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FORMULATION AND STABILITY TESTING OF NATURAL SUNSCREEN WITH DIFFERENT ESSENTIAL OILS AGAINST *Escherichia coli (E. coli)*

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Abstract:

The widespread use of synthetic sunscreen agents such as oxybenzone and avobenzone has raised significant environmental and health concerns, including coral reef bleaching and potential endocrine disruption. In response, this study aims to formulate a natural sunscreen incorporating essential oils with known antimicrobial properties, and to evaluate the antibacterial effectiveness of each formulation against *Escherichia coli* (*E. coli*). Three formulations were developed using tea tree oil (*Melaleuca alternifolia*), lavender oil (*Lavandula angustifolia*), and peppermint oil (*Mentha piperita*) at a concentration of 2% (w/w), with zinc oxide (20% w/w) serving as the physical UV blocker. All samples were tested using the agar well diffusion method, and the zones of inhibition were measured after 24 hours of incubation at 37°C to assess antibacterial activity. Preliminary findings suggest that tea tree oil exhibits the strongest inhibition zone against *E. coli*, while peppermint oil showed limited or negligible antibacterial effects. This study contributes to the growing body of research on natural cosmeceutical alternatives and supports the advancement of microbiologically stable, plant-based sunscreen formulation.

Keywords:

Natural Sunscreen, Essential Oil, Antimicrobial

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Introduction

Sunscreen development and application are essential for safeguarding the skin against the detrimental impacts of ultraviolet (UV) radiation. Solar ultraviolet (UV) radiation can lead to a range of skin problems such as early ageing, sunburn, and an elevated susceptibility to skin cancer (Agin et al. 2002). Conventional sunscreens generally include artificial chemical filters like oxybenzone, $C_{14}H_{12}O_3$ and avobenzone, $C_{20}H_{22}O_3$ which effectively block UV rays but have generated considerable environmental and health considerations (Downs et al. 2016). For instance, oxybenzone, $C_{14}H_{12}O_3$ has been associated with the bleaching of coral reefs and possible disturbance of the human endocrine system (Downs et al. 2016).

As a reaction to these concerns, there has been an increasing fascination with natural substitutes for synthetic sunscreens. Plant-derived essential oils have attracted interest for their inherent UV-blocking qualities and antibacterial actions, which may be advantageous in skincare products. Essential oils can serve as natural preservatives, providing antimicrobial benefits and extending the shelf life of cosmetics (Santos et al. 2019). Informed by earlier findings, it was found that essential oils such as carrot seed and jojoba oil increase the SPF of natural sunscreens when combined with physical blockers like zinc oxide (Safer et al. 2013). Examples of essential oils that have shown different levels of antibacterial activity include *Melaleuca alternifolia* (tea tree oil), *Lavandula angustifolia* (lavender oil), and *Mentha piperita* (peppermint oil). These oils can be advantageous in improving the stability and safety of natural skincare products (Cavanagh and Wilkinson 2005). Furthermore, these oils not only offer possible UV protection but also decrease microbial contamination, a crucial factor in reducing the proliferation of bacteria that could jeopardize the safety of the product (Hammer et al. 1999). In particular, tea tree oil has been intensively researched for its potent antibacterial effects against pathogens like *Escherichia coli* (*E. coli*) (Carson et al. 2006). In addition to its modest antibacterial properties, lavender oil has been included into some skincare products to reduce irritation and provide protection to the skin (Cavanagh and Wilkinson 2005). Peppermint oil, although not extensively researched in relation to sunscreens, has demonstrated certain antibacterial characteristics, however its impact on bacterial proliferation can vary (Singh et al. 2015).

By integrating these vital oils into sunscreen compositions, it is possible to effectively tackle the environmental and health issues linked to synthetic chemicals. The objective of this work is to investigate the development of a homemade sunscreen utilizing various essential oils and to evaluate their effects on the proliferation of microorganisms, particularly *E. coli*. This study is important in offering a more environmentally friendly and less risk substitute for conventional sunscreen and adds to the wider domain of natural cosmetic compositions.

Literature Review

The use of compounds such as oxybenzone and avobenzone in sunscreens, which are essential for skincare routines, has been subject to criticism. Although these chemicals effectively block UV radiation, they also present hazards to human health and the environment (Downs et al. 2016). Specifically, oxybenzone has been associated with the deterioration of coral reefs and has demonstrated the capacity to disturb the endocrine system in people (Downs et al. 2016). Responding to these concerns, scientists have initiated investigations into natural alternatives for sunscreen formulations, with a specific emphasis on plant-derived components such essential oils. Essential oils have shown its ability to block UV radiation and possess antibacterial qualities, which makes them very interesting options for the development of natural sunscreen formulations (Carson et al. 2006). The effectiveness of oils such as tea tree, lavender, and peppermint in inhibiting microbial development has been demonstrated to vary,

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suggesting their potential as substitutes for synthetic preservatives (Cavanagh and Wilkinson 2005).

Sunscreens are essential in safeguarding the skin against the detrimental impacts of UV radiation, encompassing both UVA and UVB wavelengths. UVA and UVB are two categories of ultraviolet (UV) radiation emitted by the sun, each impacting the skin in distinct ways. The main measure of sunscreen effectiveness is its Sun Protection Factor (SPF), which indicates the product's capacity to shield against UVB rays (Agin et al. 2002). While synthetic sunscreens that include chemical filters such as oxybenzone and octinoxate are indeed effective, there have been concerns expressed about their possible adverse effects on human health and marine ecosystems (Downs et al. 2016). Plant-derived essential oils are volatile chemicals that have been extensively researched for their therapeutic, antibacterial, and antioxidant characteristics (Cavanagh and Wilkinson 2005). Its natural origin and varied advantages have led to an increase in its use in cosmetic compositions. Multiple essential oils have shown effectiveness in both UV protection and antibacterial properties, as well as in enhancing the general stability of formulations. For instance, the strong antibacterial capabilities of tea tree oil have made it a promising option for enhancing microbial stability in sunscreens (Carson et al. 2006). Although lavender oil has modest antibacterial benefits, it is commonly used in skincare for its calming and anti-inflammatory qualities, which can be advantageous for those with sensitive skin (Kim and Lee 2002). However, the antibacterial benefits of peppermint oil have been demonstrated to be inconsistent and it may even stimulate the growth of certain bacteria (Singh et al. 2015). The oils not only provide further skin advantages but also have the ability to exhibit antibacterial properties, therefore enhancing the preservation of natural formulations that do not contain artificial preservatives.

Antimicrobial Activity of Essential Oils in Sunscreen Formulations
The microbial stability of natural skincare products is a notable issue due to their often absence of synthetic preservatives commonly used in commercial formulations. Integrating essential oils into sunscreens can potentially rectify this problem by utilising their antibacterial characteristics. The documented evidence supports the efficacy of essential oils in combating common bacteria, such as *E. coli*. Experiments have shown that tea tree oil has potent antibacterial effects, successfully suppressing the proliferation of *E. coli* (Hammer et al. 1999). As indicated by earlier studies that lavender oil has a moderate level of antibacterial action, providing a certain level of protection against bacterial infection (Cavanagh and Wilkinson 2005). However, peppermint oil, while advantageous for other purposes, may not induce the same degree of antibacterial effectiveness and could perhaps facilitate bacterial proliferation (Singh et al. 2015). Figure 1 shows the image of *E. Coli* swabbed on an agar plate. It is crucial to comprehend the interactions between these oils and bacteria, especially in sunscreen formulations, in order to create products that are both efficient in UV protection and resistant to microbiological infections.

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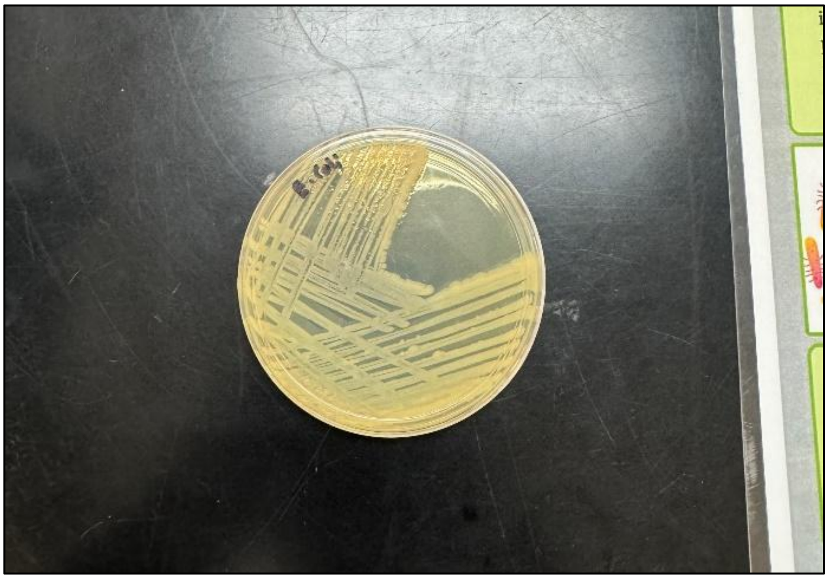

Figure 1: *Escherichia Coli* Swabbed on an Agar Plate
Source: Scientific Exploration III 2024 by Dr. Mimi Iryani Mat Taib, Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu.

According to prior research, it was found that natural ingredients, such as zinc oxide and titanium dioxide, are effective UV filters but need formulation enhancements to match the stability of synthetic sunscreens (Nightingale et al. 2005). Other study showed that essential oils such as rosemary and eucalyptus have potent antimicrobial effects, suitable for use as natural preservatives (Mannucci et al. 2018). Meanwhile, Eucalyptus oil has strong antibacterial and antifungal properties, making it useful in skincare formulations and rosemary and sage oils were found to be highly effective at preventing microbial contamination, suggesting their potential as natural preservatives (Boukhatem et al. 2013 and Djenane et al. 2012). In accordance with past research proved that tea tree oil showed strong antibacterial effects, particularly against E. coli, and reduced skin inflammation, demonstrated that botanical extracts like green tea and grape seed provide antioxidant protection and aid in UV-blocking, improving skin health making it ideal for natural formulations (Lee et al. 2017 and Gehring et al. 2011). In order to contribute significant insights into the development of safer and more sustainable skincare products, this work will build on existing research to investigate the stability and antibacterial activity of essential oils in a sunscreen formulation.

Materials and Method

There were four distinct formulations were prepared, one serving as the control and the remaining three incorporating different essential oils known for their potential antimicrobial properties. Each formulation was carefully developed to maintain consistency in base ingredients, with variations solely in the type of essential oil used. The antibacterial activity of these formulations was then assessed using the agar well diffusion method, a widely recognized microbiological technique for evaluating antimicrobial properties. This method involved introducing the prepared formulations into wells created in agar plates inoculated with E. coli, allowing for the diffusion of the active ingredients and subsequent observation of bacterial inhibition zones. The experiment was conducted in replicates to ensure the accuracy and reproducibility of results. Quantitative data on the diameter of the inhibition zones were meticulously recorded and analysed to compare the effectiveness of each formulation.

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Preparation of Sunscreens

Active Ingredients: The primary physical UV filter employed was zinc oxide (20% w/w) included. The essential oils employed at a concentration of 2% (w/w) were peppermint oil (*Mentha piperita*), lavender oil (*Lavandula angustifolia*), and tea tree oil (*Melaleuca alternifolia*).

Essential Components: Shea butter (30 g), coconut oil (20 g), beeswax (5 g), and vitamin E oil (5 drops) constituted the sunscreen foundation.

Shea butter, coconut oil, beeswax, and zinc oxide were initially heated and amalgamated over a controlled heat source to formulate the sunscreen base. The base was divided into four equal portions once it attained a temperature suitable for handling without compromising the integrity of the active components. A portion devoid of essential oil was designated as the control sample. This control served as a benchmark for assessing the antibacterial efficacy of the essential oils. An individual 2% (w/w) essential oil was included into each of the final three segments of the sunscreen base. To ensure consistent distribution of essential oils, each mixture was thoroughly agitated. The mixtures were transferred into sterile, labelled containers and allowed to solidify at ambient temperature.

Cultivation of Escherichia coli (E. coli)

Bacterial preparation was carried out following established standard inoculation procedures to ensure consistency and accuracy in the experimental setup. A loopful of *E. coli* culture was first transferred using a sterile inoculating loop into a test tube that held 10 cm^3 of sterile nutritional broth. The inoculated broth was then incubated at 37°C for a period of 24 hours to promote the multiplication of the bacteria and allow them to reach the logarithmic phase of growth, during which bacterial cells are most metabolically active and responsive to antibacterial agents.

Nutrient agar was prepared and poured into sterilised Petri dishes under aseptic conditions to avoid contamination. Sterile cotton swabs were dipped into the bacterial suspension and gently streaked over the entire surface of each agar plate using a consistent motion to ensure even distribution. This prepared setup served as the foundational stage for evaluating the effectiveness of natural sunscreen samples incorporated with essential oils.

Antibacterial Testing

The bactericidal activity of each sunscreen product was assessed using the agar well diffusion method, a standard technique for evaluating antimicrobial properties. To prevent cross-contamination and ensure reliable results, each sample control, tea tree, lavender, and peppermint was assigned to separate agar plates that had been uniformly inoculated with E. coli. Sterile equipment, such as micropipettes or spatulas, was used to apply approximately equal amounts of each sunscreen formulation to the centre of the respective agar surfaces. Care was taken to ensure even application and to avoid spillage beyond the designated area. Following sample application, the inoculated plates were incubated at 37°C for twenty-four hours to allow for sufficient interaction between the bacteria and the active compounds in the sunscreen. After the incubation period, the plates were observed for zones of inhibition, translucent circular areas surrounding the sample site that indicate suppression of bacterial growth.

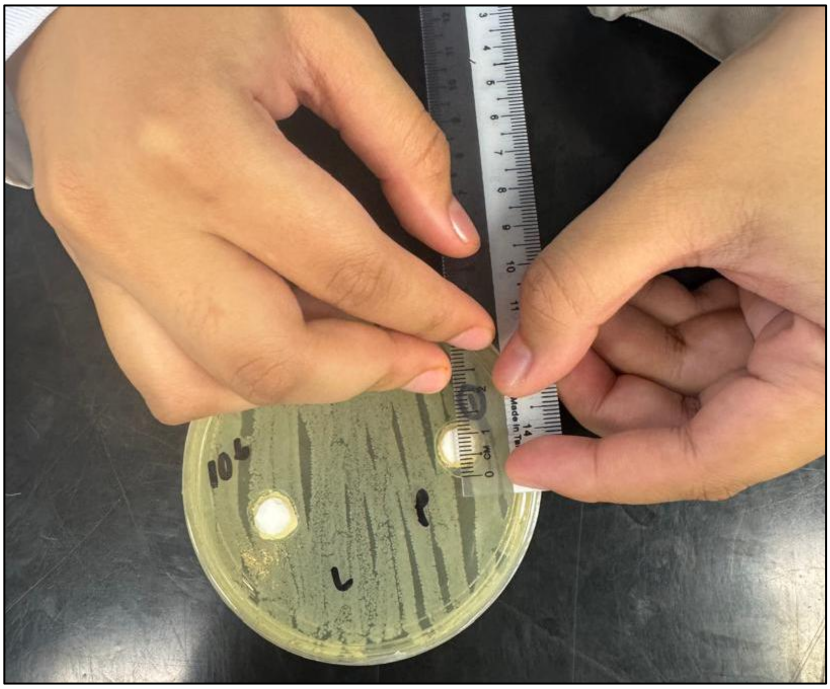

Figure 2: Measurement of the Inhibition Zone

A standard 15 cm ruler with millimetre markings was employed to measure the diameter of each inhibitory zone as shown in Figure 2. The control sample served as the baseline for comparison when data analysis is made. The data underwent descriptive analysis to evaluate the relative antibacterial effectiveness of the three essential oils. Enhanced antibacterial efficacy was assessed by the presence of bigger inhibition zones.

Two *E. Coli* bacterial concentrations were prepared:

- 6 µM – to represent moderate contamination levels commonly encountered during handling, swabbing and inoculating or post-production exposure.
- 8 µM – to simulate higher contamination levels that may occur due to inadequate preservation.

The efficacy of essential oils as natural preservatives in sunscreen formulations was evaluated by comparing the experimental findings with existing literature.

Result and Discussion

The antibacterial efficacy of sunscreen formulations incorporating three distinct essential oils, peppermint oil (*Mentha piperita*), lavender oil (*Lavandula angustifolia*), and tea tree oil (*Melaleuca alternifolia*) in comparison to a control formulation devoid of essential oils are discussed. The *E. coli*, a Gram-negative bacterium frequently associated with cosmetic contamination, was the subject of the antibacterial assessment. The evaluation was conducted under two distinct bacterial concentrations, 6 µM and 8 µM, to simulate diverse microbial contamination scenarios.

Stability Test: Agar Well Diffusion Method

Each inoculated agar plate was incubated for 24 hours at 37°C under standard microbiological conditions. This incubation period and temperature ensured optimal bacterial growth and effective diffusion of the formulation's active components into the surrounding medium. To

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ensure consistent and accurate quantification across all samples and conditions, the inhibitory zone diameters were measured in millimetres (mm) manually as illustrated in Figure 3.

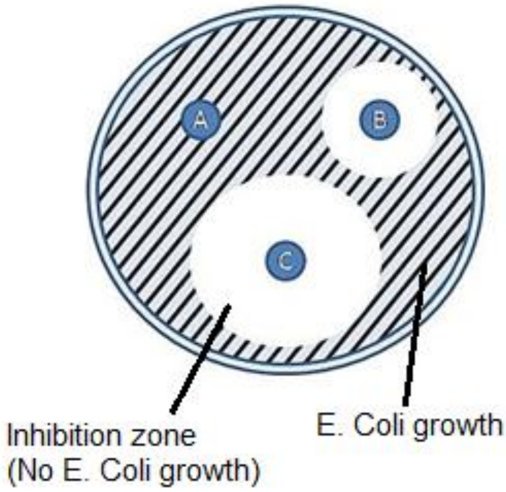

Figure 3: The Inhibition Zone Diameters Measured in Millimetres

The data obtained from the three independent experimental trials are compiled and presented below. The mean inhibition zone values and standard deviations are calculated for each sunscreen formulation to assess the repeatability of the results. The data representation provides an empirical basis for comparative analysis of antibacterial efficacy among the tested essential oils.

Table 1: Inhibition Zone Data for 6 µM *E. coli*

Sunscreen	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean (mm)	Standard Deviation (SD)
Control sample	0.00	0.00	0.00	0.00	0.00
Tea Tree Oil	16.10	15.80	16.00	15.97	0.15
Lavender Oil	9.20	9.50	9.00	9.23	0.25
Peppermint Oil	3.50	3.20	3.60	3.43	0.21

The antimicrobial screening results of the sunscreen formulations disclosed numerous significant findings. Among all evaluated formulations as shown in Table 1 and Table 2, the sunscreen with Tea Tree Oil demonstrated the highest significant bactericidal efficacy at both 6 µM and 8 µM concentrations of Escherichia coli. A minor decrease in the dimensions of the inhibitory zones was noted at elevated concentrations, indicating that the effectiveness of Tea Tree Oil is somewhat influenced by an increased bacterial load.

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Table 2: Inhibition Zone Data for 8 µM *E. coli*

Sunscreen	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean (mm)	Standard Deviation (SD)
Control sample	0.00	0.00	0.00	0.00	0.00
Tea Tree Oil	14.80	14.50	14.70	14.67	0.15
Lavender Oil	7.80	8.10	7.50	7.80	0.30
Peppermint Oil	2.90	2.80	3.00	2.90	0.10

Lavender Oil exhibited moderate and consistent bactericidal action at both doses, however a minor reduction in inhibition was observed at 8 µM. The Peppermint Oil formulation had little antibacterial efficacy, with negligible inhibitory zones observed in both circumstances. The control formulation, devoid of essential oils, consistently exhibited no inhibition, so indicating that the observed antibacterial effects were clearly linked to the incorporation of essential oils in the formulations.

Data were gathered at both 6 µM and 8 µM concentrations to determine the impact of bacterial load on preservation performance; however, the 6 µM concentration has been designated as the reference point for the comparative evaluation of the essential oils. This decision is underpinned by two principal elements. The 6 µM concentration accurately reflects the average levels of microbial contamination found in cosmetic items during normal use and storage. Secondly, inhibitory zone measurements at this dose demonstrated clearer separation among the studied formulations, so facilitating a more accurate assessment of their relative antibacterial efficacy. Consequently, to ascertain the most appropriate essential oil as a natural preservative in sunscreen formulations, the results at 6 µM are deemed the most pertinent and serve as the foundation for formulation recommendations in the ensuing analysis.

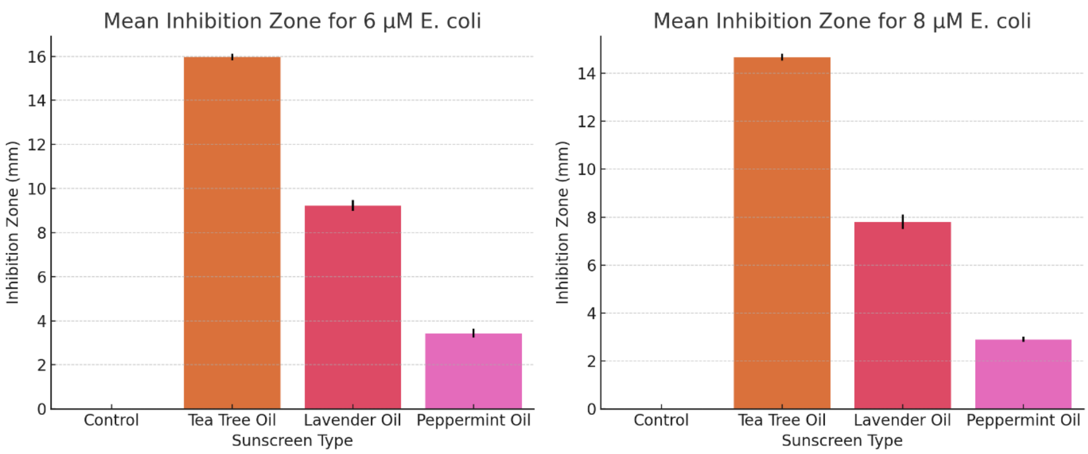

Figure 4: The Mean Inhibition Zone at 6 µM and 8 µM E. Coli

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Figure 4 shows the bar graphs illustrate the comparative effectiveness of each sunscreen formulation at both bacterial concentrations at 6 µM and 8 µM. The comparative investigation *E. coli* concentrations indicated a consistent trend: all essential oil-based formulations exhibited superior antibacterial activity at the lower bacterial concentration (6 µM). The inhibitory zones were markedly larger at this dosage, suggesting that essential oils are more efficacious in reducing microbial contamination under moderate circumstances. In contrast, when evaluated against the elevated bacterial concentration (8 µM), the inhibitory zones for all formulations diminished in size. The inverse relationship indicates that the fixed concentration of essential oil (2% w/w) may be inadequate against elevated microbial loads, highlighting the necessity for either increased dosages or the implementation of synergistic antimicrobial combinations to preserve efficacy in more challenging and strong bacterial contamination situations.

Conclusion

This study presents solid evidence that Tea Tree Oil is the most efficacious natural antibacterial agent among the essential oils evaluated for use into sunscreen formulations. Its capacity to suppress E. coli proliferation at both moderate and high bacterial densities substantiates its potential as a good natural preservative. The findings also illustrate the constraints of Lavender and Peppermint Oils as independent preservatives. Peppermint oil is not recommended for preservation applications because of its insufficient antibacterial qualities, but lavender oil can be used in supportive capacities. Future study should concentrate on assessing synergistic interactions among essential oils and investigating alternative natural chemicals to enhance antibacterial efficacy in cosmetic goods. Furthermore, broadening microbiological screening, incorporating SPF and stability evaluations, and performing dermal safety assessments would be essential stages in converting our findings into feasible commercial formulations.

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