CHARTULAE JOURNAL
OFFICIAL DEPARTMENTAL RESEARCH PUBLICATION OF THE COLLEGE OF PHARMACY
ISSN:3082-4354 MAIDEN ISSUE VOL. 1 NO. 1

EVALUATION AND FORMULATION OF LUBEG (Syzygium lineatum) LEAVES BASED TOPICAL CREAM: A STUDY FOR SUPPLEMENT ANTIMICROBIAL MANAGEMENT AND WOUND HEALING AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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ABSTRACT

Escherichia coli and Staphylococcus aureus is one the of the leading causative agent of skin infection worldwide. The Philippine Bureau of Plant Industry promotes Lubeg which has been reported to have a possible antimicrobial activity and wound healing property. The purpose of this study is developed and evaluate the antimicrobial activity and wound healing property of Lubeg Topical Cream against Escherichia coli and Staphylococcus aureus in Sprague Dawley rats a total of 15 rats were divided in three equal groups for the treatment distribution: the negative control group, the positive control group, and the treatment group which received the formulated lubeg topical cream. The lubeg extracts were subjected for microbial assaying to determine the two highest eliciting inhibitory percentage concentrations. The two highest in inbitory effect against Escherichia coli and Staphylococcus aureus is the 75% and 100% Lubeg extract. The two concentration was subjected for the formulation of topical cream. The formulation group, on average had a significant difference in concentration and across time when compared to the positive controls when it comes to its inhibitory effect. While observing the wound healing there is a significant difference between the formulation group and the positive control which post-hoc analysis revealed that the formulation group has a potential wound healing after 14 days of post administration of treatments. Therefore, the Lubeg topical cream can be an antimicrobial supplement management against Staphylococcus aureus and Escherichia coli.

Key words: *Staphylococcus aureus, Escherichia coli, Sprague Dawley Rats*

INTRODUCTION

One of the major problems that the world is facing is the antimicrobial resistance. The misuse of antibiotics accelerates the emergence of development of new antibiotics. Thus, there is an increased

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interest in medicinal plants as alternative antibiotics (Mimoune et al.,2013). Topical application has many potential advantages over systemic therapy, including high and sustained concentrations of drug directly at the infected site, low quantity of antibiotic needed, better compliance, fewer systemic side effects and potentially less chance of antimicrobial resistance (Sigh et al.,2019)

However, worldwide are still experiencing an increase of Staphylococcus aureus as the main cause of skin and soft tissue infection (Tong et al., 2015; Dayan et al., 2016; Peterson & Schora, 2016). Infections are more troublesome in patients with disrupted epidermal stratum corneum, a common occurrence in atopic dermatitis where colonization reaches almost 100% (Kobayashi et al., 2015; Tauber et al., 2016; Meylan et al., 2017).Recurrent S. aureus skin infections are reported in 39% of patients within three months and >50% within six months of initial infection, necessitating repeated courses of antibiotics and increasing the risk of antibiotic resistance (Miller et al., 2015a; Geoghegan et al., 2018).

The study's objective is to evaluate and formulate Lubeg base topical as a supplement antimicrobial management and wound healing against S. aureus and E. coli. This research aims to develop a topical cream that could supplement the management of antimicrobial resistance. The findings will give opportunity to the different government institutions concerned like the Department of Science and Technology; the study will provide information that could help the institution to promote and do additional thorough investigation about the antimicrobial effectiveness of Lubeg leaves extract as a topical cream for antimicrobial management and wound healing property against staphylococcus aureus and Escherichia coli.

METHODOLOGY

The methodology used in this study was presented in this part. It included the research design, participants of the study, data gathering tool, data gathering procedures, and the statistical analysis for the data collected.

Research Design

The research design was pure experimental research design. It includes the extraction of ethanolic extracts from the leaves of Lubeg, evaluation of the antimicrobial activity of the extracts against Staphylococcus aureus and Escherichia coli, and formulation of Lubeg leaf-based topical cream to determine the constituents of Syzygium lineatum leaves. The extraction was conducted at Laboratory Sciences of St. Paul University Philippines, and phytochemical screening was conducted at the Department of Science and Technology.

Subject of the Study

The researcher used Staphylococcus aureus and Escherichia coli to measure the antimicrobial activity of the formulated lubeg topical cream.

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Data Gathering Tool
The researcher used a observational study through experiments in assessing the antimicrobial activity of the formulated Lubeg topical cream and its wound healing activity

Data Gathering Procedure
Prior to conducting the study, the researcher secure different permits like the animal permit for the used Sprague Dawleys Rats in assessing the wound healing activity of the formulated topical cream. The researchers collected and prepared the lubeg leaves for extraction and after the extraction of the lubeg leaves, the extract was subjected for the formulation of the topical and underwent antimicrobial assay with the different concentration that is being made and the two highest concentrations that showed inhibitory effect and were compared to the commercially available topical cream**.**

RESULT
The results and discussion of all the data accomplishing the different methods of experimentation that evaluate the Lubeg leaves Topical cream in antimicrobial management and wound healing against S. aureus and E. coli after the microbial assaying and treatment of extract and controls.

TABLE 1 **ORGANOLEPTIC PROPERTIES**

ORGANOLEPTIC PROPERTY	**OBSERVATION**
Odor	**Odorless**
Texture	**Soft**
Color	**Brownish-yellow**

Table 1 showed the organoleptic test results wherein it was stated in the study of Fitriani et al., (2020) that the senses, which are used to define the qualities of crude pharmaceuticals based on form, smell, colour, and taste, are employed to specify organoleptic criteria. According to the results, the formulated Lubeg-based topical cream exhibited no significant smell or odourlessness with a brownish-yellow colour. On the other hand, the formulated Lubeg-based topical cream has a watery texture with a transparent opacity

ZONE OF INHIBITION OF THE DIFFERENT CONCENTRATION AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

ANTIBACTERIAL ANALYSIS									
SAMPLE CODE	**SAMPLE DESCRIPTION**	**ZONE OF INHIBITION (MM)**							
		STAPHYLOCOCCUS AUREUS				***ESCHERICHIA COLI***			
		R1	**R2**	**R3**	**MEAN**	**R1**	**R2**	**R3**	**MEAN**
MIC-0957	**25% lubeg extract**	10	9	8	9	8	7	9	8
MIC-0958	**50% lubeg extract**	11	12	12	12	10	11	9	10
MIC-0959	**75% lubeg extract**	15	16	15	15	14	12	13	13
MIC-0960	**100% lubeg extract**	16	17	15	16	15	15	17	16

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Negative control	**Distilled water**	6	6	6	6	6	6	6	6

The tables showed the differentiation of the mean of the zone of inhibition against E. coli and S. Aureus. The S. aureus, 25% Lubeg extract has a mean of 9 which clarify that it is inactive, 50% Lubeg extract has a mean of 12 which clarify that it is partially active, the 75% Lubeg extract has a mean of 15 which clarify that it is active. The E. coli 25% Lubeg extract has a mean of 8 which inference the result inactive, the 50% Lubeg extract mean is 10 which clarify it is partially active, the 75% Lubeg extract is 13 and it is also partially active and the 100% Lubeg extract is 16 which resulted to be active.

The table showed that the two highest concentrations having the highest zone of inhibition in Staphylococcus aureus are the 75% Lubeg extract with a mean of 15 and the 100% Lubeg extract with a mean of 16, the interference of these two is that they are both active. The two highest concentrations having the highest zone of inhibition in Escherichia coli are the 75% Lubeg extract with a mean of 13, which inference is partially active and the 100% Lubeg extract with a mean of 16 which inference is active.

It is agreeable with this study conducted by Haghgoo et.al 2017 that base on the result of their study that the antibacterial effect increased with an increase in the concentration of the extract.

TABLE 3 **ZONE OF INHIBITION OF 75% LUBEG CREAM, 100% LUBEG CREAM, POSITIVE CONTROL AND NEGATIVE CONTROL**

ANTIBACTERIAL ANALYSIS									
Sample Code	**Sample Description**	**ZONE OF INHIBITION (mm)**							
		Staphylococcus aureus				***Escherichia coli***			
		R1	**R2**	**R3**	**MEAN**	**R1**	**R2**	**R3**	**MEAN**
MIC-1098	**75% LUBEG EXTRACT**	12	13	15	13	10	11	12	11
MIC-1099	**100% LUBEG EXTRACT**	19	17	20	18	14	15	16	15
Positive Control	**Mupirocin**	34	35	37	35	33	33	32	32
	Trimycin	36	37	38	37	35	35	34	34
Negative Control	**Distilled Water**	6	6	6	6	6	6	6	6

The table showed that the commercially available products which is Mupirocin and Trimycin, exemplified a great effect in inhibiting the growth of Staphylococcus aureus and Escherichia coli with an inference of very active and the 100% Lubeg extract also elicit an inhibitory effect in inhibiting the growth of Staphylococcus aureus and Escherichia coli with an inference of active and the 75% Lubeg extract has the inference of partially active based on the inferences made by the Department of Science and Technology. See the Appendices for the basis of inference based on the result of the test result from the Department of Science and Technology.

TABLE 4 **DIFFERENCES ON THE ANTIMICROBIAL EFFECT OF THE LUBEG TOPICAL CREAM AS COMPARED TO *STAPHYLOCOCCUS AUREUS***

VARIABLES	**f – value**	**p - value**	**DECISION**

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Concentration	**16.311**	**.000**	**Significant**
Across time	**589.461**	**.000**	**Significant**
Interaction	**1.565**	**.103**	**Not Significant**

The study made use of Two-way Analysis of Variance (ANOVA) which is a statistical method used to analyze the influence of two different categorical independent variables on a dependent variable. In the context of the study on the wound healing of the Lubeg topical cream compared to Staphylococcus aureus, two independent variables were investigated: Concentration (presumably of the topical cream) and Time. The dependent variable was the antimicrobial effect.

The F-values and p-values in the table indicate the results of the ANOVA for each variable. The F-value is a measure of the ratio of the variance between groups to the variance within groups. The p-value represents the probability of obtaining the observed results if the null hypothesis is true.

For the variable Concentration, the F-value is 16.311 with a p-value of .000. This indicates a significant difference in the wound healing activity across different concentrations of the Lubeg topical cream. The decision is to reject the null hypothesis, suggesting that the concentration of the cream has a significant impact on its antimicrobial effectiveness.

For the variable Across Time, the F-value is 589.461 with a p-value of .000, again indicating a significant difference in the wound healing over time. The decision is to reject the null hypothesis, suggesting that the passage of time significantly influences the wound healing effectiveness of the Lubeg topical cream.

The Interaction term assesses whether the effect of one independent variable depends on the level of another. In this case, the Interaction F-value is 1.565 with a p-value of .103. The p-value is greater than the conventional significance level of .05, leading to a decision of non-significance. This suggests that the interaction between Concentration and Time is not statistically significant, implying that the effect of concentration on wound healing effectiveness does not depend on the time duration.

TABLE 5 **DIFFERENCES ON THE WOUND HEALING OF THE LUBEG TOPICAL CREAM AS COMPARED TO *ESCHERICHIA COLI***

VARIABLES	f – value	p – value	DECISION
Concentration	9.979	.000	**Significant**
Across Time	355.238	.000	**Significant**
Interaction	1.454	.144	**Not Significant**

The study investigates the wound-healing activity of Lubeg topical cream compared to Escherichia coli (E. coli) across different concentrations and over time. The analysis of variance (ANOVA) results revealed significant differences in the wound-healing activity y based on concentration (F = 9.979, p =

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0.000). This indicates that varying concentrations of Lubeg cream result in distinct wound-healing activity compared to E. coli. The decision to reject the null hypothesis is made due to the significance level.

Moreover, the study found a highly significant difference in wound-healing activity across time ($F = 355.238$, $p = 0.000$). This suggests that the efficacy of Lubeg cream varies significantly over different time intervals compared to E. coli. The p-value of 0.000 is below the standard alpha level of 0.05, further supporting the rejection of the null hypothesis based on the observed significance in the differences over time.

On the other hand, the interaction between concentration and time did not show significant differences ($F = 1.454$, $p = 0.144$). The decision to accept the null hypothesis in this case suggests that the combined effect of concentration and time on the wound-healing activity of Lubeg cream is not statistically different from the effect of E. coli alone. This implies that the interaction between concentration and time does not significantly influence the wound-healing efficacy of Lubeg cream compared to Escherichia coli.

DISCUSSIONS

The study reveals that based on the organoleptic evaluation of the formulated cream cream exhibited no significant smell or odourlessness with a brownish yellow color. On the other hand, the formulated Lubeg-based topical cream has a watery texture with a transparent opacity.

The mean scores of 15 for the 75% Lubeg extract and 16 for the 100% Lubeg extract indicate a high inhibitory in Staphylococcus aureus with an inference of active and the 75% Lubeg extract with a mean of 13, which inference is partially active and the 100% Lubeg extract with a mean of 16 which inference is active for the Escherichia coli. Thus, this suggests that the two concentrations are supplements for antimicrobial management. And it proves the study of Haghgoo et.al 2017 that base on the result of their study that the antimicrobial effect increased with an increase in the concentration of the extract

The comparison of the inhibitory effect of the 75% and100% Lubeg cream with the commercially available topical cream like mupirocin and trimycin reveals that the commercially available exemplified a great inhibitory effect but the two concentrations of the Lubeg cream also shows antimicrobial activity.

CONCLUSION

It can be concluded that the formulated lubeg-based topical cream was a potential treatment since the mean of the zone of inhibition is classified as active. It also could heal wounds based on the result of the wound healing property test for the formulated lubeg-based topical cream. Therefore, the formulated Lubeg-based topical cream was a potential antimicrobial supplement management against Staphylococcus aureus and Escherichia coli..

RECOMMENDATIONS

The researchers recommend replicating the study by using the different parts of the plant, this will help broaden the possibilities of formulating better treatments with promising result. Also, the future

researchers can also isolate for more specific metabolites leucoanthocyanidin present in the plant extract which is the usual metabolite with antimicrobial effect. Researchers can also use other organic solvent for extraction such as methanol and water and utilize varying concentration. Lastly, the researchers recommend the use of different cream mixers for better outcome of the formulated cream.

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ISSN:3082-4354 MAIDEN ISSUE VOL. 1 NO. 1