Development of Potential Antimicrobial Cream Incorporating with *Cassia senna* Infused Oil

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

_____

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I certify that the report of this final year project entitled "Development Of Potential Anti-Microbial Cream Incorporating with *Cassia senna* Infused Oil" by _____, matric number F15A0298 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Product Development Technology) with Honors,

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^{o}C	Celsius
g	Gram
SPSS	Statstical Pavkaging for the social Science
ml	Milliliter
mm	Milimeter

Development of Potential Antimicrobial Cream Incorporating with *Cassia senna* Infused Oil

ABSTRACT

Cassia senna (Fabaceae) which commonly known as *gelenggang* is used to cure the skin diseases by applying directly to the damaged skins. The facts that the *C. senna* has the anti-microbial properties that kills or inhibit the growth of microorganisms draws many researchers to study this plant. Thus, this study aims to evaluate the quality of the developed cream using infused oil of *C. senna* leaves in terms of physicochemical, stability, antimicrobial and finally establish sensory evaluation. The essential oil of *C. senna* leaves was infused using olive oil using low heating technique and incorporated in a cream. The cream when mixed with infused oil of *C. senna* and the petroleum jelly (Vaseline) shows the suitable pH value for the skin of 5. The developed cream should have moderate quality attributes in terms which are accepted aroma, colour, and texture in terms of its physicochemical properties. The developed cream also found to be stable at 25˚C for 3 weeks with distinct changes. An antimicrobial activity was conducted to identify the potential of the cream in inhibiting *Escherichia coli* and *Staphylococcus aureus* using disk diffusion method. The developed cream was found to significance ($P<0.05$) inhibiting the two tested bacteria. Finally, a sensory evaluation conducted using 30 amateur evaluators showed no irritation and good acceptance of the developed cream. From the above mentioned findings, it show that the developed cream incorporated with *C. senna* infused oil would be further analysed and have a potential to be commercialized as another alternative for those who prefer another alternative for those who prefer natural based antimicrobial cream with mild effects.

Keywords: *Cassia senna*, antimicrobial activity, infused oil, developed antimicrobial cream, sensory evaluation, disk diffusion method

Potensi Pembangunan Antimikrobial Krim dengan Pati Minyak daripada *Cassia senna*

ABSTRAK

Cassia senna (Fabaceae) yang selalu dikenali sebagai gelenggang digunakan untuk menyembuhkan penyakit kulit dengan menyapukannya kepada kulit yang rosak. Fakta bahawa *C. senna* mempunyai sifat anti mikrob yang membunuh atau menghalang pertumbuhan mikroorganisma menarik banyak penyelidik untuk mengkaji tumbuhan ini. Dalam kajian ini, minyak pati daun *C. senna* telah dicampurkan dengan minyak zaitun menggunakan teknik haba yang rendah dan dicampurkan ke dalam krim. Krim yang dihasilkan dengan campuran minyak yang dari *C. senna* dan jeli petroleum (Vaseline) menunjukkan nilai pH yang sesuai untuk kulit iaitu 5. Krim yang dihasilkan harus mempunyai sifat-sifat kualiti yang sederhana dari segi aroma, warna, dan tekstur sederhana. Krim yang dihasilkan juga didapati stabil pada 25˚C selama 3 minggu. Tiada perubahan yang berbeza. Aktiviti antimikrob telah dijalankan untuk mengenal pasti potensi krim dalam menghalang *Escherichia coli* dan *Staphylococcus aureus* menggunakan kaedah penyebaran cakera. Krim yang terbukti didapati penting ($P < 0.05$) menghalang kedua-dua bakteria diuji. Akhirnya, penilaian deria yang dilakukan menggunakan 30 penilai amatur tidak menunjukkan penerimaan krim yang maju. Dari penemuan yang disebutkan di atas, ia menunjukkan bahawa krim yang dihasilkan setelah digabungkan dengan minyak yang dicampur *C. senna* akan menjadi alternatif lain bagi mereka yang memilih krim antimikroba berasaskan semulajadi.

Kata kunci: *Cassia senna*, aktiviti antimikrobial, minyak yang disemai, krim antimikrob, penilaian deria, kaedah penyebaran cakera

CHAPTER 1

INTRODUCTION

1.0 Research Background

Nowadays, there are many researches about the traditional medicinal plants used as potential of the anti-microbial, anti-oxidant, and anti-bacterial agent. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (World Health Organization, 2011). *Cassia* species have been of medical interest due to their good therapeutic value in folk medicine (Abu et. al., 2000). *Cassia senna* is the Fabaceae family which is also be called Leguminosae. The common name of *C. senna* is *gelenggang*. *C. senna* is a kind of shrub easily found on the edge of the road and bush. This tree usually reaches a height of 6 to 25 feet. It is a tropical and subtropical plant. The plant constituent of *C. senna* contain of water, diluted myricyl alcohol extract, glucoside, cathartic acid, anthraquinone derivatives, rhein, aloe-emedin, kempferol, isormamnetin. *C. senna* is usually used for gastrointestinal conditions as a cleanser during a fast as a very effective laxative, constipation, eases nausea and biliousness, halitosis, and increases peristaltic movements of the colon by local action on the intestinal wall.

C. senna works by irritating the lining of the upper intestines which provokes reflex muscular activity in the colon resulting in a bowel motion due to the chemical anthraquinone. Besides, the leaves of *C. senna* usually use for skin diseases such as ringworm. In Africa and Caribbean the *C. senna* is considered to be good as reducing body temperature (febrifuge) and removing sweat (diaphoretic). *C. senna* is one of the sources of the anti-microbial agent for the medicinal plants.

Antimicrobial is the agents that kills and inhibit the growth of the microorganisms. "A variety of laboratory methods can be used to evaluate or screen the in vitro anti-microbial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism" (Mounyr Balouiri, Moulay Sadiki, Saad Koraichi Ibnsouda, 2015).

This research is conducted due to the fact that *C. senna* has a high anti-microbial property which is the leaves of *C. senna* has contain no less than 2.5 percent antraquinone glucosides. The antraquinone glucosides act as active ingredients that can cure the skin diseases such as the ringworm diseases. At the same time, there is no research about the antimicrobial activity of the *C. senna* infused oil. This research can be used to develop the potential antimicrobial cream by using the *C. senna* essential oil which is commercialized the beneficial of the *C. senna* to the society. The determination concentration of the solvent and the formulation of the development of the potential anti-microbial cream that will affect the anti-microbial activity of *C. senna* essential oil are important.

1.1 Problem Statement

Cassia senna is often used traditionally for treating various diseases, especially skin diseases. It is reported as having anti-microbial, anti-oxidant and anti-bacterial properties. One of the active ingredients of the *C. senna* leaves is antraquinone glucosides. Due to this reason, *C. senna* is used by applying directly on the skin with microbes infections. So, this why the *C. senna* is traditional used to apply directly on the damaged skin. There do not have any research on the anti-microbial activity infused oil of *C. senna*. Even though there are various products being developed from *C. senna* extracts, to date, no research was done using infused oil from *C. senna* leaves on *E. coli* and *S. aureus*. Additionally, there was also a sensory evaluation to identify the acceptance of the developed cream among amateur respondents.

1.2 Hypothesis

1) H_0: The formulation of the developed cream using *Cassia senna* infused oil shows good quality in terms of physical appearance, stability, antimicrobial activity and sensory acceptance.

2) H_1: The formulation of the developed cream using *Cassia senna* infused oil does not show good quality in terms of physical appearance, stability, antimicrobial activity and sensory acceptance.

1.3 Objectives

1) To develop a potential anti- microbial cream using *Cassia senna* infused oil.
2) To evaluate the quality and stability test of the developed cream incorporated with *Cassia senna* infused oil.
3) To assess antimicrobial activity of the developed cream incorporated with *Cassia senna* infused oil by using disk diffusion method.
4) To conduct a sensory evaluation of the developed cream using amateur evaluators.

1.4 Scope of Study

The study were focused on the formulation of a potential antimicrobial cream using *Cassia senna* infused oil. The study includes the development of a potential anti-microbial cream of the *C. senna* infused oil for commercialize the benefits of the *C. senna* to the peoples. Peoples will more aware about the functions of the *C. senna* in their daily lives.

The study was also focused on evaluation of quality assessments and accelerated stability test of developed cream incorporated with *C. senna* infused oil. Then, focusing on evaluation of antimicrobial activity of the *C. senna* infused oil which is traditional use to cure the skin diseases as the active ingredients in the *C. senna* leaves is antraquinone glucosides. This study was also focused in determine the concentration of the *C. senna* infused oil that will affect the anti-microbial activity of the *C. senna* infused oil. To date, there are no product have been develop from *C. senna* infused oil.

1.5 Significance of Study

There are some potential benefits that can be gained by the society after the completion of this study. Currently, product cream usually from *Cassia senna* using the extraction. To date, there no product cream develop with *C. senna* infused oil. The society will aware the benefits of the *C. senna* in the pharmaceutical industry. Besides, this study also provides the information of the concentration of the solvent that will affect the anti-microbial activity of the *C. senna* infused oil.

Other than that, this study also able to help society about the beneficial of the *C. senna* plant in the health problem especially for the skin diseases. This study also provides the information of the anti-microbial activity of the C. *senna* infused oil. Lastly, for the future researchers, this study will be beneficial as their guide to develop more product using this plant.

CHAPTER 2

LITERATURE REVIEW

Nowadays, herbal cosmetics have been greatly used for the longest of time. There are including skin care products, hair care products, herbal bath teas, lotions, creams and powders. According to the Laxmi and Harshal (2015), herbal cosmetics are formulated, using different cosmetic ingredients to form the base in which one or more herbal ingredients are used to cure various skin ailments. Plants are highly used for development of new drug products for cosmeceuticals and pharmaceutical applications. The advantages of the herbal cosmetics over the synthetic are the herbal cosmetic is a natural product and free from the synthetic chemicals which may be harmful to the skin. Besides, it safe to use because it is prove by the dermatologist. There are various herbs that are having natural uses in the cosmetics preparation for the skin care products, as an antimicrobial, as an antioxidant and others.

2.1 *Cassia senna*

Cassia senna is the Leguminosae family and the common name of the *C. senna* is *gelenggang*. *C. senna* is aborigine of tropical Africa and Sudan while *Cassia angustifolia* is native to Arabia, Somalia, Sind and the Punjab. According to the Shah et al (n.d), *C. senna* is largely grown on light or medium loam soil, including coarse gravelly soil and on alluvial loams with adequate drainage and pH varying from 7.0 to 8.2. It can stand moderate drought conditions and has a great tolerance for salinity; the tolerance is lower at seedling stage and progressively increases (Ayoub and Yogesh, n.d). "*C. senna* plants are low branching shrubs which are 3 feet with a straight woody stem and yellow flowers. The leaflets of *C. senna* have stout petioles, entire margin lamina with an asymmetric base and an acute apex. It has a characteristic odor (faint) and bitter unpleasant taste" (Mehta, 2013).

2.1.1 Ethnobotanical Study of *Cassia Senna*

Cassia senna is a powerful cathartic used in the treatment of constipation, working through a stimulation of intestinal peristalsis. In addition, *C. senna* also can aid the body in cleaning waste and promote the excretions of toxins which are thought to contribute to fatigue and general ill-health.

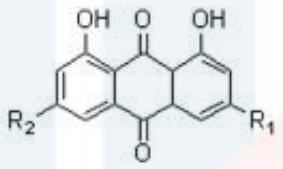
C. senna is also used for irritable bowel syndrome, hemorrhoids, and weight loss. There many benefits of the *C. senna*. Firstly, to treatment of skin conditions, the essential oils resin and tannin in *C. senna*, can alleviate skin inflammation. *C. senna* is used in Ayurvedic medicine for this purpose. It is made into a paste, which can be used as a

compress to heal ringworms, wounds, and burns. Besides, *C. senna* is used to treat the skin infections. The anti-bacterial property of *C. senna* can help in treating dermatological or skin ailments. The paste made from *C. senna* leaves is effective in treating skin infections like acne as well as inflammatory conditions like eczema

2.1.2 Phytochemical Study of *Cassia Senna*

Cassia senna contains dianthrone glycosides (1.5% – 3%), Sennosides A and B (rhein dianthrones containing the aglycone Sennidin A and Sennidin B respectively), Sennosides C and D (gylcosides of heterodianthrones rhein and aloe emodin). Besides, the free anthraquinones are also present and several other glycosides such as palmidin A and aloe-emodin dianthrone diglycosides are also present. *C. senna* also contains flavanols such as kaempferol (yellow color) and isorhamnetin. Traces of chrysophanic acid, saponin, salicylic acid and volatile oils have also been found. The phytochemical activity of the *C. senna* depends on the climate. Rai and Shok in 1983 shown that "the roots contain rhein and aloe-emodin, both free and glycosidic (Fig 1.0). Two new bis (tetrahydro) anthracene derivatives, occidentalol-I (IV, R1=Me and R2=H) and occidentalol-II (III, R1=R2=H) were isolated (Fig. 2.0) from the roots of *Cassia* sp along with chrysophanol, emodin, pinselin, questin, germichrysone, methylgermitorosone and singueanolI (I, R1=R2=Me). The structures were established on the basis of spectral evidence. A toxic albumin besides chrysophanol has been detected in the seeds of *Cassia* sp. From the seeds carbohydrates: maltose, lactose, sucrose and raffinose are also detected. A mixture of C-flavonoids of apigenin (Fig. 3.0), among them probably vitexin and a 7-heteroside of vitexin, chrysophanol and emodin as well as their glycosides and free physcion have been reported from the leaves of *Cassia* sp". The laxative effect

exerted by *senna* is mainly attributed to the anthraquinones which includes dianthrone glycosides, sennosides A and B, sennosides C and D.



Trivial Name	R1	R2
Rhein	COOH	H
Aloe-emodin	CH2OH	H
Chrysophanol	CH3	H
Emodin	CH3	OH
Physcion	CH3	OCH3

Figure 1.0: Anthraquinones glycones from *Cassia sp.* plant.

Source: Rai and Shok, (1983)

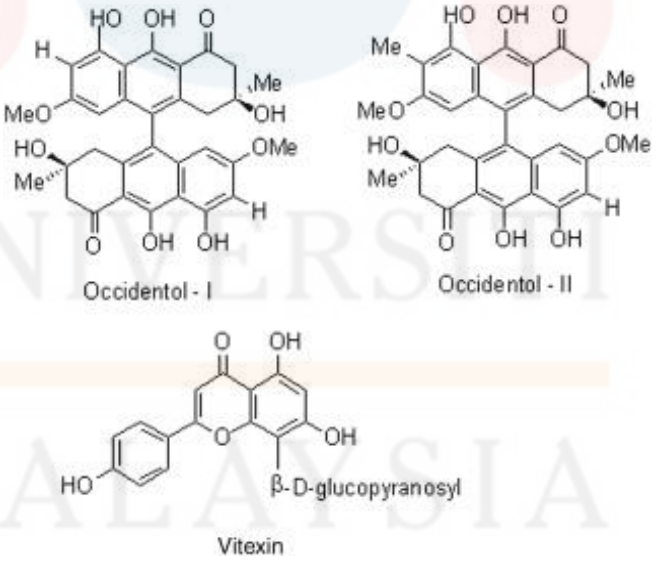


Figure 2.0: Structures of Occidentol-I, II and Vitexin isolated from *Cassia sp* plant.

Source: Rai and Shok, (1983)

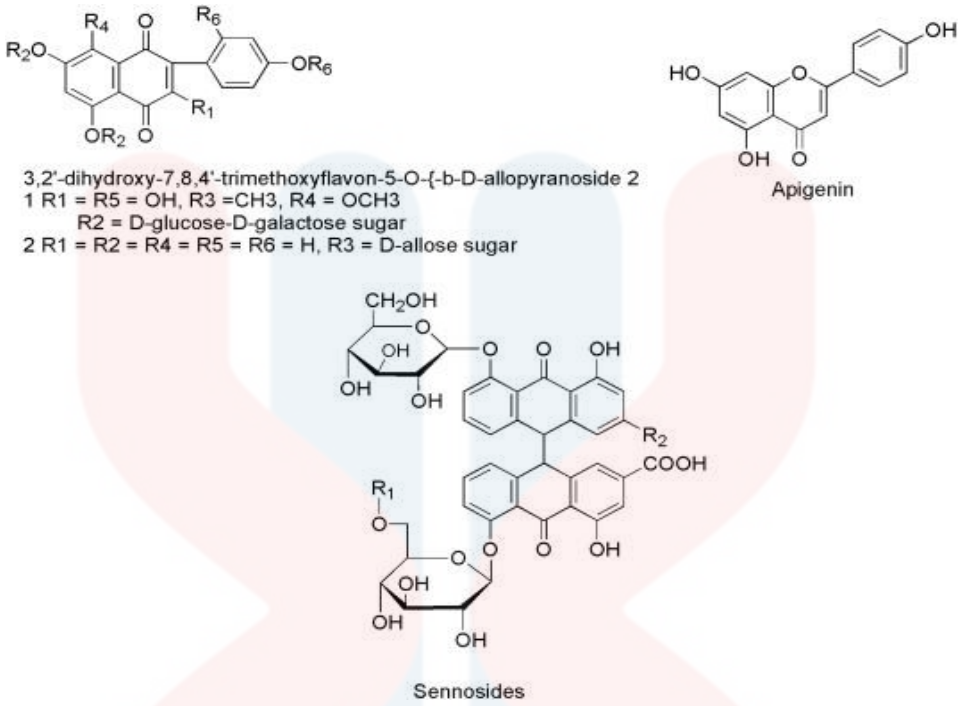


Figure 3.0: Phytochemicals isolated from *Cassia sp* plant.

Source: Rai and Shok, (1983)

2.1.3 Pharmacological Study of *Cassia Senna*

2.1.3.1 Antimicrobial Activity

According to the Sadiq et al (2016), there are different types of microorganisms (*Corynebacterium diphtheriae, Mucor sp. Neisseria sp. Salmonella sp., Aspergillus niger*) were found to be active against the leaf extracts of this plant. The leaf extract of *Cassia senna* when tested against different pathogenic bacteria was found to be active against *Salmonella enteritidis* and *Staphylococcus aureus* while a negative effect was observed against *Escherichia coli* and *Shigella dysenteriae.* Leaves of this plant were extracted with ethanol and water by Sadiq et al. The extracts were used to carry out

antimicrobial screening in vitro on *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, shigella spp*. Chromatographic separation was carried out on the active extracts, and the efficacy of the resulting fractions was tested against the susceptible organism. Some of the extracts indicated significant inhibitory activity against the tested organisms.

General phytochemical screening was done on the ethanol, water extracts and fractions. Ethanol extract revealed the presence of tannins, saponins, cardiac glycoside, terpenoids and anthraquinones while the fraction revealed the presence of tannins, terpenoid and anthraquinones. This result might explain the ethnobotanical use of the plant for the treatment of dysentery, gastro internal disorder, constipation and Typhoid fever. Besides, the seeds of this plant possess a strong antibacterial activity against *S. aureus, B. subtilis, B. proteus* and *Vibrio cholerae* and against fungi *A. flavus, A. niger* and *Trichophyton mentagrophytes.* In this study, *Escherichia coli* and *Staphylococcus aureus* was chosen because having the different effect towards the extraction of the *C. senna* leaf. Different pathogenic bacteria was found to be active against *Staphylococcus aureus* while a negative effect was observed against *E. coli.*

2.1.3.2 Antioxidant Activity

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. Antioxidants cause protective effect by neutralizing free radicals, which are toxic by product of natural cell metabolism. Nuhu et al studied *Cassia senna* to ascertain the hepatoprotective potential of the plant extract. Hypoproteinaemic effects and increase in ALT, AST and ALP were indications that the crude extract of *C. senna* leaves may be

slightly toxic as concoction for liver ailments. Their research concluded that infusion of *C. senna* leaves is used as an effective treatment for hepatitis among the rural dwellers in northern part of Nigeria. Vadnere et al evaluated "the antioxidant potential of different fractions of whole plant of *C. senna* using various in vitro assays including 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), nitric oxide scavenging activity, hydrogen peroxide scavenging activity, reducing power assay. The results of the study indicate that ethyl acetate fraction of ethanol extract of whole plant of *C. senna* possess the significant antioxidant activity. Ethyl acetate fraction of ethanol extract of whole plant of *C. senna* must contain some principles, which possess antioxidant activity".

Infusion of the leaves of *C. senna* is used as an effective treatment for hepatitis. The potential of the leaf extract of *C. senna* may be related to its antioxidant activity. Torell et al. and Faure et al. have shown that flavonoids inhibit peroxidation of the hepatoprotective activity of aqueous-ethanolic (50% v/v) extract of leaves of this plant was studied on rat liver damage induced by paracetamol and ethyl alcohol by monitoring serum transaminase, alkaline phosphatase, serum cholesterol, serum total lipids and histopathological alterations. The prepared extract produced significant hepatoprotection.

2.1.3.3 Anti-Inflammatory Activity

Leaves of this plant have good anti-inflammatory activity as assayed by Sadique et al. They have used Carrageenan induced paw edema and cotton pellet granuloma assay and found that *C. senna* was maximally active at a dose of 2000 mg/kg. They have also noted the ability of these extracts to lower the lipid peroxide content, gamma-glutamyl

transpeptidase and phospholipase A2 activity in the exudates of cotton pellet granuloma, resulting in the reduced availability of arachidonic acid, a precursor of prostaglandin biosynthesis, and/or by stabilization of the lysosomal membrane system.

2.2 Products of *Cassia senna*

Nowadays, many researches had been conducted to explore benefits. Thus, there are many rising cosmetics product using the *C. senna* as an ingredient. "*C. senna* leaves and pods have been shown to have laxative activity. It is useful in habitual constipation" (Balasankar, Vanilarasu, Selva, Rajeswari, Debjit , 2013).

C. senna has been approved by Food and Drug Administration (FDA) as a non-prescription laxative. It is used to treat constipation and also to clear the bowel before diagnostic tests such as colonoscopy. In addition, *C. senna* also uses for the strong hair. *C. senna* can be applied topically to get smooth, shiny, and strong hair. *C. senna* can be used in a similar manner as henna for healthy hair and treatment of hair problems. Furthermore, *C. senna* can be used as a conditioner. *C. senna* can be used as a conditioner to impart shine besides strengthening and thickening your hair. It is a great option to minimize the adverse impacts of chemical treatments.

Moreover, *C. senna* also found to be beneficial to humans health. *C. senna* is use for the constipation. *C. senna* is effective in relieving constipation. It has been approved by the US FDA as a non-prescription drug to treat constipation. *C. senna* stimulates the muscles of the colon to push fecal matter through more quickly. *C. senna* leaf acts on the intestinal walls to cause contractions that lead to bowel movements. It softens stool by

enabling the colon to absorb water. It can effectively cure even the most severe cases of constipation. The glycosides in it help transport electrolytes, causing bowel movements within 6 to 12 hours of its intake. At the same time, the essential oils, tannins and other compounds in *C. senna* possess anti-bacterial properties. These can inhibit the growth and proliferation of microorganisms such as bacteria, fungi and parasites. Chewing *C. senna* leaf like tobacco can cure mouth infections and gingivitis. It also possesses mild inflammatory properties, which can soothe internal and external swelling. Then, *C. senna* has been found to be effective in providing relief from heartburn, nausea, gas, bloating and belching associated with dyspepsia. *C. senna*, when taken with aromatic herbs like cardamom, fennel, ginger and peppermint, can diminish the buildup of gas in the stomach through its strong purgative actions. To date, no products were found using the infused oil of *C. senna*.

2.3 Infused Oil

According Kasthuri et al. (2010) oils are one of the most ancient forms of natural herbal medicines. Furthermore, the extraction of fat soluble from the fresh or dried herbs should be done by using an oil because oil is a good medium and can be used as the basis for creams, salves, rubs and lip gloss. According Akçar and Gümüşkesen (2011) there are many types of oil that can be used to infuse the herbs such as sunflower oil, almond oil, olive oil, jojoba oil, safflower oil, grapeseed oil and castor oil. In recent years, olive oil gain attention by macerating the aromatic herbs in the oil. This is because olive oil less expensive than most other oils and last longer. The process of making infused oil is extracting the herbs into the oil instead water

There some method recommended by Dana (2010) to infuse oil which are by using the countertop method, low heat method, slow cooker method and solar method. The countertop method placing the herbs with oil in canning jar and leave it on the countertop for 3-4 weeks while shaking the canning jar daily. Meanwhile, the low heat method placing the herbs with oil in a double boiler and heating for 2-4 hours or using the oven at 65˚C for 4-6 hours. Then, slow cooker method using the canning jar to fill the herbs with oil and heating on low heat for 10-12 hours. The solar method is done by placing the herbs with oil in the jar and leave it under the sun for 2-4 weeks while shaking it daily. The sensorial characteristics of infused oil do not affected by the infusion time.

In this study, the olive oil is chosen to be mixed together with the *Cassia senna* leaves to form the infused oil and develop the potential antimicrobial cream. The olive oil is chosen because is less expensive, last longer and more aromatic fragrance than other oils. The low heat method is used in this study because more convenient and save the time.

2.4 Development of Cream Product

According Safriani, Sugihartini, Yuliani (2015), creams are semisolid dosage from containing one or more drug substances dissolved or dispersed in a suitable base. Creams may be considered pharmaceutical products as even cosmetic creams are based on techniques developed by pharmacy and unmedicated creams are highly used in a variety of skin conditions. Creams are divided into two types: oil-in-water (O/W) creams which are composed of small droplets of oil dispersed in a continuous water phase, and water-in-oil (W/O) creams which are composed of small droplets of water dispersed in a

continuous oily phase. Oil-in-water creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. Water-in-oil creams are more difficult to handle but many drugs which are incorporated into creams are hydrophobic and will be released more readily from a water-in-oil cream than an oil-in-water cream. Water-in-oil creams are also more moisturising as they provide an oily barrier which reduces water loss from the stratum corneum, the outermost layer of the skin.

In the making of cream, there have aqueous phase and oil phase. The functions of the stearic acid in develop cream product as a fragrance ingredient, surfactant and emulsifier. The stearic acid is a fatty acid found primarily in animal derivatives, but in vegetable fats as well. It is used in a variety of cosmetics and personal care products. When stearic acid used in cosmetic products, it is primarily fulfills the role of a thickener or hardener. The studies of Al-Rimawi, Yateem, and Afaneh, (2014) recommend usage rate of the stearic acid in develop the product is 5%.

Then, the wax helps keep emulsions from separating into their oil and liquid components. Waxes are harder, less greasy and more brittle than fats, and are very resistant to moisture, oxidization and microbial attack. The waxes that usually uses in the cosmetic products are beeswax and petroleum jelly (Vaseline). Beeswax is used primarily as thickener and emollient, but has also emulsifying properties. Vaseline is an excellent emollient and lubricant but is also used as bodify agent to add viscosity to cosmetic products.

Besides, the buffering agent such as triethanolamine has masking and fragrance ingredient, and surfactant, in addition as a pH adjuster. Next, the emollient, emulsifier or thickening agent that usually in the manufacture of skin creams and lotions is cetyl

alcohol. The Cosmetic Ingredient Review (CIR) Expert Panel recommended usage rate of the cetyl alcohol in the products for the external functions is usual concentration 0.5-6%. The safety of cetyl alcohol has been assessed by the Cosmetic Ingredient Review (CIR) Expert Panel.

In cosmetics to keep products from melting in high heat or from freezing usually used propylene glycol. It also helps active ingredients penetrate skin. In 2012, the U.S. Cosmetic Ingredient Review (CIR) Expert Panel reviewed the safety data for propylene glycol used in cosmetics and personal care products. Water forms emulsions in which the oil and water component of the product are combined to form the product. In addition, mineral oil has long been recognized as an important part of many cosmetic formulations. Mineral oil that usually used in cosmetic product are almond oil and olive oil. The U.S. Food and Drug Administration (FDA) has reviewed the safety of Mineral Oil and permits its use as an active ingredient in the following OTC drug product categories: anorectal drugs, skin protectants and ophthalmic emollients.

Normally, the cream product will use the preservatives such as methyl paraben or propyl paraben because of the ability to be solvents, antimicrobial and antifungal. However, in this study, there do not using the preservatives to develop the cream because of the presence of infused oil from *Cassia senna* that acts as an antimicrobial agent. At the same time, this study only used olive oil and petroleum jelly (Vaseline) as it is the homemade ingredients to develop the potential antimicrobial cream. Moreover, homemade ingredients are more convenient and safely to be used without having negative effect to consumer for apply on the skin.

2.5 Quality Assessment of Cream Product

The quality control and standards of the develop cream are as per the FDA norms specification which is the cream should liquefy at body temperature. For the cream to easily spreading the viscosity should be low but at the same time the viscosity should be high enough in order to retain in suspension particles of dirt and insoluble foreign matter. The cream should contain enough light oils in order to permit flushing the pores, thus, the creams can penetrate the epidermis. Besides, the cream should be an emulsion type with a small percentage of water.

The evaluation of the develop cream from *Cassia senna* using the physiochemical, consistency and microbial test. In the physiochemical test, the pH of the formulated cream is observed. The range pH of the cream which is good for the skin to be in 5-6 (Rajendra and Ram, 2011). Besides, the viscosity of the formulated cream should be in the range of 27001-27089 cps which is indicating that the cream is easily to spread by a small amount of shear. There no significant variation in the stability studies of the various parameters such as appearance, nature, pH of the formulation (Akash, 2014). There are many researches about the development of antimicrobial cream from *C. senna* extract. However, there is no research about the development antimicrobial cream incorporating with *C. senna* infused oil yet.

2.6 Antimicrobial Activity of *Cassia Senna*

Antimicrobial activity is an agent that kills microorganisms or stops growth of microorganisms. There are many way to test the antimicrobial activity such as agar disk-diffusion method, antimicrobial gradient method, broth diffusion method and agar dilution method. In this study, use agar streak method to test the microbial activity of the *Cassia senna.* Streak agar method used to isolate a pure strain from a single species of microorganism, often bacteria. Samples can then be taken from the resulting colonies and a microbiological culture can be grown on a new plate so that the organism can be identified, studied, or tested. In the streaking procedure, a sterile loop or swab is used to obtain an uncontaminated microbial culture. Methods for an antimicrobial susceptibility testing and discovering novel anti-microbial agents have been extensively used and continue to be developed due to the development of microbial resistance to the existing antimicrobial agents. "The use of the solvent may affect the growth of the microorganism, and making minor methodological adaptation to standardized protocols can be a solution to ensure accurate experimental approach" (Mounyr, Moulay and Saad, 2015).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Chemicals

Nutrient agar powder, petroleum jelly (Vaseline), olive oil, leaves of *Cassia senna*, *Escherichia coli*, *Staphylococcus aureus*, Nutrient broth, Tryptic Soy Broth, ethanol, blender, hot plate, beakers, glass rod, spatula, weighing scale, digital pH meter (Hanna), Viscometer (Sine wave Vibro SV-10/SV-100), colorimeter (Konica Minolta CR-400/CR-410), measuring cylinder, incubator, incubator shaker, oven, texture analyzer (Ametek Bookfield CT-3), autoclave, thermometer, sterile container, aluminum foil, petri dish, hockey stick, , falcon tube, Bunsen burner, gloves, plate disc, loop pipette tip, pipette and parafilm.

3.2 Preparation of Plant Material

The sample of *Cassia senna* leaves were collected from the local area of Bagan Datoh, Perak. The sample is identified by Mr. Benny Franklin Hartheman as Managing Director at Bioexpert Agrotechnology Centre, Kota Kinabalu, in Sabah. The samples

were washed thoroughly before drying the sample completely at the open air under the shade for 1 week. The 100 g of dried sample were weighed and blended completely by using the blender.

3.3 Preparation of Infused Oil of *Cassia Senna*

The preparation of infused oil and the method of preparing the infused oil was adopted by pharmacopoeia of India (1966), and British Pharmacopoeia (1973). Firstly, the sample that have been blend were weighed 10 g, 5 g and 1 g respectively by using the weighed scale. The 80 ml of olive oil was measured by using measuring cylinder. Then, 80ml of olive oil was mixed together with 10 g of samples in the beaker. This step was repeated by using the 5g and 1 g of sample. The beaker was covered with the aluminium foil to avoid the mixture to be evaporate completely. The mixture of the sample and olive oil was heated in the oven at 65˚C for 5 hours.

3.4 Development of Potential Antimicrobial Cream Incorporating with *Cassia Senna* Infused Oil

The development of potential antimicrobial cream was prepared with the mixture of infused oil of *C. senna* and petroleum jelly (Vaseline). The composition and ingredients of the formulation was shown in table 3.4.1 and table 3.4.2**.** The petroleum jelly (Vaseline) and infused oil of *C. senna* was dissolved in the oil phase and heated at 75 ˚C. After that the mixture of infused oil of *C. senna* and petroleum jelly (Vaseline)

was continuous stirred for 15 minutes and poured the mixture into the sterile container. The developed cream was cooled down after a few minutes.

Table 3.4.1: Formulations of the cream incorporated with *C. senna* infused oil

Ingredients	Amount of Ingredients			
	Sample control	Sample A	Sample B	Sample C
Olive oil	-	80 ml	80 ml	80 ml
C. senna leaves	-	10 g	5 g	1 g

Table 3.4.2: Composition and ingredients of the cream incorporated with *C. senna* infused oil

Ingredients	Amount of Ingredients			
	Sample control	Sample A (12.5%)	Sample B (6.25%)	Sample C (1.25%)
Infused oil of *Cassia senna*	-	5 ml	5 ml	5 ml
Vaseline	3.8 g	3.8 g	3.8 g	3.8 g

3.5 Quality Assessments and Accelerated Stability Test

3.5.1 Quality Assessments

3.5.1.1 Physical Properties

The developed cream was observed qualitative in terms of the colour, and appearance of the cream. The colour of the developed cream was measured based on lightness (*L), greenness (*a) and yellowness (*b) by using the colorimeter (Konica Minolta CR-400/CR-410).

3.5.1.2 Determination of pH

0.5 g of cream was weighed accurately in the beaker by using the weighing scale. The cream was dissolved in the 50 ml of distilled water. The pH of the cream was recorded by using the digital pH meter (Hanna).

3.5.1.3 Viscosity of Cream

The viscosity of the developed cream was determined by using the Viscometer (Sine wave Vibro SV-10/SV-100). 20g cream was put in the provided glass container and put on the stand. The height of the stand was adjusted until reach sensor plate.

3.5.1.4 Texture Analysis of Cream

The texture analysis of the developed cream is measures based on hardness, adhesiveness and cohesiveness. The texture was analysed by using cylinder probe (2mm diameter, 20mm length). The texture analysed by texture analyzer (Ametek Bookfield CT-3).

3.5.2 Stability Test

2.0 g of the 3 formulation (n=3) of developed cream (cream A, cream B and cream C) were stored at the different temperatures which is 4 ˚C, 25 ˚C and 50 ˚C for 3 weeks. After that, the stability of the cream was observed week by week based on pH, colour and texture.

3.6 Test for Microbial Growth in Develop Cream

Microbial analysis was carried out for all the develop cream by a procedure of Indian Pharmacopeia 2010 and WHO Guideline. In this study, *Escherichia coli* and *Staphylococcus aureus* have been used to test the antimicrobial activity because there were study by the Sadiq et al (2016) stated that the pathogenic bacteria was found to be active against *S. aureus* while a negative effect was observed against *E. coli*. The pure culture of *E. coli* and *S. aureus* were obtained from Faculty of Agro Based Industry of UMK. The pure culture of *E. coli* and *S. aureus* were inoculated on the plate of Nutrient Agar by streaking method and incubate for 24 hours at 37˚C. Then, preparation of culture broth was done by the isolation of the *E. coli* and *S. aureus* and mixed with the tryptic soy broth (TSB) for the *S. aureus* while nutrient broth (NB) for the *E. coli* after autoclave the TSB and NB for 2 hours. Then, the culture broth were put in the incubator shaker for 24 hours at 37˚C. After that the test of antimicrobial was prepared by using the disk diffusion method to determine the antimicrobial activity of the develop cream. The plates were placed into the incubator at 37˚C for 24 hours. After the incubation period, the plates were taken out and checked the microbial growth by comparing with the control.

3.7 Sensory Evaluation of the Develop Cream

A questionnaire was distributed to 30 amateur evaluators who are students at UMK Campus Jeli. The questionnaire (Appendix) consisted of the product data. The questions of the product data were about the acceptability of the develop cream in terms of colour, aroma, texture, softness, greasiness and overall acceptance of the product. The consent letter were distributed and signed by the evaluators.

3.7.1 Irritation Test

The samples of the develop cream was applied to the specified area on the skin such as on the hand of the volunteers. The 30 volunteers were provided and signed the consent letter before applied the develop cream on the skin. The irritancy was checked every hour up to 24 hours.

3.8 Statistical Analysis

The cross tabulation was used in statistical analysis to analyse the data. The data is interpreted in Mean± Sd. The One way ANOVA in SPSS used in statistical analysis to analyse the data.

CHAPTER 4

RESULTS & DISCUSSION

4.1 Develop Antimicrobial Cream from Infused Oil of *Cassia Senna*

Table 4.1: Formulation of antimicrobial cream.

	Cream A	**Cream B**	**Cream C**
Concentration (%)	12.5	6.25	1.25
Yield (%)	53.3	64.7	74.07
Infused oil of *Cassia senna* (ml)	5	5	5
Petroleum jelly (g)	3.8	3.8	3.8

In this study, the ingredients to develop antimicrobial cream using which are mineral oil, the waxes as an thickener, emollient, and the infused oil of *Cassia senna*. There are three different developed cream which are cream A, cream B and cream C that have different composition of ingredients used. Cream A has highest percentage of concentration for the infused oil of *C. senna*, while cream C has the lowest percentage of concentration for the infused oil of *C. senna*. In the same time, the composition of ingredients such as Vaseline and infused oil of *C. senna* used the same amount among three samples of developed cream.

4.2 Quality Assessments and Stability Test

4.2.1 Quality Assessments

Table 4.2: Quality assessments of developed cream week 0.

	Cream A (Mean±S.D) (mm)	Cream B (Mean±S.D) (mm)	Cream C (Mean±S.D) (mm)
pH	5.66±0.02	5.46±0.02	5.25±0.01
Lightness	23.91±2.18	31.59±1.02	41.61±0.0
Greenness	1.66±0.05	0.95±0.03	0.58±0.05
Yellowness	12.09±1.82	24.5±1.33	33±0.20
Viscosity	13.03±0.84	10.81±0.57	6.9±0.20
Hardness	1260±10.58	1058±5.00	1036±30.32
Adhesiveness	0.03±0.06	0.13±0.06	0.2±0.20
Cohesiveness	1.19±0.05	1.08±0.08	1.01±0.11

Based on the data that shown in table 4.1 shows that the pH value of developed creams are in the range 5.25-5.68. In this study, the colour of developed cream determine based on lightness (*L), greenness (*a) and yellowness (*b). Cream C has the highest value of lightness compared to others developed cream. Cream A has the highest value of greenness compared to the other developed creams. However, cream A has the lowest yellowness value compare to the other developed creams. Based on the data for the viscosity of cream A has the highest viscosity value. The texture analysis of developed cream based on hardness, adhesiveness and cohesiveness. Cream A has the highest value of hardness compared to the other developed creams. Based on the adhesiveness, cream A has the lowest value but the highest cohesiveness value compared to the other developed creams.

4.2.2 Stability Test

The stability of developed creams in this study based on the pH value, colour analysis, texture analysis and the viscosity within 3 weeks in three different temperatures which is 4˚C, 25˚C and 50˚C.

4.2.2.1 pH Value of Develop Cream

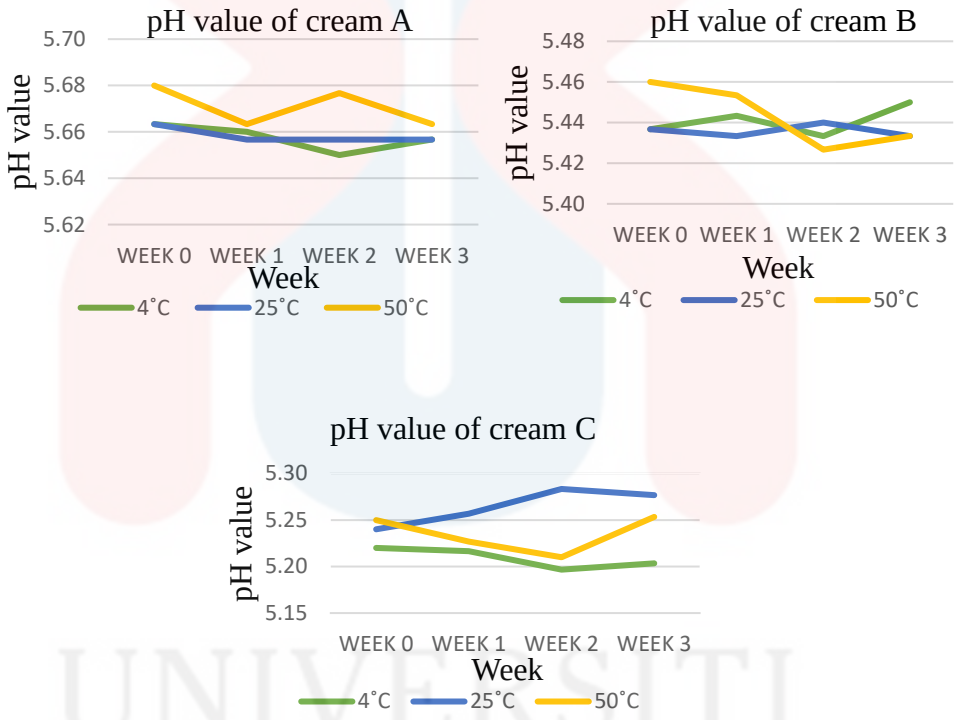


Figure 4.1: pH value of cream A, B and C at week 0, 1, 2 and 3.

The pH value of the developed creams are decreasing within 3 weeks. The pH value do not have distinct changes in three weeks. The pH value of cream A, cream B and cream C that stored at 4˚C has slightly decreases.

4.2.2.2 Colour Analysis of Developed Cream

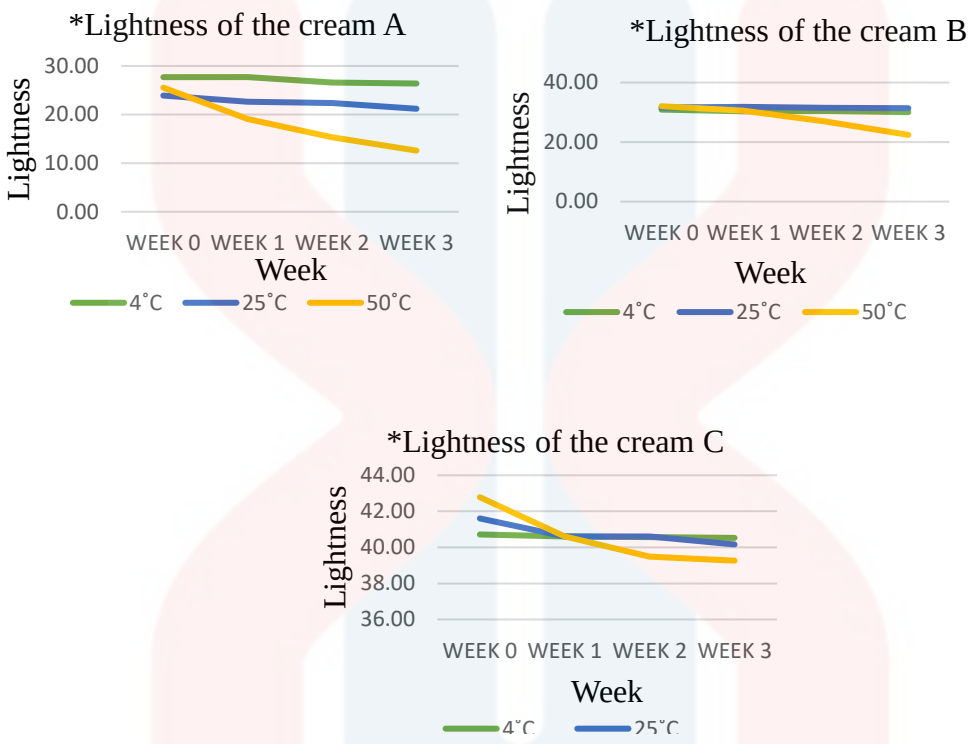


Figure 4.2: Lightness of cream A, B and C at week 0, 1, 2 and 3.

The lightness value of the cream A, cream B and cream C that stored in three different temperature are decreasing within three weeks. The lightness value for cream A and cream B that stored at 4°C has slightly decreases within three weeks. However, lightness value of the cream C that stored at 4°C and 25°C slightly decreases in three weeks.

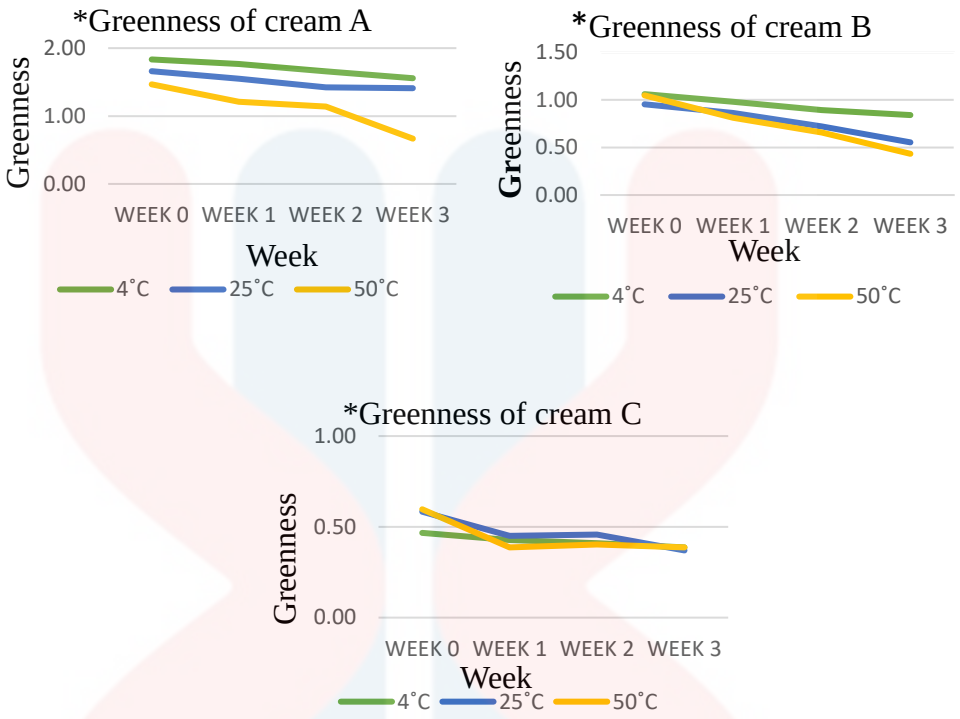


Figure 4.3: Greenness of cream A, B and C at week 0, 1, 2 and 3.

The greenness value for cream A that stored at 4°C has slightly decreases in between within three weeks. Cream A that stored at 50°C shows decreases between within three weeks. The greenness value for cream B that stored at 4°C and 25°C slightly decreases within three weeks. Cream B that stored at 50°C shows decreasing between within three weeks. The greenness value of the cream C that stored in three different temperature are decreasing within three weeks. The greenness value for cream C that stored at 4°C and 25°C also slightly decreases within three weeks.

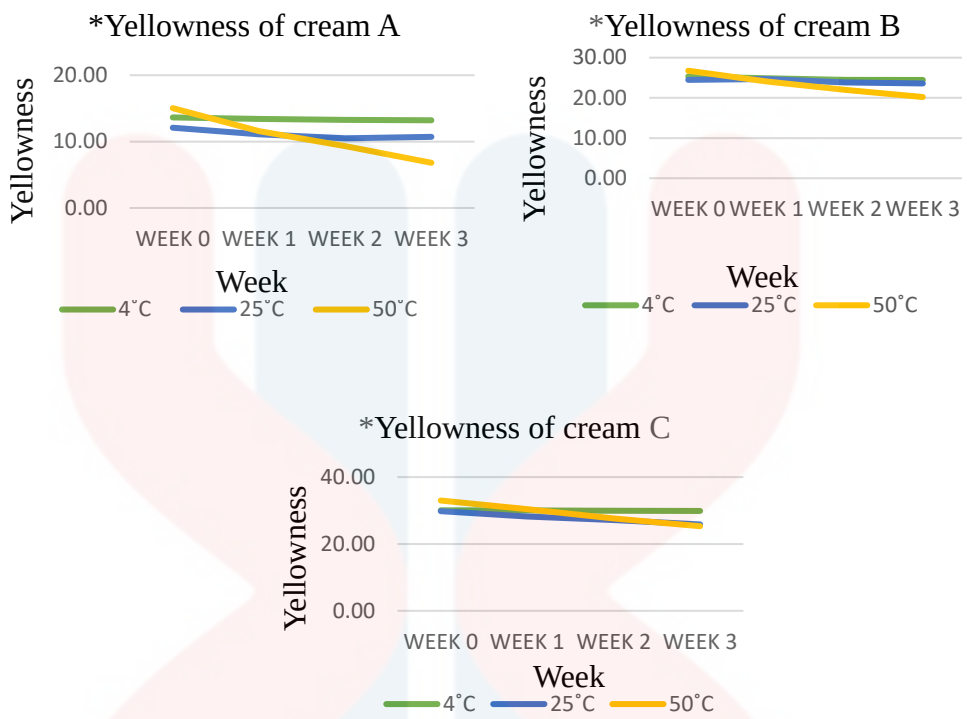


Figure 4.4: Yellowness of cream A, B and C at week 0, 1, 2 and 3.

The yellowness value for cream A that stored at 4°C and 25°C slightly decreases within three weeks. The yellowness value of the cream B that stored in three different temperature are decreasing within three weeks. The yellowness value for cream B that stored at 4°C and 25°C slightly decreases within three weeks. The yellowness value of the cream C that stored in three different temperature are decreasing within three weeks. The yellowness value for cream C that stored at 4°C and 25°C slightly decreases within three weeks.

4.2.2.3 Viscosity Value of Developed Cream

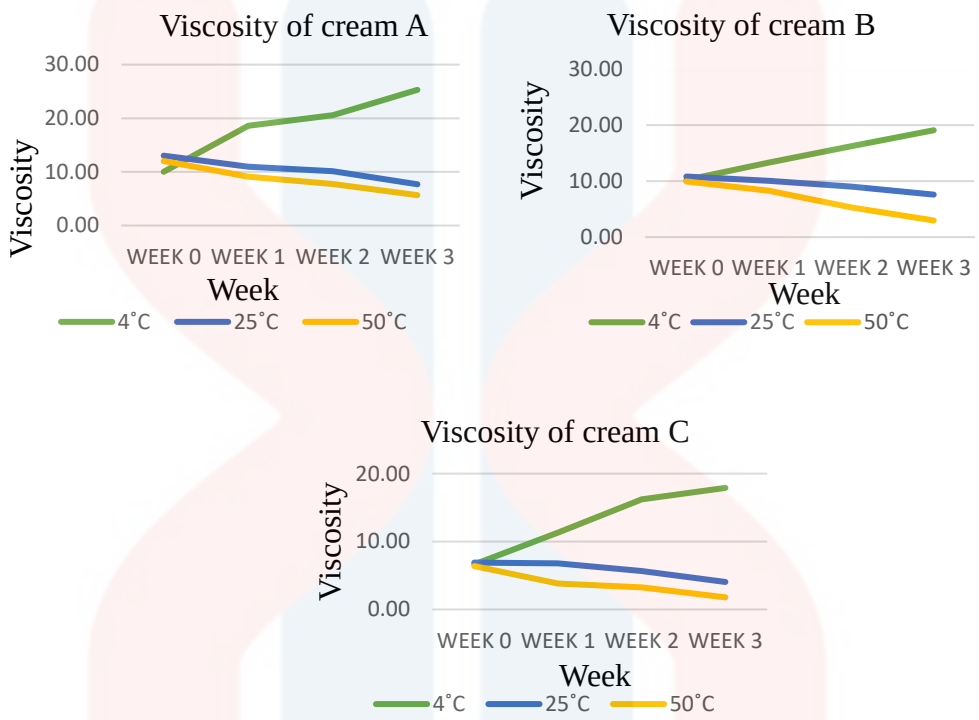


Figure 4.5: Viscosity of cream A, B and C at week 0, 1, 2 and 3.

The viscosity value of the cream A that stored at 4°C has increasing within three weeks. Cream A that stored at 25°C also slightly decreases. The viscosity value of the cream B that stored at 4°C has increasing but cream B that stored at 25°C decreases. Cream B that stored at 50°C shows decreases within three weeks. The viscosity value of the cream C that stored at 4°C has increasing in between three weeks. However, cream C that stored at 25°C also decreases.

4.2.2.4 Texture Analysis of Developed Cream

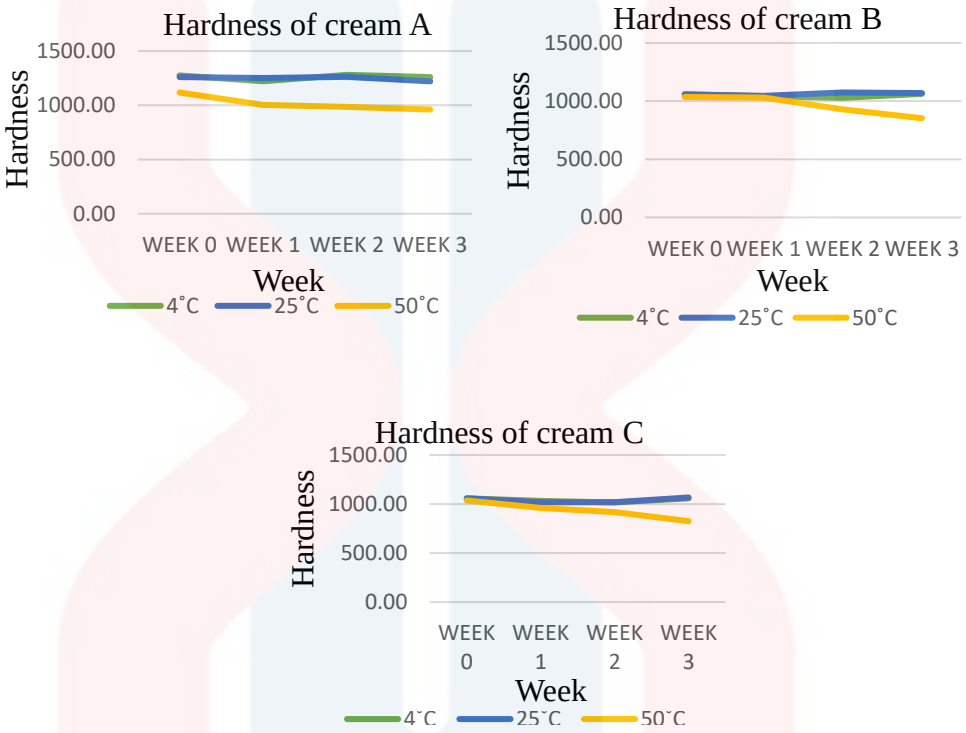


Figure 4.6: Hardness of cream A, B and C at week 0, 1, 2 and 3.

The hardness of the cream A that stored at 4˚C and 25˚C has slightly difference of value within 3 weeks. Meanwhile, hardness of the cream A that stored at 50˚C is decreasing within 3 weeks. The hardness of the cream B that stored at 4˚C and 25˚C has slightly increase within 3 weeks. Meanwhile, hardness of the cream B that stored at 50˚C is decreasing within 3 weeks. The hardness of the cream C that stored at 4˚C and 25˚C has slightly increase within 3 weeks. Meanwhile, hardness of the cream C that stored at 50˚C is decreasing within 3 weeks.

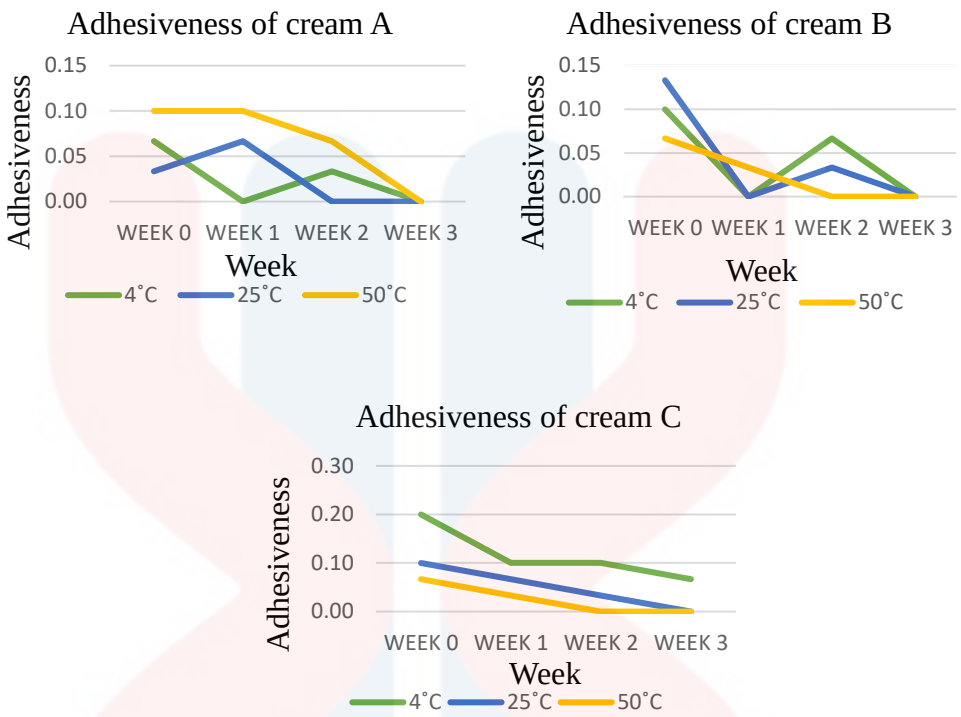


Figure 4.7: Adhesiveness of cream A, B and C at week 0, 1, 2 and 3.

The adhesiveness of the developed creams at three different temperature are drastically decreases. The adhesiveness of cream A at 25˚C is increasing in first week and decreasing in second week. The adhesiveness of cream A at 4˚C is decreasing in first week but increasing in second week. The adhesiveness of cream B at 4˚C, 25˚C, and 50˚C is decreasing within first week and increasing in second week. The adhesiveness of cream C at 4˚C, 25˚C and 50˚C are decreasing within 3 weeks.

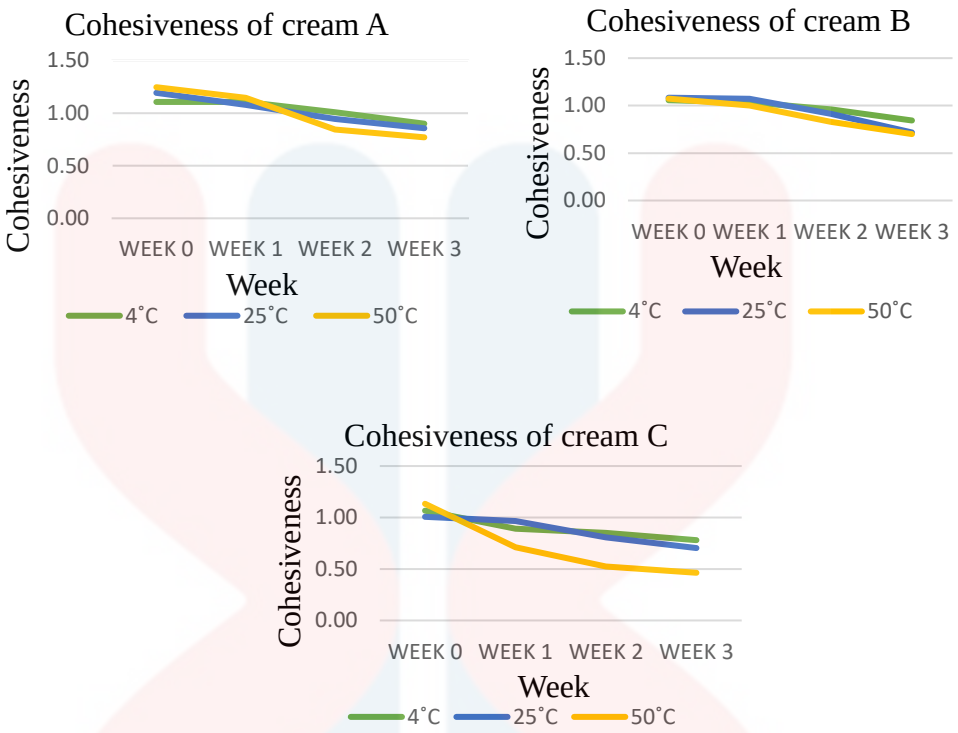


Figure 4.8: Cohesiveness of cream A, B and C at week 0, 1, 2 and 3.

The cohesiveness of the cream A that stored at 4˚C, 25˚C and 50 ˚C has slightly decreasing within 3 weeks. The cohesiveness of the cream A that stored at 4˚C, 25˚C and 50 ˚C has slightly decreasing within 3 weeks. At 4˚C and 25˚C, the cohesiveness of cream A have no difference. The cohesiveness of cream B at three different temperature had slightly decreasing within 3 weeks. The cohesiveness of cream B at 50 ˚C has the lowest value in the third week. The cohesiveness of the cream B that stored at 4˚C, 25˚C and 50 ˚C has slightly decreasing within 3 weeks.

4.3 Antimicrobial Assay

In this study, the antimicrobial that has been conducted using disk diffusion method against *E. coli* and *S. aureus*. Table 4.3 showed the inhibition zone of developed cream against *E. coli* and *S. aureus* using disk diffusion method.

Table 4.3: Inhibition zone (mm) of developed cream against *E. coli* and *S. aureus* using disk diffusion method.

	Blank Control (Mean±S.D) (mm)	Cream A (Mean±S.D) (mm)	Cream B (Mean±S.D) (mm)	Cream C (Mean±S.D) (mm)
E. coli	18.67± 1.15	11.33±1.53	0	0
S. aureus	0	0	0	0

Among the three developed creams, the cream A showed the strongest inhibition against *E. coli* at 11.33±1.53 mm while no inhibition showed off by cream B and cream C. However, cream A showed no inhibition against *S. aureus* at 0 mm as well as the other two developed creams.

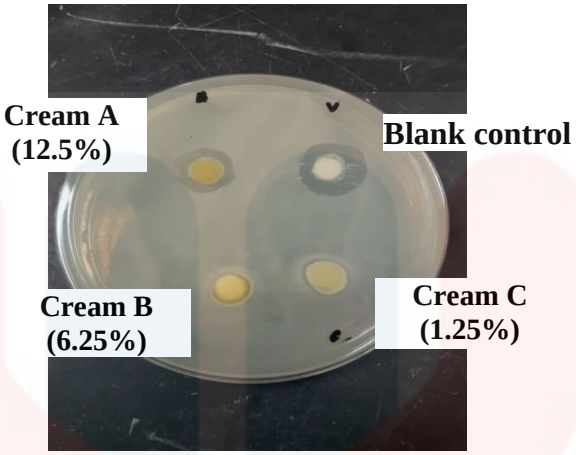


Figure 4.9: Zone of inhibition (mm) of developed cream against *E. coli*

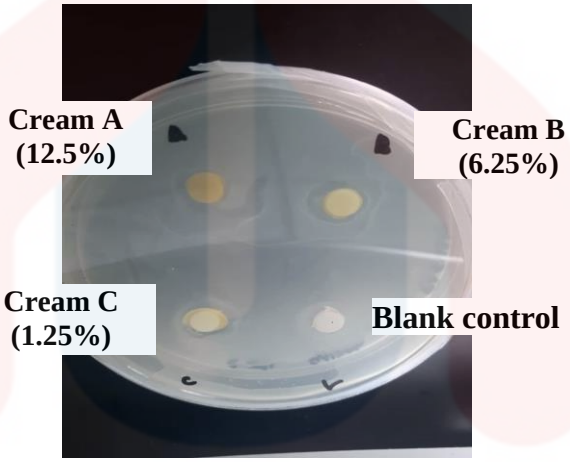


Figure 4.10: Zone of inhibition (mm) of developed cream against *S. aureus*

The antimicrobial activity of developed creams showed cream A and cream blank control against *E. coli* have the inhibition zone while there no inhibition zone showed off by cream B and cream C. The statistically analysis indicates that interaction between concentration infused oil of *Cassia senna* and antimicrobial activity has significance effect ($P<0.05$).There are no inhibition zone showed off by three developed creams from the *C. senna* infused oil against the *S. aureus.*

4.4 Sensory Evaluation

Sensory evaluation was conducted using 30 amateur evaluators at UMK, Jeli Campus. The attributes evaluated were aroma, colour, texture, softness, greasiness and overall acceptance. The evaluators were asked to rate as dislike very much, dislike, dislike slightly, neither dislike nor like, like slightly, like and like very much according to a questionnaire in Appendix.

4.4.1.1 Evaluation of Aroma of the Developed Cream

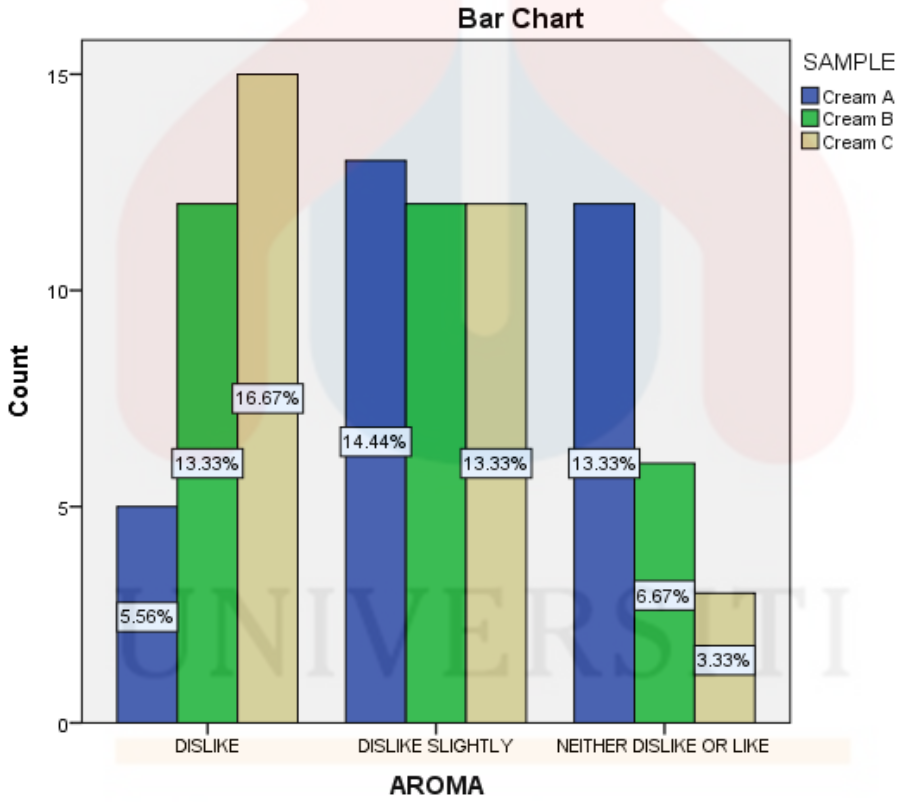


Figure 4.11: Acceptability for the aroma of the developed cream.

In this section, most of the evaluators dislike the aroma from cream C compared to other developed creams. This is because cream C has less aroma of the *Cassia senna* because the aroma of olive oil covered it. Mostly evaluators dislike slightly the aroma of

the cream A. Meanwhile, cream B has the same percentage either the evaluators dislike or slightly dislike the aroma.

4.4.1.2 Evaluation of Colour of the Developed Cream

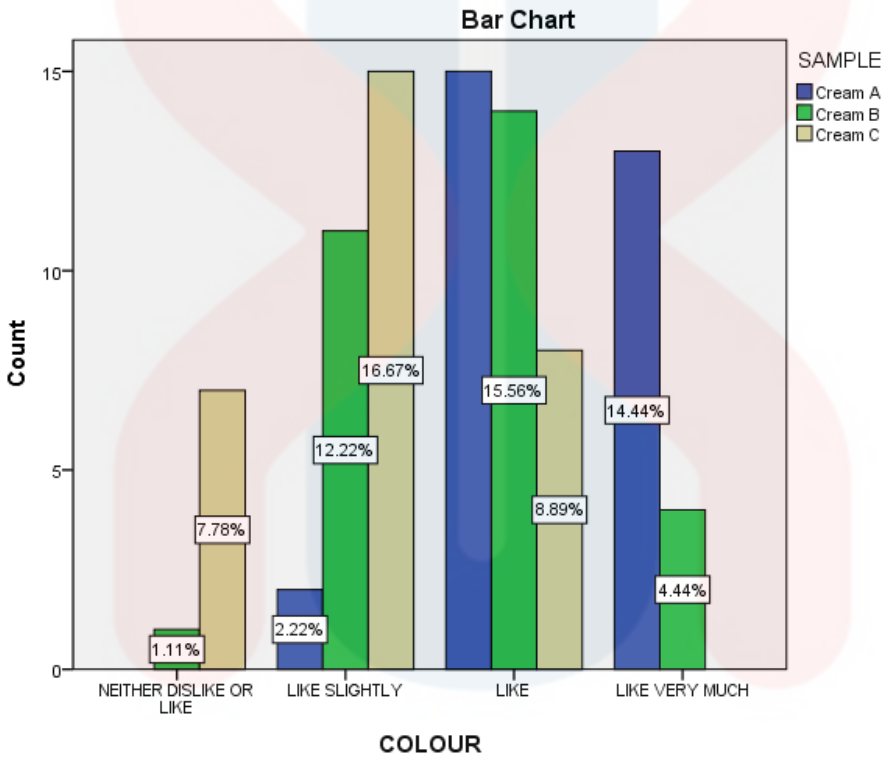


Figure 4.12: Acceptability for the colour of developed cream.

Evaluation of colour section, most of the evaluators like the colour of cream A compared to the other developed creams. However, mostly evaluators slightly like the colour of cream C and evaluators also like the colour of cream B. Cream A has the highest percentage for evaluators to like very much of the colour while cream C has the highest percentage for neither dislike or like the colour.

4.4.1.3 Evaluation of Texture of the Developed Cream

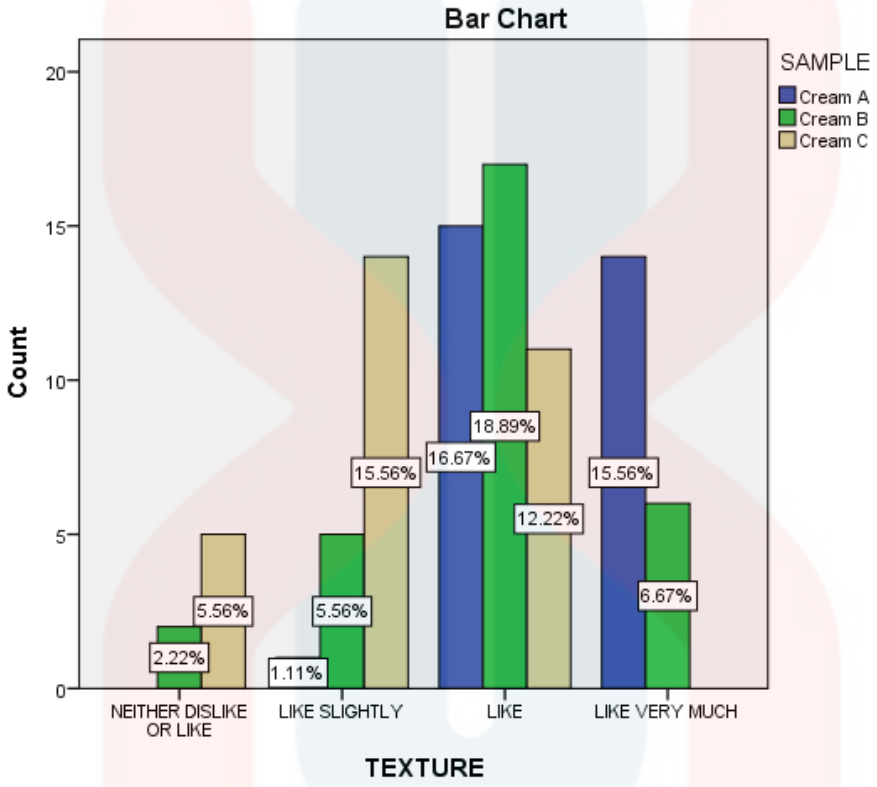


Figure 4.13: Acceptability for the texture of developed cream.

In this section, most of the evaluators like the texture of cream B while some of the evaluators choose neither dislike nor like the texture of cream B. Cream A has high percentage of the evaluators that choose like very much for the texture of cream and only a few choose slightly like. This is because the texture of cream A is smoother and good appearance. Most of the evaluators choose like for the softness of the cream A and cream B.

4.4.1.4 Evaluation of Softness of the Developed Cream

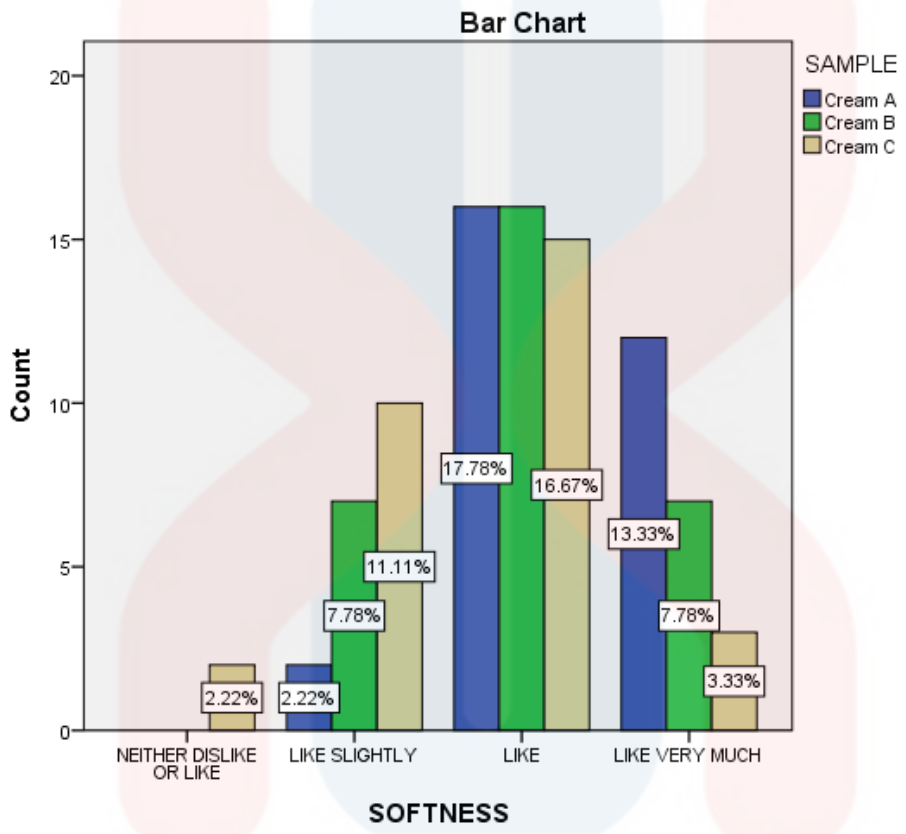


Figure 4.14: Acceptability for the softness of developed cream

Most the evaluators choose like very much for the softness of the cream A while a few of evaluators like very much the softness of the cream C. However, most of the evaluators choose like slightly the softness of the cream C. None of the evaluators choose neither dislike nor like for the softness of cream A and cream B.

4.4.1.5 Evaluation of Greasiness of the Developed Cream

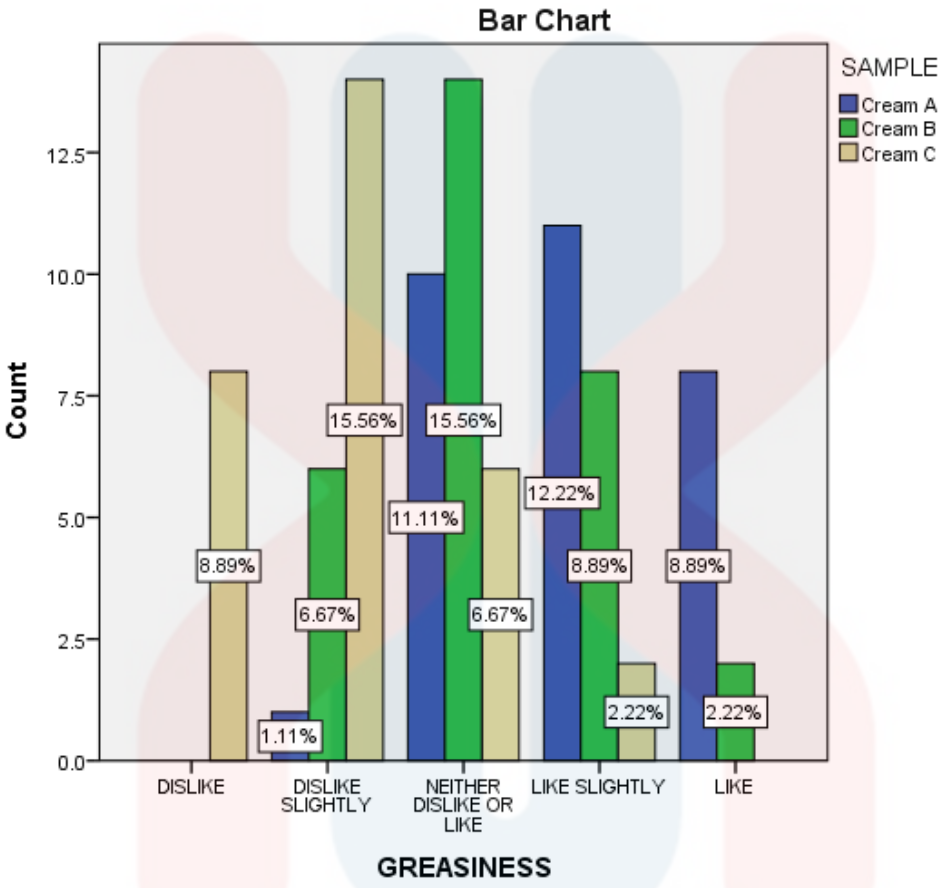


Figure 4.15: Acceptability for the greasiness of developed cream.

Most of the evaluators choose neither dislike nor like the greasiness of the cream B while most of the evaluators choose dislike slightly the greasiness of the cream C. Meanwhile, 12.22% of the evaluators choose like slightly the greasiness of the cream A. Cream C has 8.89% of the evaluators that dislike greasiness of the cream

4.4.1.6 Evaluation of Overall Acceptance of the Developed Cream

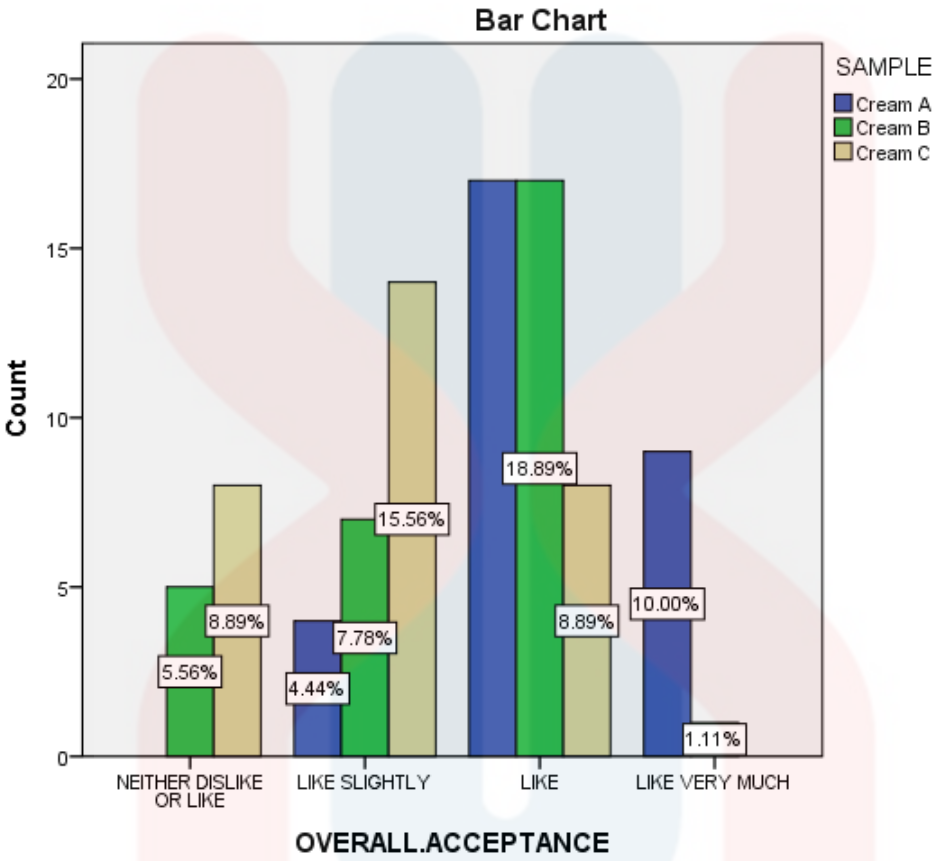


Figure 4.16: Overall acceptance of developed cream

Mostly of the evaluators choose like for the acceptance of overall attributes for cream A and cream B while a few of the evaluators like the acceptance of overall attributes for the cream C. Meanwhile, a few of the evaluators neither dislike nor like for the acceptance of overall attributes for cream C and none of the evaluator like very much for the acceptance of overall attributes for the cream C. Most of the evaluators like very much the acceptance of overall attributes for the developed creams.

4.4.2 Irritation Test

Irritation test was conducted among 30 amateur evaluators at UMK, Jeli Campus and evaluated qualitative by the observation of the irritation on the respondent's skin.

Table 4.4: Cross tabulation of the irritation test for developed cream

Irritation * Sample Cross tabulation					
Count					
		Sample			Total
		A	B	C	
Irritation	No	30	30	30	90
Total		30	30	30	90

Table 4.5: One way ANOVA of the irritation test of developed cream.

ANOVA					
Irritation					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	2	.000	.	.
Within Groups	.000	87	.000		
Total	.000	89			

There are no irritation were detected after applying the developed creams on the skin during the irritation test among 30 respondents. Irritation was described as the appearing of the redness after applying on the skin. The developed creams showed no irritation was observed as well as no redness detected after applying the developed creams on 30 respondent's skin.

In this study, the ingredients to develop antimicrobial cream using which are mineral oil, the waxes as an thickener, emollient, and the infused oil of *Cassia senna*. The olive oil used in this study because of the less expensive than others oil, last longer and have the aromatic fragrance. Then, petroleum jelly (Vaseline) used as thickener, emollient because it is an excellent emollient and lubricant but is also used as bodify agent to add viscosity to cosmetic products.

According to the Rajendra and Ram, (2011), the pH of the cream was found to be in range of 5-6 is good for skin. Based on the data shows that the pH value of developed creams have the suitable pH for the skin. As the percentage of concentration for the infused oil of *C. senna* increases, the pH value of developed creams increases.

In this study, the data of the colour analysis shows the concentration of the *C. senna* infused oil affects the colour analysis of the developed cream. The highest the concentration of the *C. senna* infused oil, the lowest the lightness value of the developed cream. In the same time, the higher the concentration of the *C. senna* infused oil, the higher the greenness of the developed cream while the lower the concentration of the *C. senna* infused oil, the highest the value of yellowness for the developed creams. This is because the amount of *C. senna* leaves in the infused oil affects the colour of developed creams.

The viscosity value refers the thickness of a fluid for the developed cream. The concentration for the infused oil of *C. senna* affects the viscosity value of the developed cream. The lower the percentage of concentration for the infused oil of *C. senna*, the lower the viscosity value. According Akash (2014), the viscosity of the developed cream should be in the range of 27001-27089 cps which is indicating that the cream is easily to spread by a small amount of shear. However, the data shows the viscosity of developed

creams in this study are among 1000 cps. This is because the addition of petroleum jelly influence the viscosity of the developed cream.

The data of the texture analysis shows the concentration of the *C. senna* infused oil affects the texture analysis of the developed creams. The higher the concentration of the *C. senna* infused oil, the higher the hardness of the developed cream. Based on the data, the lower the concentration of the *C. senna* infused oil, the higher the adhesiveness value of developed cream. However, the higher the concentration of the *C. senna* infused oil, the higher the cohesiveness value of developed creams.

The pH value of the developed creams that stored in three different temperature are decreasing within 3 weeks. Although the pH of developed creams decreasing, the pH value considered acceptable to avoid the risk of irritation upon application to the skin. In addition, data for the stability test of colour analysis shows that there a little difference within three weeks. This show the different temperature not affected the colour analysis of the developed cream. The data shows the different temperature affects the viscosity of the developed cream.

The texture analysis of the developed cream based on the hardness, adhesiveness and cohesiveness. In this study, the stability test is observed in 3 weeks because the texture and appearance of the water- in-oil creams can be affected easily because it is more greasier and moisturiser than the oil-in-water phase cream. The temperature affects the hardness of the developed creams. The developed creams not suitable to be placed in the high temperature and the suitable temperature to maintain the texture of the developed creams in the cool and room temperature. The duration of the stability test and the temperature affects the adhesiveness and cohesiveness of the developed creams. Then, the longer the duration to store the developed creams, the cohesiveness of the developed

creams decreasing. In this study, the developed cream A was more stable, whilst remaining developed creams were not stable. Although there were changes of attributes value of cream A in terms of pH, colour analysis, viscosity and texture analysis, there was still considered acceptable to avoid the irritation during application of the cream.

The antimicrobial activity of developed creams were tested against *E. coli* and *S. aureus*. According to the Sadiq et al (2016), the bacteria found to be active against the leaf extracts of this plant. The leaf extract of *C. senna* when tested against different pathogenic bacteria was found to be active against *S. aureus* while a negative effect was observed against *E. coli*. The data shows that there were inhibition zone of developed cream for cream A against *E.coli* but no inhibition zone among three developed creams against *S. aureus*. Furthermore, the different concentration of the *C. senna* infused oil can affects the antimicrobial activity of the developed creams.

The sensory evaluation of developed creams conducted among 30 amateur evaluators using the hedonic scale to determine the acceptability of the developed creams based on aroma, colour, texture, softness, greasiness and overall acceptance. In this section, cream A was more preferable in terms of aroma, colour, texture, softness, greasiness and acceptance for overall attributes of the developed cream compared with other two developed creams. This is because cream A has good aroma, a nice colour, good texture, more softness and less greasiness which the evaluator acceptance the overall attributes of the cream A. Cream C was the least preferable because has less aroma, not attractive colour, moderate texture, less softness and more greasiness which make the evaluator do not acceptance the overall attributes of cream C.

In this study, the developed creams do not used the chemical ingredients instead used olive oil and Vaseline which are safe to be apply on the skin. As Vaseline is the

popular product in the pharmaceutical industry and usually use to moisturise the skin. In the same time, olive oil also popular product in the food industry and safely can be consumed or apply on the surface of the skin. So, there no irritation were observed during the application of the developed creams on the respondents' skin as well as no redness were observed when applied the developed creams on the skin.

It can be summarized that the developed cream exhibited moderated quality and stability. Besides, it also showed moderate antimicrobial activity against *E. coli* and *S. aureus*. Sensory evaluation conducted among 30 evaluators revealed that good overall acceptance.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, the development of potential antimicrobial cream incorporated with *Cassia senna* infused oil has a good pH value that suitable for the skin which is 5. This includes the different concentrations of infused oil from *C. senna* affects the quality assessments of developed antimicrobial cream. Developed antimicrobial creams do not have any irritation toward the skin. The developed cream using *C. senna* infused oil shows good quality in terms of physical appearance based on aroma, colour and texture appearance. The evaluation of the quality and stability test of the developed cream incorporated with *C. senna* infused oil shows the moderate quality and moderate stability. The assess of antimicrobial activity of the developed cream incorporated with *C. senna* infused oil using disk diffusion shows the moderate antimicrobial activity against *E. coli* and *S. aureus.* The sensory acceptance of the developed cream using *C. senna* infused oil revealed that good overall acceptance.

5.2 Recommendations

For the future research of the *Cassia senna* plant, more products should have been developed and commercialise for the awareness of the peoples about the benefits a function of the *C. senna* in the pharmaceutical industry. This is because *C. senna* has more benefits in treat the constipation, treatment of skin infections and can ease the skin inflammation. The product using *C. senna* infused oil should have been develop and commercialise in the market industry.

In the future, the stability test for the developed cream should longer to obtain the good result because usually cosmetics product have expired in 3 months after. The developed creams incorporated with *C. senna* infused oil should have another alternatives who prefers the natural antimicrobial creams. Lastly, the evaluation of quality and stability test for the developed cream incorporated with *C. senna* infused oil should using Cronbach's alpha analysis to obtain the good results.

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APPENDIX

Table A1: the stability of the developed creams

The pH of cream A within 3 weeks

WEEK	TEMPERATURE	pH
WEEK 0	4˚C	5.66 ± 0.015
	25˚C	5.66 ± 0.015
	50˚C	5.68 ± 0.02
WEEK 1	4˚C	5.66 ± 0.01
	25˚C	5.66 ± 0.011
	50˚C	5.66 ± 0.005
WEEK 2	4˚C	5.65 ± 0.01
	25˚C	5.66 ± 0.038
	50˚C	5.68 ± 0.021
WEEK 3	4˚C	5.66 ± 0.025
	25˚C	5.66 ± 0.021
	50˚C	5.66 ± 0.015

The pH of cream B within 3 weeks

WEEK	TEMPERATURE	pH
WEEK 0	4˚C	5.44 ± 0.021
	25˚C	5.44 ± 0.021
	50˚C	5.46 ± 0.02
WEEK 1	4˚C	5.44 ± 0.012
	25˚C	5.43 ± 0.023
	50˚C	5.45 ± 0.012
WEEK 2	4˚C	5.43 ± 0.006

	25˚C	5.44 ± 0.035
	50˚C	5.42 ± 0.012
WEEK 3	4˚C	5.45 ± 0.017
	25˚C	5.43 ± 0.042
	50˚C	5.43 ± 0.042

The pH of cream C within 3 weeks

WEEK	**TEMPERATURE**	**pH**
WEEK 0	4˚C	5.22 ± 0.035
	25˚C	5.24 ± 0.035
	50˚C	5.25 ± 0.01
WEEK 1	4˚C	5.22 ± 0.006
	25˚C	5.26 ± 0.055
	50˚C	5.23 ± 0.023
WEEK 2	4˚C	5.2 ± 0.015
	25˚C	5.28 ± 0.025
	50˚C	5.21 ± 0.01
WEEK 3	4˚C	5.2 ± 0.006
	25˚C	5.28 ± 0.006
	50˚C	5.25 ± 0.023

The viscosity of cream A within 3 weeks

WEEK	**TEMPERATURE**	**VISCOSITY**
WEEK 0	4˚C	10.02 ± 3.33
	25˚C	13.03 ± 0.842
	50˚C	12.02 ± 1.531
WEEK 1	4˚C	18.63 ± 1.211
	25˚C	10.97 ± 0.26

	50˚C	9.14 ± 0.242
WEEK 2	4˚C	20.55 ± 0.913
	25˚C	10.15 ± 0.239
	50˚C	7.72 ± 1.114
WEEK 3	4˚C	25.3 ± 0.677
	25˚C	7.69 ± 0.737
	50˚C	5.66 ± 0.813

The viscosity of cream B within 3 weeks

WEEK	**TEMPERATURE**	**VISCOSITY**
WEEK 0	4˚C	10.21 ± 0.13
	25˚C	10.81 ± 0.57
	50˚C	9.94 ± 0.19
WEEK 1	4˚C	13.33 ± 1.08
	25˚C	10.04 ± 0.20
	50˚C	8.29 ± 0.49
WEEK 2	4˚C	16.21 ± 0.88
	25˚C	9 ± 0.24
	50˚C	5.30 ± 0.52
WEEK 3	4˚C	19.08 ± 0.87
	25˚C	7.59 ± 1.25
	50˚C	2.97 ± 0.87

The viscosity of cream C within 3 weeks

WEEK	**TEMPERATURE**	**VISCOSITY**
WEEK 0	4˚C	6.63 ± 0.37
	25˚C	6.9 ± 0.20
	50˚C	6.39 ± 0.42

WEEK 1	4˚C	11.30 ± 1.30
	25˚C	6.78 ± 0.17
	50˚C	3.80 ± 0.60
WEEK 2	4˚C	13.77 ± 0.31
	25˚C	5.67 ± 0.52
	50˚C	3.25 ± 1.02
WEEK 3	4˚C	17.90 ± 1.97
	25˚C	4.05 ± 0.27
	50˚C	1.78 ± 0.69

The lightness of the colour for cream A within 3 weeks

WEEK	TEMPERATURE	LIGHTNESS
WEEK 0	4˚C	27.70 ± 0.43
	25˚C	23.91 ± 2.18
	50˚C	25.55 ± 1.34
WEEK 1	4˚C	27.69 ± 0.14
	25˚C	22.61 ± 0.97
	50˚C	19.06 ± 1.15
WEEK 2	4˚C	26.62 ± 0.54
	25˚C	22.35 ± 1.44
	50˚C	15.35 ± 0.53
WEEK 3	4˚C	26.39 ± 0.48
	25˚C	21.19 ± 0.54
	50˚C	12.58 ± 2.13

The lightness of the colour for cream B within 3 weeks

WEEK	**TEMPERATURE**	**LIGHTNESS**
WEEK 0	4˚C	30.92 ± 0.77
	25˚C	31.59 ± 1.02
	50˚C	32.05 ± 0.56
WEEK 1	4˚C	30.37 ± 0.63
	25˚C	31.84 ± 0.85

	50˚C	30.60 ± 0.26
WEEK 2	4˚C	30.43 ± 0.56
	25˚C	31.55 ± 0.61
	50˚C	26.94 ± 1.57
WEEK 3	4˚C	30.07 ± 0.04
	25˚C	31.39 ± 0.43
	50˚C	22.42 ± 2.05

Table 4.8: The lightness of the colour for cream C within 3 weeks

WEEK	TEMPERATURE	LIGHTNESS
WEEK 0	4˚C	40.72 ± 0.13
	25˚C	41.61 ± 0.03
	50˚C	42.78 ± 0.40
WEEK 1	4˚C	40.61 ± 0.10
	25˚C	41.27 ± 0.37
	50˚C	40.61 ± 0.38
WEEK 2	4˚C	40.57 ± 0.10
	25˚C	40.6 ± 0.32
	50˚C	39.49 ± 1.12
WEEK 3	4˚C	40.53 ± 0.11
	25˚C	40.16 ± 0.15
	50˚C	39.26 ± 1.35

The greeness of the colour for cream A within 3 weeks

WEEK	TEMPERATURE	GREENESS
WEEK 0	4˚C	1.83±0.03
	25˚C	1.66±0.05
	50˚C	1.47±0.05

WEEK 1	4˚C	1.77±0.04
	25˚C	1.55±0.07
	50˚C	1.21±00.03
WEEK 2	4˚C	1.66±0.04
	25˚C	1.42±0.03
	50˚C	1.14±0.05
WEEK 3	4˚C	1.56±0.07
	25˚C	1.41±0.01
	50˚C	0.67±0.49

The greenness of the colour for cream B within 3 weeks

WEEK	**TEMPERATURE**	**GREENNESS**
WEEK 0	4˚C	1.06 ±0.05
	25˚C	0.95±0.03
	50˚C	1.05±0.05
WEEK 1	4˚C	0.98±0.08
	25˚C	0.86±0.03
	50˚C	0.81±0.10
WEEK 2	4˚C	0.89±0.09
	25˚C	0.72±0.08
	50˚C	0.66±0.08
WEEK 3	4˚C	0.84±0.07
	25˚C	0.55±0.12
	50˚C	0.43±0.08

The greenness of the colour for cream C within 3 weeks.

WEEK	TEMPERATURE	GREENNESS
WEEK 0	4˚C	0.47±0.02
	25˚C	0.58±0.05
	50˚C	0.60±0.11
WEEK 1	4˚C	0.43±0.01
	25˚C	0.45±0.07
	50˚C	0.39±0.07
WEEK 2	4˚C	0.41±0.01
	25˚C	0.46±0.06
	50˚C	0.40±0.03
WEEK 3	4˚C	0.39±0.01
	25˚C	0.37±0.09
	50˚C	0.39±0.02

The yellowness of the colour for cream A within 3 weeks.

WEEK	TEMPERATURE	YELLOWNESS
WEEK 0	4˚C	13.64±0.08
	25˚C	12.09±1.82
	50˚C	15.04±0.73
WEEK 1	4˚C	13.43±0.06
	25˚C	11.12±0.53
	50˚C	11.56±1.90
WEEK 2	4˚C	13.27±0.04
	25˚C	10.50±0.43
	50˚C	9.33±0.52
WEEK 3	4˚C	13.21±0.01
	25˚C	10.71±0.90

	50˚C	6.81±0.50

The yellowness of the colour for cream B within 3 weeks

WEEK	TEMPERATURE	YELLOWNESS
WEEK 0	4˚C	25.32±0.36
	25˚C	24.5±1.33
	50˚C	26.75±0.12
WEEK 1	4˚C	24.85±0.13
	25˚C	24.69±0.21
	50˚C	24.11±0.81
WEEK 2	4˚C	24.49±0.50
	25˚C	23.88±0.27
	50˚C	22.0±0.12
WEEK 3	4˚C	24.45±0.07
	25˚C	23.59±0.50
	50˚C	20.21±0.31

The yellowness of the colour for cream C within 3 weeks

WEEK	TEMPERATURE	YELLOWNESS
WEEK 0	4˚C	30.06±0.03
	25˚C	29.81±0.26
	50˚C	33.00±0.2
WEEK 1	4˚C	30.01±0.01
	25˚C	28.21±1.13
	50˚C	30.42±0.32
WEEK 2	4˚C	29.91±0.06
	25˚C	27.18±0.61
	50˚C	27.61±0.78

WEEK 3	4˚C	29.87±0.07
	25˚C	25.85±0.32
	50˚C	25.30±0.21

The hardness for cream A within 3 weeks

WEEK	TEMPERATURE	HARDNESS
WEEK 0	4˚C	1276.33± 43.41
	25˚C	1260 ± 10.58
	50˚C	1117.67 ± 107.58
WEEK 1	4˚C	1222± 36.76
	25˚C	1251 ± 9.54
	50˚C	1002.67 ± 4.62
WEEK 2	4˚C	1280.33±9.02
	25˚C	1262.33±34.36
	50˚C	986.33±11.50
WEEK 3	4˚C	1261.67±22.94
	25˚C	1220.33±15.31
	50˚C	960.67±27.15

The hardness for cream B within 3 weeks.

WEEK	TEMPERATURE	HARDNESS
WEEK 0	4˚C	1058±5.00
	25˚C	1058±14.00
	50˚C	1036±30.32
WEEK 1	4˚C	1041.67±21.03
	25˚C	1045.33±10.60
	50˚C	1029.67±28.57
WEEK 2	4˚C	1028.33±7.64

	25˚C	1074.33±10.02
	50˚C	928 ±61.65
WEEK 3	4˚C	1062±32.70
	25˚C	1068.33±9.29
	50˚C	853±54.84

The hardness for cream C within 3 weeks.

WEEK	**TEMPERATURE**	**HARDNESS**
WEEK 0	4˚C	1058±5.00
	25˚C	1058±14.00
	50˚C	1036±30.32
WEEK 1	4˚C	1041.67±21.03
	25˚C	1045.33±10.60
	50˚C	1029.67±28.57
WEEK 2	4˚C	1028.33±7.64
	25˚C	1074.33±10.02
	50˚C	928 ±61.65
WEEK 3	4˚C	1062±32.70
	25˚C	1068.33±9.29
	50˚C	853±54.84

The adhesiveness for cream A within 3 weeks

WEEK	**TEMPERATURE**	**ADHESIVENESS**
WEEK 0	4˚C	0.07±0.06
	25˚C	0.03±0.06
	50˚C	0.10 ±0.10
WEEK 1	4˚C	0±0
	25˚C	0.07±0.06

	50˚C	0.1±0.17
WEEK 2	4˚C	0.03±0.06
	25˚C	0±0
	50˚C	0.07±0.06
WEEK 3	4˚C	0±0
	25˚C	0±0
	50˚C	0±0

The adhesiveness for cream B within 3 weeks

WEEK	**TEMPERATURE**	**ADHESIVENESS**
WEEK 0	4˚C	0.1±0.1
	25˚C	0.13±0.06
	50˚C	0.07±0.06
WEEK 1	4˚C	0±0
	25˚C	0±0
	50˚C	0.03±0.06
WEEK 2	4˚C	0.07±0.06
	25˚C	0.03±0.06
	50˚C	0±0
WEEK 3	4˚C	0±0
	25˚C	0±0
	50˚C	0±0

The adhesiveness for cream C within 3 weeks

WEEK	**TEMPERATURE**	**ADHESIVENESS**
WEEK 0	4˚C	0.20±0.20
	25˚C	0.10±0.17
	50˚C	0.07±0.06

WEEK 1	4˚C	0.10±0.10
	25˚C	0.07±0.06
	50˚C	0.03±0.06
WEEK 2	4˚C	0.10±0.10
	25˚C	0.03±0.06
	50˚C	0±0
WEEK 3	4˚C	0.07±0.06
	25˚C	0±0
	50˚C	0±0

The cohesiveness for cream A within 3 weeks

WEEK	**TEMPERATURE**	**COHESIVENESS**
WEEK 0	4˚C	1.11±0.10
	25˚C	1.19±0.05
	50˚C	1.25±0.11
WEEK 1	4˚C	1.11±0.04
	25˚C	1.08±0.07
	50˚C	1.15±0.09
WEEK 2	4˚C	1.01±0.02
	25˚C	0.94±0.03
	50˚C	0.84±0.06
WEEK 3	4˚C	0.9±0.01
	25˚C	0.86±0.03
	50˚C	0.77±0.02

The cohesiveness for cream B within 3 weeks

WEEK	TEMPERATURE	COHESIVENESS
WEEK 0	4˚C	1.06±0.07
	25˚C	1.08±0.08
	50˚C	1.07±0.06
WEEK 1	4˚C	1.03±0.06
	25˚C	1.07±0.06
	50˚C	1.00±0
WEEK 2	4˚C	0.96±0.05
	25˚C	0.91±0.06
	50˚C	0.83±0.03
WEEK 3	4˚C	0.84±0.06
	25˚C	0.72±0.10
	50˚C	0.70±0.05

The cohesiveness for cream C within 3 weeks

WEEK	TEMPERATURE	COHESIVENESS
WEEK 0	4˚C	1.07±0.12
	25˚C	1.01±0.11
	50˚C	1.13±0.12
WEEK 1	4˚C	0.89±0.02
	25˚C	0.97±0.03
	50˚C	0.71±0.19
WEEK 2	4˚C	0.85±0.03
	25˚C	0.81±0.05
	50˚C	0.52±0.06
WEEK 3	4˚C	0.78±0.02
	25˚C	0.70±0.09

50˚C	0.46±0.09

Table A2: antimicrobial assay

Groups	Count	Sum	Average	Variance
Blank	3	56	18.66667	1.333333
Cream A	3	34	11.33333	2.333333

ANOVA for the zone inhibition on *E-coli*

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	80.66667	1	80.66667	44	0.00268	7.708647
Within Groups	7.333333	4	1.833333			
Total	88	5				

SENSORY EVALUATION FORM OF ANTIMICROBIAL CREAM

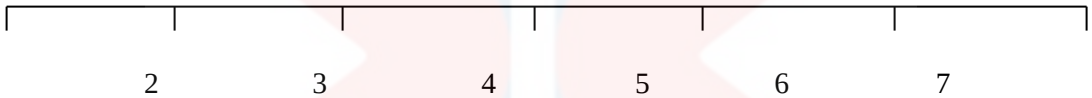
Panel No: Sample Code: Date:

Directions:
You are given some samples for test of attributes. Please state your degree of likeness based on the characteristics below at the mark ( ) provided. Circle which is appropriate. Do rinse your hand before and after testing each sample.

1. Colour

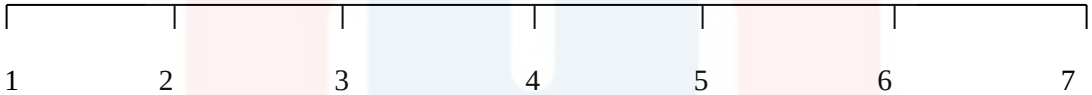
1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

2. Aroma



1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

3. Texture



1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

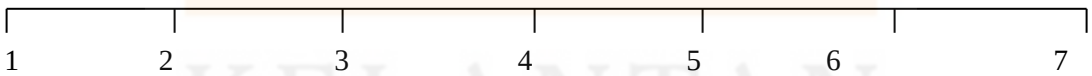
4. Softness

1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

5. Greasiness

1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

6. Overall acceptance



1 2 3 4 5 6 7
Dislike very much Neither like nor dislike Like very

CONSENT LETTER FOR THE IRRITATION TEST

Purpose of the irritation test: The primary purpose of this irritation test is to determine the effects of developed creams to the skin.

In consideration of your participation in this irritation test, you hereby agree to assume all risks of injury to yourself. You also understand that your results are intended to be used for educational purposes only and are not designed to replace the care or advice of a medical provider. If you have a disease condition, fall into certain high health risk categories, and/or receive abnormal laboratory tests, you should promptly consult with a physician and obtain his or her approval prior to engaging in any health improvement program or lifestyle change activity.

To agree to participate in this irritation test, please sign and date this consent and release. The process your questionnaire cannot be done unless you have signed and dated below. Thank you.

_____ _____

Signature of Participant Name of Participant

_____ _____

Date

Table A3: sensory evaluation of the developed creams

AROMA * SAMPLE Cross tabulation

		SAMPLE			Total
		A	B	C	
Aroma	Dislike	5	12	15	32
	Dislike Slightly	13	12	12	37
	Neither Dislike Or Like	12	6	3	21
	Total	30	30	30	90

COLOUR * SAMPLE Cross tabulation

		SAMPLE			Total
		A	B	C	
COLOUR	Neither Dislike Or Like	0	1	7	8
	Like Slightly	2	11	15	28
	Like	15	14	8	37
	Like Very Much	13	4	0	17
	Total	30	30	30	90

TEXTURE * SAMPLE Crosstabulation

Count

		SAMPLE			Total
		A	B	C	
TEXTURE	Neither Dislike Or Like	0	2	5	7
	Like Slightly	1	5	14	20
	Like	15	17	11	43
	Like Very Much	14	6	0	20
	Total	30	30	30	90

SOFTNESS * SAMPLE Crosstabulation

		SAMPLE			Total
		A	B	C	
SOFTNESS	Neither Dislike Or Like	0	0	2	2
	Like Slightly	2	7	10	19
	Like	16	16	15	47
	Like Very Much	12	7	3	22
	Total	30	30	30	90

GREASINESS * SAMPLE Cross tabulation

		SAMPLE			Total
		A	B	C	
GREASINESS	Dislike	0	0	8	8
	Dislike Slightly	1	6	14	21
	Neither Dislike Or Like	10	14	6	30
	Like Slightly	11	8	2	21
	Like	8	2	0	10
	Total	30	30	30	90

OVERALL.ACCEPTANCE * SAMPLE Crosstabulation

Count

		SAMPLE			Total
		A	B	C	
OVERALL.ACCEPTANCE	Neither Dislike Or Like	0	5	8	13
	Like Slightly	4	7	14	25
	Like	17	17	8	42
	Like Very Much	9	1	0	10
	Total	30	30	30	90