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EFFECT OF STABILITY AND BACTERIAL INHIBITION ACTIVITY OF MELASTOMA MALABATHRICUM LINN LEAVES PLANT EXTRACT IN A WATER-BASED EMULSION

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ABSTRACT: *Melastoma malabathricum Linn*. (MB) is a herbal plant that has benefits in treating scars from smallpox, pimples and black spots on the skin. The active ingredient in MB leaves can produce a quality and effective product which can inhibit bacterial development. Therefore, this study was to identify the effect of stability and bacterial inhibition activity in based-water emulsion after it had been added with the extraction of MB leaves. The water-based emulsion containing 1% of MB extraction in different storage conditions showed its stability when viscosity and pH at 4 °C, 25 °C, and 58 °C slightly changed much from the initial after 21 days. The colour of emulsion MB extract at 58 °C changes from yellowish to brown due to the degradation of chlorophyll in extract when it exposes to high temperature. Viscosity from different pH showed that pH 6 was the most stable viscosity during the storage period which this pH was in the range of skin pH. This study shows that the emulsion MB extract was most stable at lower temperature, 4 °C and pH around 6. Thus, this emulsion MB extract suitable to use on the skin. Besides that, the emulsion MB extraction did not show antibacterial activity against *Escherichia coli.*

KEYWORDS: *emulsion MB extract, stability, viscosity, pH, bacterial*

1. INTRODUCTION

Melastoma malabathricum Linn. is a widespread herb or shrub that originates primarily in the moist tropics, specifically India, Thailand, and Malaysia, where it grows as a small tree between 12 and 13 feet in height, occasionally reaching 20 feet. The leaves of this plant have been used for a variety of medicinal purposes, including the treatment of dysentery and diarrhea, the reduction of the appearance of scars, and as an anti-infection [1]. Additionally, herbs can be utilised to treat dehydration and skin irritation. Therefore, we will use the active ingredient of *Melastoma malabathricum Linn*. leaves because this plant is well known in Malaysia for its advantages in the treatment of different diseases and ailments as the leaves of this plant can treat scars, pimples, and black spots on the skin [2].

Plant extracts from herbs are widely used in cosmetics products as people seek a more holistic, natural approach to health and wellness. Water-based emulsions like emollients and moisturisers are used to treat and prevent dry skin because they help seal moisture deep inside the skin, which can keep it soft and supple. Moisturizing creams with natural ingredients are currently popular since they are likely to be effective, harmless, and skin-friendly. According to a study by Leelapornpisid et al. [3], the extraction of the freshwater macroalgae in a cream base proved safe and showed the same moisturising properties as hyaluronic acid via humectants, followed by an occlusive effect that can keep skin hydration

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for a longer period of time. Aside from that, a cream containing *Moringa oleifera* leaves extract demonstrated efficiency in enhancing skin hydration by avoiding UV radiation due to ascorbic acid in *Moringa oleifera* leaves, which provides photoprotection and inhibits inflammation, and UVB-induced immune suppression when applied topically [4].

The warm humid conditions of a tropical environment are ideal for the development of microorganisms, which are the causes of several infectious diseases and the spoilage of cosmetics and medicines. There is a possibility that if there are too many bacteria at any point during the product's shelf life, they could cause the product's physical state to get worse. The risk of this occurring is higher with many present-day moisturizing creams and lotions, which contain special additives (including plant extracts, fatty acids and vitamins) that could increase the growth of bacteria [3]. In order to obtain a stable extract cream, a physical stability test must be conducted so that the cream is safe to use and does not cause skin damage such as irritation, allergies, dark spots, and discolouration. A stable cream is one whose physical qualities and characterization remain within acceptable limits during the storage and use period. Several factors influence the stability of cream, including the stability of the active ingredient, the interaction of the active ingredient with additives, and the temperature at which additives and raw materials are heated during production. For bacterial inhibition activity, tannins and other phytochemicals have been shown to have antibacterial activity, suggesting they can greatly reduce the risk of wound infections. Tannins, an astringent and antibacterial compound found in the plant, may help accelerate the wound-healing process by reducing inflammation and promoting the formation of new skin cells (epithelium) [5]. Active compounds like flavonoids, alkaloids, tannins and saponins have antibacterial activity against various bacteria, one of which is *Staphylococcus aureus*. This Gram-positive bacterium can cause skin infection, resulting in a variety of skin diseases, including acne [6].

From previous studies, the active ingredient the *Melastoma malabathricum Linn*. plant can be used for a variety of beneficial purposes. Che Omar et al. [1] conducted a study on the growth of pathogenic bacteria from flower and fruit extract of *Melastoma malabathricum Linn.* Whereas a study on leaves of *Melastoma malabathricum Linn*. to evaluate the antioxidant, antihyperlipidemic and antidiabetic activity has been conducted by Kumar et al. [7]. Therefore, this study was done to identify the effect of leaves extraction of *Melastoma malabathricum Linn.* (MB) in the water-based emulsion on stability and bacterial inhibition activity.

2. MATERIALS AND METHODS

2.1. Materials

Leaves of *Melastoma malabathricum Linn*. as Fig. 1 were collected from Kampung Balok, Kuantan, Pahang, Malaysia. The leaves were cleaned with tap water and a portion was dried in a hot oven at 100 ◦C for 1h 30 minutes. The dried samples are ground and passed through a sieve (30 mesh). The powders were kept in sealed containers and protected from light until used. The water-based emulsion was purchased from a local vendor for use in this research project (Fig. 2).

2.2 Soxhlet extraction of dried leaves

The dried powdered leaves were separately placed into a thimble and extracted with 60% ethanol (1:20, w/v) in a Soxhlet apparatus [8]. Extraction is carried out for 7 to 8 h. The combined extract from the extraction method is separately filtered through a Whatman No. 1

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filter paper. The filtrate was dried under reduced pressure at 50 °C using a rotary vacuum evaporator. The crude extract is weighed and kept in a tight container protected from light [9].

Fig. 1. *Melastoma malabathricum Linn.* plant

Fig. 2. Water-based emulsion

2.3. Phytochemical screening

In a test of tannin, 0.5 g of the extract is heated in 10 mL of distilled water and filtered. The filtrate was diluted with distilled water until it was colourless and adds 1-2 drops of $FeCl_3$ 1% to form a blue/dark blue or blackish-green colour [10].

2.4. Stability test at different temperatures and pH

10g of an emulsion 1% MB extract mixture of was stored for 21 days for rapid stability tests. For this purpose, the samples were kept at different temperatures: 4 °C, 25 °C (at room temperature) and 58 °C. At the same time, 10g of an emulsion 1% MB extract was stored at room temperature, 25 °C. The pH of the sample was adjusted to pH 5, 6, 7, and 8, respectively by using 0.3M HCl and 0.1M NaOH solution [3]. These tests were to observe the change of colour by using a chromameter (Konica Minolta Chroma meter), pH by using a pH meter and viscosity by using a rheometer (HAAKE RheoStress 6000) during the storage period.

2.5. Antibacterial activity

The agar diffusion method was used to determine bacterial inhibition activity. Petri dishes containing nutrient agar (Tryptone Soya Broth) were inoculated with bacteria (*Escherichia coli*). Then, filter paper disks with a diameter of 6mm, containing MB extract and mixture of water-based extract were placed on the agar surface. The petri dishes were then incubated at 37°C for 24 h and observed for growth or inhibition. The diameter of zone inhibition presented in discs is recorded [1].

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3. RESULT AND DISCUSSION

3.1. Phytochemical screening

The colour change of MB extract solution from yellow to blackish-green colour (Fig. 3b). The colour change of MB extract solution from yellow to deep yellow colour then gradually becomes colourless showed the presence of flavonoids (Fig. 4c). *Melastoma Malabathricum Linn* leaves extract was found to contain tannins and flavonoids. Tannins can help protect the skin from damage caused by free radicals [5]. Free radicals are unstable molecules that can damage cells and contribute to the ageing process. According to Shamsudin et al. [6], pyrogallol structure in the flavonoids is an important structural feature for the manifestation of good antibacterial activity. Pyrogallol work by disrupting the cell membrane of bacteria which cause them to die [11].

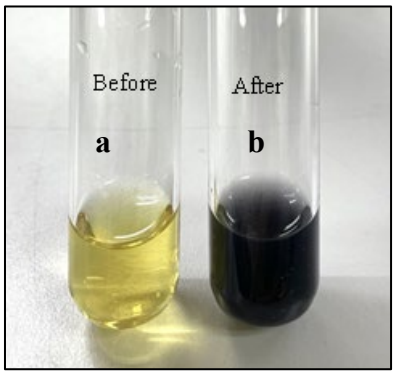

Fig. 3. Melastoma extract before add $FeCl_3$ (a) and presence of tannins after add $FeCl_3$ (b)

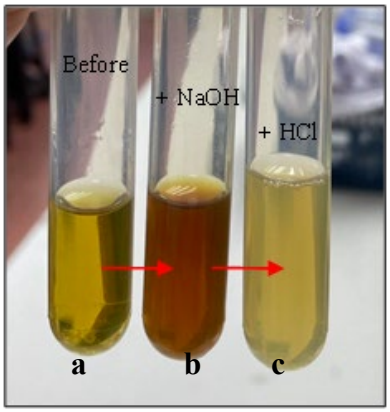

Fig. 4. Melastoma extract before add NaOH (a), after add NaOH (b) and the presence of flavonoids after gradually add HCl (c)

3.2. Stability test at different temperatures

The stability test for water-based emulsions MB extract was examined and it was found that the emulsion MB extract was stable at storage conditions (4 °C, 25 °C and 58 °C) for 21 days. Table 1 showed the value pH of emulsion MB extract for these three conditions was between 6.47-6.65. According to Muhammad et al. (2014) pH of human skin typically ranges from 4.5 to 6 but for skin care product like moisturizer, the common pH range between 5-7 [12]. Therefore, the emulsion MB extract must be in moisturizer pH range because this range is preferable for dry and sensitive skin [13].

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The viscosity of emulsion MB extract at these different storage conditions is represented in Fig. 5. Viscosity is a useful process to indicate the quality and check the stability of the emulsion [14]. The viscosity of emulsion MB extract maintained stable conditions for 21 days without liquefaction. However, the emulsion MB extract at 4 °C showed an increase in viscosity while the emulsion MB extract for 58°C had the lowest viscosity after 21 days. Increasing the temperature would decrease the viscosity of the emulsion MB extract. Baily and Davey [15] stated that high temperatures can cause an increased frequency of droplet collisions due to an increase in kinetic or thermal energy. Therefore, when the attractive binding energy in the emulsion decreases, the viscosity decreases. Meanwhile, the viscosity increases due to the less mobile of molecules in the oil droplets at lower temperatures, so they are more likely to stick together.

Table 1: Stability test of water-based emulsion 1% MB extract and without extract at 4 °C, 25 °C and 58 °C for 21 days.

Temperature			0 Day	7 Day	14 Day	21 Day
4 °C	pH	WOE	6.58	6.35	6.41	6.62
		WE	6.47	6.31	6.38	6.65
	Viscosity	WOE	4.804	4.073	5.073	5.8
		WE	4.615	4.758	5.327	5.334
	Colour	WOE	white	white	white	white
		WE	yellowish	yellowish	yellowish	yellowish
25 °C	pH	WOE	6.58	6.55	6.61	6.49
		WE	6.47	6.53	6.43	6.53
	Viscosity	WOE	4.804	4.740	4.72	4.729
		WE	4.615	4.453	4.886	4.668
	Colour	WOE	white	white	white	white
		WE	yellowish	yellowish	yellowish	yellowish
58 °C	pH	WOE	6.58	6.44	6.6	6.45
		WE	6.47	6.36	6.5	6.6
	Viscosity	WOE	4.804	4.5	5.381	5.514
		WE	4.615	3.817	4.527	4.159
	Colour	WOE	white	white	white	white
		WE	yellowish	Olive-brown	Olive-brown	Brown

* WOE = without extract, WE = with extract

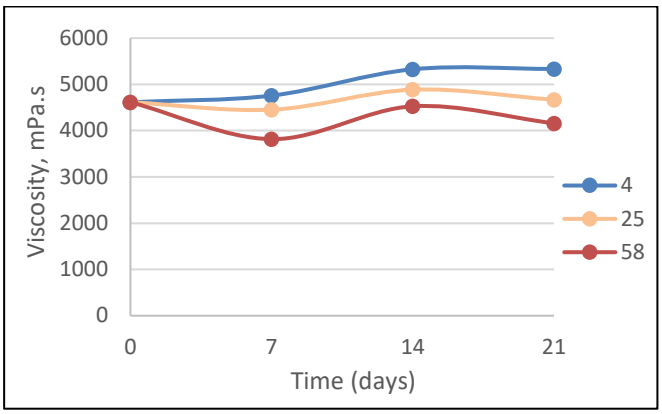

Fig. 5.Viscosity of emulsion MB extract at 4 °C, 25 °C and 58 °C

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The colour of the emulsion MB extract after 21 days showed yellowish colour for samples that store at 4 °C and 25 °C. Meanwhile, the sample at 58 °C became brown colour after 21 days. The colour of the sample changes at high temperature due to the degradation of chlorophyll in the MB extract. When chlorophyll is exposed to high temperatures, it can be degraded [16]. The loss of the green colour in the degradation process of chlorophyll results in the formation of other pigments, such as pheophytin. Pheophytin is a brown pigment that is responsible for the brown colour [17]. The colour change has been shown in Fig. 6a, 6b, 6c and 6d.

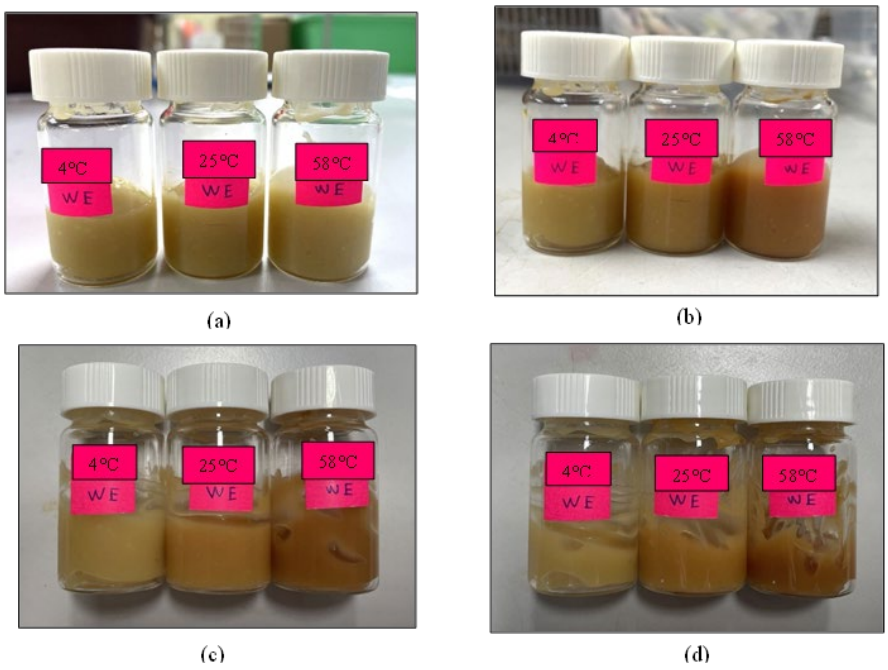

Fig. 6. (a) Colour of emulsion at initial. (b) Colour of emulsion after 7 days. (c) Colour of emulsion after 14 days. (d) Colour of emulsion after 21 days.

3.3. Stability test at different pH

At different values of pH 8,7,6 and 5, the viscosity was vary (Fig. 7). At pH 8 and 7, the viscosity was higher compared to acidic samples, pH 6 and 5. Viscosity at pH 6 and pH 5 become lower due to the presence of water when the sample mix with the HCl. Ingredients in emulsion like polyoxyethylene make the hydroxyl groups (-OH) react with the hydrogen ions (H+) of HCl to form ester which then loses a water molecule [18]. pH 8 and 7 had higher viscosity because the interaction between NaOH and surfactant in the emulsion can cause the surfactant molecule to clump together [19]. All samples of emulsion MB extract at pH 8,7 and 6 stable during storing period because still in range of standard lotion which is 2000-50000 mPa.s except sample at pH 5 [20].

The sample of emulsion MB extraction for pH 8,7,6 and 5 was stored at 25 °C (room temperature). The colour of the sample at pH 8,7,6 and 5 on day 21 slightly changed from the initial (Fig. 8a and 8b).

3.4. Antibacterial test

On the antibacterial activity test, the MB extract, and emulsion MB extract showed no inhibition zone against *E. coli*. This illustrated that *Melastoma malabathricum Linn* leaves were not effective in inhibiting the growth of *E. coli* bacteria (Fig. 9). The concentration of the

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extract in the water-based emulsion is 1%. The result studied by Fares et al. [21] showed that no inhibition zones were found for the lower concentrations (25% or 50%) of the extract. Therefore, the concentration must be more than 50% of the extract or higher. Other than that, *E. coli* is gram-negative bacteria. According to Biswas et al. [10], the Gram-negative bacteria's resistance can be attributed to its unique cell wall structure, which includes an effective permeability barrier. This barrier comprised a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding plant extract. The study from Cortis Maigoda & Nisak, [22] stated that the antibacterial action of *Melastoma malabathricum Linn* leaves extract was more effective against Gram-positive bacteria than Gram-negative bacteria.

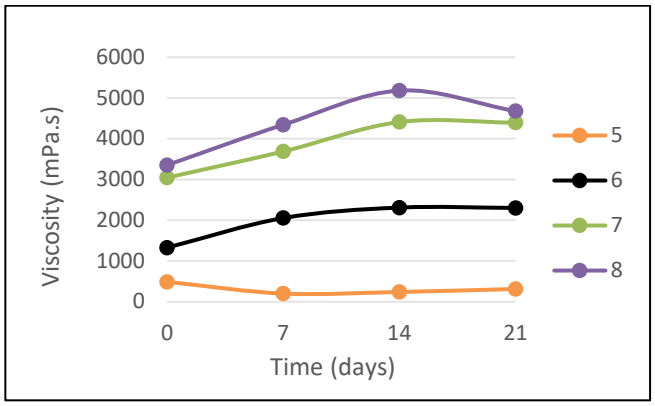
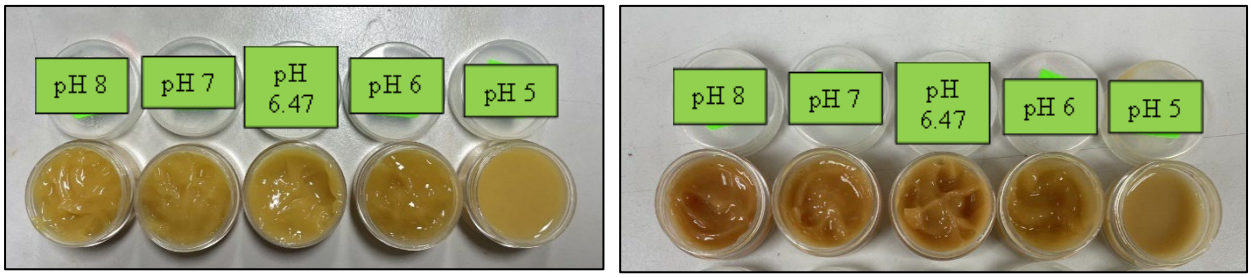


Fig. 7.Viscosity of emulsion MB extract at pH 8,7,6,5



(a) (b)

Fig. 8. (a) Colour emulsion at different pH at initial. (b) Colour emulsion at different pH after 21 days

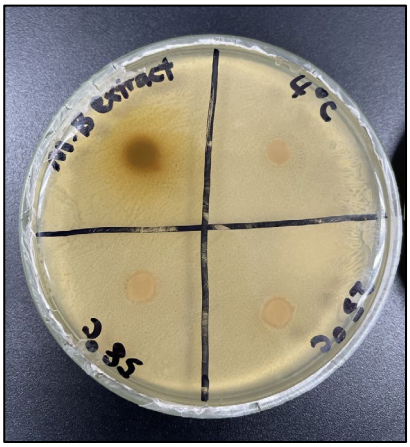


Fig. 9. Emulsion MB extract against E. coli.

4. CONCLUSION

This study showed that an emulsion containing 1% *Melatoma Malabathricum* (MB) extract maintained its viscosity, pH, and colour stability at various storage conditions for 21 days. No significant change in pH indicates that it is safe to use on the skin. The colour of emulsion MB extract at 4 °C after 21 days did not change from its initial colour. The suitable pH for the emulsion MB extract that maintained its viscosity for 21 days was pH 6. The antibacterial test showed no inhibition zone in MB extract and emulsion MB extract against Escherichia coli. From the above study, the most stable emulsion MB extract was the emulsion at pH around 6 and to keep its effectiveness of emulsion must be stored at 4 °C.

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