PREPARATION, CHARACTERISATION AND EFFECTIVENESS OF ESSENTIAL OIL-ENCAPSULATED BIOPOLYMER BASED NANOEMULSIONS AS MOSQUITO SPRAY FORMULATIONS FOR FABRIC FINISHES

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UNIVERISITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

This research was conducted to prepare, characterise and determine the effectiveness of nanoemulsion formulations consisting of biopolymers and plant essential oils. Anionic tragacanth gum and cationic guar gum act as nanocarriers for betel, clove and lemongrass essential oils, which are active ingredients in nanoemulsion systems. The characterisation studies were carried out using Fourier transform infrared (FTIR) spectrometer, thermogravimetric analyser (TGA), differential scanning calorimeter (DSC), high resolution transmission electron microscope (HR-TEM), field emission scanning electron microscope (FESEM), zeta potential and particle size analyser. The ability of nanoemulsion formulations to load and release essential oils in vitro in rainwater was determined using an ultraviolet-visible (UV-Vis) spectrophotometer. The Excito chamber study was performed to evaluate the effectiveness of the fabrics treated with nanoemulsion formulations to repel Aedes aegypti mosquitoes. Based on FTIR analysis, there were changes in absoprtion and prominent peaks that indicate the presence of biopolymers and essential oils functional group in each nanoemulsion formulation, confirming the successful encapsulation of essential oils into nanocarriers. All nanoemulsion formulations exhibit 61% to 96% encapsulation efficiency with a range of -16.8 mV to -55.5 mV zeta potential and 0.21 to 0.74 polydispersity index. Based on effectiveness studies, following 5 cycles of washing and heating, most nanoemulsion formulations were able to retain more than 50% on cotton, while more than 30% of the nanoemulsion formulations retained on polyester. The Excito chamber study shown 60% to 98% of *Ae. Aegypti* mosquitoes were successfully repelled from cotton, whereas 30% to 90% of mosquitoes were repelled from polyester. In conclusion, both anionic tragacanth gum and cationic guar gum have potential as nanocarriers for essential oils in the development of nanoemulsions for fabric finishes. In implication, the application of biopolymers as alternatives to synthetic binders for fabric finishes could produce eco-friendly mosquito repellent spray formulations.

PENYEDIAAN, PENCIRIAN DAN KEBERKESANAN MINYAK PATI TERKAPSUL BERASASKAN BIOPOLIMER NANOEMULSI SEBAGAI FORMULASI SEMBURAN NYAMUK UNTUK KEMASAN FABRIK

ABSTRAK

Penyelidikan ini dijalankan untuk menyediakan, mencirikan dan menentukan keberkesanan formulasi nanoemulsi yang terdiri daripada biopolimer dan minyak pati tumbuhan. Gam tragacanth anion dan gam guar kation bertindak sebagai nanopembawa untuk minyak pati sirih, cengkih dan serai, yang mana merupakan bahan aktif dalam sistem nanoemulsi. Kajian pencirian dijalankan menggunakan spektrometer penyerapan inframerah transformasi Fourier (FTIR), penganalisis termogravimetri (TGA), kalorimeter pengimbasan pembezaan (DSC), mikroskop elektron penghantaran resolusi tinggi (HR-TEM), mikroskop elektron pengimbasan pancaran medan (FESEM), penganalisis keupayaan zeta dan zarah saiz. Keupayaan formulasi nanoemulsi untuk memuatkan dan membebaskan minyak pati secara *in vitro* dalam air hujan ditentukan menggunakan spektrofotometer ultralembayung-nampak (UV-Vis). Kajian kebuk Excito dijalankan untuk menilai keberkesanan fabrik yang dirawat dengan formulasi nanoemulsi untuk menghalau nyamuk *Aedes aegypti*. Berdasarkan analisis FTIR, terdapat perubahan pada jalur penyerapan dan puncak utama yang menunjukkan kehadiran kumpulan berfungsi bagi biopolimer dan minyak pati dalam setiap formulasi nanoemulsi, mengesahkan kejayaan pengkapsulan minyak pati ke dalam nanopembawa. Semua formulasi nanoemulsi mempamerkan kecekapan pengkapsulan 61% hingga 96% dengan julat keupayaan zeta -16.8 mV hingga -55.5 mV dan indeks poliserakan 0.21 hingga 0.74. Berdasarkan kajian keberkesanan, selepas 5 kitaran pencucian dan pemanasan, kebanyakan formulasi nanoemulsi dapat mengekalkan lebih daripada 50% kelekatan pada kapas, manakala lebih daripada 30% kelekatan formulasi nanoemulsi dikekalkan pada poliester. Kajian kebuk Excito menunjukkan 60% hingga 98% nyamuk *Ae. Aegypti* berjaya dihalau daripada kapas, manakala 30% hingga 90% nyamuk telah dihalau daripada poliester. Kesimpulannya, kedua-dua gam tragacanth anion dan gam guar kation mempunyai potensi sebagai nanopembawa kepada minyak pati dalam pembangunan nanoemulsi untuk kemasan fabrik. Implikasinya, penggunaan biopolimer sebagai alternatif kepada pengikat sintetik untuk kemasan fabrik boleh menghasilkan formulasi semburan penghalau nyamuk yang mesra alam.

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LIST OF ABBREVIATIONS

BEO	Betel essential oil
GG	Cationic guar gum
GGBEO	Cationic guar gum loaded with betel essential oil
GGCEO	Cationic guar gum loaded with clove essential oil
GGLEO	Cationic guar gum loaded with lemongrass essential oil
CEO	Clove essential oil
DSC	Differential Scanning Calorimetry
DLS	Dynamic Light Scattering
EE	Encapsulation Efficiency
ECDC	European Centre for Disease Prevention and Control
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared spectroscopy
HR-TEM	High Resolution Transmission Electron Microscopy
ISO	International Organization for Standardization
LEO	Lemongrass essential oil
NPs	Nanoparticle
PDI	Polydispersity index
TGA	Thermogravimetric Analysis
TG	Tragacanth gum
TGBEO	Tragacanth gum loaded with betel essential oil
TGCEO	Tragacanth gum loaded with clove essential oil

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TGLEO	Tragacanth gum loaded with lemongrass essential oil
UV-Vis	Ultra-violet Visible Spectroscopy
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Insect Repellent

The field of repellent science and research studies is at a pivotal point. In-depth knowledge of how repellents function could lead to the development of novel with an improve formulations, lead to whole new applications for insect repellents. The present global focus on preventing infection from vector-borne diseases has posed a challenge for repellent researchers to determine how to use repellents for this purpose (Mapossa et al., 2021). New formulations may result in goods that are not only more effective but also more user-friendly (Coetzee et al., 2022). Taken together, current advances may pave the door for entirely new and more effective repellent applications in the future.

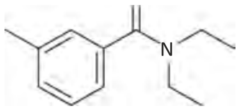
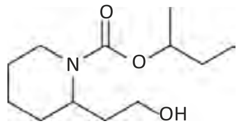
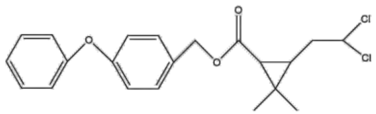
The word "repellent" can be used as either a noun or an adjective, with its origin in the Latin verb "repellere," which means "to drive back". This action involves a sense of repulsion, characterised by moving away from something. The term itself originates from the Latin word "antem," meaning "agent of action" (Gordh & Headrick, 2001; Katz et al., 2008; Sanga et al., 2023). Repellents are categorised as stimuli that elicit an avoidance reaction and are differentiated between contact repellents, which require physical touch by the insect, and vapor repellents, which can be detected in the air (Debboun et al., 2015; Sanga et al., 2023). Repellents play a crucial role in protecting individuals from insect-borne diseases by creating a barrier that insects are driven away from human.

Insect repellents can affect insect behaviour without the need for physical contact. Previous research has focused on developing tools to understand how repellents work at a neuromolecular level (Hazarika et al., 2022). In 2013, studies delved into the responses of gustatory receptor neurons (GRN) in mosquitoes to insect repellents (Sanford et al., 2013; Yamany & Abdel-Gaber, 2022). Lemongrass oil and para-Methane-3,8-diol (PMD) repelled *Anopheles coluzzii* mosquitoes, while *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes were repelled by lemongrass oil, PMD, eugenol, and diethyltoluamide (DEET). Higher concentrations of active ingredients resulted in better repellent activity and triggered more olfactory receptor neurons (ORN) in the *An. coluzzii* antennae compared to lower concentrations which led to changes in their repulsive behaviours (Affify & Potter, 2020: Ghosh et al., 2023). These studies have provided valuable insights into the effectiveness of different insect repellents and the mechanisms behind their repellent activity.

During the 1940s and 1950s, as synthetic chemicals started to replace botanicals as the preferred repellent, there was an increase in scientific rigor within the traditional repellent industry. Post-World War II, the repellent business prospered and underwent rapid evolution due to extensive research efforts aimed at discovering and creating repellents for military purposes (Debboun et al., 2015; Anwar et al., 2023). Consumer insect repellents are readily available in various retail outlets including camping and travel stores, pharmacies, and supermarkets. Table 1.1 presents a list of commercial insect repellents along with their active ingredients. A wide range of commercial insect repellents are available, each containing different active ingredients that have been scientifically tested and proven to effectively repel insects (Mapossa et al., 2021).

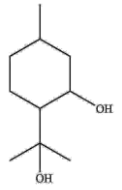
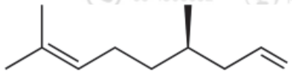
Table 1.1

An overview of commercially available insect repellents and their active ingredients

Repellent	Active ingredient	Repellency	Safety/Toxicity	Origin
DEET	*N*,*N*-diethyl-3-methyl-benzamide. (*N*, *N*-diethyl-m-toluamide) 	6.65% DEET provides about 5 hours of protection against mosquitoes.	Potential neuro toxicity if applied under sunscreen. EPA toxicity category III (slightly toxic)	Not reported
Picaridin	(2-(2-hydroxyethyl)-1-piperidine-carboxylic acid 1-methylpropyl-ester) 	Picaridin 20% works as well as DEET 20%.	Possible skin irritation. EPA toxicity category III (slightly toxic)	Not reported
Permethrin	(3-phenoxybenzyl (1RS)- cis, trans-3-(2, 2- dichlorovinyl)-2, 2- dimethyl-cyclo-propanecarboxylate) 	Effective as DEET	Not useful on skin. Possible skin irritation. (EPA, likely human carcinogen)	*Chrysanthemum* spp.

(Continue)

Table 1.1 *(Continued)*

Repellent	Active ingredient	Repellency	Safety/Toxicity	Origin
PMD	(p-menthane-3, 8-diol) 	PMD is as effective as DEET when used in like quantities. PMD provides about 2 hours of protection against mosquito bites	Potential skin irritation in atopic individuals. EPA toxicity category I (highly toxic)	*Lemon eucalyptus*
Citronella	(3, 7-dimethyloct-6-en-1-al) 	The complete protection time (CPT) of DEET (360 min) was much longer than the CPTs of citronella (10.5 min) from mosquitoes	Potential eye and skin irritation and allergies for Ceylon type EPA toxicity category IV (practically non-toxic)	*Cymbopogon* spp.

Adapted: Lee (2018)

1.1.1 Characteristic of Insect Repellent Formulations

The discomfort caused by an allergic response, including itching, swelling and skin irritations, as well as the risk of disease transmission, can result from the bite of a blood-feeding insect (Elsayed & Hassabo, 2021). Therefore, using a safe and efficient topical repellent at home or work or while outdoors during the day or night could minimise biting incidents. Repellents are substances that when applied to clothing or skin repel insects from landing and thus prevent them from biting (Mapossa et al., 2021). Table 1.2 provides the key attributes of an effective insect repellent formulation.

Table 1.2

Optimal features of an insect repellent active formulation

Characteristic	Description
1	Low toxicity, minimal skin absorption and non-irritating to the skin, mucous membranes and eyes.
2	Unpleasant scent.
3	Effective long-lasting protection to prevent the need for frequent reapplications.
4	Insects such as mosquitoes, flies, ticks, bees and wasps are the primary targets for this wide-ranging approach.
5	Excellent visual and sensory qualities without causing an oily feeling on the skin or leaving any residue.
6	Resistance to water and heavy perspiration.
7	Exhibits high chemical stability and does not react with plastics, synthetic fibers, acrylics, or glass.
8	Must not cause discolouration on fabrics and clothing.
9	Economically feasible at a low cost

Source: Tavares et al. (2018)

In experiments conducted on skin permeation in a laboratory setting, researchers aim to assess the extent of penetration of the active ingredient into the skin. The goal is for the active ingredient to remain primarily in the upper layers of the skin, while only minimal penetration is observed in the receptor solution used during in vitro permeation studies. This holds particular significance for insect repellents designed for topical use, as outlined by Pinto et al. (2017) and Gosh et al. (2023). Furthermore, efficacy evaluations involving in vivo repellence experiments are carried out using laboratory-bred mosquitoes certified to be free from arboviruses, as demonstrated by Ho et al. (2019) and Parvez et al. (2023).

1.1.2 Chemical Signalling in Insects

Insects have both olfactory and gustatory sensilla in their chemical sensing system. Mosquitoes possess olfactory sensilla on their antennae and maxillary palps as illustrated in Figure 1.1 (a). However, there have been limited observations of olfactory sensilla on the proboscis (Wheelright et al., 2021). The olfactory receptor neuron (ORN) transmits its axons to the antennal lobe, where clusters called glomeruli receive input from ORNs as depicted in Figure 1.1 (b). The dendrites of multiple ORNs are housed in the multiporous olfactory sensilla present in Figure 1.1 (c). Many ions and some important proteins involved in the olfactory process can be found in the fluid surrounding the dendrites of sensilla. Odorant-binding proteins (OBP) exhibit a high level of selectivity for odorants and are believed to help deliver odours to olfactory receptor neurons on the outer membrane of dendrites (Cuoto et al., 2005; Cassau & Krieger, 2020). ORNs, along with the olfactory receptor coreceptor Orco, form ligand-gated ion channels that largely determine their specificity when expressed. Pheromonebinding proteins (PBP) may bind to sensory neuron membrane protein 1, transporting pheromone molecules to an olfactory receptor. Additionally, various enzymes called odorant-degrading enzymes are responsible for deactivating odorants in the sensillum lymph (Nogueira et al., 2016; Legeay et al., 2018).

Gustatory sensilla are structures that contain a terminal pore through which non-volatile substances can be accessed. These substances interact with the gustatory receptors (GR) located at the tips of the dendrites of the gustatory receptor neurons (GRN) associated with each sensillum. GRs can interact with a variety of molecules, and their function is not yet fully understood. Some studies suggest that they may be G protein-coupled receptors or ligand-gated ion channels, but this is still a matter of debate (Shimdt & Benton, 2020). Gustatory receptor neurons are unique in that they can express multiple gustatory receptors, allowing them to code for a range of tastes, such as salt, sugar and bitter or unpleasant substances. Each gustatory receptor neuron sends axons to different parts of the suboesophageal ganglion, as demonstrated by Islam et al. (2017).

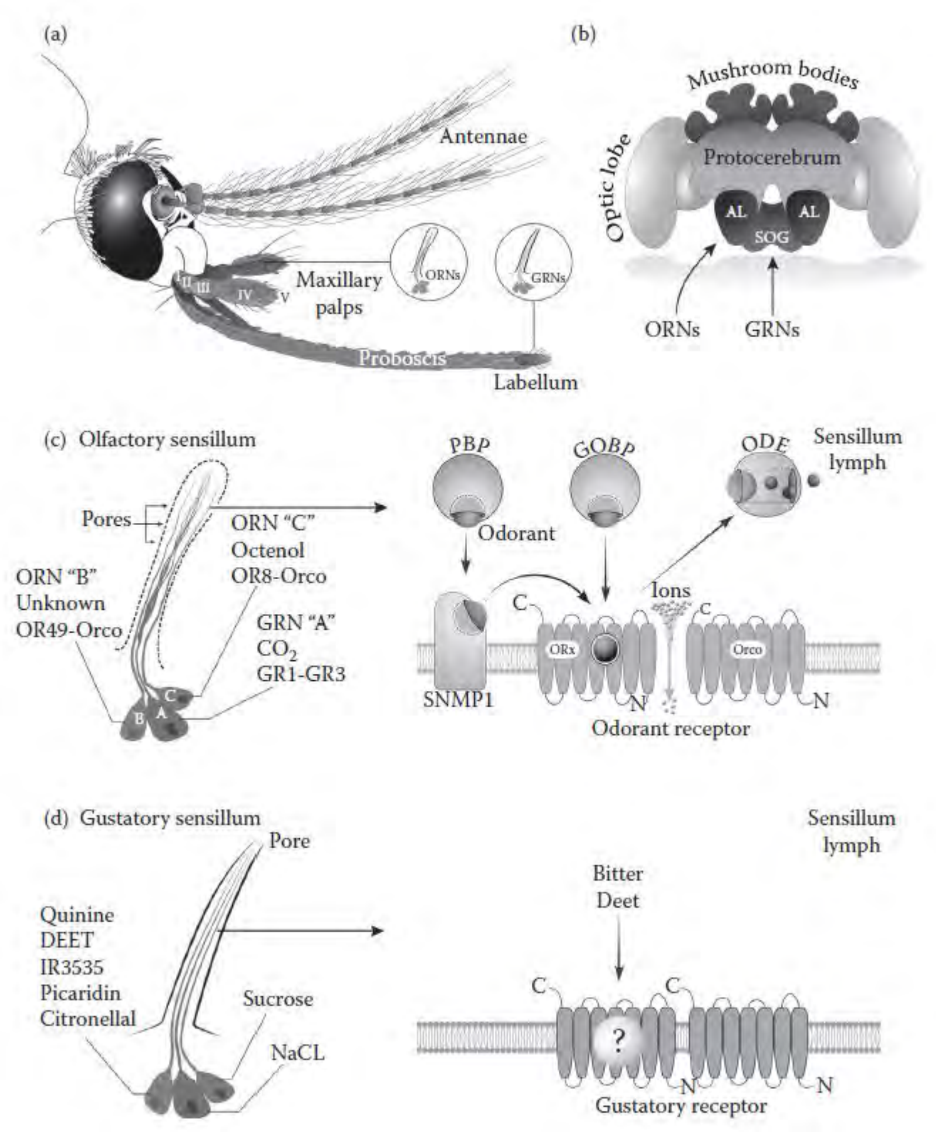


Figure 1.1. Chemical signalling in insect. Adapted: Debboun et al. (2015)

1.1.3 Application of Insect Repellent

Insect repellents are typically applied to the skin; however, they can also be applied to mosquito netting, tents and clothing. They generate a vapor barrier that prevents insects from coming into contact with human skin, which is unpleasant to them (Nogueira Barradas et al., 2016; Mapossa et al., 2021). An insect repellent is a chemical that creates a barrier to prevent insects from biting human or animal skin locally or at a distance. The repellent creates a vapor barrier that keeps mosquitoes from coming into contact with the skin (Legeay et al., 2018). Mosquito bites can cause an allergic reaction with symptoms such as itching, swelling and local redness, as well as the potential for disease transmission. Therefore, using safe and effective topical repellent compositions can prevent bites at home, work, or in open day and evening locations.

In general, a repellent is a type of chemical that deters arthropods such as mosquitoes from landing on or stinging human skin (Lee, 2018; da-Silva and Ricci-Junior, 2020). According to observations of insect behavior, there are five different categories of repellents namely: (a) genuine repellents, also known as spatial repellents or expellers, which push mosquitoes away from the source of the odor without any physical contact; (b) contact irritants, also known as landing inhibitions, which cause insects to move away from the source after coming into direct contact; (c) deterrents, which are substances that prevent specific behaviors such as blood feeding or oviposition; (d) odor-masking agents, which reduce the attractiveness of the host or disrupt the host location signal to block insect attraction; and (e) visual masking agents, which disrupt the visual signal and prevent the insects from locating the source (Islam et al., 2017; da-Silva and Ricci-Junior, 2020; Mapossa et al., 2021).

1.1.4 Global Insect Repellent Market

According to the projections by The Statistics Portal and Statista (2023), the global insect repellent market is expected to reach a value of USD 4.6 billion by 2023. This increase in market value can be attributed to the growing use of repellents in society.

In light of this trend, the insect repellent production industry presents a promising opportunity for economic growth in developing countries. The development of this sector will benefit other areas of the economy contributing to manufacture a product that is subject to the natural resources and promote it on a worldwide scale. As a result of this research, the use of chemical and natural products sourced locally will contribute to the development of new goods. During the advancement phase, the product will go through a scientific process to generate a product that is effective, environmentally safe, low in toxicity and affordable to people from all walks of life in the country.

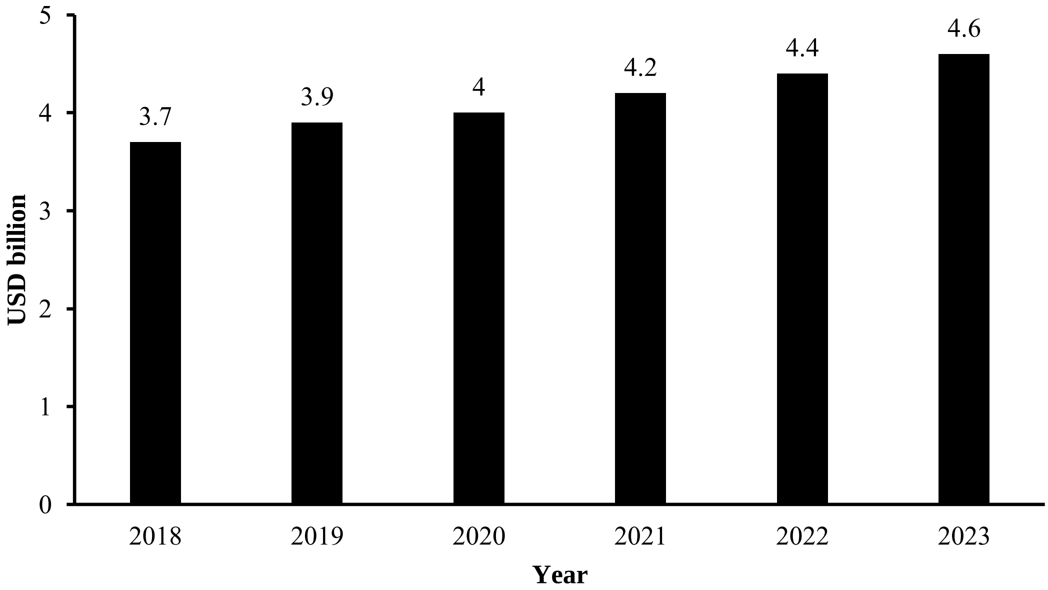


Figure 1.2. Global insect repellent market value from 2018 to 2023 (USD billion).
Source: The Statistics Portal, Statista (2023)

The high prevalence of mosquito-borne diseases has a major impact on the growth of the Asia Pacific body worn insect repellents market. Due to people are becoming more aware of insect repellents, the region has a lot of potential for insect repellent sales. The International Monetary Fund (IMF) reported that the Asia-Pacific region had the highest GDP growth rate globally in 2017 and this trend is anticipated to persist in the coming years (IMF, 2023).

1.2 Essential Oil

Flowers, roots, bark, leaves, seeds, peels, fruits, wood and whole plants are all sources of essential oils (EO), which are fragrant and volatile liquids extracted from plant material (Esmaili et al., 2021). Throughout history, these oils have captivated people's interest and while many of their applications have waned over time, it is widely believed that humans have been extracting them from fragrant plants since the beginning of time. Essential oils serve a variety of functions, ranging from enhancing the flavor and nutritional value of food in the kitchen to producing fragrances and cosmetics. Furthermore, essential oils have found applications in the pharmaceutical industry for their potential therapeutic benefits.

The ancient Egyptians utilised essential oils for medicinal, perfume and embalming purposes. The Vedas documented the use of fragrances and aromatics for religious and therapeutic reasons in ancient Asia. Many civilizations throughout history have used essential oils and fragrances for various reasons, including religious rites, perfume production and disease treatment. The Phoenicians, Jews, Greeks, Romans, and other Mediterranean cultures, as well as the Mayas and Aztecs in the Americas, had sophisticated fragrance cultures. Alchemists aimed to create the "elixir of life" for eternal life, while monks employed essential oils to produce medical items and fragrant derivatives such as soap and perfume. Essential oils were used in perfumes and cosmetics during the Renaissance period, as documented by Sonwa (2000) and Esmaili et al. (2021). Their popularity declined in the modern era, but in recent years there has been a resurgence of interest in essential oils for their natural and holistic properties.

1.2.1 Properties of Essential Oils

Essential oils can be extracted from various plant parts and they are complex mixtures that can contain up to 20 different components in different proportions. The main components of essential oils include terpenes, terpenoids and both aromatic and aliphatic compounds, which make up 20-70% of the total concentration. The remaining components make up the balance. The biological properties of essential oils are determined by the relative concentration of these main components (Singh and Pulikkal, 2022).

Essential oils are complex mixtures made up of various ingredients that are usually liquid but can also be solid. When first distilled, these mixtures range in color from colorless to slightly yellowish at room temperature, have a fragrant smell and are easily absorbed by the skin. Unlike fatty vegetable, animal, or mineral oils, a drop of essential oil on paper vanishes quickly, taking anywhere from a few minutes to a few days, depending on the temperature (Rios, 2016). Additionally, essential oils have a low density, except for cinnamon, clove and sassafras oils, which are denser than water. They are soluble in most common organic solvents, including ethanol and diethyl ether and mix well with vegetable oils, fats and waxes, despite their limited solubility in water (Gosh, Ranja and Gupta, 2023). Therefore, the distribution of major and minor components within essential oils is an important factor to consider when studying their properties and potential applications.

1.2.2 Essential Oil Industry

In 2018, the global essential oil market demand was 226.8 kilotonnes. From 2019 to 2025, it is predicted to grow at an annual rate of 8.6% (Statista, 2023). Essential oils are increasingly used in areas such as beverage, cuisine, personal care, aromatherapy and cosmetics. Essential oils provide a variety of health-related benefits and they are touted as the anticipated fuel, with growing demand in medical and pharmaceutical applications. Most conventional medications have no negative side effects. The increased popularity of organic and natural products among customers is contributing to a growth in the usage of essential oils in the beverage, food and cosmetics industries (Irshad et al., 2019).

As the world's population grows, so does the number of people suffer from various health problems. Essential oils are utilised in aromatherapy products and as a result, the global market for essential oils is growing every day. In the textile, paint and plastic industries, as well as pharmaceutical formulations, essential oils are frequently utilised in perfumes, personal hygiene products and aromatherapy, including inhalation, massage and masking agents to prevent undesirable odours (Mapossa et al., 2021; Singh & Pulikkal, 2022). Essential oils are also used in various kinds of cereals, antimicrobial packing of food items, edible thin film, nanoemulsion, preservation of fruits and vegetables, soft drinks, as flavouring agents in carbonated drinks, as major ingredients in soda/citrus concentrates, seafood preservations, fish, and so on (Mahato et al., 2019).

1.3 Triton X-100

Triton X-100 is a non-ionic surfactant that combines a hydrophilic polyethylene oxide chain with an aromatic hydrocarbon lipophilic or hydrophobic group, as depicted in Figure 1.3. This surfactant ($C_{16}H_{26}O_2$) has a molecular weight of 646.86 and is widely utilised in the biotechnology industry for laboratory operations, including cell lysis to extract proteins and increasing cell membrane permeability by dissolving lipids. This surfactant will be used to create micelles in this study. Micelles are colloidal suspensions made up of surfactant phospholipid molecules distributed in a liquid (Pavoni et al., 2020).

A surfactant is used to decrease the interfacial tension between two layers that cannot mix. The energies associated with adsorption and micelle formation are often analysed in relation to the dispersion attractions among the hydrocarbon chains, the electrostatic and van der Waals interactions between the head groups and the hydrophobic interaction occurring at the outer edge of the hydrocarbon chain of the surfactant monomer (El-Aila, 2009). Triton X-100 is a surfactant where the polyoxyethylene chains remain on the outside of the micelle's core and can move easily in the solvent. This arrangement helps to maintain the stability of the micelles in solution. Surfactants are frequently employed in practical applications where formulations consist of many chemicals and it is common to see synergistic effects. Synergism is defined as the state in which the characteristics of a mixture are superior to those achievable with the individual components in isolation (Pavoni et al., 2020). Understanding the interactions and properties of surfactants is crucial in various industries and applications where mixtures and formulations are involved.

The concentration at which surfactant solutions start to form micelles in large amounts is critical in oil-water interfacial tension reduction, rock wetting properties modification and emulsion generation. The minimum energy required for emulsion formation is lower in the presence of surfactants. A considerable reduction in oil-water interfacial tension and degree of water-wetness is also expected when there is sufficient adsorption and aggregation of the surfactant's molecules at the liquid-liquid and liquid-solid interfaces. Surfactants with lower CMC (critical micelle concentration) are expected to demonstrate significant potential for easy emulsion generation. However, the eventual stability of the emulsion will depend on the extent of surfactant aggregation at the liquid-liquid interface of the emulsion (Yekeen et al., 2020).

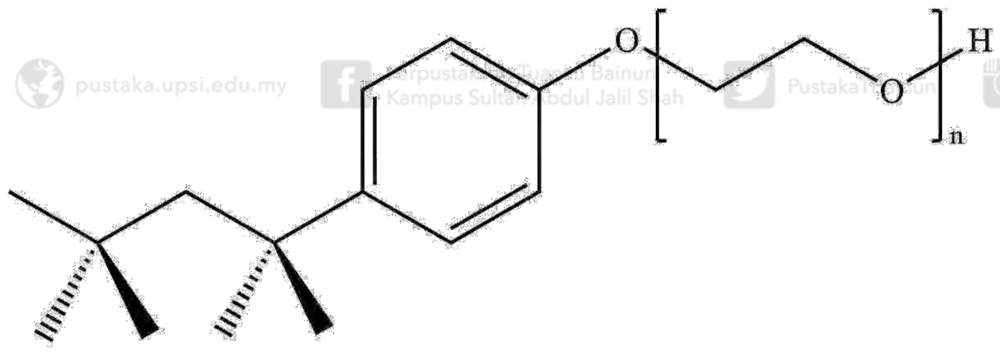


Figure 1.3. Chemical structure of Triton X-100

1.4 Biopolymer

Biopolymers have become a viable alternative to petrochemical polymers, which have been the traditional source of polymers since the 1930s. These biopolymers are produced by living organisms, such as plants and microbes, which are renewable sources. The widespread usage of petroleum-based polymers has led to serious environmental consequences, which have prompted scientists to look for alternative solutions. As a result, biopolymers are gradually displacing synthetic polymers and playing a larger role in both basic and applied research. Although they currently make up only a small percentage of the polymer market, their share is growing year after year. In terms of environmental benefits, biopolymers offer a more sustainable option by using renewable energy resources and reducing greenhouse gas emissions. Additionally, biopolymers have been found to improve soil organic content, increase water and nutrient retention, reduce chemical inputs, and suppress plant diseases (Ibrahim et al., 2019).

Gum serves as a biopolymer in the production of various items. When a tree's bark is injured, it produces a sticky substance that solidifies upon contact with air. Gums are the substance that adheres to the tree and are commonly utilised because they are eco-friendly, non-toxic, biodegradable and renewable. The gums can be divided into two categories based on their origin: natural gums and chemically modified natural gums, as well as synthetic gums that are chemically synthesised. Acacia Arabica, Acacia Catechu, mangosteen, Ghati, guar, tragacanth and a few other unidentifiable gums are among the most well-known types of gums used in industry (Taghavizadeh et al., 2021; Garg & Gupta, 2023).

1.4.1 Tragacanth Gum

Gum tragacanth, obtained from the *Astragalus* spp. plant, has a historical record of being utilised as a stabilising and viscosity-enhancing ingredient in food emulsions.

The gum is mostly manufactured in the Middle East and is approved for food consumption in both the United States and Europe (designated as E-number E413). Gum tragacanth is a hydrophilic polysaccharide that is extremely branched and heterogeneous. The gum consists of pectinaceous arabinogalactans and fucose-substituted xylogalacturonans (Gavlighi, 2013) as shown in Figure 1.4.

This heterogeneous polysaccharide made up of two subunits that occurs naturally. The primary subunit of tragacanth gum, known as tragacanthin, is water soluble, but the other subunit, known as bassorin, is somewhat less soluble in water and swells in water (Taheri & Jafari, 2019). Tragacanthin, a soluble polysaccharide, has been identified as the active ingredient in the exudate. Bassorin, which comprises 60-70% of the exudate, is the main component and contains methyl ether groups, causing it to swell in water and form gel particles. In addition to cellulose fibers and protein, the exudate contains other components. Gum tragacanth creates high-viscosity solutions, with the highest viscosity among the exudate gums, but maximum viscosity requires up to 24 hours of hydration. Due to its high shelf life, heat stability and broad pH range, gum tragacanth is used in various industries, as reported by Taghavizadeh et al. (2021). Overall, gum tragacanth is a versatile and effective ingredient due to its high viscosity, stability and compatibility with different pH levels.

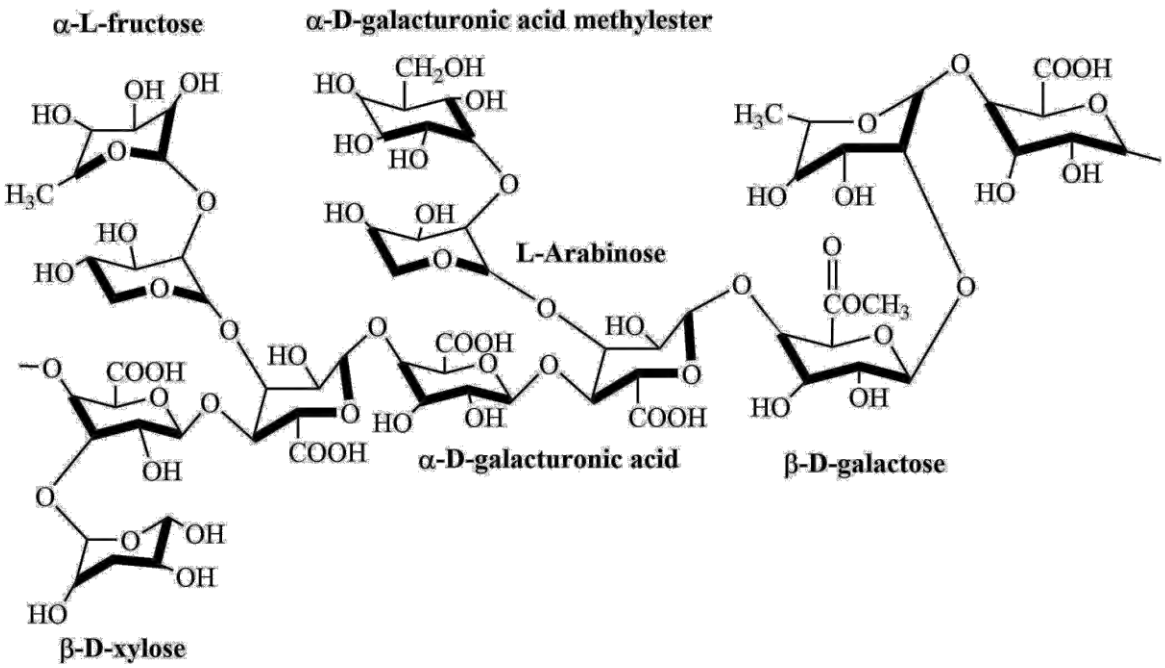


Figure 1.4. Chemical structure of tragacanth gum

1.4.2 Guar Gum

Guar gum is a polysaccharide derived from the endosperm of the *Cyamopsis tetragonoloba* seed plant (Mansa & Detellier, 2013). It consists of a chain of (1–4) β-D-manno-pyranosyl units, with a (1–6) α-D-galacto-pyranosyl unit present on every second unit as shown in Figure 1.5. Cationic guar gum is a modified version of the naturally occurring substance, in which hydroxyl groups are replaced with hydroxypropyltrimonium groups, as described by Grządka et al. (2021). According to Grządka et al. (2021), cationic guar gum has demonstrated exceptional properties as a non-gelling thickener, effectively enhancing both viscosity and volume. Moreover, when compared to conventional guar gum, cationic guar gum demonstrates enhanced solubility and thermal stability. The production of guar gum and its derivatives occurs on a large scale within the industrial sector, where they principally function as food hydrocolloids with high viscosity. However, cationic guar gum is widely employed in the field of cosmetics due to its ability to enhance viscosity, volume and foam properties (Bernal-Cháve et al., 2023).

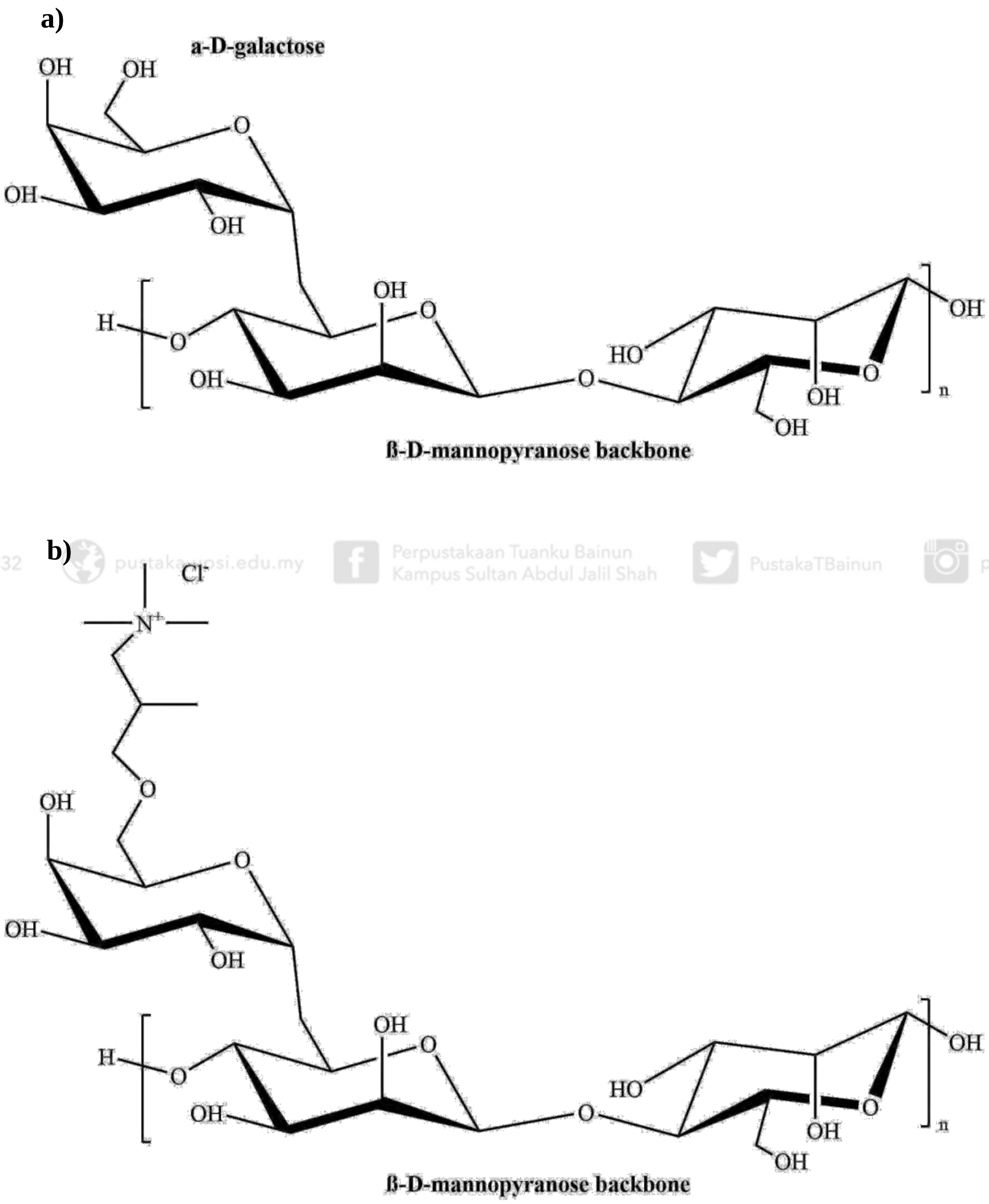


Figure 1.5. Chemical structure of (a) guar gum and (b) cationic guar gum

1.5 Types of Fabric

Textiles are materials that possess various properties such as strength, flexibility, elasticity, softness, durability, heat insulation, lightweight, water absorbency/repellence, dyeability and resistance to chemicals. These materials exhibit complex and non-linear behavior and their properties can be influenced by factors such as temperature, humidity and time. According to Denton and Daniels (2002), a textile fiber is a type of raw material used in textiles that is known for its flexibility, fineness and high length-to-thickness ratio. Approximately 90% of all fibers are first transformed into yarns before being turned into fabrics. Only about 7% of fibers are directly used in the production of final products (Grishanov, 2011). Textile production processes can be grouped into four primary categories namely: (1) fiber production, which can be either natural or man-made, (2) yarn production, where there are technical differences in spinning cotton, wool, synthetic fibers, and fiber blends, (3) fabric manufacturing, which involves the production of woven, knitted and nonwoven fabrics, carpets, webs and other sheet materials and (4) fabric finishing, which involves treating the final product to impart specific properties such as water repellency, anti-bacterial properties and fire-retardant properties through processes such as bleaching, dyeing, printing and specific treatments. These processes play a crucial role in determining the final characteristics and performance of textile materials.

1.5.1 Cotton

Cotton, flax, jute, ramie, and sisal are all-natural plant-based cellulose fibers that have been widely used in various industries, especially in the textile sector. These fibers possess exceptional qualities such as excellent water absorption, softness, hygroscopicity and natural durability, making them suitable for a wide range of applications. Moreover, they can be processed to produce a variety of colours due to their ability to be dyed in multiple ways. However, imparting high-performance properties to natural fibers by functionalising their absorption or penetration of functional polymeric materials onto/into the fiber surface can be beneficial. Cotton, in particular, is a superior fiber with the highest molecular weight and degree of structural organisation among all plant fibers. Its cellulose exhibits high crystallinity, orientation, and fibrillar arrangement, making it an excellent material for various applications (Hamdy & Hassabo, 2021). Cotton fibers are also known for their softness, breathability and ability to absorb water. They can hold up to 24-27 times their own weight in water, making them ideal for applications that require moisture retention.

1.5.2 Polyester

Polyester is a synthetic fiber that is made from a chemical reaction between acid and alcohol, which combines to form a large and stable molecule. This material is derived from resources such as coal, air, water and petroleum. It is a widely used plastic with a market share of 18%, following polypropylene and polyethylene. While natural polyesters are biodegradable, most synthetic polyesters are not (Satti & Shah, 2020). Polyester is commonly used in clothing and fabrics due to its excellent mechanical properties and dimensional stability (Palacios-Mateo et al., 2021). However, it is also hydrophobic and has low moisture absorption, which can lead to electric shocks and fire hazards. To address this issue, antistatic finishes have been applied to polyester fabric. Hydrophilic materials with antistatic properties can absorb moisture from the air to decrease static charge accumulations. However, these coatings can be easily hydrolysed during washing. Developing a polyester that is both resistant to water and static charge is challenging due to the counteracting mechanisms of water repellency and antistatic properties (Aizamddin & Mahat, 2023).

1.6 Research Background

1.6.1 Issues Related to Mosquito-Borne Diseases

Infections spread by the bite of infected mosquitoes are known as mosquito-borne disease (MBD). Mosquitoes serve as vectors in disease transmission, carrying parasites that cause human disease. Some parasite species are found in the midguts of mosquitos, and the infective stages of the parasites are transferred to humans during blood feeding. The mosquito is known as an intermediate host while parasites are growing in the midguts of mosquitos. By mitosis and meiosis, parasites evolve in the human system, making the human the definitive host. The abundance of parasites in the human body system could be harmful if they are not treated early on. Table 1.3 lists of non-exhaustive MBD according to mosquito species.

Table 1.3

List of mosquito-borne disease and its mosquito species

Mosquito	Disease	Type of pathogen
***Aedes* spp.**	Chikungunya	Virus
	Dengue	Virus
	Lymphatic filariasis	Thread-like worm
	Rift Valley fever	Virus
	Yellow Fever	Virus
	Zika	Virus
***Anopheles* spp.**	Lymphatic filariasis	Thread-like worm
	Malaria	Protozoan
***Culex* spp.**	Japanese encephalitis	Virus
	Lymphatic filariasis	Thread-like worm
	West Nile fever	Virus

The incidence of diseases such as dengue, chikungunya, yellow fever and Zika has been particularly high in tropical and subtropical regions, where they disproportionately affect the poorest populations (WHO, 2023). The outbreaks of these diseases have put a strain on healthcare systems in many countries since 2014. The distribution of mosquito-borne diseases is influenced by a variety of complex demographic, environmental and social factors, which can result in their appearance in areas where they were previously unknown (Fonseca et al., 2019). The symptoms of mosquito-borne diseases can vary depending on the specific pathogen and the severity of the symptoms can also be influenced by the individual's immunity level. MBD symptoms can be divided into two phases namely: mild symptoms and severe symptoms. The common symptoms of mosquito-borne diseases are summarised in Table 1.4.

Table 1.4

Mosquito-borne diseases and its symptoms

Mosquito-borne disease	Symptoms
Chikungunya	Fever, joint pain, headache, muscle pain, joint swelling, rash. Does not often result in death.
Dengue	Nausea, rash, aches, and pains (typically behind the eyes, muscle, joint or bone pain) – mild symptoms. Belly pain, vomiting, bleeding from nose or gums, vomiting blood - severe or blood in the stool, feeling tired, restless – severe symptoms.
Lymphatic filariasis	Swelling (Lymphedema, hydrocele), hardening and thickening of the skin (elephantiasis).
Rift Valley fever	Fever, weakness, back pain, dizziness – mild symptoms. Ocular disease (lesions on the eyes), encephalitis, haemorrhagic fever – severe symptoms.
Yellow Fever	Headache, muscle pain, joint pain, fever, flushing, loss of appetite, shivers, backpain – mild symptoms.
Zika	Fever, rash, headache, joint pain, conjunctivitis, muscle pain.
Malaria	Fever, chills, general feeling discomfort, headache, nausea, diarrhoea, abdominal paint, muscle or joint pain, fatigue, rapid breathing, rapid heart rate, cough.
Japanese Encephalitis	Fever, headache, vomiting, mental status changes, neurologic symptoms, weakness, movement disorder, seizures.
West Nile Fever	High fever, headache, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, vision loss, numbness, paralysis.

1.6.2 Mosquito-Borne Disease: Worldwide Perspective

Since the rise of arbovirus epidemics in urban cities, mosquito-borne viral diseases have been making headlines around the world. Vector-borne infections account for more than 17 % of all infectious diseases documented worldwide, according to the WHO

(2023) and they cause more than 700,000 fatalities each year. The spread of infectious diseases was ranked second in the top ten hazards in terms of impact in the Global Risks 2015 report due to this scenario of increasing case numbers and growth into new areas.

Bhatt et al (2013) estimated that 96 million dengue infections occur each year around the world and this number includes infections that emerge at any level of disease severity. In recent years, other regions have seen a spike in arboviral disease incidence, such as the Western Pacific, where more than 375,000 suspected dengue cases were reported in 2016 (WHO, 2019). Some estimates of the economic consequences of arboviral illnesses have been made, particularly for dengue infections, the median cost of all recorded dengue hospital admissions filed in a Brazilian municipality was estimated to be US$ 259.9 per hospitalisation (Machado et al., 2014).

Dengue and chikungunya are two arboviral diseases that are on the list of neglected tropical diseases by the WHO. It is estimated that 390 million people are infected with the dengue virus every year, with 96 million showing clinical symptoms. According to another study, 3.9 billion people are at risk of contracting the virus. Although there is a risk of infection in 129 countries, 70% of the burden falls on Asia. Over the past 20 years, there has been an 8-fold increase in reported dengue cases to the WHO, from 505,430 in 2000 to over 2.4 million in 2010 and 5.2 million in 2019. In the same period, the number of deaths reported increased from 960 to 4032, with younger age groups being the hardest hit. However, the total number of cases and reported deaths decreased in 2020 and 2021, but the information is incomplete due to the COVID-19 pandemic's impact on case reporting in some countries (WHO, 2022).

1.6.3 Mosquito-Borne Disease: Malaysian Perspective

Dengue fever has become a major problem in society. Dengue fever has been a problem in Malaysia since 1902. With its first big outbreak in 1973, the disease became a public health concern in the 1970s. Dengue fever has increased in prevalence from 32 cases per 100,000 people in 2000 to 361 cases per 100,000 people in 2014. Most dengue patients are between the ages of 15 to 49 and 80 % occur in metropolitan areas. Dengue fever is a virus spread by mosquitos that is both common and dangerous. Dengue virus (DENV), which has four serotypes, causes it (DENV-1, DENV-2, DENV-3 and DENV-4). In Malaysia, all serotypes are present and the predominant serotype varies from year to year. Selangor is a Malaysian state with a densely populated and urban population, accounting for 5.79 million of the country's 31.53 million residents and accounting for 90 % dengue cases (MOH, 2020).

1.7 Problem Statement

Mosquito-borne disease outbreaks pose threats to global communities. According to WHO, each year mosquito-borne diseases particularly dengue and malaria have caused approximately 1 million deaths globally. In the context of Malaysia, the number of deaths due to dengue has shown a steady increase from 147 cases in 2018 to 182 cases in 2019. As a matter of fact, 72,952 dengue cases were reported in Malaysia in 2020. Based on statistics data provided by Ministry of Health Malaysia, as of 4 March 2021 a total of 5,459 dengue cases have been reported nationwide. To mitigate mosquito-borne diseases, mosquito surveillance and control are imperative.

Chemical insecticides, such as those containing N,N-diethyl-3-methyl benzamide (DEET), have been widely used to repel mosquitoes. However, DEET is known to be toxic and there have been reports of negative reactions in people who have used repellents containing DEET excessively. These adverse effects include seizures, uncoordinated movements, agitation, aggressive behavior, low blood pressure and skin irritation. As a result, plant essential oils have been suggested as an alternative to DEET. While essential oils are highly effective in repelling mosquitoes, their active ingredients are volatile, which reduces their effectiveness for this purpose. Previous studies have suggested that using fixative materials such as liquid paraffin, vanillin, salicyluric acid, mustard and coconut oils can increase the repellent efficiency of essential oils. However, there is an urgent need to develop an innovative agent that can control the volatility and release of active ingredients in essential oils. Therefore, this research aims to formulate an effective, innovative and eco-friendly nanoemulsion that can control the release of essential oils' active ingredients in a nanoemulsion colloidal system to repel mosquitoes.

1.8 Hypothesis

Research indicates that polymer-containing nanometric structures exhibit strong adhesion when interacting with either the skin or mucosa. Consequently, we proposed the idea that this adhesive quality could enhance the duration of insect repellent application on fabrics. To put it another way, nanoemulsions may present a viable approach for creating adhesive sprays for textiles.

1.9 Research Gaps

Based on literature review, there are studies had successfully developed and assessed the potential of essential oil-based nanoemulsions for mosquito repellent in fabric finishes, yet several significant research gaps have been found and they are relevant to be investigated.

i. Most studies utilise only one type of fabric, it is crucial to investigate the efficacy of mosquito spray formulations on a variety of fabrics. Two types of fabrics were used in this study, namely cotton and polyester.
ii. In many cases, researchers normally used one type of essential oil in mosquito spray formulation. It is important to evaluate several types of essential oils. As a result, three types of essential oils were employed in this study, namely betel, clove and lemongrass.
iii. Typically, researchers employ essential oils that are not available in their own country. Due to their availability in large amount, it is advisable to use essential oil most widely available in the country.
iv. The frequent utilisation of synthetic polymers as binders in nanoemulsions is well-known. However, the novelty aspect of this study is to emphasise the connection between plant-based polymers, or biopolymers, serving as wall materials, along with the incorporation of essential oils as active ingredients, resulting in the formation of a stable nanoemulsion.
v. Researchers normally focused on the use of a single type of polymer, however it is necessary to study the potential of other type of polymers such as tragacanth gum and arabic gum were used in this study.

vi. When it comes to performance studies for fabric finishes, the effects of washing are scarcely studied. In addition to washing and heating several assesements such as essential oil-based nanoemulsion retention and repellency test were evaluated in this research.

vii. In some research projects, nanoemulsions were characterised using a few scientific analyses. This results in insufficient data to describe the chemical and physical features of the nanoemulsions. Therefore, the novelty and uniqueness of the research project were unable to be highlighted. In this study, several important characterisation analyses were carried out such as Dynamic light scattering (DLS), High resolution transmission electron microscopy (HR-TEM), Fourier Transform Infrared spectroscopy (FTIR), Ultraviolet-visible (UV-Vis) spectroscopy, Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Encapsulation efficiency (EE) and in vitro release study to investigate the chemical and physical properties of nanoemulsions.

1.10 Research Significance

This research is both relevant and significant to the National Dengue Strategic Plan and the National Malaria Elimination Strategic Plan, particularly in the area of Integrated Vector Management. Additionally, it is also pertinent to the National Policy on the Environment, with a focus on Continuous Improvement in the Quality of the Environment, specifically in extended controlled release mosquito repellency studies. The aim of this research is to develop and apply essential oils-based nanoemulsions as a mosquito spray formulation in textiles, in order to control mosquito biting and the transmission of disease vectors. If successful, this approach could potentially reduce the number of mosquitoes, leading to a healthier environment for the community. Therefore, this research is relevant to Integrated Vector Management, as it has the potential to reduce expenditure related to mosquito breeding and disease control. Additionally, it may also create opportunities for the production of effective, innovative and eco-friendly mosquito spray formulations.

1.11 Research Objectives

1. To prepare essential oil-based nanoemulsion by using high energy method.
2. To characterise physical and chemical properties essential oil-based nanoemulsions using analytical technique.
3. To identify the adherence mechanisms of essential oil-based nanoemulsions on two types of fabrics namely cotton and polyester fabrics.
4. To evaluate the effectiveness of fabrics treated by essential oil-based nanoemulsion to repel mosquito.

1.12 Research Overview

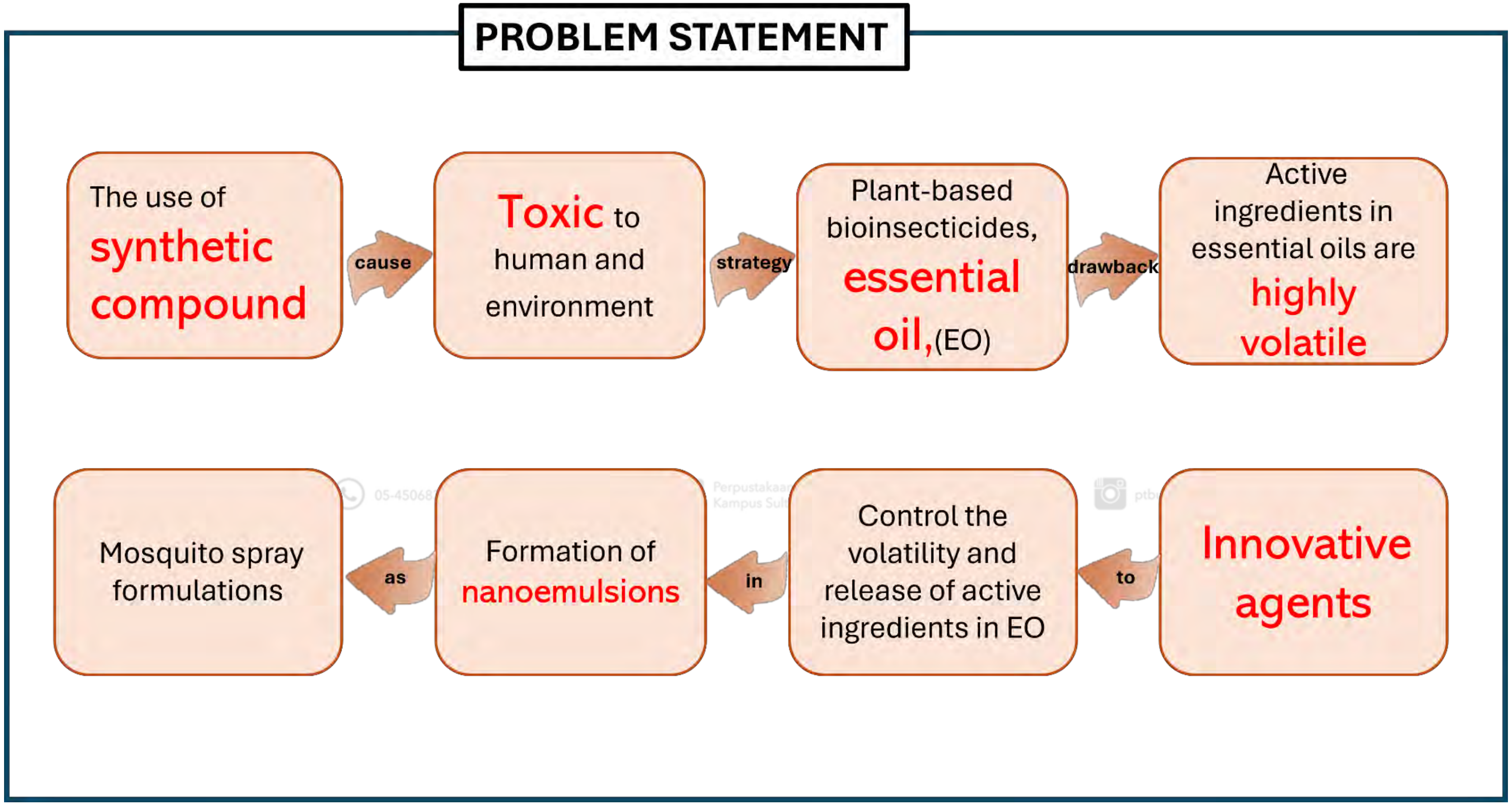


Figure 1.6. Overview of research study

1.13 Thesis Organisation

The thesis is divided into five sections and is organised as follows:

The first chapter offers a comprehensive introduction to insect repellent products, including their research history and the diseases transmitted by mosquitoes and other insects. This chapter also identifies the research gaps, the importance of the study and the specific goals and objectives of the research.

The second chapter summarises the literature review on mosquitoes, controlled-release formulation, nanotechnology and application study in textiles industry.

The approach used to attain the research objectives is discussed in third chapter. It includes a full explanation of the materials, procedures, apparatus and equipment utilised in the experiments, as well as other relevant information.

The fourth chapter showcases the results of the experiments and offers an in-depth discussion of the research findings. This chapter also provides a comparison of the current study's findings with those of previous studies, offering a comprehensive explanation of the results.

The fifth chapter draws the conclusions of the current research and suggests recommendations for future studies.