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ORIGINAL PAPER

A promising approach to wound healing – *in-vivo* study of carbon nanodots infused PVA hydrogel with Kamias extract as antibacterial wound dressing

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ABSTRACT

Introduction and aim. The use of carbon nanodots (C-nanodots) synthesized from Kamias leaves for developing antibacterial wound dressings has gained attention due to their potential in promoting wound healing and contraction. To extract Kamias leaves, synthesize C-nanodots through microwave-assisted pyrolysis, characterize the synthesized C-nanodots, and test the polyvinyl alcohol (PVA) hydrogel infused with C-nanodots for antibacterial activity and wound contraction in Sprague Dawley rats.

Material and methods. Kamias leaves extract was used to synthesize C-nanodots with varying amounts of monoethanolamine. The C-nanodots were characterized using UV-Vis spectrophotometer, electron microscope, and the paper disk method. The PVA hydrogel infused with C-nanodots was tested for antibacterial activity and wound contraction in Sprague Dawley rats.

Results. The synthesized C-nanodots exhibited antibacterial properties against Staphylococcus aureus and Subtilis bacillus, with a zone of inhibition ranging from 15 mm to 23.6 mm at different concentrations. The carbon nanodots-PVA hydrogel patch showed potential wound healing ability, with significant differences in wound contraction compared to the positive and negative controls.

Conclusion. C-nanodots synthesized from Kamias extract have potential applications in antibacterial and wound healing fields. However, further studies are required to investigate the mechanism of action and potential side effects of using carbon nanodots in these applications.

Keywords. antibacterial, carbon nanodots, Kamias leaves, PVA hydrogel, wound dressing, wound healing

Introduction

A wound is a breakdown in the protective function of the skin and the loss of continuity of epithelium, with or without loss of underlying connective tissue following injury to the skin or underlying tissues. When it happens, bacteria will invade easily and start to form colonies thereby leading to severe wound infection and delay wound healing.[1,2] Therefore, substantial efforts are being made to develop new materials for protecting damaged skin from infections and dehydration. For this purpose, traditional dry dressings are used during the initial stages of wound healing, because they are dry and cannot provide a moist environment, however, they are also often liable to adhere to desiccated wound surfaces and finally induce trauma upon removal.[2] To overcome these drawbacks, inspired by the concept of moist wound healing, various wet dressings have been developed. Among them, special attentions have been paid to hydrogels because they can maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms, remove excess exudates, have excellent biocompatibility, promote a rapid heal-

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ing of wound, and be easily removed without trauma.[2]

Hydrogels are able to store a lot of water due to their three-dimensional hydrophilic network.[3] The demand for efficient, biodegradable hydrogels is increasing as a result of synthetic polymers' non-biodegradability. Polyvinyl alcohol (PVA) is a well-liked polymer for hydrogel applications because of its water solubility, biocompatibility, non-toxicity, biodegradability, and film-forming properties.[4] PVA can be chemically modified and crosslinked with other polymers, such starch, for use in biomedical applications. Since 10 years ago, carbon nanodots (C-nanodots) have gained popularity because to their antimicrobial, wound-healing, and disinfectant properties as well as their biocompatibility, stability, low toxicity, and environmental friendliness. When utilised in dressings, their optical characteristics can be changed by varying pH levels, making them appropriate for monitoring wound pH.[5] In this study, C-nanodots were obtained from Kamias (*Averrhoa bilimbi*). The Oxalidaceae family includes the medicinal plant known as bilimbi, which is widely cultivated and has many different names. It is the perfect option for the synthesis of C-nanodots for use in treatments for wound healing because of its medicinal qualities and accessibility.[6] *A. bilimbi* is a small tree which grows up to 15 m high with sparsely arranged branches. It has compound leaves with twenty–forty leaflets each and 5–10 cm long. The leaves are hairy with pinnate shapes and form clusters at the end of branches. The tree is cauliflorous with 18–68 flowers in panicles that form on the trunk and other branches. The flowers are heterotricycles with petal 10–30 m long, yellowish green to reddish purple. The fruits are produced on the bare stem and trunk. The fruits are greenish in color with a firm and juicy flesh which becomes soft on ripening. The fruit juice is sour and extremely acidic. *A. bilimbi* holds great value in complementary medicine as evidenced by the substantial amount of research on it.[6]

The leaves ethanol extract of *A. bilimbi* was reported to exhibit appreciable antimicrobial activity against six pathogenic microorganisms, namely two Gram-positive bacteria (*Bacillus cereus* and *Bacillus megaterium*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and two fungi (*Aspergillus ochraceous* and *Cryptococcus neoformans*). The aqueous and chloroform extracts of *A. bilimbi*'s leaves and fruits (100 mg/ml) showed a positive antibacterial activity against *S. aureus*, *Staphylococcus epidermis*, *B. cereus*, *Salmonella typhi*, *Citrobacter freundii*, *Aeromonas hydrophila*, *Proteus vulgaris*, and *Kocuria rhizophila*. Whole bilimbi fruit and blended bilimbi juice (not filtered) at a concentration of 1:2 and 1:4 w/v, respectively, displayed a significant activity against *Listeria monocytogenes* Scott A and *S. typhimurium* in an *in vitro* antibacterial assay. The fruit preparations were also found to reduce the microbial load of *L. monocytogenes* Scott A and *S. typhimurium* on raw shrimps after washing and during storage (4°C). This demonstrated the potential of *A. bilimbi* fruits to be adopted as a natural method of decontaminating shrimps just before preparation and consumption. In another study, fruits and roots extracts of *A. bilimbi* were also found to exhibit the positive activity against *Mycobacterium tuberculosis* with MIC of 1600 µg/ml. The leaves extracts have also been reported to display moderate antifungal activity against *Blastomyces dermatitidis*, *Candida albicans*, *Cryptococcus neoformans*, *Pityrosporum ovale*, and *Trichophyton* spp. with MIC values ranging from 15.65 to 62.50 µg/ml. Kamias is considered as antibacterial, astringent, antiscorbutic, febrifuge, antidiabetic, stomachic, refrigerant. Its Fruits are considered as astringent, refrigerant, and stomachic.[6]

During the purification of single-walled carbon nanotubes, carbon nanodots (C-nanodots), a family of carbon nanomaterials smaller than 15 nm, were initially identified. They are useful for a variety of nanobiotechnology applications, including biosensors, due to their bright fluorescence, high aqueous solubility, chemical inertness, ease of functionalization, resistance to photobleaching, low toxicity, and biocompatibility.[7-10] One study investigated C-nanodots' optical properties for potential use in optical biosensors.[11] Carbon nanodots can be prepared from organic matter or small organic molecules and typically possess numerous functional groups on their surface.[12] Two approaches to prepare carbon nanodots are the top-down and bottom-up approaches. Top-down preparations include arc-discharge method, electrochemical oxidation, and laser ablation, while bottom-up approaches involve polymerization reactions for small molecules to form nanoscale C-nanodots, including hydrothermal method, microwave-assisted pyrolysis method, ultrasonic method, acid dehydration method, and pyrolysis method.[13] The most widely used methods are the hydrothermal method and microwave-assisted pyrolysis method, which can prepare fluorescent C-nanodots in a single step.[14-16] Some synthesis methods use passivating agents to improve the quantum yield and photoluminescence emission of synthesized C-nanodots. These agents are usually amino-containing molecules or polymers, including TTDDA, 1-hexadecylamine, octadecylamine, PEG, and N-(β-aminoethyl)-γ-aminopropyl methyldimethoxy silane.[11,17] The need for passivating agents results in a rich surface-functional group presence on C-dots, making them highly hydrophilic and easily functionalized with various organic, polymeric, inorganic, or biological species. Surface passivation forms a thin insulating capping layer that protects C-nanodots from impurities, since they are vulnerable to contamination due to the endogenous nature of carbon and oxygen to react with organic molecules.[18] After synthesizing C-nanodots, their pho-

toluminescence emission can be measured using a UV-Vis spectrophotometer. High-resolution transmission electron microscopy (HR-TEM) images can be obtained using a JEOL JEM-2100 Electron Microscope, operating at 80kV. Samples are prepared by evaporating droplets placed on Formvar/Carbon coated TEM grids and allowing the solvent to evaporate under atmospheric conditions. Scanning electron microscopy (SEM) can also be used to investigate the surface morphology and size distribution of C-nanodots.[11]

A wound is a disruption in the skin or mucosa's continuity caused by physical or thermal damage. Wounds are categorized as acute or chronic based on the healing process duration and nature. Acute wounds result from accidents or surgery and heal predictably within 8-12 weeks. Chronic wounds fail to progress through normal healing stages and often stem from decubitis ulcers, leg ulcers, or burns. Wound healing is a complex, dynamic process involving four overlapping phases: coagulation and hemostasis, inflammation, proliferation, and maturation. The kind of the wound, any accompanying pathological problems, and the material being used as a dressing all affect how quickly it heals. Ideal wound dressings depend on wound type and should be selected based on their ability to: a) maintain a moist environment, b) enhance epidermal migration, c) promote angiogenesis and connective tissue synthesis, d) allow gas exchange, e) maintain appropriate tissue temperature, f) protect against bacterial infection, g) be non-adherent and easy to remove, h) provide debridement action, and i) be sterile, non-toxic, and non-allergic.[19]

Hydrogels consist of three-dimensional hydrophilic network, which is responsible for the water holding capacity of the gel and usually used for scaffolding and wound healing management.[3] Hydrocolloid dressings are among the most widely used hydrogel wound dressings. The role of hydrocolloid dressings, their properties, mechanism of action and the range of wounds for which they are useful have been reviewed. The term 'hydrocolloid' describes the family of wound management products obtained from colloidal (gel forming agents) materials combined with other materials such as elastomers and adhesives. Typical gel forming agents include carboxymethylcellulose (CMC), gelatin and pectin. Examples of hydro- colloid dressings include Granuflex™ and Aqua- cel™ (Conva Tec, Hounslow, UK), Comfeel™ (Coloplast, Peterborough, UK) and Tegasorb™ (3M Healthcare, Loughborough, UK). Hydrocolloid dressings occur in the form of thin films and sheets or as composite dressings in combination with other materials such as alginates.[20] The dressing combines moisture vapour permeability with absorbency and conformability, and its transparency allows for wound observation. Hydrocolloid dressings are useful clinically because unlike other dressings, they adhere to both moist and dry sites. Hydrocolloid dressings are used for light to moderately exuding wounds such as pressure sores, minor burns and traumatic injuries. In their intact state, hydrocolloid dressings are impermeable to water vapour but on absorption of wound exudate, a change in physical state occurs with the formation of a gel covering the wound. They become progressively more permeable to water and air as the gel forms. As they do not cause pain on removal, they are particularly useful in pediatric wound care for management of both acute and chronic wounds.[21]

Duoderm dressing is a modern hydrocolloid dressing for the management of light to moderately exuding wounds. It is versatile, easy to use and are suitable for managing different stages of wound healing and multiple wound types in a protocol of care.

The Kirby-Bauer test for antibiotic susceptibility (also called the *disc diffusion test*) is a standard that has been used for years. First developed in the 1950s, it was refined and by W. Kirby and A. Bauer, then standardized by the World Health Organization in 1961. However, the K-B is still used in some labs, or used with certain bacteria that automation does not work well with. This test is used to determine the resistance or sensitivity of aerobes or facultative anaerobes to specific chemicals, which can then be used by the clinician for treatment of patients with bacterial infections. Table 1 shows the summary of SIR table for target organisms in this study.

Table 1. Summary of SIR table for test microorganisms (Clutterbuck, Cochrane, Dolman, Percival, 2007)

Microorganisms	Zone of inhibition in mm		
	Susceptible	Intermediate	Resistance
Subtilis bacillus	>/=22	>/=12	</=11
Staphylococcus aureus	>/=21	>/=15	</=14

Aim

Hence, this study aimed to produce an alternative way to promote and enhance wound healing and produce hydrogels with C-nanodots that can be applied as an antibacterial agent. The study gave knowledge and ideas in the release behaviour of the wound patch that will help in wound management and in fulfilling the conditions such as maintaining a local moist environment, protecting the wound from side infection, absorbing the wound fluids and exudates, minimizing the wound surface necrosis, preventing the wound dryness, depressing the bacterial growth rate, and provide a non-toxic, non-antigenic, biocompatible and biodegradable dressing materials.

This study contributes to the community by developing a wound dressing that can prevent bacterial infections and promote wound healing, potentially serving as an alternative to expensive, synthetically prepared wound dressings on the market. Furthermore, the research provides additional knowledge on C-nanodots

applications, which may serve as a reference for future researchers interested in these nanoparticles. The study involved the extraction of Kamias leaves, synthesis of C-nanodots, preparation of hydrogels, antibacterial and *in-vivo* assays of the C-nanodots infused hydrogel at various institutions in the Philippines. The study was limited to synthesizing C-nanodots from Averrhoa bilimbi using microwave-assisted pyrolysis with varying amounts of monoethanolamine as a passivating agent. Characterization of the synthesized C-nanodots was limited to SEM for determining nanostructure and particle size distribution, and UV-vis for photoluminescence emission measurement. The antibacterial activity was tested only against *Staphylococcus aureus* and *Bacillus subtilis*. The application of C-nanodots was limited to their infusion in PVA hydrogels for antibacterial wound dressing that promotes healing and contraction on Sprague Dawley rats over a 10-day treatment period.

The main focus of this study was to analyze in-vivo the effectiveness of PVA hydrogel infused with C-nanodots, which were synthesized from Kamias leaves through microwave-assisted pyrolysis, as an antibacterial wound dressing. The study aimed to extract Kamias leaves using 80% ethanol, synthesize C-nanodots through microwave-assisted pyrolysis using Averrhoa bilimbi or Kamias leaves extract at varying amounts of monoethanolamine, and characterize the synthesized C-nanodots in terms of photoluminescence emission using UV-Vis spectrophotometer, nanostructure and size distribution of C-nanodots using SEM, and antibacterial activity using paper disk method. Furthermore, the study aimed to prepare PVA hydrogel infused with C-nanodots, test the antibacterial activity at different concentrations of PVA hydrogel infused with C-nanodots using Agar disk-diffusion method, measure wound contraction in Sprague Dawley Rats applied with C-nanodots-PVA hydrogel at 3rd, 7th, and 10th day after application, and compare the contraction of wound applied with C-nanodots-PVA hydrogel and duoderm at 3rd, 7th, and 10th day after application.'

Material and methods

Ethical approval

This study was conducted per the ethical guidelines and was approved by the College of Arts and Sciences at Nueva Ecija University of Science and Technology (2018-008). All procedures involving the use of animals were performed in compliance with the institution's guidelines for the care and use of laboratory animals.

Sample collection and preparation

The leaves of Kamias was collected from Cabanatuan City local market and served as the raw material in this study. The sample was washed and air-dried. A 40g of the powdered plant material was soaked in 400 mL of 85% ethanol for 72hr. The resultant extracts was filtered through Whatman filter paper No. 1. The filtrate was concentrated under reduced pressure using rotary evaporator at 50°C. The crude extracts was collected and allowed to dry at room temperature. Kamias extract solution with concentration of 100 µL/ml (crude extract:water) was prepared.

Synthesis of carbon nanodots

The methods used in the synthesis of C-Nanodots is the Bottom-up Approach's microwave assisted pyrolysis adopted from the previous study.[22] C-nanodots was synthesized using 630W power condition of microwave. Three (3) mL of Kamias extract solution with varying amount of 1.5 ml, 3 ml, 4.5 ml, and 6 ml of monoethanolamine was poured in a 50 ml erlenmeyer flask separately and heated in 2 minutes and repeated until reddish brown solutions are obtained. Four treatments were observed in the synthesis of C-nanodots, considering the varying amount of monoethanolamine as passivating agent:

$$T_1 = 1.5ml;\ T_2 = 3ml;\ T_3 = 4.5ml;\ \text{and}\ T_4 = 6ml.$$

Characterization of synthesized carbon nanodots from Kamias

The synthesized carbon nanodots was characterized by determining its nanostructure and size distribution using SEM. The SEM that was used is a Hitachi SU8230 Field-Emission Scanning Electron Microscope at 500nm px. Also, the photoluminescence emission synthesized C-nanodots was measured by UV-Vis (U-2900UV/VIS spectrophotometer) at 200V and 630nm wavelength.

Antibacterial assay

The synthesized C-nanodots from Kamias was subjected to individual microbiological tests to ascertain its antibacterial activity against gram negative bacteria and gram positive bacteria at different concentrations (1%, 0.75%, 0.50%, 0.25% and 0%). The antibacterial activity of the C-nanodots was determined by measuring the diameter of the zone of inhibition (ZI). Antibacterial activity was determined against *S. aureus* and *S. bacillus* using the paper disk assay method.[23,24] Whatman No. 1 filter paper disk of 6-mm diameter was sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with the different concentrations (1%, 0.75%, 0.50%, 0.25% and 0%) of C-nanodots. Agar plates were surface-inoculated uniformly from the broth culture of the tested microorganisms. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37°C for 24 h.

Preparation of antibacterial C-dots-PVA hydrogel

Analytical Grade of PVA produced by Sigma-Aldrich was used to form hydrogel. Five percent (5% w/v) solu-

tion of PVA was prepared using 100 ml distilled water.[25] The mixture was heated with continues stirring until all powder polymers were dissolved. Ten ml of PVA solution was combined with 10 ml distilled water and was heated. One and a half gram of agar was dissolved into the heated solution with continuous stirring. Three ml of C-nanodots solution was added drop by drop with continuous stirring. Subsequently, the mixture was injected into a square mould for the formation of hydrogel patches. The moulds were freezed and thawed to complete the moulding process.

Analgesia injection and wound excision

Table 2 presents the key aspects of the major components of the methods used to induce and excision wounds in a laboratory rat model. Sharp mayo scissors and a scalpel were used to make 1x1 cm full-thickness cuts on the dorsal region of the rats' backs for the study. Lidocaine was used as an anesthetic for pain alleviation and to ensure the animals were treated humanely throughout the procedure. This information is presented in a clear and ordered way in the table, which can be helpful in comprehending the methodology as well as analyzing the findings of the study.

Table 2. Analgesia injection and inducing excision wounds

Aspect	Details
Species	Laboratory rats
Location	Dorsal area (back)
Anesthesia	Lidocaine (23mg/kg subcutaneously)
Wound size	1x1 cm
Wound depth	Full-thickness
Instruments	Sharp mayo scissors and scalpel
Humane treatment	Method carried out in the most humane way

Evaluation of wound healing

The laboratory rats were equally distributed into three treatment groups: $T_{1=}$ Daily Topical application of antibacterial C-dots-PVA Hydrogel at 3 hours after wound creation and daily; T_2= Commercial wound patch changed daily, first application at 3 hours after wound creation and daily; and T_3= group that did not receive treatment after wound creation. All the laboratory rats were housed in a clean and well-ventilated area to observe the healing process of wound. Wound surface area on days 3, 7, and 10 after wound creation was evaluated (in cm^2 unit) and the percentage of healing was normalized by the following formula:

$$\text{Percentage wound contraction (\%WC)} = \\ = \frac{\textit{wound surface on day 1- wound surface on day}}{\textit{wound surface on day 1}} \times 100$$

where *x* is the day when the wound surface is evaluated.

Statistical analysis

The research design used in this study was completely randomized design (CRD) for the antibacterial assay of the C-nanodots and PVA-C-nanodots hydrogel while Randomized complete randomized block design was used in the evaluation of wound healing in Sprague Dawley rats. One-way ANOVA was used to determine the significant differences in the zone of inhibition and in the wound contraction of Sprague Dawley Rats. Comparison of means using Duncan's multiple range test or DMRT was also used in treatments that was found to be significantly different using ANOVA.

Results

Synthesis of carbon nanodots from Kamias

Varying amount of monoethanolamine were used as a passivating agent in synthesizing C-nanodots from Kamias extract solution through microwave assisted pyrolysis in 2-minute repetitive heating time as shown in Figure 1. A total of 18 minutes of heating time was needed before a reddish brown solution of C-nanodots was acquired. The acquired solution was slightly viscous.[22]

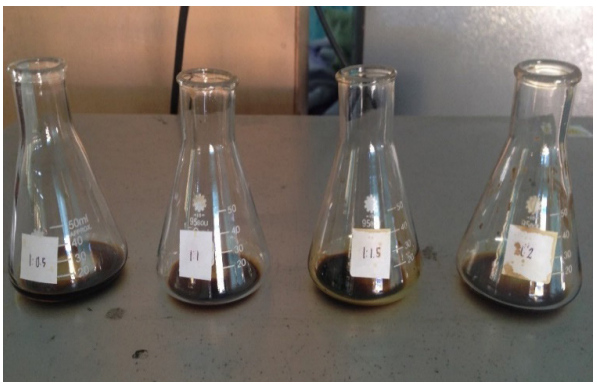

Fig. 1. Synthesized C-Nanodots from Kamias at varying amount of monoethanolamine

Characterization of synthesized carbon nanodo

The synthesized carbon nanodots from Kamias extract at different amount of passivating agent (monoethanolamine) was characterized by observing its photomuniscence (PL) absorption using UV-Vis Spectrophotometer at a wavelength of 630 nm. The absorbance of the C-nanodots at different amount of passivating agent (T1, T2, T3, T4) is presented in Table 3.

Table 3. PL emission of synthesized carbon nanodots from Kamias at varying monoethanolamide

Treatment	Monoethanolamide (ml)	Absorbance at 630nm
1	1.5	0.477
2	3	0.480
3	4.5	0.670
4	6	0.325

Shown in Table 3, photoluminiscence (PL) intensity illustrated by the absorbance at 630 nm increases with increasing amount of monoethanolamine and peaked at T3, with 4.5 ml of monoethanolamine and then decreased with the next increment of amines. This indicates that T3 was the optimum concentration in the synthesis of C-nanodots. PL intensity was improved upon surface passivation using monoethanolamine. Adding proper amount of amine into the reaction system was beneficial in improving and increasing the PL intensity resulting to a rich surface-functional groups of C-nanodots which are responsible for its high hydrophilicity and readiness for functionalization with a polymer and other inorganic and biological species. Surface passivation formed a thin insulating capping layer that protects C-nanodots from impurities and results for a stable and long life usage of C-nanodots. It has been shown that diverse oxygen functionalities may shift the PL emission wavelength. Thus, experimental and theoretical analyses associate the sp^2-hybridized carbon network with the increasing PL emission at short wavelength. Even though it was used worldwide, the exact mechanism of photoluminescence property of C-nanodots is not yet revealed because of the complicity of its structure.[18] The synthesized C-nanodots was also viewed under UV-light where its photoluminescence property were observed as shown in Figure 2. Blue photoluminescent C-nanodots with increasing intensity were observed in where T3 has the highest intensity but decreases with further addition of monoethanolamine at T4, which corresponds with most of the C-nanodots that has been synthesized that emit blue luminescence under UV irradiation. Surface passivation forms a thin insulating capping layer that protects C-nanodots from impurities and ensures stable, long-lasting usage. It has been shown that diverse oxygen functionalities may shift the PL emission wavelength.[26] Experimental and theoretical analyses associate the sp^2-hybridized carbon network with increasing PL emission at short wavelengths.[27] Although widely used, the exact mechanism of C-nanodots' photoluminescence property has not yet been revealed due to the complexity of their structure.[26] The synthesized C-nanodots were also viewed under UV-light, where their photoluminescence properties were observed (Fig. 2). Blue photoluminescent C-nanodots with increasing intensity were observed, with T3 having the highest intensity. However, the intensity decreases with further addition of monoethanolamine at T4, corresponding to most synthesized C-nanodots that emit blue luminescence under UV irradiation.[28]

The nanostructure and size distribution of the synthesized C-nanodots from Kamias was characterized using SEM and the micrograph is shown in Figure 3. The scanning electron micrograph of C-nanodots shows 14.96 nm as the computed particle size. The nanostructure form of the synthesized C-nanodots from Kamias was found to be spherical.[17]

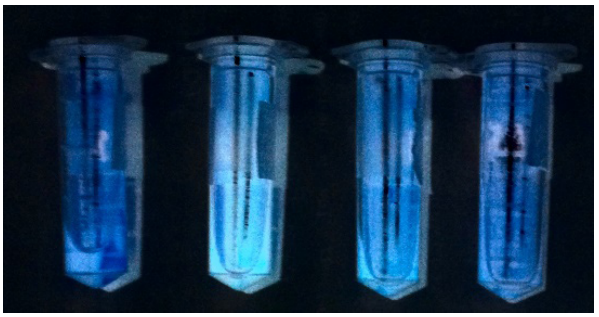

Fig. 2. Synthesized C-nanodots under UV light

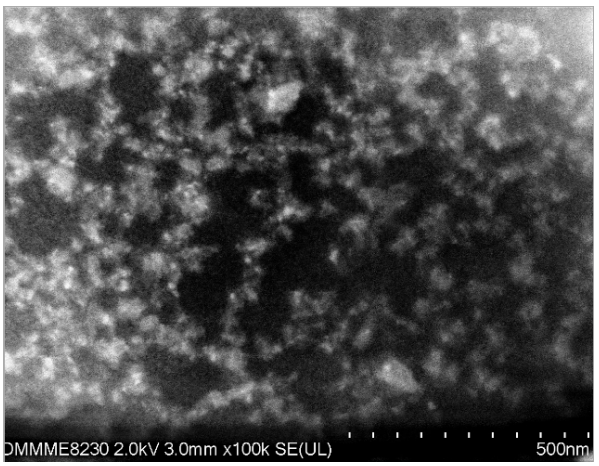

Fig. 3. Scanning electron micrograph of CD from Kamias

Antibacterial assay

The antibacterial activities of C-nanodots solutions were investigated against *Staphylococcus aureus* and *Subtilis bacillus* using paper disc assay method. In Table 4, it shows the average zone of inhibition (ZI) in millimeter exhibited by C-nanodots at different concentrations of 0.25%, 0.50%, 0.75%, and 1%.

Table 4. Antibacterial zone of inhibition of different concentrations of C-nanodots from Kamias against *Subtilis bacillus*

Treatment	0.25%	0.50%	0.75%	1%
1	8	7	10	8
2	8	8	9	8
3	9	8	8	7
4	10	9	8	7

The zone of inhibition ranges from 7 mm to 10 mm. According to the SIR Table for test microorganisms, zone of inhibition with less than or equal to 11 is described as "resistance".[29] *S. bacillus* is therefore resistance to C-nanodots. Table 5. Antibacterial zone of inhibition of different concentrations of C-nanodots from Kamias against *S. aureus.*

Table 5. Antibacterial zone of inhibition of different concentrations of C-nanodots from Kamias against *S. aureus*

Treatment	0.25%	0.50%	0.75%	1%
1	12	17.5	18	15
2	16	18	18	17.9
3	16	23.6	21	18
4	15	22	16	16.5

Table 5, it shows the average zone of inhibition in millimeter exhibited by different concentrations of C-nanodots at 0.25%, 0.50%, 0.75%, and 1%. The average zone of inhibition ranges from 15 mm to 23.6 mm. According to the SIR Table for test microorganisms, the zone of inhibition for *S. aureus* with greater than or equal to 15 is described as intermediate.[29] If the measurement is greater than or equal to 21, then the result is susceptible. *S. aureus* is therefore susceptible to 0.50% and 0.75% C-nanodots. The antibacterial properties of the C-nanodots against *S. aureus* were confirmed.[5] The average zone of inhibition ranges from 15 mm to 23.6 mm. According to the SIR Table for test microorganisms, a zone of inhibition greater than or equal to 15 is described as intermediate.[30] If the measurement is greater than or equal to 21, then the result is susceptible. Staphylococcus aureus is therefore susceptible to 0.50% and 0.75% C-nanodots.[31] This confirms the antibacterial properties of the C-nanodots against *S. aureus.*

Wound healing

In order to investigate the percentage wound size reduction using the formulated C-nanodots-PVA hydrogel patch, and duoderm, wound excisional on rat model was used. The mean percentage of the wound contraction at days 3, 7, and 10 is shown in Table 6.

Table 6. Mean Percentage of the wound contraction on 3rd, 7th, and 10th day*

Treatment	3rd day	7th day	10th day
T_A - C-dots-PVA	27.2 [a]	69.4 [a]	90.8 [a]
T_B - Duoderm	16.8 [b]	59.4 [b]	80.2 [b]
T_C - Untreated	9.0 [c]	29.0 [c]	51.4 [c]

* a – wound contraction percentage for TA- C-dots-PVA at specific time points, with the highest rate compared to other treatments; b – wound contraction percentage for TB- Duoderm at specific time points, lower than TA- C-dots-PVA but higher than TC- Untreated; c – wound contraction percentage for TC- Untreated at specific time points, with the lowest rate among all groups.

Table 6 presents the mean percentage of wound contraction for three treatment groups at specific time points (3rd day, 7th day, and 10th day). The letters a, b, and c are used to indicate significant differences between these groups, as determined by statistical tests such as ANOVA and a post hoc test like Duncan Multiple Range Test. For the TA- C-dots-PVA treatment group, the letter 'a' denotes that this group had the highest wound contraction rate at each time point when compared to the other two treatments. The TB- Duoderm treatment group, represented by the letter 'b,' had a lower wound contraction rate than the TA- C-dots-PVA group but higher than the TC- Untreated group. Finally, the letter 'c' signifies the TC- Untreated (negative control) group, which had the lowest wound contraction rate among all three groups at each time point. The presence of different letters (a, b, and c) for each treatment group at a specific time point indicates that there are statistically significant differences between the mean wound contraction rates of these groups. If two or more groups had shared the same letter, this would have implied no statistically significant difference between their mean wound contraction rates at that time point.

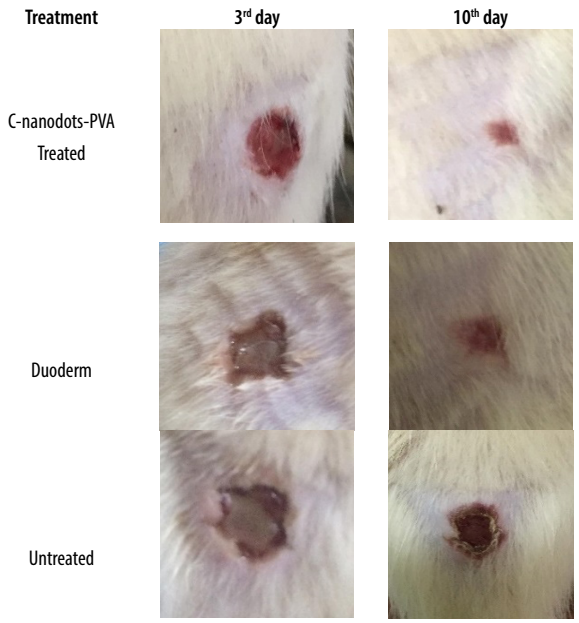

Fig. 4. Visual effect of C-nanodots-PVA Hydrogel and Duoderm vs. natural wound healing on 3rd and 10th day of evaluation

Analysis of variance at α=0.05 showed significant differences in the observed wound contraction applied with C-nanodots-PVA hydrogel, positive control (duoderm), and negative control in all days of observation. In the 3rd day, the wound contraction was reduced by 27.2% for T_A, 16.8% for T_B, 9.0% for T_c. In the 7th day, the wound contraction was reduced by 69.4% for T_A, 59.4% for T_B, 29.0% for T_c. In the 10th day, the wound contraction was reduced by 90.8% for T_A, 80.2% for T_B, 51.4% for T_c. Comparison of means using Duncan Multiple Range Test (Table 5) showed that the mean percentage of wound applied with C-nanodots-PVA hydrogel was higher compared to that of positive control and negative control at day 3. Similar trend was observed at day

7 and day 10. The effective performance of the C-nanodots-PVA hydrogel was shown visually in Figure 4. Due to its strong antibacterial and nontoxicity properties, C-nanodots can be applied as an effective wound-dressing material for enhancing the healing and pH monitoring of wounds at the same time.[5,30]

Discussion

The successful synthesis of C-nanodots from Kamias extract using microwave-assisted pyrolysis and the optimization of monoethanolamine as a passivating agent contribute to the growing body of research on carbon nanodots. C-nanodots have drawn interest in a number of sectors, including optoelectronics, bioimaging, and drug administration, as was previously highlighted in the literature review.[18] This is because of their distinctive features, such as photoluminescence, biocompatibility, and low toxicity. In comparison to current procedures, the findings of this study suggest that Kamias extract may be used as an economical and environmentally friendly carbon nanodot synthesis alternative.[22]

Characterizing the synthesized C-nanodots revealed their spherical shape and a particle size of 14.96 nm.[17] The photoluminescence intensity was found to be optimal at a specific concentration of the passivating agent, monoethanolamine.[26] This supports the hypothesis that surface passivation plays a critical role in the stability and photoluminescence properties of C-nanodots.[27] Further studies on the surface passivation and the exact mechanism of photoluminescence properties of C-nanodots are essential, considering the widespread use of C-nanodots and the complexity of their structure.[18,26]

S. aureus and *S. bacillus* were used as test organisms to see if the synthesised C-nanodots had any antibacterial characteristics. The findings demonstrated that C-nanodots had moderate sensitivity to *S. while S. aureus, S. bacillus* demonstrated C-nanodot resistance.[5, 29,30] According to these results, C-nanodots may be used as an antibacterial agent, notably against *S. aureus*, a bacteria that is known to infect wounds.[5]

The wound-healing potential of the synthesized C-nanodots was also evaluated using a rat model, where C-nanodots were incorporated into a polyvinyl alcohol (PVA) hydrogel patch. The results demonstrated that the C-nanodots-PVA hydrogel patch significantly improved the wound-healing process compared to the positive control (Duoderm) and the negative control (untreated) groups.[5,30] This finding indicates that C-nanodots, due to their strong antibacterial and non-toxic properties, can be effectively used as a wound-dressing material for enhancing wound healing and pH monitoring simultaneously.[5,30]

This study contributes to the existing knowledge on carbon nanodot synthesis, characterization, and potential applications. The findings show that Kamias extract may be used as an inexpensive and environmentally acceptable substitute for making C-nanodots, and that using monoethanolamine at the right dosage as a passivating agent enhances the photoluminescence capabilities. Furthermore, C-nanodots' antibacterial qualities suggest that they might be used as an antibacterial agent, notably against *S. aureus*. Additionally, the addition of C-nanodots to a PVA hydrogel patch accelerates the healing of the lesion, indicating a prospective use for the substance in wound-dressing materials.

Although the study's findings seem promising, more investigation is required to pinpoint the precise process underlying C-nanodots' photoluminescence capabilities and to examine their potential for use in other fields. Investigating C-nanodots' effectiveness against different bacterial strains and their potential as antifungal and antiviral medicines, for instance, might be advantageous. Furthermore, a more thorough investigation of the interactions between C-nanodots and different biological systems may provide important details about their biocompatibility and potential negative consequences. Exploring other approaches to include C-nanodots into wound dressings, such as putting them into various kinds of hydrogels, fibrous materials, or films, might also be beneficial. These studies could help optimize the wound-dressing materials and maximize the benefits of C-nanodots in promoting wound healing. It would also be crucial to carry out research on C-nanodots' long-term stability in various settings and storage circumstances, as well as how well they function when put through various sterilisation processes. Investigating C-nanodots' possible uses in other industries, such as optoelectronics, bioimaging, and drug delivery systems, would be another interesting topic. For instance, C-nanodots' photoluminescent characteristics might be used to create sensors and imaging tools, and their biocompatibility and surface functionalization could make them appropriate for customised drug delivery systems. Finally, to assess the safety and effectiveness of C-nanodots-based wound dressings in people, it would be crucial to carry out more in vivo research and eventually clinical trials. These studies would help determine the ideal concentration of C-nanodots and the optimal formulation for wound dressings, ensuring their safety and effectiveness in promoting wound healing in clinical settings. This study has demonstrated the potential of C-nanodots synthesized from Kamias extract in enhancing wound healing and their antibacterial properties. To explore the full potential of C-nanodots in diverse applications and to get a deeper comprehension of their characteristics and mechanisms of action, more study is essential. Researchers may contribute to the creation of novel and efficient solutions for a variety of applications by continuing to look into the prospective applications of C-nanodots, which will eventually enhance human health and wellbeing.

Conclusion

The research findings provide valuable insights into the potential use of C-nanodots for antibacterial and wound healing applications. The first conclusion highlights the high antibacterial activity of the synthesized C-nanodots against gram (+) bacteria. This finding is significant as gram (+) bacteria are known to cause various infections, including skin infections, pneumonia, and sepsis. C-nanodot's high antibacterial activity indicates their potential as an effective treatment option for such infections. The second conclusion reveals that the formulated C-nanodots-PVA hydrogel patch is more efficient than the standard Duoderm regarding wound healing and contraction. This finding suggests that the C-nanodots-PVA hydrogel patch could be a more effective and faster-acting wound healing option than current treatments. The third conclusion highlights the potential of the C-nanodots-PVA hydrogel patch as an alternative patch with wound healing activity against gram (+) bacteria. This is significant as it suggests that the patch could treat various infections caused by gram (+) bacteria, including skin infections, surgical site infections, and wound infections. Moving forward, there are several recommendations for further research to enhance the potential use of C-nanodots for antibacterial and wound healing applications. Firstly, using other amines as passivating agents for synthesizing C-nanodots could improve surface morphology and potentially enhance antibacterial activity. Secondly, increasing the concentration of C-nanodots in UV-vis analysis could provide more accurate and detailed information about the physicochemical properties of the C-nanodots. Thirdly, further characterization of synthesized CD using TEM and XRD could provide additional information about the morphology and crystal structure of C-nanodots. Finally, analysis of the C-nanodots-PVA hydrogel patch's tensile strength and bio adhesive strength could provide valuable insights into its physical properties and potential use as a wound healing treatment. Overall, the findings and recommendations provide promising avenues for further research into using C-nanodots for antibacterial and wound healing applications. With further investigation and development, C-nanodots could be a more effective and efficient treatment option for various infections caused by gram (+) bacteria.

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Author contributions

Conceptualization, M.C.H.; Methodology, M.C.H.; Software, M.C.H.; Validation, M.C.H.; Formal Analysis, M.C.H.; Investigation, M.C.H.; Resources, M.C.H.; Data Curation, M.C.H.; Writing – Original Draft Preparation, M.C.H.; Writing – Review & Editing, M.C.H.; Visualization, M.C.H.; Supervision, M.C.H.; Project Administration, M.C.H.; Funding Acquisition, M.C.H.

Conflicts of interest

The author of this work, hereby declare that we have no competing interests to disclose.

Data availability

Data is available on request of the author.

Ethics approval

Permission for the study was obtained from the College Ars and Sciences Ethics and Research Committee of Nueva Ecija University of Science and Technology (2018-008).

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