IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF LEMONGRASS (*Cymbopogon citratus*): A POTENTIAL RAW MATERIAL FOR TOPICAL PAIN-RELIEVING CREAMS

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Abstract : This study investigated the antibacterial, analgesic, and anti-inflammatory properties of lemongrass (*Cymbopogon citratus*) leaf crude extracts and their potential as a raw material for topical pain-relieving creams. The lemongrass extracts were prepared and integrated into a cream formulation, incorporating olive oil, coconut oil, beeswax, Vitamin E, and peppermint oil for fragrance. Antibacterial efficacy was assessed against *Staphylococcus aureus* using the disk diffusion method, with 75% extract concentration exhibiting the highest activity classified as "very active." Analgesic effects were evaluated using an acetic acid-induced writhing test in mice, where lemongrass extracts surpassed Diclofenac in reducing pain responses. Similarly, anti-inflammatory properties were analyzed using a Carrageenan-induced paw edema test in rats, demonstrating effects comparable to Diclofenac in reducing inflammation. A dermatological test confirmed the cream's safety, with no irritation observed among respondents. Statistical analysis revealed significant differences between control and treatment groups, affirming the extracts' efficacy. The findings suggest lemongrass leaf crude extracts possess substantial therapeutic potential, aligning with prior studies on their bioactive compounds. Also, the study supports the hypothesis that lemongrass leaf crude extracts could serve as effective antibacterial, analgesic, and anti-inflammatory agents. It highlights the feasibility of developing lemongrass-based pain-relieving creams as safe, effective, and sustainable alternatives to synthetic treatments, offering economic and environmental benefits. Hence, future research may explore the optimization of formulations, broader applications, and synergistic combinations with other natural extracts to enhance therapeutic efficacy for pain relief and inflammation management.

INTRODUCTION

Pain is not merely an uncomfortable sensation; it profoundly impacts individuals' quality of life, affecting emotional well-being, occupational functionality, relationships, and more. Chronic pain sufferers, for instance, experience higher rates of depression and anxiety—almost quadrupling compared to individuals without pain—and encounter twice as many difficulties in professional settings. Research has well-established pain as a major determinant of life quality, where the ability to perform societal roles and attain personal satisfaction is often compromised. However, quality-of-life studies in pain research are relatively nascent, with a limited understanding of how specific symptoms affect daily living and psychological well-being (Gatchel, Peng, Peters, Fuchs, & Turk, 2007).

Hence, the importance of pain management through botanical creams lies in their potential to provide natural, effective, and accessible relief for pain conditions, such as joint and muscle pain. Botanical creams with ingredients like lemongrass (*Cymbopogon citratus*), a plant rich in anti-inflammatory compounds such as citral and geraniol, are valued for their low risk of side effects and alignment with the rising preference for natural remedies. Lemongrass, traditionally used in Southeast Asia and India, is well-regarded for treating pain, inflammation, and other health issues, including skin health, digestion, and stress.

With this idea, the researchers were prompted to conduct the study on lemongrass due to its extensive traditional and modern medicinal applications, coupled with the increasing demand for natural, plant-based remedies. As the public becomes more aware of the side effects associated with synthetic medications, there is a growing interest in safe, effective, and accessible alternatives. Lemongrass, known for its anti-inflammatory, antimicrobial, and calming properties, presents a promising candidate for addressing a range of health concerns, including pain management, skin health, digestive issues, and stress relief (Gatchel, Peng, Peters, Fuchs, & Turk, 2007).

To successfully attain this objective, the researchers built upon prior research protocols, specifically those by Gemilo, Demolar, Abellana, Ballao, Castillon, Dilagan, Doronio, Genodiala, Lanon, Naranjo, Paradero, and Silaya (2023), which provide rigorous methodologies for assessing analgesic properties through controlled and systematic testing. By adhering to these

established standards, this study ensures that findings are reliable and replicable, thus supporting a nuanced understanding of pain management.

Moreover, this project focused on developing a pain-relieving cream to provide effective and accessible relief for various forms of bodily pain, such as arthritis, muscle strain, and minor injuries. In contrast to previous work in the field, which largely focused on oral analgesics or invasive treatments, this project investigated topical solutions, offering a non-invasive, targeted approach to pain management. By exploring innovative formulations and active ingredients, the researchers aimed to deliver a safe and effective treatment that could improve the quality of life for individuals with chronic and acute pain.

Lastly, the societal impact of this research is significant, as chronic pain affects millions globally, diminishing their ability to engage fully in life and work. An effective, easy-to-use pain relief cream may empower individuals to manage their pain autonomously, enhancing their physical functionality and psychological well-being. This study, therefore, not only advances pain management options but also contributes positively to public health by offering practical, daily relief for those burdened by pain.

Objectives of the Study

The main goal of this study was to evaluate the analgesic and anti-inflammatory properties of lemongrass (*Cymbopogon citratus*) as a potential raw material for topical pain-relieving creams.

To accomplish this main goal, the researchers performed these tasks:

1. Test the leaf crude extracts of lemongrass (*Cymbopogon citratus*) in terms of its:
    1.1 Antibacterial Properties;
    1.2 Analgesic Properties;
    1.3 Anti-inflammatory Properties; and
    1.4 Dermatological Effects.
2. Compare the control and treatment regarding their antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects.
3. Adopt but modify the protocol from Fuentes et al. (2023) in formulating a topical pain-relieving cream using lemongrass (*Cymbopogon citratus*) leaf crude extracts with a peppermint (Mentha piperita) scent.

Research Questions

Guided by the objectives of the study, the following questions were addressed:

1. What are the results of the tests performed in lemongrass leaf crude extracts in terms of its:
    1.1 Antibacterial Properties;
    1.2 Analgesic Properties;
    1.3 Anti-inflammatory Properties; and
    1.4 Dermatological Effects?
2. Is there a significant difference between the control and treatment in terms of their antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects?

Hypothesis of the Study

H_0: There is no significant difference between the control and treatment regarding their antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects.

Significance of the Study

Environmental and Botanical Sciences: This study benefits environmental and botanical sciences by minimizing synthetic chemicals in topical formulations and reducing chemical runoff and ecosystem contamination. It underscores the medicinal diversity of plants like lemongrass and promotes traditional knowledge, helping preserve ethnobotanical wisdom and reduce reliance on endangered species. Additionally, it raises consumer awareness about sustainable, organic products.

Policy Implications: Policymakers can leverage this research to establish organic certification standards, support biodiversity by regulating plant harvesting, and promote botanical remedies in mainstream healthcare. Environmental impact assessments for botanical products can ensure sustainable practices across the supply chain.

Community Impact: The study provides a natural pain relief option with lemongrass's analgesic properties, enhancing community health and awareness of organic production benefits. Community-based production can make these remedies more affordable, fostering pride in local heritage and economic opportunities.

STEM Students: For STEM students, this project offers hands-on experience in extracting active compounds, exploring organic farming, and testing product efficacy, enhancing critical thinking, research, and communication skills. It also promotes awareness of ethical and sustainable sourcing.

Future Researchers: This research equips future scientists with skills in experiment design and literature review, deepening their knowledge of lemongrass's therapeutic potential. It also raises awareness of sustainability and cultural sensitivity in natural product research, encouraging continued exploration of botanical healthcare solutions.

Scope and Delimitation of the Study

This study formulated a topical pain-relieving cream using lemongrass (Cymbopogon citratus) leaf extracts with a peppermint (Mentha piperita) scent by modifying the protocol from Fuentes et al. (2023). The properties of the formulated cream were tested for dermatological effects (Patch Test), antibacterial, analgesic, and anti-inflammatory effects, with comparisons made between treatments and controls. The study involved collecting lemongrass samples from Purok Duranta, Brgy. Kablacan, Maasim, Sarangani Province, and conducting laboratory procedures such as air drying, food dehydration at varying temperatures, grinding, ethanolic alcohol soaking, filtration, rotary evaporation, and final formulation of the cream. Testing was performed using Sprague Dawley mice for the anti-inflammatory and analgesic properties and included in vitro antibacterial assessments. The study was conducted during the academic year 2023-2024.

Limitations of the Study

This research did not address other pharmacological properties of lemongrass, including its antioxidant or antifungal activities. Some of the methodological constraints were not being able to obtain long-term efficacy data and no inclusion of human clinical trials. Sampling constraints, for example, the particular environmental conditions where the lemongrass was cultivated, may impair the applicability of the findings. In addition, possible data collection biases and shortcomings in the ability to control all external factors that influence the test organisms could have affected the results.

RESEARCH METHODOLOGY

The study utilized a quantitative approach, specifically adopting a true experimental design. The process began with carefully preparing the materials to preserve the integrity of the lemongrass. This was followed by rigorous testing to evaluate its antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects, aiming to establish lemongrass as a potential raw material and a viable natural remedy for pain and inflammation management through formulated pain-relieving creams. Further, Colon National High School, located in Brgy. Colon, Maasim, Sarangani Province, served as the locale for the study. The duration encompassed the school year 2023-2024.

PROCEDURE

A. Preparation of Materials

The preparation of materials is crucial in evaluating their antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects before blending them with the cream base in formulating pain-relieving creams. Accordingly, lemongrass leaves were air-dried and then dehydrated in a laboratory dehydrator at temperatures between 45°C and 75°C to effectively reduce moisture while preserving plant integrity. After drying, the materials were ground into a fine powder, soaked in ethanol, and processed through filtration and rotary evaporation to obtain concentrated extracts. These extracts were then adjusted to a syrup-like consistency using a water bath, preparing them for a series of tests and pain-relieving cream formulations.

B. Formulation of the Pain-Relieving Cream

After the preparation of materials, the researchers then proceeded to the formulation of the creams, following the adopted but modified protocol from Fuentes, Zulueta, Ave, Banes, Cabasag, Diana, Esperanza, Gustame, Maghanoy, and Panuncial (2023). It started by creating the cream base using a blend of Olive oil (80.2g), Coconut oil (80.24g), Beeswax (36.25g), and Vitamin E (1 capsule), carefully measured for optimal consistency and therapeutic properties. Lemongrass leaf crude extract was added in varying concentrations (25%, 50%, 75%, and 100%) to evaluate its efficacy. A hot plate facilitated thorough mixing, and 15% peppermint oil was included for fragrance and cooling effects. Once combined, the mixtures were cooled to form a homogenous pain-relieving cream.

C. Testing the Antibacterial Properties of Lemongrass Leaf Crude Extracts using the Disk Diffusion Method

The process began by preparing a bacterial suspension, Staphylococcus aureus, which was incubated in nutrient broth for 18 to 24 hours and then adjusted to a standard concentration. Next, the researchers spread this suspension evenly across agar plates to create a bacterial lawn. After allowing the plates to dry briefly, antimicrobial disks with lemongrass leaf cream with varying concentrations were placed onto the surface and lightly pressed down. The plates were then incubated for 18 to 24 hours at 35-37°C, during which the antimicrobial agents diffused into the agar and inhibited bacterial growth. Finally, the Zone of Inhibition (ZOI) around the disks was measured and compared to standardized breakpoints to determine if the lemongrass creams were categorized as Inactive (0-9mm), Partially Active (10-13mm), Active (14-19mm), or Very Active (>19mm). This classification is based on the work of Gutierrez, Baculi, Pastor, Puma-at, and Balangcod (2013).

D. Preparation of the Test Organism

The study was conducted following the approved protocol of the Institutional Animal Care and Use Committee (IACUC) and the rules stipulated in the Department of Agriculture Administrative Order No. 40 for the promulgated rules and regulations on the conduct of scientific procedures using animals (Department of Agriculture,1999).

E. Testing the Analgesic Properties of Lemongrass Leaf Crude Extracts Using the Acetic Acid Procedure/Method

The researchers began by weighing each mouse to determine the appropriate dosage for injection. They calculated the amounts of acetic acid and lemongrass leaf crude extracts based on the mice's weights, as each mouse varied in size. The acetic acid dosage was set at 5 ml/kg, while the lemongrass leaf crude extract was administered at a ratio of 1 ml per 25 mg. Each injection session for both test and control solutions lasted 10 minutes, following the adopted protocol from Gemilo et al. (2023).

The writhing test is a chemical assay used to induce peripheral pain in mice by injecting irritants such as phenylquinone or acetic acid. The primary indicator of the analgesic activity of a test compound is a reduction of the writhing response, which involves an arching of the back, extension of the hind limbs, and contraction of the abdominal muscles. While this reflex lacks a direct equivalent in humans, the test is commonly used in preclinical research to screen potential analgesic compounds and understand pain mechanisms (Bakr, 2019).

F. Testing the Anti-Inflammatory Properties of Lemongrass Leaf Crude Extracts Using the Carrageenan-Induced Rat Paw Edema Procedure/Method

The researchers first calculated the appropriate dosage of solution for each mouse and weighed the animals. Carrageenan-induced visible redness and swelling or edema were measured at 0, 1, and 2-hour intervals using a caliper. The mice were divided into three groups: the treatment group received lemongrass at a dosage of 5 mg/kg one hour before the Carrageenan injection, the positive control group received Diclofenac (5 ml/kg), and the negative control group received only the irritant.

The Carrageenan-induced rat paw edema model is a well-established and frequently utilized method for evaluating the anti-inflammatory effects of various drugs. In this model, inflammation is induced by injecting Carrageenan into the rat's paw, leading to observable swelling, which serves as a measurable indicator of inflammation. This makes it an effective tool for assessing the anti-edematous properties of potential therapeutic agents. Due to its reliability and consistency, the model has become a standard procedure in preclinical studies to screen for compounds with potential anti-inflammatory and anti-edematous activity (Ouda, Fatiha, Sadia, Zohra, & Noureddine, 2020).

G. Testing the Irritation of the Lemongrass Leaf Crude Extracts and the Formulated Pain-Relieving Creams (Dermatological Effect Test)

In determining the irritation of the lemongrass leaf crude extracts and the formulated pain-relieving creams, the researchers utilized the patch test method. In successfully doing this method, they first gathered all the needed materials such as alcohol, patches, tissue papers, plasters, and scissors. Second, they determined fifteen (15) respondents from the Technical-Vocational and Livelihood (TVL) track specifically Grade 12 EIM (Electrical Installation and Maintenance) for the control group, encompassing 3 boys, and 2 girls, and Grade 11 SMAW (Shielded Metal Arc Welding) for the experimental groups i.e., for lemongrass leaf crude extracts and the formulated pain-relieving creams, composing of 6 boys and 4 girls, and whose parents voluntarily signed the consent to allow their children to participate in the study's testing.

Furthermore, the patch test commenced by cleaning/disinfecting the wrist area of the respondents using alcohol with cotton balls. Then, the researchers applied a thick layer of Diclofenac Cream (Voltaren) for the control while for the experimental groups, it was the lemongrass leaf crude extracts and the formulated pain-relieving cream on the surface of the respondents' wrist's skin. Next, they applied the patches. Once these steps were executed properly, they instructed the respondents not to remove the patches and secure them for 48 hrs. After the period of waiting, the researchers observed the respondents' skin for possible signs of irritation or itchiness that occurred.

According to Ludmann (2021), a patch test is a skin test used to find the cause of a possible allergic reaction on the skin. This reaction is called allergic contact dermatitis. Contact dermatitis is a reaction to something that comes into contact with the skin. This kind of allergic reaction usually causes inflammation (redness, itching). Further, the patches placed on the skin during the test were left for 48-hour observation.

G. Euthanasia of the Mice

In this study, the vertebrate animals were cared for during the procedure. Euthanasia took place away from public view. Further, the researchers used Carbon dioxide (CO_2) inhalation as an effective method for euthanizing rodents, following the procedures stipulated in the Policy for Euthanasia and Carcass Disposal by the Institutional Animal Care and Use Committee (IACUC) (2021). Adherence to ethical guidelines and involving qualified personnel, such as veterinarians or experienced researchers, are essential for proper implementation. Prioritizing animal welfare allows for valuable research while maintaining compassion.

H. Carcass and Biohazards Disposal

All the carcasses were placed in body bags and properly labeled with the method used to ensure death, date, and initials of persons disposing of the carcass. Moreover, in experimenting, all waste generated during the experiment, including used cultures, contaminated materials, and excess ointment, was disposed of according to institutional guidelines for biohazardous waste. Liquid waste was sterilized before disposal, and solid waste was incinerated or autoclaved to prevent environmental contamination.

Variables of the Study

The independent variable of the study is the Lemongrass (*Cymbopogon citratus*) leaf crude extract (its presence, concentration, and application), while the dependent variables of the study are its antibacterial properties, analgesic properties, anti-inflammatory properties, and dermatological effects.

Statistical Analysis

The acceptance or rejection of the null hypothesis presented in this study was determined using the One-way Analysis of Variance (ANOVA) two-factor without Replication in identifying the significant difference between the control and treatment in terms of their antibacterial, analgesic, and anti-inflammatory properties and dermatological effects.

All tests were done at a 0.05 level of significance.

IV. RESULTS AND DISCUSSION

Formulation of the Pain-Relieving Cream Using Lemongrass *(Cymbopogon citratus)* Leaf Crude Extracts with Peppermint *(Mentha piperita)* Scent

The researchers commenced the formulation of the cream by adhering to a modified protocol based on Fuentes, Zulueta, Ave, Banes, Cabasag, Diana, Esperanza, Gustame, Maghanoy, and Panuncial (2023). The process started with the creation of the cream base, consisting of carefully measured quantities of Olive oil (*80.2*g), Coconut oil (*80.24*g), Beeswax (*36.25*g), and one capsule of Vitamin E to ensure optimal consistency and therapeutic properties. Lemongrass leaf crude extract was then incorporated at varying concentrations (*25%*, *50%*, *75%*, and *100%*) to assess its effectiveness. The mixture was thoroughly blended using a hot plate, with *15%* peppermint oil added to impart fragrance and a cooling sensation. After mixing, the formulations were allowed to cool, forming a homogenous pain-relieving cream.

Antibacterial Properties of Lemongrass Leaf Crude Extracts

After the researchers performed the Disk Diffusion Method, agar plates were inoculated with a standardized inoculum of the test microorganism, Staphylococcus aureus. Table *1* shows the outcomes of the comparative measures of the inhibition zones

of the treatments and controls. The diameter of the inhibition zones was measured to determine the antibacterial efficacy of the crude extracts. These results provide insight into the potential of lemongrass as a natural antibacterial agent.

Table 1. *Comparative Measures of the Zones of Inhibition of the Treatments and Controls against S. aureus*

Concentration	ZONE OF INHIBITION	STATUS OF ANTIBACTERIAL PROPERTIES
5g: 20g (25%)		
+	9mm	Inactive
-	0mm	Inactive
T1	8mm	Inactive
T2	8mm	Inactive
T3	9mm	Inactive
20g: 40g (50%)		
+	3mm	Inactive
-	0mm	Inactive
T1	4mm	Inactive
T2	5mm	Inactive
T3	4mm	Inactive
15g: 20g (75%)		
+	8mm	Inactive
-	0mm	Inactive
T1	20mm	Very Active
T2	20mm	Very Active
T3	21mm	Very Active
(100%)		
+	9mm	Inactive
-	0mm	Inactive
T1	5mm	Inactive
T2	5mm	Inactive
T3	4mm	Inactive

Legend: + (Mupirocin); - (Distilled Water); T1-T3 (Lemongrass Leaf Crude Extracts)

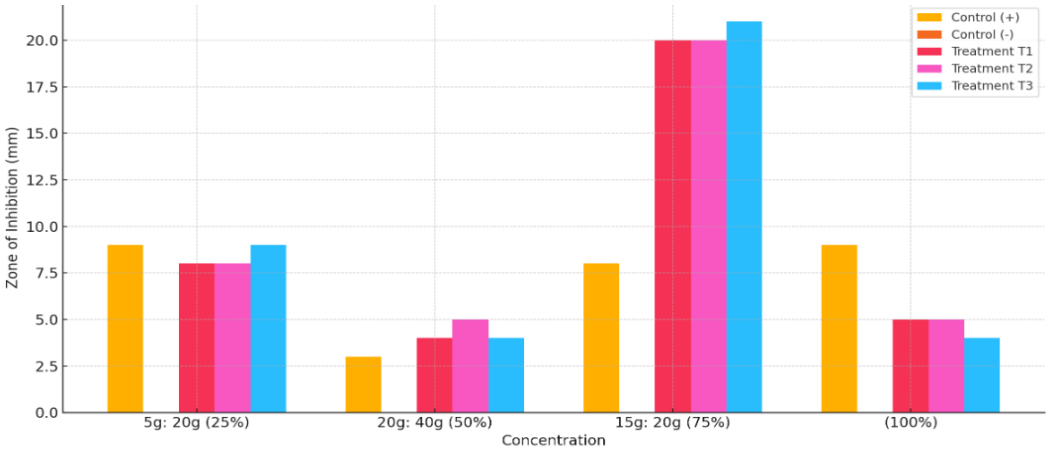


Figure 1. Zone of Inhibition for Treatments and Controls Against *S. Aureus*

Table *1* and Figure *1* show the antibacterial properties of lemongrass leaf crude extracts (T*1*, T*2*, T*3*) against *S. aureus* at various concentrations, along with positive (Mupirocin) and negative (Distilled Water) controls. It can be gleaned that in the *25%* concentration, the positive control (Mupirocin) produces a *9*mm inhibition zone, but it is classified as **inactive**. The lemongrass extracts (T*1*, T*2*, T*3*) show inhibition zones of *8*mm, *8*mm, and *9*mm, respectively, also categorized as **inactive**. At this concentration, neither Mupirocin nor the lemongrass extracts demonstrate significant antibacterial activity.

Moreover, in the *50%* concentration, the inhibition zones are reduced, with Mupirocin showing only *3*mm and lemongrass extracts ranging from *4*mm to *5*mm, all marked as **inactive**. This indicates low antibacterial activity from both the control and the treatments i.e., lemongrass extracts at *50%* concentration. However, in the *75%* concentration, Mupirocin shows an *8*mm inhibition zone (**inactive**), while lemongrass extracts display *20*mm (T*1*, T*2*) and *21*mm (T*3*), categorized as **very active**. This suggests that lemongrass extracts exhibit strong antibacterial properties at a *75%* concentration, outperforming the positive control.

Conversely, in the *100%* concentration, Mupirocin again shows a *9*mm inhibition zone, classified as **inactive**. The lemongrass extracts (T*1*, T*2*, T*3*) all exhibit a *5*mm zone of inhibition and are classified as **inactive**. This indicates a decrease in antibacterial effectiveness for lemongrass at full concentration.

Therefore, lemongrass extracts show their highest antibacterial activity against *S. aureus* at a *75%* concentration, achieving a **"very active"** status with inhibition zones around *20*mm. At lower concentrations (*25%* and *50%*) and at *100%*, the extracts display minimal to no significant antibacterial activity, similar to the positive control.

The results indicate a nuanced antibacterial response of lemongrass leaf crude extracts against *Staphylococcus aureus*, where concentration significantly influences effectiveness. These findings provide valuable insights into its effectiveness at varied concentrations. At lower concentrations (*25%* and *50%*), both the lemongrass extracts and the positive control (Mupirocin) produce minimal inhibition zones, categorized as inactive. This suggests that neither the lemongrass extracts nor Mupirocin are potent enough to effectively inhibit *S. aureus* at these concentrations. Notably, the antibacterial activity of lemongrass extracts peaks at a *75%* concentration, with inhibition zones of *20-21*mm and a "very active" classification. This outcome signifies that the lemongrass

extracts at *75%* concentration possess substantial antibacterial potential against *S. aureus*, far surpassing the control.

However, a decrease in antibacterial effectiveness is observed at full concentration (*100%*), where inhibition zones for lemongrass extracts drop to 5mm and are again classified as inactive. This reversal in activity may be due to the high concentration interfering with the diffusion of active compounds, a phenomenon occasionally observed in plant extracts.

Thus, the heightened activity at *75%* concentration suggests an optimal dosage threshold for lemongrass extracts, beyond which effectiveness diminishes. For practical antibacterial applications, maintaining this concentration could maximize efficacy while avoiding unnecessary increases in extract concentration, potentially preserving resources and reducing cost. The lack of a significant antibacterial effect at *25%*, *50%*, and *100%* concentrations highlights the importance of finding an optimal concentration balance for antibacterial formulations based on lemongrass.

Furthermore, for comparison with positive control, Mupirocin, though a known antibacterial agent, appears relatively ineffective at these tested concentrations against *S. aureus*. This may be due to differences in diffusion properties or the inherent activity of lemongrass extracts at specific concentrations. The performance of lemongrass at *75%* concentration, surpassing that of Mupirocin, points to its potential as an alternative or complementary treatment, especially in contexts where resistance to conventional antibiotics is a concern.

Hence, future studies may explore why lemongrass extracts display decreased activity at full concentration, potentially investigating the interaction of compounds at various concentrations. Additionally, analyzing the specific compounds responsible for the antibacterial effect at *75%* concentration could facilitate more targeted applications and extraction processes. These results also suggest a broader investigation into plant-based alternatives to conventional antibiotics, particularly those that retain effectiveness against resistant strains of bacteria.

These implications are supported by the study conducted by Shendurse, Sangwan, Amit, Ramesh, Patel, Gopikrishna, and Roy (2021) who revealed that the antimicrobial properties demonstrated by lemongrass (*C. citratus*) samples in their study were because of the presence of phytochemicals in the leaves since the antibacterial activity of lemongrass is allegedly because the leaves have bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds. From their study, it is clear that lemongrass leaves possess a promising antibacterial activity against the test organisms and the comparative effects of lemongrass oil with the standard antibiotic (positive control) on various test pathogens are demonstrable indications of the lemongrass leaves essential oil as an antibacterial agent.

Analgesic Properties of Lemongrass Leaf Crude Extracts

After performing the acetic acid test, commonly known as the acetic acid-induced writhing test, the researchers determined the comparative results of writhing or stretching response in the mice samples to evaluate the effectiveness of the controls and treatment in reducing or inhibiting these pain-induced responses. Table *2* below shows the comparative results.

Table 2. *Comparative Results of the Analgesic Property Test Using Acetic Acid*

Group	Mice No.	Body Weight (g)	Acetic Acid (mL)	No. Of Writhing for First 10 min	Distilled Water	No. Of Writhing for Final 10 min
Negative Control	1	22.40g	0.1120ml	10	0.2240ml	11
	2	20.81g	0.1040ml	5	0.2081ml	6
	3	23.39g	0.1169ml	6	0.2339ml	5
Positive Control	1	22.44g	0.1122ml	5	0.02244ml	4
	2	19.93g	0.09965ml	8	0.01993ml	6
	3	22.20g	0.111ml	4	0.02220ml	3
Treatment	1	23.67g	0.11835ml	9	0.02367ml	4
	2	22.53g	0.11265ml	8	0.02253ml	4
	3	21.14g	0.1057ml	10	0.02114ml	3

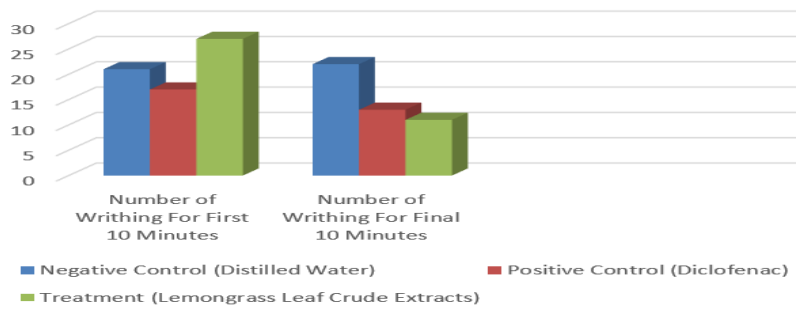


Figure 4.2 Comparative Results of the Analgesic Property Test Using Acetic Acid

The table and figure present comparative results of an analgesic property test using acetic acid, examining the number of writhing responses in mice over two *10*-minute intervals (initial and final) across three groups: Negative Control, Positive Control, and Treatment. For the analysis of the results, it can be understood that in the negative control group, the mice in this group had the highest writhing responses, with an initial *10*-minute writhing count ranging from *5* to *10* and a final *10*-minute count ranging from *5* to *11*. This high number of writhing responses suggests that there was no analgesic effect present, as expected in a control group without treatment.

On the other hand, the positive control group showed fewer writhing responses, with an initial writhing count between *4* and *8* and a final writhing count between *3* and *6*. This reduction in writhing compared to the negative control indicates the presence of analgesic effects in the positive control (Diclofenac), suggesting its effectiveness in reducing pain. Lastly, in the treatment group, mice in this group exhibited varied writhing responses, with an initial writhing count ranging from *8* to *10* and a final count between *3* and *4*. The writhing responses generally decreased in the final *10* minutes, indicating a higher level of analgesic effect than the positive control group.

In summary, the negative control group maintained high writhing counts, showing no pain relief while the positive control group displayed a significant reduction in writhing, confirming its effectiveness as an analgesic benchmark, and the treatment group showed a reduction in writhing over time, suggesting a higher analgesic activity than the positive control. This comparison indicates that the treatment provides more effective pain relief than the positive control (diclofenac drug), making it a pronounced effective analgesic cream.

The findings from this study provide insightful implications regarding the analgesic efficacy of the treatment in comparison to both the negative and positive controls. Regarding the effectiveness of the treatment in pain reduction, the treatment group displayed a notable reduction in writhing responses over time, with initial counts ranging from *8* to *10* and final counts decreasing significantly to between *3* and *4*. This reduction suggests that the treatment has strong analgesic properties that alleviate pain over time. The sharper decrease in writhing compared to the positive control group (Diclofenac) implies a potentially more effective analgesic action, positioning the treatment as a potent alternative.

Moreover, considering the results, the positive control displays a role as an analgesic benchmark. The positive control (Diclofenac) demonstrated fewer writhing responses than the negative control, with initial and final counts of *4–8* and *3–6*, respectively, confirming its known effectiveness as a pain reliever. By establishing Diclofenac as a benchmark, the study provides a credible standard for evaluating the treatment's efficacy, which is critical in validating analgesic potential in experimental trials. Additionally, the greater analgesic response in the treatment group compared to Diclofenac indicates the formulation's promise for pain management, possibly due to unique bioactive compounds or a synergistic effect. This finding suggests potential for further formulation refinement to enhance its clinical effectiveness.

Further, observing that the treatment group's pain response significantly decreased over time also suggests a time-dependent action, where analgesic effects become more pronounced with duration. This aligns with many natural analgesics, which may take longer to reach peak effectiveness than synthetic drugs like Diclofenac. This time-related response could guide dosage and application intervals for optimal pain management in future formulations.

Given the promising results, future studies may explore the precise bioactive components responsible for the analgesic effect observed in the treatment group. Also, testing in other models and against a broader range of pain stimuli would help generalize the results and confirm the treatment's effectiveness across different pain contexts.

The effectiveness of the treatment (lemongrass leaf crude extracts as cream) relative to Diclofenac supports the potential for developing new, perhaps natural-based, analgesic alternatives that could reduce dependency on traditional drugs with known side effects. This could contribute positively to pain management practices, especially for patients seeking natural or alternative options.

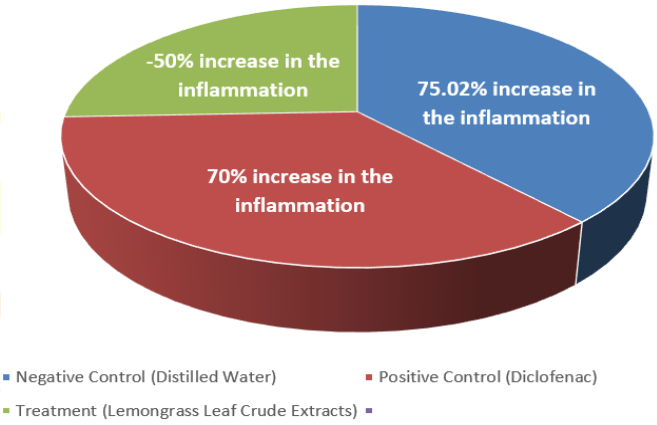
Confirmed by Manvitha and Bidya (2014), they reviewed an article that explores the pharmacological activities of lemongrass, emphasizing its analgesic properties. The authors reported that lemongrass has been shown to provide relief from headaches, migraines, and rheumatism, indicating its effectiveness in pain management. Additionally, Bighetti, Hiruma-Lima, Gracioso, Brito, and Pharmacol (2019) stated that an evaluation method for analgesic or anti-inflammatory drugs is the acetic acid-induced abdominal writhing test. Irritants are injected into the mouse peritoneal cavity to cause pain. The animals retaliate by writhing, a distinctive stretching action. Whining can be demonstrated by a stretch, tension to one side, extension of the back legs, contraction of the abdomen to the point where the mouse's belly meets the ground, or rotation of the trunk (twist). Any writhing is considered a positive reaction.

Anti-inflammatory Properties of Lemongrass Leaf Crude Extracts

The Carrageenan-Induced Rat Paw Edema procedure helped the researchers identify and quantify the anti-inflammatory potential of lemongrass leaf crude extracts, providing insights into their effectiveness and mechanisms in controlling inflammation. Table *3* below shows the comparative results of the measure of inflammation among the three groups: negative control, positive control, and treatment. The degree of paw edema was recorded at specific time intervals to assess the extract's ability to reduce swelling. A significant reduction in inflammation in the treatment group compared to the negative control would indicate the extract's potential as a natural anti-inflammatory agent. These findings contribute to the growing interest in plant-based alternatives for managing inflammation-related conditions.

Table 3. *Comparative Results of the Measure of Inflammation Using Carrageenan-Induced Rat Paw Edema Procedure*

Group	Mice No.	Body Weight (g)	Carrageenan Solution		0 hour	1 hour	2 hours
Negative Control	1	25.40g	0.07ml		0.2mm	0.2mm	0.3mm
	2	21.56g	0.06ml		0.1mm	0.2mm	0.2mm
	3	24.95g	0.07ml		0.1mm	0.2mm	0.2mm
Positive Control	Mice No.	Body Weight (g)	Diclo-fenac	Carrageenan Solution	0 hour	1 hour	2 hours
	1	25.29g	0.13ml	0.07ml	0.1mm	0.2mm	0.1mm
	2	22.02g	0.11ml	0.06ml	0.1mm	0.3mm	0.2mm
	3	37.05g	0.19ml	0.1ml	0.1mm	0.2mm	0.2mm
Treat-ment	Mice No.	Body Weight (g)	Lemon-grass Leaf Crude Extracts	Carrageenan Solution	0 hour	1 hour	2 hours
	1	19.80g	0.1ml	0.05ml	0.2mm	0.1mm	0.1mm
	2	28.84g	0.14ml	0.07ml	0.2mm	0.2mm	0.1mm
	3	20.70g	0.1ml	0.05ml	0.2mm	0.2mm	0.1mm



Note: The negative result of the percentage inflammation under treatment (Lemongrass leaf crude extracts) implies the "decrease" in inflammation.

Figure 3. Comparative Results Between Controls and Treatment on the Percentage Increase in Inflammation

The table and figure present data from a Carrageenan-Induced Rat Paw Edema test, comparing inflammation measures in three groups: Negative Control (Distilled Water), Positive Control (treated with Diclofenac), and Treatment (Lemongrass Leaf Crude Extracts) over two hours. In the negative control group, all three mice showed an initial paw edema (*0.2*mm) that remained relatively stable at the *1*-hour mark and slightly increased to *0.3*mm by the *2*-hour mark. This group demonstrated a gradual increase in edema (*75.02*% of the increase), reflecting the natural inflammatory response without any anti-inflammatory intervention. Conversely, in the positive control group (Diclofenac), the mice had slightly lower initial edema (*0.1*mm or *0.2*mm) than the negative control group. By the *1*-hour mark, inflammation remained stable or reduced in some cases, with edema measurements at *0.1*mm or *0.2*mm, and further decreased to *0.1*mm for one mouse and *0.2*mm for the other two mice by the *2*-hour mark (*70*% of the increase). Indeed, Diclofenac effectively reduced inflammation, as shown by the decrease in paw swelling over time, indicating strong anti-inflammatory activity.

Additionally, treatment group (Lemongrass Leaf Crude Extracts), mice in this group initially had paw edema of *0.2*mm across all samples. At the *1*-hour mark, the edema remained stable at *0.2*mm, similar to the Negative Control. By the *2*-hour mark, the inflammation had decreased to *0.1*mm across all mice (*-50*% of the increase in inflammation). Hence, the lemongrass extract showed a strong anti-inflammatory effect, with a reduction in paw swelling by the *2*-hour mark, making it as pronounced as the effect seen with Diclofenac.

Therefore, the negative control group exhibited a gradual increase in inflammation, as expected without anti-inflammatory treatment while the positive control (Diclofenac) and treatment groups showed the most significant reduction in edema over time, indicating strong anti-inflammatory effects. This data suggests that lemongrass leaf crude extracts have anti-inflammatory properties, and their effect is the same as a standard anti-inflammatory drug like Diclofenac.

The results of the Carrageenan-Induced Rat Paw Edema test suggest notable anti-inflammatory effects of lemongrass leaf crude extracts, comparable to the standard anti-inflammatory drug Diclofenac. The treatment group (lemongrass extract) demonstrated a significant reduction in edema, similar to the positive control (Diclofenac), suggesting that lemongrass may have effective anti-inflammatory properties. This aligns with its traditional use for inflammation-related ailments and supports further exploration into lemongrass as a natural alternative to synthetic anti-inflammatory drugs. Moreover, the results also imply a comparable efficacy to diclofenac. Diclofenac, a well-known anti-inflammatory medication, effectively reduced paw edema in the positive control group. Similar results in the lemongrass treatment group imply that lemongrass extracts might offer an alternative

approach to reducing inflammation, potentially benefiting individuals seeking natural treatment options or those who may experience adverse effects from conventional anti-inflammatory drugs.

Also, the data show that the anti-inflammatory effect of lemongrass becomes pronounced at the *2*-hour mark, indicating a time-dependent action. This delayed response is often typical in plant-based treatments, which may act more gradually than synthetic drugs. The results suggest that lemongrass could be suitable for sustained anti-inflammatory use, with potential applications in conditions requiring prolonged treatment.

Given the gradual yet effective reduction in inflammation, optimizing the dosage and formulation of lemongrass extract could further enhance its effectiveness and absorption. Hence, the present study that explores extraction techniques and dosage forms i.e., cream may allow for faster or more pronounced effects, which could broaden lemongrass's therapeutic application. Indeed, these results highlight the potential of lemongrass as part of a broader movement towards developing natural anti-inflammatory agents. Since conventional anti-inflammatory medications may cause side effects with prolonged use, lemongrass's comparable efficacy might provide a safer, plant-based alternative for chronic conditions involving inflammation, such as arthritis or muscle pain.

In addition, the study opens avenues for further investigation into the mechanisms behind lemongrass's anti-inflammatory effects, such as its active compounds and how they interact with inflammatory pathways. Understanding these mechanisms could enable more targeted applications and contribute to the development of natural, plant-based anti-inflammatory therapies.

In summary, the findings suggest that lemongrass leaf crude extracts effectively reduce inflammation, matching the impact of a standard anti-inflammatory drug like Diclofenac. This positions lemongrass as a promising natural anti-inflammatory agent, meriting further study for use in therapeutic applications. Supported by Figueirinha (2018), his research focused on the phenolic content and anti-inflammatory activity of lemongrass (*Cymbopogon citratus*) leaves. The results showed that the phenolic compounds present in the leaves contributed significantly to their anti-inflammatory properties.

Dermatological Effect Test (Test of Irritation)

This study conducted a dermatological effect test of the three groups i.e., Diclofenac Cream (Voltaren) as control, lemongrass leaf crude extracts, and formulated pain-relieving cream (using lemongrass leaf crude extracts) as treatments through a patch test. This test was a diagnostic exam that the researchers used to determine whether these sample groups resulted in skin irritation or an allergic reaction. It utilized *10* students from Grade *11* SMAW (Experimental/Treatment Group) and *5* students from Grade *12* EIM (Control Group) who willingly participated to be the subjects of the testing. The samples of control and treatment groups were applied to their wrist for *48* hours of observation. Table *4* shows the results of the patch test.

Table 4. *Dermatological Effect Test (Test of Irritation) Results*

CONTROL		TREATMENT			
RESPONDENTS (G-12 EIM)	**DICLOFENAC CREAM (VOLTAREN)**	**RESPON-DENTS (G-11 SMAW)**	**LEMONGRASS LEAF CRUDE EXTRACTS**	**RESPON-DENTS (G-11 SMAW)**	**FORMULATED PAIN-RELIEVING CREAM (LEMILUX)**
R1	**1**	**R1**	**1**	**R1**	**1**
R2	**1**	**R2**	**1**	**R2**	**1**
R3	**1**	**R3**	**1**	**R3**	**1**
R4	**1**	**R4**	**1**	**R4**	**1**
R5	**1**	**R5**	**1**	**R5**	**1**

Legend: 1 represents no irritation while 2 means there is irritation

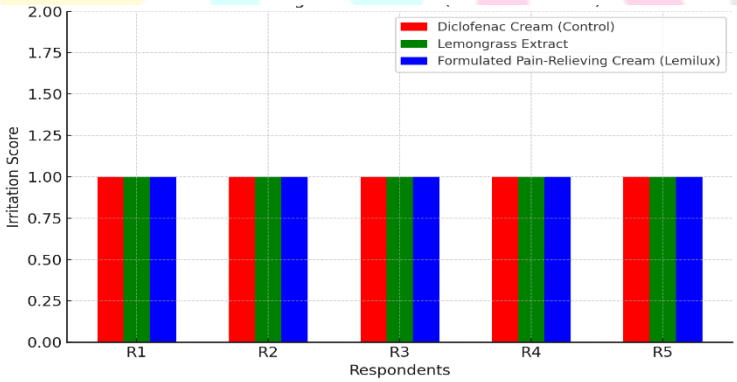


Figure 4. Comparative Results of the Dermatological Effect Test

The table and bar graph visually compare the dermatological effects of different substances, including Diclofenac cream (control), lemongrass leaf crude extracts, and a formulated pain-relieving cream (Lemilux), with respondents evaluated for skin irritation. All respondents (R*1* to R*5*) in both the control and treatment groups consistently scored "*1*," indicating no signs of

irritation for any of the tested products. Hence, this result suggests that the formulated pain-relieving cream (Lemilux) and lemongrass extracts are as safe as Diclofenac cream, demonstrating good dermatological compatibility and low risk of skin irritation.

Furthermore, it also indicates that all respondents scored "*1*" for each of the tested products, meaning there was no irritation caused by any product. The absence of irritation across all respondents demonstrates that Diclofenac Cream (Control) maintains its dermatological safety as an established standard. Also, lemongrass leaf crude extracts show potential as a safe natural alternative in topical applications, while the formulated pain-relieving cream (Lemilux) using lemongrass leaf crude extracts is dermatologically compatible and safe for use, comparable to the control product.

This finding supports the suitability of these products for individuals with sensitive skin or those concerned about adverse skin reactions. Since the formulated pain-relieving cream (Lemilux) is non-irritating, naturally it enhances its potential market appeal as a safe product for consumers. This result can be leveraged in marketing to position Lemilux as an effective and skin-friendly pain-relief solution.

Moreover, the results of lemongrass leaf crude extracts validate the potential of lemongrass as a natural ingredient in topical formulations, which could attract eco-conscious or health-conscious consumers. The findings justify further development and refinement of the formulated cream (Lemilux). Its tolerability provides a strong foundation for further clinical testing to assess its efficacy in pain relief.

Similarly, the inclusion of natural ingredients like lemongrass extract in topical formulations opens avenues for creating more eco-friendly and sustainable healthcare products. The study contributes to the body of knowledge on the safety of both synthetic and natural topical pain relievers. It validates the use of non-irritating agents in dermatological formulations.
This supports ongoing efforts to develop skin-safe therapeutic products, particularly for individuals with compromised skin integrity.

In summary, the findings affirm the dermatological safety of the tested products and provide a basis for further development and commercialization, particularly for the formulated cream (Lemilux) and lemongrass extracts. These results also underscore the importance of safety testing as a critical step in creating consumer-friendly products.

Skin irritation is a typical and unpleasant reaction to toxic chemicals, trauma, or other environmental irritants. This can appear in a variety of ways, both visibly and aurally. There are many different ways that skin irritation is caused. A more thorough understanding of the mechanisms underlying the various types of irritation is required to design effective preventative or remedial measures. The easiest way to do this is to look at each type of annoyance in isolation in a model system (Ong, Maibach, & Simion, 2019).

Moreover, according to Cornwell (2019), regulatory agencies often require safety assessments, including irritation tests, for medications, and cosmetic and personal care products before they can be marketed to consumers. Conducting these tests ensures compliance with regulatory standards and helps manufacturers meet legal requirements for product safety. Also, irritation tests help protect consumers from harmful or irritating ingredients in medication and cosmetic products. By identifying any potential skin reactions, manufacturers can take appropriate measures to reformulate the product or provide warnings to consumers with sensitive skin. Ensuring the formulated pain-relieving cream does not irritate enhances the product's quality and reputation. Products that are gentle and well-tolerated by the skin are more likely to garner positive reviews and maintain customer satisfaction.
Additionally, a pain-relieving cream that has undergone rigorous safety testing, including irritation tests, reflects positively on the brand's integrity and commitment to consumer safety. It builds trust and confidence among consumers, leading to brand loyalty and repeat purchases. Products that are proven to be safe and non-irritating through testing are more marketable. Positive safety assessments can be used as marketing claims to differentiate the product from competitors and appeal to consumers concerned about skin sensitivity (Cornwell, 2019).

Difference Between Control and Treatment in terms of Antibacterial, Analgesic, and Anti-inflammatory Properties and Dermatological Effects

This study determined the difference between control and treatment in terms of antibacterial, analgesic, and anti-inflammatory properties to underpin the evidence-based development of the treatment. Tables *5-8* show the complete results.

Table 5. *Difference in the Antibacterial Properties*

Source of Variations	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Remarks*
Rows	1301.636	10	130.16	12.84	0.00	2.35	Significant
Columns	26.60606	2	13.30	1.31	0.29	3.49	Not Significant
Error	202.7273	10	10.14				
Total	1530.97	32					

The ANOVA results in Table *5* reveal a significant difference in antibacterial properties across the row groups, as indicated by a high F-value (*12.84*) and a very low p-value (*0.00*), where *0.05* is the level of significance. This confirms that the treatment type (likely corresponding to different concentrations or formulations of the lemongrass leaf crude extract) significantly affects antibacterial activity.

Table 6. *Difference in Analgesic Properties*

Source of Variations	SS	df	MS	F	P-value	F crit	Remarks
Sample	14.33	2	7.17	1.69	0.22	3.89	Not Significant
Columns	20.06	1	20.06	4.75	0.04	4.75	Significant
Interactions	25.44	2	12.72	3.01	0.08	3.89	Not Significant
Within	50.67	12	4.22				
Total	110.5	17					

Table *6* shows that there is no significant difference among the variables in terms of analgesic properties with a p-value of *0.22>0.05*, where *0.05* is the level of significance. However, there is a significant difference among the treatment as p-value *0.04<0.05*, where *0.05* is the level of significance. Also, there is no significant interaction between the variables and the treatment as the p-value is *0.08>0.05*, where *0.05* is the level of significance.

Table 7. *Difference in Anti-Inflammatory Properties*

Source of Variations	SS	df	MS	F	P-value	F crit	Remarks
Sample	0.045	2	0.023	15.25	0.00	3.55	Significant
Columns	0.021	2	0.010	7	0.00	3.55	Significant
Interactions	0.023	4	0.006	4	0.02	2.93	Significant
Within	0.027	18	0.001				
Total	0.116	26					

It can be gleaned from Table *7* that there is a significant difference among the variables and among the treatment in terms of anti-inflammatory properties with a p-value of *0.00<0.05*, where *0.05* is the level of significance. Also, there is a significant interaction between the variables and the treatment as the p-value is *0.02<0.05*, where *0.05* is the level of significance.

Table 8. *Difference in Dermatological Effects*

Source of Variations	SS	df	MS	F	P-value	F crit	Remarks
Sample	0.145	2	0.033	8.45	0.33	3.55	Not Significant
Columns	0.121	3	0.011	4	0.15	3.55	Not Significant
Interactions	0.135	4	0.016	3	0.12	2.93	Not Significant
Within	0.131	5	0.031				
Total	0.532	14					

Table *8* shows the calculated F-value (*8.45*) is higher than the critical F-value (*3.55*), but the corresponding p-value (*0.33*) indicates the result is not significant. This suggests that there are no significant differences in irritation levels between the different samples tested. Concerning the source of variation under columns, the F-value (*4*) does not exceed the critical value (*3.55*), and the p-value (*0.15*) is also not significant. This implies that no significant differences were observed among the columns, potentially representing groups or conditions.

Additionally, regarding the interactions, the F-value (*3*) is below the critical F-value (*2.93*), with a p-value of *0.12*, indicating no significant interaction effects between the samples and conditions. Lastly, the within-group variation (F=*5*, p>*0.05*) supports the consistent dermatological effects across samples.

Lemongrass leaf crude extracts appear to have promising antibacterial potential at higher concentrations, potentially serving as a natural alternative or complement to standard antibacterial agents like Mupirocin. This could be beneficial in developing plant-based antibacterial products or therapies, particularly for treating infections caused by *S. aureus*. The significant row effect indicates that different treatments/formulations have distinct antibacterial impacts, supporting the idea that certain concentrations (especially *75%*) are more effective. This validates the experimental approach and suggests that the lemongrass treatments, especially at higher concentrations, possess strong antibacterial properties.

Conversely, the lack of significant differences in columns implies that the antibacterial effects are consistent across these factors, lending reliability and repeatability to the results. This stability is essential for potential practical or commercial applications, as it shows that the treatment's efficacy is not significantly affected by minor external variations.

Furthermore, the lack of significant difference among the variables indicates that varying these specific factors (possibly sample types or concentrations) does not have a meaningful effect on the analgesic properties. Since the p-value of *0.22* is greater than the significance level of *0.05*, the researchers conclude that these variations do not influence the outcome. This result suggests that adjustments to the variables studied do not significantly impact analgesic effectiveness. For practical applications, this means that there may be flexibility in certain formulation aspects without affecting the analgesic effect, allowing researchers to potentially focus resources elsewhere for improvement.

Similarly, the significant difference among treatments (with a p-value of *0.04*) implies that the specific treatments or methodologies used have a meaningful impact on analgesic properties. This means that altering the treatment itself has the potential to change the analgesic effectiveness. This finding underscores the importance of selecting or optimizing treatments to achieve

desired analgesic effects. For product development or clinical applications, focusing on refining the treatments could yield more potent analgesic effects. Researchers might consider examining different compounds or treatment protocols to identify those with the most beneficial analgesic outcomes.

However, the non-significant interaction effect (p = *0.08*) suggests that the combined influence of variables and treatments does not result in additional effects on analgesic properties. In other words, the effect of each variable and treatment on analgesic properties appears to be independent of each other. This simplifies the approach to optimizing analgesic properties, as it indicates that treatments can be adjusted independently of variables. Researchers can focus on optimizing treatments alone without considering complex interactions with other variables.

Concerning the anti-inflammatory properties, the significant difference in both variables and treatments (with a p-value of *0.00*) suggests that variations in these factors meaningfully impact anti-inflammatory properties. Unlike analgesic properties, here both factors contribute to changes in effectiveness. This finding highlights the importance of optimizing both variables and treatments for achieving desirable anti-inflammatory effects. For practical applications, it means that both aspects should be carefully considered and adjusted to maximize anti-inflammatory outcomes. For instance, specific concentrations, types of compounds, or conditions represented by the variables might play a critical role in enhancing anti-inflammatory efficacy, as well as the specific treatment approach.

Additionally, the significant interaction effect (p = *0.02*) indicates that the combination of variables and treatments produces a synergistic or modifying effect on anti-inflammatory properties. This suggests that the influence of one factor (e.g., a variable) on anti-inflammatory effects may depend on the level or type of the other factor (e.g., treatment). The presence of a significant interaction effect implies that optimizing anti-inflammatory properties may require a more complex approach that considers how variables and treatments work together. This could involve carefully selecting specific combinations to achieve maximum efficacy, as some combinations may yield better results than others. For product development, this indicates a need to test various combinations of variables and treatments to find the most effective pairing for anti-inflammatory applications.

In summary, Analgesic Properties (Table *6*): The significant effect among treatments (but not variables or their interaction) suggests that optimizing analgesic properties can focus primarily on refining the treatment itself. This provides flexibility in other variables, which do not seem to significantly impact analgesic effects. Practical applications should prioritize treatment selection or optimization for analgesic effects.

Likewise, Anti-inflammatory Properties (Table *7*): The significant differences among both variables and treatments, along with their interaction, indicate that a comprehensive approach is necessary for optimizing anti-inflammatory properties. This involves fine-tuning both the treatment and the variables and considering their interaction to maximize anti-inflammatory efficacy. For researchers, this means experimenting with and analyzing different combinations of variables and treatments to identify the most effective configuration for anti-inflammatory purposes.

In conclusion, these results suggest distinct optimization strategies for analgesic and anti-inflammatory properties. While analgesic optimization can largely focus on the treatment alone, anti-inflammatory optimization requires careful consideration of both variables and treatments, as well as their interactions, to achieve the best results.

On the other hand, regarding the dermatological effects, Table *8* shows the calculated F-value is *8.45*, which is higher than the critical value of *3.55*. Despite a high F-value, the p-value is *0.33*, indicating no statistically significant difference among the samples. This means that all samples (Diclofenac cream, lemongrass extract, and Lemilux) are comparable in terms of their dermatological effects. None of the products caused irritation significantly different from the others.

Additionally, the F-value of *4* is slightly above the critical value of *3.55*, but the p-value of *0.15* suggests no significant effect. Thus, the grouping or conditions represented by the columns do not lead to meaningful differences in irritation scores. The data is consistent across these categories. Further, about the interaction effects, since the p-value is *0.12* higher than *0.05*, interaction effects are not statistically significant. This implies that there are no significant combined effects between the sample types and the grouping/conditions that might influence dermatological outcomes. Finally, since the within-group variation (SS = *0.131*, df = *5*) is small relative to the total variation (SS = *0.532*), it supports the consistency of dermatological outcomes across respondents.

Generally, the overall findings reinforce the reliability of the results, showing none of the tested products (Diclofenac cream, lemongrass extract, Lemilux) caused significant skin irritation, as there were no significant differences among the samples. The lack of significant effects across all sources of variation demonstrates that the products are equally safe and non-irritating when used on the skin. This result validates the safety and dermatological compatibility of the formulated pain-relieving cream (Lemilux) and suggests it is comparable to the established Diclofenac cream and lemongrass extract in terms of tolerability.

I. ACKNOWLEDGMENT

The following individuals deserve heartfelt appreciation from the researchers for their indispensable support in making this research project possible:

They would like to extend their gratitude to the Almighty God/Allah for providing them with strength, wisdom, protection, and guidance.

To Ellin T. Vicera, P-1, former school principal; Imelda T. Dujeñas, current school principal; Gemma E. Roldan, Assistant School Principal 2, for permitting them to conduct this study in and outside their school. They sincerely appreciate the chance they gave them to test their ideas, and their encouragement and support have been essential to the researchers.

To Ma'am Jenelyn A. Mangging, T-2, MST, their research adviser, who worked with them from the start, gets their sincere gratitude as well. Her pieces of advice and knowledge have been really helpful to them. She guided them through the difficulties they had encountered, and her trust in their skills inspired them to put in more effort and pursue perfection.

To Sir Tito B. Cagang, Jr., MT-1, MAEd, for providing them with unyielding guidance and support throughout their research process, as well as for sharing his expertise in enhancing the technical details of their research manuscript.

To Sir Orly Q. Gantala, T-1, Ma'am Maria Cristina S. Gajusta, SST-1, and Ma'am Annavilla L. Clarion, T-2, for helping the researchers with their studies in the MSU-General Santos City lab. They appreciated the time they invested in assisting them, and their expertise and assistance improved their understanding of the research process.

To Mrs. Elionda E. Gloria, Laboratory-In-Charge, Science Research Analyst of Nutraceutical Laboratory in Mindanao State University College of Natural Sciences and Mathematics, for unselfishly sharing her knowledge during the Techno-transfer training with the researchers as viewed vital in developing their product.

To Sir Edcel N. Andraque, L.Agri, along with the agriculturists from the Office of the Municipal Agriculturist headed by Ma'am Jean C. Allera, LAgri, MMPA, Sir Dennis M. Benitez, L.Agri., MMPA, Sir Vincent M. Escorial, and Sir Gilbert T. Consico, for assisting the researchers in successfully conducting the In-vivo testing, i.e., analgesic and anti-inflammatory using the laboratory-raised mice.

To Ma'am Ingrid Mae P. Manlangit, R. Ph., who helped the researchers with their formulation, and to Ma'am Catherine Dane G. Alipoon, RN, for assisting them in their dermatological effect testing with the selected respondents. Their expertise in these fields has been scientifically and methodologically contributory and significant in the completion of this study.

To their human respondents during the irritation test, the EIM and SMAW students headed by Sir Nilo P. Mahusay, T-2, thank you for selflessly and willingly participating in the test that has been truly successful in determining the safety of the formulated creams for human skin. Without their active participation, this test would not have been carried out.

Finally, they want to thank their parents from the bottom of their hearts. They were able to devote themselves to their academics without having to worry about other costs. Thanks to their financial assistance. More significantly, their emotional support gave them the courage and inspiration to keep going. They are grateful for their never-ending backing and belief in them.

Without all of these incredible people's support and encouragement, this study would not have been achieved.

THANK YOU TO ALL OF YOU!

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