| Extract + MCT | 10 |

**(b) Carbopol composition**

| Acronym/Composition (%) | Carbopol |
|---|---|
| Carbopol | 2.0 |
| Water | 98.0 |

**(c) Xanthan gum (Xan) enhance nanoemulgel composition**

| Acronym/Composition (%) | XA | XB | XC |
|---|---|---|---|
| Xanthan gum (Xan) | 1.5 | 2.0 | 2.5 |
| Water | 98.5 | 98.0 | 97.5 |

**(d) Fish gelatin enhanced nanoemulgel composition**

| Acronym/Composition (%) | GA | GB | GC | GD |
|---|---|---|---|---|
| Fish gelatin (Gel) | 2.5 | 5.0 | 7.5 | 10.0 |
| Water | 97.5 | 95.0 | 92.5 | 90.0 |

**(e) Xan-Gel enhanced nanoemulgel composition**

| Acronym/Composition (%) | MA | MB | MC | MD | ME |
|---|---|---|---|---|---|
| Xanthan gum (Xan) | 2.0 | 1.5 | 1.25 | 1.0 | 0.5 |
| Fish gelatin (Gel) | 0.5 | 1.0 | 1.25 | 1.5 | 2.0 |
| Water | 97.5 | 97.5 | 97.5 | 97.5 | 97.5 |

## 3.12 Characterisation and Stability of Nanoemulsion and Nanoemulgel

Characterisation and stability study on nanoemulsion include particle size, polydispersity index, zeta potential, pH, thermodynamic stability studies, and rheological behavior measurement. The size and morphology of optimal nanoemulsion was analysed using transmission electron microscopy (TEM).

### 3.12.1 Mean Particle Size and Polydispersity Index (PDI)

The particle size and polydispersity index (PDI) of samples were measured using dynamic light scattering (DLS) droplet size analyser (Zetasizer Nano ZS90, Malvern Instruments, UK). The samples were diluted 100-fold with deionized water before being filled into cuvette capillary cells to avoid multiple scattering effects. Measurements were made with an angle 173° at room temperature (25 ± 0.5 °C). The polydispersity index (PDI), which gives an indication of the width of the droplet size distribution, was determined (Donsi et al., 2012).

### 3.12.2 Measurement of Zeta Potential (ζ potential)

The zeta potential of samples was analysed by dynamic light scattering technique (DLS) using nanoparticle analyser (Zetasizer Nano ZS90, Malvern Instruments, UK) as mentioned by Elmataeeshy et al. (2018). Samples were diluted at ratio 1:100 with distilled water. Measurement of each sample was reported in triplicate with mean ± SD.

### 3.12.3 Determination of pH

pH values of nanoemulsion and nanoemulgels were determined using portable pH meter (Milwaukee, USA) according to Elmataeeshy et al. (2018). The pH measurement is important to assess whether the sample is compatible with human skin. The measurements were made in triplicate.

### 3.12.4 Thermodynamic Stability Studies

The prepared nanoemulsion and nanoemulgels were subjected to different thermodynamic stability tests; freeze thaw cycle, centrifugation and heating and cooling (Elmataeeshy et al., 2018)

#### 3.12.4.1 Freeze Thaw Cycle

All formulations were subjected to three freeze thaw cycles in between -13 °C and 25 °C for 24 hours at each temperature to monitor for the phase separation.

#### 3.12.4.2 Centrifugation

Centrifugation test was conducted on samples for 30 min at 5000 rpm. Any formula that shows any sign of phase separation were not preceded to rheology characterisation.

#### 3.12.4.3 Heating and Cooling

All formulations were subjected to six cooling, heating cycles between 4 °C and 45 °C by storing at each temperature for 48 h. Samples were then observed for precipitation or separation.

### 3.12.5 Rheological Characterisation

The viscosity of the optimised nanoemulsion was analysed using a dynamic rheometer (AR-G2, Thermo Electron Corporation, TA Instruments, USA). The measurement was performed with 1° angle/60 mm cone and plate geometry steel with gap set at 30 µm at the rheometer base (peltier plate) at temperature 25 °C controlled by temperature

control unit (AWC 100, Julabo, Germany). The steady rheological behavior of the nanoemulsion was measured at a controlled rate from 0.1 to 100 $s^{-1}$. Prior to measurement, the sample was allowed to stand for 10 minutes after being loaded in order to reach equilibration (Elmateeshy et al., 2018). Measurements were made in triplicate. The characterisation of rheology was performed in three stages: steady state flow, strain sweep test and frequency test.

#### 3.12.5.1 Steady State Flow Measurement (Viscosity)

The measurement of steady state flow was conducted to investigate on the viscosity of formulations. The experiments were carried out in continuous mode in a shear rate at a controlled rate range between 0.01 and 100 $s^{-1}$. Prior to measurement, the sample was allowed to stand for 10 minutes after being loaded in order to reach equilibration. Measurements were made in triplicate. The relationship between the shear stress and the shear rate of each formulation was evaluated using the Power law equation (Equation 3.9).

$$\tau = K\sigma\hat{}n \qquad [3.9]$$

where τ is shear stress, κ is the flow consistency index (Pa.s), σ is shear rate or the velocity gradient perpendicular to the plane of shear, and *n* is the flow behavior index (dimensionless).

#### 3.12.5.2 Strain Sweep Test (Oscillatory Test)

The linear region of linear viscoelasticity (LVR) by strain sweep assessment at fixed frequency, 1 Hz. The linearity limit can be analysed when elastic (G') and viscous

(G") show variation after and interval in which it remains constant. Strain rate corresponding to the LVR was chosen at 10 Pa. The linearity limit was determined according to ISO 6721 – 10:2015 (E) and EN/DIN EN 14770.

#### 3.12.5.3 Frequency Sweep Test

The nanoemulgel viscous (G") and elastic (G') were determined with frequency variation ranging from 1 to 100 Hz, under constant strain stress 10 Pa with the strain set within the LVR identified from strain sweep test. Each measurement were made in triplicate.

### 3.12.6 Spreadability Test

Spreadability test was conducted according to Dhawan et al. (2014) with modifications. A sample of 0.1 g of each formula was pressed between two slides (100 g weight was placed on top of upper slide) and left for about1 min where no more spreading was expected. Diameters of spreaded circles were measured in centimeter and were taken as comparative values for spreadability. The time (in seconds) was recorded and spreadability was calculated as stated in Equation 3.10.

$$S = \frac{(m\ x\ l)}{t} \qquad [3.10]$$

where $S$ is the spreadability (g cm·s$^{-1}$), $m$ is the mass placed on the pan (0.1 kg), $l$ is the length of the slide (cm), and $t$ is the time (in seconds). The test was performed in triplicates and data are presented as mean ± standard deviation (SD).

### 3.12.7 Transmission Electron Microscopy (TEM)

Structure of nanoemulsion and nanoemulgel samples were examined using TEM (Jeol, JEM-100 CX electron microscope, Japan). Diluted samples (1:25) were placed on carbon-coated copper grid and stained with 3% uranyl acetate aqueous solution (Elmataeeshy et al., 2018).

## 3.13 *In vitro* Release Testing Study (Permeation Study)

Permeation study was evaluated using Franz diffusion cell model (Perme Gear) (Figure 3.2). The model composed of semisynthetic cellulose acetate membrane sandwiched with a donor compartment and receptor compartment with effective diffusion area (orifice area) of 0.64 $cm^2$. A 20 µl samples was added and covered with parafilm to avoid any water losses. Five milliliter (ml) of pH 7.4 phosphate buffer saline (PBS) solution was filled with needle syringe into the receptor compartment and all experiment set up was performed at 37 °C. Sample withdrawal (0.4 ml) was taken at hourly interval and refilled immediately with equal volume to compensate the buffer solution. The collected samples were analysed by high performance liquid chromatography (HPLC) to quantify the amount of drug released and as well as to determine the permeation rate and permeability coefficient of drug permeated. The samples were performed in triplicate measurements.

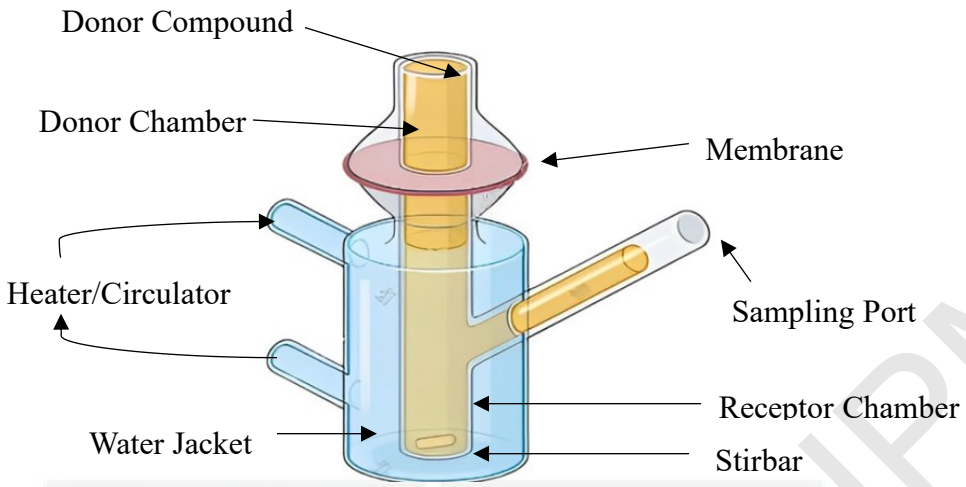



**Figure 3.2 : Franz Diffusion Cell Apparatus**

### 3.13.1 Quantification of Artocarpin by HPLC

Direct measurement of the collected samples from *in vitro* release testing procedure was analysed for the artocarpin content in using Shimadzu HPLC system, equipped with a UV/VIS detector (SPD-10AV) (i.d 4.6 x 250 mm, 5 µm column). The measurement of artocarpin applied the same procedures as stated in Section 3.8.3.

### 3.13.2 Permeation Rate

The cumulative amount of artocarpin permeated through cellulose acetate membrane was plotted as a function of time as mentioned by Salim et al. (2012). The permeation rates of artocarpin at a steady state, flux (Jss, mg/cm²/h) were calculated from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot.

### 3.13.3 Permeability Coefficient

Permeability coefficient ($K_p$) was calculated using the Equation 3.11 (Salim et al., 2012):

$$\mathrm{Kp} = \frac{j}{C_0} \quad [3.11]$$

### 3.13.4 Kinetic Modelling

The sample diffusion coefficient through the semipermeable membrane can be measured by fitting the data into release kinetic modeling methods (Table 3.6) (Sharma et al., 2023) when the concentrations in the donor and acceptor compartments are checked. Diffusion coefficients can be defined by using Fick's first (Zero order) and second (First order) laws of diffusion, which are appropriate when a membrane acts as a rate-controlling step for the release of bioactive molecules.

**Table 3.6 : Equations for Release Kinetics**

| Model | Equation |
|---|---|
| Zero order | $M = kt$ |
| First order | Ln $M = kt$ |
| Higuchi | $M = kt^{1/2}$ |
| Korsmeyer-Peppas | $M_t / M_\infty = k.\ t^n$ |
| Hixson Crowell | $M_0^{1/3} - M_t^{1/3} = k\ x\ t$ |

M - the fraction of active compound released from semisolid, $M_0$ – initial amount of drug release, $M_t$ - amount of drug release in time, t, *k*—the release constant, and *t*—time

#### 3.13.4.1 Fick's First Law (Zero Order)

Dissolution of drug from dosage forms and slowly drug release can be represented by the Equation 3.12.

$$M_0 - M_1 = K_0\, t \quad [3.12]$$

Rearrangement of Equation 3.12 yields

$$M_1 - M_0 = K_0\, t \qquad [3.13]$$

where $M_1$ is the amount of drug dissolved in time *t*, $M_0$ is the initial amount of drug in the solution, and $K_0$ is the zero order release constant expressed in units of concentration/time

### 3.13.4.2 Fick's Second Law (First Order)

Fick's second law (first order) been used to describe absorption and/or elimination of some drugs. The relase of drug which followed first order kinetics can be expressed by the Equation 3.14.

$$\frac{dC}{dt} = -Kt \qquad [3.14]$$

where *K* is the first order rate constant, *t* is the time. Equation 3.14 can be expressed as:

$$Ln\, M = Kt \qquad [3.15]$$

where *M* is the concentration of drug, K is the first order rate constant, *t* is the time. The data obtained are plotted as log cumulative percentage of drug remaining versus time that yields a straight line.

### 3.13.4.3 Higuchi Model

The amount of the released active compound changes with the appropriate rate during the analysis. The release of water-soluble and poorly water-soluble drugs from

semisolids was studied by Higuchi (1962). Today, the Higuchi equation (Bruschi, 2015) is considered to be one of the widely used and the most well-known controlled release equations. The release of a drug depends upon its diffusion. The general equation is given in the Equation 3.15:

$$M = kt^{1/2} \quad [3.16]$$

where *M*—amount of drug released in time *t*, *k* – release constant, and *t*—time required.

#### 3.13.4.4 Korsmeyer-Peppas Model

Korsmeyer-Peppas was also known as 'Power law' model describing the drug release from a polymeric system It was proposed by Korsmeyer and Peppas (1983) studied on the drug release mechanism describe simultaneously such as water diffusion into the matrix, swelling of the matrix and as well as dissolution of the matrix. The drug release data was plotted to fit the model. The log cumulative percentage of fraction of the released drug was plotted against log time to define the release pattern. Value of diffusion exponent (n) is expressed in the following Equation 3.16:

$$\frac{M_t}{M_\infty} = k.t^n \quad [3.17]$$

where $M_t/M_\infty$—amount of drug released in time *t*, *k*—release constant, and *n*—diffusion exponent.

#### 3.13.4.5 Hixson Crowell Model

The Hixson and Crowell model were developed in 1931 explain the drug release from the system where there is change in diameter of the particles and surface area of the particles. This model states that the particle of the drug and dissolution rate are assumed as the rate of drug release is limited and not by the diffusion. Hence, the equation is stated in the Equation 3.17:

$$M_0^{1/3} - M_t^{1/3} = K\,x\,t \qquad [3.18]$$

where $M_t$—amount of drug released in time $t$,$M_0$—initial amount of the drug released, and $t$—time required.

### 3.14 Statistical Analysis

All data were analysed using Minitab 16 software (Minitab Inc., State College, PA, USA). Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between samples. Values were expressed as means ± standard deviations. Differences were considered significant at $p < 0.05$. All analyses were carried out in triplicates.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Effect of Drying Methods on Quality of Jackfruit Leaves Extract

In this study, the aim of the drying of jackfruit leaves was to find out the drying behaviour, drying kinetic and the impact on colour attributes and antioxidant properties of three drying conditions, sun, shade and oven drying. Dried jackfruit leaves (JL) were proceeded into different extraction using ethanol-water (E/W) ratio. The effect of E/W solvent ratios extraction were further investigated on the influence on antioxidant properties and phytochemical compounds by using high performance liquid chromatography (HPLC) and gas chromatograpy (GC).

### 4.1.1 Impact of Drying Treatments on Drying Curve of Jackfruit Leaves

Fresh jackfruit leaves (Figure 2.3) were processed upon receiving in the laboratory. The cleaned jackfruit leaves were then divided into three groups for further drying treatments.

Heat treated jackfruit leaves resulted in losses in moisture content under shade, sun and oven are depicted in Figure 4.1. The initial moisture content of jackfruit leaves was found to be 60.44 – 62.69 %. The drying time for sun treatment was 5.4 times and 1.6 faster than shade and oven conditions, respectively. The reduced drying time distributed by the external parameters that may affect the drying process include wind speed, humidity, temperature, air stream velocity, heat supply and the contact between hot surfaces and the wet solid, and solar radiation (Babu et al., 2018; Prasad, 2009; and Jain and Tiwari, 2003). In addition, open space under sun drying allows higher

diffusion coefficient of product per unit exposed area while oven treatment has slower drying rate due to lower diffusion coefficient of the oven resulting in an increase of relative humidity in the oven chamber. (Arslan and Özcan, 2010; Kumar and Tiwari, 2007). The highest temperature that was recorded during sun drying the sample was approximately 38°C during mid-day with the average relative humidity reach 44.3 ± 0.5 %. The lower relative humidy in sun-drying as compared to shade drying (61.5 ± 0.7 %) and oven drying (47.5 ± 0.3 %) resulted in higher drying rate and faster drying time as can be observed in Figure 4.1. Although the temperature recorded in sun drying treatment, slightly lower than oven drying at 40 °C, the evaporation of moisture is faster since it is in larger space or exposed area and receiving bright sunshine at the time of the experiment was conducted. Sun drying also assisted by the movement of air surrounding that facilitates the migration of moisture from the mass inside to the surface (Belessiotis and Delyannis, 2011). The heat penetrates inside the sample, causing the increment in temperature and the water vapor starts to form. Diffusion of moisture and water vapor takes place from the interior replacing the loss of moisture by evaporation on the surface. Similar results have been obtained by other researchers in relation to other fruits and vegetables namely (Alara et al. 2018 and Tellez et al. 2018). Although sun drying method showed the shortest time compared to shade and oven method but due to fluctuating sunlight, the drawbacks of this treatment are the weather changes and unhygienic condition such as dust particle, microbial attack and prone to damage and loss to nutritional attributes at the peak of time during daytime (Roshanak et al. 2016). Whilst shade drying is able to prevent damage and loss of compound of interest, the drawback of this shade treatment, this treatment requires the longest time in order to achieve its constant weight. Furthermore, natural drying

treatments (shade and sun drying) also unable to achieve consistent quality standards (Babu et al. 2018; Roshanak et al. 2016).

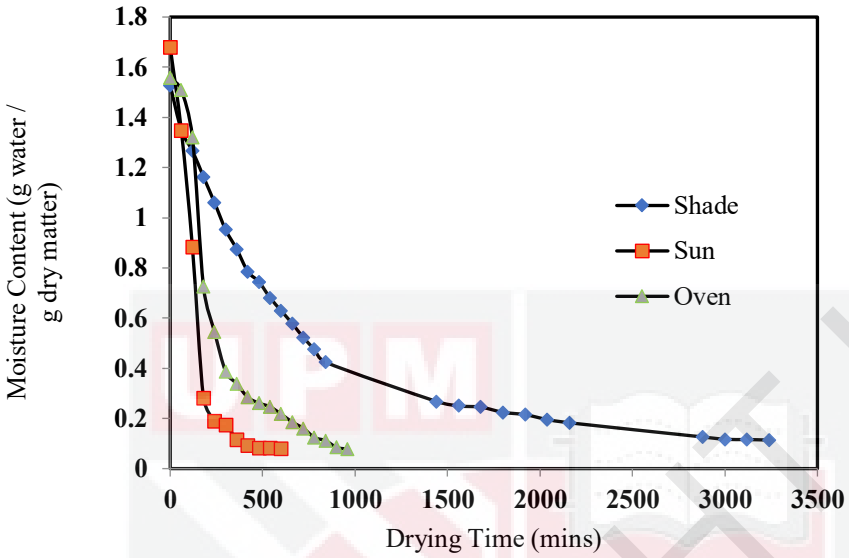



**Figure 4.1 : Drying Curve for Jackfruit Leaves. -♦- Shade, -■- Sun and -▲- Oven. The Data Given Are the Mean Values of Three Replications**

### 4.1.2 Drying Kinetic of Sun, Shade and Oven Dried Jackfruit Leaves

Seven different drying models of moisture ratio (MR) were used to predict the moisture content as a function of drying time. The statistical analyses of curve fitting coefficient determination ($R^2$), reduced root mean square error (RMSE) and reduced chi-square, used for jackfruit leaves dried under shade, sun and oven conditions are shown in Table 4.1. According to the result of present study, the coefficient determination values ($R^2$) ranged from 0.8718-0.9999 within all used models that represent the suitability of the used methods to the tested models. In the modelling of drying kinetics, Newton model is the best suited for all drying methods with highest $R^2$, and lower RMSE and $\chi^2$. It can be seen that Logaritmic, Verma, Henderson and Pabis, Midili and Page models presented very good fits. Two Term kinetic model

showed the lowest $R^2$ for sun drying method. Drying constant, k quantifies the rate at which moisture is removed from the material. This study suggested Newton model for drying kinetics which gave the best simulation of the curves of dried jackfruit leaves based on the demonstrated variances for sun, shade and oven drying, therefore, exhibiting high concordance between experimental and predicted moisture ratio.

The suitability of the Newton model for studying behaviour can be due to its simplicity and effectiveness in describing the moisture removal process during falling rate period, which typical in drying leaves in representing the drying conditions studied. This model assumes a constant drying rate, making it straightforward to apply and interpret in experimental settings and it is relevant for materials such as leaves, which often exhibit uniform drying (Kadam et al., 2011). Its predictive accuracy for moisture loss has been validated in previous study, establishing it as an effective tool for initial kinetic evaluation of process engineering (Ambawat et al., 2022). This result is in agreement with previous work by Behera et al. (2021) which shows Newton model best fitted for the performance of drying method studied. This study revealed that many factors can contribute to the time required for dehydration depends on humidity, exposed area, temperature, air stream velocity and the contact between hot surfaces and the wet solid. This could be corroborated with the fact that the elevation of the heat transfer which enhances the heat and mass transfer rate by the temperature difference between jackfruit leaves with the surrounding medium (air) (Agbede et al., 2020). The humidity of the surrounding environment directly impacts the drying process. When the ambient air has low humidity, it enhances drying rate by creating larger moisture gradient between sample and air. This increased gradient promotes faster evaporation of water from the sample. On the other hand, high humidity levels

reduce the air's ability to absorb moisture, thereby slowing the drying process. The surface area of the sample exposed to the drying medium is crucial in determining the rate of moisture removal. A larger exposed area (sun drying) allows more water to evaporate simultaneously, making it drying quicker. Other factor that contribute to faster drying is temperature. Theoretically, higher temperatures accelerates the evaporation of moisture. The increase in heat elevate the air capacity to hold water vapour, promoting a more efficient drying process. However, excessive heat can compromise the quality of heat sensitive bioactive compounds. Therefore optimising drying condition is essential to maintain product integrity (Song et al., 2023).

Other factor that may contribute to a faster dehydration in the air stream velocity. The speed of air flowing over sample affects how quickly moisture is removed. Faster air movement helps clear saturated air layer that form near the sample's surface. It is worth to note that higher air velocities can increase drying efficiency, but they must be controlled to prevent surface hardening or uneven drying. The contact between sample and hot surface improves the transfer of heat to the sample which speeds up the drying process. This is commonly known as conductive drying, effective for materials that can tolerate direct heat exposure without compromising their quality. However, poor distribution of heat may eventually caused overheating and will damaged the materials and their quality attributes (Putra and Ajiwiguna., 2017, and Liu et al., 2017).

The predicted and experimental moisture ratio in comparison to Newton model versus drying time for jackfruit leaves is shown in Figure 4.2. The longest time was observed

in JL dried in shade, followed by oven and sun. The result shows that drying air temperature is an effective parameter for the drying of jackfruit leaves.

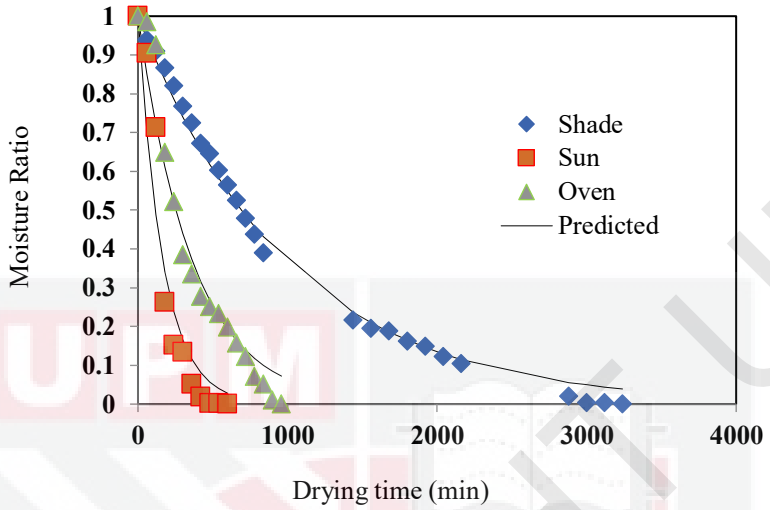



**Figure 4.2 : Variation of Moisture Ratio of Experiment and Predicted of Various Drying Conditions for Jackfruit Leaves. -♦- Shade, -■- Sun, -▲- Oven, - Newton Model. The Data Given Are the Mean Values of Three Replications**

**Table 4.1 : Curve Fitting for Shade, Sun and Oven Drying**

| Model name | Drying condition | Constants | $R^2$ | RMSE | $\chi^2$ |
|---|---|---|---|---|---|
| | Shade | k = 0.0010 | 0.9948 | 0.1676 | 0.3480 |
| Newton* | Sun | k = 0.0059 | 0.9999 | 0.1002 | 0.2450 |
| | Oven | k = 0.0027 | 0.9999 | 0.0974 | 0.2208 |
| | Shade | a = 0.9909 k = 0.0011 c = 0.1206 | 0.9460 | 0.1515 | 0.3147 |
| Logarithmic | Sun | a = 0.0009 k = 1.0000 c = 0.3015 | 0.9963 | 0.0644 | 0.1459 |
| | Oven | a = 0.8840 k = 1.0000 c = 0.3014 | 0.9998 | 0.0974 | 0.2208 |
| | Shade | a = 1.0700 k = 0.0012 g = 1.3309 | 0.9906 | 0.1662 | 0.3451 |
| Verma | Sun | a = 1.6497 k = 0.0089 g = 0.7500 | 0.9772 | 0.0619 | 0.1404 |
| | Oven | a = 1.2200 k = 0.0033 g = 0.5000 | 0.9847 | 0.0945 | 0.2141 |
| | Shade | a = 0.5510 $k_0$ = 0.0010 b = 0.4700 $k_1$ = 0.0012 | 0.9934 | 0.1671 | 0.1431 |
| Two term | Sun | a = 0.5000 $k_0$ = 0.0085 b = 0.5799 $k_1$ = 0.0033 | 0.8718 | 0.0493 | 0.1117 |
| | Oven | a = 0.5500 $k_0$= 0.0030 b = 0.5760 $k_1$ = 0.0033 | 0.9692 | 0.0915 | 0.2074 |
| | Shade | a = 1.0490 k = 0.0010 | 0.9935 | 0.1600 | 0.3471 |
| Henderson and Pabis | Sun | a = 1.0987 k = 0.0059 | 0.9292 | 0.0560 | 0.1269 |
| | Oven | a = 1.2000 k = 0.0034 | 0.9620 | 0.0901 | 0.2043 |
| | Shade | a = 0.9359 k = 0.0018 n = 0.9040 b = 0 | 0.9681 | 0.1587 | 0.0684 |
| Midilli | Sun | a = 0.9965 k = 0.0060 n = 0.9990 b = 0 | 0.9168 | 0.0545 | 0.1236 |
| | Oven | a = 1.0997 k = 0.0031 n = 0.9990 b = 0 | 0.9687 | 0.0914 | 0.2073 |
| | Shade | k = 0.0010 n = 1.0010 | 0.9945 | 0.1677 | 0.3481 |
| Page | Sun | k = 0.0035 n = 1.0999 | 0.9357 | 0.0567 | 0.1287 |
| | Oven | k = 0.0025 n = 1.0249 | 0.9563 | 0.0890 | 0.2019 |

*best fitted model
$R^2$ : Coefficient determination
RMSE: Root mean square error
$\chi^2$: Reduced chi square
k : Drying rate constant
a, b and c: dimensionless constant
**g** : Drying rate constant for the second phase of drying,

### 4.1.3 Effective Diffusivity

The effective diffusivity ($D_{eff}$) values ($m^2/s$) of dried jackfruit leaves for shade, sun and oven drying treatments were 2.9203 x $10^{-10}$, 6.8141 x $10^{-10,}$ and 6.5707 x $10^{-10}$, respectively. Sun drying possessed the highest $D_{eff}$ values which were approximately 2.3-fold than that of shade drying. In the case of shade and sun drying, it is expected that $D_{eff}$ values increased with the increase in temperature. $D_{eff}$ value for the sun drying was 1.04-fold greater than that for oven, which reveals that the process of open sun drying had better mass transfer efficiency than oven drying treatment studied. This can be associated with the free air circulation during open drying process. In general, $D_{eff}$ values fall within the range of $10^{-11}$ – $10^{-9}$ for dried food materials (Arslan and Ozcan, 2010).

## 4.2 Colour Determination of Dried Jackfruit Leaves

Drying treatments possessed significant a significant effect on the colour changes of jackfruit leaves. Therefore, colour assessment is another parameter observed during drying the leaves. Table 4.2 shows the colour measurement of dried jackfruit leaves. From the table, the results revealed that the L* value of shade drying treatment was the highest among all dried leaves (15.8), followed by oven drying (13.4), while sun drying revealed the lowest L* value (11.1) among dried treatments ($p< 0.05$). Exposure to direct sunlight is the most effective factors in colour damage during drying (Rahimmalek and Goli, 2013). Similar result obtained for b* values. Sun drying treatment showed the highest b* value (39.5) in comparison to others. For a* value (greenness), shade drying treatment had the highest value (more negative), while sun drying showed the most discolouration of green pigment. Sun drying caused a

significant increment of a* values than the other treatments, which suggests that the degradation of green colour in the final product occurred in a greater ratio. This could be due to the loss of chlorophyll content that contributes to the green colour of plant leaf and enzymatic browning (Sarpong et al., 2018). Browning process involved the action of enzymatic and nonezymatic resulted from Maillard reaction and caramelisation. Therefore, sun drying is known to be the least desirable for the final dried product. Overall, sun and shade treatments were considered the least and the most desirable drying methods, respectively regarding the final colour of jackfruit leaves.

**Table 4.2 : Colour Measurement for Shade, Sun and Oven Drying**

| Colour Measurement | Oven drying | Sun drying | Shade drying |
|---|---|---|---|
| L* (lightness) | 13.4 ± 0.1 | 11.1 ± 0.3 | 15.8 ± 0.1 |
| a* (greenness/ redness) | -7.24 ± 0.4 | -2.42 ± 0.5 | -10.53 ± 0.7 |
| b* (yellowness/blueness) | 22.2 ± 0.3 | 39.5 ± 0.3 | 12.62 ± 1.0 |

Drying cause changes to the properties of final product including discolouring, textural changes and physical appearance. Drying leaves at higher temperature may reduce time but it can resulted in poor product quality. On the other hand, at milder temperature it canimprove quality product but decrease in drying rate thus it may lengthen the drying time. Colour measurement is one of the first quality that customer perceived when they purchase a product apart from other physicochemical such as texture (Ali et al., 2014). Colour is one of the quality attributes that give an impact on the fnal product of nanoemulgel. Often, for nanoemulsion and nanoemulgel, their appearance can be related to colour, homogeneity, opacity and others such as size and concentration of oil droplet (Ahmadi et al., 2021).

## 4.3 Extraction of Dried Jackfruit Leaves

Dried JL was ground into fine powder with particle distribution passing through siever with mesh size of < 100 μm as shown in Figure 4.3. After maceration of the samples, they were dried using rotary evaporator until a thick and sticky mass extract of JL obtained. Based on the Table 4.3, it can be concluded that extraction using 80% of ethanol yielded higher percentage as compared to 100% and 50% ethanol water composition, while water extraction of all drying methods showed the least percentage yield. The acquired yield of extract increased with the increasing of ethanol concentration in the extraction medium up to 80% and then began to decline with the further increase of ethanol ratio (100% ethanol). Water is a strong polar solvent, while ethanol a low polar solvent. When water content exceeds 20%, the acquired, the acquired yield extract was reduced.


**Figure 4.3 : Photograph of Fine Powder of JL through: (a) Oven Drying (OV), (b) Shade Drying (SD) and (c) Sun Drying (SN)**

**Table 4.3 : Percentage Extraction Yield of Samples**

| Drying method | Acronym | % Extraction yield |
|---|---|---|
| Shade (SD) | SD100 | 16.8 |
| | SD80 | 32.8 |
| | SD50 | 20.0 |
| | SD-W | 12.8 |
| Sun (SN) | SN100 | 12.4 |
| | SN80 | 18.6 |
| | SN50 | 14.6 |
| | SN-W | 5.8 |
| Oven at 40 ℃ (OV) | OV100 | 13.8 |
| | OV80 | 22.8 |
| | OV50 | 16.6 |
| | OV-W | 5.6 |

The percentage yield extraction depends on several factors including solubility of the desired compound in the solvent, the selectivity of the solvent and the efficiency of the extraction method. The solvent mixture selectivity at certain percentage of ethanol/water composition can be advantageous for extracting specific compounds. Ethanol and water may selectively dissolve different types of compound due to differences in polarity and chemical interactions, leading to a more efficient extraction of a target compound (Hikmawanti et al., 2021). Some compounds may have higher solubility in a mixed solvent system compared to a single solvent (ethanol or water only). Ethanol and water can have complementary solubilising properties, allowing for the extraction of a broader range of compounds (Lim et al., 2019).

The antioxidant of plant extracts are influenced by the solvent used during extraction. While mixture of ethanol and water is commonly employed to optimise the extraction of both hydrophilic and hydrophobic compounds, some studies have explored the efficacy of pure solvents. Research on *Limnophila aromatica* extracts demonstrated that using 100 % ethanol yielded the highest total antioxidant activity, reducing power and DPPH radical scavenging activity. This extract also exhibited high TPC and TFC,

suggesting that pure ethanol can be highly effective in extracting antioxidant compounds in certain plant materials. In some cases, water have been found to be more effective (Do et al., 2014). For instance, a study on starch-derived product indicated that using water as the extraction solvent resulted in superior reducing capability, highlighting that pure water can sometimes be more efficient in extracting samples (Chen et al., 2019).

The effectiveness of absolute solvents in contrast to E/W mixtures in extracting antioxidants depends on several factors. Firstly, solubility of compounds. Antioxidant compounds vary in their polarity. Pure ethanol is more effective for non-polar compounds, while absolute water is better for polar compounds. While for mixtures can target a broader range of antioxidant. The specific composition of plant materials is one of the factors that influence which solvent is more effective. For example *Limnophila aromatic* may contain antioxidant that are more soluble in ethanol, while other plants may have compounds better extracted with water or specific E/W mixtures. Extraction efficiency also contributed to factors to extracting antioxidants. Absolute solvents may extract higher quantities of certain compounds, but mixtures can sometimes achieve a more comprehensive extraction profile leading to a higher antioxidant activity. There is no universal solvent or solvent mixture that is optimal for extracting antioxidant from all plant materials. The choice between absolute solvents and E/W mixtures should be tailored to specific plant matrix and the target antioxidant compounds. Empirical testing is often necessary to determine the most effective extraction conditions for each unique condition (Claux et al., 2021).

## 4.4 Antioxidant Properties

Determination of antioxidant was conducted on total phenolic content (TPC), total flavonoid content (TFC) and their antioxidant activities namely scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP).

### 4.4.1 Total Phenolic Content and Total Flavonoid Content

The impact of drying treatment on TPC and TFC are shown in Figure 4.4 and Figure 4.5, respectively. It was found that there was significant increase in total phenolic content and total flavonoid content observed over the extraction drying treatments (SD, SN and OV) and fresh leaves (FL), and the total phenolic content and total flavonoid content reached maximum values around 195.05 mg GAE/g and 11.02 mg AE/g of jackfruit leave extracts, respectively both dried in the oven. At different drying treatments, there were significant percentage increment for the OV80 was 1.33 and 1.28 fold higher than that for shade and sun. The TFC was observed the highest value of 11.02 mg AE/g of jackfruit leave extracts (JLE) dried in the oven, 1.48 fold and 1.20 fold significantly greater than shade and sun, respectively. The increase in TPC and TFC as affected by drying treatments could be due to the liberation of the cell constituent from the plant cell may accelerate the movement of bound phenolic and flavonoid compounds due to the thermal treatment as the heat exerts adjustments to the plant tissue microstructure, thus rupture the structure of cell the plant tissue microstructure, thus rupture the structure of cell integrity, thereby allow the migration of phenolic compounds from the plant cells (Chua et al., 2019). Longer time required for shade drying and the exposure to UV under open sun may affects the sensitive

phytochemical compounds leading to breakdown by various factors of chemical reaction comprises of light, oxygen and enzymes (Martinez-Las Heras et al. 2014).

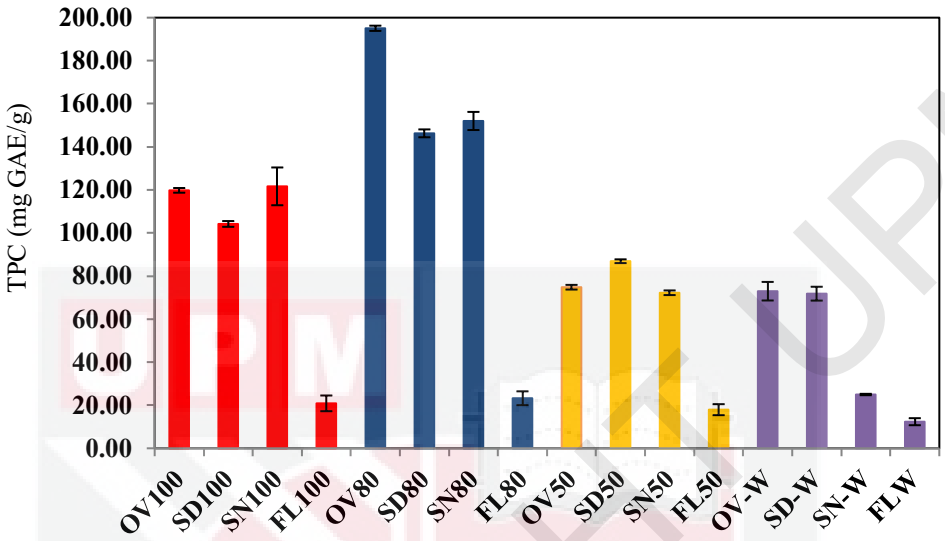



**Figure 4.4 : Effect of Different Drying Treatments and E/W (0 % - 100%) Ratios on Total Phenolic Content (TPC) of Jackfruit (A. heterophyllus) Leaves Extract**
Note: Shade (SD), Sun (SN), Oven (OV), and Fresh Leaves (FL)

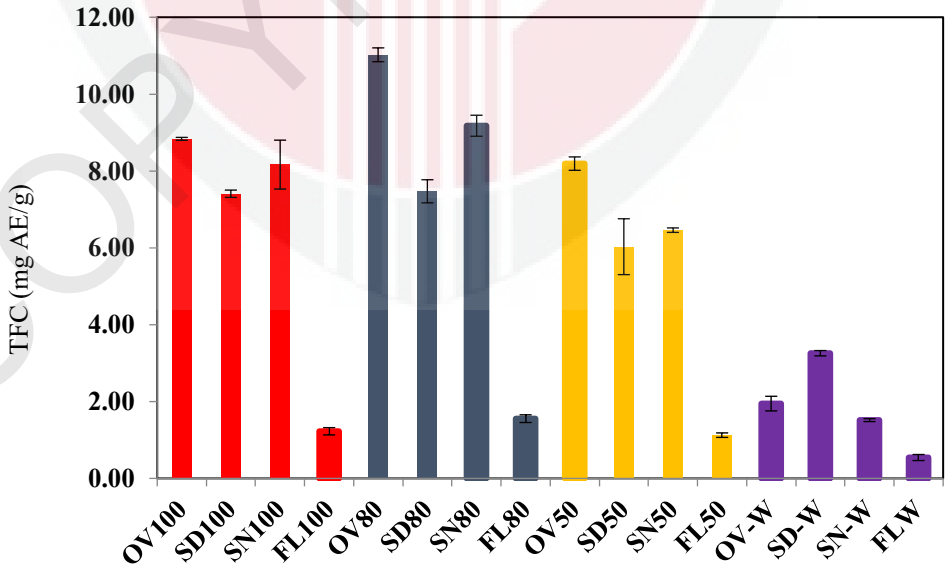



**Figure 4.5 : Effect of Different Drying Treatments and E/W (0 % - 100%) Ratios on Total Flavonoid Content (TFC) of Jackfruit (A. *heterophyllus*) Leaves Extract**
Note: Shade (SD), Sun (SN), Oven (OV), and Fresh Leaves (FL)

Drying reduces the moisture content of plant material, which can increase the concentration of bioactive compounds relative to fresh leaves. Dried or dehydrated plant matrix can be easily penetrated by solvents, breaking down cell walls and releasing bioactives phenolics and flavonoids (Azwanida, 2015). Drying can also disrupt cellular structures, exposing bound phenolics and flavonoids that might be less accessible in fresh leaves (Figueiredo et al., 2008). Ethanol/water (E/W) mixtures are effective in extracting compounds in dried leaves as they can dissolve both polar and non-polar bioactives compounds from the more porous dried materials.

While drying can enhance the release of certain compounds, the process can degrade thermolabile bioactive compounds if temperature are too high. However, mild drying methods, such as shade and oven drying at controlled temperatures, typically preserve antioxidant activity, resulting higher yields than fresh leaves (Kadam et al., 2013). Similarly, drying jackfruit leaves (JL) can preserve bioactive compounds by stabilising them in the absence of enzymatic activity, which is more prevalent in fresh leaves. Drying is one of the process that promote browning reaction. It is a process that involved ezmatic and non-enzymatic reaction. Enzymatic browning can resulted in the activity of enzyme polyphenol oxidase, peroxidase, and ascorbic acid oxidase in the jackfruit leaves. Many researchers have look into methods for inactivation of these enzyme namely sulfites that is used as reducing agent. On the other hands, non-enzymatic browning has been recognised to be most detrimental chemical reactions that leads to poor quality product. Heat accumulation in sample may resulted in the accumulating of reducing sugars and free amino acids. Therefore, a controlled drying condition maybe essential when conducting a research on drying as a pre-treatment step. Worth to mention that condition of drying in a controlled conditions such as using

oven may have advantageous effect such as preserving bioactive compounds (Sarpong et al., 2018)

Dried JL generally yield better extraction outcomes using E/W mixtures due to the structural and compositional changes induced by drying, which enhance the solvent accessability to release desired compounds. However, care must be taken to use suitable drying methods that minimise thermal degradation. The agreement in previous research supports the use of dried leaves for extracting phenolics and flavonoids, provided the drying process is optimised for bioactive compounds stability.

At different ratios of ethanol ranging from 0% to 80% for the same drying treatment the TPC and TFC showed that there was an increase in their respective values. There were significant increments at 80% ethanol/water (E/W) composition which 2.67 fold and 5.68 fold higher than water extraction for TPC and TFC assays. However, when ethanol concentration reached 100% (v/v), the TPC and TFC values in JLE decreased. The decline in values for the jackfruit leaves extract could be due to solvent polarity and lipid extraction for extracting antioxidants. Ethanol, a solvent with intermediate polarity is capable of extracting both polar and certain non-polar compounds from plant materials including jackfruit leaves. This characteristic enable ethanol to solubilise a diverse range of phytochemical, such as phenolic compounds, flavonoids and some lipid constituents. Previous study by Mustafa et al. (2020) analysed the nutritional and phytochemical composition of JL have documented the presence of lipids as part of their proximate profile. While lipids are not primary focus when

evaluating antioxidant of plant leaves, their presence within the matrix may hinder the extraction of hydrophilic compounds, particularly phenolics and flavonoids.

The polarity of a solvent significantly influences its extraction efficiency for various compounds. Non-polar solvents, like hexane, are traditionally employed to extract non-polar lipids, including triacylglycerols. In contrast, polar solvents such as ethanol are more effective in extracting polar lipids, including phospholipid and glycolipids. Ethanol's intermediate polarity allows it to interact with both hydrophilic and lipophilic substances, facilitating the extraction of broader spectrum of compounds. In jackfruit leaves, utilising ethanol as an extraction solvent can lead to co-extraction of lipid components alongside target hydrophilic antioxidant.

Extraction with water alone may not allow the optimum extraction of phenolic and flavonoid compounds. Water extraction exhibited the lowest amount of phenolic and flavonoid content in this result. However, the addition of certain percentage of ethanol into water could improve extraction efficiency as the solubility of carbohydrates and proteins in ethanol is higher than water (Alara et al. 2018). Ethanol/water (E/W) mixtures offer a tunable polarity, making them effective for extracting bioactive compounds with intermediate polarity, such as phenolics and flavonoids (Do et al., 2014). At 80 % ethanol concentration, it provide a balance between water's high polarity and ethanol's lower polarity, optimising the solubility of these compounds. In contrast, either absolute water or ethanol is less effective for certain phenolic compounds due to optimal solubility. The solvent at 80 % E/W effectively break down cell walls, allowing better release of phenolics and flavonoids from plant matrix. Both polar and moderately non-polar compounds are efficiently dissolved.

Phenolic compounds, which often contain hydroxyl groups, exhibit varies solubility based on their molecular weight and structural characterisation. In a highly aqueous medium (100 % water), phenolic compounds with lower water solubility may not be effectively extracted. Conversely, pure ethanol (100 % ethanol) may not sufficiently disrupt the hydrogen bonding in the plant matrix, limiting extraction efficiency (Lim et al., 2019).

While JL do contain lipid components, their low abundance, likely minimises the effect on the extraction of hydrophilic compounds. Nonetheless, to overcome the challenges, researcher often employ defatting step prior to antioxidant extraction. The process of defatting remove certain lipid components from the plant materials, thereby improving the accessability and solubility of hydrophilic antioxidants. Therefore, defatting procedure could be considered as precautionary measures to improve yield of antioxidant, aligning with findings from studies on other plant-based materials (Sahu et al., 2021). In conclusion, to enhance the extraction efficiency of specific compounds from JL, it is crucial to select an appropriate solvent or solvent mixtures that aligns with the polarity of the target compounds. In this research, employing E/W mixture at varying ratios can be a strategic approach to modulate solvent polarity, thereby optimising the extraction process for desired phytochemicals while minimising the co-extraction of undesired compounds.

### 4.4.2 Antioxidant Activities

The impact of drying treatment on ferric reducing absorption power (FRAP) and free radical scavenging activities (DPPH) are shown in Figure 4.6 and Figure 4.7. The results showed that the FRAP and DPPH of jackfruit leaves extract (JLE) were attained

highest in oven treatment with values of 1043.80 μM TE/g EW and 90.08%, respectively. Meanwhile, samples dried by shade drying and sun drying show the lowest FRAP and DPPH and which were 197.50 μM TE/g EW and 26.30%, respectively.

Temperature and drying time were considered as two crucial parameters for interpreting sample extract yield variation using different techniques (Hamrouni-Sellami et al. 2013). Higher reducing capacity of an extract serves as an indicator of the ability of its higher potential antioxidant activity. In this study, the oven drying treatment may still be able to preserve the heat sensitive bioactive compound present in JL extract. In previous study, it has reported that antioxidant activities and polyphenol contents in heat-treated various herb leaves increased, indicating that phenolic compounds at a partial state of oxidation are exposed to oxygen and may exert a high antioxidant activity (Chua et al. 2019).

However, under shade treatment, the lengthy process of exposure to oxygen may imply a greater exposure of the material to enzymatic and oxidation reactions thus accelerating the degradation of active compounds. Meanwhile, the antioxidant potential in sun treatment was found to be lower than oven treatment could be due to direct exposure to sunlight that might degrade the compounds. Evidently, the shorter time in sun treatment did not compensate the deterioration of the antioxidant compounds that could be beneficial to human health (Barimah et al. 2017; Martinez-Las Heras et al. 2014).

At different solvent ratios, it was found that extraction using 80% ethanol/water (SD80, SN80 and OV80) exhibit a significant higher ($p< 0.05$) FRAP (1043.84 μM TE/g EW) and DPPH (90% scavenging effect) values compared to other ratios. OV80 were found to possess 2.85-fold (FRAP value) and 1.43 fold (free radical scavenging effect) higher than water extracted jackfruit leaves extract (365.72 μM TE/g EW and 64.7% scavenging effect, respectively). This could be due to the ability of the majority of the phenols to dissolve in a wide range of mixtures of ethanol aqueous compared to water extraction alone. Solvent polarity plays an important role in the extractability and solubility of bioactive compounds from plant materials. Several studies have been reported on extracting bioactive compounds using different single solvent, solvent mixtures and solvent ratios (Bento et al., 2018; Dube et al. 2017; and Hafid et al., 2016). Alothman et al. (2009) reported on the highest antioxidant capacities of FRAP at 70% ethanol-water mixtures by increase the effectiveness of the swelling power of plants with the presence of water and provide high surface area of solute-solvent contact. The addition of water will lower the mixture viscosity and thus will ameliorate the mass transfer of extract (Hemwimol, Pavasant, and Shotipruk, 2006).

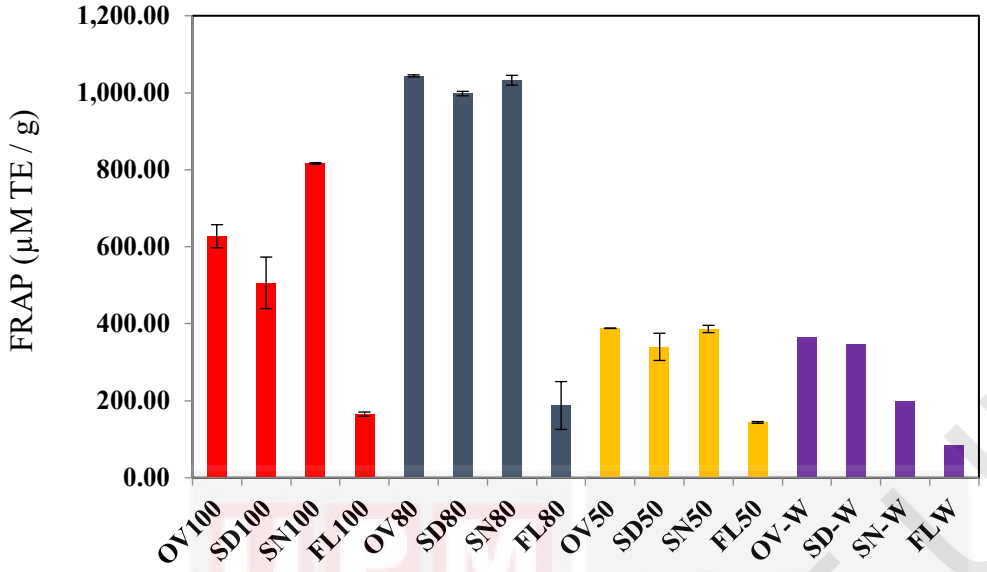



**Figure 4.6 : Effect of Different Drying Treatments and E/W (0 % - 100%) Ratios on FRAP of Jackfruit (A. *heterophyllus*) Leaves Extract**
Note: Shade (SD), Sun (SN), Oven (OV) and Fresh Leaves (FL)

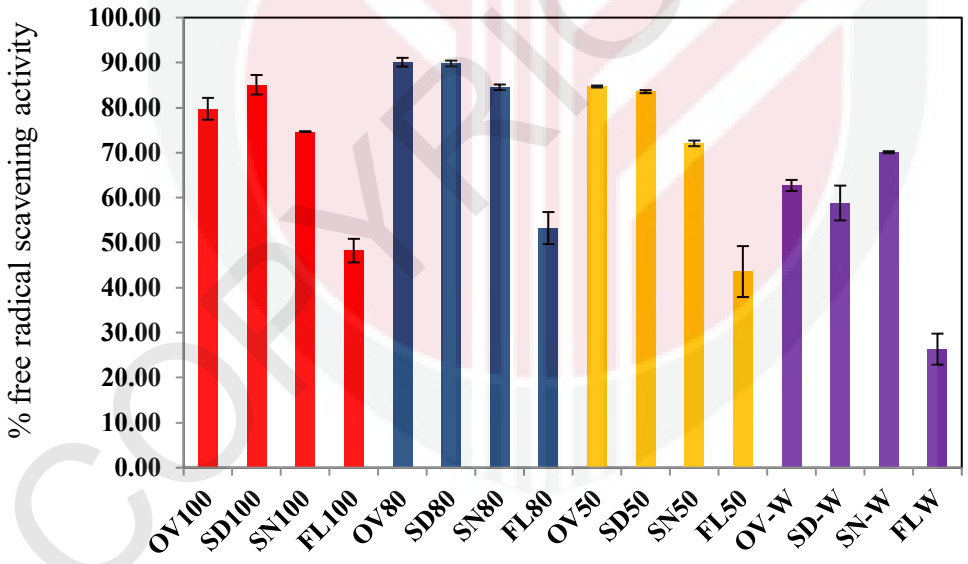



**Figure 4.7 : Effect of Different Drying Treatments and E/W (0 %-100%) Ratios on DPPH of Jackfruit (A. *heterophyllus*) Leaves Extract**
Note: Shade (SD), Sun (SN), Oven (OV) and Fresh Leaves (FL)

### 4.4.3 Correlation between Antioxidant Contents and Their Activities

It was demonstrated that SD80, SN80 and OV80 exhibited the highest ferric reducing antioxidant power (FRAP) and free radical scavenging activity (DPPH) values compared to other ethanol aqueous compositions. FRAP and DPPH assays showed the same orientations having the highest values of antioxidant activities in comparison to other ethanol/water compositions. In all treatment studied, the total phenolic content (TPC) and its related antioxidant activities exhibit high coefficient correlation ($R^2$) values at 0.9220 and 0.6680, significant level at $P < 0.05$ as presented in Table 4.4. In accordance with TPC investigated, i.e., the highest content (80% E/W), demonstrated the highest in antioxidant activities in FRAP assay than other compositions. However, as this correlation is inverted in the latter assay (DPPH), it is suggested that other constituents (e.g. non-phenolics) could be attributed to the antioxidant ability by donating hydrogen atom of *A. heterophyllus* leaves extract (Catarino et al. 2018).

**Table 4.4 : Correlation Values of TPC and TFC and Their Respective Antioxidant Activities (FRAP and DPPH)**

| Antioxidant assay(s) | Antioxidant activities | |
|---|---|---|
| | FRAP | DPPH |
| TPC | 0.9220 | 0.6680 |
| TFC | 0.7180 | 0.743 |

Meanwhile, the correlation between total flavonoid contents (TFC) and its associated antioxidant activities (FRAP and DPPH) yielded $R^2$ values of 0.7180 and 0.7430, respectively. These correlations exert their different mechanisms and ability to reduce certain free radicals to form a stable compound and also metal ions chelation (Maisarah et al., 2013). The mechanism of FRAP and DPPH employed the antioxidant ability to diminish certain radicals (ferric iron and DPPH radical). Another significant

correlation between TPC and antioxidant capacity of plant extracts (FRAP and DPPH values) was attained. These correlations can be assumed to show that the phenolic compounds are predominant in contributing to the antioxidant activities of these ethanolic compositions.

## 4.5 Artocarpin and Volatile Compounds of Jackfruit Leaves Extract

The artocarpin, squalene and β-sitosterol content of shade, sun and oven dried at 80% ethanol/water ratio (highest values of antioxidant properties) are given in Table 4.5. All crude samples assayed contain 4.71 ± 0.59 mg/g extract (SD80), 2.53 ± 0.29 mg/g extract (SN80), 8.35 ± 1.78 mg/g extract (OV80), where OV80 showed significantly higher ($p < 0.05$) artocarpin content as compared to other drying treatments. Meanwhile, the same results for volatile organic compounds were observed for squalene and β-sitosterol. Squalene content of OV80 (1.47 mg/g) was found higher than other drying treatments. However, the β-sitosterol content of SD80 and OV80 was found not statistically significant. Sun drying showed the lowest values of both volatile compounds. In this study, oven drying at low temperature with 80% ethanol/water ratio has led the bound phenolic and flavonoid contents to be released from the plant matrix and recovered from the thick mass extract during the extraction. Previous study also reported that oven drying at low temperature was suitable in preserving the antioxidant values among other dried leave methods such as shade and sun drying). Jackfruit leaves have been studied for their presence of secondary metabolites such as flavonoids and phenols for pharmaceutical and cosmeceutical applications. The application of jackfruit leaves have been extensively studied for the medicinal purposes. Sousa et al. (2021) corroborated that in their findings, JLE possessed bactericidal effect against foodborne pathogen. A new potential source of

whitening agent from young JLE has been found to be potent against treatment of irregular hyperpigmentation studied by Rayendra et al. (2016). New phenolic compounds elucidated and discovered by Wang et al. (2017) in the jackfruit leaves extract has been found to exhibit a potential source for anti-cancer treatment.

To the best of our knowledge, there is no report on profiling to detect the presence of bioactive compounds of interest in the effect of different drying methods and ethanol compositions on quality attributes on jackfruit leaves extract from *Mastura* species for the nanoemulgel application.

**Table 4.5 : Artocarpin, Squalene and β-sitosterol Content in JL Extract**

| Sample | Artocarpin | Squalene (mg/g) | β-sitosterol |
|---|---|---|---|
| SD80 | $4.71 \pm 0.59^{b}$ | $1.12 \pm 0.09^{b}$ | $0.32 \pm 0.02^{a}$ |
| SN80 | $2.53 \pm 0.29^{c}$ | $0.55 \pm 0.06^{c}$ | $0.11 \pm 0.01^{b}$ |
| OV80 | $8.35 \pm 1.78^{a}$ | $1.47 \pm 0.01^{a}$ | $0.28 \pm 0.02^{a}$ |

Note: $^{abc}$ Significant difference ($p < 0.05$) of one sample over the other(s) if values in the same column carry different superscript. The chromatogram of standards and samples are presented in Appendix D, E, F and G

## 4.6 Formulation of Nanomemulsion

### 4.6.1 Solubility Study

Development of nanoemulsion system requires a stable and good solubility of the components as solubilised drug can efficiently permeates through the skin barrier. The solubility of jackfruit leaves extract (JLE) in various excipients such as oils, surfactants and mix surfactants were investigated in Table 4.6. Various oil phase was screened namely Palmester (medium chain triglyceride), coconut oil and palm kernel oil. Among all the screened oils, JLE had the highest solubility in Palmester ($28.76 \pm 0.31$ mg/g followed by coconut oil and palm kernel oil. Palmester is categorised as

medium chain triglyceride (MCT) and able to solubilise variety of drugs. Medium chain triglyceride is widely used in nanoemulsion system and has better stability when used in formulation. Among the selection of surfactant and mix surfactants used, Tween 80 (T80) paired with Span 80 (S80) at ratio 8:2 possessed the highest solubility (8.38 ± 0.01 mg/g). In general, small molecular surfactants such as Tweens and Spans are used for nanoemulsion preparation using low energy method, such as spontaneous emulsification (Walia et al., 2022). Combination T80 and S80 has been reported to enhance the stability of oil in water (O/W) emulsion system (Salim et al., 2016).

**Table 4.6 : Solubility of JLE in Selected Surfactants, Surfactant Mixtures and Selected Oils**

| Components (surfactants) | Solubility (mg/ml) | Components (oils) | Solubility (mg/ml) |
|---|---|---|---|
| T40 | 7.13 ± 0.07 | Coconut oil | 24.11 ± 1.89 |
| T80 | 7.27 ± 0.07 | Palm kernel oil | 21.91 ± 0.21 |
| S80 | 3.07± 0.07 | Palmester | 28.76 ± 0.31 |
| T40:S80 | 7.99 ± 0.13 | | |
| T80:S80 | 8.39 ± 0.01 | | |

### 4.6.2 Surfactant to Oil Ratio (SOR)

Formation of kinetically stable nanoemulsion with small droplet radius it is essential to obtain careful emulsification. Influence of surfactant and oil ratio on the formation of JLE nanoemulsion is one of the crucial factor in obtaining the smallest mean size. In this research, T80:S80 with ratio of 8:2 was used as surfactants produce small droplets using the spontaneous emulsification method. Evaluation of the JLE naoemulsion was based on different surfactant to oil ratio (SOR) varies from 0.5 to 3.0. It was observed that as the SOR increase, the mean size average start to decrease. The higher the SOR used (1.0 to 3.0) at fix water content, the average nanoemusion size were observed below 200 nm as shown in Figure 4.8. For SOR lower than 1, it

was observed that the particle size start to increase. The final emulsion was became highly viscous and gel like suggesting that liquid crystalline phase may have been formed. There was no significant difference between particle size for the SOR 1 and above. This finding was similar to Komaiko and McClements (2014) suggesting that the effect of SOR particularly 1 and above producing small droplet size of nanoemulsion. Similar finding was reported by Nejadmansouri et al. (2016), an increase in SOR led to a decrease in the particle size which attributed to the presence of high concentration of surfactant molecules that increase in coating the disperse phase droplets. It was reported that in nanoemulsion could be produce with size dimension less than 100 nm are related to the high surfactant ratio (SOR > 1). The fine droplets formed caused by the diffusion of the surfactant and is even quicker the the turbulence generated. However in this study, this implementation of high surfactant to oil ratio would be impractical for manufacturing purpose due to high cost of surfactants. The use of high amount of surfactant, however, will cause toxic to the skin. Although at higher SOR, small droplet size can be formed, it requires high cost to produce nanoemulsion system. Higher SOR (more than SOR = 1) in this study with utilisation of Tween 80 (T80) increases raw material costs (Roldan-Cruz et al., 2016)

In industrial application, higher production cost are typically associated with raw material cost and energy cost. Emulsion with higher surfactant concentration might require more energy to mix effectively. Therefore, a sufficient amount and surfactant blends with lower SOR especially SOR =1, may also result in producing desirable and stable nano-sized droplets which also able to achieve good solubilisation capacity making it comparable to higher SOR. With the optimum amount of surfactant blends (T80 and S80), the selection of lower SOR are compatible, achieve good solubilisation

capacity and also efficient in emulsification of the droplets in formulating nanoemulsion (Huang et al., 2010). Due to safety and economic reason, in this research, SOR=1 was chosen for the fabrication of nanoemulsion.

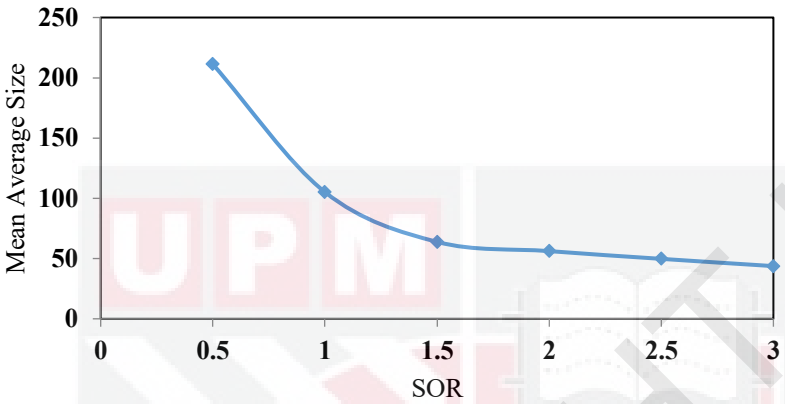



**Figure 4.8 : Nanoemulsion Average Size Based on Different Surfactant to Oil Ratio (SOR)**

### 4.6.3 Hydrophilic Lipophilic Balance (HLB) Values

HLB is an empirical classification of surfactants, described by Griffin (1949) in a study reported by Rahaman et al. (2023) which can determine the type of emulsion that will form, a surfactant with low HLB tend to stabilise w/o emulsions, while a surfactant with high HLB and high affinity for the aqueous phase, will stabilise o/w emulsions.The flexibility of surfactant film, its affinity for water, and the interfacial tension are suggested as important factors in the formation of nanoemulsions. HLB values of surfactant blends of T80:S80 were screened and calculated. Different HLB values from 10.72 to 13.9 produce small droplet size of developed namoemulsion. As the HLB value increase at 15, the droplets start to become larger (227 nm). Figure 4.9 exhibited the smallest mean size average (114.5 nm) for HLB value of 12.86 at SOR=1. The blend of Tween 80/Span 80 surfactant at the ratio 8:2 and HLB value of

12.86 solubilise slighly higher of the JLE in the formulation and produce stable nanoemulsion was attributed to the polyoxythylene, a hydrophilic head group in the used surfactant. The selection of surfactant is one of the important component in formulating an emulsion. Optimal surfactant or blends of surfactant selection could reduce surface tension and stabilise droplet against recoalescence. In this research, the system in which surfactant was at low concentration (10 wt %) in the single phase region according to their HLB values and phase behavior as well was chosen for the preparation of the final nanoemulsion.

Previous finding was reported by Mahdi et al. (2011) where blend of Tween 80/Span 80 at the ratio 9:1 resulted in higher solubility of the extract studied. Tavano et al. (2011) compared the different amount of Tween 80/Span blends with HLB values ranging from 6 to 15. It can be observed that stable nanoemulsion produced at higher HLB values (HLB 10 and above) with no separation, clear and isotropic was HLB 12 and the lowest obtained was for HLB 6.

Based on the selection drug solubility in selected oil and surfactant, surfactant to oil ratio (SOR), HLB values and screening of surfactant blends, a pseudoternary phase diagram was constructed and prepared prepared using various compositions of Palmester, surfactant blends (T80:S80) at ratio 8:2 and water to produce optimum formulation of JLE loaded nanoemulsion.

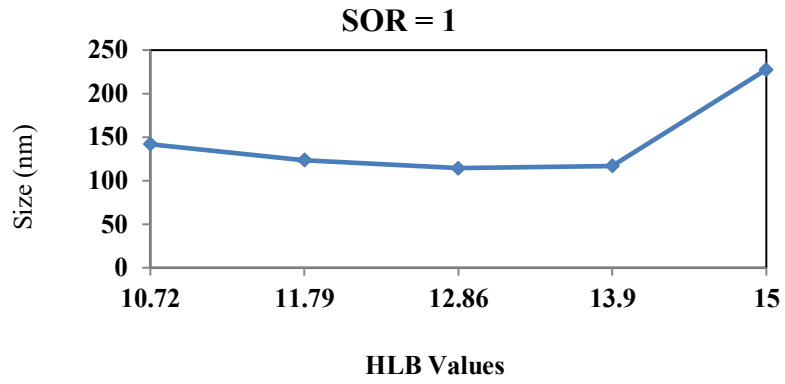



**Figure 4.9 : Nanoemulsion Average Size Based on Selected SOR = 1 with Different Hydrophilic Lipophilic Balance (HLB) Values**

### 4.6.4 Construction of Pseudoternary Phase Diagram

Evaluating the area of NE region in the phase diagram is essential for a successful development of an optimum NE (Sharma and Tailang, 2020). Hence, constructing a pseudoternary phase diagram (PTD) is vital to determine the concentration range of components for the existence range of NEs. Pseudoternary phase diagram was constructed with Palmester as an oil phase, Tween 80 and Span 80 as surfactant mixture (smix) and water as the aqueous phase to develop the most suitable composition to form nanoemulsion.

In order to obtain a desired formulation, it is critical to make surfactant selection. Each of surfactant provides specific HLB. The Tween 80:Span 80 (T80:S80) blends at the ratio of 8:2 and HLB 12.86 resulted in oil in water nanoemulsion area as presented in single phase observed as transparent/translucent that can flow easily as presented in Figure 4.10. The formation of a single-phase system in the diagram confirm by visual inspection appeared

to be clear solution with no separation and isotropic suggests the mixture of surfactants was able to decrease the surface tension between aqueous and oily phase, thus promoting the formation of nanoemulsion. Oil was added drop by drop and during the titration, samples were magnetically stirred in order to reach the equilibrium quickly. The phase boundary was determined by observing the changes of the sample appearance from turbid to transparent or from transparent to turbid. Liquid crystalline was found to be in the one phase (monophase) region.

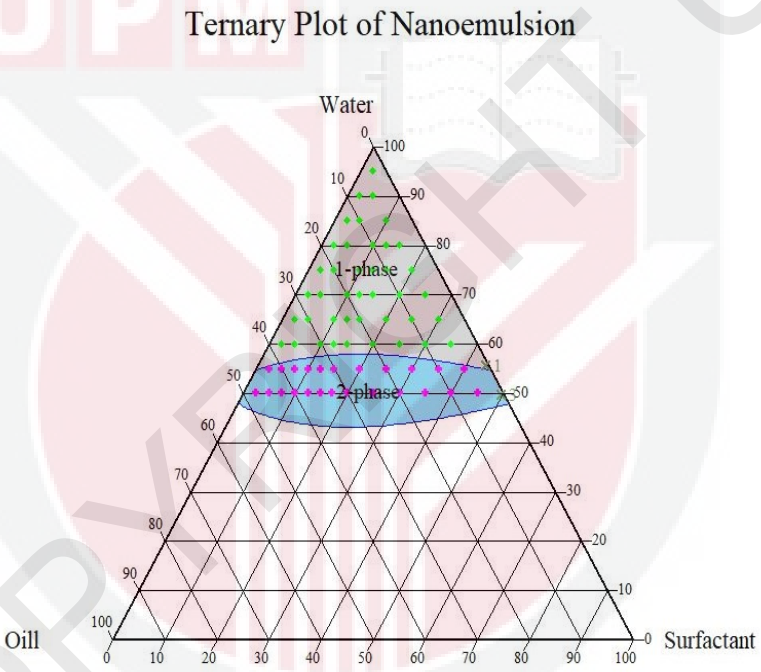



**Figure 4.10 : Pseudoternary Phase Diagram of Tween 80 and Span 80 at the Ratio 8:2 Dispersed in Water**

It has been reported by Caldero et al. (2016) that the factor of the formation of nanoemulsion was the presence of liquid crystalline along the emulsification path. Liquid crystalline layer formed due to hydrogen bonding between hydroxyl group of oxyethylene group of T80 and hydroxyl group of water which lead to the formation of gel network. Normally the hydrogen bonding ruptured when there is dilution of water.

The ruptured of liquid crystalline system when more water is added indicates the presence and role of hydrogen bonding in the formation of liquid crystalline structure where there is lack of OH- group with oxyethylene moiety compared to the excess of water gradually added.

Oil in water region area formed by the system due to dissolution behavior of the bulky polyoxyethylene group that give rise to dependent of the systems on spontaneous emulsification. Tween 80 derived from the polyoxyethylene sorbitan (head group) and oleic acid (tail group) (Figure 4.11). The tail group comprised of a long chain $C_{18}$ of unsaturated oleic acid. Maximum solubilisation capacity of water depends on the oxyethylene chain and configuration of the polar head group and hydrocarbon moiety of nonionic surfactant and types of oil. The solubilisation capacity is subjected to the importance of unsaturated trioleate because more lipophilic tail group that is structurally similar to octyl group (substituent group from octane) of Palmester which enable the surfactant to be well packed at the interface. Palmester interact less with high amount of lipophilic surfactant or surfactant blends with low HLB values. It shows the importance of better selection of surfactant blends showing strong solubilisation capacity that gives stronger stabililty. However, as the amount of water starts to decrease at the water-surfactant line of the PTD to less than 60%, formation of two phase start to occur. The two phase (bi-phasic) region was visually appears to be turbid followed by a phase separation. In order to prepare nanoemulsion, several conditions must take into considerations that include the selection of surfactant(s) should be enough to provide low interfacial tension that can be attained at the oil in water interface, concentration of surfactant needed to provide stabilisation to the droplets formed and sufficient adsorption of surfactant (fluidity) of the interface to

promote nanoemulsion (Bergfreund et al., 2021). Barradas et al. (2015) reported that addition of water from 20 wt % to 75wt % reduced the transparency, obtaining milky appearance of the nanoemulsion. Study of transformation of phases and the effect of adding water content in the system forming microemulsion system was also studied by Gandhi et al. (2021) and Abbasi and Radi (2016).

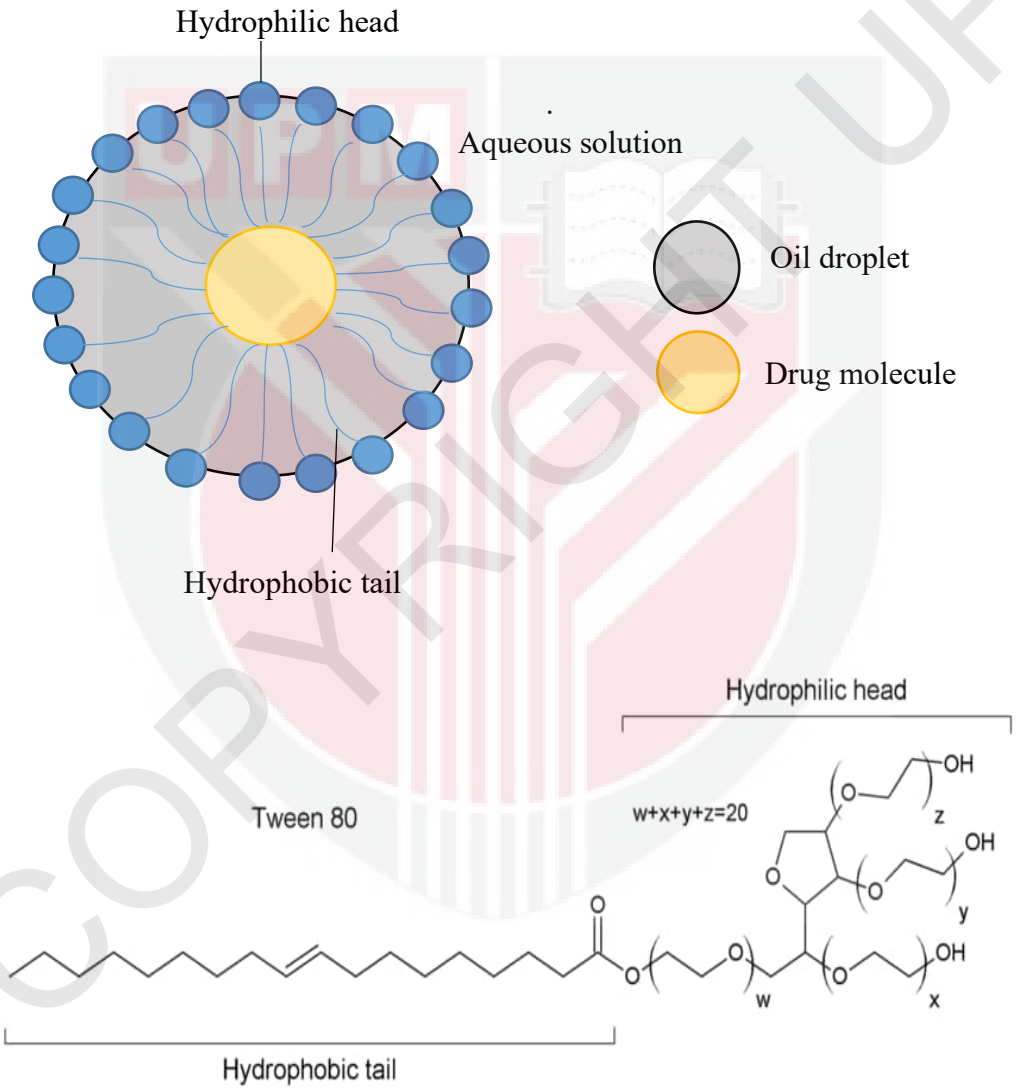



**Figure 4.11 : Oil in Water Nanoemulsion with Tween 80 Molecule Structure as Surfactant**

## 4.7 Development of Nanoemulsion

Based on the construction of the pseudoternary phase diagram, an optimum JLE nanoemulsion was developed using composition as presented in Table 4.7.

**Table 4.7 : Composition of Nanoemulsion**

| Composition | Ratio (%) |
|---|---|
| Water | 80 |
| Surfactant (T80:S80) (8:2) | 10 |
| Extract + Palmester | 10 |

It was found that after mixing all the components, the nanoemulsion (NE) visibly appeared to be translucent. The nanoemulsion portrayed acceptable droplet size (< 200 nm) with low PDI (< 0.400) indicates that NE is in nanosize range and provide narrow distribution of the particle size (Table 4.8). The NE was further characterised for the thermodynamic stability and the outcome will be discussed along with the formulated nanoemulgels in Section 4.8.

## 4.8 Development of Nanoemulgel

Nanoemulgel was developed by enhancing the nanoemulsion (NE) system with xanthan gum (Xan), gelatin (Gel) as well as xanthan gum-gelatin (Xan-Gel) blends and carbopol as a control system using spontaneous emulsification method (Figure 2.7). The prepared JLE incorporated with nanoemulgel has smooth, clear and homogenous texture. Nanoemulgels prepared incorporated with xanthan gum only labelled as XA-XC, gelatin only (GA-GD), mixtures of xanthan gum-gelatin polymers (MA-ME) produced average nano size (< 250 nm). However, the carbopol was observed to be the largest size among all formulations recorded. The nanoemulgels

were characterised based on the droplet size, polydispersity index (PDI) and ζ-potential as presented in Table 4.8.

### 4.8.1 Droplet Size

Xanthan gum has high molecular weight ($2 \times 10^6 - 2 \times 10^7$) linear molecule that have large hydrodynamic volume such that at lower concentration, it can form intermolecular entanglements leading to high viscosity. Addition of xanthan gum provide higher charge densities thus, it enhance stability and formed densed polymer network. Therefore, increasing xanthan gum percentage in the formulation (XA to XC) may increase the particle size. It can be seen that the addition of xanthan gum in the formulation produces larger droplet in comparison to the nanoemulsion (Table 4.8). The higher the amount of xanthan gum added from XA to XC, the droplet size become bigger. As the percentage of

**Table 4.8 : Size, PDI, δ Potential of NE, Xan (XA-XC), Gel (GA-GD) and Mixture of Xan-Gel (MA-ME) Formulations**

| Composition (%) | | Formulation | Size | PDI | ζ-potential |
|---|---|---|---|---|---|
| Xan | Gel | | (nm) | | (mV) |
| - | - | NE | 130.9 | 0.225 | -27.32 |
| 1.5 | - | XA | 171.6 | 0.298 | -46.33 |
| 2.0 | - | XB | 199.7 | 0.314 | -46.97 |
| 2.5 | - | XC | 222.9 | 0.328 | -47.34 |
| - | 2.5 | GA | 137.5 | 0.246 | -31.24 |
| - | 5.0 | GB | 140.0 | 0.246 | -32.92 |
| - | 7.5 | GC | 142.1 | 0.250 | -34.57 |
| - | 10.0 | GD | 143.1 | 0.258 | -41.75 |
| 2.0 | 0.5 | MA | 214.5 | 0.319 | -46.39 |
| 1.5 | 1.0 | MB | 185.8 | 0.314 | -45.72 |
| 1.25 | 1.25 | MC | 167.6 | 0.309 | -43.09 |
| 1.0 | 1.5 | MD | 164.5 | 0.298 | -40.76 |
| 0.5 | 2.0 | ME | 155.0 | 0.252 | -38.62 |
| - | - | Carbopol | 461.1 | 0.305 | -41.21 |

Note: PDI: Polydispersity index
ζ- potential: Zeta potential

xanthan gum added in the formulation from ME-MA, the droplet become larger in size. Meanwhile, fish gelatin has a high molecular polypeptide polymer. It has different gel strength from mammalian gelatin as a result of its lower content of proline (Pro) and hydroxyproline (Hyp). The formulation added with fish gelatin (GA – GD) was found to have similar size to nanoemulsion even though similar weight percentage was added to xanthan gum (XA-XC) nanoemulgel formulations. This is due to lower content of Pro and Hyp gives fish gelatin low gel modulus, low gelling and low melting temperature (Karim and Bhat, 2009).

Nanoemulgel formulation added with Xan-Gel blends (MA-ME) exhibited similar trend with XA –XC where MA with the highest percentage of xanthan gum added in the mixture provides higher particle size followed by MB to ME being the lowest percentage addition of xanthan gum. This behaviour could be associated to the effect of polymer bridging and neutralisation charge. When the fish gelatin present in higher percentage for ME formulation (2.0% Gel) as compared to MA (0.5% Gel), the viscosity of the system is dominated by the Gel-Gel association. With the reinforcement of xanthan gum (from MD to MA) the xanthan gum (Xan-Xan) associations become dominant. This could be due to at lower xanthan gum concentration, adsorption of available cationic molecules are less to the negatively charged droplets. Therefore, the cationic molecules are shared among droplets gelatin whereby it supposed to adsorbed independently on the individual droplets itself. Furthermore, cationic molecules tends to neutralise the negative charge where increase of xanthan gum concentration resulted in the partial or complete charge neutralisation. Droplets charge neutralisation will results in the decreased repulsive forces, thus, droplets come closer to each other. This could lead to aggregation and thus, increase

the particle size of the droplets (Abbas et al., 2015). Apart from the structural features of the xanthan gum backbone, side chains and conformational of molecules, the association of Xan-Gel complexes could also affected by the temperature and pH. Previous researchers (Yahoum et al., 2023; and Rahmati et al., 2018) reported that as the xanthan gum concentration increased, the particle size of the droplets also increase. In this study, carbopol used exhibited higher droplet size at concentration 2% (wt/wt) among other formulations. It could be due to at higher concentration using low energy method, it tends to agglomerate. It may require long mixing time and vigorous agitation in order the polymer to be fully dispersed. However, prolonged exposure to high shear may damage the polymer, thus reducing the viscosity. Nevertheless, the particle size of carbopol was still within nano size range (20 -500 nm) (Arianto et al., 2020).

The selection of optimum condition as presented in Table 4.7 to formulate nanoemulsion incorporated with gelling agents (xanthan gum and fish gelatin) plays a distinct and critical role in achieving droplet size and stability. The oil phase is vital in determining the droplet size of a base of nanoemulgel. Medium chain triglyceride (MCT) oil used in this study is suited for nanoemulsions due to its relatively low viscosity and shorter chain length. These characteristics facilitate the formation of smaller droplets during emulsification using spontaneous emulsification method by requiring less energy to disrupt the oil phase into the droplets. Previous study by Jaiswal et al. (2015) indicate that nanoemulsion formulated with MCT oil achieved finer droplet size than those long chain triglycerides (LCT). The compatibility of MCT and surfactants also enhances stabilisation. Ensuring that interfacial tension is effectively reduced during emulsification.

Surfactant blends also plays a critical part in reding interfacial tension, which is essential for forming smaller droplets. Combination of T80 and S80 at a ratio 8:2 able to achieve an optimal HLB in this research forming stabilised oil in water nanoemulsion. The concentration of surfactants should be able to have enough coverage of the interface which able to produce smaller droplet size of nanoemulsion. Gupta et al. (2016) found that appropriate amount of T80 and S80 significantly reduced droplet size, hence, improved the long term stability of nanoemulsion. Their findings highlight the importance of selecting surfactants with complementary HLB values to optimise emulsification.

The interplay between oil phase, surfactant blends and aqueous phase that include gelling agents in this study create optimal conditions for forming nano-sized droplets. The low viscosity of MCT oil reduces energy requirements for emulsification, while the surfactant blend minimises interfacial tensionand stabilises droplets. The viscosity-enhancing properties of xanthan gum and fish gelatin further improve stability and prevent from droplet coalescence (Kaur et al., 2017). Together, these components ensure the production of a nanoemulsion based gel with small, uniform droplet sizes and enhanced stability, making this formulation suitable for application in drug delivery system.

### 4.8.2 Polydispersity Index

The polydispersity index (PDI) is a measure of homogeneity of particles and it varies from 0.0 to 1.0. The lower the PDI value, the higher the homogeneity of the particles in the formulation (Danaei et al., 2018). All formulations were evaluated during storage period in order to analyse any changes in particle size of the dispersed droplet.

It can be observed that all formulation displayed a relatively uniform narrow size distribution globule range with PDI value less than 0.4 (Table 4.8), suggesting that production of nanoemulsion and nanoemulgels through spontaneous emulsification was well succeeded, in which results of all formulations were able to maintain in nanorange size during at least studied storage period.

### 4.8.3 Zeta Potential

Zeta potential (ζ-potential) is a function of the surface charge that repels between each other due to inner strong repulsive force and the ζ-potential value can be related to the physical stability of an emulsion system. The ζ-potential results show that the formulations were negatively charged and the values ranges between -27.32 mV and -47.34 mV (Table 4.8), indicating the system offer good stable system and well separated emulsion globules. Increase in xanthan gum ratio in XA-XC and Xan-Gel complexes reduces the zeta potential of both single and mix formulations as xanthan gum was strongly negative charge while fish gelatin is weakly positive charge (Yin et al., 2021). The reduced ζ-potential of formulation of fish gelation with addition of xanthan gum caused by the electrostatic interaction. Electrostatic interaction could form between the negative surface charge of polar group in xanthan gum (carboxyl: $COO^-$ and glucoronic acid residue which may contain pyruvate group) and positive charge of amino acid residue may increase the zeta potential of the systems as presented in Figure 4.12 (Yin et al., 2021). The negative charge of polar groups in xanthan gum enhanced the negative charge at the nanoemulsion surface and thus increased the negatively charged zeta potential values. Stability of the nanoemulgel system also related to the selection of nonionic surfactant in this study, Tween 80 (T80) and Span 80 (S80). The low molecular weight emulsifiers provide stabile

nanoemulsion by penetrating more oil region of o/w interface and electrostatistically attached to more oil droplets. The combination of these non-ionic emulsifier will increase the accessibility in water and oil phase region (Wahgiman et al., 2020).

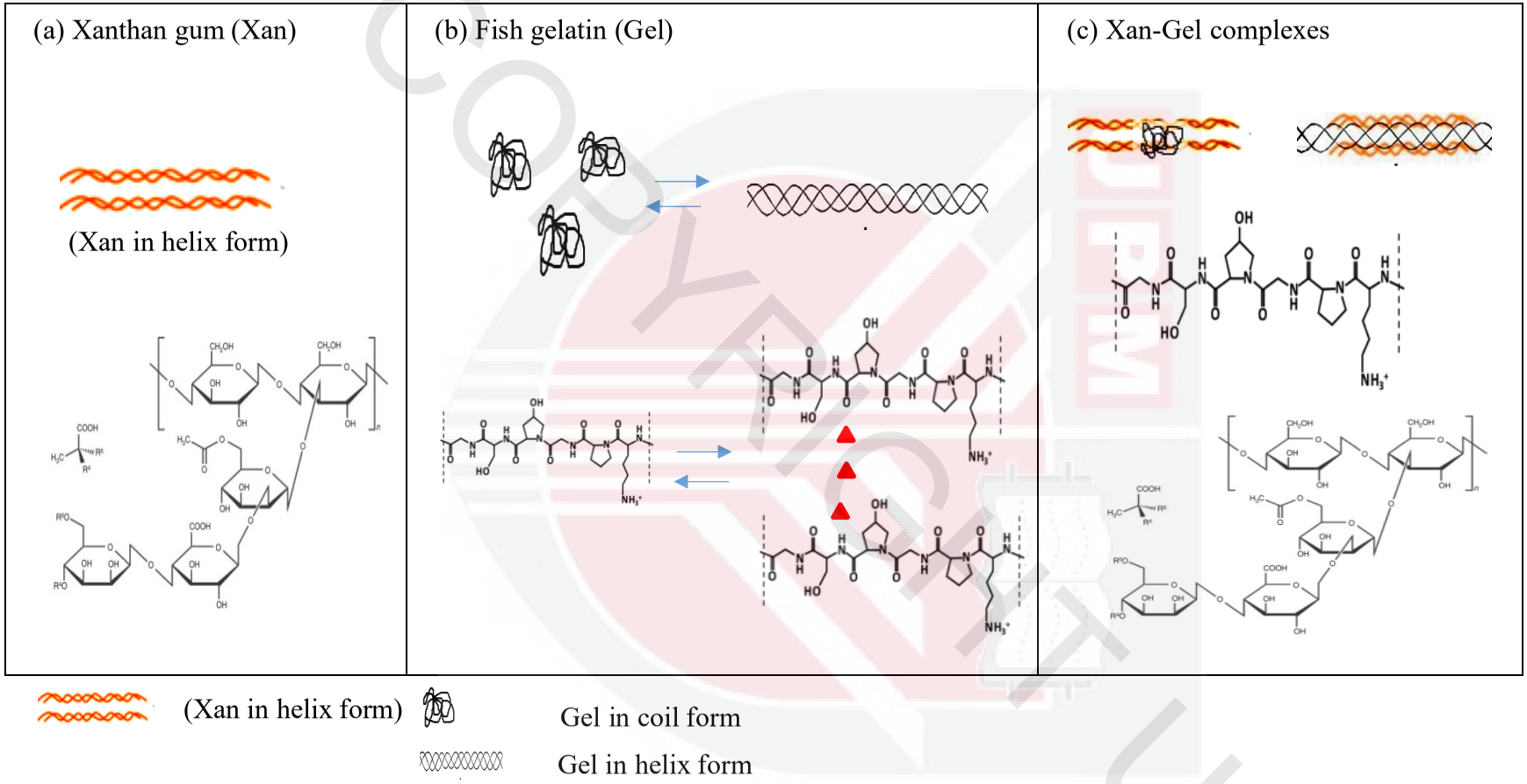



**Figure 4.12 : Schematic diagram illustrating the (a) Xanthan gum (Xan), (b) Fish gelatin (Gel) and (c) the Xan-Gel complexes formed by electrostatic interaction**

### 4.8.4 pH Measurement

Table 4.9 shows the formulated nanoemulsion, nanoemulgels and carbopol with pH range between 4.8–5.7. pH is an important characteristic when a pharmaceutical formulation is intended to apply on an open wound, as it affects the cellular processes in the wound in a direct and indirect manner (Jones et al., 2015). Acidic environment is favourable in formulating drug delivery system for dermal application. It had been reported that the application of acid onto the wounds greatly lowers the pH which then makes growth and proliferation of bacteria unfavourable (Nagoba et al., 2021). The pH of all formulations were between 4 and 6, which ideal values for the aqeuous phase of a topical dermal application and does not cause skin irritation (Lukic et al., 2021).

**Table 4.9 : pH Values of NE, Carbopol, Xan (XA-XC), Gel (GA-GD) and Blends of Xan-Gel (MA-ME)**

| Formulation | pH | Formulation | pH |
|---|---|---|---|
| NE | 5.5 ± 0.10 | MA | 5.1 ± 0.10 |
| Carbopol | 5.3 ± 0.06 | MB | 5.3 ± 0.06 |
| XA | 4.8 ± 0.06 | MC | 5.4 ± 0.00 |
| XB | 5.1 ± 0.10 | MD | 5.4 ± 0.06 |
| XC | 4.9 ± 0.06 | ME | 5.4 ± 0.06 |
| GA | 5.2 ± 0.00 | | |
| GB | 5.5 ± 0.10 | | |
| GC | 5.2 ± 0.06 | | |
| GD | 5.7 ± 0.06 | | |

## 4.9 Thermodynamic Stability Tests

Formation of metastable often occurs during storage. Therefore, thermodynamic stability tests were conducted to test observe any changes to the formulations. The nanoemulgel formulations were subjected to different stress tests, such as centrifugation, heating-cooling cycle, and freeze-thaw cycle tests. Table 4.10 revealed that JLE loaded nanoemulgel with at least in the ratio of 1:1 (1.25:1.25 % w/w) of

Xan-Gel biopolymers were remain unchanged based on transparency and any sign of phase separation. These formulations showed negative sign of instability, i.e. there were no sign of phase separation, turbidity or drug precipitation observed. However, mixtures of formulation; MD (1.0%: 1.5% w/w) and ME (0.5%: 2.0% w/w) showed changes when passed through all the stability tests with obvious signs of phase separation and cracking.Higher percentage of Gel added into MD and ME formulations that supposed to serve as gelling agent in the formulations (MD – ME) showed separation of phases (gel and liquid) that explains the extraction of a liquid from a gel medium or may also known as syneresis (Patil et al., 2019).

The presence of Gel in both formulations showed kinetically unstable could be due to the attraction of hydrophilic group to the water whereby the penetration of water in excess amount into the bulk of oil causing interfacial disruption of droplets into the bulk aqeuous phase and thus lead to phase separation. The increase in xanthan gum concentration causes the stability of nanoemulgel. When protein concentration added gradually, the nanoemulgel mixture involved in the structural transition in the formation of dual complex. The complexes remain in stable region with no apparent phase separation up until the amount of protein added in the formulation at the same ratio with Xan concentration. At this point, the mixtures enter a partially stable region of intermolecular soluble complex. There is physical formation of single phase separation observed in the formulation. A single phase system exist when two biopolymers exist separately, distributed thoroughly in the medium. When the amount of Gel exceeds Xan, the system exhibited a two-distinct phase or a phased separated system, where an unstable region of intramolecular insoluble complex formed. A phenomenon of a phase separation system is where the biopolymer complexes become

thermodynamically incompatible with each other that a protein-rich phase starts to occur (Maphosa and Jideani, 2018). Over storage period, nanoemulgel formulations, MD and ME also exhibited signs of cracking. These studied formulations (MD and and ME) were considered to be physically unstable. Other formulations that were subjected to the thermodynamic stability tests that shows no phase separation, creaming or cracking can be corfirmed stable when subject to mechanical force. In this stage, droplet size, PDI, and surface charge are consistent and no change throughout cycle period. The formulations that passed the thermodynamic stability tests were subjected to further characterisation, such as viscosity determination, and TEM.

**Table 4.10 : Thermodynamic Stability Tests of NE, Carbopol, Xan (XA-XC), Gel (GA-GD) and Blends of Xan-Gel (MA-ME) or Mulations**

| Composition Xan (%) | Gel (%) | Formulation | Centrifugation | Heating and cooling | Freeze Thaw Cycle |
|---|---|---|---|---|---|
| - | - | NE | √ | √ | √ |
| - | - | Carbopol | √ | √ | √ |
| 1.5 | - | XA | √ | √ | √ |
| 2.0 | - | XB | √ | √ | √ |
| 2.5 | - | XC | √ | √ | √ |
| - | 2.5 | GA | √ | √ | √ |
| - | 5.0 | GB | √ | √ | √ |
| - | 7.5 | GC | √ | √ | √ |
| - | 10.0 | GD | √ | √ | √ |
| 2.0 | 0.5 | MA | √ | √ | √ |
| 1.5 | 1.0 | MB | √ | √ | √ |
| 1.25 | 1.25 | MC | √ | √ | √ |
| 1.0 | 1.5 | MD | X | X | X |
| 0.5 | 2.0 | ME | X | X | X |

Note √: Stable

X: Unstable (phase separation, creaming or cracking) occur

## 4.10 Rheological Properties

The rheological characteristics of nanoemulgel formulations were investigated to give understanding on its flow and viscoelastic properties. In particular, the effects of the addition of mixture of natural polymers (xanthan gum-gelatin) concentration (0.5% up to maximum 2.0% (w/v)) on rheological properties of nanoemulgels were studied.

### 4.10.1 Flow Properties (Viscosity)

Apparent viscosity as a function of shear rate of carbopol, nanoemulgels added with studied xanthan gum (Xan, XA – XC at a mass ratio 1.25 -2.5% w/w), blends of Xan - Gel (MA – MC) were depicted in Figure 4.13, while nanoemulsion (NE) and fish gelatin (Gel, GA – GD) at a mass ratio between 2.5 – 10.0 % (w/w) were depicted in Figure 4.14, respectively. Based on these figures, XA – XC and Xan-Gel blends (MA – MC) demonstrated a shear thinning behavior. Increase in xanthan gum concentration in the systems XA –XC and MC – MA showed an increase in viscosity.

Addition of fish gelatin in the Xan-Gel blends (MA – MC) enhanced the nanoemulgels systems and had a significant effect of the formulation by having more homogenous structure of the nanoemulgel formulated (Yin et al., 2021) and synergistic gelation mechanism by forming interpolymer complexes between Xan-Gel (Wang et al., 2018) . The incorporation of biopolymer blends provide lower flow index indicate the enhancement of flow properties of gel in comparison to single xanthan gum formulations (XA-XC). Addition of fish gelatin at 0.5% in MA nanoemulgel mixtures showed similar viscosity results to that single xanthan gum formulation at 2.5% w/v. Xanthan gum have had a positive influence on the viscosity of the nanoemulgels produced. Previous research also reported that the impact of application of low amount

of xanthan gum increased the viscosity of the formulated nanoemulgels (Alfaro-Rodríguez et al., 2022). Syed Azhar et al. (2018) reported that xanthan gum added enhanced the formation stronger network by generation of greater bonds surrounding the oil droplets creating stable droplets and that of increasing the viscosity of the nanoemulgels.

The shear-thinning viscosity in the nanoemulgel systems  implying the existence of network entanglements (physical nodes) between the added natural polymers chains. The physical nodes or the formation of the entanglements of the polymer networks hindering the free flow of the systems which responsible for the increasing viscosity. However as the shear rate increased, it can be seen that the viscosity of the systems decreased. It can be associated with the polymer chains or the physical nodes that are broken and being aligned in the direction of the applied deformation leading to reduced viscosity. This is desirable behaviour for the pharmaceutical application as their high apparent viscosity at low shear stresses is required to descend the dispersed phase mobility. It is important to note also that when agitation occurred, the system exhibit free flow movement. Thus, providing low viscosity against high shear stresses. Inversely, in this findings it can be observed that plain nanoemulsion (NE) and fish gelatin formulations (GA – GD) presented Newtonian behavior indicated by the viscosity of independent from the applied shear rate as presented in Figure 4.14.

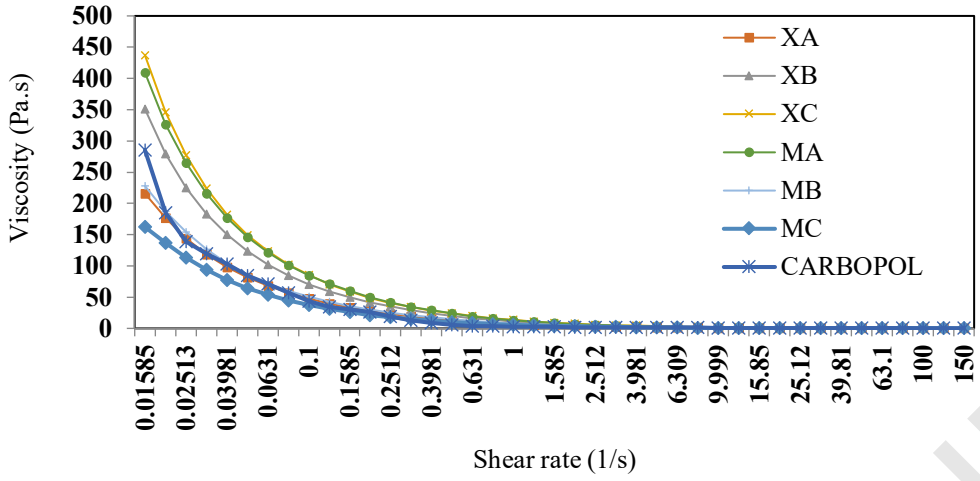



**Figure 4.13 : Viscosity of Carbopol, Xanthan Gum (XA-XC) and Xan-Gel Blend (MA-MC) Formulations**

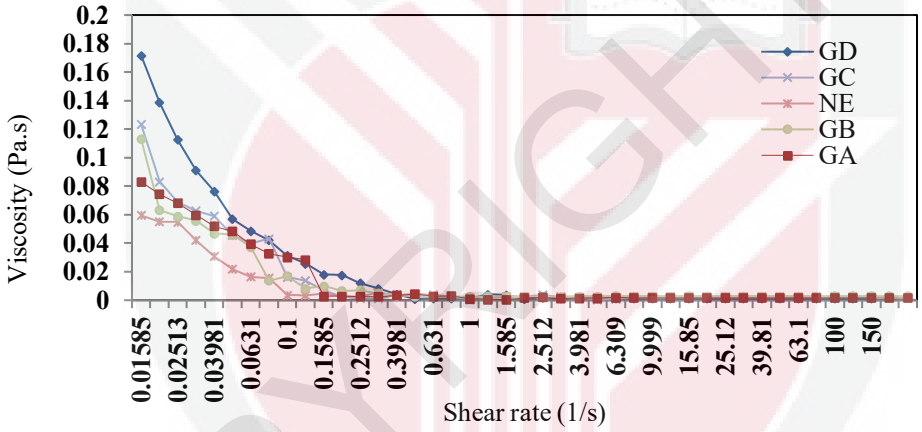



**Figure 4.14 : Viscosity of Nanoemulsion (NE) and Fish Gelatin (GA-GD) Formulations**

Table 4.11 shows flow behavior properties of the XA – XC, MA – MC and GA – GD fitted to the Power Law model. Theoretically, the *k*-value should be in parallel with the viscosity trend. Table 4.11 shows that the effect of the independent variables on *k*-value has similar pattern with the viscosity (Figure 4.13 and Figure 4.14). The *k*-value (> 12) were obtained in XC and MA formulation. A higher *k*-value indicated better nanoemulsion structure (Musa et al., 2017). Therefore, it can be concluded that carbopol, XA – XC and MA – MC nanoemulgel formulations with n<1 showed the fluid had shear thinning or non-Newtonian behaviour while plain NE and GA – GD

showed an indication of flow behaviour indices n ≥ 1 with shear thickening or Newtonian properties regardless the the shear rate applied.

**Table 4.11 : Power Law Model Parameters for NE, Carbopol, Xan (XA-XC), Gel (GA-GD), Xan-Gel Blends (MA-MC) Formulations**

| Formulation | Consistency coefficients $k$(Pa $s^n$) | Flow behavior indices $n$ |
|---|---|---|
| NE | $1.29 \times 10^{-3}$ | 1.0000 |
| Carbopol | 9.96 | 0.1407 |
| XA | 6.861 | 0.1389 |
| XB | 11.95 | 0.1358 |
| XC | 12.69 | 0.1245 |
| MA | 12.33 | 0.1260 |
| MB | 7.261 | 0.1422 |
| MC | 5.421 | 0.1686 |
| GA | $1.63 \times 10^{-3}$ | 1.0050 |
| GB | $1.75 \times 10^{-3}$ | 1.0310 |
| GC | $2.41 \times 10^{-3}$ | 1.0060 |
| GD | $2.81 \times 10^{-3}$ | 1.0060 |

### 4.10.2 Viscoelastic Properties–Strain Sweep

Strain sweep test was conducted to measure the linear viscoelasticity range (LVR), determining the system behavior under maximum deformation stress that the sample can hold with structure remain unchanged. The linearity of the region determined by viscous modulus (G") and elastic modulus (G') was performed at a fixed frequency (1 hertz). In the oscillatory test, the characterisations of nanoemulgels were contributed by the structural pattern of their viscous and elastic properties. The nanoemulsion (NE) and Gel formulations (GA – GD) that were assessed for their viscosity (Figure 4.14) were excluded from this step, since they do not show gel-like behavior, therefore, measurement of G' and G" would be inappropriate. Figure 4.15 – Figure 4.17 presents the results for JLE loaded Carbopol, XA- XC and MA –MC, respectively. It was observed that at 10 % strain stress rate, they were independent of the strain stress,

suggesting that the system had attained the linear viscoelasticity. Thus, this LVR value was selected to ensure that they are conducted within the same region to be applied for the frequency test. The storage modulus (G') of nanoemulgels over the LVR always higher than that G" (G'>G"). This indicates that all the suspensions are elastic, solid like behavior. The storage modulus (G') give information about the amount of structure present in a material (Xu and Gupta, 2018), which represents the energy storage capability of the material. If it is higher than G", the materials can be regarded as mainly elastic. A softer materials with lower modulus of elasticity deforms quickly but recover its shape just as fast. Conversely, a higher modulus imbues the substance with stiffer characteristics, a dense form that can absorb heavy loads. After the applied force being removed, the material flow recovery will be more than a smaller storage modulus value towards its original state. In this research, G' values were observed to be in ascending order for Xan only formulations in Figure 4.16 (a) to Figure to 4.16 (c) and for Xan-Gel blends (MC to MA) in Figure 4.17 (c) to Figure 4.17 (a). Addition of high polymeric network of xanthan gum in increasing concentration manner from XA to XC (1.5 – 2.5%) and complexes in MC to MA (1.25 -2.0%) nanoemulgel formulations, affect the nanoemulgel by modifying the texture becomes thicker and an indication of good spreadability characteristics (Rawat et al., 2019).

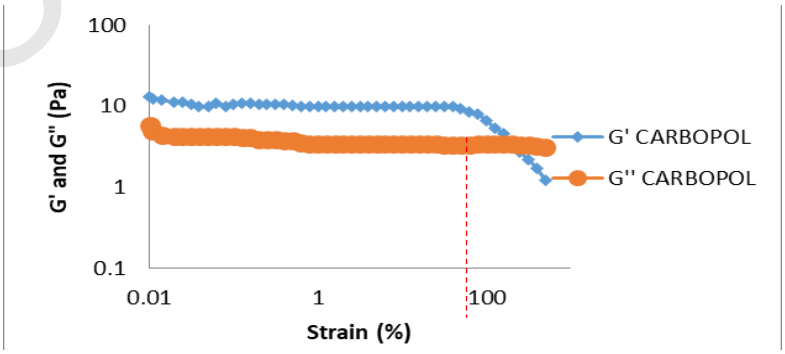
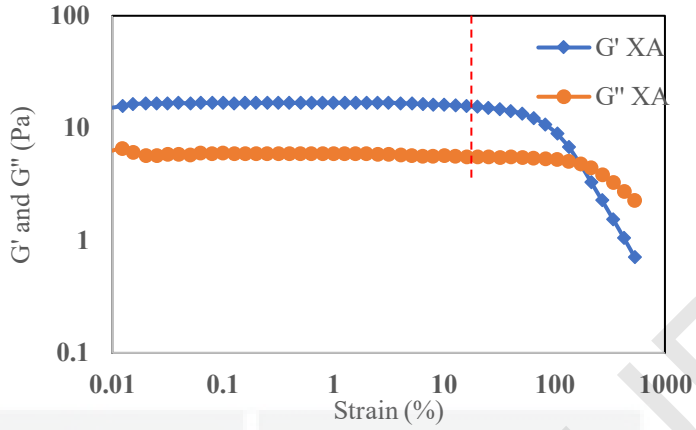
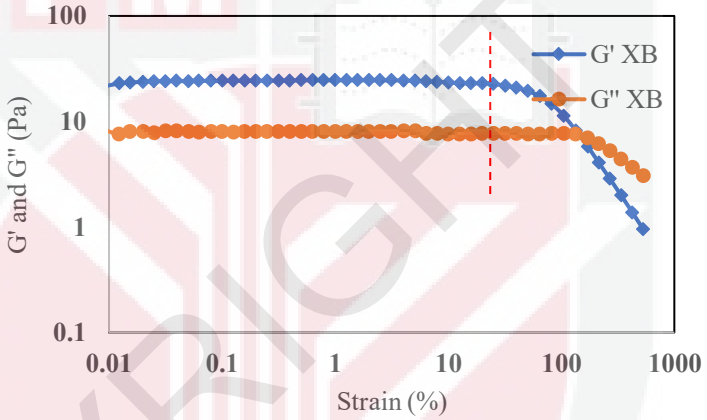



**Figure 4.15 : Strain Sweep for Carbopol**

(a)




(b)




(c)

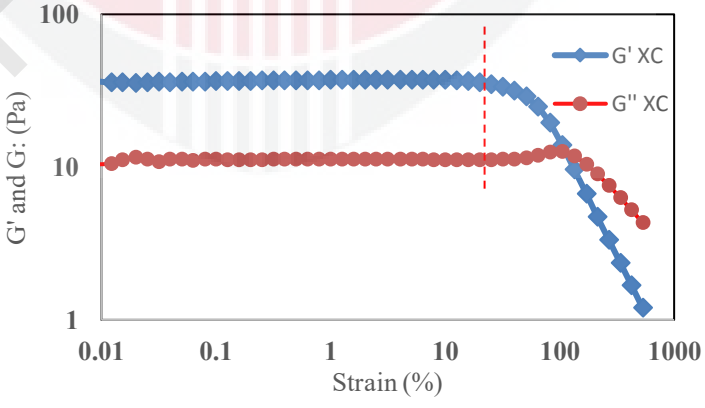
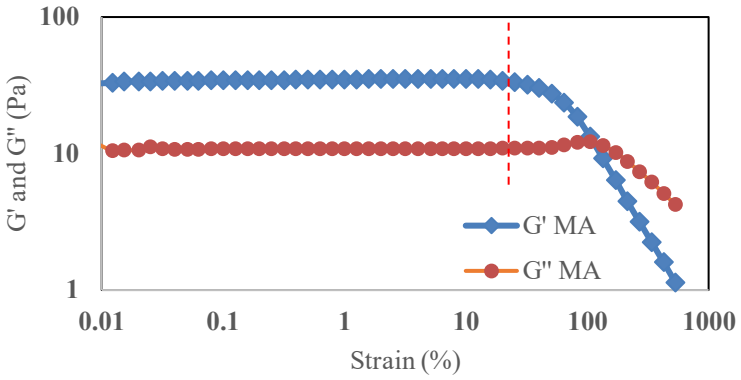
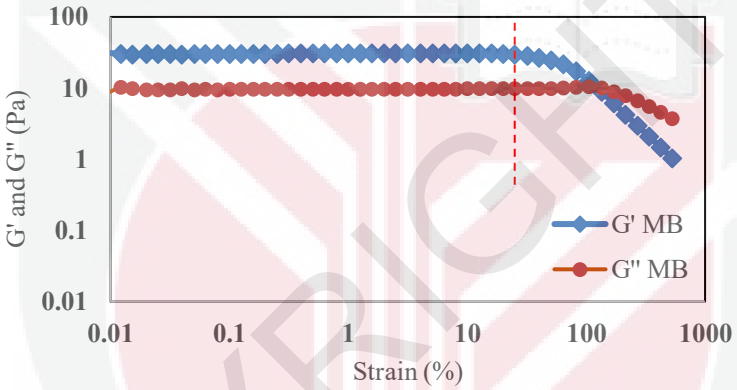



**Figure 4.16 : Strain Sweep for (a) XA, (b) XB and, (c) XC**

(a)




(b)




(c)

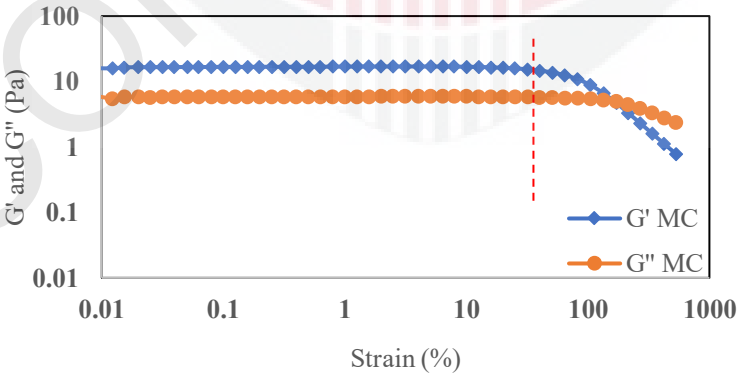



**Figure 4.17 : Strain Sweep for (a) MA, (b) MB and, (c) MC**

Table 4.12 presents the points of which the LVR, linearity limit and crossover values were determined. The point in which stress strain relationship deviate from linear behavior using derivative of logarithmic relationship is known as critical strain or linearity limit. The critical strain point is calculated at which storage modulus (G') tolerance range of deviation of ± 5% deviation from its plateau (linear) value (according to ISO 6721 – 10:2015 (E) and EN/DIN EN 14770). Therefore the linearity limits are presented in all rheograms (Figure 4.15 –Figure 4.17) as indicated by vertical red lines. The G' and G" at some point in the non LVR will crossover each other. The cross over values (G'=G") as presented in Table 4.12 were observed to be at different values for each of the formulations.

**Table 4.12 : Determination of LVR, Linearity Limit and Crossover for Carbopol, Xan (XA- XC) and Xan-Gel Blends (MA-MC)**

| Sample | LVR | Linearity limit | | Crossover (G' = G") |
|---|---|---|---|---|
| | | G' in LVR | G" in LVR | |
| Carbopol | 13.02 - 3.61 | 9.27 | 3.35 | 3.51 |
| XA | 15.00 - 6.80 | 13.44 | 5.46 | 4.80 |
| XB | 21.52 - 8.20 | 20.82 | 7.62 | 7.86 |
| XC | 36.41 - 13.89 | 31.65 | 11.23 | 13.23 |
| MA | 32.57 - 13.2 | 30.25 | 10.97 | 12.77 |
| MB | 31.60 - 17.7 | 26.83 | 9.84 | 9.43 |
| MC | 15.98 - 6.59 | 13.76 | 5.72 | 4.83 |

According to Gravelle et al. (2017) and Ibanescu et al. (2010), the crossover value can be an indication for the how much the product spreadability can be predicted where the lower crossover indicated better spreadability. When strain crosses the critical strain, the structure of the system starts to break down, and the relationship becomes nonlinear. The impact of strain on individual viscous and elastic component breakdown of a formulation can be measured from the nonlinear region. Beyond this

cross over, the G" was greater than G', indicating that the transition from elastic, solid-like behaviour to a viscous, liquid-like behavior.

Based on the observation, nanoemulgels studied behave like a solid at low stress. As the shear strain increases, both G′ and G″ decrease, and the material becomes progressively more fluid-like, and eventually G″ becomes greater than G′. The point where G′ is decreasing represents the onset of flow corresponding to the material's dynamic yield stress ($\sigma_0$). Therefore, in this research, all samples tested for the strains sweep for their viscoelastic properties showed a gel-like behavior indicating it is suitable for topical gel application.

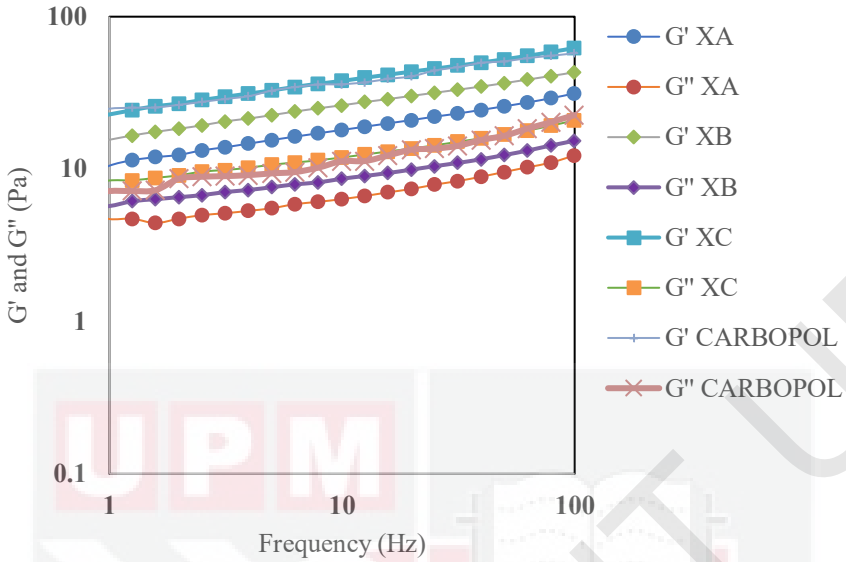
### 4.10.3 Viscoelastic Properties–Frequency Sweep

The frequency sweep tests were performed on nanoemulgels that form gel like namely, XA – XC and MA – MC, frequency set from 0.1 to 10 Hz. Figure 4.18 shows G' for all formulations were greater than G" throughout the frequency range test indicating the formulations were elastic, solid-like behavior was superior than the elastic, liquid like behavior. As the concentration of xanthan gum increased, both G' and G" became less frequency dependent indicating that a stable gel-like suspensions were formed. The XC has greater gel –like behavior and followed by MA, Carbopol, XB, MB, XA and MC. The gel properties imparted by the Xan-Gel complexes form a gel network that entraps the emulsion droplets in the continuous phase, thereby giving the high elastic properties to the emulsions.

Similar results were observed by Fagioli et al. (2018) who reported that xanthan gum in their research showed typical gel-like behaviour among many polysaccharide gums

used (glucomannan, tara gum, guar gum, konjac gum and gellan gum). The storage modulus (G') greater than loss modulus (G") upon frequency over the whole range tested. Liu et al. (2023) also concluded that xanthan gum stabilised sodium stearoyl lactylatein (SSL) at 0.5 - 4.0% concentrations used in their study exhibited viscoelastic properties with storage modulus (G') significantly higher than loss modulus (G") at frequencies tested.

(a)




(b)

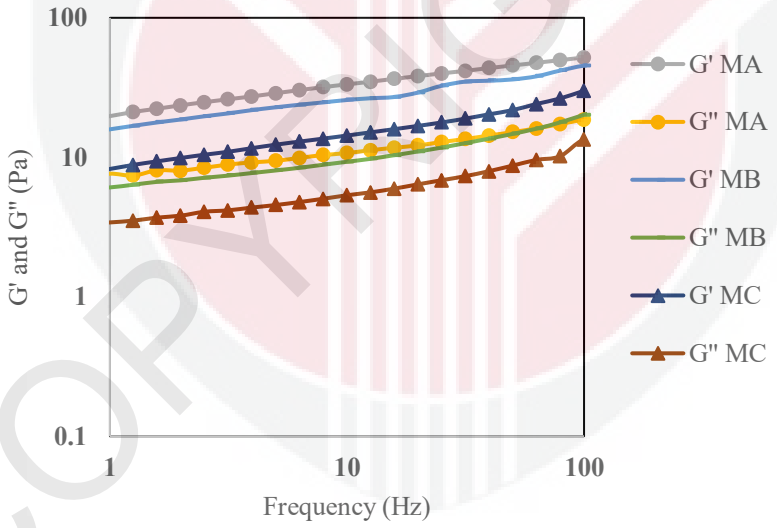



**Figure 4.18 : Frequency Sweep for (a) Carbopol, XA-XC and, (b) MA-MC**

## 4.11 Spreadability Test

Spreadability is one of the important feature for topical gel formulation as it determine how the gel readily spread upon application onto skin. Based on the result in Figure 4.19, it could be interpreted that the increment of gel concentration especially incorporated with xanthan gum as gelling agent concentration, the spreadability of the formulation decrease. This can be observed in carbopol, XA, XB, XC and MA formulations. Meanwhile, MB and MC were found to be significanly higher spreadability values in comparison to XC. Incorporation of higher percentage of xanthan gum having higher viscosity lowers the spreadability area. Generally, the viscosity of gel formulations reflects the consistency. The viscosity of these gels reduces as the shear rate increased, showed with non-Newtonian flow behaviour. This type of behaviour is preferred due to low flow resistance when there is high shear conditions applied. The pseudoplastic behaviour possessed as the viscosity decreased observed in all formulations when applying forces and able to remain at the application site without drain (Dantas et al., 2016).

This finding is congruent stated that a gel possess a spreadability more than 2.4 cm can be classified as fluid gel. From this finding it could be inferred that formulated nanoemulgel are in the form of fluid gel which could be easily spread over the skin area.

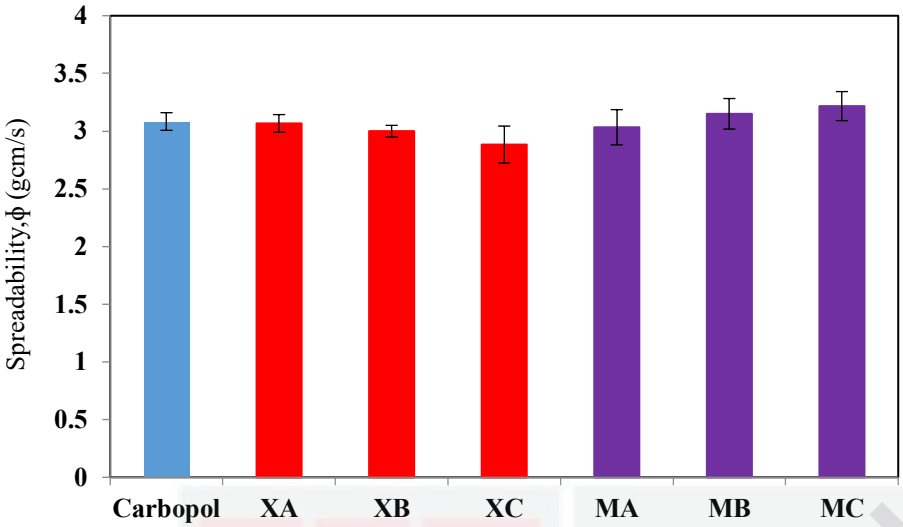



**Figure 4.19 : Spreadability of Carbopol and Nanoemulgel Formulations**

## 4.12 Transmission Electron Microscopy (TEM) Imaging

The physical properties of oil droplets in nanoemulsion (control) and nanoemulgel were microscopically investigated. Figure 4.20 (a) shows the positive image of oil droplet found in nanoemulsion appeared dark with bright surroundings. The regular spherical shape observed in the sample in the range 30 -50 nm and revealed a good size distribution. Figure 4.20 (b) – (d) represent the TEM image of JLE incorporated with Xan, Xan-Gel blend (MA) and Gel polymers, respectively. In Figure 4.20 (b) and Figure 4.20 (c), xanthan gum and MA were found to encapsulate the oil droplets and resulted in non-free movement in the polymer networks, respectively. The nanoemulsion added with the biopolymers remains within the three dimensional network.

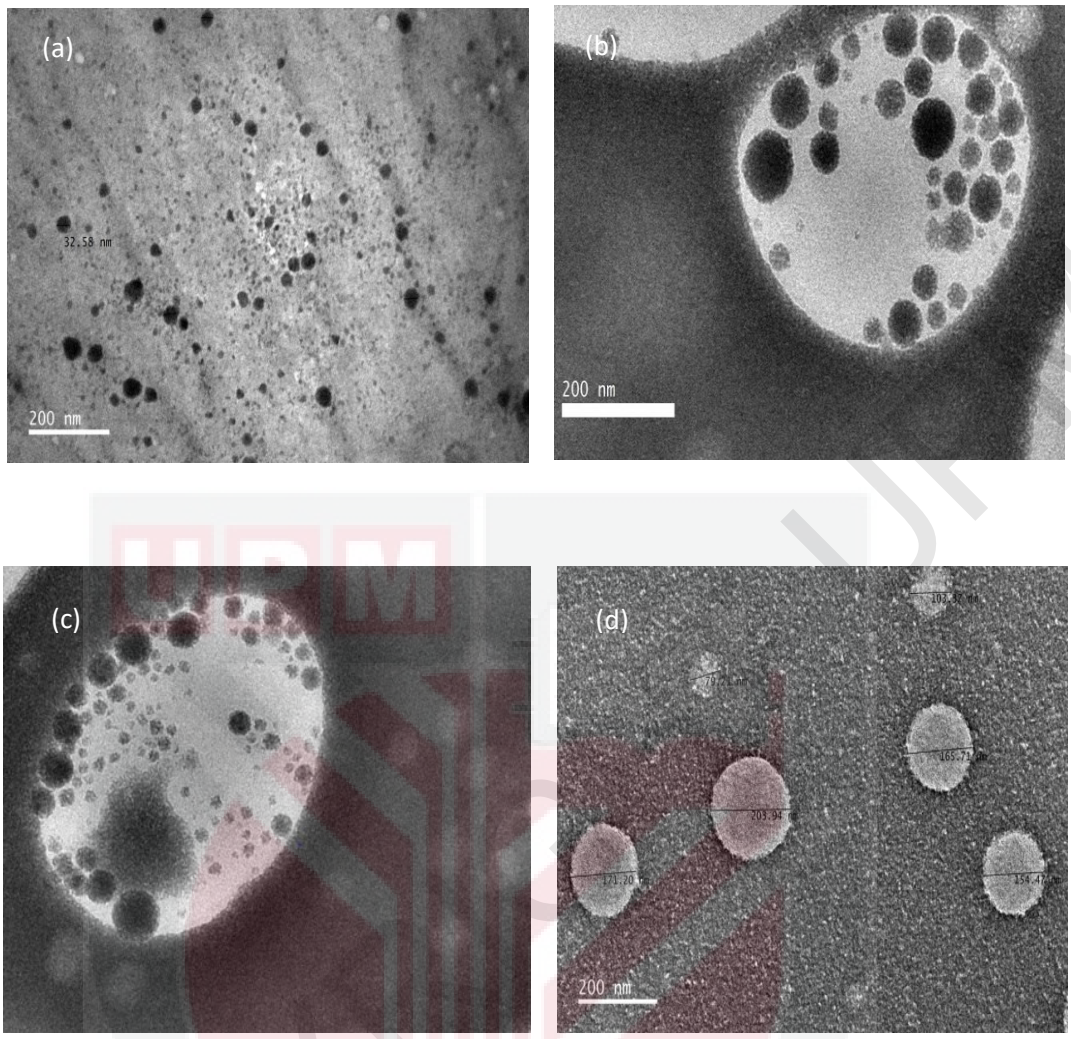



**Figure 4.20 : The TEM Images of (a) Nanoemulsion (NE), (b) XC, (c) MA and (d) Gel**

## 4.13 *In vitro* Release Testing Study (Permeation Study)

An investigation of JLE drug release from nanoemulgel was carried out using a semisynthetic cellulose acetate membrane with UV detection at 285 nm. The profile of drug release from the developed JLE-loaded formulations and suspension of JLE in phosphate buffer pH 7.4 was evaluated and the results are shown in Figure 4.21. The *in vitro* study exhibited permeation of 0.4534 ± 0.0069 mg/cm² in NE significantly higher ($p < 0.05$) than MA (0.4294 ± 0.0062 mg/cm²), followed by carbopol (0.3881 ± 0.0018 mg/cm²), XA (0.3859 ± 0.0038 mg/cm²), XB (0.3936 ± 0.0073 mg/cm²), MC

(0.3792 ± 0.0093 mg/cm²), MB (0.3758 ± 0.0036 mg/cm²) and XC (0.3674 ± 0.0014 mg/cm²) of drug permeation in 8 h. The enhancement the dissolution of bioactive compound into the aqueous phase could be due to the nanosize of the droplets which provide a large surface area for the drug, thus promotes the drug release from the formulation. Although the fabricated nanoemulgel formulations found to exhibit lower percentage release in comparison to the nanoemulsion, the gel network in nanoemulgel promote sustain release of drug profile due to the three dimensional hydrogel matrix and high viscosity of the gel (Mulleria et al., 2021).

Comparative study shows that xanthan gum concentration increases (XA –XC) the cumulative amount of drug release decreases ($p < 0.05$). As the xanthan gum content increase, the water decreases its diffusibility into polymeric network and thus the release rate of drug also decrease. In this study, the oil phase (Palmester) also used was employed as an integral component of the formulation which can help in permeate within skin lipid bilayer by disrupting their order and management. The enhancement mechanism by the Palmester increased the fluidity of lipid portion of the stratum corneum (Dhawan et al., 2014).

There is a significantly higher artocarpin drug release was released from MA compared to that released from XA – XC nanoemulgels and carbopol ($p < 0.05$) observed, owing to the polymer ratio used (2.0% Xan/ 0.5% Gel) used, which eases the release of the drug into the release media. Alternatively, the higher viscosity of XC, due to the lower water content, could lead to slow diffusion of the entrapped drug thus causing its slower permeation rate. This illustrates the significant lower release of artocarpin from XC compared to the release from other gel formulations studied

(XA-XB). In the case of Xan-Gel mixture, it can be found that with the addition of 0.5% fish gelatin into the formulation (MA), it increases the release rate of drug studied. Co-polymerization of the adequate amount fish gelatin into xanthan gum enhanced the increase of swelling behavior of the formulation. Thus it helps to release the drug at higher rate in comparison to MB and MC. Slower drug release rate was exhibited for MB and MC in this 8 hours drug permeation study could be assumed that by adding more gelatin, it could result in the aggregation complexes. Previous study (Bejrapha et al. 2011) studied the role of gelatin in the stability of capsicum oleoresin nanocapsules in gelatin matrix. The authors verified that an adequate amount of gelatin should be used because, a relatively low gelatin concentration or a relatively high could not be suitable due to its limited solubility, resulting in the aggregation of complexes when gelatin concentration increased.

Fabricated nanoemulgel, MA has the highest drug release rate value among other formulated nanoemulgels could be favoured over NE, due to sustain release effect and increased viscosity from the point of view of its skin application. This mechanism is desirable for topical formulation that provide better spreadability as the effect of dosing of the drug by rubbing action when thus lead to better absorption when applied onto skin (Dhawan et al., 2014).

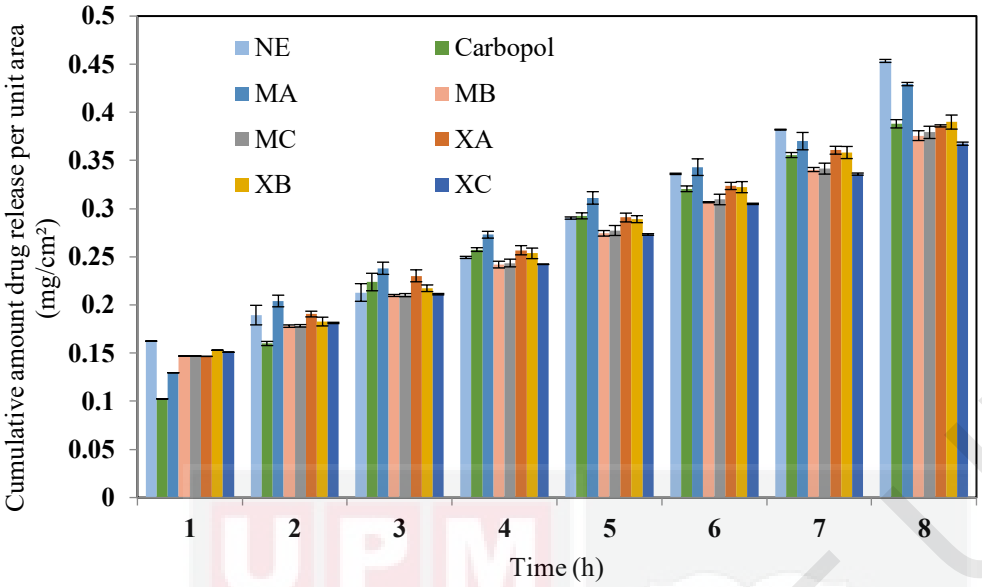



**Figure 4.21 : Cumulative Amount Drug Release Per Unit Area**

Data from Figure 4.21 were extracted to tabulate the permeation parameters; flux and permeability coefficient as presented in Table 4.13. It was observed that the drug release (JLE containing artocarpin) varied on changing the Xan-Gel ratio released in the receptor compartment at the 8 hours of drug permeation. Statistical comparison of the flux showed NE has significantly higher flux, Jss (0.050 ± 0.002 mg/cm²/h) and permeability coefficient, Kp (7.142 x $10^{-4}$ cm/h) ($p < 0.05$) as compared to formulated nanoemulgels and carbopol. Among six formulated nanoemulgels (MA-MC and XA-XC), MA possessed the highest flux and permeability coefficient with values of 0.037 ± 0.002 mg/cm²/h and 5.285 x $10^{-4}$ cm/h respectively.

**Table 4.13 : Permeation Parameters of the Nanoemulsion, Carbopol and Formulated Nanoemulgels**

| Formulations | Flux, Jss mg/cm²/h | Permeability Coefficient, Kp ($10^{-4}$, cm/h) |
|---|---|---|
| NE | 0.050 ± 0.002 | 7.142 |
| Carbopol | 0.032 ± 0.000 | 4.571 |
| MA | 0.037 ± 0.002 | 5.285 |
| MB | 0.033 ± 0.001 | 4.714 |
| MC | 0.033 ± 0.001 | 4.714 |
| XA | 0.032 ± 0.001 | 4.571 |
| XB | 0.034 ± 0.001 | 4.857 |
| XC | 0.031 ± 0.000 | 4.428 |

It was reported in previous works (Elmateeshy et al., 2018 and Dhawan et al., 2014) that nanoemulgel could provide as a drug reservoir where the drug was released from inner to outer phase and further into human skin. The drug delivery enhancement may be resulted from different mechanism, which include the enhancement of potential different components added (types of oil, polymer and surfactants) of nanoemulgels permeated. Reduced penetration rate release of nanoemulgels with MA might be preferred over NE owing to it's prolong contact time and greater viscosity when applied to the skin.

Comparing the cumulative drug release outcome between the six nanoemulgels (XA-XC and MA-MC), it could be inferred that there is a decreasing trend of drug release with increasing concentration of xanthan gums. This is especially notable at the 8 h of drug release. It could be noted that the cumulative drug release decreases from XA to XC correspond to their increasing xanthan gum concentration (Figure 4.21). However, the results of permeation rates in nanoemulgels containing polymer mixtures (MA-MC) were in contrast of XA-XC in which the xanthan gum added in increasing manner. It can be observed that MA containing 2.0% xanthan gum and 0.5% gelatin releases drug higher than XC (2.5% xanthan gum added) based on the total amount of

polymer added at maximum 2.5% which could be correlated to the interaction between polysaccharide-protein polymer exhibit a lower viscosity than XC and thus, is able to showcase a higher drug release (Yeo et al., 2021 and Dhawan et al., 2014). The decrease in release rate in XC as compared to MA could be explained by the fact that the drug-loaded oil droplets are coated by the polymeric surface, and thus the drug had to travel a long and tortuous pathway to reach to the release media, thereby the release of the formulations was decreased in successive formulations. Due to highest permeation rates among formulated gel studied, MA was chosen to fit into mathematical models for kinetic release rate.

Mathematical modeling is an important aspect to analyse the release kinetic of a drug from formulation. In determining the suitable model for fitting dissolution data, it is crucial not only for quantitative evaluation of drug release characteristics but also for comparison of drug release profiles using model-dependent approaches. Using 5 mathematical models for determining the drug release profile, it provides statistical criteria for evaluating the goodness of fit of a model. The result obtained from MA was employed to five different kinetic models namely zero order model, first order model, Higuchi model, Korsmeyer-Peppas and Hixson Crowell models (Figure 4.22 – Figure 4.26).

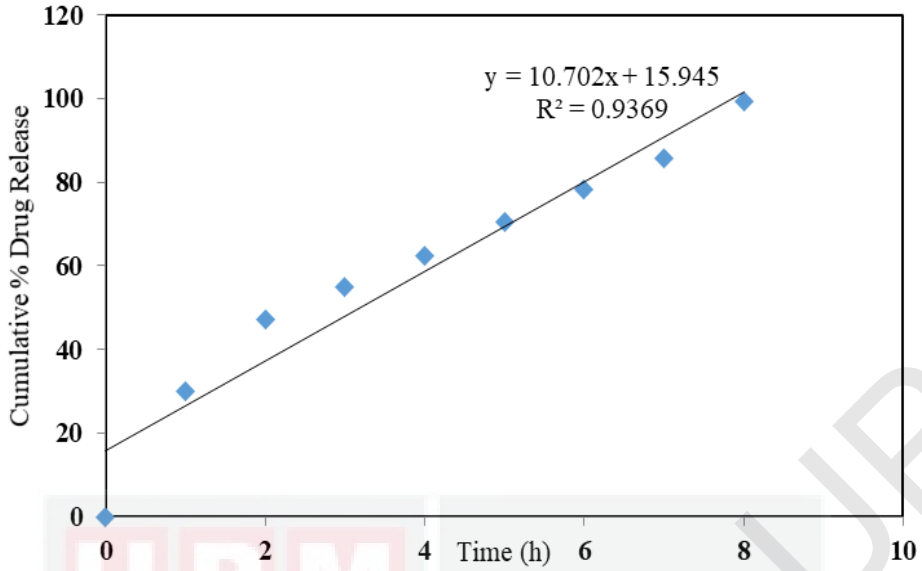



**Figure 4.22 : Graph of Zero Order Model of MA**

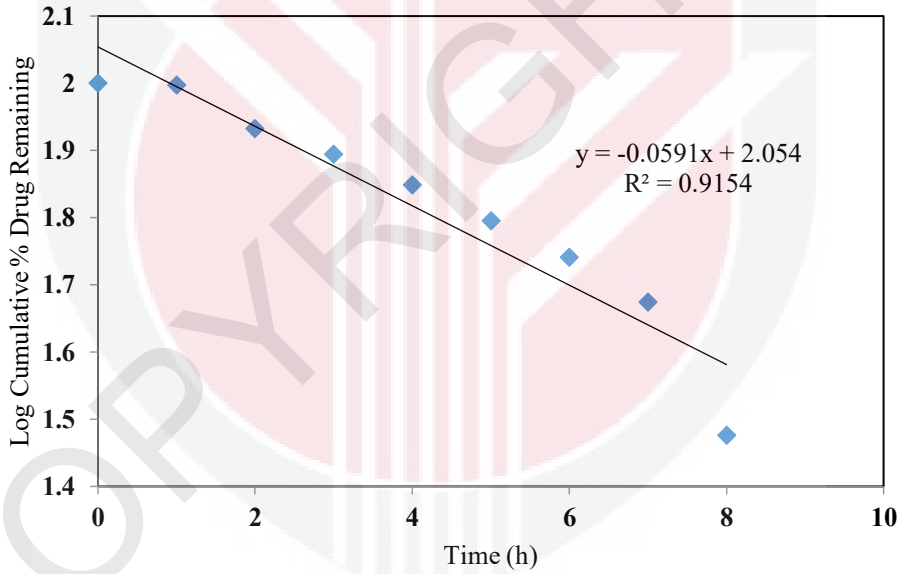



**Figure 4.23 : Graph of First Order Model of MA**

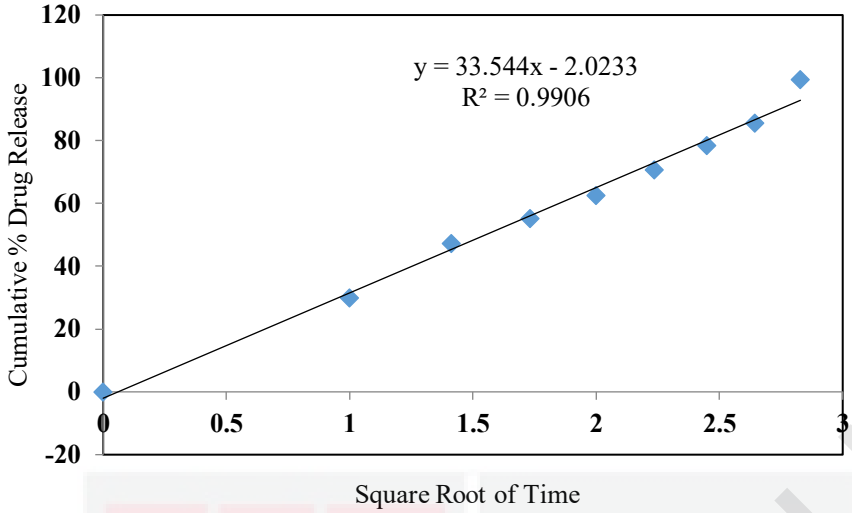



**Figure 4.24 : Graph of Higuchi Model of MA**

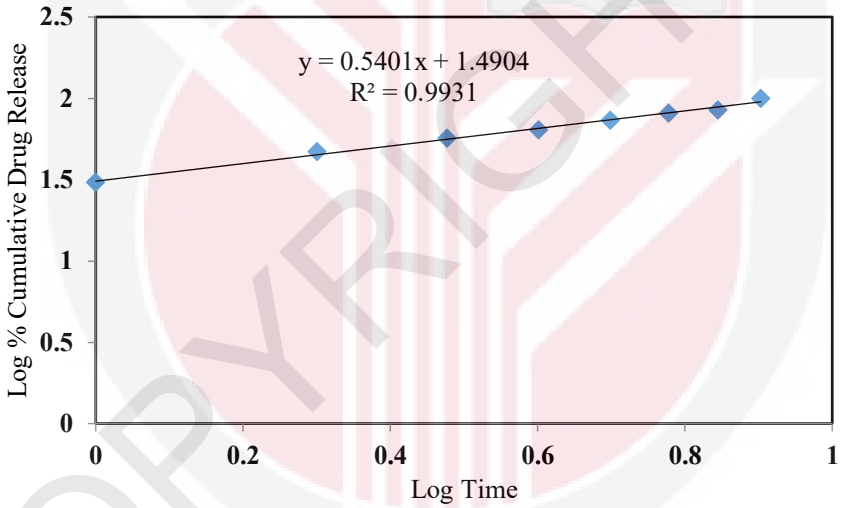



**Figure 4.25 : Graph of Korsmeyer-Peppas Model of MA**

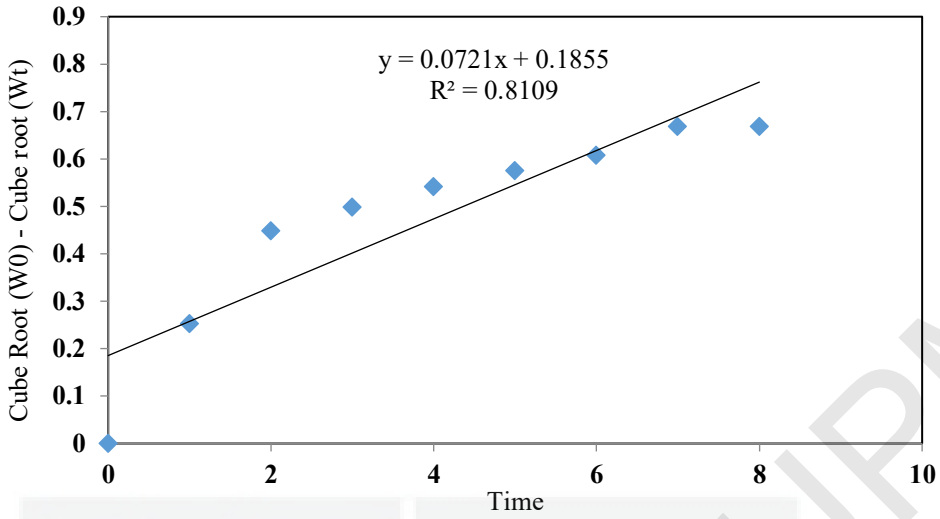



**Figure 4.26 : Graph of Hixson-Crowell Model of MA**

Based on Table 4.14 and graphs (Figure 4.22 - Figure 4.26) generated, it can be concluded that graph MA obeyed the Korsmeyer-Peppas model with $R^2$ value nearer to one (0.9930) indicating a good fit (Abdelwahd et al., 2022). An initial "burst release" is often observed in polymer matrix-based formulation, where the drug release usually occur either through diffusion or erosion of the matrix (Lu et al., 2011). The mentioned initial "burst release" is observed in Figure 4.21 in the first 1 h of drug release. Korsmeyer-Peppas model is one of the kinetic models that could be used to analyse or interpret a drug release mechanism in which diffusion dominates the drug release pattern (Siepmann and Peppas, 2011). To further confirm the diffusion behaviour and mechanism, data that was fitted into the Korsmeyer-Peppas model, generated "n value" in Equation 3.16 which helps to determine the release pattern. This depends on the release exponent "n" which was estimated from the linear regression, whereby $n = 0.45$ indicates Fickian diffusion, $0.45 < n < 0.89$ as non-Fickian transport, $n = 0.89$ as case-II transport and $n > 0.89$ indicates super case-II transport (Dash et al., 2010).

**Table 4.14 : $R^2$ Values of MA Fitted into 5 Mathematical Models**

| Mathematical model | $R^2$ |
|---|---|
| Zero order | 0.9360 |
| First order | 0.9150 |
| Higuchi | 0.9900 |
| Korsmeyer-Peppas | 0.9930 |
| Hixson-Crowell | 0.8100 |

The present results showed that MA follows non-Fickian release mechanism with n value of 0.540 obtained from the slope of the graph in Figure 4.25 which represent the release of artocarpin from MA will be through diffusion and erosion (Yeo et al., 2021; and Jones et al., 2019). Thus on the basis of the release rate studies, it can be said that the drug is governed by the diffusional path length and the gel strength ratio of xanthan gum and fish gelatin formed in of the polymer matrix. Diffusional path length of being contributed by the swelling of Xan-Gel polymer complexes. The matrix of polymer is swelled and degraded and the latter part of the drug release is governed by the erosion of the polymers (Rabin and Siege, 2012). The mechanism of diffusion and erosion of polymer matrix were also reported by Agarwal and Murthy (2015) who observed the effect of the polymer ratio used and obeyed Korsmeyer-Peppas model suggesting the drug release rate exhibited diffusion and erosion characteristics of the polymer matrix. They concluded that the drug release rate occurs through the gel barrier depends on the formation and viscosity of gel layer, the extent of swelling and the erosion of the polymer chains. A predictive drug loaded nanoemulgel model illustrating the behavior of drug diffusion and polymer erosion characteristics is shown in Figure 4.27.

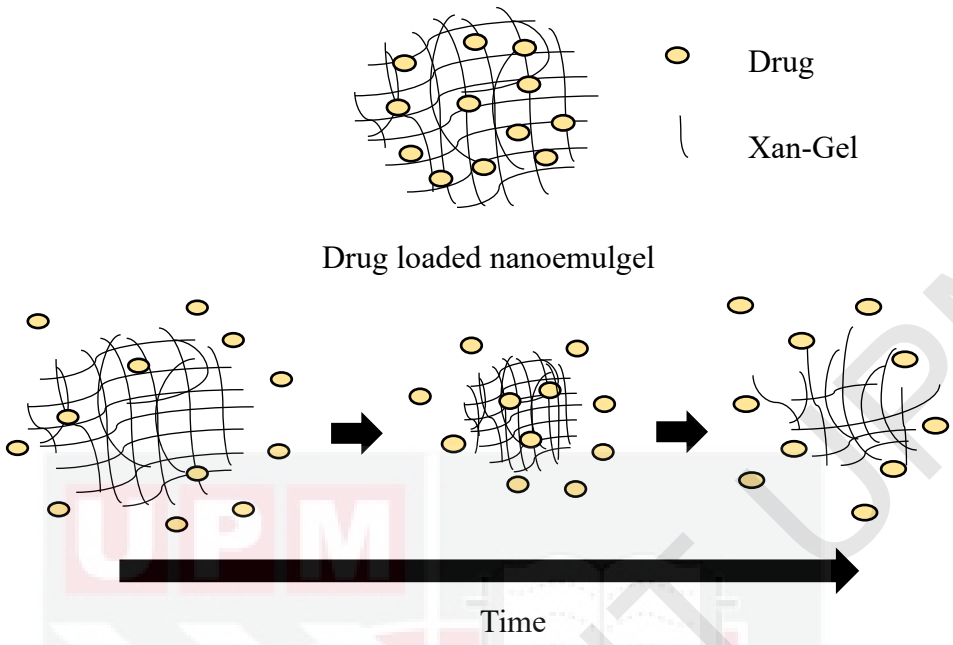



**Figure 4.27 : Predictive Model of Drug Diffusion and Polymer Erosion of MA**

Although the interaction of nanoemulsion with human skin depends on the electrical charge of the droplets, other factors such as composition of the formulation could have also enhanced the permeation of JLE drug through the skin. Therefore, the enhancement of permeation attributed by integration of the composition of the nanoemulgel system (such as the Xan-Gel ratio, Palmester and surfactants) used will help to consider best formulated gel, MA for future studies.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH

## 5.1 Conclusion

The impact on drying kinetic, colour and antioxidant properties of jackfruit leaves was observed. The *Artocarpus heterophyllus* Lam. leaves were dried under three conditions namely, shade (SD), sun (SN), and oven (OV). Results showed that Newton model was found to be the best fitted mathematical model for the jackfruit leaves (JL) drying kinetics. The moisture effective diffusivities ranged from 2.920 x $10^{-10}$ to 6.814 x $10^{-10}$ $m^2/s$ over the temperature range studied. Shade drying was able to preserve the green pigment better than OV and SN drying treatment. Ethanol/water ratio at 80% dried in oven (OV80) revealed the highest antioxidant properties of TPC (195.05 ± 1.21 mg GAE/g EW), TFC (11.02 ± 0.17 mg AE/g EW), DPPH (90% scavenging activity) and FRAP (1043.84 ± 5.28 μM TE/g EW) as compared to SD and SN treatments. OV80 also possessed the highest artocarpin, squalene and β-sitosterol contents determined.

Nanoemulsion based gel or known as nanoemulgel was developed by adding 1:1 ratio of nanoemulsion reinforced with xanthan gum/fish gelatin polymers studied. Based on the screening of components to formulate nanoemulsion, jackfruit leaves extract (JLE) works best in 10% Palmester oil, 10% surfactant mixture (smix) of T80:S80 and the remaining is water. Development of nanoemulsion system requires a stable and good solubility of the components which then further development of nanoemulgel. Result showed that nanoemulgel produced have nano size emulsion (<500 nm), low PDI (<0.3), low ζ-potential values and present in mild acid condition that is good for

topical application. Nanoemulgel also able to remain unchanged thorughout stability test period. Nanoemulgel exhibited desirable rheological properties with a shear thinning behavior (n<1) and throughout the frequency range test with G' for all formulations were greater than G" indicating the formulations were elastic, solid-like behavior was superior to the elastic, liquid like behavior. Nanemulsion added with polymer mixture, MA produces better spreadability followed by single xanthan gum formulation. Better spreadability indicates better spreading and rubbing experience of the topical products on the skin surface, thus creating a better absorption into the skin. Under microscopic view, oil droplet was observed to be surrounded by the gel promoting stability and hinder free movement formulation.

The nanoemugels were tested for the ability of permeation and results showed that nanoemulgel exhibited a sustain release profile in comparison to nanoemulsion. This property is desirable for the pharmaceutical industry such as gel and cream. The drug kinetic release result revealed that the best formulation, MA obeys the Korsmeyer-Peppas model for topical drug delivery system in this study. The MA also possessed highest amount artocarpin released ($0.4294 \pm 0.0062$ mg/cm$^2$), permeation rate, flux ($0.037 \pm 0.002$ mg/cm$^2$/h) and permeability coefficient ($5.285 \times 10^{-4}$, cm/h) among other developed nanoemulgel formulations.

## 5.2 Recommendations for Future Work

1. Drying treatments could be extended to other method such as indoor drying method that similar to industrial application for the jackfruit leaves such as tray drying. Isolating the antioxidant component and incorporate into the drug formulation can be applied for future works. On the other hand, the extraction of JL could be explored with other techniques such as solid-phase

extraction (SPE). It would be worth to explore other drying methods and extraction methods in order to compare and contrast on the yield and valuable properties such as the antioxidant properties.

2. Production of nanoemulsion can be discover through high energy methods such as using high pressure homogeniser (HPH) and high shear homogeniser (HSH) as it require less time to produce one. In this study, nanoemulgel was successfully developed using one of the low energy methods; spontaneous emulsification. Though it may not require a high technology equipment, however, by manipulation the nanoemulgel components such as surfactant, oil and water content, other low energy method could be applied as it may give valuable information on its characteristics that worth to be explored.

3. The permeability of drug can be further studied using rat skin as a model to know in depth of the permeation and absorption information when applied onto skin. The microbiology and toxicity test also recommended for future work towards commercialisation of product.

## REFERENCES


Abbas, S., Karangwa, E., Bashari, M., Hayat, K., Hong, X., Sharif, H. R., and Zhang, X. (2015). Fabrication of polymeric nanocapsules from curcumin-loaded nanoemulsion templates by self-assembly. *Ultrasonics Sonochemistry* 23: 81-92.

Abbasi, S., and Radi, M. (2016). Food grade microemulsion systems: Canola oil/lecithin: n-propanol/water. *Food Chemistry* 194: 972-979.

Abbasian Chaleshtari, Z., Zhou, M., and Foudazi, R. (2022). Nanoemulsion polymerization and templating: Potentials and perspectives. *Journal of Applied Physics* 131(15).

Abd Aziz N.B., Milan A.R., Razali M.Z. (2016) Fruit morphology description of seven jackfruit clones from farmers collection. In: Yacob N., Mohamed M., Megat Hanafiah M. (eds) Regional Conference on Science, Technology and Social Sciences (RCSTSS 2014). Springer, Singapore.

Abdelwahd, A., and Rasool, B. K. A. (2022). Optimising and evaluating the transdermal permeation of hydrocortisone transfersomes formulation based on digital analysis of the *in vitro* drug release and ex vivo studies. *Recent Advances in Drug Delivery and Formulation* 16(2): 122.

Agarwal, S., and Murthy, R. S. R. (2015). Effect of different polymer concentration on drug release rate and physicochemical properties of mucoadhesive gastroretentive tablets. *Indian Journal of Pharmaceutical Sciences* 77(6), 705.

Agbede, O.O., Oke, E.O., Akinfenwa, S.I., Wahab, K.T., Ogundipe, S., Aworanti, O.A., Arinkoola, A.O., Agarry, S.E., Ogunleye, O.O., Osuolale, F.N., Babatunde, K.A., 2020. Thin layer drying of green microalgae (Chlorella sp.) paste biomass: drying characteristics, energy requirement and mathematical modeling. *Bioresource Technology Reports* 11: 100467.

Ahmad, J., Gautam, A., Komath, S., Bano, M., Garg, A., and Jain, K. (2019). Topical nano-emulgel for skin disorders: Formulation approach and characterisation. *Recent Patents on Anti-infective Drug Discovery* 14 (1): 36-48.

Ahmad, M. Z., Mohammed, A. A., and Mokhtar Ibrahim, M. (2017). Technology overview and drug delivery application of proniosome. *Pharmaceutical Development and Technology* 22(3): 302-311.

Ahmadi, O., and Jafarizadeh-Malmiri, H. (2021). Intensification process in thyme essential oil nanoemulsion preparation based on subcritical water as green solvent and six different emulsifiers. *Green Processing and Synthesis* 10(1): 430-439.

Akpinar, E.K., Bicer, Y. and Yildiz, C. (2003) Thin-layer drying of red pepper. *Journal of Food Engineering* 59: 99-104.

Alara, O. R., Abdurahman, N. H., Kholijah, S., and Mudalip, A. (2018). "Mathematical modeling of thin layer drying using open sun and shade of *Vernonia amygdalina* leaves. *Agriculture and Natural Resources* 52(1): 53–58.

Alfaro-Rodríguez, M. C., Prieto, P., García, M. C., Martín-Piñero, M. J., and Munoz, J. (2022). Influence of nanoemulsion/gum ratio on droplet size distribution, rheology and physical stability of nanoemulgels containing inulin and omega-3 fatty acids. *Journal of the Science of Food and Agriculture* 102(14): 6397-6403.

Ali, M. A., Yusof, Y. A., Chin, N. L., Ibrahim, M. N., and Basra, S. M. A. (2014). Drying kinetics and colour analysis of Moringa oleifera leaves. *Agriculture and Agricultural Science Procedia* 2: 394-400.

Ali, M. S., Alam, M. S., Alam, N., Anwer, T., and Safhi, M. M. A. (2013). Accelerated stability testing of a clobetasol propionate-loaded nanoemulsion as per ICH guidelines. *Scientia Pharmaceutica* 81(4): 1089-1100.

Ali, M., Mir, S., Ajlouni, A., Alvi, S. G., and Bibi, S. (2023). Applications of chitosan based bionanocomposites in drug-delivery and anticancer treatment-a review. *European Polymer Journal* 201: 112576.

Alothman, M., Bhat, R., and Karim, A. A. (2009). "Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry* 115(3): 785–788.

Alves, T. F. R., Morsink, M., Batain, F., Chaud, M. V., Almeida, T., Fernandes, D. A., da Silva, C. F., et al. (2020). Applications of natural, semi-synthetic, and synthetic polymers in cosmetic formulations. *Cosmetics* 7(4): 75.

Amadi, J. A., Ihemeje, A., and Afam-Anene, O. C. (2018). Nutrient and phytochemical composition of jackfruit (*Artocarpus heterophyllus*) pulp, seeds and leaves. *International Journal of Innovative Food, Nutrition and Sustainable Agriculture* 6(3): 27-32.

Ambawat, S., Sharma, A., and Saini, R. K. (2022). Mathematical modelling of thin layer drying kinetics and moisture diffusivity study of pretreated moringa oleifera leaves using fluidised bed dryer. *Processes* 10(11): 2464.

Ammar, H. O., Ghorab, M. M., Mostafa, D. M., Abd El-Alim, S. H., Kassem, A. A., Salah, S., and Shalaby, E. S. (2020). Development of folic acid–loaded nanostructured lipid carriers for topical delivery: preparation, characterisation and ex vivo investigation. *Journal of Microencapsulation* 37(5): 366-383.

Anand, K., Ray, S., Rahman, M., Shaharyar, A., Bhowmik, R., Bera, R., and Karmakar, S. (2019). Nano-emulgel: emerging as a smarter topical lipidic emulsion-based nanocarrier for skin healthcare applications. *Recent Patents on Anti-Infective Drug Discovery* 14(1): 16-35.

Anita, C., Munira, M., Mural, Q., and Shaily, L. (2021). Topical nanocarriers for management of Rheumatoid Arthritis: A review. *Biomedicine and Pharmacotherapy* 141: 111880.

Anton, N., Akram, S., and Vandamme, T. F. 2018. Transitional nanoemulsification methods. In *Nanoemulsions*, pp. 77-100. Academic Press.

Anvari, M., and Chung, D. (2016). Dynamic rheological and structural characterisation of fish gelatin–gum Arabic coacervate gels cross-linked by tannic acid. *Food Hydrocolloids* 60: 516-524.

Arianto, A., Lie, D. Y. L., and Bangun, H. B. (2020). Preparation and evaluation of nanoemulgels containing a combination of grape seed oil and anisotriazine as sunscreen. *Open Access Macedonian Journal of Medical Sciences* 8(B): 994-999.

Arora, R., Aggarwal, G., Harikumar, S. L., and Kaur, K. (2014). Nanoemulsion based hydrogel for enhanced transdermal delivery of ketoprofen. *Advances in Pharmaceutics* 2014: 1-12.

Arora, S., Sharma, N., Mahajan, A., Kaur, J., and Singh, S. (2015). Development, physicochemical characterisation and in-vitro evaluation of herbal sunscreen lotion. *Journal of Pharmaceutical Technology, Research and Management* 3(2): 113-125.

Arslan, D., and Ozcan, M. M. (2010). Study the effect of sun, oven and microwave drying on quality of onion slices. *LWT – Food Science and Technology* 43: 1121-1127.

Ay Şenyiğit, Z., Coşkunmeriç, N., Çağlar, E. Ş., Öztürk, İ., Atlıhan Gündoğdu, E., Siafaka, P. I., and Üstündağ Okur, N. (2021). Chitosan-bovine serum albumin-Carbopol 940 nanogels for mupirocin dermal delivery: ex-vivo permeation and evaluation of cellular binding capacity via radio labeling. *Pharmaceutical Development and Technology* 26(8): 852-866.

Ayensu, A. (1997). Dehydration of food crops using a solar dryer with convective heat flow. *Solar Energy* 59(4-6): 121-126.

Babu, A. K., Kumaresan, G., Aroul, V. A., and Velraj, R. (2018). Review of leaf drying: Mechanism and influencing parameters, drying methods, nutrient preservation, and mathematical models. *Renewable and Sustainable Energy Reviews* 90: 536–556.

Baliga, M. S., Shivashankara, A. R., Haniadka, R., Dsouza, J., and Bhat, H. P. (2011). Phytochemistry, nutritional and pharmacological properties of *Artocarpus heterophyllus* Lam (jackfruit): A review. *Food Research International* 44(7): 1800-1811.

Barclay, T. G., Day, C. M., Petrovsky, N., and Garg, S. (2019). Review of polysaccharide particle-based functional drug delivery. *Carbohydrate Polymers* 221: 94-112.

Barimah, J., Yanney, P., Laryea, D., and Quarcoo, C. (2017). Effect of drying methods on phytochemicals, antioxidant activity and total phenolic content of dandelion leaves. *American Journal of Food and Nutrition* 5(4): 136-141.

Barradas, T. N., de Campos, V. E. B., Senna, J. P., Coutinho, C. D. S. C., Tebaldi, B. S., e Silva, K. G. D. H., and Mansur, C. R. E. (2015). Development and characterisation of promising o/w nanoemulsions containing sweet fennel essential oil and non-ionic sufactants. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 480: 214-221.

Barradas, T. N., Senna, J. P., Cardoso, S. A., e Silva, K. G. D. H., and Mansur, C. R. E. (2018). Formulation characterisation and *in vitro* drug release of hydrogel-thickened nanoemulsions for topical delivery of 8-methoxypsoralen. *Materials Science and Engineering: C* 92: 245-253.

Behera, B., and Balasubramanian, P. (2021). Experimental and modelling studies of convective and microwave drying kinetics for microalgae. *Bioresource Technology* 340: 125721.

Bejrapha, P., Surassmo, S., Choi, M. J., Nakagawa, K., and Min, S. G. (2011). Studies on the role of gelatin as a cryo-and lyo-protectant in the stability of capsicum oleoresin nanocapsules in gelatin matrix. *Journal of Food Engineering* 105(2): 320-331.

Belessiotis, V., and Delyannis, E. (2011). Solar drying. *Solar Energy* 85(8): 1665 – 1691.

Bento, E. B., de Brito Júnior, F. E., de Oliveira, D. R., Fernandes, C. N., de Araújo Delmondes, G., Cesário, F. R. A. S., and Kerntopf, M. R. (2018). Antiulcerogenic activity of the hydroalcoholic extract of leaves of *Annona muricata Linnaeus* in mice. *Saudi Journal of Biological Sciences* 25(4): 609-621.

Bergfreund, J., Siegenthaler, S., Lutz-Bueno, V., Bertsch, P., and Fischer, P. (2021). Surfactant adsorption to different fluid interfaces. *Langmuir* 37(22): 6722-6727.

Bhattacharjee, S. (2016). DLS and zeta potential–what they are and what they are not?. *Journal of Controlled Release* 235: 337-351.

Biai, C. (2022). *Initiatives to Develop the Jackfruit as A Crop For Income Generation In Malaysia'*. Prepared by Crop Industry Development Division, Department of Agriculture, Malaysia in Report on The International Webinar On 'Developing The Jackfruit for Global Consumption and Markets', International Tropical Fruits Network (TFNet).

Bouyer, E., Mekhloufi, G., Rosilio, V., Grossiord, J. L., and Agnely, F. (2012). Proteins, polysaccharides, and their complexes used as stabilisers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field. *International Journal of Pharmaceutics* 436(1-2): 359-378.

Bruschi, M. L. (2015). Mathematical Models of Drug Release In Strategies to Modify The Drug Release From Pharmaceutical Systems (pp 64-86). Woodhead Publishing.

Calderó, G., Montes, R., Llinàs, M., García-Celma, M. J., Porras, M., and Solans, C. (2016). Studies on the formation of polymeric nano-emulsions obtained via low-energy emulsification and their use as templates for drug delivery nanoparticle dispersions. *Colloids and Surfaces B: Biointerfaces* 145: 922-931.

Calderón-Chiu, C., Calderón-Santoyo, M., Damasceno-Gomes, S., and Ragazzo-Sánchez, J. A. (2021). Use of jackfruit leaf (*Artocarpus heterophyllus* L.) protein hydrolysates as a stabiliser of the nanoemulsions loaded with extract-rich in pentacyclic triterpenes obtained from *Coccoloba uvifera* L. leaf. *Food Chemistry* 12: 100138.

Campani, V., Biondi, M., Mayol, L., Cilurzo, F., Pitaro, M., and De Rosa, G. (2016). Development of nanoemulsions for topical delivery of vitamin K1. *International Journal of Pharmaceutics* 511(1): 170-177.

Cardoso, S. A., and Barradas, T. N. (2020). Developing formulations for drug follicular targeting: Nanoemulsions loaded with minoxidil and clove oil. *Journal of Drug Delivery Science and Technology* 59: 101908.

Carter, P., Narasimhan, B., and Wang, Q. (2019). Biocompatible nanoparticles and vesicular systems in transdermal drug delivery for various skin diseases. *International Journal of Pharmaceutics* 555: 49-62.

Catarino, M. D., Silva, A. M. S., Saraiva, S. C., Sobral, A. J. F. N., and Cardoso, S. M. (2018). Characterisation of phenolic constituents and evaluation of antioxidant properties of leaves and stems of *Eriocephalus africanus*. *Arabian Journal of Chemistry* 11(1): 62–69.

Chakravarthi, S. S., De, S., Miller, D. W., and Robinson, D. H. (2010). Comparison of anti-tumor efficacy of paclitaxel delivered in nano-and microparticles. *International journal of pharmaceutics* 383(1-2): 37-44.

Chan, E. W. C., Wong, S. K., Tangah, J., and Chan, H. T. (2018). Chemistry and pharmacology of Artocarpin: An isoprenyl flavone from Artocarpus species. *Systematic Reviews in Pharmacy* 9(1): 58-63.

Chandra, A., Arya, R. K. K., Pal, G. R., and Tewari, B. (2019). Formulation and evaluation of ginger extract loaded nanoemulgel for the treatment of rheumatoid arthritis. *Journal of Drug Delivery and Therapeutics* 9(4): 559-570.

Chang, Y., and McClements, D. J. (2014). Optimisation of orange oil nanoemulsion formation by isothermal low-energy methods: influence of the oil phase, surfactant, and temperature. *Journal of Agricultural and Food Chemistry* 62(10): 2306-2312.

Che Sulaiman, I. S., Basri, M., Fard Masoumi, H. R., Ashari, S. E., Basri, H., and Ismail, M. (2017). Predicting the optimum compositions of a transdermal nanoemulsion system containing an extract of *Clinacanthus nutans* leaves (L.) for skin antiaging by artificial neural network model. *Journal of Chemometrics* 31(7): 1-13.

Cheenkachorn, K., Paulraj, M. G., Tantayotai, P., Phakeenuya, V., and Sriariyanun, M. (2022). Characterization of biologically active compounds from different herbs: Influence of drying and extraction methods. *Journal of the Indian Chemical Society* 99(1): 100297.

Chen, F., Huang, G., Yang, Z., and Hou, Y. (2019). Antioxidant activity of Momordica charantia polysaccharide and its derivaties. *International Journal of Biological Macromolecules* 138: 673 -680.

Cheng, Z., Chen, X., Zhai, D., Gao, F., Guo, T., Li, W., and Wang, B. (2018) Development of keratin nanoparticles for controlled gastric mucoadhesion and drug release. *Journal of Nanobiotechnology* 16: 1-13 .

Choudhury, H., Gorain, B., Pandey, M., Chatterjee, L. A., Sengupta, P., Das, A., and Kesharwani, P. (2017). Recent update on nanoemulgel as topical drug delivery system. *Journal of Pharmaceutical sciences* 106 (7): 1736-1751.

Chua, L. Y. W., Chua, B. L., Figiel, A., Chong, C. H., Wojdyło, A., Szumny, A., and Choong, T. S. Y. (2019). Antioxidant activity, and volatile and phytosterol contents of *Strobilanthes crispus* dehydrated using conventional and vacuum microwave drying methods. *Molecules* 24: 1–21.

Chua, L. Y. W., Chua, B. L., Figiel, A., Chong, C. H., Wojdyło, A., Szumny, A., and Łyczko, J. (2019). Drying of *phyla nodiflora* leaves: antioxidant activity, volatile and phytosterol content, energy consumption, and quality studies. *Processes* 7(210): 24.

Chuesiang, P., Siripatrawan, U., Sanguandeekul, R., McLandsborough, L., and McClements, D. J. (2018). Optimisation of cinnamon oil nanoemulsions using phase inversion temperature method: Impact of oil phase composition and surfactant concentration. *Journal of Colloid and Interface Science* 514: 208-216.

Claux, O., Santerre, C., Abert-Vian, M., Touboul, D., Vallet, N., and Chemat, F. (2021). Alternative and sustainable solvents for green and analytical chemistry. *Current Opinion in Green Sustainable Chemistry* 31: 100510.

Combrinck, J., Otto, A., and Du Plessis, J. (2014). Whey protein/polysaccharide-stabilised emulsions: Effect of polymer type and pH on release and topical delivery of salicylic acid. *AAPS Pharmscitech* 15(3): 588-600.

Coondoo, A., Phiske, M., Verma, S., andLahiri, K. (2014). Side-effects of topical steroids: A long overdue revisit. *Indian Dermatology Online Journal* 5(4): 416–425.

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., and Mozafari, M. R. (2018). Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics* 10(2): 57.

Dantas, M. G. B., Reis, S. A. G. B., Damasceno, C. M. D., Rolim, L. A., Rolim-Neto, P. J., Carvalho, F. O., and Almeida, J. R. G. D. S. (2016). Development and evaluation of stability of a gel formulation containing the monoterpene borneol. *The Scientific World Journal* 2016(1): 7394685.

Dardak, R. A. (2019, August 26). FFTC Agricultural Policy Platform. Retrieved from Trends in Production, Trade, and Consumption of Tropical Fruit in Malaysia: https://ap.fftc.org.tw/article/1381

Das, M., and Mishra, C. (2019). Jackfruit leaf as an adsorbent of malachite green: recovery and reuse of the dye. *Springer Nature Applied Sciences* 1(5): 483.

Dasgupta, N., Ranjan, S., Mundra, S., Ramalingam, C., and Kumar, A. (2016). Fabrication of food grade vitamin E nanoemulsion by low energy approach, characterisation and its application. *International Journal of Food Properties* 19(3): 700-708.

Dash, S., Murthy, P. N., Nath, L., and Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica* 67(3): 217-223.

Daud, M. N. H., Fatanah, D. N., Abdullah, N., and Ahmad, R. (2017). Evaluation of antioxidant potential of *Artocarpus heterophyllus* L. J33 variety fruit waste from different extraction methods and identification of phenolic constituents by LCMS. *Food chemistry* 232: 621-632.

Debele, T. A., Mekuria, S. L., and Tsai, H. C. (2016). Polysaccharide based nanogels in the drug delivery system: Application as the carrier of pharmaceutical agents. *Materials Science and Engineering: C* 68: 964-981.

Department of Agriculture (2020). *Laporan Tahunan 2020 Jabatan Pertanian Malaysia*. (First Edition 2021). ISSN 1511-0591.

Derkach, S. R., Ilyin, S. O., Maklakova, A. A., Kulichikhin, V. G., and Malkin, A. Y. (2015). The rheology of gelatin hydrogels modified by κ-carrageenan. *LWT-Food Science and Technology* 63(1): 612-619.

Devi, N., Sarmah, M., Khatun, B., and Maji, T. K. (2017). Encapsulation of active ingredients in polysaccharide–protein complex coacervates. *Advances in Colloid and Interface Science* 239: 136-145.

Dhawan, B., Aggarwal, G., and Harikumar, S. L. (2014). Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. *International Journal of Pharmaceutical Investigation* 4(2): 65.

DIN EN 14770, Bitumen and bituminous binders - Determination of complex shear modulus and phase angle - Dynamic Shear Rheometer (DSR)

Djekic, L., Martinovic, M., Stepanović-Petrović, R., Micov, A., Tomić, M., andPrimorac, M. (2016). Formulation of hydrogel-thickened nonionic microemulsions with enhanced percutaneous delivery of ibuprofen assessed in vivo in rats. *European Journal of Pharmaceutical Sciences* 92: 255-265.

Dmour, I., and Taha, M. O. (2018). Natural and Semisynthetic Polymers in Pharmaceutical Nanotechnology in Organic Materials as *Smart Nanocarriers for Drug Delivery* (pp. 35-100). William Andrew Publishing.

Do, Q. D., Angkawijaya, A. E., Ttan-Nguyen, P. L., Hyunh, L. H., Soetaredjo, F. E., Ismadji, S., and Ju, Y. H. (2014). Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis* 22(3): 296 – 302.

Donkor, A. M., Nashirudeen, Y., and Suurbaar Jennifer. (2016). Stability evaluation and degradation kinetics of rutin in *Ficus pumila* leaves formulated with oil extracted from *Moringa oleifera* seeds. *Journal of Analytical and Pharmaceutical Research* 3(2): 1–7.

Donsì, F., Annunziata, M., Vincensi, M., and Ferrari, G. (2012). Design of nanoemulsion-based delivery systems of natural antimicrobials: effect of the emulsifier. *Journal of Biotechnology* 159(4) 342-350.

Doymaz, I., and Kipcak, A. S. (2018). Effect of pretreatment and air temperature on the drying time of cherry tomato. *Journal of Thermal Engineering* 4(1): 1648–1655.

Draelos, Z. D. (2011). The art and science of new advances in cosmeceuticals. *Clinics in Plastic Surgery* 38(3): 397-407.

Dube, P., Meyer, S., and Marnewick, J. L. (2017). Antimicrobial and antioxidant activities of different solvent extracts from fermented and green honeybush (*Cyclopia intermedia*) plant material. *South African Journal of Botany* 110: 184–193.

Eid, A. M., El-Enshasy, H. A., Aziz, R., andElmarzugi, N. A. (2014). Preparation, characterisation and anti-inflammatory activity of *Swietenia macrophylla* nanoemulgel. *Journal of Nanomedicine and Nanotechnology* 5(2): 1.

Ekenna, I. C., and Abali, S. O. (2022). Comparison of the Use of Kinetic Model Plots and DD Solver Software to Evaluate the Drug Release from Griseofulvin Tablets. *Journal of Drug Delivery and Therapeutics* 12(2-5): 5-13.

Elhussein, E. A. A., and Şahin, S. (2018). Drying behaviour, effective diffusivity and energy of activation of olive leaves dried by microwave, vacuum and oven drying methods. *Heat and Mass Transfer* 54: 1901-1911.

Elmataeeshy, M. E., Sokar, M. S., Bahey-El-Din, M., and Shaker, D. S. (2018). Enhanced transdermal permeability of Terbinafine through novel nanoemulgel formulation; Development, *in vitro* and *in vivo* characterisation. *Future Journal of Pharmaceutical Sciences* 4(1): 18-28.

Erramreddy, V. V., Qi, W., Bowles, R. K., and Ghosh, S. (2019). Modeling the influence of effective oil volume fraction and droplet repulsive interaction on nanoemulsion gelation. *Journal of Food Engineering* 249: 25-33.

Erramreddy, V. V., Tu, S., andGhosh, S. (2017). Rheological reversibility and long-term stability of repulsive and attractive nanoemulsion gels. *RSC Advances* 7(75): 47818-47832.

Fagioli, L., Pavoni, L., Logrippo, S., Pelucchini, C., Rampoldi, L., Cespi, M., ... and Casettari, L. (2019). Linear viscoelastic properties of selected polysaccharide gums as function of concentration, pH, and temperature. *Journal of Food Science* 84(1): 65-72.

Fan, W., Yu, Z., Peng, H., He, H., Lu, Y., Qi, J., ... and Wu, W. (2020). Effect of particle size on the pharmacokinetics and biodistribution of parenteral nanoemulsions. *International Journal of Pharmaceutics 586*: 119551.

Feng, J., Esquena, J., Rodriguez-Abreu, C., and Solans, C. (2020). Key features of nano-emulsion formation by the phase inversion temperature method. *Journal of Dispersion Science and Technology*, 1-9.

Fredenberg, S., Wahlgren, M., Reslow, M., and Axelsson, A. (2011). The mechanisms of drug release in poly (lactic-co-glycolic acid)-based drug delivery systems—a review. *International Journal of Pharmaceutics* 415(1-2): 34-52.

Freitas, F., Alves, V. D., and Reis, M. A. (2014). Bacterial polysaccharides: production and applications in cosmetic industry. Polysaccharides: Bioactivity and Biotechnology. Cham*: Springer International Publishing*, 1-24.

Froelich, A., Osmałek, T., Snela, A., Kunstman, P., Jadach, B., Olejniczak, M., and Białas, W. (2017). Novel microemulsion-based gels for topical delivery of indomethacin: Formulation, physicochemical properties and *in vitro* drug release studies. *Journal of Colloid and Interface Science* 507: 323-336.

Gandhi, S. M., Khan, A. K., Rathod, S., Jain, R., Dubey, S. K., Ray, D., ... and Tiwari, S. (2021). Water driven transformation of a nonionic microemulsion into liquid crystalline phase: Structural characterisations and drug release behavior. *Journal of Molecular Liquids* 326: 115239.

Ghate, V. M., Lewis, S. A., Prabhu, P., Dubey, A., and Patel, N. (2016). Nanostructured lipid carriers for the topical delivery of tretinoin. *European Journal of Pharmaceutics and Biopharmaceutics* 108: 253-261.

González, A., Tártara, L. I., Palma, S. D., andIgarzabal, C. I. A. (2015). Crosslinked soy protein films and their application as ophthalmic drug delivery system. *Materials Science and Engineering: C* 51: 73-79.

Gravelle, A. J., Davidovich-Pinhas, M., Barbut, S., and Marangoni, A. G. (2017). Influencing the crystallization behavior of binary mixtures of stearyl alcohol and stearic acid (SOSA) using ethyl cellulose. *Food Research International* 91: 1-10.

Griffin, W. C. (1949). Classification of surface-active agents by HLB. *Journal of the Society of Cosmetic Chemists* 1: 311-325.

Gulotta, A., Saberi, A. H., Nicoli, M. C., and McClements, D. J. (2014). Nanoemulsion-based delivery systems for polyunsaturated (ω-3) oils: formation using a spontaneous emulsification method. *Journal of Agricultural and Food Chemistry* 62(7): 1720-1725.

Gunasekaran, S., and Ak, M. M. (2000). Dynamic oscillatory shear testing of foods—selected applications. *Trends in Food Science and Technology* 11(3): 115-127.

Gupta, A., Badruddoza, A. Z. M., and Doyle, P. S. (2017). A general route for nanoemulsion synthesis using low-energy methods at constant temperature. *Langmuir* 33(28): 7118-7123.

Gupta, A., Narsimhan, V., Hatton, T. A., and Doyle, P. S. (2016). Kinetics of the change in droplet size during nanoemulsion formation. *Langmuir* 32(44): 11551-11559.

Gupta, N., Jain, U. K., and Pathak, A. K. (2009). Wound healing properties of *Artocarpus heterophyllus* Lam. *Ancient Science of Life* 28(4): 36.

Hafid, A. F., Septiani, R. P., Fabriana, L. H., Febrianty, N., Ranggaditya, D., and Widyawaruyanti, A. (2016). Antimalarial activity of crude extracts of *Artocarpus heterophyllus, Artocarpus altilis, and Artocarpus camansi*. *Asian Journal of Pharmaceutical and Clinical Research* 9(1): 279-281.

Hamrouni-Sellami, I., Rahali, F. Z., Rebey, I. B., Bourgou, S., Limam, F., and Marzouk, B. (2013). Total phenolics, flavonoids, and antioxidant activity of sage (*Salvia officinalis* L.) plants as affected by different drying methods. *Food Bioprocess Technology* 6: 806-817.

Hasenhuettl, G. L. (2019). Synthesis and Commercial Preparation of Food Emulsifiers in *Food Emulsifiers and Their Applications* (pp. 11-39). Second Edition. New York: Springer Science Business Media

Hategekimana, J., Chamba, M. V., Shoemaker, C. F., Majeed, H., and Zhong, F. (2015). Vitamin E nanoemulsions by emulsion phase inversion: Effect of environmental stress and long-term storage on stability and degradation in different carrier oil types. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 483: 70-80.

Hemwimol, S., Pavasant, P., and Shotipruk, A. (2006). Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. *Ultrasonics Sonochemistry* 13: 543–548.

Higuchi, W. I. (1962). Analysis of data on the medicament release from ointments. *Journal of Pharmaceutical Sciences* 51(8): 802-804.

Hii, C. L., Law, C. L., and Cloke, M. (2008). Modelling of thin layer drying kinetics of cocoa beans during artificial and natural drying. *Journal of Engineering Science and Technology* 3(1) 1-10.

Hikmawanti, N. P. E., Fatmawati, S., and Asri, A. W. (2021, April). The effect of ethanol concentrations as the extraction solvent on antioxidant activity of Katuk (*Sauropus androgynus* (L.) Merr.) leaves extracts. In *IOP Conference Series: Earth and Environmental Science* (Vol. 755, No. 1, p. 012060). IOP Publishing.

Hixson, A. W., and Crowell, J. H. (1931). Dependence of reaction velocity upon surface and agitation. *Industrial and Engineering Chemistry* 23(8): 923-931.

Hong, L., Zhou, C. L., Chen, F. P., Han, D., Wang, C. Y., Li, J. X., ...and Liu, C. G. (2017). Development of a carboxymethyl chitosan functionalized nanoemulsion formulation for increasing aqueous solubility, stability and skin permeability of astaxanthin using low-energy method. *Journal of Microencapsulation* 34(8): 707-721.

Huang, Q., Yu, H., and Ru, Q. (2010). Bioavailability and delivery of nutraceuticals using nanotechnology. *Journal of Food Science* 75(1): R50 – R57.

Hussain, A., Singh, V. K., Singh, O. P., Shafaat, K., Kumar, S., and Ahmad, F. J. (2016). Formulation and optimisation of nanoemulsion using antifungal lipid and surfactant for accentuated topical delivery of Amphotericin B. *Drug Delivery* 23(8): 3101-3110.

Ibanescu, C., Danu, M., Nanu, A., Lungu, M., and Simionescu, B. C. (2010). Stability of disperse systems estimated using rheological oscillatory shear tests. *Revue Roumaine de Chimie* 55(11-12): 933-940.

Imeson, A. (Ed.). (2011). *Food Stabilisers, Thickeners and Gelling Agents*. John Wiley and Sons.

Inyang, U. E., Oboh, I. O., and Etuk, B. R. (2018). Kinetic models for drying techniques—food materials. *Advances in Chemical Engineering and Science* 8(02): 27.

Iqbal, S., Bhanger, M. I., and Anwar, F. (2007) Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. *LWT* 40: 361–367.

Ismail, N., and Kaur, B. (2013). Consumer preference for jackfruit varieties in Malaysia. *Journal of Agribusiness Marketing* 6: 37-51.

ISO 6721-10:2015, Plastics — Determination of dynamic mechanical properties — Part 10: Complex shear viscosity using a parallel-plate oscillatory rheometer.

Jacob, J., Haponiuk, J. T., Thomas, S., and Gopi, S. (2018). Biopolymer based nanomaterials in drug delivery systems: A review. *Materials Today Chemistry 9*: 43-55.

Jacobs, C., Kayser, O., and Müller, R. H. (2000). Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. *International Journal of Pharmaceutics* 196(2): 161-164.

Jagtap, U. B., and Bapat, V. A. (2010). Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology,* 129(2): 142-166.

Jain, D., and Tiwari, G. N. (2003). Thermal aspects of open sun drying of various crops. *Energy* 28: 37–54.

Jaiswal, M., Dudhe, R., and Doyle, P. S. (2016). Nanoemulsions: Formation, properties, and applications. *Soft Matter* 12(11): 2826 – 2841.

Jao, D., Xue, Y., Medina, J., and Hu, X. (2017). Protein-based drug-delivery materials. *Materials* 10(5): 517.

Jeon, S., Yoo, C. Y., and Park, S. N. (2015). Improved stability and skin permeability of sodium hyaluronate-chitosan multilayered liposomes by Layer-by-Layer electrostatic deposition for quercetin delivery. *Colloids and Surfaces B: Biointerfaces* 129: 7-14.

Johari, A. M., AbdRahman, N. A., Baharuddin, A. S., Basha, R. K., Mohammed, M. A. P., Parida, D. M., ... and Wakisaka, M. (2020). Effects of different low temperature storage conditions on the physico-chemical properties of *Mastura* (J37) jackfruit bulbs. *Journal of Agricultural and Food Engineering* 1*:* 0009.

Jones, D. S., Yu, T., and Andrews, G. P. (2019). A statistical determination of the contribution of viscoelasticity of aqueous carbohydrate polymer networks to drug release. *Carbohydrate Polymers* 206: 511-519.

Jones, E. M., Cochrane, C. A., and Percival, S. L. (2015). The effect of pH on the extracellular matrix and biofilms. *Advances in Wound Care* 4(7): 431-439.

Jyothi, D., and Koland, M. (2016). Formulation and evaluation of an herbal antiinflammatory gel containing *Trigonella foenum greacum* seed extract. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(1): 41-4.

Kadam, D. M., Goyal, R. K., and Gupta, M. K (2011). Mathematical modelling of convective thin layer drying of basil leaves. *Journal of Medicinal Plants Research* 5(19): 4721-4730.

Kale, S. N., and Deore, S. L. (2017). Emulsion micro emulsion and nano emulsion: a review. *Systematic Reviews in Pharmacy* 8(1): 39 – 47.

Kang, B., Opatz, T., Landfester, K., and Wurm, F. R. (2015). Carbohydrate nanocarriers in biomedical applications: functionalization and construction. *Chemical Society Reviews* 44(22): 8301-8325.

Kaplan, A. B. U., Cetin, M., Orgul, D., Taghizadehghalehjoughi, A., Hacımuftuoglu, A., and Hekimoglu, S. (2019). Formulation and *in vitro* evaluation of topical nanoemulsion and nanoemulsion-based gels containing daidzein. *Journal of Drug Delivery Science and Technology* 52: 189-203.

Karami, Z., Zanjani, M. R. S., and Hamidi, M. (2019). Nanoemulsions in CNS drug delivery: recent developments, impacts and challenges. *Drug Discovery Today* 24(5): 1104-1115.

Karim, A. A., and Bhat, R. (2009). Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food hydrocolloids* 23(3): 563-576.

Kaur, G., and Mehta, S. K. (2017). Developments of Polysorbate (Tween) based microemulsions: Preclinical drug delivery, toxicity and antimicrobial applications. *International Journal of Pharmaceutics* 529(1-2): 134-160.

Kaur, K., Kumar, N., and Aggarwal, P. (2017). Role of natural gumsin nanoemulsion formation and stabilisation. *International Journal of Biological Macromolecules* 102: 903 – 940.

Khan, M. R., Omoloso, A. D., and Kihara, M. (2003). Antibacterial activity of *Artocarpus heterophyllus*. *Fitoterapia* 74(5): 501-505.

Kichou, H., Dancik, Y., Eklouh-Molinier, C., Huang, N., Soucé, M., Gressin, L., ... and Bonnier, F. (2022). Highlighting the efficiency of ultrasound-based emulsifier-free emulsions to penetrate reconstructed human skin. *International Journal of Cosmetic Science* 44(2): 262-270.

Kim, J. S. (2016). Liposomal drug delivery system. *Journal of Pharmaceutical Investigation* 46(4): 387-392.

Kim, S., Jeong, M., Lee, J. W., Kim, S. Y., Choi, C. K., and Kang, Y. T. (2018). Development of nanoemulsion $CO_2$ absorbents for mass transfer performance enhancement. *International Communications in Heat and Mass Transfer* 94: 24-31.

Komaiko, J., and McClements, D. J. (2014). Optimisation of isothermal low-energy nanoemulsion formation: Hydrocarbon oil, non-ionic surfactant, and water systems. *Journal of Colloid and Interface Science* 425: 59-66.

Komaiko, J., and McClements, D. J. (2015). Low-energy formation of edible nanoemulsions by spontaneous emulsification: Factors influencing particle size. *Journal of Food Engineering* 146: 122-128.

Koroleva, M. Y., andYurtov, E. V. (2012). Nanoemulsions: the properties, methods of preparation and promising applications. *Russian Chemical Reviews* 81(1): 21.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., and Peppas, N.A. (1983). Mechanisms of solute release from porous hydrophilic polymers. *International Journal of Pharmaceutics* 15(1): 25–35.

Krull, I. S., Sebag, A., and Stevenson, R. (2000). Specific applications of capillary electrochromatography to biopolymers, including proteins, nucleic acids, peptide mapping, antibodies, and so forth. *Journal of Chromatography A* 887(1-2): 137-163.

Kumar, A., Kandasamy, P., Chakraborty, I., andHangshing, L. (2022). Analysis of energy consumption, heat and mass transfer, drying kinetics and effective moisture diffusivity during foam-mat drying of mango in a convective hot-air dryer. *Biosystems Engineering* 219: 85-102.

Lao, L. L., Peppas, N. A., Boey, F. Y. C., and Venkatraman, S. S. (2011). Modeling of drug release from bulk-degrading polymers. *International Journal of Pharmaceutics* 418(1): 28-41.

Li, X., Qin, Y., Liu, C., Jiang, S., Xiong, L., and Sun, Q. (2016). Size-controlled starch nanoparticles prepared by self-assembly with different green surfactant: The effect of electrostatic repulsion or steric hindrance. *Food Chemistry* 199: 356-363.

Liang, J., Qian, Y., Yuan, X., Leng, L., Zeng, G., Jiang, L., and Li, X. (2018). Span 80/Tween 80 stabilised bio-oil-in-diesel microemulsion: Formation and combustion. *Renewable Energy* 126: 774-782.

Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Taboada, E. B., and Lacks, D. J. (2019). Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol–water binary solvent systems. *Journal of Food Science and Technology* 56: 2536-2544.

Lim, T. K. (2012). *Artocarpus heterophyllus* In *Edible Medicinal and Non Medicinal Plants* (pp. 318-336). Springer, Dordrecht.

Ling, A. L. M., Yasir, S., Matanjun, P., and Abu Bakar, M. F. (2015). Effect of different drying techniques on the phytochemical content and antioxidant activity of *Kappaphycus alvarezii*. *Journal of Applied Phycology* 27: 1717-1723.

Liu, C., Li, Y., Liang, R., Sun, H., Wu, L., Yang, C., and Liu, Y. (2023). Development and characterisation of ultrastable emulsion gels based on synergistic interactions of xanthan and sodium stearoyl lactylate. *Food Chemistry*, *400*, 133957.

Liu, L., Sun, Y., Laura, T., Liang, X., Ye, H., and Zeng, X. (2009). Determination of polyphenolic content and antioxidant activity of kudingcha made from Ilex kudingcha C. J. Tseng. *Food Chemistry* 112(1): 35-41.

Liu, Q., Huang, H., Chen, H., Lin, J., and Wang, Q. (2019). Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds. *Molecules* 24(23): 4242.

Liu, Y., Sun, Y., Yu, H., Yin, Y., Li, X., and Duan, X. (2017). Hot air drying of purple fleshed sweet potato with contact ultrasound assistance. *Drying Technology* 35(5): 564-576.

Liu, Z., Liu, W., Lang, C., and Li, Y. (2019). Effect of surfactant HLB value on Methane hydrate formation in non-ionic surfactant-oil water emulsions systems. *Energy Procedia* 158: 5275-5280.

Loizzo, M. R., Tundis, R., Chandrika, U. G., Abeysekera, A. M., Menichini, F., and Frega, N. G. (2010). Antioxidant and antibacterial activities on foodborne pathogens of *Artocarpus heterophyllus* Lam (Moraceae) leaves extracts. *Journal of Food Science* 75(5): 291-295.

Lu, X. Y., Wu, D. C., Li, Z. J., and Chen, G. Q. (2011). Polymer nanoparticles. *Progress in Molecular Biology and Translational Science* 104: 299-323.

Lu, Y., Wu, N., Fang, Y., Shaheen, N., and Wei, Y. (2017). An automatic on-line 2, 2-diphenyl-1-picrylhydrazyl-high performance liquid chromatography method for high-throughput screening of antioxidants from natural products. *Journal of Chromatography A* 1521: 100-109.

Lukić, M., Pantelić, I., and Savić, S. D. (2021). Towards optimal ph of the skin and topical formulations: From the current state of the art to tailored products. *Cosmetics* 8(3): 69.

Lupi, F. R., Gabriele, D., Seta, L., Baldino, N., de Cindio, B., and Marino, R. (2015). Rheological investigation of pectin-based emulsion gels for pharmaceutical and cosmetic uses. *Rheologica Acta* 54(1): 41-52.

Magangana, T. P., Makunga, N. P., Fawole, O. A., and Opara, U. L. (2020). Processing factors affecting the phytochemical and nutritional properties of pomegranate (Punica granatum L.) peel waste: A review. *Molecules* 25(20): 4690.

Mahdi, E. S., Noor, A. M., Sakeena, M. H., Abdullah, G. Z., Abdulkarim, M. F., and Sattar, M. A. (2011). Formulation and *in vitro* release evaluation of newly synthesized palm kernel oil esters-based nanoemulsion delivery system for 30% ethanolic dried extract derived from local *Phyllanthus urinaria* for skin antiaging. *International Journal of Nanomedicine* 6: 2499 - 2512.

Mahdi, E. S., Sakeena, M. H., Abdulkarim, M. F., Abdullah, G. Z., Sattar, M. A., and Noor, A. M. (2011). Effect of surfactant and surfactant blends on pseudoternary phase diagram behavior of newly synthesized palm kernel oil esters. *Drug Design, Development and Therapy*: 311-323.

Maisarah, M. H., Noriham, A., Zainon, M. N. (2013). Quantification of polyphenolic acids and antioxidant capacity of palm puree from different tenera breeds of *Elaeis Guineensis*. *International Journal of Bioscience, Biochemistry and Bioinformatics* 3(4): 349–353.

Mandal, S., and Vishvakarma, P. (2023). Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. *Indian Journal of Pharmaceutical Education and Research* 57(3): 481-498.

Maphosa, Y., and Jideani, V. A. (2018). Factors Affecting the Stability of Emulsions Stabilised By Biopolymers. In *Science and Technology behind Nanoemulsions* (pp. 65-81). Intech Open.

Martinez-Las Heras, R., Heredia, A., Castello, M. L., and Andre, A. (2014). Influence of drying method and extraction variables on the antioxidant properties of persimmon leaves. *Food Bioscience* 6: 1–8.

Mason, T. G., Wilking, J. N., Meleson, K., Chang, C. B., and Graves, S. M. (2006). Nanoemulsions: formation, structure, and physical properties. *Journal of Physics: Condensed Matter* 18(41): R635.

McClements, D. J. (2012). Nanoemulsions versus microemulsions: terminology, differences, and similarities. *Soft Matter* 8(6): 1719-1729.

McClements, D. J. (2015). Food Emulsions: Principles, Practices, and Techniques. CRC Press.

McClements, D. J., and Jafari, S. M. (2018a). General aspects of nanoemulsions and their formulation. In *Nanoemulsions* (pp. 3-20). Academic Press.

McClements, D. J., and Jafari, S. M. (2018b). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science* 251: 55-79.

McClements, D. J., and Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition* 51(4): 285-330.

Mehrnia, M. A., Jafari, S. M., Makhmal-Zadeh, B. S., and Maghsoudlou, Y. (2016). Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification. *International Journal of Biological Macromolecules* 84*:* 261-267.

Meon, Z (2014). Nutrient Content Analysis of Rust-Affected Jackfruits. *Innovative Plant Productivity and Quality* 22: 95- 99.

Midilli, A., Kucuk, H. and Yapar, Z. A. (2002). A new model for single-layer drying. *Drying Technology* 20: 1503 – 1513.

Mitri, K., Shegokar, R., Gohla, S., Anselmi, C., and Müller, R. H. (2011). Lipid nanocarriers for dermal delivery of lutein: preparation, characterisation, stability and performance. *International Journal of Pharmaceutic* 414(1-2): 267-275.

Morsy, M. A., Abdel-Latif, R. G., Nair, A. B., Venugopala, K. N., Ahmed, A. F., Elsewedy, H. S., and Shehata, T. M. (2019). Preparation and evaluation of

atorvastatin -loaded nanoemulgel on wound-healing efficacy. *Pharmaceutics* 11(11): 609.

Moulai Mostefa, N., HadjSadok, A., Sabri, N., and Hadji, A. (2006). Determination of optimal cream formulation from long-term stability investigation using a surface response modelling. *International Journal of Cosmetic Science* 28(3) 211-218.

Mulleria, S. S., Marina, K., and Ghetia, S. M. (2021). Formulation, optimisation and *in vitro* evaluation of apremilast nanoemulgel for topical delivery. *International Journal of Pharmaceutical Investigation* 11(2): 230-237.

Musa, S. H., Basri, M., Fard Masoumi, H. R., Shamsudin, N., and Salim, N. (2017). Enhancement of physicochemical properties of nanocolloidal carrier loaded with cyclosporine for topical treatment of psoriasis: *in vitro* diffusion and in vivo hydrating action. *International Journal of Nanomedicine*: 2427-2441.

Mustafa, H. M., Abdullah, N., and Noor, Z. M. (2016). Quantitative analysis of hydrophilic and lipophilic antioxidant components in palm puree. In Regional Conference on Science, Technology and Social Sciences (RCSTSS 2014): Science and Technology (RCSTSS 2014), N. Yacob, M. Mohamed, and M. Megat Hanafiah (ed.), Springer, Singapore, pp. 147-156.

Mustafa, H. M., Amin, N. A. M., Zakaria, R., Anuar, M. S., Baharuddin, A. S., Hafid, H. S., and Omar, F. N. (2020). Dual impact of different drying treatments and ethanol/water ratios on antioxidant properties and colour attribute of jackfruit leaves (*Artocarpus heterophyllus* Lam.) *Mastura* Variety (J35). *BioResources* 15(3): 5122-5140.

Nagoba, B., Gavkare, A., Rayate, A., Mumbre, S., Rao, A., Warad, B., ... and Jamadar, N. (2021). Role of an acidic environment in the treatment of diabetic foot infections: A review. *World Journal of Diabetes* 12(9): 1539.

Nahar, L., and Sarker, S. D. (2019). *Chemistry for Pharmacy Students: General, Organic and Natural Product Chemistry*. John Wiley and Sons.

Nayak, A. K., and Das, B. (2018). Introduction to Polymeric Gels. In *Polymeric Gels* (pp. 3-27). Woodhead Publishing.

Nazri, M. S. M., Tawakkal, I. S. M. A., Khairuddin, N., Talib, R. A., and Othman, S. H. (2019). Characterisation of jackfruit straw-based films: effect of starch and plasticizer contents. *Adapting to Challenges* 1: 1-14.

Noor, N. A. M., Islam, M., Hamid, N., and Raseetha, S. (2024). Phytochemical Composition of jackfruit (*Artocarpus Heterophyllus*) and Indian jujube (*Ziziphus Mauritiana*) leaves extracted using different ethanol concentrations. *Journal of Sustainability Science and Management* 19(9): 146-162.

Ojha, B., Jain, V. K., Gupta, S., Talegaonkar, S., and Jain, K. (2022). Nanoemulgel: A promising novel formulation for treatment of skin ailments. *Polymer Bulletin* 79(7): 4441-4465.

Ojwang, R. A., Muge, E. K., Mbatia, B., Mwanza, B., and Ogoyi, D. O. (2017). Comparative analysis of phytochemical composition and antioxidant activities of methanolic extracts of leaves, roots and bark of jackfruit (*Artocapus heterophyllus*) from selected regions in Kenya and Uganda. *Journal of Advances in Biology and Biotechnology* 16: 1–13.

Omar, H. S., El-Beshbishy, H. A., Moussa, Z., Taha, K. F., and Singab, A. N. B. (2011). Antioxidant activity of *Artocarpus heterophyllus* Lam. (Jackfruit) leaf extracts: remarkable attenuations of hyperglycemia and hyperlipidemia in streptozotocin-diabetic rats. *The Scientific World Journal* 11: 788-800.

Ong, B. T., Nazimah, S. A. H., Osman, A., Quek, S. Y., Voon, Y. Y., Hashim, D. M., and Kong, Y. W. (2006). Chemical and flavour changes in jackfruit (*Artocarpus heterophyllus* Lam.) cultivar J3 during ripening. *Postharvest Biology and Technology* 40(3): 279-286.

Ooi, Z. X., Ismail, H., Abu Bakar, A., and Aziz, N. A. (2011). Effects of jackfruit waste flour on the properties of poly (vinyl alcohol) film. *Journal of Vinyl and Additive Technology* 17(3): 198-208.

Páez-Hernández, G., Mondragón-Cortez, P., and Espinosa-Andrews, H. (2019). Developing curcumin nanoemulsions by high-intensity methods: Impact of ultrasonication and microfluidisation parameters. *LWT* 111: 291-300.

Paliwal, H., Solanki, R. S., Chauhan, C. S., and Dwivedi, J. (2019). Pharmaceutical considerations of microemulsion as a drug delivery system. *Journal of Drug Delivery and Therapeutics* 9(4-s): 661-665.

Parhi, R. (2022). Recent advances in microneedle designs and their applications in drug and cosmeceutical delivery. *Journal of Drug Delivery Science and Technology*: 103639.

Patel, A. R., and Velikov, K. P. (2014). Zein as a source of functional colloidal nano- and microstructures. *Current Opinion in Colloid and Interface Science* 19(5): 450-458.

Patel, M. J., Patel, S. S., Patel, N. M., and Patel, M. M. (2010). A self-microemulsifying drug delivery system (SMEDDS). *International Journal of Pharmaceutical Sciences Review and Research* 4(3): 29-35.

Patel, R. B., Patel, M. R., Bhatt, K. K., Patel, B. G., andGaikwad, R. V. (2016). Microemulsion-based drug delivery system for transnasal delivery of Carbamazepine: preliminary brain-targeting study. *Drug Delivery* 23(1): 207-213.

Patel, S.N. and Patel, D M and Patel, C.N. and Patel, T.D. and Prajapati, P.H. and Parikh, B.N. (2010). Self emulsifying drug delivery system. *Journal of Global Pharma Technology* 2: 29-37.

Patil, P. B., Datir, S. K., and Saudagar, R. B. (2019). A review on topical gels as drug delivery system. *Journal of Drug Delivery and Therapeutics* 9(3-s): 989-994.

Periyanayagam, K., and Karthikeyan, V. (2013). Wound healing activity of the leaves of Artocarpusheterophyllus Lam. (Moraceae) on ex-vivo porcine skin wound healing model. *Innovare Journal of Life Sciences* 1(1): 28-33.

Perva-uzunalic, A., Otto, F., and Gru, S. (2006). Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine. *Food Chemistry* 96: 597–605.

Pin, K. Y., Chuah, A. L., Rashih, A. A., Mazura, M. P., Fadzureena, J., Vimala, S., and Rasadah, M. A. (2010). Antioxidant and anti-inflammatory activities of extracts of betel leaves (*Piper betle*) from solvents with different polarities. *Journal of Tropical Forest Science* 22(4): 448-455.

Prakash, O., Jyoti, A. K., and Gupta, R. (2013). Evaluation of anticonvulsant activity of *Artocarpus heterophyllus* Lam. leaves (Jackfruit) in mice. *Der Pharmacia Lettre* 5(1): 217-220.

Prakash, O., Kumar, A., and Kumar, P. (2013). Screening of analgesic and immunomodulator activity of *Artocarpus heterophyllus* Lam. leaves (Jackfruit) in mice. *Journal of Pharmacognosy and Phytochemistry* 1(6): 33-36.

Prakash, O., Kumar, R., Mishra, A., and Gupta, R. (2009). *Artocarpus heterophyllus* (Jackfruit): an overview. *Pharmacognosy Reviews* 3(6): 353.

Prakash, O., Srivastava, R., Kumar, R., Mishra, S., and Srivastavam, S. (2017). Preliminary pharmacognostic and phytochemical studies on leaves of *Artocarpus heterophyllus*. *International Journal of Natural Products and Marine Biology* 1(1): 35 – 40.

Prasad, J. A. (2009). Convective heat transfer in herb and spices during open sun drying. *International Journal of Food Science and Technology* 44: 657–665.

Premachandra, J. K., Manoj, B. S., Aberathne, S. N. P. P. G. P. E., and Warnapura, L. H. P. (2017). Proceeding from *Moratuwa Engineering Research Conference (MERCon)* IEEE.

Putra., R. N., and Ajiwiguna, T. A. (2017). Influence of air temperature and velocity for drying process. *Procedia Engineering* 170: 516-519.

Putro, J. N., Soetaredjo, F. E., Lunardi, V. B., Irawaty, W., Yuliana, M., Santoso, S. P., …and Ismadji, S. (2023). Polysaccharides gums in drug delivery systems: A review. *International Journal of Biological Macromolecules*: 127020.

Qiu, C., Zhao, M., and McClements, D. J. (2015). Improving the stability of wheat protein-stabilised emulsions: Effect of pectin and xanthan gum addition. *Food Hydrocolloids* 43: 377-387.

Rabin, C. R., and Siegel, S. J. (2012). Delivery systems and dosing for antipsychotics. *Current Antipsychotics*: 267-298.

Rahaman, S. M., Bhattarai, A., Kumar, D., Singh, B., and Saha, B. (2023). Application of Biosurfactants as Emulsifiers in the Processing of Food Products with Diverse Utilisation in the Baked Goods. In *Applications of Next Generation Biosurfactants in the Food Sector* (pp. 203-237). Academic Press.

Rahimmalek, M., and Goli, S. A. H. (2013). Evaluation of six drying treatments with respect to essential oil yield, composition and colour characteristics of *Thymys daenensis* subsp. daenensis. Celak leaves. *Industrial Crops and Products* 42*:* 613–619.

Rahmati, N. F., Koocheki, A., Varidi, M., and Kadkhodaee, R. (2018). Thermodynamic compatibility and interactions between Speckled Sugar bean protein and xanthan gum for production of multilayer O/W emulsion. *Journal of Food Science and Technology* 55(3): 1143–1153.

Rai, V. K., Mishra, N., Yadav, K. S., andYadav, N. P. (2018). Nanoemulsion as pharmaceutical carrier for dermal and transdermal drug delivery: Formulation development, stability issues, basic considerations and applications. *Journal of Controlled Release* 270*:* 203-225.

Ranasinghe, R. A. S. N., Maduwanthi, S. D. T., and Marapana, R. A. U. J. (2019). Nutritional and health benefits of jackfruit (*Artocarpus heterophyllus* Lam.): A review. *International Journal of Food Science*: 1-12.

Rathod, H. J., and Mehta, D. P. (2015). A review on pharmaceutical gel. International *Journal of Pharmaceutical Sciences* 1(1): 33-47.

Ravera, F., Dziza, K., Santini, E., Cristofolini, L., and Liggieri, L. (2021). Emulsification and emulsion stability: The role of the interfacial properties. *Advances in Colloid and Interface Science* 288: 102344.

Rawat, A., Gupta, S. S., Kalluri, H., Lowenborg, M., Bhatia, K., and Warner, K. (2019). Rheological characterisation in the development of topical drug products. *The Role of Microstructure in Topical Drug Product Development*: 3-45.

Rayendra, R., Wientarsih, L., Priosoeryanto, B. P., and Gunawan, H. (2016). Potency of jack fruit leaves as tyrosinase inhibitor. *International Journal of Sciences: Basic and Applied Research* 30(4): 351-357.

Ren, G., Sun, Z., Wang, Z., Zheng, X., Xu, Z., and Sun, D. (2019). Nanoemulsion formation by the phase inversion temperature method using polyoxypropylene surfactants. *Journal of Colloid and Interface Science* 540: 177-184.

Reyes, A., Vasquez, J., Pailahueque, N., and Mahn, A. (2019). Effect of drying using solar energy and phase change material on kiwifruit properties. *Drying Technology* 37(2): 232-244.

Ribeiro, A. S., Estanqueiro, M., Oliveira, M. B., and Sousa Lobo, J. M. (2015). Main benefits and applicability of plant extracts in skin care products. *Cosmetics* 2(2): 48-65.

Ribeiro, L. N., Alcantara, A., Rodrigues da Silva, G. H., Franz-Montan, M., Nista, S. V., Castro, S. R., and de Paula, E. (2017). Advances in hybrid polymer-based materials for sustained drug release. *International Journal of Polymer Science*: 1-16.

Roldan-Cruz, C., Vernon-Carter, E. J., and Alvarez-Ramirez, J. (2016). Assesing the stability of Tween 80-based O/W emulsions with cyclic voltammetry and electrical impedance spectroscopy. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 511: 145-152.

Roshanak, S., Rahimmalek, M., and Goli, S. A. H. (2016). Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and colour of green tea (*Camellia sinensis* or *C. assamica*) leaves. *Food Science Technology* 53(1): 721–729.

Saani, S. M., Abdolalizadeh, J., and Heris, S. Z. (2019). Ultrasonic/sonochemical synthesis and evaluation of nanostructured oil in water emulsions for topical delivery of protein drugs. *Ultrasonics Sonochemistry* 55: 86-95.

Saberi, A. H., Fang, Y., and McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: factors affecting particle size using spontaneous emulsification. *Journal of Colloid and Interface Science* 391: 95-102.

Sabet, S., George, M.A., El-Shorbagy, H.M., Bassiony, H., Farroh, K.Y., Youssef, T. and Salaheldin, T.A., 2017. Gelatin nanoparticles enhance delivery of hepatitis C virus recombinant NS2 gene. *PLoS One* 12(7) 1-15.

Safdar, M. N., Kausar, T., and Nadeem, M. (2017). Comparison of ultrasound and maceration techniques for the extraction of polyphenols from the mango peel. *Journal of Food Processing and Preservation* 41(4): e13028.

Sahu, R., Kundu, P., and Sethi, A. (2021). *In vitro* amtioxidant activity and enzyme inhibition properties of wheat whole grain, bran and flour defatted with hexane and supercritical fluid extraction. *LWT* 146: 111376.

Saidin, N. M., Anuar, N. K., and Affandi, M. M. M. (2018). Roles of polysaccharides in transdermal drug delivery system and future prospects. *Journal of Applied Pharmaceutical Science* 8(3): 141-157.

Saifullah, M., Ahsan, A., and Shishir, M. R. I. (2016). Production, stability and application of micro-and nanoemulsion in food production and the food processing industry. In *Emulsions* (pp. 405-442). Academic Press.

Salim, N., Ahmad, N., Musa, S. H., Hashim, R., Tadros, T. F., and Basri, M. (2016). Nanoemulsion as a topical delivery system of antipsoriatic drugs. *RSC Advances* 6*(*8): 6234-6250.

Salim, N., Basri, M., Rahman, M. B., Abdullah, D. K., and Basri, H. (2012). Modification of palm kernel oil esters nanoemulsions with hydrocolloid gum for enhanced topical delivery of ibuprofen. *International Journal of Nanomedicine*: 4739-4747.

Samala, MadhaviLatha, and G. Sridevi. (2016). Role of polymers as gelling agents in the formulation of emulgels. *Polymer Sciences* 1: 2.

Sankar, V., Praveen, C., Prasanth, K. G., Srinivas, C. R., andRuckmann, K. (2009). Formulation and evaluation of a proniosome hydrocortisone gel in comparison with a commercial cream. Die Pharmazie-An *International Journal of Pharmaceutical Sciences* 64(11): 731-734.

Sarheed, O., Shouqair, D., Ramesh, K. V. R. N. S., Khaleel, T., Amin, M., Boateng, J., and Drechsler, M. (2020). Formation of stable nanoemulsions by ultrasound-assisted two-step emulsification process for topical drug delivery: effect of oil phase composition and surfactant concentration and loratadine as ripening inhibitor. *International Journal of Pharmaceutics* 576: 118952.

Sarpong, F., Yu, X., Zhou, C., Oteng-Darko, P., Amenorfe, L. P., Wu, B., ….and Ma, H. (2018). Drying characteristics, enzyme inactivation and browning pigmentation kinetics of controlled humidity-convective drying of banana slices. *Heat and Mass Transfer* 54: 3117 – 3130.

Saxena, A., Bawa, A. S., and Raju, P. S. (2011). Jackfruit *(Artocarpus heterophyllus* Lam.). In *Postharvest biology and technology of tropical and subtropical fruits* (pp. 275-299). Woodhead Publishing.

Sayuti, N. H., Kamarudin, A. A., Razak, N. A. A., Saad, N., Dek, M. S. P., and Esa, N. M. (2020). Optimised aqueous extraction conditions for maximal phenolics, flavonoids and antioxidant capacity from artocarpus heterophyllus (jackfruit) leaves by response surface methodology (RSM). *Malaysian Journal of Medicine and Health Sciences* 16(2): 135-144

Schmid, E. M., Farahnaky, A., Adhikari, B., and Torley, P. J. (2022). High moisture extrusion cooking of meat analogs: A review of mechanisms of protein texturization. *Comprehensive Reviews in Food Science and Food Safety* 21(6): 4573-4609.

Sengupta, P., and Chatterjee, B. (2017). Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. *International Journal of Pharmaceutics* 526(1-2): 353-365.

Septama, A. W., and Panichayupakaranant, P. (2016). "Simultaneous HPLC analysis of three flavonoids in the extracts of *Artocarpus heterophyllus* heartwoods," *Natural Product Sciences* 22(2): 77-81.

Setiowati, A. D., Wijaya, W., and Van der Meeren, P. (2020). Whey protein-polysaccharide conjugates obtained via dry heat treatment to improve the heat stability of whey protein stabilised emulsions. *Trends in Food Science and Technology* 98: 150-161.

Ševčíková, P., Kašpárková, V., Vltavská, P., andKrejčí, J. (2012). On the preparation and characterisation of nanoemulsions produced by phase inversion emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 410: 130-135.

Shao, Y., Wu, C., Wu, T., Li, Y., Chen, S., Yuan, C., and Hu, Y. (2018). Eugenol-chitosan nanoemulsions by ultrasound-mediated emulsification: Formulation, characterisation and antimicrobial activity. *Carbohydrate Polymers* 193: 144-152.

Shariatinia, Z. (2019). Pharmaceutical Applications of Natural Polysaccharides. In *Natural Polysaccharides in Drug Delivery and Biomedical Applications* (pp. 15-57). Academic Press.

Sharma, P., and Tailang, M. (2020). Design, optimisation, and evaluation of hydrogel of primaquine loaded nanoemulsion for malaria therapy. *Future Journal of Pharmaceutical Sciences* 6: 1-11.

Sharma, R., Awasthi, R., and Malviya, R. (2023). Formulation of Cocos nucifera husk fiber reinforced Acacia chundra gum based hydrogel composite for controlled drug release. *Journal of Drug Delivery Science and Technology* 89: 105005.

Sharma, V., Nayak, S. K., Paul, S. R., Choudhary, B., Ray, S. S., and Pal, K. (2018). Emulgels. In *Polymeric Gels* (pp. 251-264). Woodhead Publishing.

Sidhu, J. S., and Zafar, T. A. (2020). Fruits of Indian Subcontinent and Their Health Benefits. In *Herbal Medicine in India* (pp. 451-478). Springer, Singapore.

Siepmann, J., and Peppas, N. A. (2011). Higuchi equation: Derivation, applications, use and misuse. *International Journal of Pharmaceutics* 418(1): 6-12.

Sodha, M. S., Dang, A., Bansal, P. K., and Sharman, S. B. (1985). An analytical and experimental study of open sun drying and a cabinet tyre drier. *Energy Conversion and Management* 25(3): 263-271.

Solanki, R., Gupta, A., Tripathy, A., Soni, D., and Jana, G. K. (2011). Pharmacognostic, phytochemical and physiochemical studies of *Artocarpus hetrophyllus* leaf (Moracae). *Journal of Natural Product Plant Resources* 1(4): 20-26.

Solans, C., and Solé, I. (2012). Nano-emulsions: formation by low-energy methods. *Current Opinion in Colloid and Interface Science* (5): 246-254.

Somashekhar, M., Nayeem, N., and Sonnad, B. (2013). A review on family Moraceae (Mulberry) with a focus on Artocarpus species. *World Journal of Pharmacy and Pharmaceutical Science* 2: 2614-21.

Song, X., Wei, J., Mao, Z., Chi, X., Zhu, Z., Han, G., and Cheng, W. (2023). Effect of hot air-drying conditions on the drying efficiency and performance of a waterborne coating on pinewood. *Forests* 14(9): 1752.

Sonneville-Aubrun, O., Yukuyama, M. N., and Pizzino, A. (2018). Application of Nanoemulsions in Cosmetics. In *Nanoemulsions* (pp. 435-475). Academic Press.

Sousa, D. F. D., Campos Filho, P. C., and Conceicao, A. O. D. (2021). Antibacterial activity of jackfruit leaves extracts and the interference on antimicrobial susceptibility of enteropathogen. *Food Science and Technology* 42*: 1-5.*

Sulaiman, I. S. C., Basri, M., Masoumi, H. R. F., Ashari, S. E., and Ismail, M. (2016). Design and development of a nanoemulsion system containing extract of *Clinacanthus nutans* (L.) leaves for transdermal delivery system by D-optimal mixture design and evaluation of its physicochemical properties. *RSC Advances* 6(71): 67378-67388.

*Summary of External Trade Agrofood and Selected Agricultural Products* (2022). Policy and Strategic Planning Division, Ministry of Agriculture and Food Security Malaysia (2022): Putrajaya.

Swami, S. B., Thakor, N. J., Haldankar, P. M., and Kalse, S. B. (2012). Jackfruit and its many functional components as related to human health: a review. *Comprehensive Reviews in Food Science and Food Safety* 11(6): 565-576.

Syed Azhar, S. N. A., Ashari, S. E., and Salim, N. (2018). Development of a kojic monooleate-enriched oil-in-water nanoemulsion as a potential carrier for hyperpigmentation treatment. *International Journal of Nanomedicine*: 6465-6479.

Tamnak, S., Mirhosseini, H., Tan, C. P., Amid, B. T., Kazemi, M., and Hedayatnia, S. (2016). Encapsulation properties, release behavior and physicochemical characteristics of water-in-oil-in-water (W/O/W) emulsion stabilised with pectin–pea protein isolate conjugate and Tween 80. *Food hydrocolloids* 61: 599-608.

Tan, S. M., Teoh, X. Y., Le Hwang, J., Khong, Z. P., Sejare, R., Almashhadani, A. Q., ... and Chan, S. Y. (2022). Electrospinning and its potential in fabricating pharmaceutical dosage form. *Journal of Drug Delivery Science and Technology* 76: 103761.

Tan, X., Feldman, S. R.., Chang, J., and Balkrishnan, R. (2012). Topical drug delivery systems in dermatology: a review of patient adherence issues. *Expert Opinion Drug Delivery* 9(10): 1263-1271.

Tavano, L., Alfano, P., Muzzalupo, R., and de Cindio, B. (2011). Niosomes vs microemulsions: new carriers for topical delivery of capsaicin. *Colloids and Surfaces B: Biointerfaces* 87(2): 333-339.

Tellez, C. M., Figueroa, P. I., Tellez, C. B., Vidana, E. C. L., and Ortiz, A. L. (2018). Solar drying of Stevia (*Rebaudiana Bertoni)* leaves using direct and indirect technologies. *Solar Energy* 159: 898–907.

Toğrul, İ. T., and Pehlivan, D. (2004). Modelling of thin layer drying kinetics of some fruits under open-air sun drying process. *Journal of Food Engineering* 65(3) 413-425.

Tong, X., Pan, W., Su, T., Zhang, M., Dong, W., and Qi, X. (2020). Recent advances in natural polymer-based drug delivery systems. *Reactive and Functional Polymers* 148: 104501.

Trujillo-Cayado, L. A., Santos, J., Calero, N., Alfaro-Rodríguez, M. C., and Muñoz, J. (2020). Strategies for reducing Ostwald ripening phenomenon in nanoemulsions based on thyme essential oil. *Journal of the Science of Food and Agriculture* 100(4): 1671-1677.

Vazhacharickal, P. J., Sajeshkumar, N. K., Mathew, J. J., Kuriakose, A. C., Abraham, B., Mathew, R. J., ... and Jose, S. (2015). Chemistry and medicinal properties of jackfruit (*Artocarpus heterophyllus*): A review on current status of knowledge. *International Journal of Innovative Research and Review* 3(2): 83-95.

Verma, D., Gulati, N., Kaul, S., Mukherjee, S., and Nagaich, U. (2018). Protein based nanostructures for drug delivery. *Journal of Pharmaceutics*: 1-18.

Verma, L. R., Bucklin, R. A., Endan, J. B., and Wratten, F. T. (1985). Effects of drying air parameters on rice drying models. *Transactions of the ASAE* 28(1): 296-0301.

Wahgiman, N. A., Salim, N., and Rahman, M. B. A. (2020). Phase behaviour study on medium-chain triglyceride/surfactant/water systems containing gemcitabine using phase inversion composition technique. *Malaysian Journal of Analytical Sciences* 24(3): 363-372.

Walia, N., Zhang, S., Wismer, W., and Chen, L. (2022). A low energy approach to develop nanoemulsion by combining pea protein and Tween 80 and its application for vitamin D delivery. *Food Hydrocolloids for Health* 2: 100078.

Wang, C. S., Virgilio, N., Wood-Adams, P. M., and Heuzey, M. C. (2018). A gelation mechanism for gelatin/polysaccharide aqueous mixtures. *Food Hydrocolloids* 79: 462-472.

Wang, L., and Zhang, Y. (2017). Eugenol nanoemulsion stabilised with zein and sodium caseinate by self-assembly. *Journal of Agricultural and Food Chemistry* 65(14): 2990-2998.

Wang, Q., Zhang, H., Huang, J., Xia, N., Li, T., and Xia, Q. (2018). Self-double-emulsifying drug delivery system incorporated in natural hydrogels: a new way for topical application of vitamin C. *Journal of Microencapsulation* 35(1): 90-101.

Wang, S., Wang, X., Liu, M., Zhang, L., Ge, Z., Zhao, G., and Zong, W. (2020). Preparation and characterisation of *Eucommia ulmoides* seed oil O/W nanoemulsion by dynamic high-pressure microfluidisation. *LWT* 121: 108960.

Wang, X. L., Di, X. X., Shen, T., Wang, S. Q., and Wang, X. N. (2017). New phenolic compounds from the leaves of *Artocarpus heterophyllus*. *Chinese Chemical Letters* 28(1): 37-40.

Wang, Z., Guo, W., Kuang, X., Hou, S., and Liu, H. (2017). Nanopreparations for mitochondria targeting drug delivery system: current strategies and future prospective. *Asian Journal of Pharmaceutical Sciences* 12(6): 498-508.

Xu, W., Ling, P., and Zhang, T. (2013). Polymeric micelles, a promising drug delivery system to enhance bioavailability of poorly water-soluble drugs. *Journal of Drug Delivery*: 1-15.

Xu, X., and Gupta, N. (2018). Determining elastic modulus from dynamic mechanical analysis: a general model based on loss modulus data. *Materialia* 4: 221-226.

Yadav, K. S., and Kale, K. (2020). High pressure homogeniser in pharmaceuticals: understanding its critical processing parameters and applications. *Journal of Pharmaceutical Innovation* 15: 690-701.

Yahoum, M. M., Toumi, S., Tahraoui, H., Lefnaoui, S., Kebir, M., Amrane, A., Assadi, A. A., Zhang, J., and Mouni, L. (2023). Formulation and evaluation of xanthan gum microspheres for the sustained release of metformin hydrochloride. *Micromachines* 14(3): 609.

Yaldiz, O., Ertekin, C., and Uzun, H. I. (2001). Mathematical modeling of thin layer solar drying of sultana grapes. *Energy* 26(5): 457-465.

Yamaguchi, T., Takamura, H., Matoba, T., and Terao, J. (1998). "HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl," *Bioscience, Biotechnology, and Biochemistry* 62(6): 1201-1204.

Yan, J. K., Wu, L. X., Qiao, Z. R., Cai, W. D., and Ma, H. (2019). Effect of different drying methods on the product quality and bioactive polysaccharides of bitter gourd (Momordica charantia L.) slices. *Food Chemistry* 271: 588-596.

Yazid, N. S. M., Abdullah, N., and Muhammad, N. (2019, July). Comparison of Chemical, Functional and Morphological Characteristics of Jackfruit (*Artocarpus Heterophyllus* Lam.) (J33) Seed Starch and Commercial Native Starches. In *IOP Conference Series: Earth and Environmental Science* (Vol. 269, No. 1, p. 012031). IOP Publishing.

Yeo, E., Chieng, C. J. Y., Choudhury, H., Pandey, M., and ranasinghe, B. (2021). Tocotrienols-rich naringenin nanoemulgel for the management of diabetic wound: Fabrication, characterisation and comparative *in vitro* evaluations. *Current Research in Pharmacology and Drug Discovery* 2: 100019.

Yin, B., Deng, W., Xu, K., ajovalasit, L., and Yao, P. (2012). Stable nano-sized emulsions produced from soy protein and soy polysaccharide complexes. *Journal of Colloid and Interface Science* 380(1): 51-59.

Yin, M., Yang, D., Lai, S., and Yang, H. (2021). Rheological properties of xanthan-modified fish gelatin and its potential to replace mammalian gelatin in low-fat stirred yogurt. *LWT* 147: 111643.

Yukuyama, M. N., Ghisleni, D. D. M., Pinto, T. D. J. A., and Bou-Chacra, N. A. (2016). Nanoemulsion: process selection and application in cosmetics–a review. *International Journal of Cosmetic Science* 38(1): 13-24.

Zang, X., Wang, J., Yu, G., and Cheng, J. (2019). Addition of anionic polysaccharides to improve the stability of rice bran protein hydrolysate-stabilised emulsions. *LWT* 111: 573-581.

Zhang, L., Han, C., Liu, M., Yang, H., Zhang, F., Liu, B., and Meng, X. (2020). The formation, stability of DHA/EPA nanoemulsion prepared by emulsion phase inversion method and its application in apple juice. *Food Research International* 133: 109132.

Zhang, L., Tu, Z. C., Xie, X., Wang, H., Wang, H., Wang, Z. X., and Lu, Y. (2017). Jackfruit (*Artocarpus heterophyllus* Lam.) peel: A better source of antioxidants and a-glucosidase inhibitors than pulp, flake and seed, and phytochemical profile by HPLC-QTOF-MS/MS. *Food Chemistry* 234: 303-313.

Zhang, R., Zhang, Z., Kumosani, T., Khoja, S., Abualnaja, K. O., and McClements, D. J. (2016). Encapsulation of β-carotene in nanoemulsion-based delivery systems formed by spontaneous emulsification: influence of lipid composition on stability and bioaccessibility. *Food Biophysics* 11(2): 154-164.

Zhang, T., Ding, M., Tao, N., Wang, X., and Zhong, J. (2020). Effects of surfactant type and preparation pH on the droplets and emulsion forms of fish oil-loaded gelatin/surfactant-stabilised emulsions. *LWT* 117: 108654.

Zhang, W., Chen, L., and Fang, X. (2015). Optimising the preparation conditions for shea butter nanoemulsions via response surface methodology. *Journal of Dispersion Science and Technology* 36(7): 983-990.

Zhang, Z., and McClements, D. J. (2018). Overview of Nanoemulsion Properties: Stability, Rheology, and Appearance. In *Nanoemulsions* (pp. 21-49). Academic Press.

Zhang, Z., Tsai, P. C., Ramezanli, T., and Michniak-Kohn, B. B. (2013). Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 5(3): 205-218.

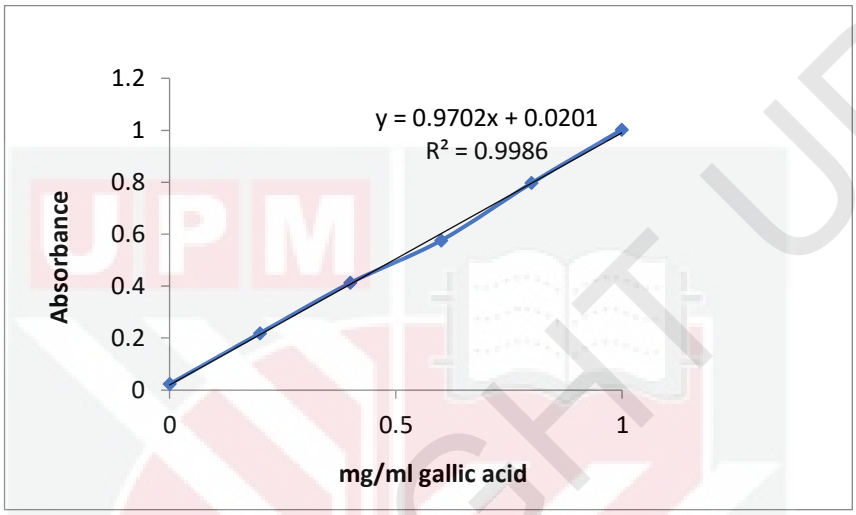
Zheng, Z. P., Xu, Y., Qin, C., Zhang, S., Gu, X., Lin, Y., Xie, G., Wang, M., and Chen, J. (2014). Characterisation of antiproliferative activity constituents from *Artocarpus heterophyllus*. *Journal of Agricultural and Food Chemistry* 62(24): 5519–5527.

# APPENDICES

## Appendix A

### Standard Curve for Total Phenolic Content (TPC)

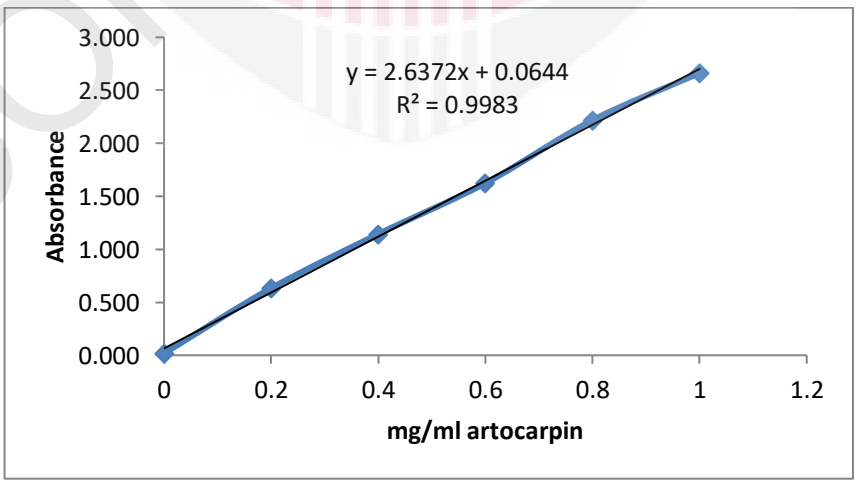
The standard curve for TPC using gallic acid as standard was shown as follows;




## Appendix B

### Standard Curve for Total Flavonoid Content (TFC)

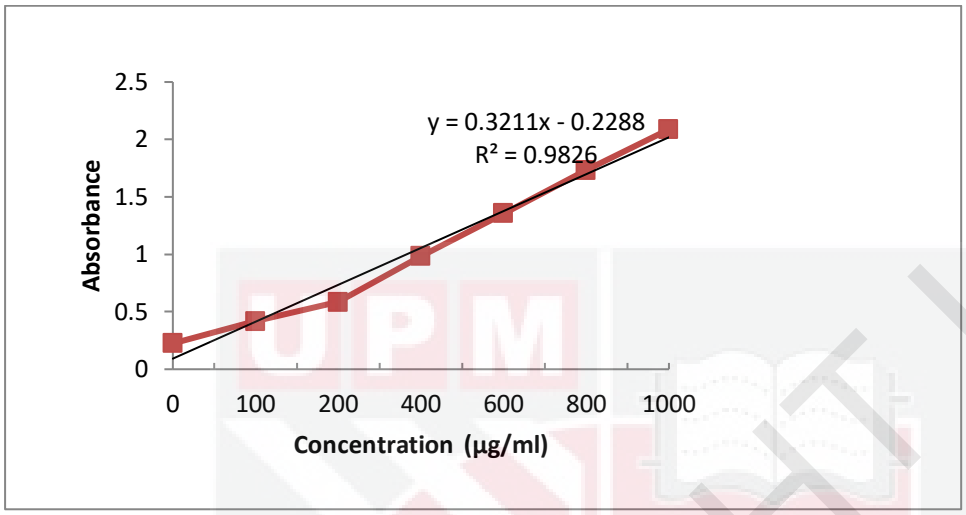
The standard curve for TFC using artocarpin as standard was shown as follows;



## Appendix C

### Standard Curve for Ferric Reducing Absorption Power (FRAP)

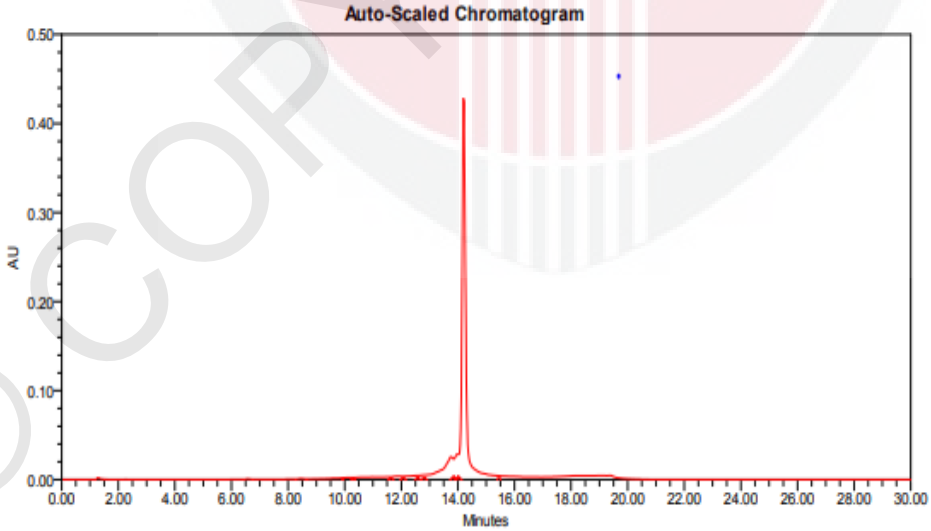
The standard curve for FRAP using Trolox as standard was shown as follows;




## Appendix D

### Chromatogram for Artocarpin

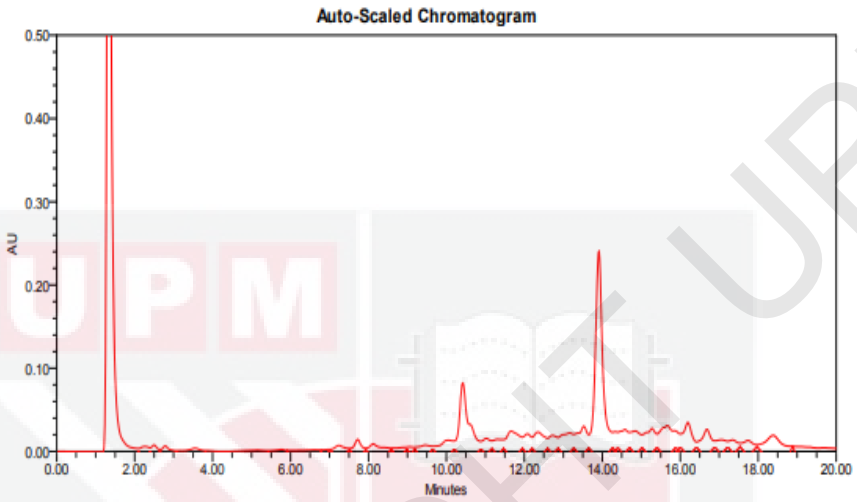
The chromatogram for artocarpin standard using HPLC was shown as follows;



## Appendix E

### Chromatogram for OV80 for Quantification of Artocarpin

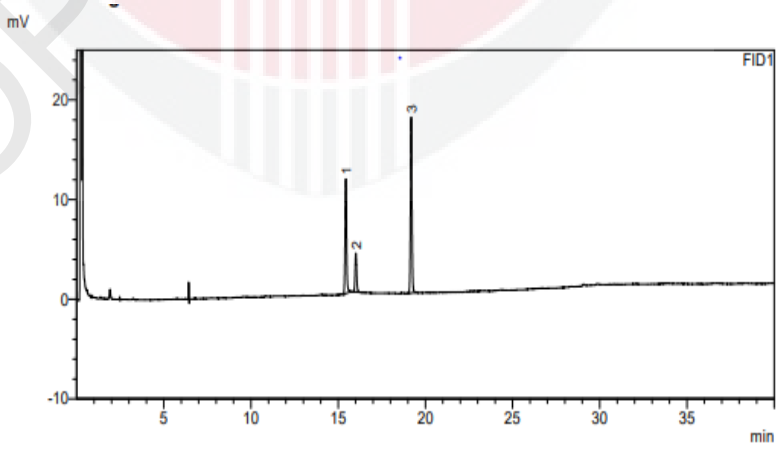
The chromatogram of 0V80 for quantification of artocarpin using HPLC was shown as follows;




## Appendix F

### Chromatogram for Squalene and β-sitosterol

The chromatogram for squalene and β-sitosterol standard using GC-FID was shown as follows;

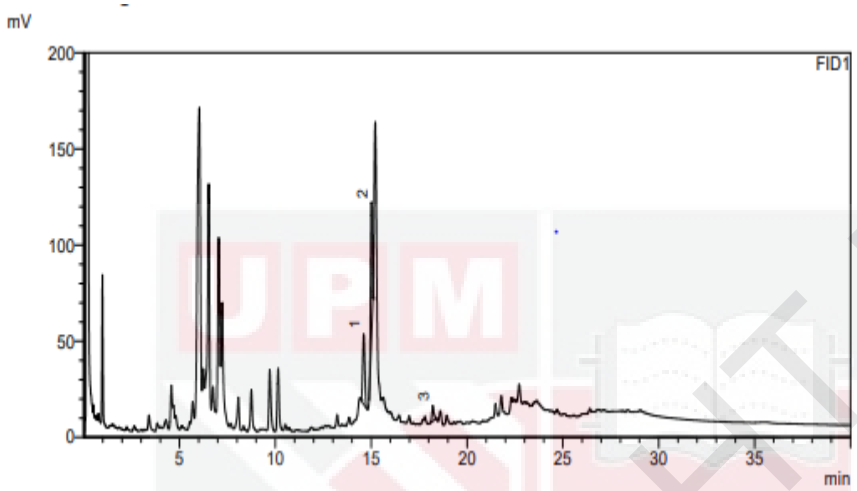



| Peak# | Retention Time | Area | Area % | Name |
|---|---|---|---|---|
| 1 | 15.434 | 70702 | 34.938 | Squalene |
| 2 | 16.013 | 24455 | 12.085 | 5α-cholestane |
| 3 | 19.189 | 107206 | 52.977 | B-sitosterol |
| Total | | 202362 | | |

## Appendix G

### Chromatogram of OV80 for Quantification of Squalene and β-sitosterol

The chromatogram of OV80 for quantification of squalene and β-sitosterol using GC-FID was shown as follows;




| Peak# | Retention Time | Area | Area % | Name |
|---|---|---|---|---|
| 1 | 14.611 | 279707 | 37.924 | Squalene |
| 2 | 15.130 | 401083 | 54.381 | 5α-cholestane |
| 3 | 18.414 | 56754 | 7.695 | B-sitosterol |
| Total | | 737543 | | |

## BIODATA OF STUDENT

Haswani Maisarah was born on 29th August 1984 at Hospital Pusrawi, Kuala Lumpur. She received her early education at S. R .K Jalan Padang Tembak 2, Kuala Lumpur before moved to Bandar Baru Bangi to continue her primary education at S.K. Jalan 2, Bandar Baru Bangi and S. R .K. Jalan 3 Bandar Baru Bangi, Selangor. She then continued her secondary education at Sekolah Menengah Kebangsaan Jalan 3, Bandar Baru Bangi, Selangor.

She was then furthered her tertiary education at Universiti Teknologi MARA, Kuala Pilah branch, Negeri Sembilan for her Diploma in Science. She pursued her Bachelor Degree (Hons.) in Food Science and Technology in 2006 and received her Master in Science at Universiti Teknologi MARA, Shah Alam in 2014.

In 2016, she continued to study in Doctor of Philosophy majoring in Food Engineering/Nanotechnology. During her study, she was actively involved in research and development activities, attending workshop and seminars to gain knowledge and experiences.

## LIST OF PUBLICATIONS

### Published

Mustafa, H. M., Amin, N. A. M., Zakaria, R., Anuar, M. S., Baharuddin, A. S., Hafid, H. S., and Omar, F. N. (2020). Dual impact of different drying treatments and ethanol/water ratios on antioxidant properties and colour attribute of jackfruit leaves (*Artocarpus heterophyllus* Lam.) *Mastura* Variety (J35). *BioResources* 15(3), 5122-5140.

Mustafa, H. M., Amin, N. A. M., Zakaria, R., Anuar, M. S., and Baharuddin, A. S. (2025). Effect of gelatin/xanthan gum ratios on jackfruit leaf extract nanoemulsion gel stability and properties. *BioResources* 20(1), 2056-2070.