

EVALUATION OF STABILITY, TANNIN CONTENT, ANTIOXIDANT ACTIVITY, AND ANTIMICROBIAL PROPERTIES OF *Melastoma Malabathricum* Linn. LEAF EXTRACT IN A WATER-BASED EMULSION

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ABSTRACT: *Melastoma malabathricum* (Linn.) (MML) is a small tree native to Southeast Asia, traditionally used in folk medicine to treat a variety of ailments, including hemorrhoids and wounds from childbirth. The leaves of MML contain several phytochemical constituents, including tannins, which are known for their antimicrobial and antioxidant properties. These properties position MML as a promising alternative to traditional antibiotics, especially in combating drug-resistant pathogens like *Staphylococcus aureus*. Traditionally, MML leaves are applied as a paste to wounds to serve as a topical antibiotic. However, this approach has proven to be impractical, prompting interest in more conventional formulations such as lotions and emulsions. To keep pace with the increasing reliance on herbal medicine, this study evaluates the potential of MML leaf extract in water-based emulsions, focusing on tannin content, antimicrobial and antioxidant activities, and emulsion stability. The crude extract was obtained using two methods—Soxhlet Extraction Method (SEM) and Cold Extraction Method (CEM)—to determine which method has the higher potential to produce the most effective antimicrobial agent. Both methods used a solvent mixture of ethanol, methanol, and water in a 70:20:10 ratio. After rotary evaporation, the yields of crude extract were 4.98 grams (SEM) and 3.62 grams (CEM), respectively. The extraction efficiency of CEM was higher in terms of tannin yield (31.63% more than SEM), while SEM exhibited superior antioxidant activity (66.94% higher than CEM). In addition, the stability tests indicated that the emulsions should be stored at 30°C to prevent dissociation, suggesting a temperature-dependent stability factor. For antimicrobial activity, the study tested emulsions containing 3%, 5%, and 7% crude leaf extract, with the 7% concentration exhibiting the most promising antimicrobial properties. Based on the results of both the extraction methods and emulsion testing, the 7% emulsion could serve as an effective formulation for developing alternative topical treatments.

KEY WORDS: *Melastoma, Leaves, Extraction, Medicinal, Water-Based Emulsion*

1. INTRODUCTION

Traditional herbal medicine, derived from plants, is widely embraced in various countries, including Malaysia, due to the rich pharmacological properties of medicinal plants. Over 80% of the global population relies on plants for basic healthcare needs, driven by growing

scepticism towards modern medicine, which is often costly, limited in availability, and has potential adverse effects. As a result, researchers are proactively turning to plant-based solutions, seeking cost-effective, safe, and sustainable medicinal alternatives with fewer side effects and a slower development of resistance [12].

Multidrug resistance occurs when bacteria, fungi, or other microorganisms undergo evolutionary changes that enable them to resist the effects of antibiotics, such as *Staphylococcus aureus*. This poses a substantial global health risk, as it can render antibiotic treatments ineffective, complicate infection control, and increase the likelihood of transmission [5]. In response, the scientific community has focused efforts on developing new antibiotics to counteract multidrug resistance. Simultaneously, there is growing recognition of the potential of herbal medicine as an effective alternative in combating multidrug-resistant pathogens, thanks to their rich phytochemical compositions and natural antimicrobial properties.

Melastoma malabathricum (Linn.), a shrub native to Southeast Asia, is known by various names, including Senduduk, Singapore Rhododendron, Indian Rhododendron, and Malabar Gooseberry. This small tree is found in tropical and monsoonal climates, including China, Australia, Singapore, Indonesia, Malaysia, and other Southeast Asian countries (Fig. 1). The leaves, flowers, and fruits of *Melastoma malabathricum* (Linn.) are the primary ethnobotanical features, though this study focuses on the leaves due to their greater availability. In Indonesia, Malaysia, and India, these leaves have been used for centuries to treat ailments such as leucorrhoea, dysentery, haemorrhoids, diarrhoea, and confinement wounds from childbirth [8].



Fig. 1: The shrub *Melastoma malabathricum* (Linn.)

Traditionally, the leaves of *Melastoma malabathricum* (Linn.) (MML) were applied as a paste onto wounds to prevent scarring and promote healing [11]. However, despite the long history of its use in folk medicine, there is limited scientific evidence supporting its potential as a source of antioxidant and antimicrobial agents. This knowledge gap has hindered the broader development and application of MML leaves for therapeutic purposes.

Recent studies have confirmed that MML leaves contain key phytochemicals, including tannins, flavonoids, and alkaloids, which are known for their antimicrobial and antioxidant properties [10, 24]. Tannins, which are polymeric phenols (e.g., ellagitannins), have demonstrated antimicrobial and antidiarrheal properties [17]. While much research has focused on the antioxidant properties of saponins, tannins are also known to possess similar benefits [2]. Focusing on extracting tannins from the leaves allows for a more targeted approach for investigation. To our knowledge, no studies have yet optimized the

antimicrobial and antioxidant efficacy of tannins derived from MML leaves. Exploring these properties could lead to the development of a stable, effective, and strong water-based emulsion, offering a more convenient alternative to traditional pastes [16].

In this work, we will investigate the antimicrobial properties and stability of MML leaf extract when incorporated into a water-based emulsion. Additionally, we will measure the tannin content and assess the antioxidant activity of the leaf extract. By combining these evaluations, we hope to provide a comprehensive understanding of the potential medicinal and functional properties of MML leaf extract in the form of a water-based emulsion lotion.

2. METHODOLOGY

2.1 Materials

The apparatus required for this study includes a laboratory grinder, glass beaker, aluminium foil, filter paper (Whatman No. 1), UV-Visible spectrophotometer, Soxhlet apparatus, micropipette, analytical balance, petri dishes, rotary evaporator, sterile swabs, and an incubator.

The materials needed for this research include *Melastoma malabathricum* leaves, 200g of store-bought water-based emulsion, 300 mL of 99% methanol, 600 mL of 99% ethanol, water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), nutrient agar, mannitol salt agar, Folin-Ciocalteu reagent, tryptone soya broth, and sodium carbonate (Na_2CO_3). All chemicals used are of analytical reagent grade to ensure that the research meets standard protocols, thereby enhancing the credibility of the results.

2.2 Pre-treatment of *Melastoma malabathricum* (Linn.) leaves

The leaves were collected from Selangor, Malaysia, and thoroughly washed with running water, followed by distilled water, to remove any dirt and contaminants. For the cold extraction method [1], the leaves were dried in a controlled, air-conditioned environment at 18°C. In contrast, for the conventional Soxhlet extraction method, the leaves were air-dried at room temperature. After drying, the leaves were ground into a fine powder to increase the surface area, facilitating more efficient extraction. As shown in Fig. 2, the total weight of the powdered leaves obtained after the pre-treatment process for the Soxhlet method was 60 g.



Fig. 2: Grounded *Melastoma malabathricum* (Linn.) leaves

2.3 Extraction of Leaves

In this study, two extraction methods were used: Soxhlet extraction and cold extraction. Both methods utilized the same solvent mixture in a ratio of 70% ethanol, 20% methanol, and 10% water [1].

2.3.1 Soxhlet Extraction Method

The Soxhlet extraction of *Melastoma malabathricum* leaves was carried out by first preparing 10 grams of powdered leaves, which were wrapped in filter paper and securely tied at both ends with thread. This wrapped material was then placed in the thimble holder of the Soxhlet apparatus. A solvent mixture consisting of 99% ethanol, 99% methanol, and water in a ratio of 70:20:10 was prepared, and 300 mL of this mixture was poured into the round-bottom flask of the Soxhlet apparatus. As the apparatus was heated to 80°C, the solvent vaporized, rose, and condensed, filling the thimble with fresh solvent. The extraction process was conducted for 6 hours, and as the solvent passed through the thimble repeatedly, it extracted compounds from the leaves. The process continued until the solvent droplets in the siphon tube became colorless, indicating that the extraction was complete.

2.3.2 Cold Extraction Method

The powdered leaves were soaked in a solvent and stored in an enclosed jar for 72 hours. The mixture was shaken periodically to ensure thorough extraction. Afterward, it was filtered through Whatman No. 1 filter paper to separate the solid matter. A portion of the filtered solution was then concentrated using a rotary evaporator at 40°C. The resulting crude leaf extract was analyzed for tannin content and antioxidant activity. These results were compared with those obtained from the Soxhlet extraction method to evaluate the differences in extraction efficiency and effectiveness.

2.4 Water-Based Emulsion Characterizations

The extracted leaves were incorporated into a water-based emulsion and tested in two categories: antimicrobial activity and stability. The tests were conducted on three different concentrations of the leaf extract (3%, 5%, and 7%), with each concentration added to 10 grams of emulsion.

2.5 Quantitative Assay of Tannin Content

Tannin compounds will be quantified calorimetrically using a combination of saturated sodium carbonate (Na_2CO_3) and Folin-Ciocalteu reagent. The absorbance will be measured with a UV-Visible Spectrophotometer at a modified wavelength of 750 nm. Standard solutions of tannic acid will be prepared by dissolving it in leaf extract at concentrations of 2%, 4%, 6%, and 8%. To each solution, 10 mL of sodium carbonate and 5 mL of Folin-Ciocalteu reagent will be added. Distilled water will then be added to each solution to bring the total volume to 100 mL. The mixture will be transferred into labeled test tubes and allowed to react for 40 minutes. A blank solution will be prepared by adding 10 mL of sodium carbonate and 5 mL of Folin-Ciocalteu reagent to 85 mL of distilled water, without any leaf extract. The absorbance of the standard tannin solutions will be measured under identical conditions, and a standard curve will be generated for quantification.

2.6 Antimicrobial Assay

2.6.1 Preparation of broth

The pathogens used for the antimicrobial assay were *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative) [17]. Each bacterium was cultured for 24 hours at 37°C in Tryptone Soya Broth (TSB) to allow for optimal growth. After incubation, a bacterial suspension was prepared to be used for inoculation onto agar plates.

2.6.2 Preparation of agar

Mannitol Salt Agar (MSA) plates were prepared for inoculation. The agar plates were autoclaved and prepared according to standard procedures, ensuring that they were ready for bacterial inoculation.

2.6.3 Inoculation and application of emulsion

Under sterile conditions in a laminar flow hood, a 1000 µL micropipette was used to inoculate the bacterial suspension onto the surface of MSA plates. The inoculated plates were left to dry briefly in a sterile environment. After the plates dried, paper disks were placed onto the agar surfaces, each soaked with water-based emulsion at different concentrations (3%, 5%, and 7%).

2.6.4 Incubation and measurement

The inoculated and treated plates were then incubated at 37°C for 24 hours. After incubation, the diameters of the inhibition zones surrounding the discs were measured to assess antibacterial activity and confirm the antimicrobial effects. For reference, see Fig. 3 in the appendix for an image of the Mannitol Salt Agar (MSA) agar before incubation.

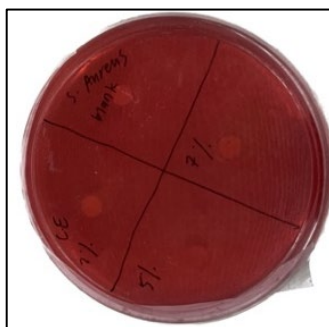


Fig. 3: MSA agar before incubation

2.7 Antioxidant Assay

2.7.1 Preparation of DPPH solution

A 24 mg quantity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder was dissolved in 100 mL of methanol to produce a 0.6 mM DPPH solution, resulting in a dark violet solution [2].

2.7.2 Sample dilution

The CEM and SEM samples were prepared by diluting them into a series of concentrations: 2%, 4%, 6%, and 8% each in 10 mL of solvent. For each concentration, 2 mL was set aside as a blank sample and 2 mL was transferred in a test tube to be mixed with DPPH solution.

2.7.3 DPPH addition and incubation

To the remaining volume of each sample, 8 mL of the DPPH solution was added. These prepared samples were then fully covered with aluminum foil to protect them from light and incubated at 37°C in a dark room for 30 minutes.

2.7.4 UV-Visible Spectrophotometry

After incubation, the absorbance of the reaction solution was measured at 515 nm using a UV-Visible Spectrophotometer. The percentage of antioxidant activity for each sample was calculated using the following formula, where A symbolizes absorbance reading from the UV-Visible Spectrophotometer [3]:

$$\% \text{ scavenging activity} = \left(\frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \right) \times 100\% \quad (1)$$

2.8 Stability Test

The base emulsion and three other emulsions incorporating different extract concentrations (3%, 5%, and 7%) were subjected to two stability tests, which are thermal and colour test.

2.8.1 Thermal Test

The thermal test was conducted in a water bath. The samples were heated in duplicate at three different temperatures: 30°C, 50°C, and 70°C. The emulsions were visually observed if there were any changes in texture or colour [3].

2.8.2 Colour Test

A colour test was conducted to quantitatively analyze the changes. A colorimeter was used to measure the colour changes in the water-based emulsion after exposure to the various temperature tests. The L*, a*, and b* values of the emulsions were recorded and discussed [14].

3. RESULTS AND DISCUSSION

The extraction of 10 grams of leaves using 300 mL of a combined and ratioed polar solvent mixture yielded different results from two methods. The Soxhlet extraction method produced approximately 4.98 grams of dark green extract, while the cold extraction method yielded 3.62 grams of sage green extract. Further analyses are discussed below.

3.1 Crude Leaf Extract

Both extraction techniques used a mixture of three polar solvents to extract compounds from the plant leaves, and these solvents were combined and found to be soluble. The extraction method can influence the physical properties of the crude leaf extract, as heat in the Soxhlet extraction method can affect solubility [1]. Therefore, selecting the appropriate solvent is crucial. Refer to Fig. 4, for the results of crude leaf extract obtained from the Soxhlet and cold extraction methods.

The Soxhlet extraction method yielded more extract (4.98 grams) compared to the cold extraction method (3.62 grams), with a yield difference of approximately 31.63%. This suggests that Soxhlet extraction is more efficient for obtaining components from leaves and is considered the most effective method for extracting crude oil from leaves [17]. However, further analysis is needed to identify the phytochemical constituents, such as tannins, in the extracts from both methods.

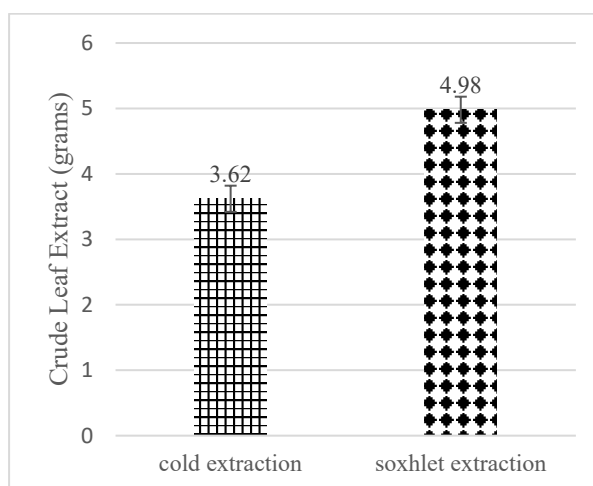


Fig. 4: Yield of different extraction methods

3.2 Tannin Content Analysis

This study confirms the presence of tannin in MML leaf extract. The extraction method significantly influences the quantity of tannins extracted from the herbal leaf [13]. To quantify the tannin content, a standard curve was constructed with a linear regression equation ($y=16.05x+0.2954$) (refer Fig. 5). This high R^2 value of 0.9942, indicates that the data is highly reliable and can be used to compare the two extraction methods. In the equation, y represents the absorbance of the sample, and x refers to the Tannic Acid Equivalent (TAE). The tannin content of the samples was calculated using this equation. The results of the tannin content for both cold extraction and Soxhlet extraction are shown in Fig. 6.

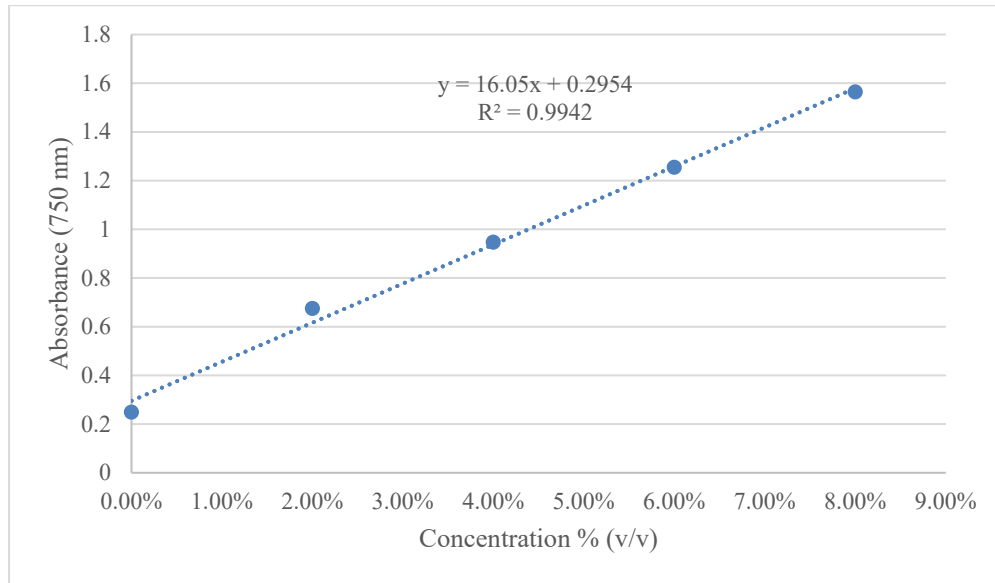


Fig. 5: Tannic acid standard curve

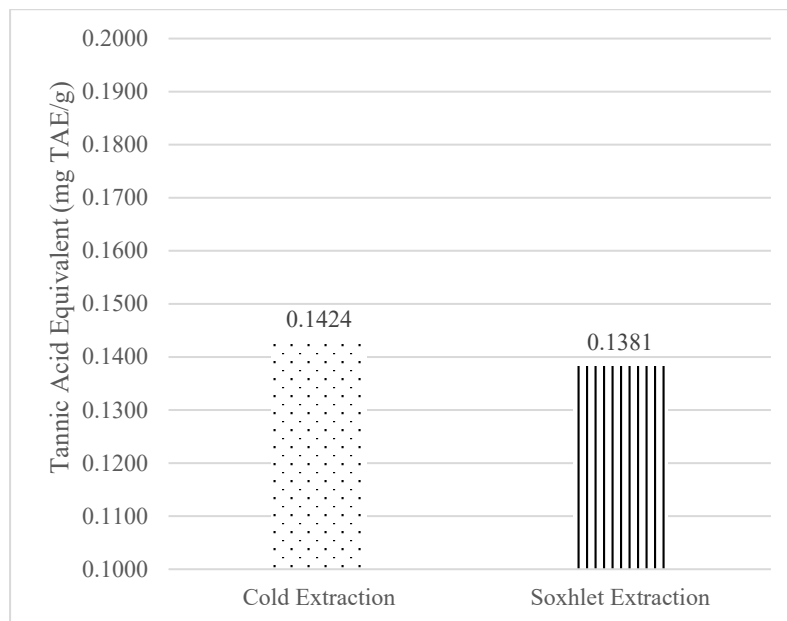


Fig. 6: Tannin content of each extraction methods

The cold extraction method yielded a higher tannin content than the Soxhlet extraction method. A t-test of two samples was used in Microsoft Excel to see if there were statistically significant differences in the amount of tannins in the two extraction methods (cold extraction and Soxhlet extraction). However, the difference between the two methods was relatively small, at only about 3.05% ($p < 0.05$); it may not be statistically significant. Nevertheless, the higher tannin content in the cold extraction method is consistent with previous research, which has shown that cold extraction is more effective than Soxhlet for extracting tannins and saponins [20]. These compounds play a crucial role in the antibacterial activity of the extracts, making cold extraction a promising method for obtaining potent antimicrobial agents.

3.3 DPPH Radical Scavenging Activity (%RSA)

The findings of this analysis focus on the antioxidant potential of each extraction method. The results of the analysis are presented in Table 1 and Table 2, while the graph is shown in Fig. 7. The highest scavenging activity for the Soxhlet method was 99.01%, while highest for cold method was 49.36%. A percentage difference in antioxidant activity between cold and Soxhlet methods is 54.65%. This significant difference in scavenging activity highlights varying antioxidant capacities, validating the obtained data.

Table 1: Soxhlet extraction %RSA data

Concentration (%v/v)	Absorbance @ 515 nm	%RSA
0	0.5943	0.0000
2	0.0776	86.940
4	0.0415	93.010
6	0.0378	93.640
8	0.0059	99.010

Table 2: Cold extraction %RSA data

Concentration (%v/v)	Absorbance @ 515 nm	%RSA
0	1.1082	0.0000
2	1.0305	7.0114
4	0.7161	35.3817
6	0.6825	38.4136
8	0.5612	49.3593

In Fig. 7 below, the cold extraction method demonstrated lower radical scavenging activity than the Soxhlet extraction method. The graph for the cold extraction method shows a significant increase in antioxidant activity between the 2% to 4% and 6% to 8% concentrations. In contrast, the Soxhlet extraction method shows a rapid increase in %RSA at 2%, followed by a steady increase from 4% to 8%. This indicates that as the concentration of the extract increases, the percentage of radical scavenging activity also increases [7].

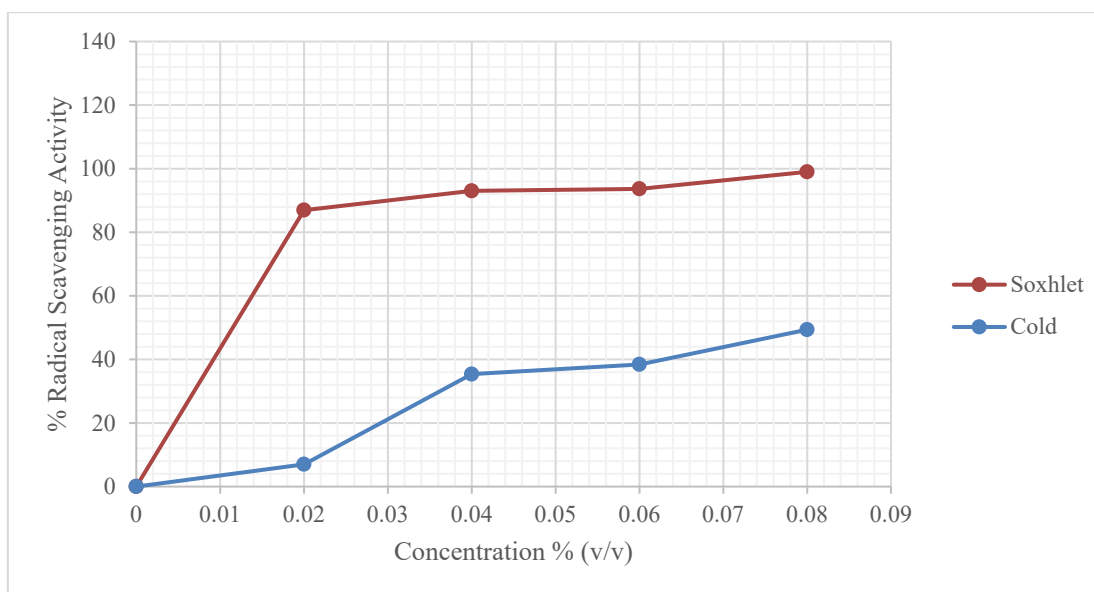


Fig. 7: DPPH radical scavenging activity on different extraction methods

Various medicinal plants in Malaysia have shown high scavenging activity, with *S. Barbata*, a plant indigenous to Malaysia, demonstrating the highest radical scavenging activity at 99.6%, validating that a significantly high %RSA is achievable [6]. Although the cold extraction method yielded a higher amount of tannins, other phenolic compounds such as flavonoids and alkaloids may contribute to the higher %RSA observed in the Soxhlet extraction method. The total phenolic content in the Soxhlet method was found to be higher than in the cold extraction method, likely due to the greater presence of alkaloids in the Soxhlet extract [1]. This finding is supported by previous studies, where the Soxhlet extraction procedure using the DPPH method yielded the highest antioxidant activity and phenolic content [4].

3.4 Thermal and Colour Stability

This test focuses on the stability of the emulsion in terms of color degradation due to thermal changes and how different extraction methods affect the thermal behavior of emulsions. The thermal test was conducted on all six emulsions, which were prepared using two distinct extraction methods at three different concentrations. Fig. 8 (a) and (b) shows the emulsion from the cold extraction method and the emulsion from the Soxhlet extraction method. Table 3 below presents the physical data of the emulsions before the thermal test. After the thermal test, color analysis was performed, and the results for L^* , a^* , and b^* values are presented in Fig. 10 through 12.

Table 3: Data before thermal test

Concentration (%v/v)	Cold Method	Soxhlet Method
Temperature	25°C	25°C
Colour (7%)	Dark Green	Pale Green

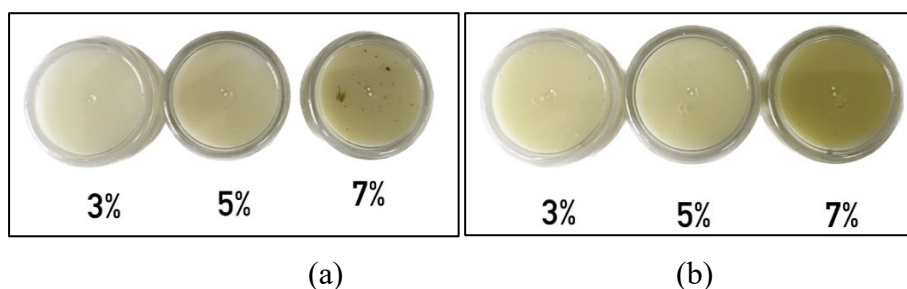


Fig. 8: (a) Soxhlet extraction emulsion and (b) cold extraction emulsion

The L value in color analysis represents lightness on a scale from 0 (black) to 100 (white) [14]. As shown in Fig. 9, at 30°C, the lightness of the emulsion remains the same for all methods and concentrations. The cold extraction method exhibits lower lightness than the Soxhlet extraction method. Additionally, the graph shows that as the concentration increases, the difference in L values decreases, indicating that the emulsion becomes darker with higher concentrations.

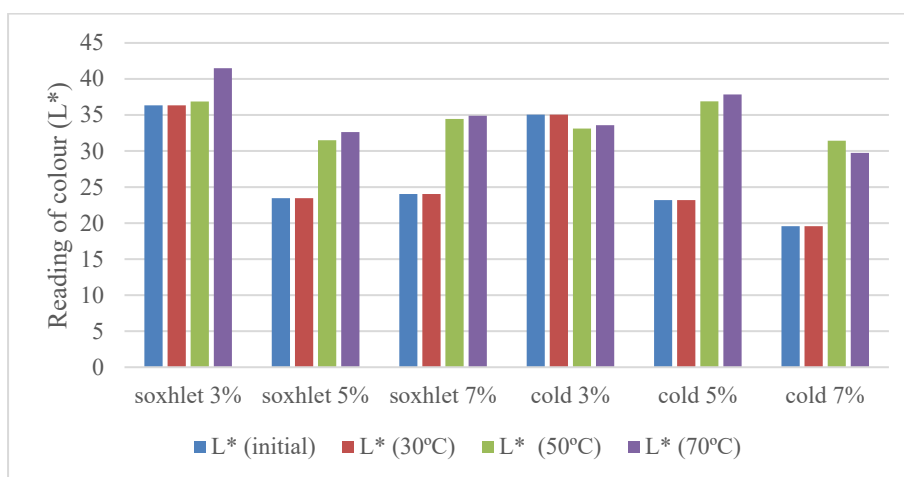


Fig. 9: L* Differences after temperature test

The average L* value of the emulsion is 31.6, indicating that it is 31.6% closer to black than to white (68.4%). In both extraction methods, the data began to deviate inconsistently after 50°C. These inconsistent deviations may be caused by the watery texture of the water-based emulsion, which suggests instability. The wet or watery texture could create a reflective surface that scatters light differently than a dry surface, leading to variations in the L value [19].

The a* value indicates the red-to-green hue, with +a* representing red and -a* representing green [14]. As shown in Fig. 10, the initial values for the 3% extract in both extraction methods showed a negative a value, indicating a greenish hue. At 5%, the a value increases as the temperature rises, which suggests that the red hue intensifies, and the color of the emulsion darkens. Additionally, the b value represents the yellow-to-blue hue [14].

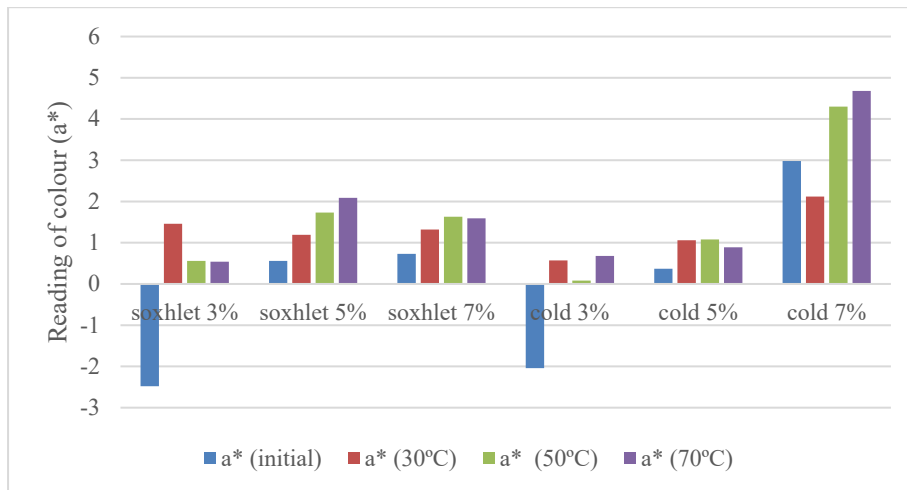


Fig. 10: a* Differences after temperature test

In Fig. 11, the b* value indicates the yellow-to-blue hue, with +b* representing yellow and -b* representing blue. At 3% Soxhlet extract, the yellow hue increases as the temperature rises, suggesting that the extracts become more saturated with yellow as they are exposed to higher temperatures. The most saturated yellow color is observed at 7% extract after undergoing the 70°C thermal test. In conclusion, the color changes exhibited by the emulsion after each temperature test indicate that heat causes instability in the emulsion [25], especially at higher extract concentrations [3]. Therefore, it is recommended to keep the emulsion below 30°C to prevent degradation.

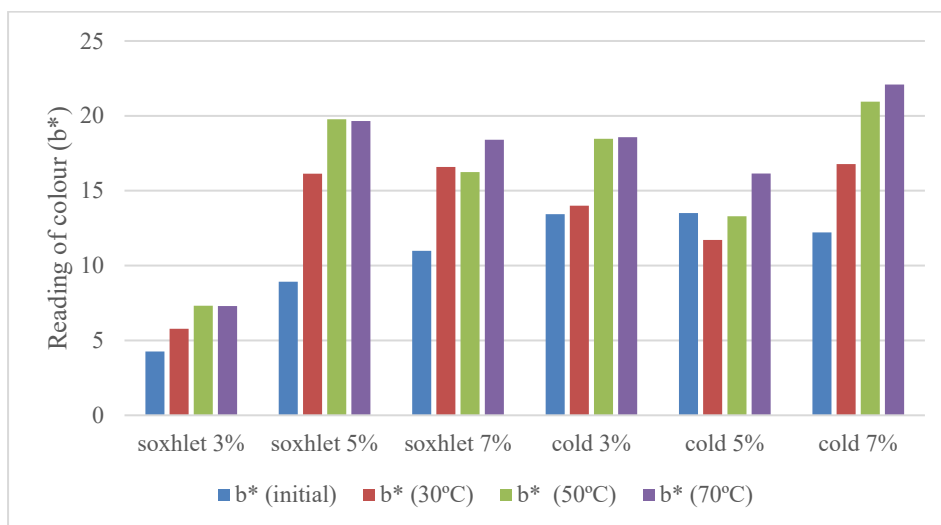


Fig. 11: b* Differences after temperature test

3.5 Antimicrobial Analysis

This test focuses on the microbial susceptibility of cold and Soxhlet extract at different emulsion concentrations.

As seen in Fig. 3, the Mannitol Salt Agar (MSA) agar changed from red to yellow (see Fig. 13 (a) and (b)), indicating the presence of *Staphylococcus aureus* [15]. Table 4 below presents the data from the results. After 24 hours, smaller inhibition zones were observed

with the Soxhlet extraction method (SEM) compared to the cold extraction method (CEM), as shown in Fig. 13 and 14.

Table 4: Data of inhibition zones of different pathogens by different extraction methods

Extract	Pathogens	Inhibition Zones (mm)			
		Control	3% Extract	5% Extract	7% Extract
Cold Extraction Method	<i>Staphylococcus Aureus</i>	0	0	±20	±50
	<i>Escherichia Coli</i>	0	0	±10	±10
Soxhlet Extraction Method	<i>Staphylococcus Aureus</i>	0	0	±10	±10
	<i>Escherichia Coli</i>	0	0	±0	±5

This difference is likely due to the higher presence of tannins in the cold extract compared to the Soxhlet extract [9]. This finding aligns with the results of [1], which showed that cold extraction exhibited higher bacterial resistance than Soxhlet extraction. The consistency across these studies reinforces the idea that extraction methods significantly influence the antimicrobial efficacy of plant extracts. The control disc containing only water-based emulsion showed no resistance to either pathogen, as expected.

As shown in Fig. 12, the largest inhibition zone was observed for *Staphylococcus aureus*, with a 50 mm zone at 7% cold extract, whereas the highest inhibition zone for Soxhlet extract was 10 mm. For *Escherichia coli*, the cold extract produced a 10 mm inhibition zone, while the Soxhlet extract resulted in a 5 mm inhibition zone.

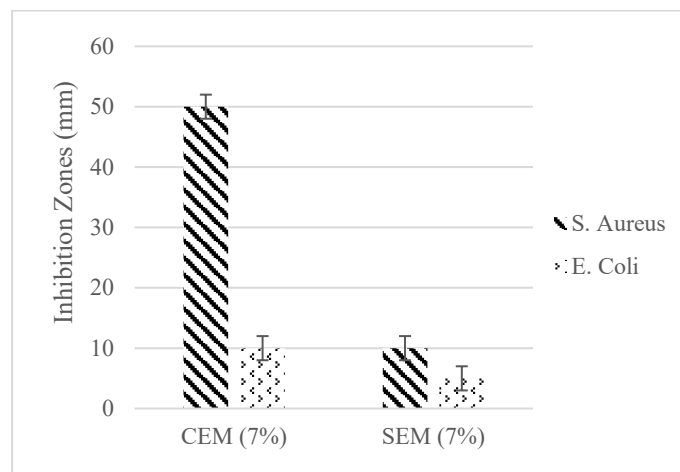


Fig. 12: Inhibition Zones of 7% Extracts

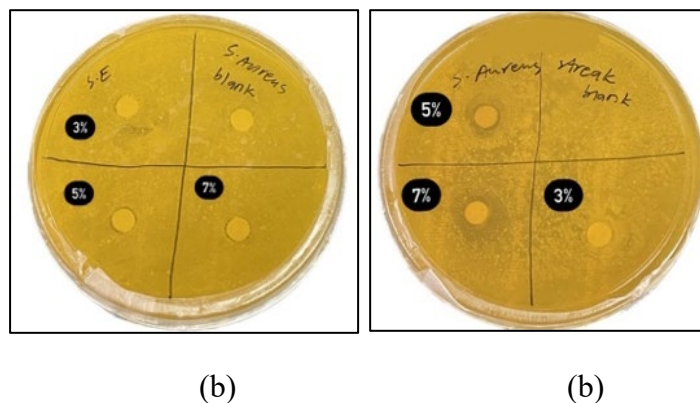


Fig. 13: *S. aureus* inhibitory region of (a) Soxhlet extraction method (SEM) and (b) cold extraction method (CEM)

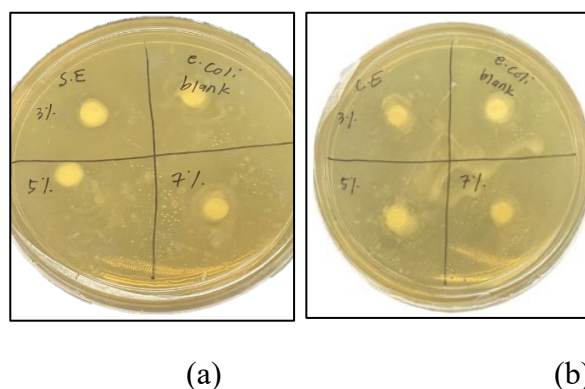


Fig. 14: *E. coli* inhibitory region of (a) Soxhlet extraction method (SEM) and (b) cold extraction method (CEM)

This indicates that the gram-negative pathogen exhibits more resistance to the plant extract compared to the gram-positive pathogen [23]. The Soxhlet extract shows a 43.33% smaller inhibition zone than the cold extract. This suggests that tannin activity is higher in the gram-positive pathogen [6]. This finding is important for the development of natural antimicrobial agents for pharmaceutical or food preservation purposes. Based on these results, it can be concluded that higher concentrations of cold extract lead to increased resistance against pathogens.

4. CONCLUSION AND REMARKS

The study concludes that Soxhlet extraction is superior in yield and antioxidant activity, whereas cold extraction is more effective for antimicrobial applications due to its higher tannin content. These findings emphasize the importance of selecting appropriate extraction methods based on specific bioactive properties for applications in healthcare and pharmaceuticals. Further research is needed to evaluate the effects on various microbes, particularly gram-negative bacteria like *Salmonella spp.*, to determine whether the lotion is effective or exhibits no effect, as seen with *E. coli*. Additionally, exploring other phenolic groups, such as alkaloids, flavonoids, and saponins, is recommended to understand the mechanisms underlying these differences and expand the applications of *Melastoma*

malabathricum. Future studies should keep integrating traditional knowledge with modern scientific methods to fully realize the plant's potential for innovative and sustainable herbal medicine solutions.

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