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Original Research Article

Formulation of Microemulsions Containing Rambutan Peel Extract and Their Antibacterial Activities

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ABSTRACT

Nowadays, numerous pathogens have become increasingly resistant to antibiotics. To address this issue, it is important to undertake additional research to develop alternative antibacterial agents. The goal of this investigation was to fabricate microemulsions from rambutan (*Nephelium lappaceum* L.) peel extract and test their antibacterial activity. The cytotoxicity of the rambutan peel extract was studied. The other assessments were conducted on the visual, physical, and electrical properties of microemulsions, which include their appearance, phase separation, viscosity, and conductivity. Moreover, the research also examined the potential antibacterial effects of microemulsions against both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria by exploring their ability to inhibit bacterial growth. The findings indicated that even when the extract was used at the highest concentration (100 µg/mL), there were no cytotoxic effects on skin keratinocyte cells. Using the pseudoternary phase diagram, a blend of rosemary oil, water, and a combination of surfactant (Tween® 80) and co-surfactant (Ethanol) were employed to create microemulsions containing different quantities of rambutan peel extract. According to the results, a stable microemulsion was observed as the ratio of Tween® 80 and ethanol was higher than 38%. The microemulsions containing extract at concentrations of 1% w/w, 5% w/w, and 10% w/w were clear and transparent, with no phase separation. All formulations were physiochemically acceptable. Microemulsions containing 1% w/w – 10% w/w rambutan peel extracts were shown to be efficient in suppressing only gram-positive *S. aureus*.

Keywords: microemulsion, rambutan peel, extract, antibacterial, cytotoxicity

Introduction

Nephelium lappaceum L., belonging to the Sapindaceae family, is commonly known as rambutan. This fruit is broadly cultivated in countries across Southeast Asia, such as Thailand, Myanmar, Malaysia, Singapore, Indonesia, and the Philippines.[1] It has now expanded to Asia, Africa, Oceania, and Central America. When harvested, the rambutan fruit has a bright red or yellow color. However, the peel and spinterns may become darker over time during storage. The edible pulp of the fruit is obtained by peeling the skin, which leaves the fruit peel as a by-product. Importantly, these by-products are produced in large quantities as a result of increased consumption and fruit processing, resulting in environmental problems caused by improper disposal. Rambutan peels, on the other hand, contain beneficial bioactive substances like flavonoids, phenolic acids, and polysaccharides.[2,3]

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Phuong and colleagues reported the phenolic content of rambutan peel methanolic extract, which included quercetin, ellagic acid, geraniin, corilagin, and rutin.[4] Studies showed, that rumbutan peel extracts exhibit anti-inflammatory, antioxidant antimicrobial, antibacterial, anti-osteoporosis, antiphotoaging, antiproliferative, antihyperglycamic and antidiabetic properties.[5] The rambutan peel contains phenolic compounds that display notable antibacterial effects against various types of bacteria. These bacteria include gram-positive varieties such as *Bacillus subtilis*, *Listeria monocytogenes*, and *Staphylococcus aureus* (*S. aureus*), as well as gram-negative types like *Salmonella* Typhi, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, *Escherichia coli* (*E. coli*), and *Proteus vulgaris*.[4] On the other hand, in accordance with the finding from Tadthong and co-workers, the rambutan peel extract showed no impact on the gram-negative bacteria *E. coli*. However, it exhibited bactericidal properties against *S. aureus* and methicillin-resistant *S. aureus* and *Streptococcus mutans*.[6] Other studies have also confirmed that the rambutan peel extract only affects gram-positive bacteria.[3,7] Although rambutan extract has been shown to be beneficial, the extracts may contain compounds that are poorly soluble. Additionally, many natural products can be unstable and prone to degradation over time. To address these issues, the use of alternative delivery systems may significantly improve the efficacy of extracts by enhancing their solubility and stability.

New discoveries about microemulsions for numerous uses are being made as nanotechnology advances. Recently, a number of research articles have been carried out with the aim of exploring the creation,

characterization, and application of these systems, owing to their numerous applications in various fields of sciences and technologies.[8] Specifically, in the pharmaceutical field, the most typical applications of microemulsions are for delivering drugs or active ingredients to the targeted drug delivery approach, e.g., via oral, parenteral, or topical routes. A microemulsion can be defined as a clear, transparent, or translucent liquid-liquid dispersion system that is highly dispersed and low in viscosity, and is spontaneously formed from the combination of oil, water, surfactant, and co-surfactant.[9,10] A homogeneous clear or slightly opalescent liquid may be generated simply by gently swirling as long as the composition of the four phases is acceptable.[9] The optimization of microemulsion components is commonly achieved through the utilization of a pseudoternary phase diagram. This diagram demonstrates the appropriate proportions of oil, water, and surfactant mixture required for the formation of a microemulsion while also indicating the microemulsion region. Generally, water-in-oil, oil-in-water, and bicontinuous microemulsions are the three different types of microemulsions. Many researchers have investigated microemulsions extensively over the last few decades due to their high potential in food and pharmaceutical applications. Microemulsions are useful not just because of their ease of production and low cost, but also because of their enhanced bioavailability. Microemulsions are interesting alternative systems for topical drug administration due to their ability to incorporate both hydrophilic and lipophilic molecules while also enhancing penetration.[11] Microemulsions have also been observed as a carrier for herbal topical medicine.[12] Due to their ability to remain thermodynamically stable, their small droplet size (5-200 nm), low viscosity with low surface tension, and ease of fabrication, microemulsion formulations can be easily absorbed into the skin, resulting in rapid absorption.[9] Therefore, topical microemulsions are anticipated to be safe and efficient method for enhancing plant extract absorption.

The objective of this research was to create microemulsions that contain rambutan peel extract. To begin the study, a cytotoxicity assessment of the rambutan peel extract was conducted. Then, the microemulsions containing the extract were prepared by using the combination of rosemary oil as an oil phase, water as an aqueous phase, Tween® 80 as a surfactant, and 95% ethanol as a co-surfactant, based on a pseudoternary phase diagram. The fabricated microemulsions were subsequently evaluated for their characteristics, mainly by microscopic observation, visual inspection, viscosity, and conductivity measurement. Subsequently, The effectiveness of antibacterial properties against both gram-negative bacteria (*E. coli*) and gram-positive bacteria (*S. aureus*) was measured using the standard agar-well diffusion technique.

Materials and Methods

Materials

According to an earlier report published by Chaiwarit and colleagues, the extract of rambutan fruit peels was obtained by maceration in ethanol.[3] Briefly, the dried powders of rambutan peel were mixed with 95% ethanol for 18 h and then the solvent was removed by a rotary evaporator. Polysorbate 80 (Tween® 80, lot number 72334437) was purchased from Caesar & Loretz GmbH, Hilden, Germany. Rosemary oil (lot number 2018112048) was received from Joh. Vögele KG, Germany. Denatured ethanol (lot number 545301) was obtained from Carl Roth GmbH & Co. KG, Karlsruhe, Germany. The keratinocyte HaCaT cells, an immortalized human skin cell line[13], were acquired from Cell Lines Service GmbH (Germany). All other excipients were of pharmaceutical grade, and other chemicals were of reagent grade.

Cytotoxicity test of rambutan peel extract

HaCaT cells, a type of long-lived and immortalized human keratinocyte cell line capable of *in vitro* differentiation, were utilized as a model for cytotoxicity testing.[14] Initially, the cells were unfrozen and reconstituted in a solution consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The HaCaT keratinocytes were cultured within an incubation chamber under 5% CO_2 at 37°C. The adherent cells were rinsed with phosphate buffer saline and then cultured at 37°C for 3 min with 4 mL of trypsin solution. The trypsinization process was terminated by adding 6 mL of DMEM containing 10% FBS. After centrifuging the cell suspension for 5 min, the supernatant was then removed. The cells were then resuspended in 5 mL of new medium. The cell number could now be determined with the electronic cell counter and analyzer system (CASY TT, Roche Innovatis AG, Reutlingen, Germany). The HaCaT cells (20,000 cells/0.2 mL) were seeded in pure medium in 96-well plates. After 48 h, the medium was discarded, and 200 µL of the respective diluted samples (100 µg/mL and 50 µg/mL) were added. The cells were exposed to the samples for 48 h in an incubator. To ensure maximal cell damage, a positive control (Triton X) was also used. A negative control was also performed, in which the cells were placed in pure cell culture media, resulting in no cell damage. The two solvents, mainly ethanol and dimethyl sulfoxide (DMSO), were used as background controls. Samples were prepared by adding 50 or 100 mg of rambutan peel extract to 1 mL of either ethanol or DMSO. MTS tetrazolium compound was then given to each well. Incubation was then performed at 37°C for 3 h. The absorbance values were then detected at 490 nm. The samples were measured with n=4.

Construction of pseudoternary phase diagram

The oil phase, surfactant, and cosurfactant were designated as rosemary oil, Tween® 80, and ethanol, respectively, for the phase diagram study, while distilled water was used as an aqueous phase. Tween® 80 and ethanol were combined at a weight ratio of 2:1 and named S_{mix}. Using titration with water, the pseudoternary phase diagram was developed. In glass vials, the oil and S_{mix} were completely mixed in varying weight ratios (Table 1). The water dilution line was drawn, indicating rising water content, and decreasing S_{mix} levels. The water was gently titrated with S_{mix}. The clarity and turbidity of the system were assessed visually. After each addition of water, the mixture was agitated on a vortex mixer. If the sample resulted in a clearly transparent mixture after stirring, it was deemed monophasic. Each monophasic emulsion composition was then assigned a point on the phase diagram. The region of existence of the microemulsion was assumed to be the area encompassed by these locations.

Preparation of microemulsions containing rambutan peel extract

The selected combination of oil, S_{mix} and water was used for preparing the microemulsions containing rambutan peel extract. Taking into account that the extract can influence the rheological characteristics of the microemulsion formulations, the proportion of oil and Smix has been kept constant to assess the impact of the extract on these features. The composition of the different formulations of microemulsions comprising various concentrations of extract is also shown in Table 1. Rambutan peel extract was dissolved in the S_{mix} using a vortex mixer. The oil and water were then added and mixed using a vortex mixer to generate a homogenous phase.

Characterization of emulsions and microemulsions

Visual and microscopic observation. The appearance of microemulsions was examined by visual evaluation of the resulting formulations. To investigate the morphology of the formulations that were obtained, an optical microscope (BA310 LED Digital, Motic Deutschland GmbH, Germany) was employed. A slide was used to apply a drop of the microemulsions, which were then covered with a cover slip. The photos captured by the software were then examined.

Conductivity measurement. The conductivity values of the microemulsions were evaluated using the conductivity meter (ECTestr, Eutech Instruments Europe B.V., Netherlands). This was accomplished by employing a conductivity cell comprised of two plates separated by a specified distance, with liquid serving as a conductor between the plates. The conductivity values were measured and then recorded in triplicate.

Viscosity Measurements. The viscosity of the microemulsion samples was investigated using a rotary viscometer (Brookfield LVDV-II+Pro, UK) with a Sc-18 spindle. Each determination was performed in triplicate.

Antibacterial activity test

The standard agar-well diffusion method was employed to perform microbiological tests against *E. coli* and *S. aureus*. This test aimed to observe the antibacterial activity of both the rambutan peel extract and the microemulsion containing rambutan peel extract. The method was adapted from the previous report with some modifications.[15] Briefly, stock cultures of gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria were inoculated on agar before creating consistent wells (holes) in the agar with a sterile Pasteur pipette. After that, 20 µL of each formulation was added into the well. Plates were refrigerated for 2 h to enhance formulation penetration on agar plates. Following that, all plates were incubated at 37°C for 18-24 h. Each assay was repeated in triplicate, and the growth inhibition zones were determined using a caliper to measure the antibacterial activity.[16] The positive control in the experiment involved using a well containing a solution of clindamycin at a concentration of 1% clindamycin base equivalent.

Statistical analysis

The statistical software Minitab 19 for Windows (SPSS Inc., USA) was employed to perform ANOVA and Levene's test to assess the homogeneity of variance. To compare the statistical significances in multiple groups, the Scheffé or Games-Howell tests were used as post hoc tests, depending on whether the results of Levene's test was significant. A p-value below 0.05 was deemed to be statistically significant.[17]

Results and Discussion

According to a previous work from Chaivarit and colleagues, rambutan peel extract contains flavonoid compounds with a total flavonoid content of 110.26 ± 9.37 mg catechin equivalent per 1 g of dry matter, and a total phenolic content of 146.17 ± 7.15 mg gallic acid equivalent per 1 g of dry matter.[3] Initially, the impact of rambutan peel extract on cell viability was evaluated using a protocol that measures the reduction of the 3-(4,5- dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) compound, which allows for quick screening of a large number of samples. To that purpose, HaCaT cells were exposed to two different doses of extract (50 and 100 µg/mL) in ethanol and DMSO for 48 h. As shown in Figure 1, incubation with extract at a lower dose (50 µg/mL in both ethanol and DMSO) had no impact on the MTS conversion rate of HaCaT cells. Higher concentrations of the extract (100 µg/mL) in both ethanol and DMSO reduced the viability of HaCaT cells marginally but not statistically significantly, as compared to cells that were not treated at all (negative control), suggesting that even 100 µg/mL of the extract did not cause a cytotoxic effect on skin keratinocyte cells. Figure 2 shows the cell viability of HaCaT cells after 48 h incubation at 37°C with different concentrations (50 and 100 µg/mL) of rambutan peel extract.

In this study, the rambutan peel extract was shown to possess significant amounts of phenolic and flavonoid compounds. Geraniin, corilagin, and ellagic acid were the primary flavonoids identified in the rambutan peel extract.[7] Rambutan peel extract is well known for being non-toxic and safe. Therefore, the concentrations of 50 and 100 µg/mL were used for the cytotoxicity test based on the previous report.[7] The present study employed the MTS assay to evaluate the cytotoxicity and cell viability in order to confirm this observation. This procedure involves the reduction of MTS tetrazolium reagent by live mammalian cells to create a soluble formazan dye, which can be observed under cell culture conditions. Our findings demonstrated that rambutan peel extract had negligible toxicity on the HaCaT cell lines (Figure 2). The decrease in cell viability observed with the higher concentration of rambutan peel extract did not exhibit a statistically significant difference compared to the lower concentration of extract and the negative control. Another research showed that the extract obtained from rambutan peel did not exhibit cytotoxicity towards human colon adenoma and normal peripheral blood mononuclear cell lines.[18] Jantapaso and Mittraparp-arthorn discovered that rambutan peel extract is non-toxic at low doses (60 µg/mL) but cytotoxic at higher concentrations on murine fibroblast and monkey kidney cell lines. The IC_{50} against a breast cancer cell line (MCF-7 cell lines) was reported to be about 94 µg/mL.[19]

Pseudoternary phase diagram

A pseudoternary phase diagram was created using rosemary oil as the oil phase, a surfactant mixture of Tween® 80 and ethanol at a 2:1 ratio as the surfactant, and water as the aqueous phase. The resulting diagram is shown in Figure 3. The phase diagram clearly showed that the microemulsion region was observed when the weight ratio percentage of the Tween® 80/ethanol mixture increased over 38%, i.e., ME-3, ME-4, and ME-5, as depicted in Table 1. At the weight ratio percentage of surfactant mixture 38% or lower, the phase separation or cloudy suspension was observed (i.e., ME-1, ME-2, ME-6). According to Table 2, when viewed under a light microscope at a magnification of 400, droplets were detected in ME-5.

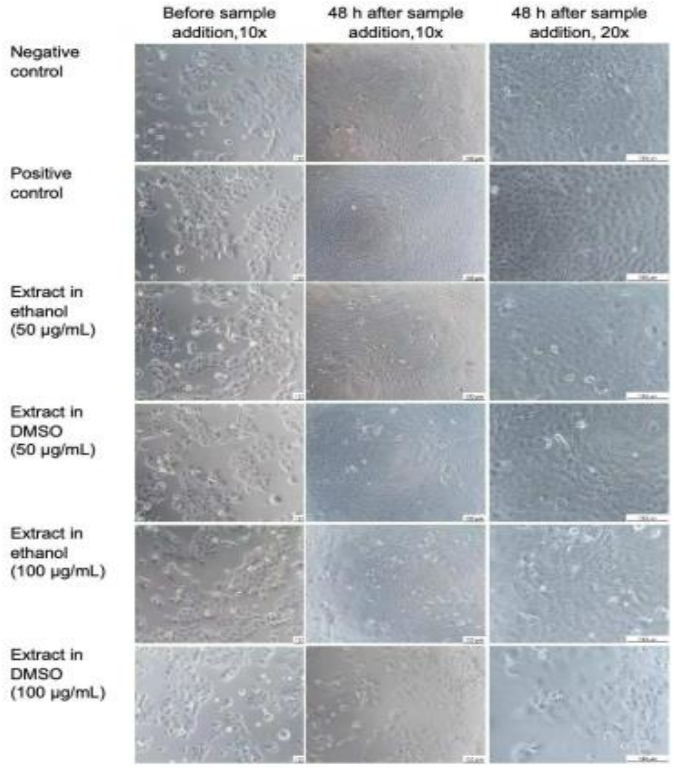

Figure 1: Microscopic images of cells before and 48 h after sample addition.

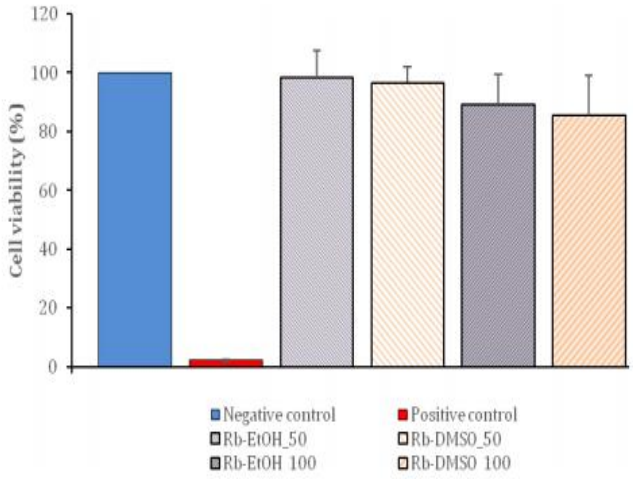

Figure 2: Viability of HaCaT cells after 48 h incubation at 37°C with different concentrations (50 and 100 µg/mL) of rambutan peel extract in ethanol and DMSO. Note: the cell viability percentages are given as mean ± standard deviations from four independent experiments.

However, no birefringence was observed, indicating that it was a regular dispersion. As the percentage of water was less than 13.3%, the samples had very low conductivities, as shown in Table 2. In this study, at a water percentage between 16.6% and 17.3%, the conductivity was still low, at 10 µS/cm. A substantial rise in conductivity (90 µS/cm) was seen when the water percentage was 65%.

The pseudoternary phase diagram was created to determine the existing microemulsion zone. From the constructed phase diagram, the microemulsion zone was seen when the concentration of the surfactant/co-surfactant mixture (i.e., Tween® 80/ethanol mixture) increased above a certain concentration, most likely due to a gradual decrease in interfacial tension.[9] Many forms of dispersions, including typical oil-in-water emulsions, oil-in-water microemulsions, and water-in-oil microemulsions, can be produced using the investigated combination of rosemary oil, water, and a Tween® 80/ethanol mixture. Microemulsions with oil droplets dispersed in water (oil-in-water microemulsions) have high conductivity due to the water phase, which is continuous and has a high conductivity. Because of the relatively poor conductivity of the oil, water-in-oil microemulsions have low conductivity.[20] In this study, the microemulsions exhibited low conductivity as long as the water component was less than 20% w/w. At large water fractions, conductivity increased dramatically. A notable change in conductivity occurs during the transformation from oil-in-water to water-in-oil configurations.[21]

Formulation and characterization of microemulsions containing rambutan peel extract

According to the pseudoternary phase diagram, a weight ratio percentage of a surfactant mixture of more than 38% could be used to prepare clear and stable microemulsions. Therefore, the selected combination of oil, S_{mix} and water (as shown in Table 1) was used for preparing the microemulsions containing different concentrations of rambutan peel extract. The microemulsions containing rambutan peel extract were clear and transparent, with no phase separation or droplets visible in the microscopic observation. As the concentration of rambutan peel extract increased, the microemulsions became darker in color. The ME-R10 microemulsion had the darkest color due to the high concentration of rambutan peel extract, yet it is still a transparent solution with no phase separation (Figure 4). The rambutan peel extract had completely dissolved in the microemulsions, and no droplets were visible, as confirmed by the optical microscopic images (Figure 5). The physicochemical characteristics of microemulsions containing extract are also shown in Table 2. The microemulsions containing rambutan peel extracts were visually examined, and there were no indications of phase separation, flocculation, or precipitation. The conductivity of these formulations was zero, and their appearance was clear with no phase separation.

The viscosities of microemulsions containing 1% w/w, 5% w/w, and 10% w/w rambutan peel extract were 35.0±1.0, 47.8±2.0 and 63.6±2.9 cPs, respectively. It was found that the viscosity of microemulsions containing different concentrations of rambutan peel extract differed marginally ($p<0.05$).

The suitable microemulsions were selected for further development with rambutan peel extract. The criteria for selecting microemulsion formulations were based on their physicochemical features, which included being clear or transparent, having no precipitation, having a low viscosity, and having no separation. The final concentrations of rambutan peel extract of 1% w/w, 5% w/w, and 10% w/w were successfully prepared. The obtained microemulsion formulations were transparent and homogenous. The visual examination revealed that the addition of rambutan peel extract did not result in precipitation or phase separation; the conductivity of microemulsions containing the extract was zero.

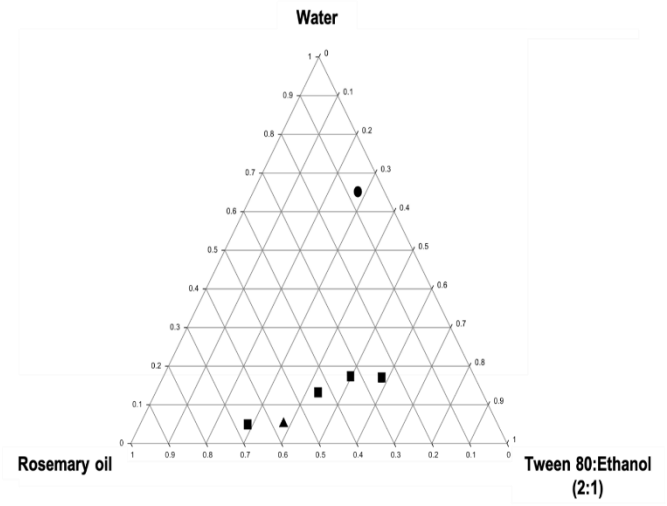

Figure 3: Pseudoternary phase diagram of Tween® 80/ethanol mixture, rosemary oil and water. [Symbol: square = clear, triangle = phase separation, circle: clear with cloudy suspension]

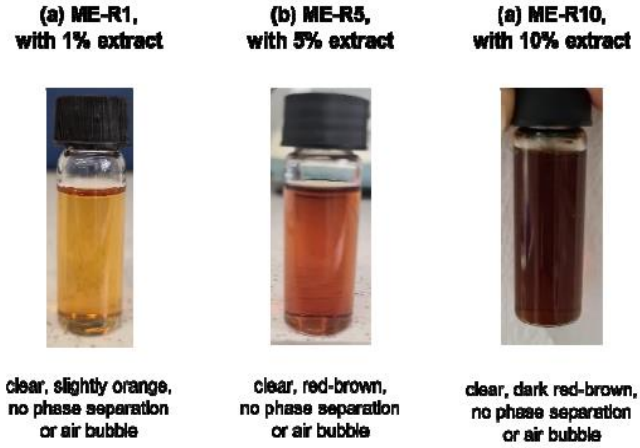

Figure 4: Appearance of microemulsions containing different concentrations of rambutan peel extract; (a) ME-R1, (b) ME-R5, and (c) ME-R10.

Table 1: Composition of different microemulsions and microemulsion containing rambutan peel extract

Formulation	Oil (% w/w)	S_{mix} (% w/w)	Water (% w/w)	Rambutan peel extract (% w/w)
ME-1	66.6	28.7	4.7	-
ME-2	56.8	38.0	5.2	-
ME-3	43.4	43.4	13.3	-
ME-4	33.1	49.6	17.3	-
ME-5	25.2	58.2	16.6	-
ME-6	7.0	27.8	65.1	-
ME-R1	33.4	46.1	19.5	1.0
ME-R5	33.1	43.4	18.6	4.8
ME-R10	31.7	41.7	17.1	9.3

Table 2: Physicochemical characteristics of blank microemulsions and microemulsions containing rambutan peel extract

Formulation	Conductivity (µS/cm)	Type of microemulsion	Phase separation	Visual inspection	Observation under microscope (400X)
ME-1	0	Water-in-oil	Yes	Two phases with blurred boundary	No droplet observed
ME-2	0	Water-in-oil	Yes	Cloudy, minimal clear layer below	No droplet observed
ME-3	0	Water-in-oil	No	Clear	No droplet observed
ME-4	10	Oil-in-water	No	Clear	No droplet observed
ME-5	10	Oil-in-water	No	Clear	Small droplets found
ME-6	90	Oil-in-water	Yes	Cloudy, foaming	No droplet observed but fibrous/branched particles founds
ME-R1	0	Water-in-oil	No	Clear	No droplet observed, extract dissolved completely
ME-R5	0	Water-in-oil	No	Clear	No droplet observed, extract dissolved completely
ME-R10	0	Water-in-oil	No	Clear	No droplet observed, extract dissolved completely

The droplets could not be seen visually based on the microscopic photos. This is most likely owing to the fact that microemulsion droplets are typically in the nanometer range. Emulsifiers can also help to minimize coalescence by producing surface potential, which can cause repulsive interactions between neighboring oil droplets.[22] The viscosity of microemulsions containing rambutan peel extract at different concentrations varied considerably. The higher the concentration of extract, the more viscous the formulation. This is most likely due to the lower quantity of S_{mix} used in the formulation (Table 1).

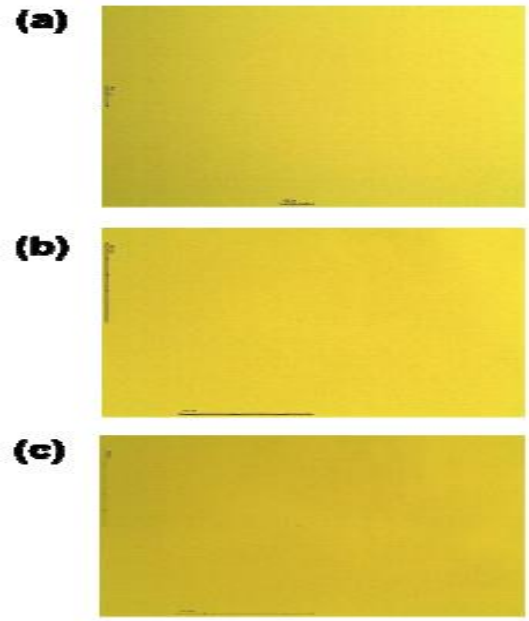

Figure 5: Microscopic images of microemulsions containing (a) 1% w/w, (b) 5% w/w and (c) 10% w/w rambutan peel extract (magnification 40X).

Antibacterial activity of microemulsions containing rambutan peel extract

To assess the antibacterial activity, the microemulsions that contained rambutan peel extract were tested against gram-negative bacteria (*E. coli*) and gram-positive bacteria (*S. aureus*) using the well diffusion method. The antibacterial activity was observed in microemulsions against *S. aureus* but not *E. coli*, indicating their effectiveness against gram-positive bacteria. The diameter zone value was used to classify the antibacterial activity, where 5 mm indicated weak, 5-9 mm indicated medium, 10-19 mm indicated strong, and >20 mm indicated very strong activity.[23] Table 3 reveals that neither blank microemulsions nor microemulsions containing rambutan peel extract exhibited antibacterial action against *E. coli*. In the case of *S. aureus*, blank microemulsions had no effect on bacterial growth, while microemulsions containing different concentrations of rambutan peel extract efficiently inhibited bacterial growth to the same extent (at the medium level). However, there was no difference in the antimicrobial action against *S. aureus* of the microemulsions containing 1% w/w – 10% w/w rambutan peel extract. This point might be described as the fact that the bioactive compounds in rambutan may reach their capacity at the tested concentrations.

Another important aspect is that the phenolic active ingredient in the rambutan peel extract interacts due to Van der Waals interaction with the agar medium used in the diffusion test ("Hemmhof-Test"). The phenolic oxygen interacts strongly with the hydrogen of the hydroxyl groups of the agar-gel (and vice versa, which is a polysaccharide containing many –OH groups). Due to this interaction, a large amount of the rambutan extract is bound to the agar gel via an electrostatic (Van der Waals) interaction. Because of this, only the formulation which comes first on the surface in contact to the microorganism, can unfold it´s antimicrobial activity. Due to diffusion within the agar, a large amount of the active component is adsorbed to the gel. Consequently, increasing the amount of dose applied does not result in a linear enlargement of the Hemmhof zone. This effect is also often observed in drug formulations, where phenolic compounds are formulated together with macrogol or other compounds and are able to interact via Van der Waals hydrogen bond formation.[24] The binding is due to the formation of hydrogen bonds and hydrophobic interactions.[25]

The microemulsions containing rambutan peel extract revealed antibacterial activity only against *S. aureus* (a gram-positive bacteria) based on the inhibitory zone diameter measurements, unlike *E. coli* (a gram-negative bacteria). The dissimilarity in the antibacterial effectiveness can be explained by the differences in the cell wall structures of the two bacterial types. Gram-negative bacteria possess two cellular membranes, while gram-positive bacteria have only a single membrane, which is simpler and mainly composed of a peptidoglycan layer with low lipid content, allowing easier penetration of bioactive molecules into the cells. The composition and structure of the cell wall can affect the antibacterial activity of chemical compounds.[23,26] The cell walls of gram-negative bacteria are comprised of three distinct layers: a lipoprotein outer layer, a lipopolysaccharide middle layer that serves as a deterrent to the penetration of antimicrobial bioactive elements, and an inner layer of peptidoglycan containing approximately 11-12% lipids.[26] This is consistent with previous findings showing phenolic compounds found in rambutan peel have strong antibacterial action against gram-positive bacteria, including *S. aureus*.[3,4]

Table 3: Antimicrobial activity, presented as inhibition zone, against gram-negative (E. coli) and gram-positive (S. aureus) bacteria of microemulsions containing various concentrations of rambutan peel extract.

Formulation	Zone of inhibition (mm ± SD), n=3	
	E. coli	*S. aureus*
ME-R1	No inhibition zone	6.00 ± 0.00
ME-R5	No inhibition zone	6.00 ± 0.00
ME-R10	No inhibition zone	6.44 ± 0.88
Blank microemulsion	No inhibition zone	No inhibition zone
Sterile water	No inhibition zone	No inhibition zone

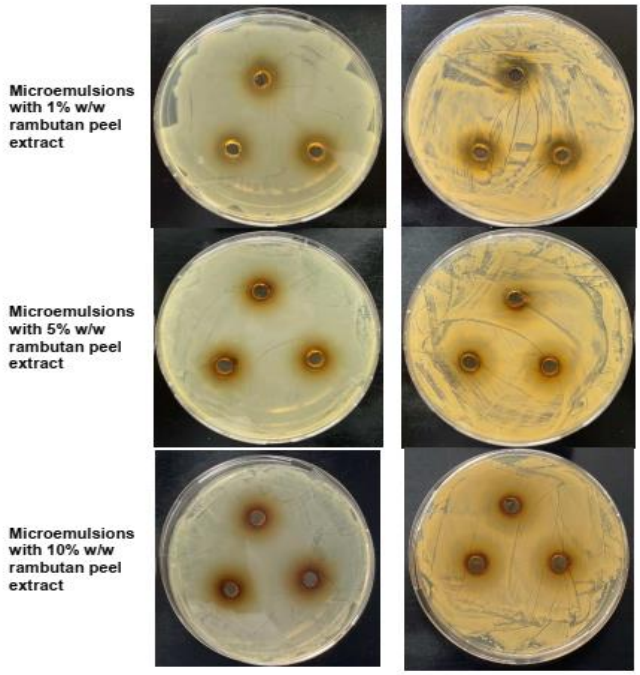

Figure 6: Antimicrobial activity against gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria of microemulsions containing different concentrations of rambutan peel extract.

Conclusion

In conclusion, microemulsions containing different concentrations of rambutan peel extract (1% w/w, 5% w/w, and 10% w/w) were successfully formulated and exhibited antibacterial activity against gram-positive *S. aureus* without being toxic to HaCaT cells. These observations provide a basis for further research in the field of natural products for the development of new antibacterial agents. Therefore, additional studies are required to optimize the formulation of microemulsions containing natural extracts to enhance and confirm their effectiveness as bioactive agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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