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DurioGel: Durian (Durio Zibethinus) Rind Pectin Synthesized with Copper Nanoparticles (Cu-NPs) as Antibacterial Hydrogel Dressing

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ABSTRACT

This study is conducted to evaluate the potentials of Durian (Durio zibethinus) rind pectin loaded with Copper Nanoparticles (CuNPs) as an innovative alternative for antebacterial hydrogels.

By using the Kirby-Bauer Test (Disc Diffusion Method) and utilizing S.aureus as the bacteria, the analysis of variance (ANOVA) confirmed that the data is significant having $F=444.875$; $p<.0001$.

Keywords: Durian rind, pectin, Copper Nanoparticles, antebacterial, hydrogel, Kirby-Bauer, S.aureus, ANOVA

I. Introduction

Durian (Durio zibethinus) is a round, oblong- shaped fruit famous with its spiky and hard green outer shell. This tropical fruit is seasonal and native to South East Asian countries (Husin et al., 2018). The Philippines' durian production reached to about 78,820 MT in 2020 with the highest production coming from the Mindanao Island (Statista, 2024). As a result, the country generates 22,000 MT of durian waste annually, which often ends up in landfills or decomposes along roads, contributing significantly to agricultural waste and environmental issues (Gamay et al., 2024). As a result, the majority of the durian plant, aside from the fruit, is wasted. There are several uses for the durian husk, which is the part of the fruit that extends from the green, prickly shell to the white interior.

Owing to its excellent gelling and thickening characteristics, Pectin has been widely employed in numerous applications (Gamay et al., 2024). It is utilized as stabilizers, thickeners, texture modifiers, and gelling agents. Pectin is derived from plant by-products and found in food, pharmaceutical, and medical industries (Hasem et al., 2019). It can extracted from durian husk using a number of steps, such as heating, filtering, concentrating, and extracting (Ghaffar et al., 2019).

Meanwhile, according to a study conducted by the Department of Health (DOH) and the Online National Electronic Injury Surveillance System (ONEISS), there were 668,179 injury cases in the Philippines between 2011 and 2018. Open wounds accounted for 37.56% of these cases, while abrasion and contusion accounted for 26.23% and 13.34% of the various types of injuries (Sumalapao et al., 2020).

Pectin-based hydrogels are effective wound dressings with a lot of compelling qualities. They reduce tissue damage, steer clear of bacteria resistant to antibiotics, and offer long- lasting antimicrobial protection. Due to its distinct structural makeup and functional groups, pectin is also capable of self-healing, which enables it to adjust to changes in body position and prevents wound reopening, which can lead to infection and slow the healing process (Kocaağa et al., 2024).

By preventing protein synthesis, peroxidizing cell membranes, and destroying bacterial and viral nucleic acids, nanoparticles such as Copper (CuNP) can stop infectious processes in their tracks. With its special bactericidal qualities and ability to speed healing, nanotechnology has opened up new avenues for wound care. Because of their affordability, stability, high surface-to-volume ratio, and safety, bioactive nanoparticles are being explored for use in clinics (Salvo & Sandoval, 2021)

A synthesis of durian-derived pectin and Copper Nanoparticles (CuNP) provide a novel approach to treating open wound-related cases and lowering the amount of biowaste generated locally. A hydrogel based on pectin is a biocompatible, biodegradable, and non-toxic alternative to traditional wound healing materials. Copper nanoparticles (CuNP), on the other hand, support an improved antibacterial and cost-effective material for wound healing. Overall, the study aims to characterize the pectin-based hydrogel loaded with CuNP by means of a standard antibacterial test to effectively quanitisize the effectiveness of the material.

II. Materials and Method

A. Preparation of Durian Rinds

The durian rinds were collected from Bankerohan Public Market, Davao City, Davao del Sur. The researchers contracted WVN Testing & Research Laboratory to perform the needed tests. The lab used chemicals and equipment, including a dehydrator, and an oven.

The preparation began by brushing the durian rinds clean to prevent them from absorbing excess water during boiling or washing. Brushing the durian rinds, instead of boiling or washing them, effectively preserves their chemical properties while minimizing moisture absorption. This gentle cleaning technique removes surface contaminants without exposing the rinds to heat or excessive water, which could otherwise alter their chemical composition or lead to increased moisture retention (Ahmad, R., 2024). The researchers then proceeded to cut the inner white rinds from the durian into pieces about 1 inch long and up to 0.5 cm thick. This size ensures the rinds will be small enough to become crispy after drying, while still being large enough for the grinder and small enough to minimize moisture absorption.

Figure 1: *Initial Weighing*

Figure 2: *Dried Durian*

B. Pectin Extraction

For the extraction of pectin, approximately 100-200 g of dried durian rind samples were weighed and ground using a blender to achieve a homogeneous consistency. The ground material was transferred to a clean container. A 0.1 M hydrochloric acid (HCl) solution was prepared by diluting concentrated HCl with distilled water, and sufficient HCl was added to fully submerge the plant material, typically at a 1:5 or 1:10 plant-to-HCl ratio.

The mixture was then stirred and allowed to macerate for 2-4 hours at room temperature with occasional stirring to promote the release of peptides (Singh & Singh, 2014).

After maceration, the mixture was strained through cheesecloth to separate the solid plant material, collecting the liquid extract. Cold ethanol or methanol, stored at -20°C, was added to the filtered extract at a 3:1 or 4:1 alcohol-to- extract ratio and kept on ice for 12-24 hours to facilitate peptide precipitation. After the cold ethanol precipitation, the filtered extract was filtration apparatus was alternatively centrifugated at 3000-5000 rpm for 10-15 minutes to filter out the precipitated peptide. The peptide precipitate was then washed with cold ethanol to remove impurities and then dried under low heat (around 40°C) or using vacuum drying (Nwachukwu & Aluko, 2019). Finally, the dried peptides were stored in a desiccator or airtight container at -20°C until further analysis or use.

Figure 3: *Extracted Pectin*

C. Preparation of Cogon Grass Washing and Drying

In preparing the Cogon grass (Imperata cylindrica), sanitation involves repeatedly washing the grass with distilled water. The washing process ensures that the plant material is free from external impurities, essential for ensuring the integrity of the phytochemicals in subsequent analyses. This step ensures that the grass is clean and reduces the moisture content before extraction (Irzaman et al, 2021).

D. Extraction of Phytochemicals

In extracting phytochemicals from the cleaned and dried cogon grass, one liter (1L) of the plant material is submerged in a 95% ethyl alcohol solution for 40 hours since ethanol, being a polar solvent, is particularly effective at extracting a wide range of phytochemicals, including flavonoids, alkaloids, and phenolic compounds, which possess stabilizing properties for nanoparticles (Bonnia et al., 2020).

After the extraction process is complete, the solution is subjected to rotary evaporation at the WVN Research Facility to remove the ethanol. This step concentrates the phytochemicals into a final volume of 100 mL of extract. The concentrated extract is then quantified to evaluate its concentration and potential effectiveness in stabilizing nanoparticles before being stored for future applications.

E. Synthesis of Copper Nanoparticles

In creating Copper Nanoparticles (CuNPs), 12g Copper Sulfate Pentahydrate (CuSO4.5HO) is dissolved in 350 mL and 400 mL distilled water of 0.1 molality, allowing copper ions to be ready for reduction by the phytochemicals from the cogon grass solution (Bonnia et al, 2020). Approximately 75 mL (10%) of the Cogon grass extract was then slowly added to the Copper Sulfate solution using a dropper. During this procedure, the solution is placed in a magnetic stirrer to ensure even distribution and heated at approximately 60°C. This process inhibits the reaction between the Cogon grass extract phytochemicals and Copper ions allowing for the creation of Copper Nanoparticles.

Figure 4: *Copper Sulfate Green Synthesis*

F. Separation of Nanoparticles

A sample of 100 mL from the synthesized copper nanoparticle mixture is extracted for analysis, while the remaining solution is centrifuged at 10,000 RPM for 10-15 minutes to separate the solid nanoparticles from the liquid phase. To eliminate any unreacted copper ions or other impurities, the researchers then added distilled water, and centrifugation was repeated for an additional 10 minutes at the same speed. If the resulting extract still exhibits a greenish tint, indicating the presence of impurities, this washing process is continued until a clear extract is obtained, ensuring the synthesized nanoparticles are pure and suitable for further applications.

The Copper Nanoparticles (CuNPs) were added at a ratio of 20 mg per 100 mL of the pectin hydrogel. The whole process is done as the hydrogel is spun around a magnetic stirrer to further allow even distribution of CuNPs and pectin hydrogel (Kazemzadeh, 2015)

Figure 5: *Centrifugation*

Figure 6: *Copper NPs after centrifugation*

G. Creation of Hydrogel and Addition of CuNPs

Approximately 5g of the dried pectin powder was dissolved in 100 mL of distilled water to achieve a pectin solution with approximately 5% concentration. In gelating the pectin solution, 0.1 M of cross-linking agents such as Calcium Chloride (CaCl2) and Sodium Alginate (NaC6H7O6) was added to produce hydrogel. The solution was heated at a constant temperature of around 40°C for approximately 10 minutes to further spur the gelation process.

Figure 7: *Pectin Hydrogel Gelation*

H. Antibacterial Testing

The antibacterial testing will utilize the Kirby- Bauer Disc Diffusion Method where the Staphylococcus aureus bacteria were swabbed onto Mueller-Hilton Agar Plates including a Normal Saline Solution as the negative control, Novobiocin as the positive and the pectin-based hydrogel with CuNPs as the third one. Three (3) replicates (T1, T2, T3) are made to ensure consistency within the results. The petri dishes were incubated for 24 hours to promote bacterial growth around the inhibition zones of the disc.

Figure 8: *Disc Diffusion Method in Bacterial Testinh*

III. Results and Discussion

SAMPLE	T1	T2	T3	Mean
Positive Control (Novobiocin)	26 mm	28 mm	26 mm	26.667 mm
Negative Control (Blank Disk)	6 mm	6 mm	6 mm	6 mm
DurioGel	10 mm	10 mm	11 mm	10.333 mm

Table 1: *Control Group Zone of Inhibition*

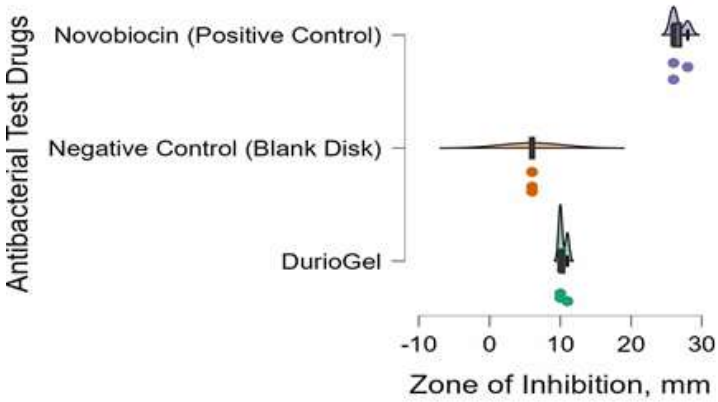

Table 2: *Antibacterial Test Results*

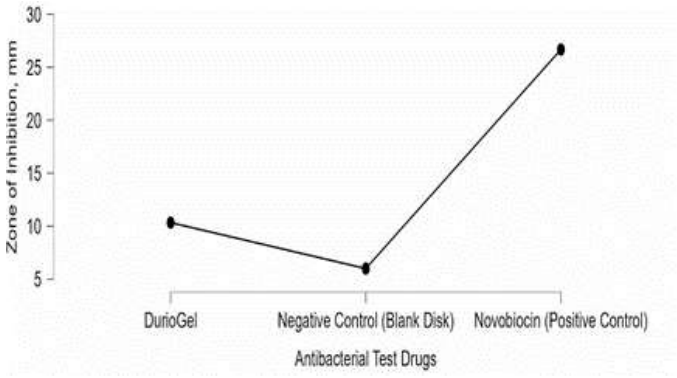

Table 3: *Descriptive Plots*

After the 24 hour incubation of the samples, results indicate a moderate inhibition of the pectin-based hydrogel loaded with CuNPs with regards to the bacterial growth. The three clusters of the hydrogel showed a zone of inhibition of 19 mm to 11 mm. The positive control Novobiocin showed a relatively up and down progress in its zone of inhibition through keeping a relatively high figure across the different samples. Meanwhile, the negative control Saline Solution kept its constant value showing its negligible inhibition rate.

Test for ANOVA - Zone of Inhibition

Results of the analysis of variance (ANOVA) for the inhibition zones against Staphylococcus aureus indicate that the antibacterial test materials, such as DurioGel, had relatively significant impact on the observed differences in inhibition zones ($F = 444.875$). The sum of squares related to the test materials (790.889) is considerably higher than that of the residuals (5.333), indicating that the variations between the test materials, including DurioGel, account for a significant portion of the observed differences. This confirms the findings through the strong evidence opposing the null hypothesis provided by a p-value that is significantly smaller than the standard significance level ($p < 0.001$).

These findings support the potential use of DurioGel with copper nanoparticles (CuNPs) in antibacterial bandages, offering effective localized protection against bacterial infections caused by S. aureus. Such bandages could play a crucial role in wound care, providing targeted antibacterial action to prevent or manage infections.

Table 4: *Test for ANOVA - Zone of Inhibition*

ANOVA - Zone of Inhibition, mm

Cases	Sum of Squares	df	Mean Square	F	p
Antibacterial Test Drugs	712.667	2	356.333	641.400	< .001
Residuals	3.333	6	0.556		

Note. Type III Sum of Squares

Test for Post Hoc Comparison - Zone of Inhibition

DurioGel, which is made from durian rind pectin and copper nanoparticles (Cu-NPs), demonstrated antibacterial activity against S. aureus, with a positive mean difference of 4.333 when compared to the Negative Control. The difference is statistically significant, as indicated by the p-value of < .001, suggesting that DurioGel provides antibacterial action. However, p-values less than 0.001 indicate that Novobiocin significantly outperformed DurioGel and the Negative Control, with a mean difference of -16.333. Despite this, DurioGel shows potential as an antibacterial hydrogel and could be further improved to enhance its effectiveness.

Table 5: *Test for Post Hoc Comparison-Zone of Inhibition*

Post Hoc Comparisons - Antibacterial Test Drugs

		Mean Difference	SE	t	p_{tukey}
DurioGel	Negative Control (Blank Disk)	4.333	0.609	7.120	< .001
	Novobiocin (Positive Control)	-16.333	0.609	-26.838	< .001
Negative Control (Blank Disk)	Novobiocin (Positive Control)	-20.667	0.609	-33.959	< .001

Note. P-value adjusted for comparing a family of 3

IV. Conclusion & Recommendation

As per the study's findings, DurioGel exhibits promising antibacterial qualities against Staphylococcus aureus, indicating that it is a viable candidate for use in antibacterial bandages. DurioGel provides a natural alternative to conventional antibiotics like Novobiocin for treating bacterial infections in wound care, even though its effectiveness may not be quite as high. Its potential as a competitive antibacterial treatment could be improved with further investigation and refinement.

For future researchers, we recommend increasing the initial amount of dried durian rinds used in pectin extraction, as the grinding process reduces the weight of the pulverized rinds, which can directly impact your pectin yield. Additionally, we suggest increasing the concentration of copper sulfate pentahydrate in your solution, as this higher concentration may yield more copper nanoparticles after synthesis.

Lastly, extending the inhibition periods of the antimicrobial process will be beneficial, allowing the hydrogel to fully demonstrate its potential as an antimicrobial agent against S. aureus. By testing the hydrogel over longer periods, you can determine its sustained effectiveness in inhibiting bacterial growth more accurately. This extended testing will provide better insights into how well the hydrogel performs over time and how effective it is in reducing or preventing bacterial contamination under various conditions.

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