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XbeeSoap: Formulation of Organic Soap from Stingless Bee Propolis

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DECLARATION

I declare that this thesis entitled “XbeeSoap: Formulation of Organic Soap from Stingless Bee Propolis” was carried out in accordance with the regulations of Universiti Malaysia Kelantan. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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XbeeSoap: Formulasi Sabun Organik daripada Stingless Bee Propolis

ABSTRAK

Minyak sayuran, sering dikenali sebagai minyak sawit, berasal daripada buah pokok kelapa sawit, yang kebanyakannya ditanam di kawasan beriklim tropika. Ia adalah minyak yang digunakan secara meluas dalam industri masakan, serta dalam industri kosmetik dan biofuel. Terdapat 3 konstituen aktif dalam kelapa sawit: asid lemak, vitamin E dan karotenoid. Matlamat penyelidikan ini adalah untuk formulasikan biosoap daripada minyak sawit dan mengekstrak propolis daripada sarang lebah tanpa sengat iaitu XbeeSoap. Dua sampel yang diformulasikan daripada XbeeSoap ekstrak propolis berbeza sebanyak 3 g dan 6 g ekstrak propolis telah dihasilkan. Pencirian XbeeSoap yang diformulasi dijalankan dengan menyiasat kumpulan fungsinya menggunakan FTIR, pH, kandungan lembapan dan sifat antimikrob. Nilai pH kedua-dua sampel XbeeSoap ialah 8. FTIR XbeeSoap menunjukkan kumpulan berfungsi berbeza wujud dalam XbeeSoap seperti Hidrokarbon Alifatik (C-H) dan Alkohol Alifatik Primer (O-H) yang menunjukkan kehadiran sebatian propolis. Manakala, kandungan lembapan menghasilkan di bawah 11% dan keputusan sifat antimikrob menunjukkan bahawa sampel 2 adalah yang paling berkesan terhadap semua organisma patogen yang diuji, dengan zon perencatan maksimum (13 mm) terhadap Bacillus subtilis dan 12 mm terhadap E.coli apabila diberikan pada kepekatan tertinggi 50 mg/mL. Kesimpulannya, pembangunan biosoap XbeeSoap ini berjaya diformulasikan dan dihasilkan.

Kata kunci: Minyak Sawit, Ekstrak, XbeeSoap, Propolis, Sarang Lebah Tanpa Sengat

XbeeSoap: Formulation of Organic Soap from Stingless Bee Propolis

ABSTRACT

Vegetable oil, often known as palm oil, is derived from the fruit of the oil palm tree, which is predominantly cultivated in tropical climates. It is a widely used oil in the culinary industry, as well as in the cosmetics and biofuels industries. There are 3 active constituents in oil palm: fatty acid, vitamin E and carotenoids. The aim of this research is to formulate the biosoap from palm oil and extract propolis from stingless beehive namely XbeeSoap. The two formulated samples from XbeeSoap different propolis extracts of 3 g and 6 g of propolis extracts were produced. Characterization of formulated XbeeSoap was carry out by investigating its functional group using FTIR, pH, moisture content and antimicrobial properties. The pH value of both sample XbeeSoap was 8. FTIR of the XbeeSoap indicated different functional group exist in the XbeeSoap like Aliphatic Hydrocarbons (C-H) and Primary Aliphatic Alcohol (O-H) which indicate the presence of propolis compound. While, the moisture content was resulted below 11% of the soap and the results of antimicrobial properties showed that sample 2 was the most efficient against all pathogenic organisms tested, with the maximum zone of inhibition (13 mm) against *Bacillus subtilis* and 12 mm against *E.coli* when administered at the highest concentration of 50 mg/mL. In conclusion, the development of this biosoap of XbeeSoap was successfully formulated and produced.

Keywords: Palm Oil, Extract, XbeeSoap, Propolis, Stingless Beehive

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Palm oil is a vegetable oil that is derived from the fruit bunch of the oil palm tree commonly its used in the food business as well as other sectors including the cosmetics and biofuels industries. Three active ingredients in oil palm which are fatty acid, vitamin E and carotenoids. Oil palm oil contains a lot of saturated and unsaturated fatty acids such palmitic acid, oleic acid, and linoleic acid. Vitamin E, a potent antioxidant that aids in defending the body against oxidative stress and inflammation thus important component of oil palm. These fatty acids are necessary for the human body and are important for supporting several bodily processes. Carotenoids including beta-carotene, alpha-carotene, and lycopene, which are organic pigments that give fruits and vegetables their vibrant colours, are found in oil palm oil. Since they are known to have antioxidant characteristics, carotenoids might be good for your health (Saini et al., 2015). Saturated fatty acids are formed during frying when the double bonds in unsaturated fatty acids are disrupted. (Tomskaya et al., 2008).

Stingless beehive or native name as madu kelulut, has have gained a prominence attention due to its health advantages. Incorporating stingless bee honey into soap offers several benefits, including its anti-inflammatory properties. According to (Thirumulu Ponnuraj et al., 2021) that stingless bee showed anti-inflammatory action. The researchers hypothesized that this might be because honey contains flavonoids and phenolic acids (Maringgal et al., 2019). The advantage of the stingless beehive that it can efficiently adsorb pollutants and contaminants from palm oil due to its high surface area and porous structure. This aids in enhancing the oil's quality and extending its shelf life. A more sustainable alternative to synthetic adsorbents is stingless beehive because it is a natural and renewable material. The material is biodegradable and simple to dispose of. It also contains natural antibacterial properties, stingless bee is well known for having antibacterial characteristics that can help combat dangerous microorganisms. Besides, it can help eliminate skin microorganisms and stop illness when used in soap. It can exfoliate the skin because natural enzymes found in honey can aid in a mild exfoliation of the skin especially the dead skin cells to make it skin soft and moist.

Propolis, a resinous substance, is produced by Apis mellifera honey bees through the combination of plant materials and their secretions. It's used in bee hives as an inner coating ingredient for keeping them hygienic (Barros et al., 2019). Propolis' colour, odour, and texture, as well as its chemical properties, are influenced by the botanical biodiversity of the location where it is generated. The botanical biodiversity of the site where propolis is produced influences its colour, odour, texture, and chemical qualities. The chemical composition of propolis varies depending on botanical diversity, seasonality, and geographic location with mainly ingredients of resin, wax, essential oils, pollen, and other chemical components such as flavonoids (flavones, flavonols, and flavonones), terpenoids, aromatic acids, and other substances (Barbosa et al., 2014). Propolis is a natural substance collected by stingless bees or honeybees like *Trigona sp*. and used as a nest, a component of protection, external immune systems, and antimicrobials. Propolis was one of the antioxidant sources because of its high concentration of bioactive components such as phenolics and flavonoids (Khairunnisa et al., 2020). Propolis contains bioactive chemicals that have pharmacological properties such as antibacterial, anti-inflammatory, antiseptic, antimutagenic, and antihepatotoxic properties (Sforcin et al., 2017). Propolis also be used as an effective antioxidant source in Xbeesoap formulation and improving product performance of Xbeesoap. Propolis contains minerals like Mg, Ca, I, K, Na, Cu, Zn, Mn, and Fe, as well as vitamins like A, B1, B2, B6, C, and E (Barros et al., 2019).

Organic soap made from oil-palm extract is a type of soap that widely used in the soap-making industry due to its excellent lathering and cleansing properties. This oil-palm biosoap is a natural and biodegradable product that is gaining popularity due to its environmentally friendly properties. In the production of oil-palm biosoap, sodium hydroxide ($NaOH$) and palm oil are combined involving the saponification process that produced glycerin as byproduct. Glycerine is a natural moisturiser that keeps skin smooth and supple. Oil-palm biosoap is a good natural and eco-friendly which can be an alternative to traditional soaps besides It is soft on the skin and free of harsh chemicals and artificial perfumes (Ahmad et al., 2021). It is gentle on the skin and does not contain any harsh chemicals or artificial fragrances. Additionally, since it is biodegradable, it is less harmful to the environment than many commercial soaps.

In this study, the XbeeSoap was produced from the formulation of palm oil was used as a raw material with addition of propolis extract reacting with NaOH. This soap of eco-friendly bar soap because was used the neutral material in making the soap.

1.2 Problem Statement

The current problem is the effectively of using palm oil while addressing to the demand for sustainable and eco-friendly cleaning methods. The problem associated to this study is how to avoid skin inflammatory problem and moisturize the skin at the same time. In this study, by using stingless beehive in the formulation of soap, it will help in avoid the inflammatory problem since it provides anti-inflammatory effects. At the same time, it will help in moisturize the skin. The problem is that stingless beehives are not currently used in the production of sustainable personal care items such as soap. While the health benefits of stingless bee honey have drawn attention, stingless beehives may have other applications that can help advance sustainable practices and lessen the need for artificial chemicals. A holistic and environmentally friendly skincare solution may be offered by this soap by utilising the special qualities of stingless bee products, such as their antibacterial and moisturising effects. Soap is still popular, probably due to the negative connotations associated with anything that is not considered "natural." It is therefore critical that both formulators and end-users understand the science underlying cleansers, particularly those designed to maintain healthy skin and control common skin disorders such as eczema (Mijaljica, Spada, & Harrison, 2022).

The global honey production was anticipated to be 1.72 million metric tonnes, with China dominating the market, followed by Turkey and Canada as other important producers. The worldwide market is very competitive since it is dependent on environmental conditions, and even little changes in the weather can severely impede manufacturing. Not only that, the price of pure honey a quite high that the reason why propolis was used as one of alternative in making the soap. However, previous study by Kurek-Gorecka et al., 2020 state the wax contains in the stingless beehive also provide moisture to the skin because beeswax is used in cosmetics as a stiffening agent, a substance that provides elasticity, and increases skin adhesiveness. Beeswax also contains lubricating and softening properties, as well as the ability to prevent transepidermal water loss from the skin (Kurek-Górecka et al., 2020).

1.3 Expected Output

The outcome of using palm oil and propolis extract from a stingless beehive to make soap is a bar of XbeeSoap with the ability to eliminate toxins and pollutants from the skin because of the propolis. Because of the propolis, the soap should be a dark brown or brown colour and may smell oil. The use of propolis extract gives the soap its detoxifying authority while the use of palm oil produces a hard, long-lasting bar of soap. Propolis is often used to treat the anti-inflammatory and antibacterial qualities provide relief from inflammatory and non-inflammatory lesions, as well as significant relief from seborrhoea and excess oiliness of the skin, with excellent tolerability.

1.4 Objectives

The objectives of this study are shown as below:

1. To extract propolis from stingless beehive.
2. To formulate the XbeeSoap from propolis extracted from stingless beehive.
3. To characterize the chemical and physical properties of formulated XbeeSoap.

1.5 Scope of Study

In this study, the procedure of propolis stingless beehive extraction was carried out using maceration technique with slight modifications (Dewantoro et al., 2022). The crude propolis was soak in 70% ethanol with a 1:3 ratio (w/v). To further characterise the formulation Xbeesoap manufacturing using stingless beehive, pH analysis, Fourier transform infrared spectroscopy (FTIR), moisture content and antimicrobial properties were used in this research project. We combine a chemical called ethanol with soaking stingless beehive for 24 hours. Then, filered the mixture and evaporated the solvent to obtain the concentrated below than 85°C using the hotplate below 2 hours. it. The oil and propolis extract stingless beehive were mixed in a 1liter beaker of solution. All the experiments were carried out using a stirred magnet and hot plate to an easier solution to mix. The solution need to dry in silicone mold and observe after 2 weeks. After that, the soap will characterize using a few test to check the quality before using the soap. Then, the result of soap was observed.

1.6 Significance of study

Due to its high output and capacity to produce a solid bar of soap, palm oil is a preferred ingredient in the production of soap. In tropical areas, stingless bees, usually referred to as meliponines. It is a common kind of bee. They are becoming increasingly famous for their honey and wax, which are utilised in many goods, including soap. However, there may be certain risks to using stingless beehive products while creating bio soap, such as cost and allergies. Because stingless bee products have a lower yield and need more time to produce, they may be more expensive than honey and wax from other sources.

CHAPTER 2

LITERATURE REVIEW

2.1 Stingless Bee

Stingless bees, also known as *meliponines* or stingless honeybees, that are found in tropical and subtropical locations all over the world. They are commonly found in Australia, Central and South America, and Southeast Asia. There are over 500 recognized species of stingless bees, belonging to various genera. A group of bees belonging to the tribe of *Meliponini* been shown in Figure 2.1.

Figure 2.1: Stingless bee

(Kwapong et al., 2010)

Unlike honeybees and other bee species, stingless bees have a unique characteristic of not possessing a stinger or having a reduced stinger, rendering them incapable of delivering a painful sting. Similar to honey bees, stingless bees are eusocial insects that live in colonies. However, their colonies are relatively smaller, typically consisting of a few hundred to a few thousand individuals. The colonies are typically housed in cavities such as hollow trees, crevices, or man-made hives. These bugs are the largest and most varied group of corbiculate bees (de Paula et al., 2021). A perennial colony of insects that produce honey is formed by stingless bees. There are more than 500 species of stingless bees, mostly Neotropical, that are found across tropical latitudes (Roubik et al., 2023).

2.1.1 Stingless Beehive

A stingless beehive, also known as a *meliponary* or *meliponine* hive, is a specially designed structure colonies of stingless bees. These hives are constructed to provide a suitable environment for the bees to live, breed, store food, and carry out their activities. Stingless bees use a resinous substance called propolis to seal and protect the hive. They collect propolis from plant sources and use it to close gaps or cracks in the hive, providing insulation and defence against pests and pathogens. The bees also use wax and other materials to build honey pots, partitions, and other structures within the hive.

As shown in Figure 2.2, have a research that is showed the small-scale meliponid culture of stingless bee colonies and stingless beehive. For thousands of years, beehive products were applied to treat a variety of diseases in different cultures (Nader et al., 2021).

Figure 2.2: A) Small-scale meliponiculture of stingless bee colonies B) Stingless beehive

(Zulhendri et al., 2022)

Beehive that is contain honey, bee venom, propolis, pollen, and royal jelly Figure 2.3, which contain a high concentration of bioactive chemicals, making them efficient against a wide range of bacterial strains. Honey and bee venom are effective antibacterial substances against a wide variety of microorganisms, including pathogenic pathogens.

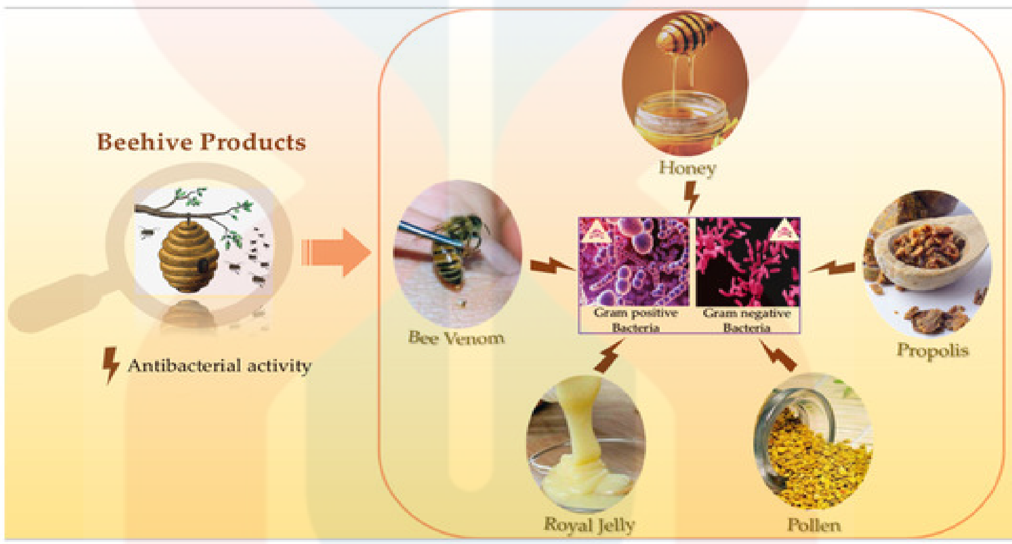

Figure 2.3: Beehive product

(Nader et al., 2021)

2.1.2 Propolis

Propolis is a resinous hive product collected from various plant materials by honey bees and is considered to be a protective barrier against the bees' enemies. The chemical composition of propolis includes flavonoids, aromatic acids, esters, aldehydes, ketones, fatty acids, terpenes, steroids, amino acids, polysaccharides, hydrocarbons, alcohols, hydroxybenzene, and several other compounds in trace amounts. The composition of propolis varies according to the plants in a specific region. Propolis has been used as a folk medicine and has been reported to possess therapeutic or preventive effects against inflammation, heart disease, diabetes mellitus, microbes hepatotoxity, and cancer (Farnesi et al., 2009). Bees use the propolis to seal and protect their hives, hardening the structure and guarding it against external threats such as bacteria, fungi, and other microbes.

2.1.3 Propolis in the Xbeesoap Formulation

Propolis used in soaps due to its potential health advantages and natural antibacterial and antifungal qualities. When used in bio or natural soaps, propolis can help to improve the cleansing and protecting properties of the product. Propolis contains bioactive chemicals that have pharmacological properties such as antibacterial, anti-inflammatory, antiseptic, antimutagenic, and antihepatotoxic properties (Sforcin et al., 2017). Propolis also be used as an effective antioxidant source in soap formulation and improving product performance of biosoap. Propolis also contains minerals like Mg, Ca, I, K, Na, Cu, Zn, Mn, and Fe, as well as vitamins like A, B1, B2, B6, C, and E (Barros et al., 2019). Thus the Table 2.1 was shown the data of propolis that can used for making a soap from different source and locations.

Table 2.1: Soap produced from different types of propolis

Propolis	Types of soap	Location	Source
Propolis wax	Transparent soap	Indonesia	(Sahlan et al., 2018)
Propolis extract	Hand cream	Bulgaria	(Tumbarski et al., 2023)
Propolis extract	Solid soap	Indonesia	(Dewantoro et al., 2022)

(Sahlan et al., 2018; Tumbarski et al., 2023; Dewantoro, et al.2022)

2.2 Oil Palm

The oil palm or scientific name *Elaeis guineensis* Jacq is a monocotyledonous plant of the *Arecaceae* palm family. It is a monoecious plant that produces unisexual male and female inflorescences alternately (Okolo et al., 2019). Refer to Table 2.2 some of the various sections of the oil palm and their utility and value to humans are summarised.

Table 2.2: Some part of oil palm with the uses to human

Oil palm	Uses to human
Fruit	Palm kernel, palm oil, palm kernel oil
Empty fruit bunch	Manufacturing of black soap, local fertilizer
Roots	Medicine

(Okolo et al., 2019)

Palm oil is typically extracted from oil palm fruits, however, other portions of the oil palm plant can be used to generate raw materials for bioproducts. Biomass from oil palm can be divided into three categories which are bioproducts, biofuels, and biopower. In terms of biofuel, biodiesel produced from palm oil will be comprehensively investigated, as will its consequences in Malaysia, one of the world's top oil palm producers. Palm oil has proven to be a viable solution for mitigating the harmful environmental effects of global warming (Mahlia et al., 2019).

An essential product for the economy is oil palm (*Elaeis guineensis* Jacq.). In comparison to oil and gas, oil palm contributes to the production of more foreign currency. One of the applications of oil palm is the production of vegetable cooking oil. Oil palm oil can produce more than seven times the oil per hectare over rapeseed (Brassica napus) and eleven times more oil per hectare than soybean. With a total production of 30 million tonnes and an average growth rate of 8% per year since 2004, oil palm has gradually emerged as a major source of vegetable oil worldwide (Febriani et al., 2020). Palm oil has also become the primary source of vegetable oil due to its higher production yield than rapeseed, sunflower, and soybean. Malaysia has a total of 4.49 million hectares which are oil palm agricultural land, that can produce around 17.73 million tonnes of palm oil per year (Liu et al., 2021).

2.2.1 Properties of Oil Palm

Southeast Asia is a major producer of palm oil, with 90% of output going towards food usage and the remaining 10% going towards non-food uses such the creation of oleo-chemicals. Vegetable oil soaps can be pyrolyzed to create products rich in hydrocarbons. It is a multipurpose oil that is made from fruit pulp and is utilised in food, the soap industry, cosmetics, and biofuels. As it accounts for around 35% of the consumption of all vegetable oils and ranks first in terms of global oil production, it is currently the most consumed oil in the world (Absalome et al., 2020).

Palm oil is an important product in countries like Malaysia and Indonesia. Palm oil is used to make a variety of products, including food, cosmetics, and biodiesel. It generates export profits and benefits toward economies, particularly Indonesia and Malaysia. Oil palm output is influenced by both climate change and the presence of pests and diseases. *Ganoderma boninense* or commonly known as white rot fungus, is now identified as a severe impediment to the immensely successful oil palm business, particularly in Southeast Asia and other production locations (Abubakar et al., 2022).

2.2.2 Oil Palm into Bio Soap

In bio-soap production, have many types of oil that can use in making a soap. For example, can use olive oil, waste coconut oil and coconut oil that can produce a solid soap or commonly known as bar soap. Table 2.3 shown a few example types of oil that can produce the soap.

Table 2.3: Types of oil, soap, and source

Types of oil	Types of soap	Source
Olive oil	Bar soap	(Abd Rahman et al., 2022)
Waste cooking oil	Bar soap	(Li et al., 2020)
Coconut oil	Bar soap	(Sukeksi & Diana et , 2020)

(Abd Rahman et al., 2022; Li et al., 2020; Sukeksi & Diana, 2020)

Palm oil is frequently applied in body soap making due to it's ability to clean and moisturise the skin. Due to its antibacterial and anti-inflammatory qualities, propolis from stingless bee hives has recently attracted interest for its potential use in cosmetics. This study of the literature intends to investigate the numerous research projects on the formulation of oil palm soap with propolis extract from stingless bee hives. A range of organic products from stingless bee colonies has long been utilised for therapeutic and cosmetic purposes. The use of stingless bee hive products in the creation of cosmetic goods, particularly soap, has recently attracted growing interest. They possess special qualities, such as a high concentration of antioxidants, antibacterial agents, and moisturising compounds.

The oil combines with lye (NaOH) and triglyceride-containing lye to produce soap through a process known as saponification reaction as been shown in Figure 2.4. Since the fatty acid content is incompatible, oils with different properties help to distinguish them from one another (Arasaretnam et al., 2019). Typically, a strong base such as sodium hydroxide combines with an animal fat or vegetable oil in an aqueous media to create soap (sodium salts of hydrolyzed free fatty acids) and glycerol, which are primarily composed of triacylglycerol esters (Baptista et al., 2019).

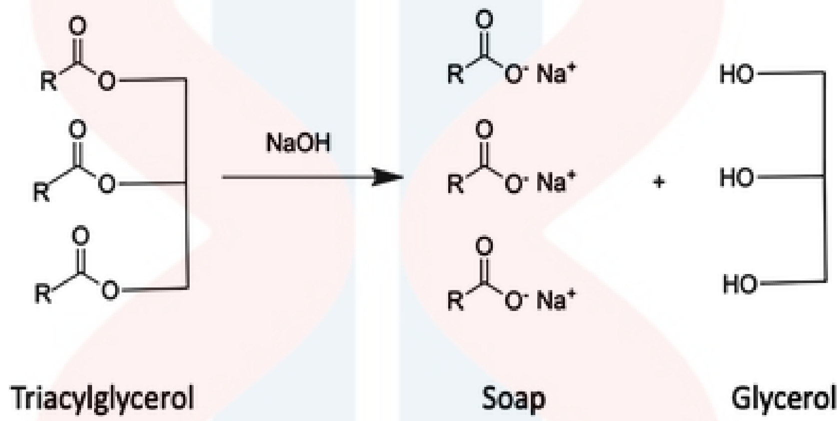

Figure 2.4: Saponification reaction

(Baptista et al., 2019)

In bio-soap production, have many types of oil that can use in making a soap. For example, can use olive oil, waste coconut oil and coconut oil that can produce a solid soap or commonly known as bar soap.

2.3 Formulation of Soap

A surfactant, such as soap, is used in combination with water to wash or clean the objects that come in both solid and liquid form. This resulted with the soap molecule's polar and non-polar groups attached to oil and water respectively, and it's ability to remove fat or other impassable dirt, soap can also be used as a cleaning agent. Triglyceride (fixed seed oil) and lye solution undergo a chemical reaction that mostly produces soap (Frank, 2004; Shahidi, 2005). In terms of chemistry, soap is a fatty acid salt. Whereas the typical method for making soap involves reacting bases with fats or oils like NaOH or KOH. The reaction that occurs when fat or triglycerides react with NaOH and KOH to generate soap is known as the saponification reaction (Apgar et al., 2010). A small amount of extra alkali is heated with fat or oil. Once the soaping process is finished, salt is added to help in solidifying the soap. Glycerol is recovered through distillation when a layer of water that contains salt, glycerol, and excess alkali is separated (Hart et al., 2003). Because of the immiscibility of oil and an alkaline solution, the lathering process takes a while to begin. The reaction speed will increase after the soap is generated, then decrease again at the end because of the less oil present. Since lathering is an exothermic reaction, it must be taken into consideration when mixing oil and alkali to prevent creating too much heat. This study is focusing on the saponification process, where an alkaline solution like KOH or NaOH is gradually added while being stirred and heated to create the soap. To produce the soap, another step that needs to be cautious is when stirring the solution. By stirring the solution properly and well will make the process more equitable and excellent (Rahayu et al., 2021).

2.4 Characterization of Formulated Soap

2.4.1 pH Analysis

The pH value is used to determine whether the soap is acidic or alkaline. The pH of the soap must also be determined in order for its basic properties of being alkaline on a healthy scale to be achieved below than 10 range (Abd Rahman et al., 2022). In make sure the finished soap is safe to use and has the appropriate qualities, pH testing must below than 10 is an important stage in the soap-making process. A substance's acidity or alkalinity can be determined using the pH scale, which ranges from 0 to 14. A pH of 7 is regarded as neutral, while values lower than 7 are acidic and higher than 7 are alkaline. For soap, a pH between 8 and 10 is optimum. In general, a standard range of soap will range between 9 to 11 (Ameh et al., 2013).

2.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy or commonly known as FTIR, is a method of analysis used to detect the compound exist and different functional group. It scans test samples and examines chemical characteristics using infrared light. When infrared light is sent through a sample by the instrument, some of it is absorbed and some of it passes through. The resultant signal at the detector is presented as a spectrum after the absorbed radiation was transformed by the sample molecules into a rotational or vibrational energy. Every chemical compound or molecule will generate a distinct spectral fingerprint. Figure 2.5 shown the example of FT-IR machine in FSB laboratory, University Malaysia Kelantan.

Figure 2.5: Fourier Transform Infrared Spectroscopy (FT-IR)

In ensure the quality of this soap, the functional group of soap will be examined using the IR spectrum of FTIR analysis to determine the proper soap components present in the soap, such as fatty acids. After the pH value and weight of the soap will be checked every week, the soap is ready for public use (Abd Rahman et al., 2022).

2.4.3 Moisture Content

The percentage of moisture in soap affects the quality and performance. In general, soap contains some water or moisture, which influences its texture, hardness, and shelf life. The moisture level of soap varies according to the soap-making process like the components used, and the duration of the solid process. Saponification is the process of combining fats or oils with a strong alkali, such as sodium hydroxide, to make a solid soap. During this chemical reaction, the oils and alkali combine to create soap and glycerin. The soap will proceed to dry in 2 weeks to observation.

The ideal moisture content in soap is normally between 6% and 15%. If the moisture level is too high, the soap may become mushy, decrease in effectiveness, or have a low lather. If the moisture content is too low, the soap may become brittle and rough on the skin. The moisture content of soap is typically stated as a percentage of the total weight of the soap.

According to the AOAC, moisture content was evaluated by drying 10g of the sample to a constant weight at 105 °C (Zauro et al., 2016). It was allowed to cool before being reweighed. The formula below was used to compute the percentage moisture content shown in equation 2.1;

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\boldsymbol{Weight\ of\ sample\ after\ drying}}{\boldsymbol{Weight\ of\ sample\ before\ drying}}\ \boldsymbol{x\ 100}\% \qquad \text{Equation 2.1}$$

2.4.4 Antimicrobial properties

The antimicrobial properties of soaps and detergents are very helpful against some pathogenic organisms such as multi-drug-resistant pathogens such as *Escherichia coli (E.Coli)* and *Bacillus*. Antibacterial soaps are considered to be more effective than beauty (plain) soaps. This study suggests that antiseptic soaps were more effective against Gram-negative and Gram-positive bacteria than plain soaps. Present work showed that plain soaps also possessed antibacterial activity although lesser than that of antibacterial soaps. Antimicrobial activity is described as a substance's ability to either kill or prevent the growth of germs. Antimicrobial action is important in avoiding illnesses and skin infections in humans. Disinfectants used in daily routines include soaps. Soaps are cleaning agents that come in the form of liquids, solids, semisolids, or powders. Bacteria are extremely diverse and can be found in soil, water, sewage, standing water, and even the human body. Bacteria that attack the human body are extremely dangerous to one's health. Antimicrobial active compounds in soaps have been found in studies to eliminate more bacteria than ordinary soap. The antimicrobial effectiveness of stingless beehive propolis soaps against *Bacillus subtilis* and *Escherichia coli* has been investigated (Bhat et al., 2011).

The soap should contain useful chemicals that may destroy bacteria while not affecting bodily tissues. A variety of bacteria, including Gramme positive and Gramme negative bacteria, are deposited on the surface of the skin and cause skin inflammation (Chaudhari et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Oil palm, extracted propolis from stingless bee hive, sodium hydroxide (NaOH), ethanol, coconut oil, inoculum loop, cotton swab, petri dish, media bottle 1L, test tube 15 mL, parafilm, test tube rack, beakers 500mL, beakers 100mL, measuring cylinder 100mL and aluminium foil.

3.2 Methods

Palm oil is a combination with addition stingless beehive to produce the bio-soap and characterize with analysis using pH, Fourier Transform Infrared Spectroscopy (FT-IR), moisture content and antimicrobial properties.

3.2.1 Propolis Extract from Stingless Beehive

The propolis stingless beehive extraction was carried out using maceration technique with slight modifications (Dewantoro et al., 2022). The crude propolis was soak in 70% ethanol with a 1:3 ratio (w/v). The mixture then macerated for 24 hours with 70% ethanol. Next, concentration below 85°C was obtained by filtrating the mixture and evaporated the solvent using the hotplate below two hours.

3.2.2 Formulation of XbeeSoap

30 palm oil and 20 mL coconut oil was measured with ratio of palm oil and coconut oil is 1:0.7. Then, 7 g of NaOH was mixed in 20 mL water. Next, NaOH solution was added with 30 mL of palm oil and 20 mL coconut oil heated to 60°C and blend until homogenized. 2 different amount of propolis of 3g and 6g were added and mixed in formulation refer Table 3.1. After that, the formulation of soap was placed in mold and leave the solution for 2 weeks.

Table 3.1: Formulation of XbeeSoap

Sample		
Ingredient	**Concentration Sample 1**	**Concentration Sampel 2**
Propolis	3 g	6 g
Sodium hydroxide	7 g	7 g
Palm oil	30 mL	30 mL
Coconut oil	20 mL	20 mL
Distilled water	20 mL	20 mL

3.2.3 pH Analysis

The small piece of 2g soap was dissolved in the distilled water. After that, the pH strip was dipped into solution and the pH result was recorded.

3.2.4 Fourier Transform Infrared Spectroscopy Analysis (FTIR)

The FT-IR analysis was used to determine the functional groups in a soap sample. It evaluates test samples and detects chemical properties using infrared light. The FTIR range utilised was 4000 cm^{-1} to 400 cm^{-1}. To avoid errors during the test, a sample location will be pre-cleaned with ethanol. A spatula will be used to place a little amount of soap in the area to be sampled. After that, infrared light was allowed to permeate the sample to identify the total beam and selected frequencies. After a few minutes, the sample result was shown on the FTIR computer, and the data will be recorded (Abd Rahman et al., 2022).

3.2.5 Moisture Content

Firstly, two grams of sample product was put on the aluminium foil and placed in a 105°C oven overnight. After 24 hours in oven, weight the sample and calculated the moisture content using the manual way. The result was recorded. The following equation 3.1 was used to calculate moisture content according (Bahari et al., 2021).

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\boldsymbol{Weight\ of\ sample\ after\ drying}}{\boldsymbol{Weight\ of\ sample\ before\ drying}}\ \boldsymbol{x}\ \mathbf{100}\% \qquad \text{Equation 3.1}$$

3.2.6 Antimicrobial Properties

In preparation of bar soap sample using dilution method, 0.1 g of bar soap was weighed and mixed with 10 mL of distilled water in test tube. The method was repeated used another sample of bar soap. All the sample was leave to homogenous for 24 hours in laboratory incubator shaker at 35°C (Balouiri et al., 2016).

In an antimicrobial test using Plate Total Count Determination, the microbiological growth in propolis extract soap may be evaluated. This method used the nutrient agar as agar in the determination of the microbial testing. The 2 of sample soap surface was used in dilution method to obtain after the agar plate had been prepared with used the *Escherichia coli (E. coli)* with *Bacillus* as bacteria.

For nutrient agar, inserted 28 g of nutrient agar powder into a media bottle and poured 1 liter of distilled water. Next, was shaken to fully dissolve all components, and autoclave the dissolved mixture at 121°C for 15 minutes. After nutrient agar had been autoclaved, was cooled it but did not solidify. When it is cool, pour nutrient agar into each petri dish on the sterile surface until the agar has solidified. Lastly, keep the nutrient agar in plastic and store it in chiller.

First, swab the *e.coli* on the nutrient agar using the cotton swab. After that, put the dis on the agar in each port selection. Then, put the antibiotic on positive port while we were put distilled water on the negative port. Next, put 20 µm sample 1 on the dis and 20 µm sample 2. The next step was incubation. Put the petri dish in the incubator for microbial development in 37°C for overnight. Next we were recorded the result and repeat the same method for 2 times (Tenover et al., 2019). This test was repeated using another bacterium which is *bacillus* species with same method step.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results and Discussion

Soaps are commonly used for cleansing and to eliminate dust and microorganisms from the skin's surface. The type of soap used varies depending on the individual, but it should not irritate delicate skin and should be effective against disease-causing bacteria found on the skin. The results were recorded from formulation of soap, pH analysis, Fourier Transform Infrared Spectroscopy (FT-IR), moisture content and antimicrobial properties. The test involved the 2 sample of soap but different weight of propolis extract in the soap to see which one more efficient in using daily day.

4.1.1 pH Analysis

The pH of a substance determines its acidity or alkalinity. This is important since the skin also carries the pH. Chemical reactions are common when substances with radically different pH levels come into contact. Soaps also can affect the pH of the skin and thus human health, especially on the skin. All of the soaps must have pH levels that are less than 10.

Table 4.1: pH Testing of Two sample of XbeeSoap

Soap	pH after 2 weeks
Sample 1	8
Sample 2	8

In this result pH was show both sample 1 and 2 is suitable to use because it alkaline level and it’s not acidic. The result was observing after 2 weeks. We can see the pH after 2 weeks less than before. Soaps with a pH of around 8 are usually gentle on the skin. They may not be as acidic as some facial cleansers or body washes targeted for more sensitive skin, but they are still gentle enough for individuals to use without causing irritation. However, alkaline soaps, like those with a pH of 8, tend to have good cleansing properties. They also can effectively remove oils and dirt from the skin, leaving it clean and refreshing (Poucher et al., 2013).

4.1.2 Fourier Transform Infrared Spectroscopy Analysis (FTIR)

The result FT-IR analysis or FT-IR spectroscopy was recorded which is pure propolis, sample 1 and sample 2. Because of the vibrations of the chemical bonds, different functional groups in a molecule present characteristic peak at specific wavenumbers in FTIR spectroscopy. The OH (hydroxyl) group often exhibits a broad and robust peak in the 3200-3600 cm^{-1} range. Figure 4.1 illustrates a FTIR analysis of the propolis. According figure 4.1, a peak at 3359 cm^{-1} indicates a stretching vibration of several hydroxyls in saponin's side chain oligosaccharides of propolis. The 2921 cm^{-1} peak corresponds to the C-H aliphatic sapogenin bond, whereas the 1647 cm^{-1} peak reflects the C=C bond at sapogenin. The 1375 cm^{-1} signal denotes the -OH bond, while the 1050 cm^{-1} peak is connected with the C-O stretching vibration (Nowrouzi et al., 2022).

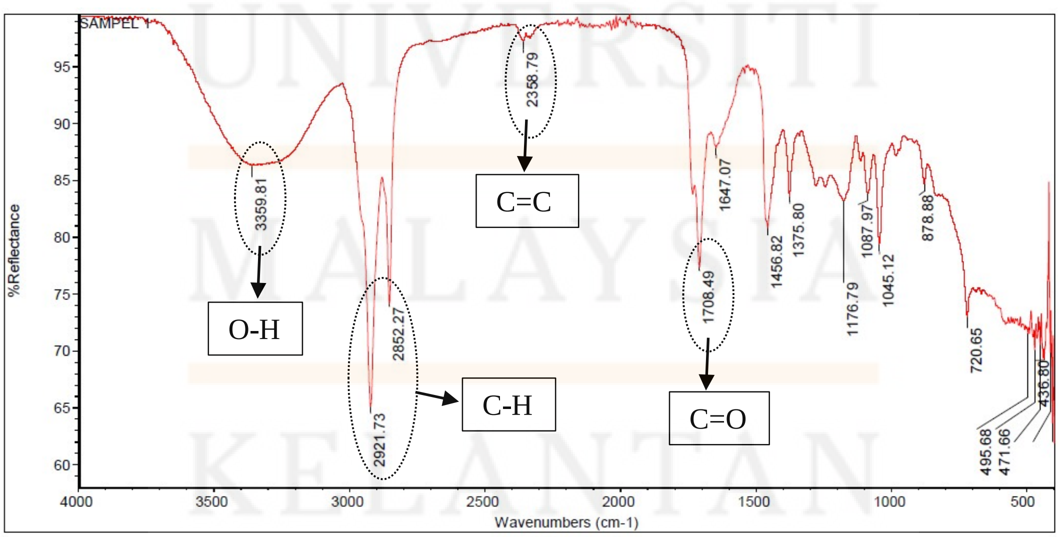

Figure 4.1: Functional group for Propolis

The outcome of the FTIR analysis of propolis are represent based on the types of chemical bond and different functional group that present characteristic the peak at specific wavenumbers in FTIR spectroscopy. The peak at 3359.81 cm^{-1} in an FTIR spectrum indicates chemical bonding between oxygen and hydrogen. This peak is commonly associated with hydroxyl (OH) groups found in alcohols, phenols, and carboxylic acids. The peak at 3359.81 cm^{-1} in an FTIR spectrum, along with the identification of the O-H stretch, indicates the existence of primary aliphatic alcohols. The exact wavenumber and shape of the peak can reveal more about the chemical environment and nature of the O-H bond. Primary aliphatic alcohols often have a strong and broad O-H stretching vibration between 3350-3200 cm^{-1}. The exact position of the peak can vary significantly depending on molecule structure and the presence of adjacent functional groups. The reading at 3359.81 cm^{-1} corresponds to primary aliphatic alcohols in an FTIR spectrum.

Table 4.2: Selected peak of functional group in Propolis

Peak list	Assignment
3359.81	O-H
2921.73	C-H
2852.27	C-H
2358.79	C=C
1708.49	C=O
1647.07	C=C
1456.82	C-H
1375.80	-OH
1176.79	C-O
1087.97	C-O

Next, the peaks at 2921.73 cm^{-1} and 2852.27 cm^{-1} in an FTIR spectrum, related with C-H stretching vibrations, indicate aliphatic C-H bonds. Specifically, 2921.73 cm^{-1} corresponds to the asymmetric stretching vibrations of C-H bonds in aliphatic molecules. Alkanes and alkyl groups frequently exhibit this type of vibration. The symmetric stretching vibrations of C-H bonds in aliphatic compounds, especially alkanes and alkyl groups were measured at 2852.27 cm^{-1}. These peaks were considered together, indicating aliphatic hydrocarbons in the analysed material. The unique wavenumbers reveal information about the nature of the C-H bonds, assisting in identifying and characterizing the molecular structure.

For the peak at 2358.79 cm^{-1} in an FTIR spectrum, associated with C=C stretching vibrations, this peak indicates the presence of carbon-carbon double bonds (C=C) in the analyzed sample. The specific wavenumber provides information about the nature of the double bond. The FTIR peak at 1708.49 cm^{-1}, is associated with C=O stretching vibrations, indicating the presence of carbonyl functional groups (C=O) in the analysed sample. The wavenumber indicates the type of the carbonyl bond. The C=O bond is found in molecules with carbonyl groups, such as ketones, aldehydes, and carboxylic acids. This information helps to identify and characterize the molecular structure, providing insights into the sorts of functional groups found in the sample. The presence of this peak at 1708.49 cm^{-1}, along with other characteristic features in the FTIR spectrum, supports the identification of an aliphatic carboxylic acid functional group in the analyzed sample.

The FTIR spectrum shows peaks at 1456.80 cm^{-1}, 1375.80 cm^{-1}, and 1045.12 cm^{-1}, indicating aliphatic hydrocarbon functional groups in the compound. These peaks especially indicate the presence of saturated hydrocarbons, in which carbon atoms are associated with hydrogen atoms in aliphatic (non-aromatic) molecular structures. The combination of these peaks, together with other features in the FTIR spectrum, provides helpful data for identifying and characterizing aliphatic hydrocarbons in the sample.

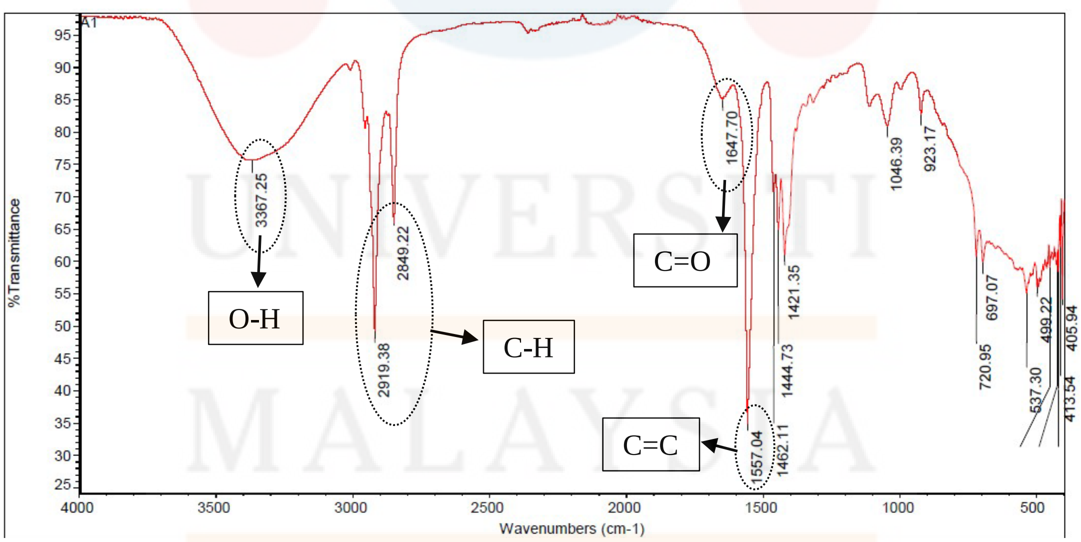

Figure 4.2: Functional group for Xbeesoap in Sample 1

Table 4.3: Selected peak of functional group in XbeeSoap (Sample 1)

Peak list	Assignment
3367.25	O-H
2919.38	C-H
2849.22	C-H
1647.70	C=O
1557.04	C=C
1462.11	C-H
1444.73	C-H
1421.35	C-H
1046.39	C-H
923.17	C-H
720.95	C-H

The functional group for sample 1 of XbeeSoap was showed like inorganic carbonate, aliphatic nitro compounds, aliphatic hydrocarbon and primary aliphatic alcohols. The peak at 3367.25 cm^{-1} in an FTIR spectrum, which indicates the O-H stretching vibrations, suggests the existence of hydroxyl (OH) groups in the analysed sample. The O-H stretching vibration is found in molecules containing hydroxyl groups, such as alcohols, phenols, and carboxylic acids. The data at 3367.25 cm^{-1} indicates O-H stretching vibrations and is associated with primary aliphatic alcohols, suggesting the presence of the hydroxyl group (-OH) in the analysed sample. A peak at 3367.25 cm^{-1} indicates a stretching vibration of several hydroxyls in saponin's side chain oligosaccharides of propolis. Primary aliphatic alcohols have the general formula R-CH2-OH, where R denotes an alkyl group. The specific wavenumber, when combined with the context of the full FTIR spectrum, aids in identifying and characterising primary aliphatic alcohols in the sample. It is important to note that the existence of other functional groups, as well as the overall molecular structure, should be taken into account in an in-depth analysis.

The peaks at 2919.38 cm^{-1} and 2849.22 cm^{-1} in an FTIR spectrum are related to C-H stretching vibrations and indicate the presence of aliphatic C-H bonds in the analysed sample. The peak at 2919.38 cm^{-1} represents asymmetric stretching vibrations of C-H bonds in aliphatic molecules. Alkanes and alkyl groups frequently exhibit this type of vibration. The peak at 2849.22 cm^{-1} represents the symmetric stretching vibrations of C-H bonds in aliphatic molecules, specifically alkanes and alkyl groups. These peaks were carried out together, indicating that the analyzed material contains aliphatic hydrocarbons or aliphatic groups. The unique wavenumbers reveal information about the nature of the C-H bonds, assisting in identifying and characterizing the molecular structure.

The peak at 1557.04 cm^{-1} in an FTIR spectrum, which indicates C=C stretching vibrations, indicates the presence of carbon-carbon double bonds (C=C) in the compound. The exact wavenumber provides information on the nature of the double bond. The existence of a C=C bond is characteristic of compounds containing alkenes or other unsaturated organic molecules. This data helps to identify and characterize the molecular structure, providing insights into the sorts of functional groups identified in the sample. The 1557.04 cm^{-1} peak, which indicates C=C stretching vibrations, tends to be associated with aliphatic nitro compounds. The nitro functional group exhibits distinctive peaks in the FTIR spectrum, including a high absorption at 1500-1600 cm^{-1} due to symmetric and asymmetric stretching vibrations of the nitro group. The 1557.04 cm^{-1} data indicates the presence of carbon-carbon double bonds (C=C), which are abundant in alkaline compounds and other unsaturated organic compounds.

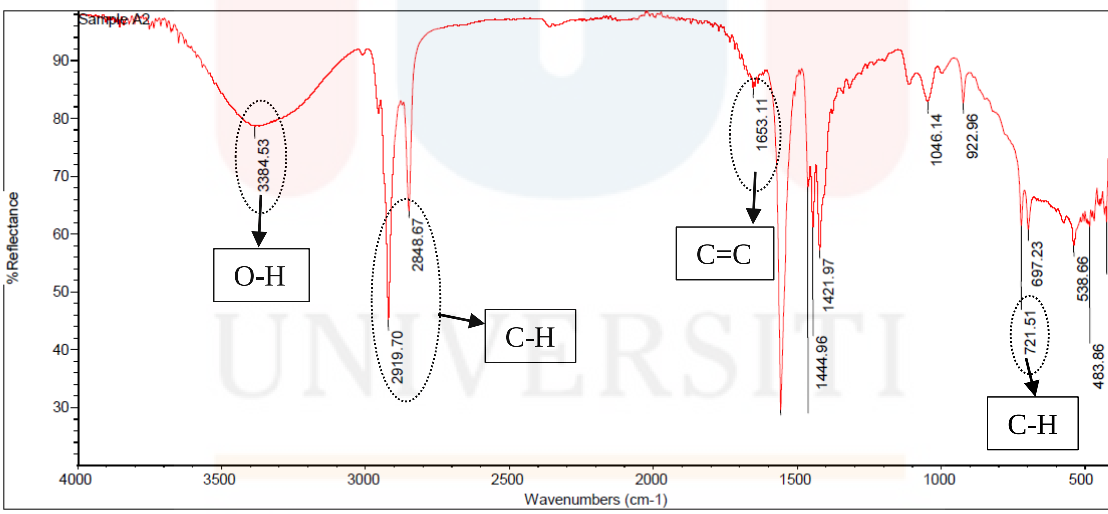

Figure 4.3: Functional group for Xbeesoap in Sample 2

Table 4.4: Selected peak of functional group in XbeeSoap (Sample 2)

Peak list	Assignment
3384.53	O-H
2919.70	C-H
2848.67	C-H
1653.11	C=C
1557.20	C=C
1461.97	C-H
1444.96	C-H
1421.97	C-H
1046.14	C-H
922.96	C-H
721.51	C-H

In an FTIR spectrum, the peaks of Table 4.4 at 1461.97 cm^{-1}, 1444.96 cm^{-1}, and 1421.97 cm^{-1} indicate C-H stretching vibrations, which are also related to the functional group of inorganic carbonate ($CO3^{2-}$). The particular wavenumber that is provided aligns with typical carbonate vibrations. The carbonate ion's symmetric stretching vibrations are represented by 1461.97 cm^{-1}. The carbonate ion's asymmetric stretching vibrations are shown at 1444.96 cm^{-1}. The peak number at 1421.97 cm^{-1} is connected with the carbonate ion and is commonly reflected in bending vibrations.

In this result of sample 2 of XbeeSoap was recorded have two functional group which are Aliphatic Nitro Compound and Aliphatic Hydrocarbons. A peak at 3384.53 cm^{-1} indicates a stretching vibration of several hydroxyls in saponin's side chain oligosaccharides of propolis. The peaks at 2919.70 cm^{-1} and 2848.67 cm^{-1} in an FTIR spectrum, which are associated with C-H stretching vibrations, show the presence of aliphatic C-H bonds in the material. The peak at 2919.70 cm^{-1} indicates the asymmetric stretching vibrations of C-H bonds in aliphatic molecules, including alkanes and alkyl groups. The peak number at 2848.67 cm^{-1} represents the symmetric stretching vibrations of C-H bonds in aliphatic molecules, specifically alkanes and alkyl groups. These peaks indicate the presence of aliphatic hydrocarbons in the sample that related with the propolis. Aliphatic hydrocarbons are chemical molecules composed of straight or branched carbon chains linked to hydrogen atoms.

The peak at 1557.20 cm^{-1}, which is associated with C=C stretching vibrations, is often indicative of aliphatic nitrogen compounds. The nitro functional group (NO2) shows distinctive peaks in the FTIR spectrum, including high absorptions at 1500-1600 cm^{-1} due to the symmetric and asymmetric stretching vibrations of the nitro group. The signal at 1557.20 cm^{-1} indicates the existence of carbon-carbon double bonds (C=C), which are typically present in alkenes and other unsaturated organic compounds.

4.1.3 Moisture Content

The moisture content calculation result was recorded with 2 sample of soap manually using the formula below. This table shows the moisture content in soap for sample 1 and sample 2. The both sample is in good quality according to standard value between between 6% to 15%.

Table 4.5: The moisture content of two sample of XbeeSoap

Soap	Moisture Content (%)
Sample 1	10.62
Sample 2	9.15

The moisture content in table 4.5 shows the moisture content of 2 solid soap after increasing the amount of propolis extracts. This is represented with negative constants for each factor. The addition of propolis extracts did not affect the increase in moisture content because they contain saponins. Saponins in propolis extracts are complex glycosides that when hydrolyzed, are separated into sugars and non-sugar components, The sugar components have hygroscopic qualities allowing them to absorb water (Dewantoro et al., 2022). According to Widyasanti et al., 2017 researched saponins in white tea extracts and found that they had a decreased influence on moisture content in transparent solid soap. This demonstrates that as additional extracts are added, the sugars in saponins absorb a large amount of water, and causing the moisture content in solid soap to decrease (Widyasanti et al., 2017). Based on table 4.5, the moisture content of sample 2 was decreased than sample 1 because in sample 2, we used 6 grams of extract propolis while in sample 1, we just used 3 grams of extract propolis. This shows more extract propolis are in soap making it can decrease the moisture content to reach a good standard value between 6% and 15% for solid soap.

Coconut oils and palm oils showed a significant effect on moisture content in soap indicated by the value of this response being less than 10%. High moisture content in soap would cause the soap to shrink faster, but low content could increase its shelf life. Besides that, the moisture content in soap was related to hardness level, which means higher content resulting in softer soap (Febriani et al., 2020). Hardian et al. explained that the hardness level of soap was affected by saturated fatty acids, such as a high amount of palmitic acids, stearic acids, myristic acids, and lauric acids (Hardian et al., 2014). Palmitic and stearic acids can harden or solidify of the soap. Coconut and palm oils contain a high concentration of saturated fatty acids, and increasing the amount of these would increase the hardness or decrease the moisture content in soap (Mardawati et al., 2022).

4.1.4 Antimicrobial Properties

The result of antimicrobial properties of Xbeesoap was shown in Figure 4.4, where the plate was divided into four sections. Those four sections included the result analysis of antimicrobial for positive content, negative content, sample 1 of Xbeesoap contain 3 g extract propolis and sample 2 contain 6 g extract propolis.

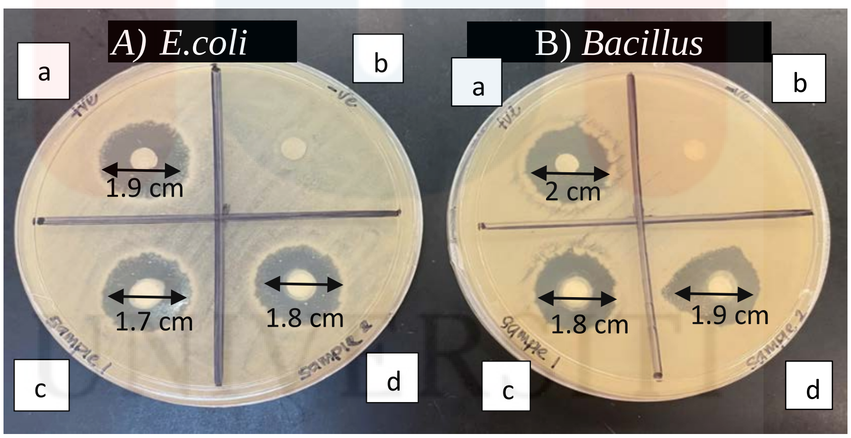

Figure 4.4: Analysis of antimicrobial for A) E.Coli and B) Bacillus. It indicate: a) positive content, b) negative content, c) sample 1, d) sample 2

Based on the figure 4.4, it one of the best picture was taking from three times repeated of antimicrobial properties on the sample 1 and sample 2 of xbeesoap.

Table 4.6: Diameter of Zone Inhibition *E.coli*

E.coli	**Diz**	**D**	**(Diz-D)/2**	**((Diz-D)/2)/d**	**Inhibition Zone (mm)**
Sample 1 (3 gram)	1.6 cm	0.5 cm	0.55 cm	1.1 cm	11
	1.6 cm	0.5 cm	0.55 cm	1.1 cm	11
	1.7 cm	0.5 cm	0.60 cm	1.2 cm	12
Sample 2 (6 gram)	1.7 cm	0.5 cm	0.60 cm	1.2 cm	12
	1.7 cm	0.5 cm	0.60 cm	1.2 cm	12
	1.8 cm	0.5 cm	0.65 cm	1.3 cm	13

Table 4.7: Diameter of Zone Inhibition *Bacillus*

Bacillus	**Diz**	**D**	**(Diz-D)/2**	**((Diz-D)/2)/d**	**Inhibition Zone (mm)**
Sample 1 (3 gram)	1.7 cm	0.5 cm	0.60 cm	1.2 cm	12
	1.8 cm	0.5 cm	0.65 cm	1.3 cm	13
	1.8 cm	0.5 cm	0.65 cm	1.3 cm	13
Sample 2 (6 gram)	1.8 cm	0.5 cm	0.65 cm	1.3 cm	13
	1.9 cm	0.5 cm	0.70 cm	1.4 cm	14
	1.9 cm	0.5 cm	0.70 cm	1.4 cm	14

Meanwhile, XbeeSoap's antimicrobial activities were measured by measuring the diameter of the zone of inhibition, and the mean results are shown in the table 4.6 and table 4.7. XbeeSoap of 3g and 6g showed antibacterial efficacy against every microorganism tested. Among all the microbial strains studied in this study, *Bacillus subtilis* was the most vulnerable and *E. coli* was the most resistant. According figure 4.5 was showed the three times repeated of antimicrobial properties against *e.coli* and *bacillus.*

The present research investigation was carried out to determine the antimicrobial efficacy of stinglees beehive soaps against skin micro flora isolates *Bacillus subtilis*, and *E. coli*. When the efficacy of antibacterial soaps was compared using the disc agar diffusion method, analysis of variance for the means of antimicrobial activity among the different soaps demonstrated favourable correlations. The results of the zone of inhibitions employing the organisms revealed that there were significant differences on the numerous microorganisms utilised in the research (Chaudhari et al., 2016). For the result, *Bacillus* have more zone of inhibition (13 mm) while *E. coli* have zone of 12 mm for sample 2 of XbeeSoap.

The results showed that sample 2 was the most efficient against all pathogenic organisms tested, with the maximum zone of inhibition (13 mm) against *Bacillus subtilis* and 12 mm against *E.coli* when administered at the highest concentration of 50 mg/ml. Following sample 1, the least zone for *Bacillus* species has occurred. For the sample 1, the maximum zone of inhibition 12 mm against *Bacillus* and 11 mm against *E.coli* at the highest concentration of 20 mg/ml per sample of XbeeSoap. In conclusion, the high propolis content which is sample 2 contain 6g have high antimicrobial properties than sample 1 that contain 3g of propolis.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The objective of the project was performed successfully and achieved. The first objective of the project was to extract propolis from stingless beehive. The second objective was to formulation XbeeSoap from oil palm and extract propolis from stingless beehive. The last objective was to characterize the chemical and physical properties of formulated XbeeSoap.

The optimal formula for sample 2 solid soap with propolis extracts addition was 6 g of propolis extracts, 20ml of coconut oil, and 30ml of palm oil. This formula produced soap with an 8 pH range and a moisture level of 9.15%. This sample 2 soap is a greater result than the 3 grams of propolis extract soap formula and even gets a similar pH but different moisture content because sample 1 which is 3 grams of extract propolis has a higher moisture content than sample 2 which is 10.62%. The soaps are cleaning substances that are commonly used for cleaning and germ removal. Soaps cause membrane disruption and protein disruption in microbial cells. Soaps evaluated in this study exhibited varying levels of effectiveness against the pathogenic microorganisms examined. XbeeSoap was successfully produced from palm oil with purified alkali derived from propolis extract using an enhanced conventional process. Thus, the properties of soaps demonstrated that using vegetable matter to produce alkali for soap manufacture is worthwhile. Aside from the fact that our environment would be free of agricultural wastes, which frequently make it messy, it would also protect the ecosystem from the potentially dangerous consequences of pollution commonly connected with these synthetic chemicals. Furthermore, if a concerted effort is undertaken to develop this local raw material supply for XbeeSoap-making, the heavy reliance on synthetic chemicals for soap manufacture will be significantly decreased. Last but not least, FTIR of the XbeeSoap indicated different functional group exist in the XbeeSoap like Aliphatic Hydrocarbons (C-H) and Primary Aliphatic Alcohol (O-H) which indicate the presence of propolis compound. While, the moisture content was resulted below 11% of the soap and the results of antimicrobial properties showed that sample 2 was the most efficient against all pathogenic organisms tested, with the maximum zone of inhibition (13 mm) against *Bacillus subtilis* and 12 mm against *E.coli* when administered at the highest concentration of 50 mg/ml.. In conclusion, the development of this biosoap of XbeeSoap was successfully formulated and produced.

5.2 Recommendations

There are a few recommendations in improve the soap preparation like adding essential oil and packaging.

5.2.1 Essential Oil

There are a few things that are recommended to improve the study. My first recommendation is to added the essential oil to get fragrance results of XbeeSoap. Essential oils are often used in aromatherapy to influence mood and emotions. For instance, citrus oils like orange can be invigorating and uplifting, while lavender and chamomile can have a calming effect. Not only that, lemongrass and peppermint also have insect-repelling properties adding these essential oils to soap can make it useful in outdoor activities. It also can less the oil smell in XbeeSoap.

5.2.2 Packaging

The second thing I would advise is making the soap container more appealing to consumers. Customers can infer from the soap's packaging that it is safe to use because we have conducted several tests on its pH and moisture content. The soap's safety and hygienic conditions must also be preserved in the packaging. It can shield soap from handling, dust, and other contaminants while it's being transported and displayed. To guarantee that the soap arrives at the end user's location spotless and ready for use, it must be packaged with care and sealed.

5.2.3 Filter Chemical Screening

Filter chemical screening detect propolis in soap through chemical screening, consider using techniques like chromatography or spectroscopy to analyze the soap composition for propolis-specific compounds. High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) can be effective methods for this research.

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APPENDIX A

CALCULATION

Total moisture content was calculated using this formula:

$$\textit{Moisture Content}\ (\%) = \frac{\textit{Different weight of sample}}{\textit{Weight of sample}}\ x\ 100\%$$

$$\textit{Moisture Content}\ (\%) = \textbf{result}$$

CALCULATION SAMPLE 1

Total moisture content was calculated using this formula:

$$\textit{Moisture Content}\ (\%) = \frac{\textit{Different weight of sample}}{\textit{Weight of sample}}\ x\ 100\%$$

$$\textit{Moisture Content}\ (\%) = \frac{0.2233}{2.1028}\ x\ 100\%$$

$$\textit{Moisture Content} = 10.62\%$$

- **10.62% is the total moisture content of sample 1.**

<u>CALCULATION SAMPLE 2</u>

Total moisture content was calculated using this formula:

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\boldsymbol{Different\ weight\ of\ sample}}{\boldsymbol{Weight\ of\ sample}}\ \boldsymbol{x\ 100}\%$$

$$\boldsymbol{Moisture\ Content}\ (\%) = \frac{\boldsymbol{0.1951}}{\boldsymbol{2.1310}}\ \boldsymbol{x\ 100}\%$$

$$\boldsymbol{Moisture\ Content} = \boldsymbol{9.15}\%$$

- **9.15% is the total moisture content of sample 2.**

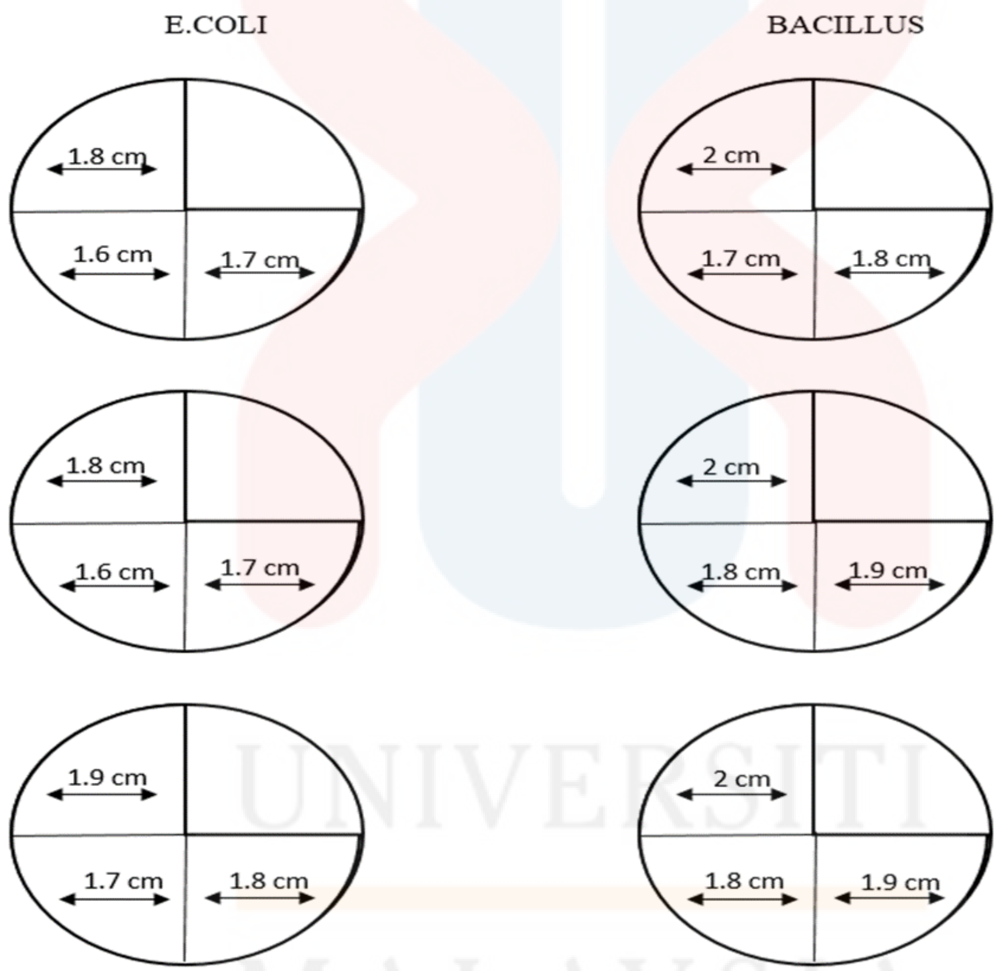
APPENDIX B

Trial Formulation Soap		
Ingredient	Concentration	pH
Propolis	6 g	
Sodium hydroxide	9 g	
Palm oil	60 mL	pH 8
Coconut oil	51.6 mL	
Distilled water	23.4 mL	
Propolis	9 g	
Sodium hydroxide	5 g	
Palm oil	15 mL	pH 11
Coconut oil	15 mL	
Distilled water	56 mL	
Propolis	6 g	
Sodium hydroxide	5 g	
Palm oil	15 mL	pH 14
Coconut oil	15 mL	
Distilled water	59 mL	
Propolis	3 g	
Sodium hydroxide	5 g	
Palm oil	15 mL	pH 7
Coconut oil	15 mL	
Distilled water	62 mL	
Propolis	3 g	
Sodium hydroxide	15 g	
Palm oil	50 mL	pH 14
Coconut oil	10 mL	
Distilled water	22 mL	
Propolis	3 g	
Sodium hydroxide	10 g	
Palm oil	50 mL	pH 13
Coconut oil	10 mL	
Distilled water	27 mL	

a) The trial formulation of soap

Soap	Initial Weight Of Soap (g)	Final Weight Of Soap (G)	Moisture Content (%)
Sample 1	2.1028	0.2233	10.62
Sample 2	2.1310	0.1951	9.15

b) The moisture content

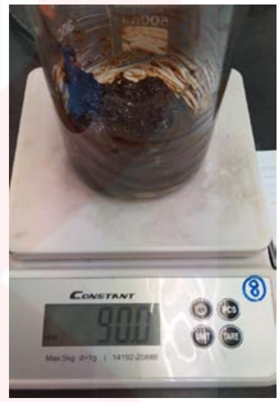


c) The three times repeated result of antimicrobial

APPENDIX C

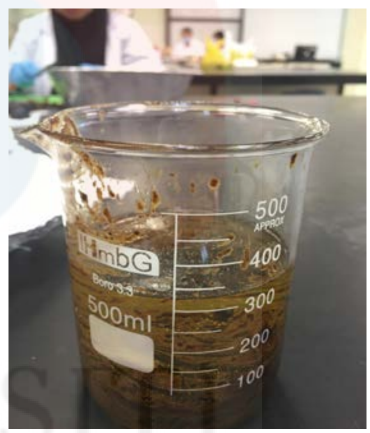
a) Preparation of extraction propolis


The propolis before extract



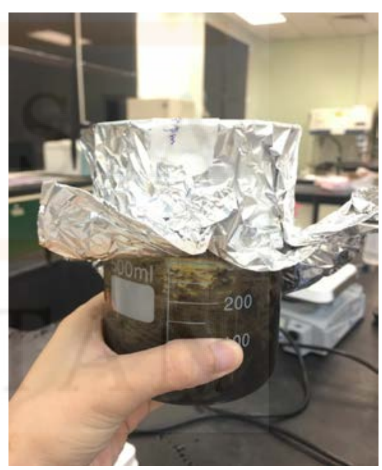
The propolis was weighed

The process of propolis extract



The process of propolis extract


The pure propolis



The process of propolis extract

b) Picture of the soap

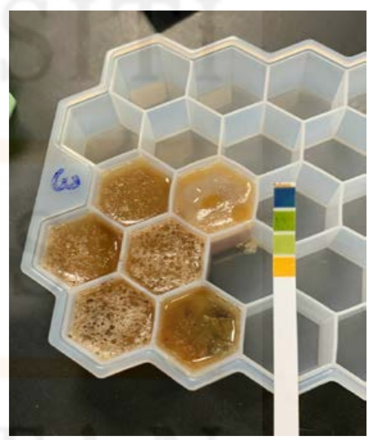

The final soap


The trial soap formulation


The trial soap formulation

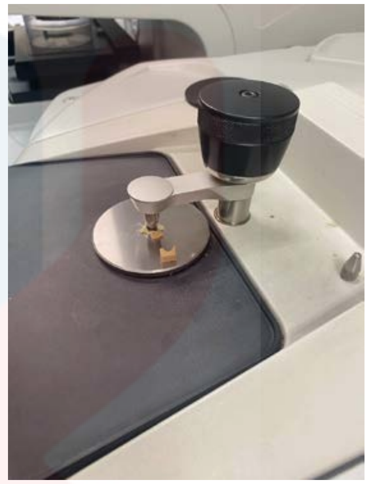

The soap process before be solid


The trial soap formulation


The pH of soap

c) Testing for FT-IR and Moisture content

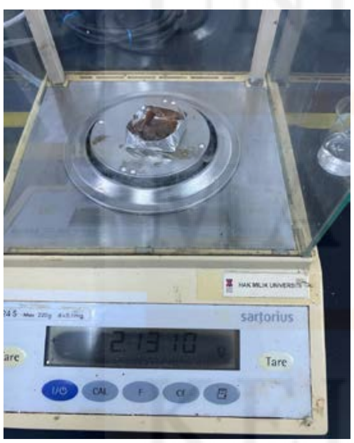
The semi liquid of propolis



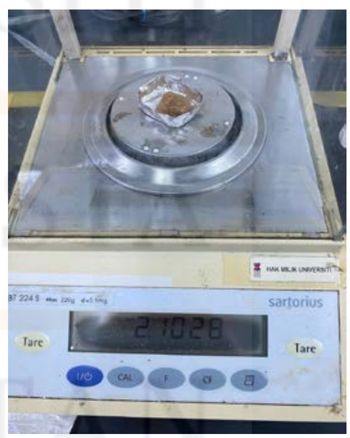
Testing FTIR of soap sample 1

Testing FTIR of soap sample 2

Oven at BAP, UMK



Weighed before put in oven



Weighed before put in oven