Jurnal Kejuruteraan 38(3) 2026: 1397-1406
https://doi.org/10.17576/jkukm-2026-38(3)-30

Optimization and Characterization of Stability and Bacterial Inhibition Activity of *Melastoma Malabathricum L.* leaf Plant Microwave Extract in a Water-Based Emulsion

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Received 5 August 2025, Received in revised form 18 February 2026
Accepted 18 March 2026, Available online 30 May 2026

ABSTRACT

The Melastoma Malabathricum L. plant contains multiple bioactive components that can inhibit the growth of bacteria and was traditionally used to cure various diseases. The main objective of this study was to optimize the extraction of Melastoma Malabathricum L. leaf by using microwave technology at varying ethanol concentrations and microwave powers. Additionally, the study aimed to characterize the stability of the extract at various temperatures and their ability to inhibit the growth of bacteria Escherichia coli (E. coli) in water-based emulsion. The extraction was done using different ethanol concentration (50%, 70%, and 95%) with varying microwave power level (300 W, 400 W, and 600 W) has been set to gain the extract. The stability of the extracts in water-based emulsion was determined by observing the pH value, color changes, and viscosity after 14 days of storage at different temperatures (4 °C, 25 °C, and 58 °C). The antibacterial activity of the extracts in water-based emulsion was tested using paper disc diffusion method against Escherichia coli (E. coli) as the bacteria. Based on the result of this study, ethanol with 95% concentration and 400 W microwave power used yields the highest amount of extract by 12.93%. The pH value and the viscosity of the extract in water-based emulsion decreases as temperature increases, and the colour turns darker when stored at high temperature of 58 °C. The biggest diameter of bacterial inhibition zone was measured at 11 mm which was obtained from the sample containing the extract (95%, 300 W). The result concludes that the extraction of Melastoma Malabathricum L. leaf by using microwave technology at various ethanol concentration and microwave power has been optimized, and the stability of the extracts at various temperatures and its ability to inhibit bacterial activity in water-based emulsion has successfully characterized.

Keywords: Melastoma Malabathricum leaf extract; microwave extraction; stability test; bacterial inhibition activity; water-based emulsion

INTRODUCTION

The *Melastoma Malabathricum L.* plants or "senduduk" is a common herb that can be found in the tropics such as shrubs, where it is mostly available in India, Thailand, and Malaysia (Zheng et al. 2021; Susanti et al. 2008). This species exhibits at least three distinct sizes of flowers: large, medium and small. The blooms are dark purple-magenta, light pink-magenta, and a very rare white colour. This herb contains saponins, the second metabolites in the plant, defending it from pathogens and herbivores. Saponins have various properties including antibacterial properties (Sparg et al. 2004). Hence, making it extensively utilized in the pharmaceutical industry today.

There are numerous extraction techniques available nowadays such as ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), enzyme-assisted extraction (EAE), and including microwave-assisted extraction (MAE). The conventional method, such as maceration, heating, boiling, or refluxing usually time taking to extract the active components and it causes the loss of bioactive constituents due to oxidation, hydrolysis, and ionization (Sitoresmi et al. 2016). The benefits of using microwave extraction are environmentally friendly, effective, requires shorter extraction time, and use lower temperatures (Purbowati & Maksum, 2019). In this present study, the microwave extraction method is used by varying the solvent concentration and microwave power level.

In the extraction process, the solvent used needs to be relevant to the method of extraction, which in this case is microwave extraction method. Polarity plays an important part in extracting the extract as much as it can. The use of a highly polar solvent could produce a high yield of extracts, however, the phenolic and flavonoid content will be low compared to the non-polar solvent (Nawaz et al. 2020). Ethanol was chosen compared to methanol as the microwave power could be absorbed by the methanol and eventually cause toxicity to humans' application (Hemwimon et al. 2007). Ethanol was known for its non-toxic properties, high polar solvent, and commonly used for extraction.

Ensuring the stability of the extract is crucial as it can impact the product's quality under varying storage conditions. There are two factors that can influence the extract's stability: external factors such as temperature and humidity, and internal factors, for instance, the extract's properties. In this study, temperature was manipulated during storage to observe the change on pH value, viscosity, and colour changes when the extract was incorporated into the water-based emulsion. Any changes in pH value can cause the formulation to be either acidic that causes dryness or alkaline which leads to irritation to the users. The viscosity needs to be maintained to ensure consistency of the emulsion does not affect due to the varying temperatures. In pharmaceutical and cosmetic industry, appearance like colour plays an important role to ensure the colour remain constant regardless of the storage conditions.

Critical factors must be taken into account during the selection of water-based emulsions, including the choice of emulsifiers, stabilisers, and manufacturing processes. Emulsifiers are critical constituents that maintain the stability of the interface between immiscible liquids, thereby preventing phase separation and guaranteeing the creation of a cohesive emulsion (Danila et al. 2019). To ensure the quality and efficacy of a product, manufacturing techniques such as high-pressure homogenization and ultrasonication are frequently utilised to attain a consistent distribution of droplet sizes. The optimization aspects mentioned above hold significant importance in the advancement of water-based emulsions in diverse sectors such as cosmetics, pharmaceuticals, and food (Fransiska et al. 2021). In these industries, the emulsion's stability, texture, and overall effectiveness are directly influenced by the choice of emulsifiers, stabilisers, and manufacturing techniques.

Nowadays, numerous methods in assessing antibacterial activity has been introduced including the paper disc diffusion method (Agyare et al. 2012; Baydar et al. 2004). This method has been well-known and mostly used in microbiology laboratories to analyze the bacterial inhibition activity of plant extracts. It has many advantages compared to other methods as it is simple, low cost, able to test colossal amount of microorganisms and antimicrobial agents, and the results are straightforward (Poikulainen et al. 2020). In this case, samples will be tested for its ability to inhibit the bacteria growth, which is *Escherichia coli, E. coli* by analyzing the diameter of inhibition zone. *E. coli* is chosen for its simplicity and ease to be studied in the laboratory.

The objective of this research is to optimize the extraction of *Melastoma Malabathricum* leaf using microwave technology at various ethanol concentrations and microwave powers. Other than that, this study aimed to characterize the stability of the extract at various temperatures and its ability to inhibit the growth of bacteria *Escherichia coli (E. coli)* in water-based emulsion.

METHODOLOGY

MATERIALS

The main material used in this study was fresh *Melastoma Malabathricum* leaf which has been collected at Pahang. Other materials used were ethanol, distilled water, and

water-based emulsion (cream base for cosmetic use only) which has been purchased from local vendor. The fresh leaves were washed with clean water and dried at 80 °C using an oven for average of 3 hours. The dried leaves were then hand-crushed into flakes form of average sizes of 0.5 mm as shown in Figure 1. Then, it was kept in the refrigerator for the next use.

(a)

(b)

FIGURE 1. *Melastoma Malabathricum L.* leaves (a) before drying (b) after drying

EXTRACTION

The extraction was conducted by using a microwave (Panasonic, NN-ST651M) with varying concentrations of ethanol (50%, 70%, and 95%) and microwave power (300 W, 400 W, and 600 W). The ratio used for the extraction is 1:10 w/v where 10 g of dried *Melastoma Malabathricum L.* flakes were mixed with 100 mL of ethanol in a beaker. The slurry was radiated in the microwave for 70 seconds and then cooled to room temperature. Next, the slurry was first filtered by using a strainer and then using filter paper (Whatman No. 1) to ensure all residues were removed. After that, the solvent in the sample was evaporated by using rotary evaporator at 70 °C. Lastly, the sample was then stored in airtight bottles in the refrigerator at 4 °C for future use. The percentage yield of the extract was determined using Equation (1):

$$Percentage\ yield\ of\ extract\ (\%) = \frac{x}{y} \times 100\% \quad (1)$$

where: x is the weight of extract and y is the weight of soaked plant material. The value was calculated and presented as percentage yield (%) (Fiardilla & Warsiki, 2020).

DETERMINATION OF SAPONINS

A total of 1 mL sample was added with 20 mL distilled water and vigorously shaken for 2 minutes. A layer of foam about one centimeter was formed on the liquid surface and was persistent for 15 minutes. This indicates that saponins were present in the samples (Gul et al. 2017;Rao et al. 2016).

DETERMINATION OF THE EFFECT OF TEMPERATURE ON STABILITY

In order to observe the stability of the extract in water-based emulsion, a ratio of 1:100 (w/w) was used, which approximately 1% of each extract was mixed with 10 g of the emulsion for each sample. After thorough mixing, the mixture was stored at three distinct temperatures: 4 °C, room temperature of 25 °C, and 58 °C for 14 days. This was done to evaluate the changes of the sample in terms of pH value, color, and viscosity after 14 days at different temperatures (Rahman & Zamanhuri, 2023). The pH test was to determine its acidity or alkalinity by using a pH meter (FP-20, Mettler-Toledo, Switzerland). The viscosity of emulsion products, particularly emollient products, is an essential parameter. The viscosity of a substance indicates its thickness as determined by a viscometer (RVDV-11+P, Wells Brookfield, United States of America). Color was evaluated with a colorimeter (CR-400, Konica Minolta, Tokyo, Japan) and results were expressed according to the lightness of the color (L*).

SCREENING OF BACTERIAL INHIBITION ACTIVITY

The observation of antibacterial activity of the sample was performed by paper disc diffusion method (Baydar et al. 2004). Petri dishes containing nutrient agar (Tryptone Soya Broth) were inoculated with bacteria *(Escherichia coli)*. Then, a filter paper disc with diameter about 6 mm was soaked into each of the samples and placed on top of the agar surface. The water-based emulsion without extract

has been set as the control. The petri dishes then were sealed, inverted and incubated at 37 °C for 24 hours. The bacterial inhibition activity was determined by measuring the diameter of the inhibition around the paper disc (Che Omar et al. 2013).

RESULTS AND DISCUSSION

EFFECT OF ETHANOL CONCENTRATION AND MICROWAVE POWER ON TOTAL YIELD OF EXTRACT

The factors affecting the microwave extraction of *Melastoma Malabathricum L.* leaf extract were chosen as ethanol concentrations and microwave power. Ethanol concentration was adjusted by adding distilled water, using concentrations of 50%, 70%, and 95%, and microwave power levels of 300 W, 400 W, and 600 W were applied. The extraction time has been set constant at 70 seconds based on the prior studies. Based on Figure 2, the ethanol concentration of 95% at 400 W yielded the most extract, while 50% yielded the least which were 12.93% and 9.27%, respectively. At 300 W, 70% ethanol concentration yields 9.81% of extract, while 95% yields 6.84% of extract, which lower than the value of 70% ethanol concentration. This shows that the ethanol concentration affects the percentage yield of the extract. The percentage yield of extract increases as the solvent concentration increases. This is due to the polarity of the solvent and the contents in the extract. Ethanol is a high polar solvent while *Melastoma Malabathricum L.* extract contains flavonoids that are semi polar, hence, it helps to produce high percentage of yield's extract.

Microwave power was also chosen as the factor that affects the percentage yield. Based on Figure 2, 50% ethanol concentration at 300 W gives a percentage yield of 9.14% that is lower than at 400 W which was 9.27%. Similarly goes to 70% concentration, the power of 400 W yields more than power of 300 W. This shows that an increase in microwave power will increase the percentage yield. Similar behaviour had been observed on investigation of optimization of phenols extraction from roselle (*hibiscus sabdariffa*) by microwave assisted extraction (Sitoresmi et al. 2016). The observed phenomenon can be attributed to the fact that increased microwave power induces a greater impact of microwave energy on biomolecules via ionic conduction and dipole rotation. Consequently, power is dissipated within the solvent and plant material, leading to the generation of molecular motion and heating (Marsoul et al. 2020).

According to Figure 2, the yield's extract percentage for 50% ethanol concentration at three distinct microwave power continues to increase, unlike 70% and 95% concentration. This can be assumed that the sample is not yet reaching the degradation point as low concentration of ethanol was used, hence it increased. However, this condition differs for 70% and 95% concentration, which the percentage yield's extract decreases when the microwave power was set at 600 W. This is due to the degradation of heat sensitive compounds in the extract. It can be concluded that 600 W is sufficient enough to destroy the cell wall of the *Melastoma Malabathricum L.* leaf at 70% and 95% concentration.

Thus, the relationship between microwave power and ethanol concentration has shown a correlation with the percentage yield of extract. An increase in both microwave power level and ethanol concentration results in a decrease of the percentage yield's extract. At higher microwave power levels (400 W to 600 W), the rate of yield's extract decelerates in comparison to lesser power levels (300 W to 400 W). This implies that increasing the power level further might not result in a substantial increase in yield of extract.

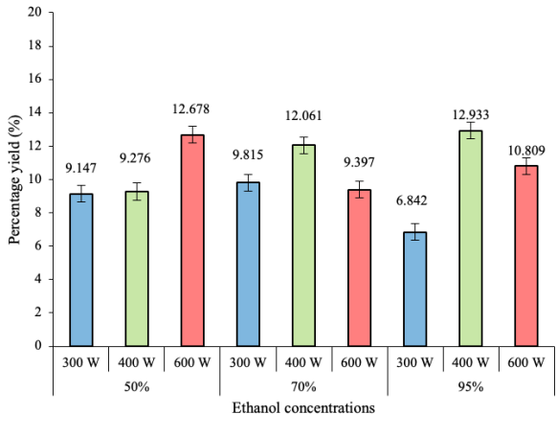


FIGURE 2. Yield's extract profile of *Melastoma Malabathricum L.* leaf obtained by microwave extraction as a function of the microwave power and ethanol concentration

SAPONINS PRESENCE

Saponins was present in the extract as 1 cm layer of foam formed during the test and was persistent for 15 minutes as shown in Figure 3. This is similar to findings with researchers, where persistence of soap-like foam for about 5 minutes indicated the presence of saponin in Rambutan leaf extract (Mohamed et al. 2021). Saponins are widely known for its antibacterial properties to inhibit the growth of bacteria, fungi, or microorganisms and it is commonly used in the pharmaceutical industry.

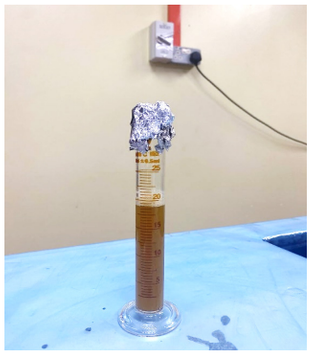


FIGURE 3. Saponins presence

Saponins are great to be used for the treatment of skin problems. It has been demonstrated that saponins possess antifungal, antibacterial, and anti-inflammatory properties, among others. The prospective applications of these properties in agriculture and medicine are currently under investigation.

STABILITY TEST: EFFECT OF TEMPERATURE ON PH VALUE

Table 1 shows pH values before and after storage for 14 days at selected temperatures: in refrigerator (4 °C), at room temperature (25 °C), and in incubator (58 °C). In this case, used concentrations of 50%, 70%, and 95%, along with microwave power levels of 300 W, 400 W, and 600 W. Before the experiment, the pH was below 7.5 under all conditions, except for the 95% absolute ethanol concentration at 400 W and 600 W, indicating a slightly high value of 7.54. The acceptable pH range of human skin is between 4.5 to 7.5 (Fransiska et al. 2021). According to Table 1, only one sample was within the acceptable pH range of 7.29 to 7.49 at three different temperatures, and it was sample 70%, 300 W. This shows that the water-based emulsion sample was stable to be kept at any temperature without drastically changing the pH value and it was safe to be used on the skin (Hanifah & Jufri, 2018). The results of the pH test on all samples of combination extract of *Clitoria ternatea* flower and dragon fruit peels showed that they met the standard not less than 4.5 and not more than 6.5. The purpose of the pH test is to ascertain whether the formulations is suitable for application on the skin (Ginting et al. 2022).

Initially, the measured pH before was mostly at the acceptable range. However, after 14 days, the pH value of each sample varied due to the temperature conditions. Any lower than the range is acidic which might cause dryness, while any higher than the range is alkaline which can cause irritation. The pH level of skin care products is an essential determinant in preserving the skin's health and stability. This acidic environment maintains the skin's barrier function and protects against harmful microorganisms. It is advised that, when selecting skin care products, one opts for formulations whose pH value is in close proximity to that of the skin's natural pH. An excess of alkaline products have the potential to disturb the acid layer of the skin, resulting in symptoms such as dehydration, irritation, and an elevated susceptibility to infections (Ginting et al. 2022). On the contrary, substances characterized by an acidic pH level may also induce irritation and inflammation. It is advisable to seek guidance from a dermatologist or a reputable skin care professional in order to ascertain the optimal pH range that is appropriate for one's particular skin type and concerns.

TABLE 1. pH value before and after 14 days storage of *Melastoma Malabathricum L.* extract in water-based emulsion

Properties		pH before	pH after at different temperature		
Conc. (%)	MP (W)		4°C	25°C	58°C
50	300	7.44	7.83	7.57	7.22
	400	7.44	7.71	7.57	7.34
	600	7.34	7.49	7.51	7.36
70	300	7.31	7.49	7.48	7.29
	400	7.27	7.5	7.57	7.39
	600	7.24	7.57	7.57	7.44

continue...

...cont.

	300	7.49	7.8	7.79	7.68
95	400	7.54	7.87	7.82	7.68
	600	7.54	7.89	7.86	7.7

STABILITY TEST: EFFECT OF TEMPERATURE ON VISCOSITY

Viscosity is part of the observation for stability test for changes in the consistency of the extract in water-based emulsion. As mentioned by Fransiska et al. (2021), the standard viscosity value for lotion is 2000-50000 cP (centipoise) which converted to 2-50 Pas (Pascal second). Based on Figure 4, it can be seen that the viscosity decreases over higher temperature, in this case the temperature was 58 °C. The (50%,300 W) and (70%, 600 W) sample shows a drastically change in consistency of the water-based emulsion when at high temperature. All of the *Melastoma Malabathricum L.* leaves extract in water-based emulsion met the standards, however sample (70%, 600 W) and (50%, 300 W) were unacceptable due to its drastic change in viscosity. Sample (50%, 300 W) as a viscosity of 10.85 Pa.s on day 0 and was decreased to 4.68 Pa.s on day 14. The high temperature emits high energy which causing the bond to break. The decrease in viscosity might also be due to the stability of the extract in which the extract was not stabled to maintain the consistency of the water-based emulsion (Wang et al. 2003;Xiao et al. 2009).

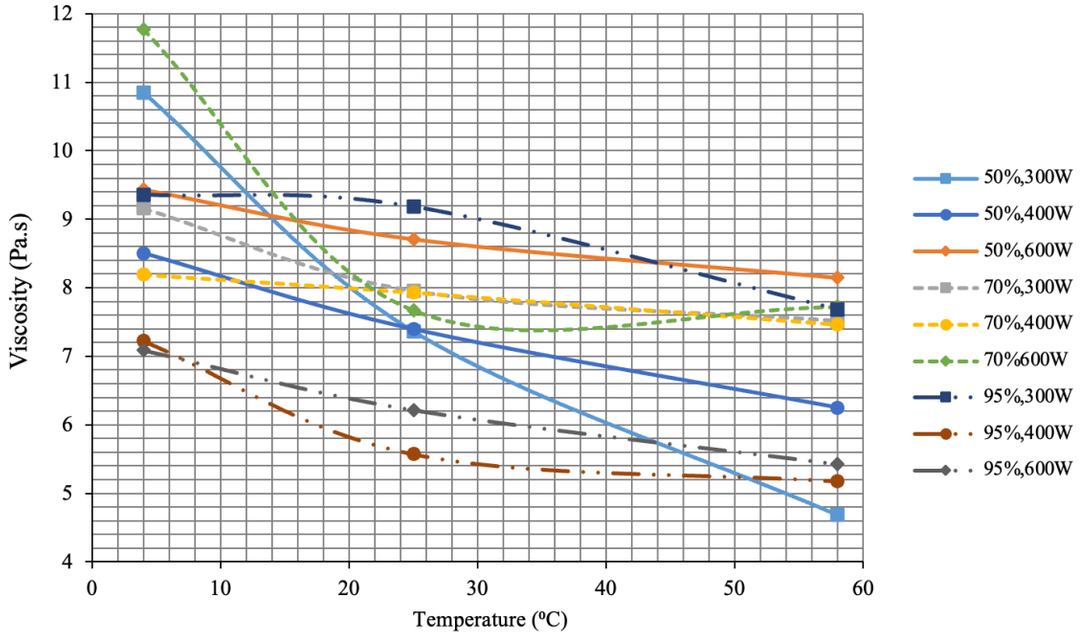
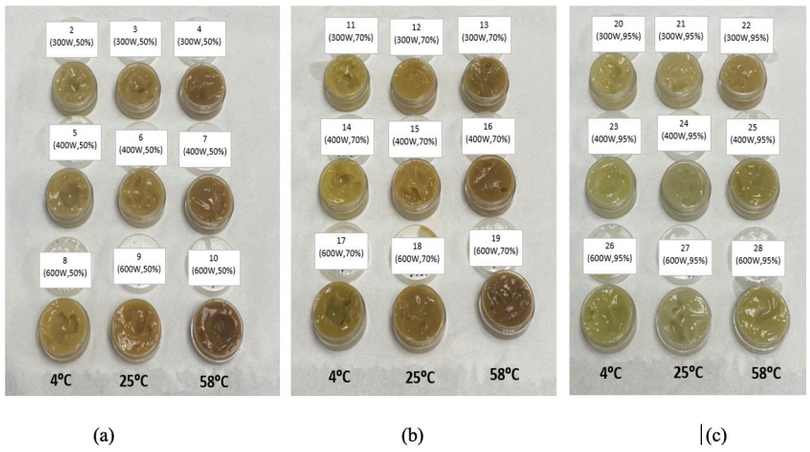


FIGURE 4. Viscosity of the *Melastoma Malabathricum L.* leaves extract in water-based emulsion after 14 days

STABILITY TEST: EFFECT OF TEMPERATURE ON COLOR CHANGES

Color changes were part of the stability test. A spectrophotometer determines the spectral properties of a colour, whereas a colorimeter assesses its visual appearance. The color intensity was obtained by using colorimeter. The data that has been considered was L*, which indicates the luminosity (white to black). L* value indicates lightness of *Melastoma Malabathricum L.* leaves extract in water-based emulsion from day 0 to day 14. The color of the samples was significantly different than the control sample where most of them were darker after being kept at different temperatures.



(a) (b) (c)

FIGURE 5. Real colours of *Melastoma Malabathricum L.* leaves extract in water-based emulsion after 14 days

The real color of the samples was shown in Figure 5. The L* axis is a gray scale with values from 0 (black) to 100 (white). The original water-based emulsion without extract has been used as control, 40.22. By referring to Figure 6, the lowest L* value was 20.46 at sample (70%, 600 W) at 58 °C which has the darkest color due to degradation of chlorophyll since it has exposed at high temperature (Rahman & Zamanhuri, 2023). The phenomenon of extracts undergoing colour degradation, resulting in a transition to a darker hue, has been extensively documented in scientific research. Elevated temperatures induce chemical reactions in extracts, which have the potential to modify both their chemical composition and physical characteristics. The degradation process frequently entails the disintegration of delicate compounds present in the extract, leading to the destruction of chlorophyll that contributes to the darkening effect of the colour .

In industries where product quality and appearance are of the utmost importance, such as food, pharmaceuticals, and cosmetics, it is vital to comprehend the effects of temperature on extract stability. In order to maintain the integrity of extracts and prevent undesired colour changes, researchers in these disciplines must meticulously monitor and regulate temperature conditions. Additional research examining the specific mechanisms of deterioration across diverse temperature ranges is imperative in order to enhance optimization and characterization of stability and bacterial inhibition activity of *Melastoma Malabathricum L.* leaves plant microwave extract in a water-based emulsion.

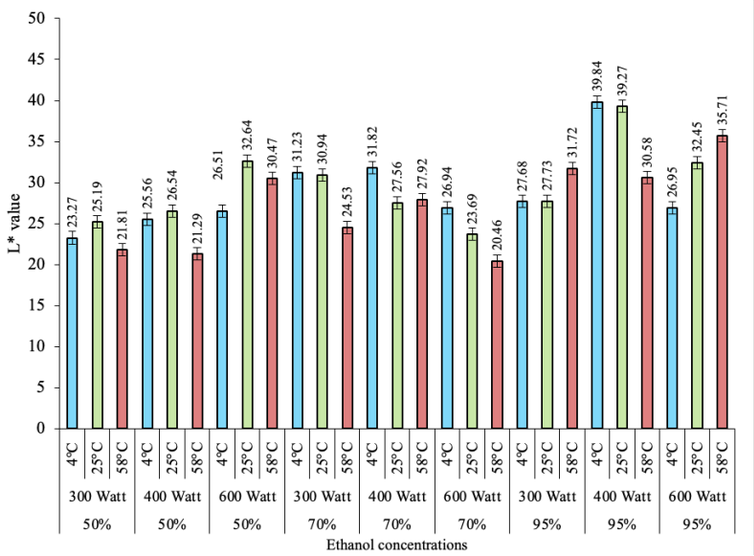


FIGURE 6. L* (brightness) value of *Melastoma Malabathricum L.* leaves extract obtained by microwave extraction as a function of the temperature, microwave power and ethanol concentration

OBSERVATION ON BACTERIAL INHIBITION ACTIVITY OF PLANT EXTRACT IN A WATER-BASED EMULSION

The bacterial inhibition activity was tested against *E. coli* which has been incubated with the samples for 24 hours at 37 °C. Water-based emulsion without extract has been used as control in this observation. Based on Figure 7, the zones of inhibition with extract ranged from 8 mm to 11 mm while without extract ranged from 7 mm to 8 mm. The biggest diameter of inhibition zones was sample (95%, 300 W) as in Figure 8 with 11 mm diameter. The control samples show inhibitory effects on the *E. coli* as there was a ring formed around the disc, however, the water-based emulsion with extracts were more powerful as it increases the inhibition activity of the bacteria by having a bigger diameter of zone inhibition (Marsoul et al. 2020). Thus, it can be postulated that the extract (95%,300 W) has high inhibition activity to inhibit bacterial activity from growing.

The findings suggested that the antimicrobial activity of the *Melastoma Malabathricum L.* leaves plant microwave extract was significantly diminished as a consequence of the water-based emulsion. Significantly, in comparison to without plant extract, the water-based emulsions demonstrated enhanced effectiveness, as evidenced by the increased inhibition zones that surrounded the discs when water-based emulsions were present up to 25%. In addition, the study showed how important water-based emulsion are for making antimicrobial agents more bioavailable and effective. In general, the results show that emphasised water-based emulsions may be able to improve the antimicrobial properties of plant extracts from *Melastoma Malabathricum L.* leaves. This is important information for the progress of antimicrobial methods in many areas.

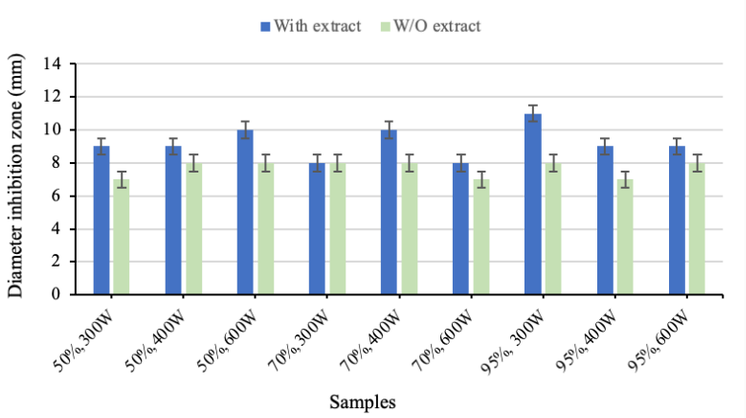


FIGURE 7. Antibacterial activity zone of inhibition (mm) of *Melastoma Malabathricum L.* leaves extract in in a water-based emulsion

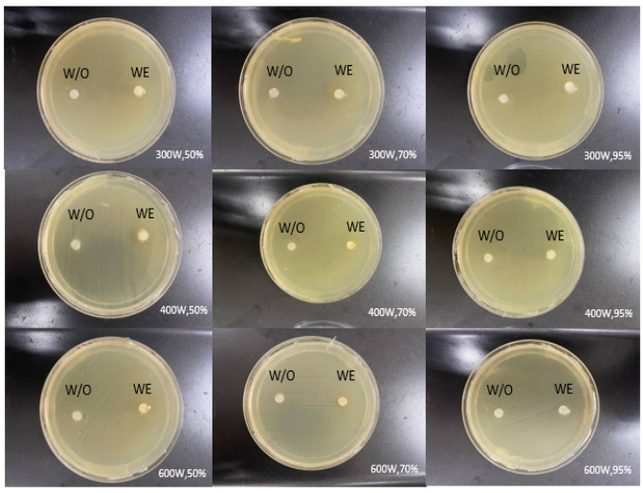


FIGURE 8. Antibacterial activity test against *Escherichia coli (E. Coli)*

CONCLUSION

In conclusion, the optimization of microwave extraction with various concentration and microwave power has been identified. The characterization of the stability of the *Melastoma Malabathricum L.* extract at various temperatures and its ability to inhibit bacterial growth in the water-based emulsion has been observed. The most effective condition for achieving the highest amount of extraction was by applying a moderate microwave power of 400 W and utilizing 95% pure ethanol concentration as high power could damage the cell wall of the extract. The most suitable sample for human's application was sample (70%, 300 W) as the pH value is within the given range during pH test for all the conditions tested. All of the extract in water-based emulsion sample has met the standard viscosity of the lotion, except for sample (70%,600 W) and (50%, 300 W). The color for all samples has significant changed depending on the temperature conditions and the darkest is sample (70%, 600 W) due to degradation of chlorophyll content in the extract at high temperature. Last but not least, the biggest inhibition zone with 11 mm diameter was from sample containing extract (95%, 300 W). Overall, the objectives of this research have been achieved successfully.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support received from Ministry of Higher Education of Malaysia through MYRA-PHD (600-RMC/GPM LPHD 5/3 (065/2023)), Research Management Centre (RMC) and School of Chemical Engineering, Universiti Teknologi MARA, Shah Alam, Selangor, Malaysia.

DECLARATION OF COMPETING INTEREST

None.

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