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Formulation and Evaluation of Antidiabetic Bio-cream Incorporated with Aqueous Extract *Albizia myriophylla Benth* (*A. myriophylla*)

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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 submitted for a higher degree to any universities or institutions.

Student

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Date: 28th February 2024

I certify that the report of this final year project entitled "Formulation and Evaluation of Antidiabetic Bio-cream incorporated with *Albizia myriophylla* (*A.myriophylla*) Aqueous Extract" by Siti Nur Dini binti Mazli, matric number J20A0620 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Bioindustrial Technology) with Honours, Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan.

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Supervisor

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ABSTRACT

The use of plants for medicinal purposes and the development of natural products is widespread, but ensuring the quality of these natural products remains a challenge. This research focuses on the extract of *Albizia myriophylla Benth*, which is traditionally used by the community in Pasir Mas, Kelantan, Malaysia, which is believed to be effective in preventing diabetes or lowering blood or body sugar levels among diabetic patients. This study mainly aimed to quantify the bioactive compounds in *A. myriophylla* and investigate its glucose-related properties while promoting skin benefits. In addition, it studied topical cream formulations combining *A. myriophylla* extract and natural ingredients, evaluating their quality through various parameters including organoleptic and physicochemical characteristics, antibacterial activity, microbial count, stability, and sensory evaluation, as well as skin irritation tests. The results showed that the formulated cream had good physical properties with a pleasant smell and exhibited antibacterial activity against *E. coli* and *S. aureus*, without the presence of colonies observed. A month-long stability test revealed that the cream remained stable under cold storage and room temperature, with no significant changes in odor, texture, or pH. User tests showed a high preference for the cream among respondents, with no skin irritation reported. Further research is warranted to evaluate the effectiveness of the cream in its antihyperglycemic and wound healing activities, potentially contributing to future developments in diabetes prevention and skin protection.

Keywords: Topical herbal cream, Quality assessment, *Albizia myriophylla Benth* aqueous extract, glucose-related properties, antibacterial activity.

ABSTRAK

Penggunaan tumbuhan untuk tujuan perubatan dan pembangunan produk semula jadi adalah meluas, namun memastikan kualiti produk semula jadi ini kekal sebagai suatu cabaran. Penyelidikan ini memfokuskan kepada ekstrak *Albizia myriophylla Benth*, yang digunakan secara tradisional oleh komuniti di Pasir Mas, Kelantan, Malaysia yang dipercayai mempunyai keberkesanannya dalam mencegah diabetes atau penurunan kadar gula dalam darah atau badan dalam kalangan pesakit diabetes. Kajian ini terutamanya bertujuan untuk mengukur sebatian bioaktif dalam *A. myriophylla* dan menyiasat sifat berkaitan glukosanya sambil menggalakkan manfaat kepada kulit. Selain itu, ia mengkaji formulasi krim tropikal yang menggabungkan ekstrak *A. myriophylla* dan bahan-bahan semula jadi, menilai kualitinya melalui pelbagai parameter termasuk ciri organoleptik dan fizikokimia, aktiviti antibakteria, kiraan mikrob, kestabilan dan penilaian deria, serta ujian kerengsaan kulit. Keputusan menunjukkan bahawa krim yang diformulasikan mempunyai sifat fizikal yang baik dengan bau yang menyenangkan dan mempamerkan aktiviti antibakteria terhadap *E. coli* dan *S. aureus*, tanpa kehadiran koloni diperhatikan. Ujian kestabilan selama sebulan mendedahkan bahawa krim kekal stabil di bawah penyimpanan sejuk dan suhu bilik, tanpa perubahan ketara dalam bau, tekstur atau pH. Ujian pengguna menunjukkan keutamaan yang tinggi untuk krim di kalangan responden, tanpa kerengsaan kulit dilaporkan. Penyelidikan lanjut adalah wajar untuk menilai keberkesanan krim dalam aktiviti antihiperglisemik dan penyembuhan luka, yang berpotensi menyumbang kepada perkembangan masa depan dalam pencegahan diabetes dan perlindungan kulit.

Kata kunci: Krim herba topikal, Penilaian kualiti, ekstrak *Albizia myriophylla Benth*, sifat berkaitan glukosa, aktiviti antibakteria.

CHAPTER 1

INTRODUCTION

1.1 Background Study

Rooted in ancient civilizations, herbal plants have historically played a vital role in treating illnesses and crafting natural remedies, as evidenced by the enduring practices of traditional Chinese medicine and Ayurveda in India (Romeu & Rosa, 2007). With the World Health Organization reporting that 80% of people globally rely on herbal medicines for primary healthcare (World Health Organization, n.d.), the therapeutic potential of these natural remedies is gaining recognition. The escalating global prevalence of diabetes mellitus poses a significant public health concern, with Malaysia, classified as an upper-middle-income country, experiencing a substantial economic burden of around 600 million US dollars annually due to the high prevalence of diabetes (Kurubaran Ganasegeran et al., 2020). Despite advances in diabetes management, the prevalence of this chronic metabolic disorder has witnessed a significant increase in Malaysia, underscoring the need for innovative and effective interventions (NHMS, 2019). One promising avenue for exploration is the potential therapeutic use of *Albizia myriophylla*, a medicinal plant native to Malaysia. A study conducted in Pasir Mas revealed that the bark of *Albizia myriophylla*, in combination with virgin coconut oil, demonstrated promising results in reducing sugar levels in individuals with diabetes (Saat et al., n.d.). While these findings highlight the potential of *Albizia myriophylla* in diabetes management, there remains a critical gap in the literature concerning the extent of its hyperglycemic control. Against this backdrop, this study aims to contribute to the existing body of knowledge by investigating the efficacy of *Albizia myriophylla* bark extract in formulating a bio-derived cream, offering a novel approach to diabetes management, and bridging the current knowledge gap in this field.

1.2 Problem Statement

The increasing global prevalence of diabetes presents a substantial health challenge, prompting the need for innovative management approaches. Despite strides in conventional treatments, there is a growing demand for alternative therapies with fewer side effects. The research endeavors to address this challenge by studying and formulating a bio cream derived from herbal plants, specifically *Albizia myriophylla* (*A.myriophylla*), for antidiabetic purposes. The formulation of a bio cream as an alternative therapy responds to concerns regarding potential side effects or limitations associated with conventional diabetes medications. By leveraging the diverse therapeutic properties of *A.myriophylla*'s bioactive compounds, the bio cream aims to offer localized benefits, improving skin health, while also potentially exerting systemic effects. Despite the rich bioactive composition of *A.myriophylla*, its application in skincare formulations targeted at diabetic patients remains underexplored. Recognizing the skin as an accessible and effective route for drug administration, the study seeks to understand the potential of *A.myriophylla's* aqueous extracts in bio cream formulation, with a focus on inherent antidiabetic properties. A comprehensive investigation into the efficacy, safety, and optimization of formulations derived from *A.myriophylla* aqueous extracts becomes imperative to bridge existing gaps and address the unmet needs of diabetic patients seeking skincare solutions aligned with their health condition. The quest for safer, effective, and more holistic approaches to diabetes management aligns with the perception that herbal remedies, with potentially fewer side effects than synthetic drugs, offer a gentler alternative. Thus, the proposed research on formulating a bio cream derived from herbal plants for antidiabetic purposes aims to contribute to a more tolerable and potentially safer skincare regimen for individuals managing diabetes.

1.3 Expected Outcomes

An optimized bio-cream may be successfully developed using natural ingredients and *A.myriophylla* aqueous extract. The optimized cream exhibits good organoleptic and physicochemical characteristics. The total number of colonies forming unit (cfu) found on the optimized cream complies with the value of National Pharmaceutical Control Bureau and British Pharmacopoeia standard. Antibacterial activity of the optimized cream shows significant inhibition zone against *E. coli* and *S.aureus*. The optimized cream exhibits good

stability at 4°C and room temperature (20–22°C) over a one–month storage period. The cream formulated has high degree of preference and acceptance among respondents and does not cause any skin irritation reaction.

1.4 Objectives

The objective of this research comprises of the following:

i. To study the optimum formulation of bio-cream using natural ingredients incorporated with *A. Myriophylla* aqueous extract.
ii. To determine the organoleptic, physicochemical characteristics and antibacterial property of the formulated bio-creams.
iii. To study the glucose reduction between *A.Myriophylla*.

1.5 Scope of Study

The study's objective was to create a bio cream in the lab at Universiti Malaysia Kelantan using an aqueous extract of *A. myriophylla*. The following are included in the project's scope: sample preparation and collection. After utilizing aqueous extraction of sample, modifications were made to the bio cream formulation process until a desired texture and set of sensory characteristics was achieved. Then, physicochemical examination of the bio cream formulation was assessed in accordance. The cream formulation was then evaluated and classified for its fundamental organoleptic qualities, such as color, fragrance, pH, and instant stickiness and greasiness. The major methods for identifying these traits were visual inspection, scent, and touch. Once the desired product has been obtained, an antimicrobial test against *E. coli* and *S. aureus* was determined by measuring the diameter of zone of inhibition. Besides the total plate count was conducted to determine the total colonies forming units (cfu/g) of the optimized. Next, an accelerated stability test was conducted to determine the physical stability of formulated cream under different storage conditions at refrigerator at 4°C and room temperature (20–22°C). The evaluation was performed on every 0^{th}, 10^{th}, 20^{th}, and 30^{th} will be performed with respect to the physicochemical parameters. Lastly, sample testing was

performed randomly on 40 respondents for the evaluation of sensorial properties and skin irritation reaction. The entire research was conducted for 4 months and 8 days.

1.6 Significance of Study

Hypoglycaemic effect in *A.myriophylla* has been proven scientifically in both vivo, where The aqueous bark extract of *Albizia myriophylla* and virgin coconut oil has anti diabetic activity as it lowers serum glucose levels in diabetic rats and significantly increases glucose tolerance (Saat et al., n.d.), additionally there is also one study showing that it has α-glucosidase inhibitory activity that were done through in vitro investigations (Tanasorn Tunsaringkarn et al., 2007). However, the potential of this plant (*Pokok Tebu Gajah*) has yet to be exploit in the development of product. Coupled with that, this research was emphasizing on formulation of bio-cream using natural ingredients and aqueous extract bark of *A.myriophylla*. On top of that, the present research also demonstrated scientific documentation on the preparation of bio-cream, assessment parameters and qualitative informative for better assurance on quality, safety, and consistency of the product. Optimistically, the present study contributed to novel uses of *A.myriophylla* plant by bringing the value of a medicinal herbs as well as references into a potential product.

CHAPTER 2

LITERATURE REVIEW

2.1 Albizia myriophylla Benth (A.myriophylla)

The *Albizia myriophylla Benth*, often known as *A.myriophylla*, or *Pokok Tebu Gajah* has a wide range of habits, including scrambling shrub and tenacious climbing plant. Grows largely in the wet tropical habitat and is armed with hook-like prickles up to 5mm long on the branchlets. Most species have a tree-like habit, but a select few, like *A. myriophylla*, are armed lianas. These species are frequently confused with lianescent *Acacia* species and *Albizia Chinensis* species, but they can be distinguished by the stems, which have bipinnate (twice divided), 15–20 cm long, bright green leaves. The plant species of *A. myriophylla* were illustrated in Figure 2. as an example.

(a) (b) (c)

Figure 2: Plant species of (a) *A. myriophylla*; (b) *Albizia Chenisia;* (c) *Acacia sp.*

Source: Google Image

2.2 Ethnobotanical Studies

Albizia myriophylla, locally known as "Cha-em Thai", commonly used in the Thai traditional medicine as antitussive (root), tonic (wood), digestant (flower), menstrual stimulant (leaves), expectorant (wood and root), and demulcent (wood and root) (Medical Registration Division 1998). According to few research studies claimed that *A. myriophylla* is one of the powerful medicinal plants that can be utilised either by itself or in conjunction with additional therapeutic plants in different herbal formulations in natural cures and traditional medicine to treat various illnesses and relieve discomforts. *A. Myriophylla*, according to reports Nazneen Bakasatae et al., 2018, is a common medicinal plant used as a folk medicine treatment for a variety of maladies in Thailand and other Asian nations. Additionally, based on recent studies have investigated the antidiabetic potential of Albizia myriophylla, revealing promising hypoglycemic effects in both in vivo and in vitro settings (Saat et al., n.d.; Tanasorn Tunsaringkarn et al., 2007). Ethnobotanical studies of *A. myriophylla* were summarized in Table 2.1.

Table 2: Certain popular uses of *A. myriophylla* in traditionally and medicinal uses

A. myriophylla uses	
Traditional uses	• An internal infusion of *A. myriophylla* roots is used to treat fever in Malaysia. • For youngsters with fever, a lotion produced from the roots is applied together with other botanicals. • An internal infusion of *A.myriophylla* bark is used to treat or prevent diabetic, commonly used by a small segment in Malaysia. • To treat earaches, a lotion is made by boiling the leaves. • The leaves are applied to wounds to stop bleeding in traditional Indo-Chinese medicine, while the bark is used to treat bronchitis and cough. The bark can also be used in place of liquorice. • Traditional Thai herbal, the wood component is traditionally used to cure fever, sore throat, and aphthous ulcers as a single herbal medicine in the form of an aqueous infusion.
	• *A. myriophylla* stem extract in methanol shown strong antifungal activity against six pathogenic *Candida* species, including *Candida*

Medicinal uses	*albicans, Candida glabrata, Candida guilliermondii, Candida krusei, Candida parapsilosis*, and *Candida tropicalis*.

Sources: *Albizia Myriophylla · MYETHBO* (2023); Surasak Limsuwan et al., (2017); Saat et al., n.d

2.2.1 Phytochemical Studies

Diabetic individuals will not be harmed if they consume *Tebu Gajah* because it contains saccharin, which has antidiabetic properties. Saccharin is commonly recommended as a sugar alternative for diabetics. This is because it is not digested by the human organism and does not have the same effect on blood sugar levels as refined sugar. Thus, consuming saccharin-containing plants, despite their high sugar content, is safe for diabetics. Nazneen Bakasatae et al. (2018) found that the ethanolic wood extract of *A.myriophylla* has significant antioxidant activity against DPPH, with an IC50 of 46.23 ± 0.11 µg/ml. Furthermore, *A. myriophylla* has been explored for its antidiabetic potential. Sa'at (2011) conducted a study on the hypoglycemic effects of *A. myriophylla* in diabetic rats produced by streptozotocin and nicotinamide. The study discovered that the aqueous bark extract of *A. myriophylla* has anti-diabetic properties and reduced serum glucose levels in diabetic rats. The extract was also discovered to have hepatoprotective and nephroprotective properties, as it regulated blood glucose levels while lowering liver enzyme and kidney function test levels. On another study, also investigated the pharmacological effects of *A. myriophylla* and its active components, the report said that *Albizia myriophylla* has been proven to have anti-inflammatory properties in a variety of animal models, including mice and rats (Zhang et al. (2021); Azmah Saat et al., (2020). The plant has been shown to inhibit the formation of inflammatory cytokines, reducing inflammation in the body. The report also highlighted *A. myriophylla's* active components, including flavonoids, which have been demonstrated to have anti-inflammatory properties. The researchers then proposed that *A. myriophylla* and its active components could be employed as a treatment for inflammatory illnesses.

2.3 Preparation of Plant Extraction

Krakowska-Sieprawska et al. (2022) presents recent advances in methods of pre-treatments of plant material for the extraction of secondary metabolites with high biological activity. The study highlights that the correct preparation of the material for extraction is as important as the selection of the extraction method. This step should prevent the degradation of bioactive compounds as well as the development of fungi and bacteria. The authors suggest that the methods of preparation are expected to modify the articles of the plant materials in such a way that will contribute to the release of bioactive compounds loosely bonded to cell wall polymers. The influence of the methods on the structure of plant material particles, the level of preserved bioactive compounds, and the possibility of their release during the extraction were highlighted. On the other hand, different solvents are available to extract active biochemical compounds such as polar and non-polar solvents. Primarily, the choice of solvents and extraction methods should consider the yield of extraction and the targeted phytochemical compounds in particular plant materials to be obtained.

2.4 Decoction

Decoction is a conventional process of plant extraction that requires boiling plant material in a solvent to extract desired components. This approach is especially useful for extracting substances from harder plant parts like roots, seeds, and bark. In decoction, the plant sample is ground and soaked in a closed container of solvent. The soaking plant material is heated, resulting in a quicker extraction time. Nurhan Uslu et al. (2022), investigated the effects of brewing methods (decoction and infusion) and time on antioxidant activity, caffeine concentration, and phenolic components in coffee brews. The study discovered that coffee brews made using the decoction method had more total phenolic, total flavonoid, and total tannin than those made using the infusion method. Another study optimized the extraction conditions of phenolic components from *Cymbopogon citratus* (lemongrass) using the decoction method. The study discovered that the decoction extraction process was successful at recovering phenolic components from *C. citratus* leaves. These findings indicate that the decoction method may effectively extract phenolic chemicals from a variety of plant materials.

However, the method's performance is dependent on several criteria, including plant part selection, solid-liquid ratio, pressure, temperature, and process time (Smith, J et al., 2020).

2.5 Glucose Removal Test

The reasoning of conducting glucose removal tests with plant extracts derives from the search for alternative or supplementary treatments for diabetic mellitus. Traditional medicinal plants have long been recognized as having potential therapeutic effects, including antidiabetic characteristics to unravel the mechanisms of action of these plants through in vitro tests, particularly in terms of influencing glucose metabolism. Understanding how these plant extracts affect glucose dispersion provides vital information about their potential as complementary therapy or dietary supplements for diabetics. Several studies have delved into the exploration of traditional antidiabetic plants and their impact on glucose diffusion in vitro. In a study conducted by A.M. Gallagher*, P.R et.al, (2002), the potential of various plants as dietary supplements for enhancing blood glucose control and mitigating long-term complications in type 2 diabetes mellitus was investigated. This study focused on ten aqueous plant extracts known for their antihyperglycemic properties. Utilizing an in vitro method, the researchers assessed the effects of these extracts, each at a concentration of 50 g, on glucose diffusion. Remarkably, they observed a reduction in the movement of glucose levels in response to *Agrimony eupatoria* (agrimony) and *Persea americana* (avocado) extracts.

2.6 Formulation of Bio-cream

Water and oil-based ingredients are commonly employed in cosmetic compositions. Animal fats and vegetable oils are either natural or created are being used in cream formulation (Nooratiqah Azmi et al., 2022). Topical cream formulation components are dissolved or distributed in a water-in-oil (W/O) or oil-in-water (O/W) mixture (Mayba & Gooderham, 2017). Water-in-Oil (W/O) emulsions have a dispersed phase and use oil as a dispersive medium. They are also more humidifying because they form an oily barrier against water loss from the skin's outermost layer of the ocular stratum. Creams have a thicker consistency and act as a barrier to keep skin hydrated, making them useful for treating and preventing dry,

cracked skin while keeping it supple and moisturized. Bhatia et al. (2019) provide an in-depth study of the formulation of bio-based creams, which are becoming increasingly popular due to their eco-friendliness and biodegradability. The authors propose that bio-based creams be formulated using natural ingredients such as plant extracts, essential oils, and waxes. The article discusses a variety of natural substances utilized in the production of bio-based creams, such as aloe vera, chamomile, lavender, and tea tree oil. The authors also cover the numerous methods for making bio-based creams, such as emulsion, microemulsion, and solid lipid nanoparticles. The review emphasizes that the formulation of bio-based creams is a complex process requiring careful consideration of the type and concentration of ingredients, the technique of production, and the stability of the finished product.

2.7 Acid value and Saponification value

According to a review paper by Mawazi et al. (2022), the acid value and saponification value are two essential indicators used to assess the quality of cosmetics. The acid value is defined as the amount of potassium hydroxide (KOH) required to neutralize fatty acids in a sample, whereas the saponification value is the amount of KOH required to saponify esters and free fatty acids in a sample. Saraf et al. (2011) found that the acid value of a cream formulation ranged from 5.98 to 14.21, while the saponification value ranged from 23.27 to 33.25. The saponification value is a measurement of the amount of free fatty acid esters in a sample, which affects the formulation's stability, pH, and cleaning properties. The relationship between acid value (AV) and saponification value (SV) in a cream formulation indicates the composition and quality of the product's fats and oils. In general, a positive correlation is expected, implying that higher acid levels may indicate a higher concentration of free fatty acids, maybe due to incomplete saponification or the presence of more hydrolyzed fats. This favorable relationship demonstrates the consistency of the fats and oils used in the cream. For example, a cream with high acid and saponification values may have been made with certain oils or fats with precise molecular qualities. As a result, formulators can use this correlation to make better decisions about formulation adjustments. Other factors to consider include fatty acid content, fat supply, and processing methods, all of which might affect the link between AV and SV. Regular monitoring and analysis of these characteristics helps with quality control, maintaining consistency and high standards in cream compositions in the industry.

2.8 Cronbach's Alpha

Reliability is an important consideration when evaluating measurement instruments. Cronbach's alpha is a useful metric for investigating the internal consistency of questions. In other words, it describes the extent to which all test items measure the same notion (Miller, 2010; Moran, 2018). Cronbach alpha values of 0.7 or higher suggest adequate internal consistency (Taber, 2017). It is typically used in conjunction with a survey or questionnaire that has many Likert scale questions. The dependability coefficient's values vary from 0 to 1. A high Cronbach's alpha value near to "1" indicates good internal consistency of scale items and the generation of reliable findings. An alpha value greater than 0.8 is thought to indicate strong reliability.

Table 2.1. Cronbach's alpha rule of thumb.

Cronbach's alpha	Internal consistency
$\alpha \geq 0.9$	Excellent
$0.9 > \alpha \geq 0.8$	Good
$0.8 > \alpha \geq 0.7$	Acceptable
$0.7 > \alpha \geq 0.6$	Questionable
$0.6 > \alpha \geq 0.5$	Poor
$0.5 > \alpha$	Unacceptable

Source: Moran, (2018)

2.9 Sensory Evaluation

Sensory evaluation is a scientific yet subjective method for identifying a product's characteristics, particularly using the human senses. Color, odor, touch, and texture are typically measured. This method is used in a variety of industries, including food, cosmetics, personal care items, and textiles, to evaluate product quality and acceptance. The evaluation can be analytical, with an effective test aiming to identify detectable differences and provide full descriptions of the samples. Affective testing, commonly known as "consumer testing," is

the process of determining acceptance or preference among untrained panels. In affective testing, the primary goal is to obtain ratings of like. Due to its simplicity and ease of use, hedonic scales are frequently employed to quantify the extent or severity of liking and disliking, making them ideal for use with untrained consumer panels. As a result, consumer testing is an important part of product development and marketing decisions to better fulfil consumer needs.

2.9.1 Skin Irritation Testing

Skin irritation tests are an important aspect of chemical and product safety studies. Skin testing for drug or chemical allergies is routinely used to assess the potential negative effects of consumer products such as cosmetics and pharmaceuticals. According to the journals studied, skin irritation is likely caused by the presence of synthetic chemicals, added scents, and preservatives (Robinson & Perkins, 2002). Mild itching, rashes, redness, and swelling of the skin are all possible reactions to an allergy test. Previously, many of these skin tests required the use of experimental animals. However, new best practices for skin corrosion and skin irritation testing and risk assessment are being developed, which eliminates the requirement for animal test techniques. As a result, it must be determined by the degree of reaction (see Tables 2.2 and 2.3). Primary Irritation Index (PII) is a useful measure for evaluating skin irritation in laboratory tests. PII is a numerical value that represents a substance's total skin irritation potential. It is commonly used in dermatological investigations and safety evaluations to measure skin irritation and compare the irritation potential of various compounds.

Table 2.2: Evaluation of skin reaction

Skin reaction	Degree of reaction	Score
Erythema (redness) formation	No erythema	0
	Very slight erythema	1
	Well-defined erythema	2
	Moderate to severe erythema	3
	Severe erythema (best redness) to slight eschar information	4
	No edema	0

Edema (swelling) formation	Very slight edema (barely perceptible)	1
	Wel-defined edema (edges of are well delineated by definite swelling)	2
	Moderate to severe edema (raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Table 2.3: Primary irritation index and irritation classification.

Primary Irritation Index	**Irritation Classification**
0 - 0.9	Non-irritant
1 - 1.9	Mild
2 - 4.9	Moderate
5 - 8	Severe

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

The total 20 pieces of bark part of plant sample (*A. myriophylla*) was successfully obtained from on August 30, 2023, at Ayer Lanas, Kelantan, Malaysia.

3.1.2 Apparatus

Laboratory apparatus used in this research were beaker (10 ml, 50 ml, 100 ml and 500 ml), measuring cylinder (5 ml), volumetric flask (50ml), spatula, thermometer, filter paper, inoculating loop, Bunsen burner, pipette tips, glass petri dish, sterile petri dish, conical shake flasks, parafilm, forceps, media bottle (500 ml, 1000 ml), round bottom flask, gloves and masks.

3.1.3 Equipment

Equipment used in this research were oven, blender, lamina flow cabinet, electronical analytical balance, autoclave machine, refrigerator, stirring hotplate, digital pH meter, incubator, homogenizer, spectrophotometer. Nevertheless, activity involved in analysing bioactive compounds in plant aqueous of *Albizia myriophylla* using Hewlett Packard 6890 series Gas Chromatograph were conducted in University Sains Malaysia, Kelantan.

3.2 Methods

3.2.1 Preparation of Plant Sample

The plant sample *Pokok Tebu Gajah* (bark part of *A. Myriophylla*) was collected from Ayer Lanas, Kelantan and washed thoroughly to remove soil sediments and foreign particles. The plant was cut into small pieces and dried using oven at 90ºC for 24 hours. Upon complete drying, the dried pieces of bark sample were cut into smaller pieces by grinder machine. Then, the sample was grinded into powder using a heavy blender and sieve and kept in zip lock bag for subsequent aqueous extraction.

3.2.2 Extraction of Plant Sample using Decoction

A. Myriophylla powder was decocted with sterile distilled water in a 1:20 ratio. Specifically, 30 g of the sample was mixed with 600 ml of sterile water, heated to 60ºC, and stirred continuously for an hour using a stirring hotplate. The mixture was then filtered via filter paper until the desired amount of aqueous extract was achieved. To prevent microbial growth, a few drops of Euxyl PE9010 were added to the solution and stored at 4ºC.

3.2.3 GC-MS Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted using a Hewlett Packard 6890 series Gas Chromatography equipped with a 5973N Mass Selective Detector and controlled by the Chemstation Data System. The ionization mode employed was Electron Impact at an energy level of 70eV. The acquisition mode utilized was Full Scan. A HP-5 crosslinked 5% phenyl methyl siloxane fused silica capillary column with dimensions of 30x0.25mm x 0.25µm film thickness was employed for separation. The oven temperature was programmed to initially maintain at 50 ºC for 5 minutes, followed by a linear increase at a rate of 25 ºC/min until reaching 300 ºC, where it was held for an additional 10 minutes. The injection port and interface temperature were set at 280 ºC. Helium was used as the carrier gas, and the injection mode was spitless with a sample volume of 1µL.

3.2.4 Glucose Removal Test

In the present study, we want to study the glucose reduction by *A. myriophylla* by preparing a stock solution of 50 mg/mL of glucose. Then, glucose solution from stock solution is placed according to the concentration set each in the ten centrifuge tubes respectively. Next, all samples were measured for absorbance using a spectrophotometer at 540nm which is set as the wavelength for glucose. Then, all data were collected to plot a standard curve to be used as a reference. To see the reduction rate of *A. myriophylla* to glucose is to prepare four types of concentration of glucose (20 - 80%) in different 250 ml beakers. Then, 10 g of sample powder were put into each beaker and left for 2 hours and data was taken and diluted with 1µl DNS reagent and was placed in a spectrophotometer at 540nm as the set wavelength. The sample then were tested by performing Benedict Test can serve as a confirmation of those results. This helps to ensure the accuracy and reliability of your findings. The steps are repeated for 10 ml of plant aqueous extract to see the level of effectiveness between powder and liquid acting on glucose. Then, all results will be put into a table, to make comparation and study of reduction of glucose more effective between solid raw and water extract of *A.myriophylla*.

3.2.5 Formulation of Finding Base

Table 1 the formulation of bio-cream to find a suitable and desired texture of bio-cream before proceeding to formulate a bio-cream with *A.myriophylla* aqueous extract. As the present study, a formulation of cream was adopted with slight modification that consists of both oil and aqueous phase (Handali et al., 2011). The composition of the formula as to produce a 100 g were presented in Table 3.

Table 3. Amount of formulation of base bio cream

Phase	Ingredients	Amount of ingredients (%)			
		F1	F2	F3	F4
Oil	Emulsifier wax	8	10	15	20
	Jojoba oil	15	10	10	10
	Coconut Oil	10	15	15	15

	Glycerin	5	5	5	5
Aqueous	Euxyl PE 9010	2	2	1	1
	Distilled water	60	58	54	49

Initially, both the oil phase and water phase ingredients were weight, mixed and heated to 60^{o}C using water bath and stirred until become a homogenous. Upon heating, both phases were all, owed to cool down, the oil phase was then slowly added to the aqueous phase and stirred well. The stirring using homogenizer at 2500 rpm process was continued for until a consistent emulsion was formed. The cream modifications were done according to calculation and formulae (Table 3) until a desirable texture and appearance properties were obtained. An optimized cream was chosen for further formulation of bio-cream with *A. myriophylla* aqueous extract (see Table 3.1).

Table 3.1: The composition and amount of ingredients used to make 100g of bio-cream incorporated with *Albizia myriophylla* plant aqueous.

Phase	**Ingredients**	**Amount of ingredients (%)**			
		F5	**F6**	**F7**	**F8**
Oil	Emulsifier wax	10	10	10	10
	Jojoba Oil	15	15	15	15
	Coconut Oil	10	10	10	10
	Glycerin	5	5	5	5
Aqueous	Euxyl PE 9010	1	1	1	1
	Plant extract	20	40	60	80
	Distilled water	34	19	-	-

Using varied quantities of aqueous extract *A. myriophylla* in cream compositions serves several goals in research and development. For starters, it enables the optimization of active ingredient concentrations, making it easier to determine the most effective dosage to accomplish desired therapeutic benefits such as antidiabetic, antihyperglycemic or wound-healing qualities. Second, varied concentrations allow for the examination of the dose-response

relationship, which provides information about how the cream's efficacy changes with different active component concentrations. Furthermore, testing multiple doses helps assess the cream's safety profile by determining the concentration that balances best efficacy with the lowest likelihood of adverse reactions or skin irritation. Furthermore, experimenting with different concentrations helps to address formulation difficulties by optimizing physical attributes like as viscosity, texture, and stability. Overall, this approach allows for the fine-tuning of cream formulations for optimal efficacy, safety, and ideal physical properties, which will guide future development and eventual clinical application.

3.2.6 Organoleptic and Physicochemical Analysis of Formulated Cream

The created cream (F5, F6, F7 & F8) was evaluated and characterized for fundamental organoleptic features such as appearance, color, odor, and immediate after-feel including stickiness and greasiness. These traits were mostly assessed by visual inspection, scent, and touch.

Irritancy tests were performed on a marked area (1 sq.cm) of the left-hand dorsal surface. The cream was applied to the indicated area and time was recorded. When the prepared cream does not cause redness, irritation, or inflammation during the test, it is safe to use.

The pH of the optimized cream was measured with a digital pH meter. Prior to the analysis, the pH meter was calibrated by weighing 0.5 g of cream, dissolving it in 50 ml of pure water, and then measuring its pH.

3.2.7 Stability Test of Formulated Cream

Stability test studies are carried out as International Council for Hominization (ICH) guidelines. The 20 g of cream is filled in the petri dish and kept in under two different storage conditions at 4^{o}C (chiller), and 23 ± 26^{o}C (room temperature) for a week. The tested parameters including texture, color, pH, odor was observed and evaluated for any changes on each 0th, 10t^{h}, 20th, and 30th days interval. The results were collected in triplicate and compared with the initial value of respectively parameters.

3.2.8 Acid value and Saponification of Formulated Creams

Acid value where 10 gm of the cream dissolved in 50 ml mixture of equal of alcohol and solvent ether. Then attach the flask with the condenser and reflux it with slow heating until the sample gets completely dissolved, then add 1 ml of phenolphthalein, and titrate it with 0.1 NaOH until it gets faint pink color appears after shaking in 20 seconds. Then, with the formula below is calculating the acid value expressed as the amount of sodium hydroxide (NaOH) in milligrams required to neutralize the free acids present in one gram of the sample.

$$Acid\ value\ (AV) = n * 5.61/w$$

Where

n = number of ml of NaOH required

w = weight of sample (g)

Saponification value where 2 gm of the substance and reflux it with the 25 ml of 0.5 N alcoholic KOH for 30 minutes. Then, add 0.1 ml of phenolphthalein as an indicator and titrate it with the 0.5 N HCL. To calculate the saponification value (SV) using the given formula below.

$$Saponification\ value\ (SV) = (b - a) * 28.05/w$$

Where

a = the volume in ml of titrant

b = the volume in ml of titrant (blank sample)

w = weight of sample (g)

3.2.9 Sample Testing for Sensory Evaluation

A sensory evaluation test was conducted with approximately 40 randomly selected participants from University Malaysia Kelantan, Campus Jeli, to assess the sensory characteristics of the optimized cream. These characteristics included color, scent, texture, appearance, greasiness, stickiness, and overall acceptability. The evaluation utilized five-point hedonic scales, with the following scores: 1 = dislike very much, 2 = dislike, 3 = neither like nor dislike, 4 = like, and 5 = like the best. Participants were instructed to provide their assessments via a Google Form.

Approximately 5 g of sample was placed in a petri dish and distributed to respondents. Prior to sample testing, participants' consent was documented. The respondents were evaluated to confirm that there was no chronic skin illness or injury on the left-hand dorsal surface, followed by a brief introduction and instructions to guarantee that the respondents' evaluation was completed professionally. Finally, each respondent is expected to complete a feedback form indicating their preference and degree of resemblance.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Quantitative Analysis of Bioactive Compounds in Solid Raw and Aqueous Extract

In the present study, the investigation into the chemical composition of *A. myriophylla*, a plant commonly utilized in Pasir Mas, Kelantan, Malaysia, known locally as *Tebu Gajah*, for its possible antidiabetic activities was successfully performed. To detect the presence of beneficial components, GC-MS analysis was performed on *A. myriophylla* aqueous extracts and solid raw bark. By combining mass spectra and retention times, individual chemical ingredients were segregated and identified. The findings demonstrated the presence of many bioactive components in both powdered bark and water extracts of *A. myriophylla*, with substances identified in total (see Appendices).

Table 4.1: GC-MS analysis of powdered bark part of *A. myriophylla* and classification.

No. Peak	Compound Name	Molecular Formula	Rt (min)	Percentage Area (%)	Biological Activity
1	Pyridine	C_5H_5N	2.299	1.58	Antioxidant, antibacterial, antidiabetic and anti-inflammatory
2	N-Serylserine	$C_6H_{12}N_2O_5$	11.577	22.13	Antioxidant, anti-hyperglycemic
3	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	12.24	5.99	Antioxidant, hypocholesterolemia, and antibacterial

4	9, 12-Octadecenoic acid, methyl ester	$C_{19}H_{34}O$	12.913	1.80	Antioxidant, antidiabetic, anti-ulcer, analgesic, anti-inflammatory, antibacterial
5	9-Octadecenaoic acid, methyl ester	$C_{19}H_{36}O_2$	12.935	3.21	Antioxidant, antibacterial, and anti-inflammatory
6	Chondrillasterol	$C_{29}H_{48}O$	17.848	10.54	Antimicrobial
7	Lupeol	$C_{30}H_{50}O$	18.519	4.01	Antioxidant, anticancer, anti-inflammatory, anti-hyperglycemic effects

Source: Abdulfatai Temitope Ajiboye et al., 2022, Mohamad et al., 2018, Marinescu & Popa, 2022; De et al., 2022, Mozirandi et al., 2019, Motoshi Kato et al., 2020, Paradee et al., 2021, "Pyridine and Its Biological Activity: A Review - ProQuest," 2024, Patil et al., 2013, Gallo et al., n.d., Lalthanpuii, C. Lalrinmawia, B. Lalruatfela, Lal Ramliana, & K. Lalchhandama, 2023, Tsai, Lin, & Wu, 2016, None Anshika et al., 2022

Figure 4.2: GC-MS analysis of aqueous extract bark part of *A. myriophylla* and classifications.

No. Peak	Compound Name	Molecular Formula	Rt (min)	Percentage Area (%)	Biological Activity
1	Octanoic acid	$C_8H_{16}O_2$	8.002	0.60	Antimicrobial, Cosmetic, Flavor
2	Benzoic acid, 3,4-dimethoxy-, methyl ester	$C_{10}H_{12}O$	10.809	0.20	Antimicrobial
3	Methyl tetradecanoate	$C_{15}H_{30}O_2$	11.357	0.67	Antimicrobial and anticancer
4	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	11.385	3.08	Antifungal, antioxidant, cancer

					preventive, hypercholesterolemic
5	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	11.823	0.32	Antibacterial, cytotoxic, antimicrobial
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	12.153	6.92	Anticancer, anti-inflammatory, antibacterial, antidiabetic, 5-Alpha reductase inhibitor
7	9- Octadecadienoic acid, methyl ester	$C_{19}H_{36}O$	12.933	2.82	Antibacterial,
8	Hexadecane	$C_{16}H_{34}$	14.607	0.07	Antioxidant, antimicrobial, hypocholesterolemia agent
9	Tetracosanoic aid, methyl ester	$C_{25}H_{50}O$	15.01	0.12	Antioxidant, antidiabetic properties
10	Heptadecane	$C_{17}H_{36}$	15.208	0.19	Anti-inflammatory, antifungal
11	Squalene	$C_{30}H_{50}$	15.338	0.24	Emollient properties, antioxidant, hydration, antitumor activities, antihyperglycemic

Source: N. Rosnani et al., 2018, Zhu et al., 2021, Bakar et al., 2017, Aparna et al., 2021, Pu et al., 2011, Farina Mujeeb et al., 2014, Njoku Ugochi Olivia et al., 2021, D. A. Mareez et al., 2021, Kavitha & Uduman Mohideen, 2017, Ahmad et al., 2023, Shilpa Kusampudi et al., 2009, Dae Hyun Kim et al., 2013, Pavani Chirumamilla, Sunitha Bai Dharavath, & Shasthree Taduri, 2022, Huang, Lin, & Fang, 2009, Europe PMC, 2016

The comparison of phenolic compounds found in powdered and water extracts of *A. myriophylla* gives important information about their possible health benefits and biological activity. Major phenolic chemicals found in powdered form include Pyridine (1.58%), N-Serylserine (22.13%), Hexadecanoic acid methyl ester (5.99%), 9,12-Octadecenoic acid methyl ester (1.80%), 9-Octadecenoic acid methyl ester (3.21%), Chondrillasterol (10.54%), and Lupeol (4.01%). On the other hand, the liquid extract comprises 68 chemicals, 11 of which are phenolic. Despite the larger total number of chemicals in the liquid extract, only a subset of these compounds has been characterized in terms of characteristics in previous research and journals.

Comparing the most prominent and lowest phenolic components in both types of extracts yields interesting findings. N-Serylserine is the most abundant phenolic component in the powdered bark, accounting for 22.13% of the extract. This molecule has been linked to a variety of biological activities, including antibacterial and antioxidant characteristics. The liquid extract, on the other hand, contains the highest phenolic component (6.92%): hexadecanoic acid methyl ester. This molecule, along with other discovered phenolics, has also been found to contain antioxidant, antibacterial, and antidiabetic effects. Additionally, while the liquid extract has a greater total number of components, including phenolics, the powdered bark extract contains some specific phenolic compounds, such as Pyridine and Lupeol, that are not present in the liquid extract. These chemicals, along with others found in solid raw form, have been shown in literature to have a variety of therapeutic benefits, including antioxidant, antibacterial, antidiabetic, and anti-inflammatory properties.

The comparison indicates the need of understanding the exact chemical composition of various plant extracts to assess their health benefits. The absence of specific chemicals in each extract could be attributed to the extraction process and solvent used, both of which influence what compounds are extracted. Due to extraction efficiency, various procedures such as Soxhlet extraction or steam distillation produce different chemical profiles. Ethanol, methanol, and water are important solvents for extracting phenolic chemicals, with each producing various profiles due to polarity and efficiency. For instance, in a study that used microwave-assisted extraction (MAE) and high-performance liquid chromatography (HPLC) for the analysis, HPLC analysis of *Albizia myriophylla* bark revealed the presence of bioflavonoids viz., naringin, quercetin and apigenin. Another study examined the phenolic, flavonoid, and saponin contents and antioxidant activity from methanol extract (ME) and its derived fractions hexane (HE), chloroform (CE), ethyl acetate (EAE), butanol (BE), and aqueous fraction of the bark of

Albizia myriophylla. Among the extracts, EAE showed the highest total phenolic content of about 0.77 mg of gallic acid equivalent/g of extract (mg GAE/mg). However, the highest flavonoid content was detected in HE at 1.04 µg retinol equivalent ((RE)/g extract), while the saponin content was highest in CE at 1.1 µg diosgenin equivalent ((DE)/g extract). Subsequently, there is no doubt that *A. myriophylla* extracts contain important phenolic compounds with a variety of health advantages, indicating potential in traditional medicine and pharmaceuticals. Additional research is required to properly comprehend their medicinal potential.

4.2 Glucose Removal Test

The evaluation of antidiabetic activity of aqueous extract of *A. myriophylla* is conducted in vito methods. The result of the glucose removal test conducted using *A. myriophylla* indicates its potential impact on reducing glucose concentrations. The test involved two different forms of the plant material: a powdered form (10 g) and an aqueous extract (10 ml).

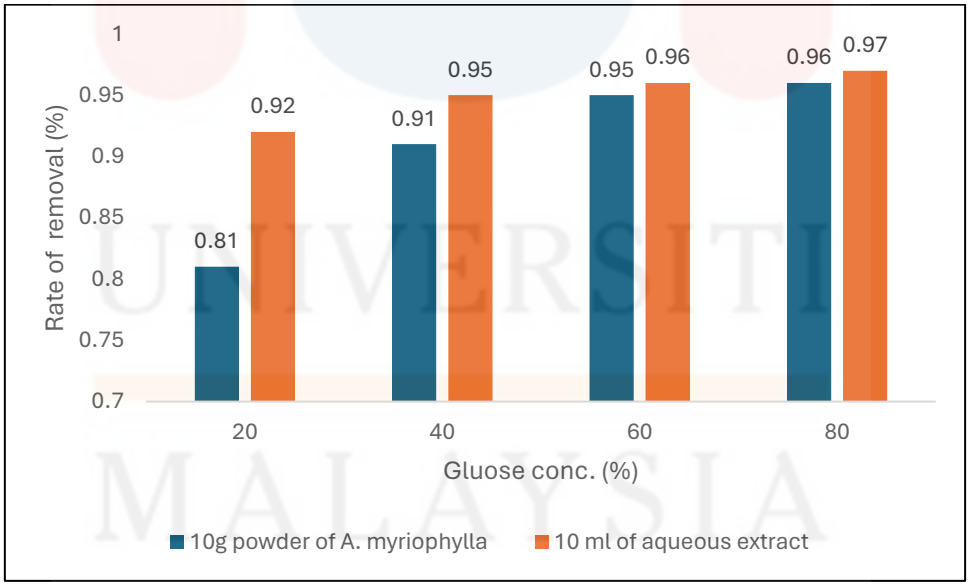


Figure 4. Removal rate of powdered sample and aqueous extract against synthetic glucose.

The glucose reduction test on powdered bark and water extract of *A. myriophylla* reveals important information about their possible antidiabetic activities. The data show varied rates of glucose elimination at different concentrations, demonstrating the efficacy of both forms in decreasing glucose levels. The observations in powdered bark show that the rate of glucose elimination increases with increasing glucose concentration. Specifically, with a glucose content of 20%, the rate of elimination is 0.81%, which subsequently increases to 0.91% at 40%, 0.95% at 60%, and 0.96% at 80%. This trend implies that the powdered bark extract has a dose-dependent effect on glucose elimination, with higher concentrations showing better efficacy. Similarly, the aqueous extract of *A. myriophylla* shows a dose-dependent response, but at slightly higher rates of glucose elimination than the powdered bark. At a glucose concentration of 20%, the water extract removes 0.92%, increasing to 0.95% at 40%, 0.96% at 60%, and 0.97% at 80%.

Moreover, by performing the Benedict test, it was observed that these samples changed color from yellow to orange, indicating the presence of reducing sugars (see in Appendices). These data indicate that the water extract may have better antidiabetic activities than the powdered bark, particularly at higher glucose concentrations. Based on the findings, assuming that the presence of these phenolic compounds in both forms of *A. myriophylla* extracts may contribute to the reported antihyperglycemic effect. Tetracosanoic aid, methyl ester, hexadecanoic acid, and squalene, all of which are often found in plant extracts, have been demonstrated to have antidiabetic characteristics by enhancing insulin sensitivity and lowering blood glucose levels (Tri Widyawati et al., 2015; Mahmood et al., 2020).

According to the findings, differentiating between the usage of medicinal herbs in raw form versus water extract is critical in experimental design. Extracting plant chemicals into aqueous solutions, such as water extracts, makes it easier to isolate and concentrate the bioactive ingredients that cause the observed pharmacological effects. Water extracts are frequently selected because they are simple, inexpensive, and relevant to traditional therapeutic practices. Furthermore, water extraction might help to normalize the concentration of active chemicals, assuring uniformity and reproducibility in experimental results. In contrast, employing raw plant materials may create fluctuation in bioactive component content, confounding the interpretation of experimental data. As a result, using water extracts allows for more controlled research and a better understanding of the therapeutic potential of medicinal plants in controlling glucose levels.

Finally, the study found that plant extracts from various sources, such as roots, fruits, vegetables, and aerial portions, have antioxidant properties. These findings are consistent with previous research on plant extracts with antihyperglycemic activities, making *A. myriophylla* an interesting candidate for further exploration. Future study should concentrate on discovering and characterizing bioactive components found in *A. myriophylla*, as well as investigating potential synergies with existing antidiabetic medications and natural substances to improve its therapeutic efficacy in glucose management. Additionally, stronger criteria are required to determine the therapeutic potential of chemicals discovered in *A. myriophylla* for diabetes treatment.

4.3 Formulation of Bio-cream

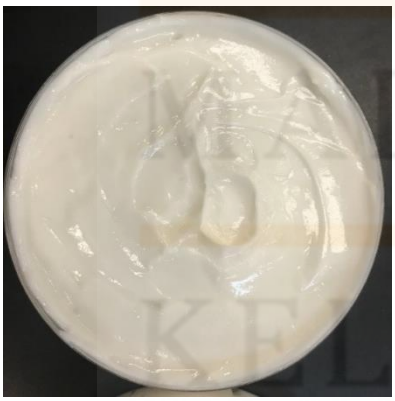
During this research, a cream formulation in Method 1 (see Table 3) was formulated by mixing oil phase and aqueous phase ingredients using Homogenizer at 2500 rpm. The basic ingredients used in the formulation of cream bases by main ingredient, emulsifier wax that act as a stabilizer and influence the consistency of the formulation, as water and oil do not mix, emulsifier assist in forming homogeneous mixtures of both. Following with grapeseed oil, coconut oil, glycerin, Euxyl PE 9010, distilled water. A total of four different formulations were prepared and the composition of each cream was given in Table 4. Particularly, F1-F4 were prepared with the same amount of oil phase with varying weight of emulsifier wax and water phase (distilled water) to obtain a desirable base cream.

Out of four formulations, F2 were selected as optimal formulation of cream based on overall physical evaluation (appearance, texture, color). Meanwhile, F1, F3 and F4 were rejected as the physical characteristics of the cream base were less desirable (e.g. overly soft texture and inadequate thickness). The issues encountered during the formulation were overly soft texture and the thickness of the cream base. Based on the test conducted, the weight of the emulsifier wax influences the texture, thickness as well as weight of the cream base. Hence, the amount of emulsifier wax is essential in formulating creams.

F2 cream foundation served as the base for formulations F5 through F8, which contained different concentrations of *A. Myriophylla* aqueous extract. The stability, texture, and appearance of these formulations were analyzed (see Table 4.2). Thickness differences were noted, with F6 being less thick and F8 much thicker compared to F5 and F7, despite similar

emulsifier wax compositions. These variations were attributed to processing factors like mixing speed and temperature. Each formulation was homogenized for an hour at 2500 rpm. The less thick texture of F6 could be due to insufficient cooling of the water phase before mixing with the oil phase, slowing down the process. On the other hand, the blending speed during manufacturing could affect final texture and thickness. Different speeds alter particle distribution inside the cream, resulting in varying consistency. Thus, the study underscores the importance of careful attention to processing parameters like mixing speed and temperature to achieve consistent texture and thickness in cream formulations. Further research and development of processing technologies are needed to optimize cream formulations for desired physical attributes.

Table 4.2: A physical evaluation on formulated cream (F5, F6, F7 and F8) based on its color, aroma, appearance, and texture.

Code Sample	Parameters	Evaluation
 **F5**	**Color**	White
	Aroma	Pleasant
	Appearance/Texture	- Light weight with smooth texture. - Opaque and glossy application.
 **F6**	**Color**	White
	Aroma	Pleasant
	Appearance	- Light weight with smooth application. - Glossy and greasy application. - Less thicker

 **F7**	**Color**	White
	Aroma	Pleasant
	Appearance/Texture	- Light weight with smooth texture. - Glossy and greasy on application.
 **F8**	**Color**	White
	Aroma	Pleasant
	Appearance/Texture	- Light weight with smooth texture. - Glossy and greasy application. - A bit thicker.

Table 4.3: pH value of the four (F5, F6, F7, F8) formulated creams on 0^{th} day

Parameter	Formulated cream (pH)	Standard (pH)
pH	6.15 ± 0.0866	5.5 – 6.8

The values were presented in mean ± standard deviation (n=4)

A product is intended for external use and applications; therefore, it should be alkaline towards skin (pH= 5 to 6.8). At the present study, the pH value of the formulated cream was found to be compatible with the skin pH and considered safe to apply. It is essential to obtain pH within the required range as products with too high or too low pH will affect skin or potentially induce irritation.

4.4 Acid value and Saponification value

4.4.1 Acid value analysis

The acid value of a cream, denoted as the milligrams of sodium hydroxide (NaOH) required to neutralize free fatty acids per gram of the sample, reflects the concentration of free fatty acids present. In recent experiment, four cream samples labelled as F5, F6, F7, and F8 were analysed for their acid values, which ranged from 0.062 to 0.16 mg KOH/g (see Table 4.4)

Table 4.4: Acid value of formulated cream codded as F5, F6, F7 and F8.

Code sample	Acid value (mg KOH/g)
F5	0.095
F6	0.062
F7	0.073
F8	0.16

Among these samples, F6 had the lowest acid value (0.062 mg KOH/g), indicating a lower concentration of free fatty acids than the other samples. F8 had the highest acid value (0.16 mg KOH/g), indicating a larger quantity of free fatty acids. Variations in acid values among cream samples could be attributed to changes in the types and amounts of fats and oils employed in the formulation. For example, formulations high in saturated fats may provide higher acid values due to the presence of more free fatty acids than formulations high in unsaturated fats. In addition, processing procedures, measurement inconsistencies during the oil phase, and the addition of additives such as preservatives (e.g., Euxyl PE 9010) may affect the measured acid values. Certain preservatives, particularly those with acidic qualities, may increase the overall acid value of the cream formulation and interact with fats and oils, thus changing composition and acidity.

4.4.2 Saponification value analysis

Table 4.5: Saponification value of sample coded as F5, F6, F7 and F8

Code sample	Saponification value (mg KOH/g)
F5	11.36
F6	11.07
F7	10.51
F8	10.93

The saponification value (SV) of a cream is calculated by the quantity of potassium hydroxide (KOH) required to saponify one gram of the sample. It provides information about the average molecular weight of the ester compounds present. In our experiment, the formulated creams (F5, F6, F7, and F8) had saponification values ranging from 10.51 to 11.36 mg KOH/g. F5 had the highest saponification value of 11.36 mg KOH/g, while F7 had the lowest, at 10.51 mg KOH/g. Variations in saponification levels between samples may reflect changes in the types and amounts of fatty acids present, which are impacted by factors such as fat and oil source, refining level, and presence of additives.

4.4.3 Implications for Stability and Quality

Cream's acid and saponification values are critical in determining their stability, quality, and shelf life. Higher acid readings may suggest an increased risk of rancidity and oxidation, which could reduce the product's quality over time. While there is no universally accepted range for "good" cream formulations, the cosmetic and pharmaceutical industries often choose acid values ranging from 0.1 to 1.0 mg KOH per gram. Analyzing acid value changes assists formulators in optimizing formulations for cream product stability and quality. High acid readings, which indicate increasing quantities of free fatty acids, might cause instability, textural changes, safety concerns, and decreased efficacy of active substances. Maintaining an optimum pH level is critical for product efficacy and skin compatibility. According to a review paper by Mawazi et al. (2022), the acid value and saponification value are two essential indicators used to assess the quality of cosmetics. Furthermore, Saraf et al. (2011) found that the acid value of a cream formulation was between 5.98 and 14.21, while the saponification

value was between 23.27 and 33.25. The saponification value is a measurement of the amount of free fatty acid esters in a sample, which affects the formulation's stability, pH, and cleaning properties. Thus, conducting acid value trials is crucial to ensuring the quality, stability, safety, efficacy, and shelf life of prepared creams. Ongoing research and optimization are required to enhance formulations, improve efficacy, and extend shelf life using the information gained from acid and saponification value inquiries.

4.5 Viscosity

The viscometer readings obtained from the vibro viscometer SV 100 for the formulated creams (F5, F6, F7, and F8) provide valuable insights into the rheological properties of each cream. Viscosity is a crucial parameter in the formulation of creams, influencing product texture, stability, and user experience.

Table 4.6: Viscosity readings obtained from vibro viscometer SV 100 for formulated creams, coded as F5, F6, F7, and F8

Sample code	Viscosity readings
F5	14.6
F6	10.9
F7	12.1
F8	13.8

The viscosity of the prepared creams coded as F5, F6, F7, and F8 was determined to be 14.6 Pa.s, 10.9 Pa.s, 12.1 Pa.s, and 13.8 Pa.s, respectively, according to the data obtained from the vibro viscometer Sv 100. F5 has the highest viscosity (14.6 Pa·s) among all formulations. This suggests that F5 has a thicker consistency, implying a cream with a stronger resistance to flow. F5's higher viscosity may be ideal for applications that require a more substantial and richer feel on the skin. In contrast, F6 has a comparatively lower viscosity of 10.9 Pa·s. This suggests a cream with a slightly less viscous texture than F5, potentially resulting in better application and easier spreadability on the skin. The moderate viscosity of F7 (12.1 Pa.s) may appeal to consumers looking for a compromise between thickness and ease of application. F7

and F8 had intermediate viscosity readings (12.1 Pa·s and 13.8 Pa·s, respectively). These formulas strike a balance between thickness and spreadability, resulting in a flexible texture that can meet a wider range of consumer preferences.

Viscosity is a critical factor that influences the stability, texture, and spreadability of cosmetic products. The differences in viscometer readings between the prepared creams (F5, F6, F7, and F8) demonstrate the formulation's ability to generate a variety of texture profiles. These variances in viscosity measurements could be due to variations in constituent composition, concentration levels, or the formulation process itself. According to Kaur et al. (2019), the viscosity of a cream formulation can be impacted by a variety of parameters, including emulsifier type and concentration, oil phase composition, and processing circumstances. In another study by Kulkarni et al. (2018), it was found that the viscosity of a cream formulation can be controlled by adjusting the concentration of the emulsifier and the oil phase composition as well as processing time or method use. As a result, although using the same amount of emulsifier wax, F6's texture and thickness differ from the other three created creams (F5, F7, and F8), most likely due to a lack of proficiency during processing processes.

4.6 Antimicrobial Activity

The antibacterial activity of the prepared creams (F5, F6, F7, and F8) against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was determined using the Agar well diffusion method for three times to determine its consistency. This approach determines the inhibitory impact of creams by measuring the diameter of halazone surrounding the wells. From the result obtained, it was shown that all formulations (F5, F6, F7 and F8) demonstrated antibacterial efficacy against *E. coli* and *S. aureus*, as shown in the appendices.

Table 4.7. Zone of inhibition of four formulated creams incorporating with water extract *A.myriophylla.*

Test Microorganism	Zone of Inhibition (mm)			
	Sample cream			
	F5	F6	F7	F8
E. coli	-	-	0.66±0.57	2.33±1.0

S. aureus	-	-	0.33±0.57	1.33±0.57

The value was presented in mean ± standard deviation (n=3) per trial, (-) represent no inhibition.

Based on the observations, all formulations cream containing 20%, 40%, 60% and 80% or aqueous extract *A.myriophylla* demonstrated a modest zone of inhibition (see in appendices) against *E.coli* and *S.aureus*. Cream formulation F8 (80% aqueous extract) demonstrated broader and more distinct zones of inhibition against *E. coli* compared to the rest. However, the zone of inhibition of *S. aureus* was rather limited for all formulation creams.

E. coli and *S. aureus* are frequent bacteria linked to wound infections. Builders et al., (2013) reported on their prevalence, while a retrospective investigation by Puca et al. (2021) identified S. aureus as the most common species in wound infections. Similarly, Kim et al. (2021) discovered *E. coli* as a common isolation in chronic wounds. Given the predominance of these bacteria in wound infections, the formulated creams' substantial antibacterial action of formulation cream F8 only shows promise for improving wound healing by preventing bacterial infections against *E. coli*.

The observed differences in antimicrobial activity against *S. aureus* could be ascribed to a variety of factors, including the concentration of extract integrated into the cream. This is likely that the concentrations utilized were insufficient to successfully prevent *S. aureus* development. Furthermore, limited research on the antimicrobial capabilities of *A. myriophylla* bark aqueous extract suggests that organic solvent extractions may produce more potent antibacterial effects. Furthermore, chemicals found in the *A. myriophylla* bark aqueous extract, such as Hexadane and 9-octadecenoic acid methyl ester, have antibacterial activities (Bilal Ahmad Ghalloo et al., 2022). Handali et al. (2011) proposed that increasing the concentration of the herbal extract may improve antibacterial activity, as seen by creams containing 80% extract against *E. coli*.

Ultimately, the creams prepared with *A. myriophylla* bark aqueous extract show dissatisfaction on antibacterial efficacy against *E. coli* and *S. aureus*. While F8 had significant action against *E. coli*, hence, additional modification of the extract concentration may be necessary to increase activity against both *E. coli* and *S. aureus*. The extract contains chemicals with established antimicrobial characteristics, which are most likely responsible for the observed antibacterial activities. Additional research and optimization efforts are required to

fully realize the therapeutic potential of *A. myriophylla* bark extract in wound care formulations.

4.7 Total Plate Count of Formulated Creams

Total plate count analysis was conducted to determine the colony-forming units (CFU) of microorganisms present in cream samples after 48 hours of incubation. The acceptance criteria for microbial count, as per guidelines from the National Pharmaceutical Control Bureau Ministry of Health Malaysia (NPCB MOH) and the British Pharmacopoeia (2012), is ≤ 100 CFU/g. Initially, the analysis of the first and second batches of the formulated creams (coded as F5, F6, F7, and F8) yielded no detectable microbial count. Several interpretations can be made based on these results. Firstly, it is possible that the containers used to preserve the creams were sterile, or the products themselves were intentionally sterilized, as is often the case in cleanroom environments or with sterilized products. Additionally, the environmental conditions within the samples (such as temperature, pH, and nutrient availability) may not have been conducive to microbial growth, resulting in no observable growth.

Furthermore, the samples may have contained effective antimicrobial agents that inhibited or eliminated microbial growth. It is also possible that errors occurred during the sampling process, such as inadequate sampling techniques or improper storage, or the use of improper selective media, leading to a lack of viable microorganisms in the analyzed portion. Additionally, some microorganisms may be slow-growing or dormant, and therefore may not grow within the typical incubation period of a total plate count. Guidelines from the FDA's Bacteriological Analytical Manual (2001) recommend incubating plates at 35±1°C, while other sources suggest different temperatures, such as 20 or 30°C, for three days (Sagar Aryal, 2022).

To detect the presence of microorganisms in the last batch (third batch), the total plate count assay was extended to three days of incubation. The results showed that only F6 (containing 40% *A. myriophylla* bark aqueous extract) contained a single colonies microorganism. The difference between F6 and the other samples (F5, F7, and F8) shows that there may have been procedural problems in terms of preparation, work area, or equipment sterility. According to Abatenh et al. (2018), shared work areas can be a source of contamination in the laboratory environment, particularly among workers who are not completely educated in aseptic practices. Cross-contamination can occur if lab tools are not

adequately cleaned after each use or if samples are stored too close together. In conclusion, while the initial batches of formulated creams had no detectable microbial count, additional analysis during the last batch indicated the presence of bacteria in one sample. This emphasizes the need to strictly adhere to suitable sampling methodologies, storage conditions, and laboratory hygiene to avoid contamination and assure reliable microbial analysis of cosmetic items.

4.8 Stability and Physical Evaluation of Formulated Creams

In this study, the stability of four formulated cream samples was assessed over a one-month period under two different storage conditions: refrigeration at 4°C and room temperature at 20-25°C. Evaluations were conducted every 30 days, focusing on various parameters including aroma, pH value, texture, appearance, viscosity, and after-removal. During the stability test, the creams were stored in petri dishes to reduce the impact of containers on formulation stability and to avoid undesired interactions between formulation and container materials. Deborah Adefunke Adejokun and Kalliopi Dodou (2020) describe a novel method for evaluating the long-term stability of cream formulations containing natural oils, stating that a stable formulation should not exhibit more than a 5% change in assays from its initial value or any degradation beyond acceptance criteria. The physicochemical parameters of samples coded F5, F6, F7, and F8 maintained at room temperature and in the refrigerator for 30 days were summarized (refer to Table 4.7).

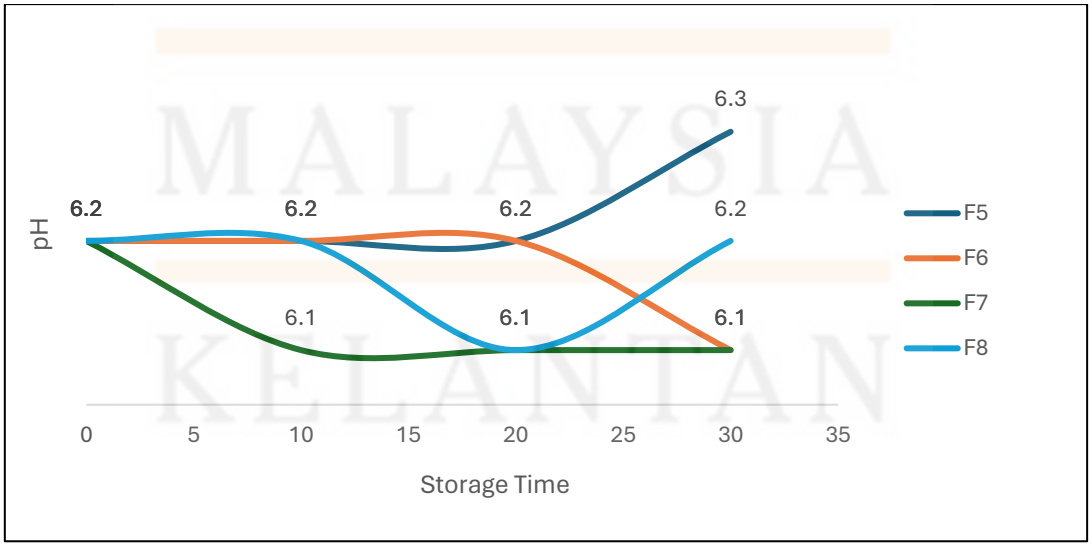


Figure 4.2: Change in the pH of four formulated creams at room temperature over 30 days

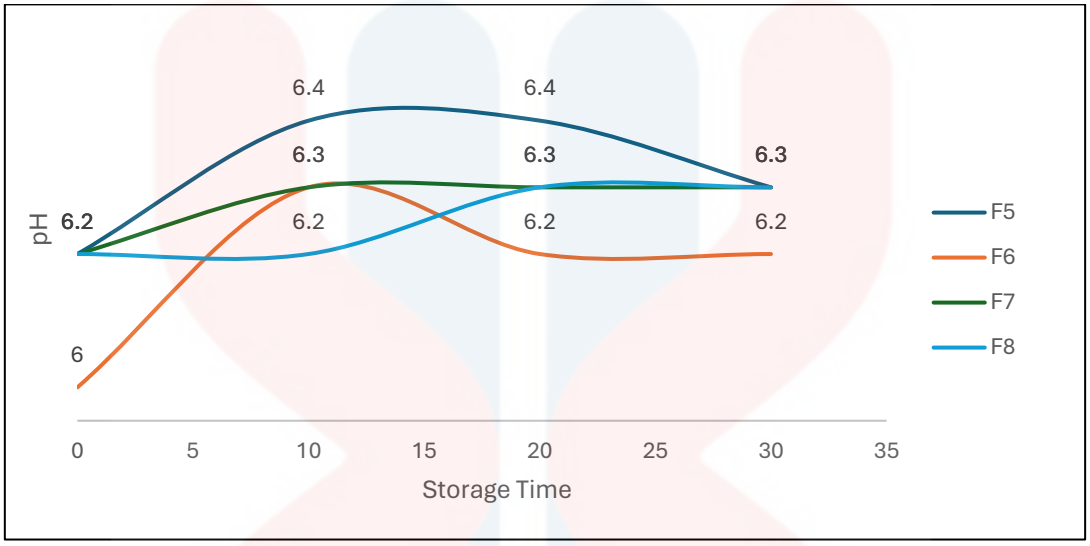


Figure 4.3: Change in the pH of four formulated creams at 4 ºC±1 ºC over 30 days

A two-way analysis of variance (ANOVA) was used to investigate the effects of storage temperature and time on pH levels. The results showed that the fluctuation in pH levels of the cream samples was not statistically significant ($p > 0.05$) throughout storage durations and temperatures. The p-values for the rows (creams) and columns (days) were both greater than 0.05, indicating that there was no significant difference in mean pH values between creams or days. As a result, there is insufficient evidence to reject the null hypothesis that the average pH values for creams and days are the same. Despite an insignificant difference in pH levels, the one-month stability investigation showed that the prepared creams remained stable at both room temperature and refrigerated. There were no indicators of phase separation, odor, color, or texture alterations, indicating that the formulations were overall stable. As a result, the prepared creams were found to be stable over a one-month period at both room temperature and refrigeration. The results indicate that the creams' quality qualities remained within acceptable ranges, confirming their appropriateness for long-term storage and prospective usage in applications.

Table 4.8: Physical properties of F5, F6. F7 and F8 cream on room temperature and accelerated temperature.

Day 0th					
Sample code	**Temperature (ºC)**	**pH**	**Appearance**	**After-feel**	**Removal**
F5	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.2	No color change	Emollient & greasy	Easy
F6	**RT**	6.0	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.0	No color change	Emollient & greasy	Easy
F7	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.2	No color change	Emollient & greasy	Easy
F8	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.2	No color change	Emollient & greasy	Easy
Day 10th					
F5	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.5	No color change	Emollient	Easy

				& greasy	
F6	**RT**	6.5	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.5	No color change	Emollient & greasy	Easy
F7	**RT**	6.1	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.5	No color change	Emollient & greasy	Easy
F8	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.2	No color change	Emollient & greasy	Easy
			Day 20th		
F5	**RT**	6.1	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.4	No color change	Emollient & greasy	Easy
F6	**RT**	6.2	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.1	No color change	Emollient & greasy	Easy
F7	**RT**	6.0	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.3	No color change	Emollient	Easy

				& greasy	
F8	**RT**	6.1	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.5	No color change	Emollient & greasy	Easy
			Day 30th		
F5	**RT**	6.3	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.2	No color change	Emollient & greasy	Easy
F6	**RT**	6.0	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.4	No color change	Emollient & greasy	Easy
F7	**RT**	6.1	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.0	No color change	Emollient & greasy	Easy
F8	**RT**	6.1	No color change	Emollient & greasy	Easy
	4 ºC±1 ºC	6.3	No color change	Emollient & greasy	Easy

Based on the observations recorded in Table 4.7, changes in the color of the formulated creams (F5, F6, F7, F8) were noted even after being stored for an extended period. Despite these color changes, all cream formulations exhibited pH levels close to the skin's natural pH, falling within the range of 6.0 to 6.5. This alignment with skin pH suggests compatibility and enhances the likelihood of user acceptance. Furthermore, the viscosity of the creams remained within a favorable range of 10.9 to 14.6 Pa.s after one month of storage. This consistent viscosity indicates that the creams maintained their intended texture and consistency over time, which is indicative of stability and quality in the formulation. Such stability contributes positively to the user experience and meets established quality standards.

Notably, formulations F7 and F8 exhibited superior spreadability compared to the other formulations, which is a desirable characteristic for ease of application. Additionally, all formulations demonstrated uniform distribution of extracts within the cream, as confirmed through visual appearance and tactile assessment during the 30-day stability test. This uniform distribution is crucial for ensuring consistent efficacy and performance of the creams over time.

4.9 Skin Irritancy and Sensory Test

This study included 40 participants chosen at random from students and staff at the University Malaysia Kelantan, Campus Jeli. Gender, native state, and a wide range of age groups were among the demographic data gathered. The statistics revealed that female respondents made up 60% of the sample, with males accounting for 40%. The age distribution spanned from 20 to 60 years and above, with the 20 to 30 age group accounting for 87.5% of all respondents. The remaining responders were divided as follows: 7.5% were 31 to 40 years old, whereas 5% were 41 to 50 years old. Kelantan had the greatest presence (35%), followed by Kedah (15%) and Sarawak (12.5%), among 13 states in Malaysia.

In the study, 40 people were asked about their familiarity with *Pokok Tebu Gajah* (*A. myriophylla*) and if they had ever used or ingested any goods derived from it. A substantial majority (see Appendices), 97.5%, responded negatively, with the remaining 2.5% answering "Maybe." One respondent from Kedah stated that a close family member had consumed *Tebu Gajah*, leaving her unaware of its purpose. Furthermore, respondents were asked to score their overall understanding of herbal treatments and traditional medicinal plants. The findings

showed that 67.5% of respondents assessed their expertise as "not good," indicating a general lack of experience with such therapies.

4.9.1 Sensory Evaluation

In this study, consumer acceptance testing for sensory attributes was conducted to gauge respondents' preferences regarding various characteristics of the optimized cream. The attributes assessed included color, aroma, texture, appearance, spreadability, stickiness, greasiness, and overall acceptance. A 5-point hedonic scale was utilized due to respondents' potential lack of proficiency in quantifying intensity attributes without specialized training.

The evaluation began with visual appearance, focusing on color, aroma, texture, and appearance, as these are the primary senses engaged upon initial contact with the cream samples (coded as F5, F6, F7, and F8). The color attribute received a rating of "4" when the color of all cream samples was deemed presentable and satisfactory upon first sight. Regarding aroma, a rating of "5" signified a pleasant smell, while "1" indicated a disagreeable smell. All formulated cream samples received a rating of "4" for pleasant smell.

Subsequently, spreadability and immediate skin feel, including stickiness and greasiness, were assessed by applying a small amount of cream sample to the back of the hand. Spreadability was rated "5" for good spreadability or "1" for poor spreadability. Immediate skin feel was rated "5" for not being greasy or sticky after application, while ratings below "3" denoted stickiness and greasiness, which were less preferable. Overall acceptability of the formulated cream samples (coded as F5, F6, F7, and F8) was rated based on respondents' individual satisfaction levels.

Table 4.9: Consumer acceptance testing of four creams (F5, F6, F7 and F8) based on sensory attributes.

Sensory Attribute	Mean Value
Color	4.02 ± 0.16
Aroma/Odor	3.96 ± 0.28
Texture/Appearance	4.01 ± 0.49
Spreadability	4.02 ± 0.22
Stickiness	4.02 ± 0.22

Greasiness	4.02 ± 0.19
Overall acceptance	4.12 ± 0.35

The value was mean ± standard deviation. N=40. Evaluation based on 5-point hedonic scale (1=dislike very much, 2=dislike, 3=Neither like nor dislike, 4=like, 5=like the best).

Consumer acceptance testing of four creams (F5, F6, F7, and F8) based on sensory qualities yielded mostly good results (Table 4). Participants scored color, spreadability, stickiness, and greasiness with mean values of roughly 4, suggesting overall satisfaction. However, there was greater variability in responses to aroma/odor and texture/appearance, as seen by slightly lower mean scores and bigger standard deviations. Despite this, participants rated the creams favorably. These findings show possible areas for development, particularly in aroma/odor and texture/appearance, while confirming the creams' overall excellent response. In this study, the Cronbach's alpha reliability coefficient exceeds the minimum standard threshold ($\alpha = 0.60$) for determining internal consistency among components. The α value of 0.84 indicates that the Likert scale components (N = 7) have satisfactory internal consistency and fit for this investigation (refer to Table 2.1).

4.9.2 Skin Irritancy Test

Following the sensory evaluation, the same set of responders underwent a skin irritation test to determine the safety of the cream compositions. Among the 40 respondents, only 3 persons experienced very minor erythema, which was barely detectable. Furthermore, one of these three responders experienced a minor itching sensation during the 30-minute application time due to a sensitive skin condition. However, most responders showed no indications of erythema, and no cases of edema were noted. The results of the skin irritation test indicated that the cream formulations were primarily non-irritant according to the PII scoring system. This finding could be due to a variety of circumstances. First, the pH of the manufactured creams (coded as F5, F6, F7, and F8) was found to be about neutral, similar to the pH of normal skin. Furthermore, the creams were made with natural components and minimum preservatives, notably Euxyl PE9010, which is known to produce less irritation or allergic responses. It is also worth mentioning that all four cream samples contained varied quantities of water extracted from *A.myriophylla*, a natural component with potential benefits in formulations. Despite this, only sample F8 included two respondents with barely visible

indications of erythema. This shows that the addition of *A.myriophylla* extract may have improved the formulations' overall skin tolerability.

In summary, the skin irritation test results (Table 4.9) confirm the conclusion that the designed creams are well tolerated and provide a low risk of skin irritation. The use of natural components, combined with the precisely controlled pH and minimum preservatives, most certainly contributed to the positive safety profile seen in this study.

Table 4.9.1: Summary individual skin reaction scores

Skin reaction	Cream sample	Individual scores				
		0	1	2	3	4
Erythema formation	F5	39	1	0	0	0
	F6	39	1	0	0	0
	F7	39	1	0	0	0
	F8	38	2	0	0	0
Edema formation	F5	40	0	0	0	0
	F6	40	0	0	0	0
	F7	40	0	0	0	0
	F8	40	0	0	0	0

Average PII score for each sample ≤ 0.025

Category of irritation: Non-irritation

CHAPTER 5

5.0 CONCLUSION

Ultimately, the examined the chemical profile of *A. myriophylla*, also known as Tebu Gajah in Malaysia, to determine its potential for diabetes management. Using GC-MS analysis on both water extracts and solid bark samples, the discovered various bioactive chemicals, particularly phenolic compounds known for their antioxidative and antibacterial effects. Both powdered bark and water extract included notable components such as Pyridine, N-Serylserine, and Lupeol, with the latter exhibiting a more diverse profile of 11 phenolic compounds, all of which may have potential antidiabetic properties. Furthermore, both powdered bark and water extract showed promising results in the glucose removal test, with the second substance slightly outperforming the former in terms of glucose reduction. This could be due to the presence of phenolic substances such as Tetracosanoic acid methyl ester and Hexadecanoic acid, which are known to have anti-diabetic properties. Furthermore, the cream formulation (F5, F6, F7, and F8) containing *A. myriophylla* extract demonstrated acceptable qualities such as appropriate viscosity, antibacterial activity against common bacteria, and favorable sensory ratings. However, modifications are required to improve the texture. Furthermore, our skin irritation test found that the creams were primarily non-irritating, most likely due to their skin-friendly pH and the use of natural components with minimum preservatives. In conclusion, the current findings give some insight into the chemical composition, antidiabetic potential, and formulation properties of creams containing *A. myriophylla* extract. These findings support the use of this traditional medicinal plants in cosmetics formulations with minimal risk, highlighting the need for additional research in this area.

5.1 RECOMMENDATION

This study primarily focused on formulating and assessing the quality of creams containing water extract of *A. myriophylla* and natural ingredients for managing diabetes. However, the specific pure compounds responsible for the observed glucose reduction in the glucose removal test were not investigated. In future research, the active compounds in the

water extract of *A. myriophylla* could be identified using spectroscopic techniques such as UV-Vis Spectroscopy, Infrared Spectroscopy (IR), or Fluorescence Spectroscopy. Employing multiple spectroscopic techniques can facilitate a comprehensive analysis of the bioactive compounds in the water extract, aiding in their characterization and potential therapeutic applications as well as during determining glucose reduction. Moreover, it is imperative to evaluate the in vivo efficacy of the antihyperglycemic and antidiabetic properties of A. *myriophylla* in formulated creams by conducting tests on laboratory animals or diabetes patients. This would also help determine the optimum concentration required for significant activity, especially in promoting wound management in the skin area.

The formulated creams demonstrated antibacterial activity against *E. coli* and *S. aureus*. Further research should be conducted to confirm and optimize the antibacterial properties of the aqueous extract alone, possibly determining the optimum concentration for significant antibacterial activity. Additionally, studying different combinations of formulated creams without preservatives and without water extract could elucidate their antibacterial activity. Enhancing the antibacterial activity of the creams is crucial to mitigate contamination issues, ensuring product safety and quality for consumers.

Furthermore, the stability evaluation of the sample creams was limited to a one-month testing period, which may not provide sufficient evidence for overall stability. Hence, it is recommended to extend the testing period to a minimum of three months to accurately predict or determine the product's shelf life in real-time conditions. Additionally, conducting texture profile analysis (TPA) can enhance the understanding of the product's thickness and firmness compared to commercial creams. Improving the consistency of the emulsion by adjusting the temperature before mixing both phases is also essential for ensuring consistency in the formulated creams.

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APPENDICES

A Chemical profile of solid raw and water extract of *A.myriophylla*

Table 7. Chemical profile of solid raw found in GC-MS

No Peak	R.T. min	Peak area	% of total	Compounds Name	% Library matching
---	-----	-------	-------		
1	2.299	4363496	1.58%	Pyridine	91
2	10.278	11967883	4.35%	unidentified	
3	10.308	7423694	2.70%	unidentified	
4	11.555	49375601	17.93%	4,6-di-O-methyl-.alpha.d-galactose	64
5	11.577	60953118	22.13%	N-Serylserine	50
6	11.829	15728212	5.71%	4,6-Di-O-methyl-.alpha.-d-galactose	53
7	11.862	32697643	11.87%	.alpha.-Methyl 4-O-methyl-D-mannoside	50
8	12.24	16491628	5.99%	Hexadecanoic acid, methyl ester	98
9	12.913	4952841	1.80%	9,12-Octadecadienoic acid, methyl ester	99
10	12.935	8835517	3.21%	9-Octadecenoic acid, methyl ester	99
11	13.018	5379638	1.95%	Methyl stearate	93
12	14.906	1695188	0.62%	unidentified	
13	14.968	1591205	0.58%	unidentified	
14	15.012	1658643	0.60%	unidentified	
15	15.152	2560843	0.93%	unidentified	
16	15.339	1691909	0.61%	4-Methyl-1,5-Heptadiene	60
17	17.848	29041420	10.54%	Chondrillasterol	93
18	18.282	7992817	2.90%	Stigmast-8(14)-en-3.beta.-ol	81
19	18.519	11038593	4.01%	Lupeol	90

Table 7. 1. Chemical profile of liquid form found in GC-MS.

No. Peak	R.T. min	Peak area	% of total	Compounds Name	% Library matching
---	-----	-------	-------		
1	2.346	26618825	1.13%	unidentified	
2	2.626	14246993	0.60%	Tetramethyl silicate	58
3	3.669	8054358	0.34%	unidentified	
4	6.67	5500357	0.23%	Germacyclobutane, 1,1-dimethyl-	60
5	7.963	6400712	0.27%	Octanoic acid, methyl ester	91
6	8.002	14194587	0.60%	Octanoic acid, methyl ester	90
7	8.224	12811691	0.54%	unidentified	
8	8.479	6826273	0.29%	Methyl pyruvate dimethyl acetal	80
9	8.524	21076928	0.89%	Methyl cis-3-chloropropenoate	50
10	8.61	5658320	0.24%	unidentified	
11	8.97	36289340	1.54%	unidentified	
12	8.993	35543766	1.51%	unidentified	
13	9.015	103562452	4.39%	unidentified	
14	9.311	5710704	0.24%	unidentified	
15	9.361	10478468	0.44%	Decanoic acid, methyl ester	93
16	9.479	5444082	0.23%	unidentified	
17	9.629	141396226	5.99%	unidentified	
18	9.655	192525706	8.15%	unidentified	
19	9.959	5238390	0.22%	unidentified	
20	10.073	9218457	0.39%	unidentified	
21	10.098	4125147	0.18%	unidentified	
22	10.132	22880111	0.97%	unidentified	
23	10.168	23986658	1.02%	unidentified	
24	10.334	20697557	0.88%	1-Dodecanamide. N,N-dimethyl-	86

25	10.437	108687781	4.60%	Dodecanoic acid, methyl ester	97
26	10.458	26043005	1.10%	l-Alanine, N-allyloxycarbonyl-, hexyl ester	50
27	10.641	15687510	0.66%	Methyl pyruvate dimethyl acetal	53
28	10.665	14733545	0.62%	Methyl pyruvate dimethyl acetal	53
29	10.688	6682092	0.28%	unidentified	
30	10.809	4802371	0.20%	Benzoic acid, 3,4-dimethoxy-, methyl ester	93
31	10.836	17548978	0.74%	unidentified	
32	11.051	8724841	0.37%	unidentified	
33	11.095	8123734	0.34%	unidentified	
34	11.125	18634160	0.79%	Propylamine, N-[9-borabicyclo[3.3.1]non-9-yl]-	53
35	11.164	9715608	0.41%	unidentified	
36	11.294	154087229	6.52%	1-Tetradecanamide, N,N-dimethyl-	86
37	11.357	15784983	0.67%	Methyl tetradecanoate	91
38	11.385	72796236	3.08%	Tetradecanoic acid, methyl ester	98
39	11.73	8379081	0.36%	Benzenesulfonamide, N-butyl-	64
40	11.823	7658329	0.32%	Pentadecanoic acid, methyl ester	97
41	11.974	13268151	0.56%	L-Proline, N-valeryl-, hexadecyl ester	62
42	12.09	5374489	0.23%	unidentified	
43	12.153	10246131	0.43%	9-Azabicyclo(6.1.0)non-4-en-9-amine,(1.alpha.,4Z,8.alpha.)-	80
44	12.241	163489053	6.92%	Hexadecanoic acid, methyl ester	99
45	12.327	21756569	0.92%	Pyrrolo(1,2-a)pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	96
46	12.386	48419712	2.05%	Pyrrolo(1,2-a)pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	80
47	12.628	11414199	0.48%	i-Propyl 14-methyl-pentadecanoate	95
48	12.864	3150904	0.13%	Cyclopentadecane	95
49	12.933	66587593	2.82%	9-Octadecenoic acid, methyl ester	99
50	13.02	136542832	5.78%	Methyl stearate	99
51	13.368	4455204	0.19%	Isopropyl stearate	87

52	13.75	24551975	1.04%	Benzaldehyde, 4-ethyl-	80
53	13.968	5072508	0.22%	Heptadecane,2,6,10,15-tetramethyl-	86
54	14.607	1593037	0.07%	Hexadecane	89
55	14.91	2693547	0.11%	Heneicosane	83
56	15.01	2828246	0.12%	Tetracosanoic acid, methyl ester	90
57	15.208	4565029	0.19%	Heptadecane	95
58	15.338	5638502	0.24%	Squalene	89
59	15.442	1434348	0.06%	unidentified	
60	15.49	664476	0.03%	unidentified	
61	15.538	1369817	0.06%	unidentified	
62	16.14	5735747	0.24%	1H-Indole, 1-methyl-2-phenyl-	52
63	16.277	237103530	10.04%	7,8-dimethoxy-5-phenyl-2-methylthiazolo(5,4-c)isoquinoline	83
64	16.384	5406267	0.23%	unidentified	
65	16.456	30578610	1.30%	unidentified	
66	16.476	38370731	1.63%	unidentified	
67	16.645	178664640	7.56%	unidentified	
68	16.747	94370914	4.00%	unidentified	

C Glucose Removal Test

Table 7.2: Absorbance from ten varies glucose concentration sample at 540nm spectrophotometer.

Glucose conc. (mg/ml)	Abs. (540nm)
0.5	0.01
1	0.013
1.5	0.017
2	0.022
2.5	0.028
3	0.033
3.5	0.037
4	0.045
4.5	0.049
5	0.051

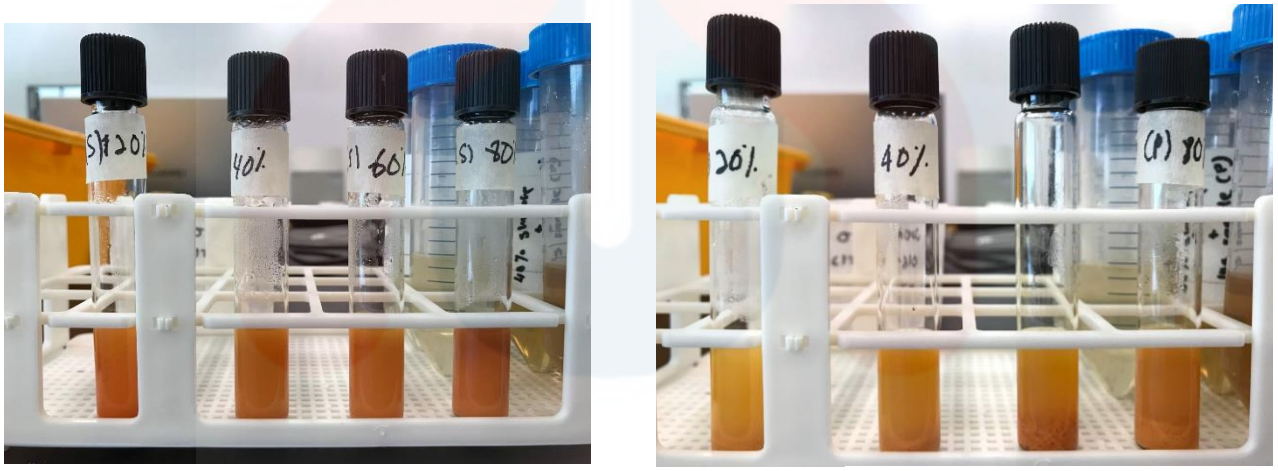
Table 7.3: Absorbance of 10 g of powdered sample dissolved into 100mL in different glucose concentrations after 2 hours.

Tube	Glucose conc. (%)	Abs. (540nm)	Conc. of unknown sample (mg/mL)
1	20	0.040	3.7
2	40	0.037	3.41
3	60	0.035	3.2
4	80	0.031	2.8

Table 7.4: Absorbance of 10 ml of aqueous plant extract dissolved into 100mL in different glucose concentrations after 2 hours.

Tube	Glucose conc. (%)	Abs. (540nm)	Conc. of unknown sample (mg/mL)
1	20	0.019	1.59
2	40	0.023	2
3	60	0.028	2.5
4	80	0.030	2.7

Figure 7.1. 10g of powdered and 10ml of liquid form *A.myriophylla* was diluted into 100ml of glucose with different concentrations



(a: 10g of powdered)

(b: 10ml of liquid form)

Figure 7.2. Benedict test on sample (a) and (b)

D Two-way ANOVA

Anova: Two-Factor Without Replication

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
F5	4	25.3	6.325	0.0225
F6	4	25.1	6.275	0.049167
F7	4	25	6.25	0.043333
F8	4	25.1	6.275	0.009167
Day 0	4	24.6	6.15	0.01
Day 10	4	25.7	6.425	0.0225
Day 20	4	25.3	6.325	0.009167
Day 30	4	24.9	6.225	0.029167

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.011875	3	0.003958	0.17757	0.908923	3.862548
Columns	0.171875	3	0.057292	2.570093	0.119178	3.862548
Error	0.200625	9	0.022292			
Total	0.384375	15				

Anova: Two-Factor Without Replication

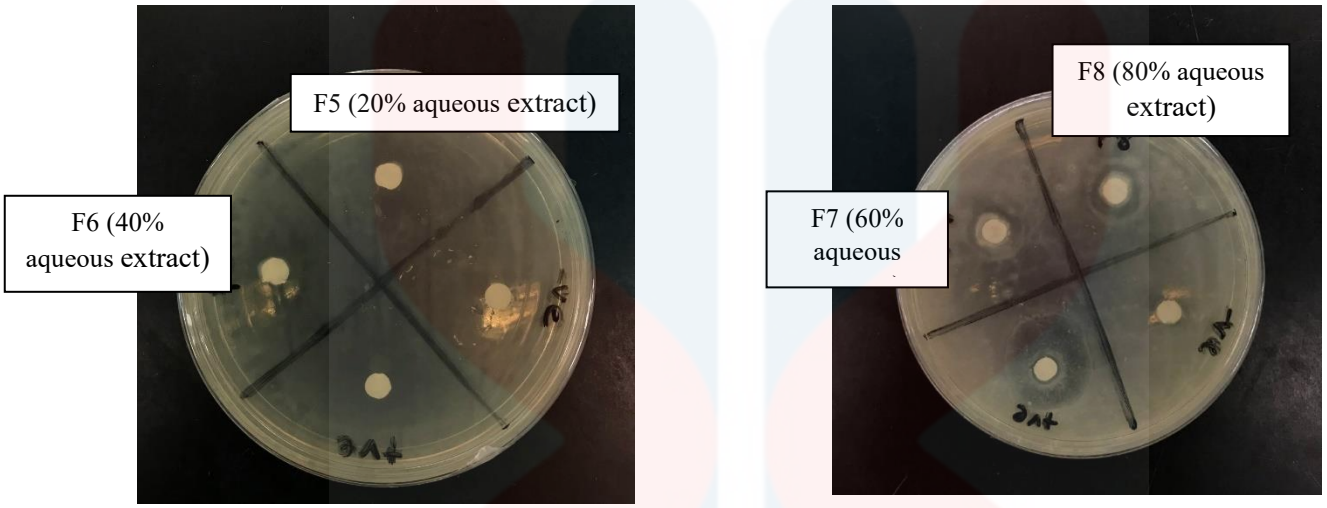
SUMMARY	*Count*	*Sum*	*Average*	*Variance*
F5	4	24.8	6.2	0.006667
F6	4	24.7	6.175	0.055833
F7	4	24.4	6.1	0.006667
F8	4	24.6	6.15	0.003333
Day 0	4	24.6	6.15	0.01
Day 10	4	25	6.25	0.03
Day 20	4	24.4	6.1	0.006667
Day 30	4	24.5	6.125	0.015833

ANOVA

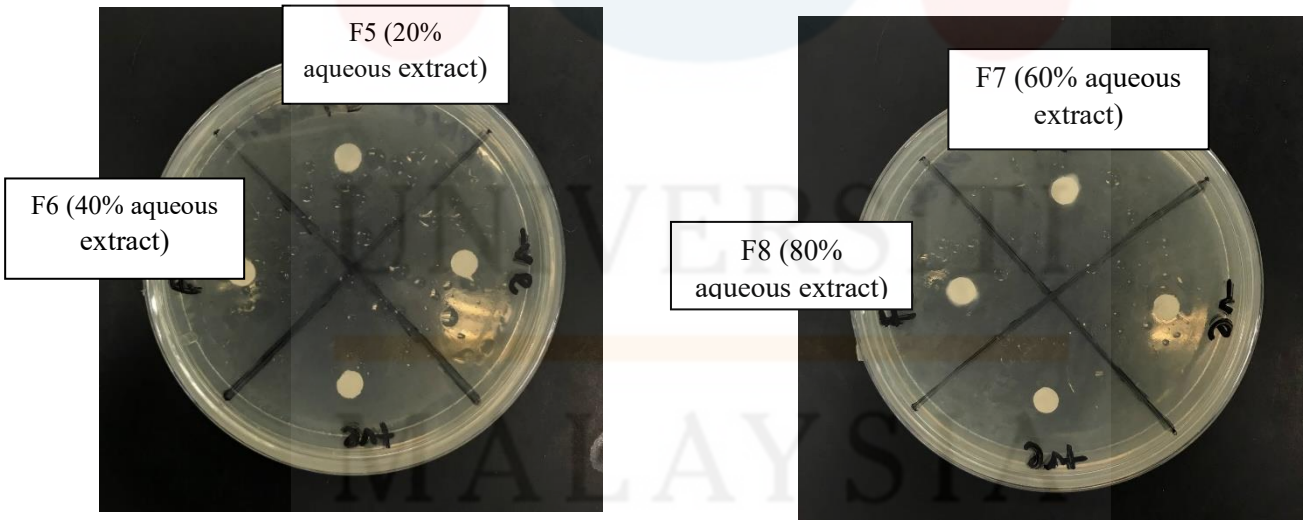
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.021875	3	0.007292	0.396226	0.758971	3.862548

Columns	0.051875	3	0.017292	0.939623	0.461104	3.862548
Error	0.165625	9	0.018403			
Total	0.239375	15				

E Antimicrobial Activity



Paper Disc Diffusion Assay of four formulted creams (F5, F6, F&and F8) incorporating A.myriophylla aqueous extract against *E.coli.*



Paper Disc Diffusion Assay of four formulted creams (F5, F6, F&and F8) incorporating A.myriophylla aqueous extract against *S.aureus.*