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*Original Research Article*

# Development of Topical Formulations with Antibacterial Efficacy from Peel Extract of *Mangifera indica*: Emulsions and Micro-emulsions

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ABSTRACT

Because some pathogenic bacteria are becoming more resistant to known antibiotics, more research into the development of new antibacterial agents is required. The main study objective was to develop several formulations containing peel extract of *Mangifera indica* (mango) and evaluate their antibacterial efficacy. The macerating extraction process was used to produce the mango peel extract. Cytotoxicity of the mango peel extract was also evaluated. The emulsions and microemulsions were prepared and assessed based on their appearance, phase separation, consistency, viscosity, and conductivity. The formulations were then evaluated for antibacterial and growth inhibitory effects against gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*) bacteria. The findings revealed that no cytotoxic effects on skin keratinocyte cells were identified. Most formulations were physico-chemically acceptable. Only *S. aureus* bacteria were inhibited by emulsions and microemulsions containing mango peel extract.

***Keywords*:** *Mangifera indica*, Mango peel, Extract, Emulsion, Micro-emulsion, Antibacterial.

## Introduction

Natural or alternative remedies have gained in popularity in recent years. More elderly individuals are using complementary and alternative medicine nutritional supplements and herbal medicines, believing that these materials will be useful. Herbal medicine, also known as phytomedicine, is the medical use of any plant's seeds, berries, roots, leaves, bark, or flowers. Mango (*Mangifera indica* L.), a member of the Anacardiaceae family, is one of the world's popular fruits, particularly in Asian countries. Mango is classified into two cultivar groups depending on their embryo type: the monoembryonic (Indian) type is found mostly in the subtropics, while the polyembryonic (Southeast Asian) type is found primarily in the tropics.[1] The Indian type has a zygotic (sexually produced) embryo, and the fruit skin is mostly red, but the Southeast Asian type contains multiple nucellar embryos (produced from the mother plant), and the skin is predominantly green to yellow.[2,3] Mango fruit processing produces two forms of waste: solid waste (peel and stones) and liquid waste (juice and wash water). Mango peel (or exocarp) is the most important byproduct of mango processing, accounting for 7-24% of total mango weight.[4] Mango peel has been studied for its phytochemical composition, which contains pectin, phenolic compounds, carotenoids, and other bioactive compounds, which have been reported to promote human health.[5,6]

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Phenolic compounds found in mango peel exhibit antibacterial action against gram-positive bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus subtilis*, as well as gram-negative bacteria such as *Salmonella typhi*, *Escherichia coli*, and *Proteus vulgaris*.[7,8] Topical formulation has the benefit of avoiding the negative effects of oral treatment while simultaneously improving drug accumulation at the appropriate layer of skin. The formulations of topical products can range from liquids (solutions, emulsions, and microemulsions) through semisolids (gels and ointments) to solid systems (powders and transdermal patches).[9]

The formal research works [10-14] based on extraction of peels or seeds as well as of characterization of compounds inside the mangos-extract. This research work concentrated on specific formulations, such as emulsions, and microemulsions. which were not studied before. Emulsions are heterogeneous systems made up of at least two immiscible liquid phases in which one liquid is disseminated as droplets in the other with the help of an emulsifier.[15,16] Because of their excellent solubilizing characteristics for both hydrophilic and lipophilic active chemicals, as well as their high acceptability due to their pleasant skin sensory properties, they are often used in cosmetic and pharmaceutical formulations.[17] Water-in-oil (W/O) emulsions are an interesting carrier in topical products due to their properties; in the case of superficial skin application, it is important to develop a carrier with minimal penetration, keeping the substance active at the skin surface. A microemulsion is a clear, stable, isotropic mixture of oil, water, and a surfactant, commonly in combination with a co-surfactant. Gently swirling can generate a homogeneous clear or slightly opalescent liquid as long as the composition of the four phases is adequate.[18] Because of its capacity to integrate both hydrophilic and lipophilic molecules while also improving penetration, microemulsion is an interesting approach for topical pharmaceutical delivery.[19] Microemulsion has also been employed as a topical herbal drug carrier.[20] Recently, antibacterial herbal ointments and creams containing extract from mango seeds have been developed and tested.[21] Another research group also demonstrated that both mango leaf extract and carbopol hydrogel containing mango leaf extract are

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effective against *Staphylococcus aureus* and can be used as an alternative to presently existing topical antiseptic/antibiotic formulations for staphylococcal infection treatment.[22] To the best of our knowledge, the emulsions and microemulsions containing mango peel extract have not been reported. Therefore, the goal of this study was to develop topical formulations using mango peel extract, such as emulsions, and microemulsions, and to assess the antibacterial efficacy of the developed formulations.

## Materials and Methods

*Materials*

Mangoes (*Mangifera indica* L.), Kent & Keitt variety, were purchased in March 2021 from a local store in Berlin, Germany. Polysorbate 80 (Tween® 80, lot number 72334437), and almond oil (lot number 19084518) were purchased from Caesar & Loretz GmbH, Germany. Ethanol (lot number 545301) was obtained from Carl Roth GmbH, Germany. Sorbitan monooleate (Span® 80, lot number T62411, Seppic GmbH, Germany) and rosemary oil (lot number 2018112048, Joh. Vögele KG, Germany) were used. HaCaT cells, spontaneously transformed aneuploid immortal keratinocyte cell line from adult human skin, were purchased from Cell Lines Service GmbH (Germany). All other chemicals were of pharmaceutical grade and were used without further purification.

*Mango peel extraction*

The ripe Kent & Keitt mangoes were carefully washed, dried, and peeled. The peels were sliced into small pieces (less than 1 cm) to increase the surface area for the subsequent extraction. They were then dried in a drying oven at 45°C for 24 h, followed by 48 h at room temperature (25°C) and 25% relative humidity. The dried mango peels had a residual moisture content of 1.3%. The crushed, dried mango peels weighing about 200 g were put to amber glass vessel, and denatured ethanol (2 L) was added. After three days, the mixture containing the mango peels was filtered. The solvent was then evaporated from the solution using a precision rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at a water bath temperature of 60°C, a pressure of 250 mbar, and a rotational speed of 85 rpm, in order to obtain the mango peel extract.

*Cytotoxicity of mango peel extract*

In this study, HaCaT cells was used as an *in vitro* cell culture model to elucidate the cytotoxicity of mango peel extract, using a ready-to-use CellTiter 96® Aqueous One Solution Cell Proliferation Assay containing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) compound.[23] The cells were first thawed and resuspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). The HaCaT keratinocytes were then grown in an incubator at 37°C with 5% $CO_2$. The confluent cells were rinsed with phosphate buffer saline before being treated with 4 mL trypsin solution for 8 min at 37°C. The addition of 6 mL of DMEM containing 10% FBS prevented trypsinization. After centrifuging the cell suspension for 5 min, the supernatant was discarded. After that, the cells were resuspended in 5 mL of fresh media. The electronic cell counter and analyzer device may now be used to determine the cell number (CASY TT, Roche Innovatis AG, Reutlingen, Germany). In 96-well plates, HaCaT cells (20,000 cells/0.2 mL) were planted in pure media. After 48 h, the medium was withdrawn and 200 µL of the respective diluted samples (100 µg/mL and 50 µg/mL) were added. The cells were incubated with the samples for 48 h. A positive control (Triton X) was also employed to assure maximum cell damage. There was also a negative control in which the cells were placed in pure cell culture media, which resulted in no cell damage. As background controls, two solvents, ethanol and dimethyl sulfoxide (DMSO), were utilized. Samples were made by dissolving 50 or 100 mg of mango peel extract in 1 mL of ethanol or DMSO. All samples were then treated with MTS, and incubated for 3 h at 37°C. The absorbance was measured at 490 nm and the percentage of viable cells was calculated. The samples were measured with n = 4.

*Preparation of emulsions containing mango peel extract*

Different compositions for emulsions containing mango peel extract are shown in Table 1. The extract, jojoba oil, and Span 80 were used as oil phase. Span 80
was used as hydrophobic surfactant and mixed with oil phase. Deionized water that mixed Tween 80 (hydrophilic surfactant) was used as water phase of emulsions. Emulsions containing mango peel extract were prepared by adding water phase to oil phase, and then mixed by mechanic stirrer until a homogenous phase formed. The obtained emulsions were then transferred to a plastic container with lid to see if phase separation happened during the next few days.

*Preparation of microemulsions containing mango peel extract*

A surfactant mixture containing 1 g of Tween 80 and 0.5 g of ethanol was combined in a small glass vessel using a vortex mixer. After that, the determined amount of mango peel extract was dissolved in rosemary oil. Finally, deionized water was added and stirred for roughly 30 s with the vortex mixer to generate a homogenous, transparent solution with no phase boundary. Table 2 lists all of the components used in the preparation of micro-emulsions.

*Characterization of emulsions and micro-emulsions*

*Visual and microscopic observation:* The appearance of emulsions and microemulsions was determined by visual evaluation. The morphology of the resulting formulations was examined using an optical microscope (BA310 LED Digital, Motic Deutschland GmbH, Germany). A drop of each emulsion or microemulsion was put to a slide, which was then covered with a cover slip. The photos captured by the software were then examined.

*Conductivity measurement:* The conductivity meter was used to measure the conductivity of the emulsions and microemulsions (ECTestr, Eutech Instruments Europe B.V., Netherlands). This was accomplished by using a conductivity cell made up of two plates separated by a predetermined distance, with liquid acting as a conductor between the plates. All measurements were conducted three times.

*Viscosity measurement:* The viscosity of the emulsions and microemulsion samples was determined using a rotary viscometer (Brookfield LVDV-II+Pro, UK) with a Sc-18 spindle. Each determination was carried out in triplicate.

**Table 1:** Formulation composition of emulsions containing mango peel extract

| Composition (% w/w) | E-M5 | E-M10 |
|---|---|---|
| Jojoba oil | 13.9 | 18.0 |
| Tween 80 | 21.5 | 22.3 |
| Span 80 | 7.7 | 8.1 |
| Deionized water | 52.2 | 41.6 |
| Mango peel extract | 4.7 | 10.0 |

**Table 2:** Formulation composition of microemulsions containing mango peel extract

| Composition (% w/w) | ME-M1 | ME-M5 | ME-M10 |
|---|---|---|---|
| Rosemary oil | 33.8 | 32.7 | 30.9 |
| Tween 80 | 29.1 | 28.6 | 27.0 |
| Ethanol | 16.1 | 14.8 | 13.7 |
| Deionized water | 20.1 | 18.9 | 18.4 |
| Mango peel extract | 1.0 | 5.0 | 10.0 |

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*Antibacterial activity test*

The antibacterial activities of the mango peel extract as well as the developed formulations those containing mango peel extract were tested. The agar well diffusion method against gram-positive *S. aureus* and gram-negative *E. coli* were performed.[24] In brief, gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria stock cultures were inoculated on agar before making uniform wells (holes) in the agar with a sterile Pasteur pipette. The well was then filled with 20 µL of each formulation. To improve formulation penetration on agar plates, plates were kept in refrigerator for 2 h. After that, all plates were incubated at 37°C for 18-24 h. Each experiment was performed in triplicate, and antibacterial activity was assessed using a caliper to determine growth inhibition zones. As a positive control, a well filled with clindamycin solution corresponding to 1% clindamycin base was employed.

*Statistical analysis*

Minitab 19 for Windows (SPSS Inc., USA) was used to run ANOVA and Levene's test for variance homogeneity. Post hoc testing ($p<0.05$) for multiple comparisons was performed using the Scheffé or Games–Howell tests, depending on whether Levene's test was insignificant or significant.[25]

## Results and Discussion

*Mango peel extraction and cytotoxicity*

The mango peel extract was successfully prepared by maceration and solvent evaporation technique. The yield obtained from the extraction was 8.62% w/w. The cell cytotoxicity of the extract was assessed using a protocol based on a reduction of MTS tetrazolium compound, which allows for quick screening. The extract was applied to HaCaT cells for this purpose. Figure 1 depicts the cell viability of HaCaT cells after 48 h of incubation at 37°C with different doses (50 and 100 µg/mL) of mango peel extract. It was discovered that extract incubation had no significant influence on the MTS conversion rate of HaCaT cells, implying that the extract did not have a cytotoxic effect on skin keratinocyte cells. These results agree with the findings by Lauricella and co-workers,[26] who explored the effect of mango peel extract on colon cancer cell lines and found that the extract affected cell viability and inhibited the colony formation trend of tumor cells, while no effects were observed in human dermal fibroblasts used as a non-cancerous cell line model.

*Emulsions containing mango peel extract*

Two emulsion formulations comprising mango peel extract (5 and 10% w/w) were developed. The macroscopic observations demonstrated that all of the formulations were consistent, had no phase separation, and had no herbal component incompatibility. Emulsions containing 5% mango peel extract were mustard yellow with a hint of green, but emulsions containing 10% mango peel extract were dark yellow and thicker in mass (Figure 2a and 2b). A microscopic study was then carried out to analyze the distribution of the droplets, as shown in Figure 3. The microscopic investigations confirmed that the emulsion systems were accomplished and that the droplets of emulsions containing 10% mango peel extract (droplet size ranged from 1.1 to 4.1 µm) were smaller and more uniform than those containing 5% extract (droplet size ranged from 1.5 to 6.5 µm). It is likely that the emulsions containing 10% extract had a little higher surfactant concentration, which could lower the interfacial tension of the emulsion, causing the droplet size of the emulsion to become smaller.[27] The conductivity of formulations is examined to show if they are water-in-oil or oil-in-water. The conductivity of a water-in-oil emulsion system cannot be measured because the oil phase may hinder conductivity. The conductivity results were obtained from formulation depends on their composition, as given in Table 3. The findings revealed that the type of both emulsion formulations was oil-in-water. The increased conductivity may be related to the number and composition of ions in the system.[28]

The viscosity changes are not unusual and may be easily explained by looking at thermodynamic fundamentals.

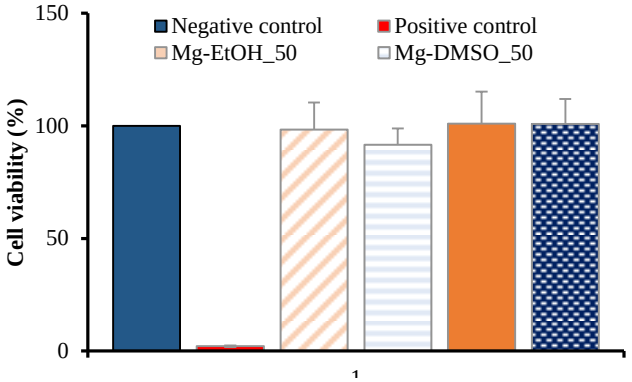


**Figure 1:** Cell viability of HaCaT cells after 48 h incubation at 37°C with different concentrations (50 and 100 µg/mL) of mango peel extract in ethanol and DMSO.

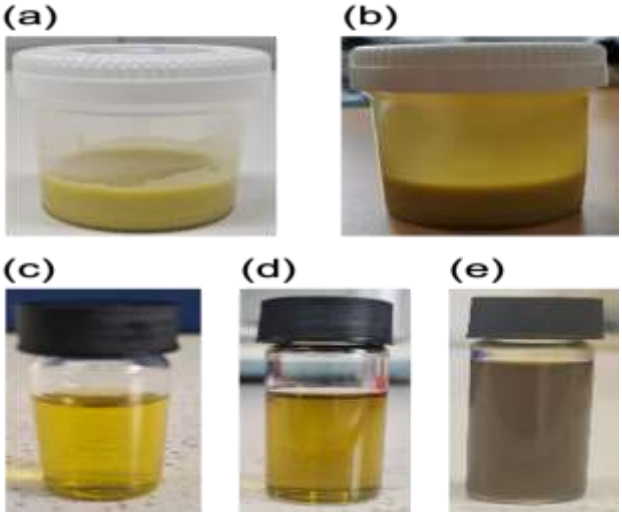


**Figure 2:** Appearance of formulations containing mango peel extract; (a) emulsion with 5% w/w extract, (b) emulsion with 10% w/w extract, (c) microemulsion with 1% w/w extract, (d) microemulsion with 5% w/w extract, and (e) microemulsion with 10% w/w extract.

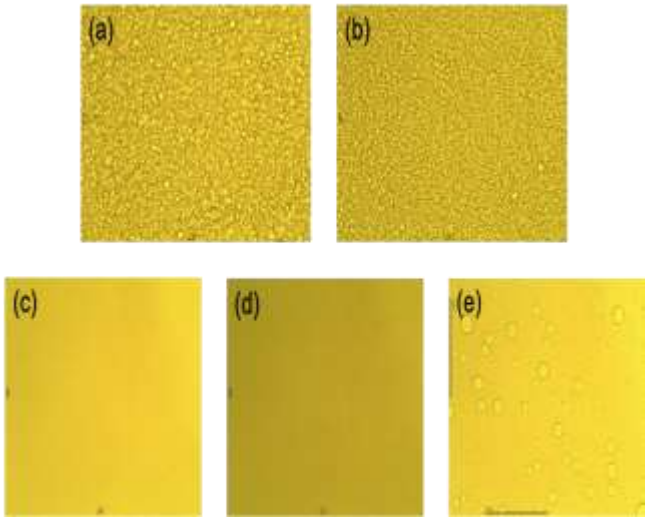


**Figure 3:** Microscopic images of formulations containing mango peel extract; (a) emulsion with 5% w/w extract, (b) emulsion with 10% w/w extract, (c) microemulsion with 1% w/w extract, (d) microemulsion with 5% w/w extract, and (e) microemulsion with 10% w/w extract. Note: magnification of 40X.

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**Table 3:** Physical characteristics of emulsions and microemulsions containing mango peel extract

| Formulation | Conductivity (μS/cm), n = 3 | Viscosity (cPs), n = 3 | Visual inspection |
|---|---|---|---|
| **Emulsions** | | | |
| E-M5 | 0.20 ± 0.01 | 193.3 ± 10.1 | Mustard yellow with a touch of green, no phase separation |
| E-M10 | 0.23 ± 0.06 | 240.0 ± 3.0 | Dark yellow, no phase separation |
| **Micro-emulsions** | | | |
| ME-M1 | 0 | 34.4 ± 1.0 | Clear, yellow color, no phase separation |
| ME-M5 | 0 | 36.5 ± 0.9 | Clear, yellow color, no phase separation |
| ME-M10 | 0 | 43.6 ± 1.2 | Somewhat cloudy, dark green to brown |

**Table 4:** Antibacterial activity of various formulations against S. aureus and E. coli, as shown by inhibition zone diameter (n = 3).

| Formulation | Zone of inhibition (mm ± SD) | |
|---|---|---|
| | ***E. coli*** | ***S. aureus*** |
| **Emulsions** | | |
| E-M5 | No inhibition zone | 1.67 ± 0.50 |
| E-M10 | No inhibition zone | 2.22 ± 0.67 |
| Blank emulsions | No inhibition zone | No inhibition zone |
| **Microemulsions** | | |
| ME-M1 | No inhibition zone | 1.56 ± 0.89 |
| ME-M5 | No inhibition zone | 2.00 ± 0.00 |
| ME-M10 | No inhibition zone | 2.44 ± 0.89 |
| Blank microemulsions | No inhibition zone | No inhibition zone |
| Sterile water (negative control) | No inhibition zone | No inhibition zone |
| Clindamycin (positive control) | No inhibition zone | 2.11 ± 0.33 |

The smaller the droplet, the lower the viscosity. Because of the larger droplet size, the viscosity of emulsions containing 10% extract was significantly higher ($p < 0.05$) than that of emulsions containing 5% extract. The viscosity of microemulsions containing 1% and 5% extract were insignificantly different ($p > 0.05$). Because of the big droplets in the formulation, microemulsions containing 10% extract have statistically significantly higher viscosity than those containing 1% and 5% extract ($p < 0.05$).

*Micro-emulsions containing mango peel extract*

The microemulsions containing mango peel extract were clear and transparent, with no obvious phase separation or droplets. The higher the concentration of mango peel extract, the darker the microemulsions (Figure 2). The microemulsion formulation containing 10% w/w extract was the darkest in color due to the largest concentration of mango peel extract, and it was fairly hazy with no phase separation. Figure 3 also shows the photomicrographs of microemulsions containing different concentrations of mango peel extract. Based on the photomicrographs, the droplets could not be seen visually in the formulations of microemulsions containing 1% w/w and 5%w/w mango peel extract. The size of microemulsion droplets is typically in the nanometer range. Emulsifiers can also help to minimize coalescence by generating surface potential, which can cause repulsive interactions between adjacent oil droplets.[29] The exception is microemulsions containing high concentration of extract (10% w/w extract), which displayed several large droplets; this formulation was most likely unstable. Microemulsion instability can cause Oswald ripening, which causes the small droplets to dissolve and the large droplets to grow in size.[30] These microemulsions have no conductivity, indicating that they are a water-in-oil system. Because the oil phase may hinder conductivity, the conductivity of a water-in-oil emulsion system cannot be measured.[31] The viscosity of all microemulsions was low, ranged from 27.2 to 53.7 cPs, as low viscosity is one of the characteristics of microemulsions. The variations amongst formulations with different concentrations of mango peel extract were not significantly different.

*Antibacterial activity of emulsions and microemulsions containing mango peel extract*

The antibacterial activity of the emulsions and microemulsions containing mango peel extract was checked against gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria by well diffusion method. Figure 4 shows the antibacterial activity of various formulations against *S. aureus* and *E. coli*, demonstrating the antibacterial activity of emulsions and microemulsions containing mango peel extract. According to the classification of antibacterial activity by Handayani et al.,[32] the value of the diameter zone <5 mm includes weak, 5-9 mm includes medium category, 10-19 mm includes strong category, and >20 mm includes very strong category. It can be seen from Tablet 4 that the formulations in the form of both emulsions and microemulsions containing mango peel extract did not have antibacterial activity against *E. coli* bacteria, while in *S. aureus* bacteria, the formulations showed a weak bacterial growth inhibition. This is most likely due to gram-positive and gram-negative bacteria having different cell wall structures. Gram-negative bacteria have two cell membranes, the outer membrane and the cytoplasmic membrane, whereas gram-positive bacteria only have the cytoplasmic membrane; differences in cell wall composition and structure can alter a chemical compound's antibacterial action.[32] The structure of simpler, single-layered gram-positive bacterial cell walls is composed of 90% peptidoglycan layer with a low lipid content (1-4%), allowing bioactive substances to enter cells.[33] Gram-negative bacteria have more complex cell walls with three layers: an outside layer of lipoprotein, a middle layer of lipopolysaccharide that functions as a barrier to the entry of antibacterial bioactive components, and an interior layer of peptidoglycan with a lipid content of 11-12%.[33]

The formulations containing mango peel extract inhibited *S. aureus* growth significantly, compared to blank emulsions and blank microemulsions ($p<0.05$). The growth inhibition was stronger with a greater concentration of mango peel extract, but not statistically significantly different ($p>0.05$). Okareh and coworkers[34] also reported that *S. aureus* bacteria exhibit a high susceptibility to mango kernel extract and ointment. Mango peel has a high concentration of phenolic components such as syringic acid, quercetin, mangiferin pentoside, and ellagic acid. The hydroxyl group (-OH) in these phenolic compounds plays a significant role in bacterial growth inhibition.[32] The group disrupts the cell wall in order to inhibit bacterial metabolism. The hydroxyl group (-OH) will most likely interact with proteins found in bacteria, preventing enzymes from working. This inhibits bacterial growth and causes microorganisms to death.

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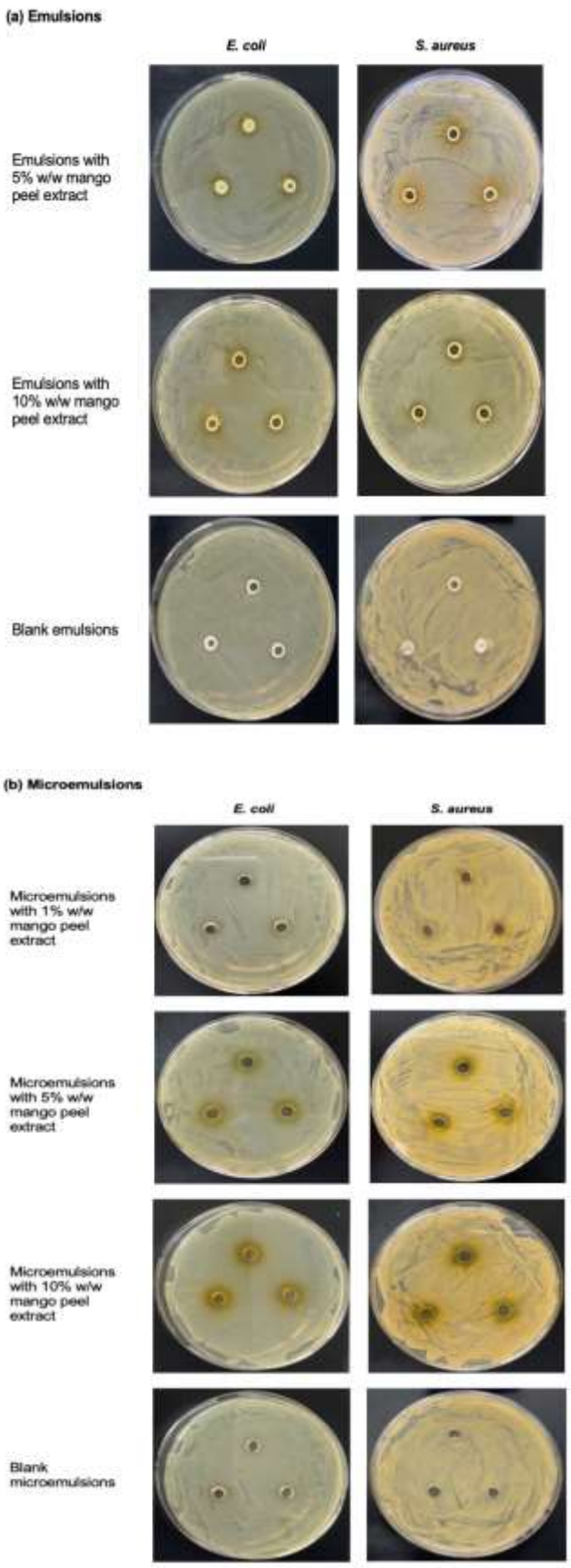


**Figure 4:** Antibacterial activity of (a) emulsions and (b) microemulsions containing mango peel extract against S. aureus and E. coli.

## Conclusion

This research work focuses on emulsion and microemulsion which were not studied before. These novel formulations can be applied as alternative products for treatment of bacterial infections. The extraction procedure employed in this research yielded mango peel extract. Then, different emulsion and microemulsion formulations comprising mango peel extract were prepared and characterized. The mango peel extract formulations have demonstrated an antibacterial and growth-inhibiting effect against only gram-positive bacteria, *S. aureus*. More testing and formulation optimization are needed, however, to offer a more accurate assessment of the effect on topical infections. In order to rule out any allergic reactions in patients, the pharmacological effect of the formulations will need to be researched in the future.

## 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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