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Carbon Dots Synthesized from Dried Mahongay Leaves Enhanced *in vitro* Antiobiotic Activity of Vancomycin against Methicillin-resistant *Staphylococcous aureus*

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ABSTRACT

This study provides scientific research on carbon dots (CDs) synthesis through carbonization techniques utilizing dried Philippine Mahogany (*Swietenia macrophylla*) leaves. The main focus is on the ability of CDs to improve the efficacy of Vancomycin (VCM), a first-line medicine for treating Methicillin-resistant *Staphylococcus aureus* (MRSA) infections, as well as the evaluation of its antibacterial properties. The synthesized CD filtrate from mahogany leaves was subjected to UV light and UV Vis spectroscopy, where the filtrate exhibited blue fluorescence under UV light and had a 2.5 AU absorbance reading under 375-425 nm wavelength under UV-Vis spectroscopy, validating the presence of CDs and success in CD synthesis. A simple centrifugation technique conjugated VCM and CDs, on which CD presence was also confirmed using UV-Vis spectroscopy. To evaluate antibacterial activity, pure CDs, bare, VCM, and VCM-conjugated CD treatments were tested against two gram-positive bacteria strains, *Staphylococcus aureus* and MRSA. Results yielded a significant result on the CD-VCM treatment tested against MRSA, which inferred that conjugating CDs with VCM enhances MRSA inhibition and showed no significant difference in *S. aureus* inhibition. An antibiotic release test was also conducted under two treatments– VCM and CD-VCM– which results statistically show have no significant difference. The CD-VCM treatment, however, achieved its peak concentration time at 4 hours, which was earlier than that of the VCM treatment, whose peak concentration was observed at 24 hours. Toxicity tests revealed that CDs are biocompatible, allowing researchers to conclude that conjugating CDs with antibiotics is not harmful and that CDs have potential as a drug carrier material in biomedical applications.

Keywords

Antibacterial properties, Antibiotic Release Time, Carbon dots, Carbonization, Mahogany Leaves, MRSA, Staphylococcus aureus, Vancomycin

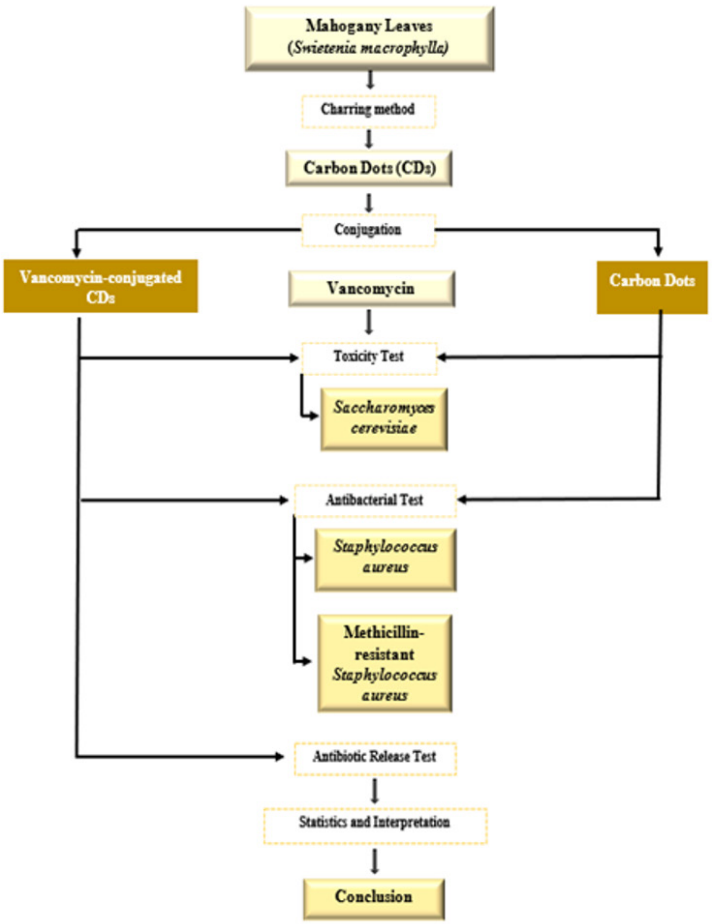
INTRODUCTION

Antibiotic resistance has become one of the world's most pressing public issues (Lessa & Sievert, 2023) as it continues to impact the treatment and diagnosis of patients significantly. The MRSA burden, which poses a threat to public health, is partly due to pathogen-specific features of developing clones resistant

to VCM. In the Philippines, methicillin-resistant *Staphylococcus aureus* (MRSA) rates remained at 50%, but the resistance to other antibiotics, including Vancomycin (VCM), is low (Masim et al., 2021). VCM is the first-line treatment for *S. aureus* and is one of the oldest antibiotics used for gram-positive bacteria, including *Staphylococci*, *Enterococci*, *Streptococci*, *Pneumococci*, *Corynebacterium*, and *Clostridia* (Cong et al., 2020). Developing nanotechnology has led to increasing attention on carbon dots (CDs) since their accidental discovery 2004 by Xu et al. Researchers have discovered many significant applications, from strong photoluminescent properties to its application in biomedicine. Conjugation of nanoparticles with elements having antibacterial properties, such as antibiotics, is one of the emerging techniques to address the increasing concern about antibiotic resistance (Jelinkova et al., 2019).

The studies conducted by Dong et al. have cited many significant sources with highly considerable inputs and tested Ciprofloxacin-conjugated CDs against gram-negative and gram-positive bacteria. It has shown that CDs' efficacy as antibacterial agents enhanced the antibacterial effect of Ciprofloxacin

Figure 1
Conceptual Framework



with CDs. This action, therefore, reduces potential toxicity and mitigates the development of antibiotic resistance. Supported by several authors indexed in their study, they have inferred that CD conjugation is an efficient way to give the desired maximal antibacterial activity at a minimal dosage. This action, therefore, reduces potential toxicity and mitigates the development of antibiotic resistance. A recent study by Albay et al. (2021) has conjugated Ciprofloxacin with CDs synthesized from dried mahogany leaves. The results were significant, showing the efficacy of conjugation in enhancing the antibacterial effect of Ciprofloxacin. As the researchers have acknowledged the limits of their work, they have recommended utilizing another antibiotic conjugated with mahogany CDs. This research also only focuses on staphylococcus strains, in contrast to the gram-negative and gram-positive strains that Albay et al. (2021) utilized.

This study examined the potential of mahogany leaves CDs in a more specific context, based on Albay et al. discussions and recommendations. Hence, this research focuses on a primarily effective antibiotic for gram-positive strains, the VCM, which tests antibacterial activity against gram-positive bacteria, precisely the two strains of *S. aureus*. The experiment targeted a resistant strain of bacteria, MRSA, to yield more specific results. As a part of a continuous effort in medical research to combat the impact of antibiotic resistance, this paper focuses on assessing the potential enhancement of the antibacterial activity of VCM when conjugated with CDs synthesized from dried mahogany leaves. The experiment involved a toxicity test, antibacterial activity test, and antibiotic release test. For the toxicity test, the researchers used *S. cerevisiae*. This study only focused on MRSA and *S. aureus,* as it aimed to mitigate the burden of antibiotic resistance by potentially enhancing the antibacterial activity. VCM, the first-line drug for MRSA, was the only antibiotic utilized for CD conjugation to reduce toxicity by providing a minimal dose with maximal efficacy in addressing drug-resistant strains of bacteria.

Considering antibiotic resistance is a global crisis, the findings of this study benefit society by providing new knowledge on the potentially significant role of CDs in enhancing VCM's in-vitro performance, notably against MRSA. Since the general population assumes that raising the dosage of a drug likewise improves its efficacy, misuse of antibiotics has been long prevalent. This research can help prevent the development of VCM resistance due to the commonly documented toxicities of VCM at higher doses (Álvarez et al., 2016). It can reduce the VCM dose while achieving the maximum desired effect.

The pressing global issue of antibiotic resistance has motivated the search for alternative and improved antimicrobial strategies through the advancement in nanoscience. The development of antimicrobial nanomedicine utilizes CDs due to their intrinsically low toxicity and high biocompatibility (Dong et al., 2017). CDs are small carbon nanoparticles (less than 10 nm in size) found to be an effective antimicrobial agent (Dong et al., 2017). Dong et al. further discuss that combining CDs and their antimicrobial reagents can effectively inhibit microbial growth while reducing potential toxicity. This combination strategy of CDs with conventional antibiotics gave a role to CDs as drug carriers. Many researchers analyzed the efficacy of CD conjugation with another antimicrobial agent, evaluating potential antibacterial activity enhancements. Jijie et al. attached ampicillin (AMP) to CDs and considered the dot-AMP "conjugates" effective visible light-triggered antibacterial agents.

Ghirardello et al.'s (2021) study also showed that CD-ampicillin (AMP) conjugates exhibited a more potent antibacterial activity than AMP alone, indicating improved drug internalization inside bacterial cells. Dong et al., in 2017, also conjugated Ciprofloxacin with CDs and found

that the conjugation yielded antimicrobial activity enhancement against both gram-positive and gram-negative bacteria. Combination strategies utilizing different antimicrobial chemicals or nanomaterials have been, by far, producing positive outcomes that significantly contributed to the efforts against multidrug resistance.

As the analysis of carbon dots continues, Rani et al. (2018) mentioned the advantage of hydrothermal treatment, which involves carbonization, as a better method of synthesizing carbon dots without adding any chemicals. This method belongs to the bottom-up processes for preparing CDs, a low-cost and easy set of techniques for CD synthesis. This process's homogeneous energy distribution can contribute to uniformly sized CDs' high luminescence. Rani et al. considered this approach sustainable and better since it yields CDs of good stability and lower toxicity.

Ciprofloxacin and CD were conjugated by adding 0.5 ml of the ciprofloxacin solution to 9.5 ml of the pure isolated CD solution and centrifuging at 1000 rpm for 1 hour (Albay et al., 2021). Data before the conjugation process indicated successful binding (2021). Results showed that the CD solution absorbed about 250 nm to 300 nm in wavelength. Reflects the observations of similar studies that cite absorption peaks at 267 nm as characteristic CD markers. The conjugation of CD to Ciprofloxacin produced optimal results in all procedures performed and, therefore, may benefit drug development for more effective results (2021).

The study conducted by Hassan et al. (2019) has explored the nanoplex delivery method's potential as a promising delivery system against MRSA infections. They used the nanoplex delivery method for VCM against MRSA using dextran sulfate sodium salt (DXT) as a polyelectrolyte complexing agent analyzed in vitro, in vivo, and in silico. In vitro studies confirmed the biosafety of nanoplex by conducting toxicity tests, in which results were low. Furthermore, Hassen et al. concluded that novel nanoplexes could be suitable for the delivery system for combating MRSA infections and improving the treatment of bacterial infections. Its development, therefore, is needed to address MRSA infections.

The specific application of *S. macrophylla* that Widiastuti mentioned is the two limonoids, two hydroxy 3-swietenolide, and two hydroxy-3-O-tigloylswietenolide; this plant possesses, which have antimicrobial activity against MDR (multiple-drug resistant) bacteria. It acts as a disinfectant and bacteriostat, preventing cellular growth due to terpenoids and limonoids, which is the significant cause of its pharmacologic activity (Widiastuti, 2021). According to Durai, the suitable diffusion method showed that *S. macrophylla* extracts in the crude form exhibit excellent antibacterial activity against *S. aureus* and *E. coli*; with higher natural extract concentrations, the zone of inhibition grows wider.

Vancomycin is an ancient drug used as the first-line agent for treating MRSA and infections caused by multidrug-resistant strain sites (Álvarez et al., 2016). In multiple studies cited by Álvarez et al., its proven effectiveness for more than five decades for treating severe health-related infections caused by MRSA. Unlike IV VCM, locally applied VCM powder has poor systemic absorption. Due to the lack of systemic absorption, a higher concentration required VCM powder to increase its antimicrobial action (Adogwa et al., 2017). Vancomycin has two toxicities: nephrotoxicity and ototoxicity. Although vancomycin-induced nephrotoxicity is generally reversible, it can be difficult to distinguish from acute interstitial nephritis and deterioration in renal function due to uncontrolled infection. Patel et al. further state that ototoxicity, a rare complication related to Vancomycin monotherapy, is common for patients who receive excessive doses of VCM. Higher dosing and frequency of administration and poor patient adherence lead to poor treatment outcomes

and the development of resistance.

Antibiotic resistance is the result of negligence in proper antibiotic use. Multidrug-resistant bacteria (MDR), known for their heterogeneity, increased their pathogenicity and spread throughout hospitals and communities, resulting in the decline in the discovery of new antibiotics, posing problems globally. Widespread antimicrobial resistance is a significant health and economic issue globally. Due to slow antibacterial discovery, demand for new antibiotics and the development of new effective strategies to inform antibiotic prescription increases (Ghirardello et al., 2021). Growing MDR among bacterial pathogens threatens the effective prevention and treatment of infections (Dong et al., 2017). This issue is eye-opening in searching for alternative antimicrobial strategies, including nanotechnology, photoactivated antimicrobial technology, and micromotor technology.

METHODOLOGY

The conducted study was over three weeks; this included the experimental stage and data analyses. The researchers utilized carbonized dried mahogany leaves as the source of CDs, then subjected to simple characterization and conjugation with an antibiotic. Vancomycin is a drug used as the first-line agent for treating MRSA and for infections caused by multidrug-resistant strain sites (Álvarez et al., 2016); then was used in the conjugation process utilized in the following tests– antibiotic release test and toxicity test. Vancomycin was the control group for testing the antibacterial effect and antibiotic release. For toxicity assessment, the organism subjected to the assessment was *Saccharomyces cerevisiae*. The test variables were CDs and Vancomycin-conjugated CDs; the positive control group was pure YEPD agar.

The materials used for the experiment included standard laboratory apparatuses. Syringe filters and UV light were purchased. Below is detailed information on the procedures used in the experiment.

The design used in the study is a quantitative experimental research design (Albay et al., 2021). The utilization of this design measured the significant difference between the variables. It determined if the collected data fell within the accepted range that either accepts or rejects the hypothesis claimed. The researchers compared the means of the zone of inhibition of the three variables in this research: CDs, VCM, and VCM-conjugated CDs-- with VCM serving as the positive control and distilled water as the negative control. The researchers carried out two tests to compare the means of the variables— the antibacterial activity and antibiotic release testing. Additional processes, such as CD characterization and toxicity testing using *S. cerevisiae*, are covered to ensure the validity of the synthesized CDs and that it is free from any contaminants or toxicity.

The researchers employed carbonized dried mahogany leaves as a source of CDs, and then the CDs were characterized and conjugated with VCM. The researchers measured the resistance of CD, CD-VCM, and VCM by subjecting the samples to antibacterial activity testing. The researchers determined the zone of inhibitions of *S. aureus* and MRSA. The antibacterial activity testing had eight samples: four samples for the *S. aureus* straining and the other four samples for the MRSA, with one sample for each variable (CD, CD-VCM, VCM, distilled water). The researchers also closely monitored the drug release time for antibiotic release testing to compare the values of CD-VCM and VCM. Pure YEPD agar, a representative organism, and *S. cerevisiae* investigated the toxicity of CDs and VCM-conjugated CDs. Each variable had three replicates (YEPD agar with VCM and YEPD agar with CD- VCM). The negative control (pure YEPD agar) was one plate of no interference, totaling 7 Petri dishes for the toxicity testing.

The data gathering was at the University of Negros

Occidental-Recoletos Biochemistry Laboratory. To be measured will be the zone of inhibition to quantify the antibacterial activity of the treatment and control groups. The zone of inhibition was assessed in diameter using a vernier caliper and was in millimeter units.

Absorbance measures determined and confirmed the presence of CDs after synthesis, conjugation, and during the evaluation of the antibiotic release time, in which readings provided evidence of the drug passing through the membrane. The absorbance measures were collected through UV-VIS spectroscopy.

The researchers did the growth or no grwoth assessment for the toxicity test in which growth indicates biocompatibility, whereas no growth indicates toxicity (Kucsera et al., 2000). The qualitative measure was the growth of *S. cerevisiae* cultured on YEPD agar media supplemented with the treatments of the study.

Synthesizing CDs utilized carbonization, a hydrothermal treatment from the bottom-up method, where dried mahogany leaves underwent 150oC carbonization for 10 minutes or until leaves were brown-black (Albay et al., 2021). Samples were allowed to cool at room temperature before fine grinding, where the powdered product was dispersed in distilled water in a 1:10 ratio by vigorous stirring (Albay et al., 2021). The solution is filtered using a 0.22um syringe filter, where the filtrate proceeds for characterization (Albay et al., 2021; Thakur et al., 2014).

Dilution of VCM was in the ratio of 0.1 g VCM powder to 10 mL distilled water. The solution was then filtered utilizing Whatman filter paper (No. 40) for further purification (Albay et al., 2021).

Fluorescence of the filtrate under 365 nm UV light and 200- 600nm UV-Vis spectroscopy for further characterization to confirm that CD was present (Albay et al., 2021; Sharma & Yun, 2020; Das et al., 2019). The emission of light varies depending on the size of CDs: small CDs emit UV light 1.2 nm at the center, medium-sized CDSs 1.5-3 nm emit visible light 400-700 nm, and large-sized CDs emit near-infrared 3.8 nm at the center (Sun & Lei, 2017).

VCM and CDs were conjugated by adding 0.5mL of the VCM solution to 9.5mL of the pure isolated CDs solution and centrifuged at 1000 rpm for 1 hour. UV-Vis spectroscopy is utilized for conjugated VCM CDs to ensure a change in the reading of 200-600nm concerning pure CDs, which indicates the success in the binding (Thakur et al., 2014; Li et al., 2016; Albay et al., 2021).

Two (2) mL of VCM-conjugated CDs were dialyzed against 1% phosphate buffer saline (PBS, pH 7.2) in a new cellophane bag at 37°C (Albay et al., 2021). The antibiotic release was measured spectrophotometrically (at 375-425 nm) for two consecutive days taken at 12 AM and 4 PM, conducted by Thakur et al. (2014), with slight modifications applied. After each sample collection, the researchers added prewarmed PBS (pH 7.2) to the dialysis chamber and maintained it at 37°C in an incubator. Pure VCM underwent exact measurements and procedures in a separate chamber as the control group for this test.

The culture media for the toxicity test was the Yeast Extract of Peptone Dextrose (YPD or YEPD) Growth Agar. In distilled water, 20g/L peptone, 10g/L yeast extract, 20g/L dextrose, and 10g agar make 500 mL of YEPD agar (Sharma et al., 2020). Five (5) mL of CD solution to every 95mL of agar for supplementation (Albay et al., 2021). The solution took the exact measurements for the plates supplemented with conjugated CDs. This test has a sample size of 7: three replicate plates of YEPD agar with pure CDs, three replicate plates of YEPD agar with conjugated CDs, and one plate of no-interference YEPD agar as a negative control (Albay et al., 2021). For three hours, the agar was allowed to cool at room temperature. *S. cerevisiae* was cultured (DIY Biotech,2020). Using the traditional streak plate and aseptic techniques, *S. cerevisiae* is

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activated (Swart et al., 2012) and inoculated into the prepared YEPD agar plates.

In the UNO-R Biochemistry laboratory, the microorganisms were subcultured and prepared. MRSA and *S. aureus* bacteria were swabbed and grown on eight plates of MH agar (Thakur et al.,2014). The researchers used the well-diffusion method for the treatment groups: CDs solution, VCM-conjugated CDs solution, pure VCM (positive control), and distilled water (negative control) (Balouiri, Sadiki, and Ibnsouda, 2015).

The CD solution, being very water-soluble, was disposed of in the sink. The researchers returned the excess reagents to the UNO-R Biochemistry Laboratory personnel, where the laboratory adviser directed the disposal instructions. All agar plates used to cultivate bacteria and yeast for the antibacterial study and toxicity test were emptied, with the medium autoclaved separately from the plates at 121 C, 15 psi, for 15 minutes. All discarded materials in the yellow biosafety bags included the autoclaved agars utilized.

In the initial characterization of the CDs, the absorbance data of CD filtrate was compared from a blank sample (distilled water), where the presence of CDs was confirmed due to the change in the reading of the two samples under 375-425 nm UV-Vis spectroscopy. Absorbance data recorded from the antibacterial release test also showed a difference in reading between the pure VCM and CD-VCM presented in a linear graph. The well-diffusion method determined the antimicrobial activity of CDs, VCM, and VCM-conjugated CDs in which zones of inhibition were measured, recorded, and further analyzed through statistics. The recovered data plotted the drug release test in the graph of absorbance versus time (in hours).

For the antibacterial test, values were given in mean ± Standard Error of the Mean (SEM) to express the variability of different parameters. The researchers performed a One-way Analysis of Variance (ANOVA) to determine the significant difference in the antibacterial activity between VCM-conjugated CDs, pure VCM, and bare CDs in terms of *S. aureus* and MRSA. ANOVA was also used to determine the significant difference in the antibiotic release time measurements between pure VCM and CD-VCM. T-test independent samples to determine the difference in the antibiotic release between CD-VCM and VCM.

RESULTS, DISCUSSION, AND IMPLICATIONS

The researchers used dried mahogany leaves as their source of CDs in this study, mainly because CDs from *S. macrophylla* exhibit excellent antibacterial activity and reported low toxicity (Durai et al., 2016) and because of many notable reasons. Mahogany (*Swietenia macrophylla* King) is prevalent in the Philippines and is an antibacterial, antifungal, and antiplasmodial agent. Several investigations on limonoids' antifungal and antimalarial activities, a key ingredient of *S. macrophylla*, have also been published (Durai et al., 2016). Several studies have used CDs from *S. macrophylla* as the subject of their experimental research (Albay et al., 2021; Durai et al., 2016)

The researchers successfully synthesized carbon dots from dried mahogany leaves using the carbonization method. Results from fluorescence and UV Vis spectroscopy tests confirmed the presence of CDs, where samples produced exhibited fluorescence and absorbance. The same set of tests also showed positive results after performing CD-VCM Conjugation, which attests to VCM and CDs' successful conjugation.

In the antibacterial study, the VCM-conjugated CDs showed a significant difference compared with pure CDs in their antibacterial activity towards MRSA only, and both treatments showed no significant difference

towards the common *S. aureus* strain. In line with the experiment's objective to measure the difference in antibiotic release time of the two different treatments, the results showed no significant difference between the VCM and CD-VCM samples. A more detailed analysis was done in the following sections.

UV-Vis Spectroscopy showed absorbance at around 375nm–425 nm wavelength in the CDs solution. The observation parallels a study that states the relative absorption range of CDs at 250–450nm (Anuar et al., 2021). The solution also showed fluorescence under UV light. The solution is clear and yellowish under natural light and exhibits a neon light blue fluorescence under 365 nm UV light. This observation reflects the findings of a similar study (Albay et al., 2021) as supplemental information to detect and confirm the presence of CDs in the conjugated solution. Through the actual absorbance readings and fluorescence test, and with ideal values taken from related studies, it can be inferred that the researchers have successfully conjugated the bare CD and pure VCM.

Beer's Law implies that the absorbance of a solution over a given duration is directly proportional to its concentration. Following this principle, results showed an increase in the concentration of the CD conjugated with VCM than that of pure VCM with a 2.425 AU difference in spectroscopy readings. This difference was observed between the peak levels of VCM and CD-VCM, which was at 375 nm wavelength, with VCM having the lower absorbance. The increase in the absorption corresponds with the observations from similar studies (Albay et al., 2021), which reaffirms that CDs were successfully conjugated with VCM, as supported by the apparent increase in absorption levels of CDVCM.

The zone of inhibition was measured after a 24-hour incubation period for both MRSA and common *S. aureus* strain. Values were compared and statistically analyzed concerning the different treatments applied. ANOVA results revealed a significant difference in the means of the different interventions applied– pure CDs, CD-VCM, bare VCM, and distilled water– tested against the MRSA strain. Post Hoc test (LSD) revealed that CD-VCM was significantly higher than the CD. Therefore, the null hypothesis is rejected since statistical data suggests a more notable antibacterial activity of CD-VCM compared to other treatments in terms of MRSA [$F(2, 6) = 18.600$, $p = 0.003$*].

As for the common *S. aureus* strain, observable antibacterial activity has been noted with CD-VCM compared with the pure CD, bare CD, and negative control of the treatments. However, the statistical analysis of the means of the treatments measured by the zone of inhibition shows no significant difference. One-way Analysis of Variance (ANOVA) was also used to determine the significant difference in the antibacterial activity between VCM-conjugated CDs, pure VCM, and bare CDs in terms of *S. aureus*. There was no significant difference in the antibacterial activity between VCM-conjugated CDs, pure VCM, and bare CDs in *S. aureus* [$F(2, 6) = 3.800$, $P = 0.086$].

Research on the antibacterial properties of pure CDs against *S. aureus* reports efficacy at a concentration of 100 μg·mL−1 (Li et al., 2018). In this study, only 1 μg·mL−1 in the concentration of CDs were subjected to the inoculated plates. The decreased quantity could contribute to the lack of inhibition of the CDs against *S. aureus*. Furthermore, a study conducted by Prybylski (2015) reported no correlation between trough Vancomycin concentration and therapeutic outcomes involving patients with *S. aureus* bacteremia.

After two consecutive days of sample observation and spectrophotometric analysis (375-425 nm wavelength), 12 values were recorded in four readings. Each reading was subjected to three different wavelengths—375 nm, 400 nm, and 425 nm. Results showed no significant difference between all the absorbance values recorded at wavelengths 375

nm, 400 nm, and 425 nm in the CD-VCM and pure VCM samples.

The significant difference in antibiotic release time measurements between the CD-VCM was determined using a one-way analysis of variance (ANOVA). There was no significant difference [F (2, 9) = 0.043, p = 0.958].

One-way Analysis of Variance (ANOVA) was also used to determine the significant difference in the antibiotic release time measurements between the pure VCM. Statistics suggest no significant difference in the antibiotic release time measurements between the pure VCM [F (2, 9) = 0.227, p = 0.802].

T-test independent samples were used to determine the difference in antibiotic release between CD-VCM and VCM. There was no significant difference, p = 0.957, which shows that both treatments have the same level of antibiotic release.

The Vancomycin-conjugated CDs reached an absorbance peak of 0.288 AU after 4 hours (at 425 nm), obtained earlier than that of bare Vancomycin, which peaked at 0.217 AU at 24 hours (at 425 nm). The CD-VCM recorded absorbance values have been shown to generally have greater absorbance than that of pure VCM. This observation corresponds to the reports of similar studies regarding intracellular drug delivery (Sun et al., 2020; Albay et al., 2021).

Strains of *S. cerevisiae* inoculated in media with different treatment groups showed growth after 24 hours of incubation at 37°C. Growth of the *S. cerevisiae* can be observed as small, creamy white, flat colonies, which corresponds to positive growth of the colonies of *S. cerevisiae* (Leeuwenheok, 2007). The growth in all media, expressed in Table 8, shows that CDs and CD-VCM were non-toxic.

The drug release study has shown that CD-VCM depicted more substantial results in greater visible absorbance and, consequently, the concentration of released drugs across the semipermeable membrane barrier. Its implications in drug delivery were further highlighted by its peak absorbance, which was observed earlier than pure VCM. Although statistics imply no significant differences in the absorbance values between VCM and CD-VCM, it can be starting evidence that CDs are effective carriers for antibiotics, as they release more concentration faster.

Moreover, it can be inferred that CDs alone have a weaker antibacterial activity on MRSA and common *S. aureus*. Contrary to this, CD-VCM has significantly enhanced the strains' antibacterial activity. This could be attributed to the synergism acted by CD as a potent antimicrobial agent (Thakur et al., 2014). Lastly, growth has been observed in all media inoculated with *S. cerevisiae*, with no observable uncharacteristic properties among the colonies formed. This implies that 26 CDs and CD-VCM are safe for clinical use and in vivo study, further suggesting the biocompatibility of CD synthesized from dried mahogany leaves.

CONCLUSION AND RECOMMENDATIONS

The researchers have successfully determined the antibacterial properties of Vancomycin-conjugated CDs regarding their activity and release measures. Observed results have also been subjected to statistical analysis for validation. A toxicity test showed evidence of its safety to support the safety of the conjugated CD-VCM. After having done all of the considerations and measures mentioned, the researchers have gathered enough data to reject the claim of their hypothesis. It can be confidently inferred that conjugation of CDs with Vancomycin produced maximum results in antibiotic activity tests, which could effectively contribute to developing drug delivery methods.

This study aimed to determine the effect of conjugating CDs with VCM antibiotic activity against MRSA and common *S. aureus*. Moreover, the potential of CD-VCM in drug delivery is extensively assessed through antibiotic release tests.

Carbon dots were successfully synthesized from dried mahogany leaves using the carbonization process, and the CDs' existence was confirmed using a UV-Vis spectrometer. The success in CD and Vancomycin conjugation was validated spectrophotometrically.

There was a significant difference in the zone of inhibition measurement of CD-VCM against MRSA, and insignificant differences were observed with CD-VCM against common *S. aureus*.

As for the antibiotic release, no significant difference was exhibited in the drug release time between CD and CD-VCM. However, CD-VCM was able to deliver drugs across the membrane at a faster rate with more concentration.

Growth of *S. cerevisiae* colonies was also observed in all media with corresponding treatment supplementation. This indicates that CD-VCM is not harmful and is safe to consume.

It is critical to characterize CDs to produce a more desirable output effectively and further establish this study's relevance. As a result, the researchers strongly advise a Transmission Electron Microscope (TEM) or Scanning Electron Microscope (SEM) analysis of the samples to obtain a complete picture of the properties of the CDs produced. Future researchers can concentrate on CDs' fluorescence properties to elicit more meaning from this technology.

Future researchers will be able to investigate drug release studies more deeply, incorporate CD characteristics, and find new applications, which will be a significant advancement. Studies cited in this paper used CDs in drug delivery imaging because of their fluorescence properties.

Future researchers can study other antibiotics and other species of plants that may be used as the carbon dots to apply in the specific antibiotic they choose to see if they can acquire a comparison of the results. They can also consider other factors that will improve their doubts about the study, such as the different antibiotic measures released to the specific treatment the researcher will be applying. Bacterial strains resistant to antibiotics may also be concerned with the interventions provided in this study.

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