Research Article

Formulation of Wound Healing Hydrogel Using Carbon Nanoparticles Synthesized from *Piper longum*

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Submission Date: 11-01-2024; Revision Date: 16-02-2024; Accepted Date: 14-04-2024.

ABSTRACT

Aim: Bacterial infection is a significant factor in the complex process of delayed wound healing. The advent of antibiotic resistance in bacterial isolates led the creation of antimicrobial drugs from new resources. Formulating the hydrogel using carbon nanoparticles synthesized from the burnt ash of Piper longum against the multidrug resistance skin infection causing pathogens were the goal of the current study. **Materials and Methods:** The dried fruit of Piper longum were collected from local market, burnt and carbon nanoparticles were synthesized from the burnt ash. The synthesized nanopartiocles were characterized and the anti-bacterial activity as well as the MIC value was assessed. **Results:** The absorption peak spectrum, size and shape were identified by UV-Vis spectrophotometry, Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD). Additionally, the functional group present in the synthesized carbon nanoparticles was detected by FTIR analysis. The synthesized carbon nanoparticles showed bactericidal action against Multidrug resistance Escherichia coli, Staphylococcus aureus and K.pneumoniae. Minimum inhibitory concentration, red blood cell hemolysis, Time kill assay and skin irritation test were performed. **Conclusion:** The developed wound healing hydrogel demonstrated both significant wound healing and antibacterial action. As a result, the formulated hydrogel has the potential to be employed as a dressing for wounds

Keywords: Anti-bacterial, Carbon nanoparticles, Hydrogel, Multidrug resistance, *Piper longum*.

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INTRODUCTION

The anatomical breakdown of a tissue brought on by microbiological, physical, chemical, thermal, or immunological harm is referred to as a wound. Wounds are classified as acute or chronic based on their healing process.[1] Acute wounds are tissue damage like burns and chemical injuries, require 8-12 weeks to recover. Chronic wounds require a lengthy healing period that goes beyond twelve weeks. The factors which affect the healing includes nutrition, patient age, diseases, size, depth, causation of wound and infection.[2] *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Proteus sp*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Coagulase-negative Staphylococci are a few of the bacterial species that affect wound healing.[3] The development of bacteria strains resistant to antibiotics as a result of the frequent use of various antimicrobial medications has complicated the research. To deal with such pathological situations, it is vital to develop more effective treatments and find new medications. Today, wounds combined with antimicrobial agents have become a realistic option to lower the rate of colonisation and infection in order to optimise the healing process.[1]

Plants have been the inspiration for new medicinal molecules since the ancient times and medications developed from them have greatly improved human

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DOI: 10.5530/ajbls.2024.13.15

health. Over the past few years, numerous initiatives have been made to identify novel antimicrobial compounds derived from various types of natural sources, including microorganisms, plants and animals. Few natural products have been approved as new antimicrobial drugs.[4]

Because of their numerous therapeutic characteristics, members of the genus Piper have been utilised in a variety of medical systems. *Piper longum*, often known as long pepper, is a slender aromatic climber with woody perennial roots and oval leaves that is a member of the Piperacea family and is prized for its therapeutic benefits.[5] The majority of the alkaloids and related compounds found in long pepper are piperine, which is followed in abundance by methyl piperine, piper-Nonaline, piperettine, asarinine, pellitorine and pipe-Rundecalidine, all of which have been shown to have antimicrobial effects on a variety of microorganisms.[6]

Nanotechnology is a special branch of science and engineering concerned with the production, strategy and management of particles spanning in size from 1 to 100 nm.[7] Nanoparticles performance is influenced by factors such as size, shape, surface chemistry which draw attention in a variety of fields, including medicine, medical diagnostic imaging, medication administration, antibacterial activity etc., due to their physicochemical and optoelectronic features.[8] The most abundant element exists in the world is thought to be carbon. Because of their special physical and chemical properties, carbon-based nanoparticles are used to combat human diseases.[9] The cell membrane of some bacterial strains can be structurally damaged by carbon nanoparticles and the chemical reactions between carbon nanomaterials and the surfaces of bacteria may lead to the creation of dangerous compounds such Reactive Oxygen Species (ROS) that result in oxidative damage in organisms.[10]

Nowadays, there is a great demand for polymeric membrane materials to be used in wound dressings. A network of hydrophilic macromolecules known as a hydrogel is created when soluble polymers are cross-linked chemically or physically. An innovative method to create multifunctional hydrogels is to incorporate diverse nanostructures and biomaterials into the hydrogels. These multifunctional hydrogels display a variety of traits and capabilities, including antibacterial, antioxidant and bio adhesive etc. Although there are several hydrogel-based dressings on the market, new solutions for wound healing are needed to address the rising incidence of acute and chronic wounds in today's culture. Thus, this study focuses on the formulation of hydrogel using carbon nanoparticles synthesized from *Piper longum* (fruit) ash.[2]

MATERIALS AND METHODS

Collection of Sample

The dried fruits of *Piper longum* were collected from the local market in Coimbatore. The obtained samples were thoroughly cleaned from dust and it was stored in an airtight containers.

Synthesis of Carbon Nanoparticles

Carbon nanoparticles were purified by modified method described by Sheena *et al*., (2013).[9] The long pepper was burnt with methanol and ash was collected by grinding them using mortar and pestle. The grinded powder was dissolved in methanol-water (3:1) solution and hand mixed for an hour. Later the mixture is incubated at 60ºC for 1 hr, again hand mixed well for 1 hr and centrifuge at rpm for 5 min. After centrifugation, the undissolved substance was discarded and the supernatant was collected. The rotary heating mantle was adjusted to 60ºC and the solvent was evaporated and the fluorescing Carbon nanoparticles was collected and processed further.

Antibiotic Sensitivity Test

The antibiotic sensitivity test was evaluated against multidrug resistance *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The sterile Petri plates were filled with sterilized Muller-Hinton agar medium, which was then left to solidify. Standardized inoculum suspension of each bacterial strain was spread on nutrient agar with sterile swab. The Antibiotic-impregnated discs were positioned on the inoculated agar surface and incubated in upside-down (inverted) position at 30ºC. The inhibitory zone was measured after 16-18 hrs of incubation.[11]

Characterization of Carbon Nanoparticles

UV-Visible Spectroscopy

The wavelength of absorbance of the synthesised carbon nanoparticles was determined using UV-Visible spectroscopy (Shimadzu-UV 1800). The spectra were captured between 200-900 nm in wavelength. The sterile distilled water was utilized as a reference.

Scanning Electron Microscopy

Scanning electron microscopy was used to determine the surface structure and shape of the carbon nanoparticles. The sample was prepared and given for analysis at Biotechnology laboratory, Bharathidasan University, Trichy. Before SEM examination, the dry samples were gold sputtered to make them electrically conductive under vacuum conditions and at an accelerated voltage of 10 kV.

X-ray Diffraction

X-ray Diffraction (XRD) studies were carried out using X-ray diffractometer. The sample was prepared and given analysis at C. N. Rao laboratory, Coimbatore. The synthesised carbon nanoparticles were subjected to X-ray Diffraction (XRD) observations using a PANalytical equipment running at 40 kV, 30 mA, with Cu K radiation.

FTIR Analysis

The functional groups present in the extracts of carbon nanoparticles are identified via FTIR analysis. A wavelength of 600-3600 cm^{-1} was used for the scanning process the result was exhibited in transmission analysis. The sample was prepared and given for analysis at C. N. Rao laboratory, Coimbatore.

Anti-Bacterial Activity of Carbon Nanoparticles

Antibacterial ability of carbon nanoparticles synthesized from the burnt ash of *Piper longum* was evaluated against multidrug resistance *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* by well diffusion method. The sterile Petri plates were filled with 20 mL of nutritional agar, which was then left to solidify. Standardized inoculum suspension of each bacterial strain was spread on nutrient agar with sterile swab. Then the agar plates were punched with a sterile cork borer size of 6 mm and 100 µL of the extract was added to the well using micropipette. The plates were incubated for 24 hr at 37ºC. On each plate containing the test pathogens, the inhibitory zones around the wells were measured to evaluate the antibacterial activity.[12]

Minimum Inhibitory Concentration

Twelve Eppendorf tubes were obtained for each organism. 100 µL of nutritional broth were added to each tube. 100 µL of extract are introduced to the first tube and serially diluted into the last tube. All of the tubes were then filled with 10 µL of culture and 50 µL with resazurin and they were incubated for 24 hr at 37ºC. Positive color changes were noted for any purple to pink or colorless transitions. The MIC value was determined by taking the lowest concentration at which a color change was seen. The MIC for the test substance and bacterial strain was determined by averaging three results.[13]

Red Blood Cells Heamolysis Assay

To 49 mL of PBS, 1 mL of RBC were added. 4 mL were obtained in 5 tubes from the 50 mL of the PBS and RBC mixture. Positive control-4 mL of blood, Negative control-4mL blood+200 microliter of NaOH. To the remaining 3 tubes 100 µL of sample and 4 mL of blood was added and incubated at 37ºC for 40 min (done in triplicates). Following the incubation period, the tubes were centrifuged for 10 min, the supernatant was removed and the Optical Density (OD) was measured at 450 nm using a UV-Vis spectrophotometer.[14] The percentage of RBC lysis was calculated using the formula:

$$\text{RBC lysis} = \frac{\text{OD} - \text{NC}}{\text{PC} - \text{NC}}$$

Time Kill Assay

The MIC concentrations of carbon nanoparticles were measured using the bacterial time kill assay. Turbidometry analysis at 600 nm was used to measure the amount of bacterial growth after the treatment at 0, 1, 3, 9, 15, 18, 24 and 30 hr. Following incubation, the optical density difference (MIC concentrations of carbon nanoparticles) between the test and control samples was observed. The decrease in Optical Density (OD) values represents the inhibitory potential of carbon nanoparticles.[15]

Preparation of Wound Dressing Hydrogel

Distilled water is combined with polyvinyl alcohol (2%) and the mixture is agitated at room temperature. Extract of carbon nanoparticles at a concentration of 2% were added drop wise while stirring. The solution is supplemented with 2% citric acid and agitated for 30 min. The films were placed in a plastic petri dish and left overnight at 60ºC.[1]

Antibacterial Activity of Hydrogel

Antibacterial activity of formulated hydrogel using carbon nanoparticles synthesized from the ash of *Piper longum* was evaluated against multidrug resistance *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* by well diffusion method. Sterilized nutrient agar was poured into the sterile Petri plates and allowed to solidify. Standardized inoculum suspension of each bacterial strain was spread on nutrient agar with sterile swab. Then the agar plates were punched with a sterile cork borer size of 6mm and 100 µL of hydrogel was added to the well using micropipette. The plates were incubated for 24 hr at 37ºC. On each plate containing the test pathogens, the inhibitory zones around the wells were measured to evaluate the antibacterial activity.[12]

Skin Irritation Test

On the first day of the Skin Assessment, patch tests were performed on each subject's forearms to determine the hydrogel's potential for primary irritation. The forearms were marked in a 5×4 cm area. 1 g of active hydrogel was used to apply the patch (Bandage disc) for the forearm together with surgical

dressing. After 1 hr, the patches were removed and using Hexameter and an experienced dermatologist, the forearms were checked for any signs of skin irritation. The skin irritation was quantified using a numeric scale that provided a numerical representation of the irritation. The average quotations acquired for each volunteer were used to determine the hydrogel's average irritating score, which allowed grading from "non-irritant to very irritant".[16]

RESULTS

Antibiotic Sensitivity Test

The bacterial strains such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* were resistant to the all tested commercial antibiotics (Ciprofloxacin, tetracycline, ampicillin, amoxicillin and streptomycin). The antibiotics such as tetracycline, ampicillin and amoxicillin were failed to produce the inhibitory zones against the *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli*. The inhibitory zone of 15mm was observed for streptomycin against the *Staphylococcus aureus* and *Escherichia coli* respectively. Likewise, the zone of inhibition was noted to be 13 mm for ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli* respectively. The ciprofloxacin showed 7mm inhibition zone and 14mm for streptomycin against the *Klebsiella pneumoniae* respectively (Table 1).

Table 1: Zone of inhibition of drugs.

Micro-organism	Cipro-floxacin	Tetra-cycline	Ampi-cillin	Amox-icillin	Strep-tomycin
MDR Klebsiella pneumoniae	7 mm Resis-tance	No zone Resis-tance	No zone Resis-tance	No zone Resis-tance	14 mm Resis-tance
MDR S. aureus	13 mm Resis-tance	No zone Resis-tance	No zone Resis-tance	No zone Resis-tance	15 mm Resis-tance
MDR E. coli	13 mm Resis-tance	No zone Resis-tance	No zone Resis-tance	No zone Resis-tance	15 mm Resis-tance

Characterization of Carbon Nanoparticles

UV-Visible Spectroscopy

UV-Vis spectral analysis was carried out to confirm the formation of carbon nanoparticles. Synthesized carbon nanoparticles were analyzed using UV-Visible spectrometer was depicted in Figure 1. The characteristic absorption peak for carbon nanoparticles synthesized from the burnt ash of *Piper longum* was observed at 284, 340, 364, 370, 376 and 654nm.

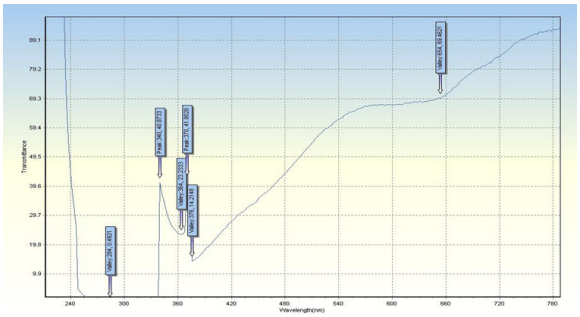


Figure 1: UV-visible spectra of synthesised carbon nanoparticles.

Scanning Electron Microscopy

The SEM analysis was carried out to examine the surface morphology and shape of the synthesized carbon nanoparticles. The synthesized carbon nanoparticles were found to be small, agglomerated and irregular in shape. Most of nanoparticles were observed to be circular. (Figure 2).

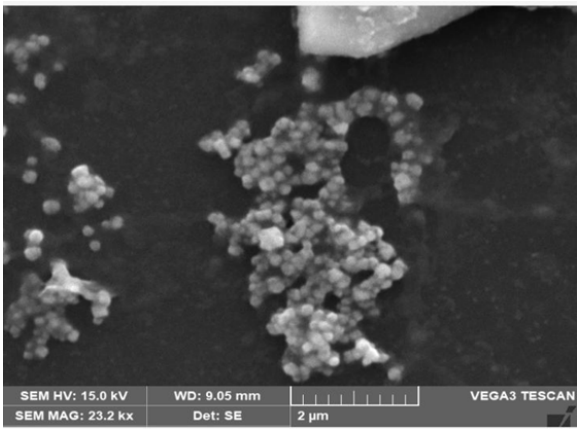
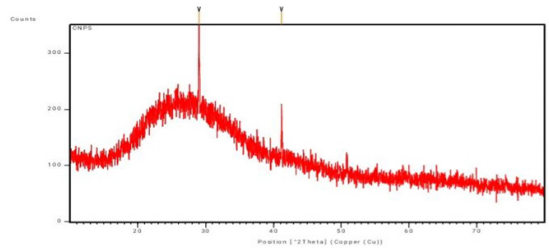


Figure 2: SEM micrograph of carbon nanoparticles

XRD analysis

The major peak for the carbon nanoparticles synthesized from burnt ash occurred at 2θ=29.03 and short peak was observed at 2θ=41.16. The XRD analysis of the carbon nanoparticles were plotted in Figure 3.



Peak List: (Bookmark 3)

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
29.0311	126.63	0.1004	3.07584	100.00
41.1642	96.77	0.0502	2.19297	76.42

Figure 3: XRD analysis of carbon nanoparticles

FTIR analysis

The functional group within the carbon nanoparticle extract was identified using FTIR analysis. The absorption band seen in the examination of carbon nanoparticles is depicted in Figure 4. Absorption band at 3842 cm^{-1} denotes alcohol group, 3340 cm^{-1} corresponds to aliphatic primary amine, 1635 cmz^{1} denoted the amine group, 1338 cm^{-1} belongs to sulfonate group, 686 cm^{-1} and 601 cm^{-1} represented the existence of halo compounds (Table 2).

Table 2: Absorption and functional group for FTIR analysis of carbon nanoparticles.

Sl. No.	Absorption peak (cm^{-1})	Functional group
1	3842.20	Alcohol
2	3340.71	Aliphatic primary amine
3	1635.64	Amine
4	1338.75	Sulfonate
5	686.66	Halo compounds
6	601.79	Halo compounds

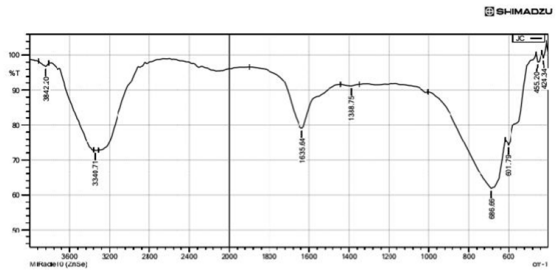


Figure 4: FTIR analysis of carbon nanoparticles.

Antibacterial Activity of Carbon Nanoparticles

The antibacterial potential of carbon nanoparticles synthesized from long pepper against the skin pathogens were tested by well diffusion method (Figure 5). Carbon nanoparticles made from Piper longum were tested for their antibacterial properties against both Gram-positive and Gram-negative bacteria. The carbon nanoparticles extract at the concentration of 100 microliter showed 19 mm diameter zone of inhibition against MDR *Klebsiella pneumoniae*, 17 mm diameter zone of inhibition against MDR Staphylococcus aureus and 18mm diameter zone of inhibition against MDR *Escherichia coli* (Table 3).

Table 3: Antibacterial activity of CNPs against MDR *K. Pneumoniae, S. aureus* and *E.coli.*

Sl. No.	Pathogens	Zone of Inhibition (mm)
1	MDR *Klebsiella pneumoniae*	19
2	MDR *S. aureus*	17
3	MDR E. *coli*	18

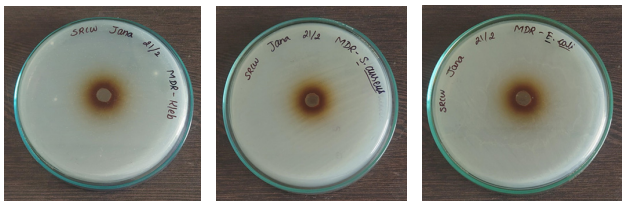


Figure 5: Antibacterial activity of CNPs against MDR *K. pneumoniae, S. aureus* and *E. coli.*

Minimum Inhibitory Concentration

The results proved that MIC value of carbon nanoparticles synthesized from *Piper longum* against selected skin pathogens was varied depending on the bacterial strains. The color shift was detected visually. Positive color changes were defined as any purple to pink or colorless transition. The lowest concentration at which color change occurred was found to be the MIC value. The MIC values of carbon nanoparticles against skin pathogens were 12.5 mg/mL (MDR K.pneumoniae), 12.5 mg/mL (MDR *S.aureus*) and 6.25 mg/mL (MDR *Escherichia coli*) (Table 4 and Figure 6).

Table 4: MIC assay results for CNPs against MDR *K. Pneumoniae, S. aureus and E.coli*

Sl. No.	Pathogens	MIC VALUE (mg)
1	MDR *Klebsiella pneumoniae*	12.5
2	MDR *S.aureus*	12.5
3	MDR *E. coli*	6.25

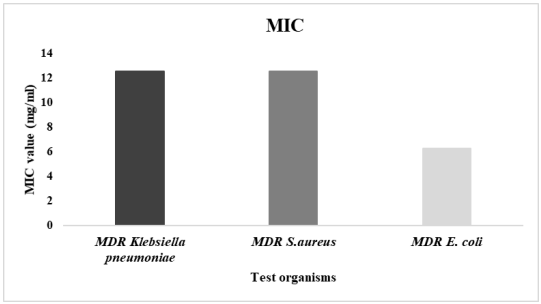


Figure 6: Graph Representing Mic Value For Carbon Nanoparticles

Red Blood Cells Hemolysis

Red blood cells haemolysis assay is done to calculate the percentage of RBC lysed by the carbon nanoparticles synthesized from the burnt ash of *Piper longum* (Figure 7). The OD value for positive control was found to be 1.8463, the OD value for negative control was determined as 0.2594, the OD value for the sample was 0.2785±0.134 and the percentage of lysis of red blood cells by the 100 µL of carbon nanoparticles synthesized from the burnt ash of *Piper longum* was calculated as 12.36±0.26 % (Table 5).

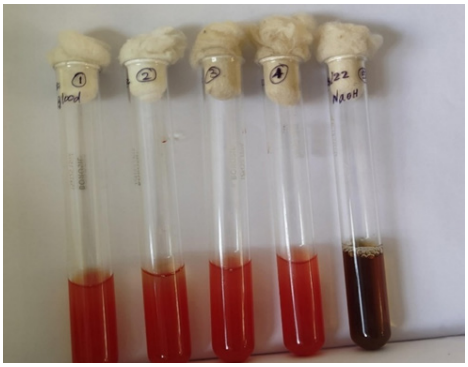


Figure 7: RBC Hemolysis of Carbon Nanoparticle

Table 5: OD value for positive control, negative control, sample OD and percentage of lysis of red blood cells by carbon nanoparticles synthesized from the burnt ash of *Piper longum*

OD Value For Positive Control	OD Value For Negative Control	Sample OD	Percentage of Lysis
1.8463	0.2594	0.2785 ± 0.134	12.36±0.26 %

Time Kill Assay

The MDR *Klebsiella pneumoniae*, MDR *Staphylococcus aureus* and MDR *Escherichia coli* were used to perform the time kill assay, which was carried out over 30 hr (Tables 6, 7 and 8). The OD values of the bacterial culture (Untreated) were found to be increased over time. The OD values of the bacterial culture treated with synthesized carbon nanoparticles (Treated) were drastically reduced than the untreated samples (Figures 8, 9 and 10).

Table 6: Bacterial time kill assay of CNPs for MDR *K. pneumoniae*

Time (Hrs)	OD at 660 nm of untreated	OD at 660 nm of treatment
0	0	0.003
1	1	0.0426
3	3	0.1804
9	9	0.3842
15	15	1.8781
18	18	0.147
24	24	0.172
30	30	0.0497

Table 7: Bacterial time kill assay of CNPs for MDR *S. aureus*

Time (Hrs)	OD at 600 nm of untreated	OD at 600 nm of treated
0	0.008	0.003
1	0.023	0.0278
3	1.1134	0.0859
9	1.5432	0.1564
15	1.212	0.4485
18	0.9176	0.5885
24	0.4137	0.2941
30	0.2097	0.1203

Table 8: Bacterial time kill assay of CNPs for MDR *E. coli.*

Time (Hrs)	OD at 600 nm of untreated	OD at 600 nm of treated
0	0.001	0
1	0.0161	0.0014
3	0.1474	0.032
9	0.4166	0.1641
15	1.1846	0.2865
18	0.6356	0.339
24	0.3791	0.3481
30	0.192	0.1826

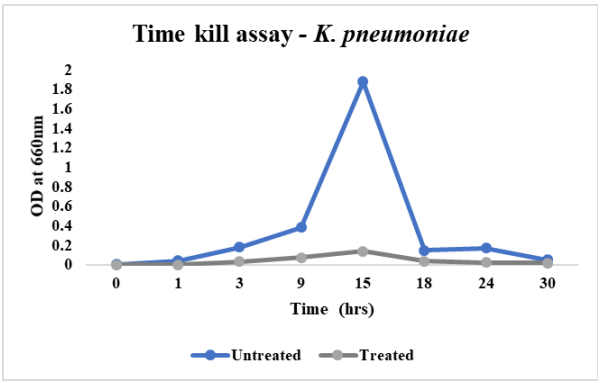


Figure 8: Graph representing the bacterial time kill assay of CNPs for MDR *Klebsiella pneumoniae.*

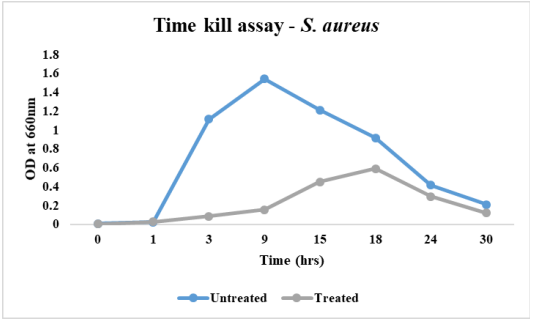


Figure 9: Graph representing the bacterial time kill assay of CNPs for MDR *S. aureus.*

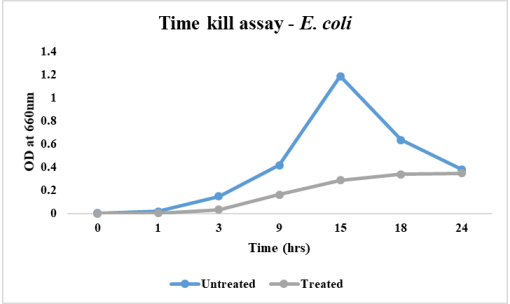


Figure 10: Graph representing the bacterial time kill assay of CNPs for MDR *E. coli*

Antibacterial Activity of Hydrogel

The antibacterial potential of hydrogel formulated from carbon nanoparticles synthesized from long pepper against the skin pathogens was examined by the well diffusion method (Figure 11). The antibacterial action of formulated hydrogel was studied against the Gram-negative and Gram-positive bacteria. The carbon nanoparticles extract at the concentration of 100 µL showed a 27 mm diameter zone of inhibition against MDR Klebsiella pneumoniae, a 25 mm diameter zone of inhibition against MDR *Staphylococcus aureus* and a 25mm diameter zone of inhibition against MDR *Escherichia coli* (Table 9).

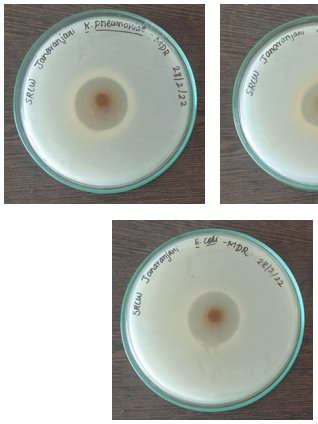


Figure 11: Antibacterial activity of formulated hydrogel against MDR *Klebsiella pneumoniae, S.aureus* and *E. coli*

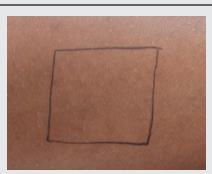
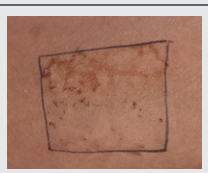
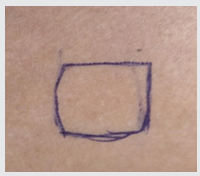
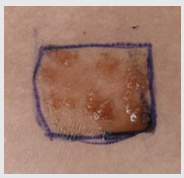
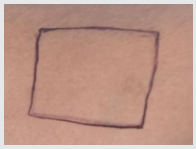
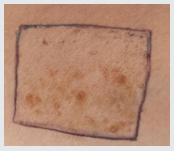
Table 9: Zone of Inhibition for Antibacterial Activity of Hydrogel Formulated from the Burnt Ash of *Piper longum*.

Organisms	Zone of Inhibition (mm)
MDR *K. pneumoniae*	27
MDR *S. aureus*	25
MDR *E. coli*	25

Skin Irritation Test

The skin irritation test was carried out by applying the hydrogel formulated from the burnt ash of Piper longum on the forearm of the subjects and observed for skin irritation after an hr. The formulated hydrogel doesn't show any irritation on the forearm of the subjects (Table 10).

Table 10: Before and After Results of Skin Irritation Test

Subjects	Before applying hydrogel	After applying hydrogel
Subject 1		
Subject 2		
Subject 3		

DISCUSSION

Nanotechnology is a vast, multidisciplinary field of study rather than a single, isolated field of knowledge and nanoparticles are found in many everyday things. Since the discovery of burning, carbon nanoparticles have been present in the atmosphere and have been in contact with people for millennia. The use of several nanomaterials kinds to treat skin conditions has been researched.[17] Natural products, primarily plants, have played an important part in healthcare for humans from the dawn of time to the present. Primary and secondary metabolites are greatly enriched in plants. Because of its great chemical diversity, plants are a promising possibility for use in modern medicine. Plant materials' flowers, fruits, seeds, stems and roots are particularly rich in a variety of bioactive compounds. Natural sources provide a precursor for the synthesis of Carbon dots, a particular type of carbon nanomaterial, because of the abundance of C (carbon), O (oxygen) and N (nitrogen) in them.[18] Since ancient times, medications have been derived from plants. Piper species are among the significant therapeutic herbs utilised in numerous medical systems. "Long pepper" is the common name for the plant *Piper longum* L. (Piperaceae). Since ancient times, medications have been derived from plants. Piper species are among the significant therapeutic herbs utilised in numerous medical systems.[19]

Hence, this study focused on the formulation of hydrogel using carbon nanoparticles synthesized from *Piper longum* (fruit) ash.

UV-Vis spectroscopy proved the existence of carbon nanoparticles by confirming wide absorbance in the range of 250-800 nm and a maximum at 280 nm, which is linked to the conjugated C=C band's -* transition and the C=O band's n-* transition.[20] SEM analysis was employed to examine the morphological characteristics of rice bran extract-derived CNPs that were synthesised. The synthetic nanoparticles had sizes that varied between 62 to 76 nm and were circular and rod-shaped[21] whereas, the synthesized

carbon nanoparticles were observed to be irregularly shaped, but some of the nanoparticles were circular and agglomerated. The primary peak of the carbon nanoparticles, which is centered at 2θ=19.85º and lines up with [311] crystal planes, can be seen in the XRD pattern of the rice bran-derived carbon nanoparticles that were synthesised. The weak broad peak belongs to the [533] set of crystalline planes, first appearing at roughly 2θ=42.6º.[21] Likewise, the XRD patterns displayed distinct, sharp peaks for nanocarbon at 26.5º and 44.3º. These peaks pointed to planes at 002 and 100, which represent graphite's face-centered cube lattice, respectively. Consequently, XRD graphs demonstrated that the carbon nanoparticles.[20] These results were correlated with our findings. The FTIR analysis of carbon nanoparticles synthesized from honey showed the stretching as well as vibration bonds, such as C-O-C, C-OH and C-H bonds could be seen in FT-IR spectra. Low intensity stretching vibrations of carboxylate groups (1200-1300 cm^{-1}) and carbonyl stretching vibrations (C=O; 1620 cm^{-1}) were also detected. These vibrations are likely the result of the residual or the microwave's oxidation or breakdown of C-OH bonds.[20] Peaks that can be seen show the chemical interactions formed by burning carbon nanoparticles.[22] Likewise, in the current study, many peaks may be seen, indicating that there are bonds between the obtained carbon atoms. The sample contains NH bonds, as evidenced by the signal 3340.71 cm^{-1}. A second peak, amine was found at 1635.64 cm^{-1}, indicating the presence of carbon in the sample.

In vitro studies can be done on the biocompatibility of nanoparticles with mammalian red blood cells and various nucleated cell types, mainly fibroblasts, osteoblast-like cell lines and mesenchymal stem cells produced from bone marrow of humans, mice, rats and rabbits.[23] Hemolysis causes red blood cells, or erythrocytes, to burst, which indicates that cytotoxic effects are being applied to the red blood cells.[24] If the test sample exhibited more than 30% hemolysis, the test samples are deemed toxic to erythrocytes.[25] Synthesized carbon nanoparticles were co-cultured with red blood cells to test for hemolysis and the test measured the amount of hemoglobin seeping from ruptured red blood cells.[26] The percentage of lysis of red blood cells by carbon nanoparticles synthesized from the burnt ash of *Piper longum* was calculated as 12.36 % and so it is considered to be non-toxic.

The anti-bacterial activity of anti-bacterial activity of the carbon nanoparticles synthesized from burnt ash of *Piper longum* fruits against multidrug-resistant bacterial species (*K. pneumonia*, *Staphylococcus aureus* and *Escherichia coli*). *Klebsiella pneumoniae* is an encapsulated gram-negative bacteria that inhabits the mouth, skin and intestines. *Staphylococcus aureus* is another major human pathogen. This bacterium can cause a variety of illnesses, including endocarditis and septic shock, wounds as well as soft tissue infections.[17] *Escherichia coli* is a gram-negative bacterium that can cause several illnesses, including infection of skin wounds.[27] The current study evidenced the significant bactericidal action of the carbon nanoparticles synthesized from burnt ash of *Piper longum* fruits against all tested multidrug-resistant bacterial species. The major components of piper fruits include alkaloids, phenols, flavonoids and tannins, which may be the cause of the fruit's antibacterial activity.[28] Additionally, the alkaloid piperine found in *Piper longum* was reported with antimicrobial properties.[6] Therefore, this might be also a reason for the anti-bacterial activity of carbon nanoparticles in the present work. The nanoparticles may penetrate cell membranes, enter inside the cell and engage in intracellular locations to stop bacterial growth and division. It produces cell lysis and so kills the bacterium. The inhibitory zone unequivocally demonstrated the potential efficacy of carbon nanoparticles derived from kitchen soot against both Gram-negative bacteria like *Proteus refrigerate* and *Pseudomonas aeruginosa* and Gram-positive bacteria like *Staphylococcus aureus* and *Streptococcus hemolyticus*.[9]

Several cytotoxicity tests employ the oxidation-reduction indicator resazurin. It is mostly used to monitor cell expansion. It is a colorless, innocuous blue dye that turns pink and bright when oxidoreductases in living cells transform it into resorufin. Reduction of resorufin to non-fluorescent state yields hydroresorufin.[29] The silver nanoparticles made from *Piper longum* had zones of inhibition of 16-24 mm and MIC values that were greater than the standard (vancomycin) of 31.25-250 μg/mL. These silver nanoparticles were effective against both Gram-positive as well as Gram-negative bacterial strains.[30] The results of the current study revealed that the MIC of *Piper longum* ranged from 0.20 to 0.0625 mg/mL, with the aqueous extract having the lowest concentration (0.5 mg/100 μL) and the methanol extract having the highest (1 mg/100 μL). Similarly, the another study showed that the aqueous had a minimal Minimum Bactericidal Concentration (MBC) of 0.5 mg/100 μL whereas methanol plant extract had a maximum MBC of 1 mg/100 μL.[31]

The antibacterial effects can be assessed by the Time Kill Assay, which is usually believed to be more practical in clinical settings. Unfortunately, the Time Kill Assay cannot be used in the vast majority of microbiological

laboratories since it is laborious, expensive and challenging to carry out. Time Kill Assay is finished in around 4 days.[32] Thus, the current study showed that the MDR *Klebsiella pneumoniae*, MDR *Staphylococcus aureus* and MDR *Escherichia coli* were used to perform the time kill assay, which was carried out over 30 hr. The OD values of the bacterial culture (Untreated) were found to be increased over time. The work done by Fahimmunisha *et al*.,[33] the bactericidal activity was shown to increase gradually and the bacteria continued to be susceptible after 8 hr of incubation in the *Aloe socotrina* extract and *Aloe socotrina*-ZnO NPs at each pathogen's specific MIC.

The high water content of hydrogels may improve the ability of anti-infective medicines to penetrate the skin and increase medication absorption. In addition, hydrogels have demonstrated excellent biocompatibility, prompt absorption of wound exudate and protection of fragile skin and are generally simple and painless to remove, all qualities that make them ideal wound dressings. A promising approach is to combine hydrogel-based anti-infective therapy with other medications or technology for other systems.[34] By combining nanoparticles with a monomer solution and gelating them, nanoparticles can be incorporated into hydrogel networks. Overall, nanoparticle-hydrogels have become a varied category of biomaterials with significant application possibilities for enhancing drug delivery.[35]

The substance's biocompatibility was demonstrated by a cutaneous acute toxicity test in which it could be classified as mildly irritating. With these benefits, it is anticipated that the Si-Cu NP-loaded hydrogel will exhibit significant potential for use in a range of clinical domains, including wound dressings and fillers.[36] The present findings evinced that the formulated hydrogel from the carbon nanoparticles of *Piper longum* doesn't show any irritation on the forearm of the subjects. Similarly, the acute cutaneous toxicity assay was performed for the iron oxide nanoparticles on different skin conditions by Coricovac *et al*.[37]

Therefore, our current study suggested that the hydrogel formulated using synthesized carbon nanoparticles had significant antibacterial activity against pathogen and found to be safe in skin irritation test.

CONCLUSION

Herbal medicine has a long history of use for numerous therapeutic objectives. In this study, wound healing hydrogel was formulated using carbon nanoparticles synthesized from the burnt ash of *Piper longum*, which were effective against certain Multi-Drug Resistance skin infection-causing bacteria. MDR is an emerging issue in the medical community since MDR bacteria develop drug resistance as a means of preserving their existence. Because of this, scientists are concentrating on developing new drugs that could be utilized to treat MDR infections. In this study, we want to identify the most effective drugs for the treatment of MDR infections. The chosen pathogens are MDR *Klebsiella pneumoniae*, MDR *Staphylococcus aureus* and MDR *Escherichia coli*. Characterization includes UV-visible spectroscopy, scanning electron microscopy, XRD and FTIR was done. The test for the antibacterial activity of carbon nanoparticles synthesized from the burnt ash of Piper longum was done. At 100 mg/mL, the inhibition zone of MDR *Klebsiella pneumoniae* was 19mm, MDR *Staphylococcus aureus* was 17mm and MDR Escherichia coli was 18mm. MIC was identified, for MDR Klebsiella pneumoniae inhibited at 12.5 mg, MDR Staphylococcus aureus at 12.5mg and MDR Escherichia coli inhibited at 6. 2 5mg. Red blood cell lysis was analysed at 12.36%. Time kill assay is done to determine the inhibition activity of carbon nanoparticles against skin pathogens. Thus, our study showed that the hydrogel formulated from carbon nanoparticles synthesized from burnt ash of *Piper longum* has good antibacterial activity against specific MDR microorganisms and lowers the risk of medical waste pollution. Its future business potential is quite great because it is biodegradable.

ACKNOWLEDGEMENT

The authors wish to gratefully acknowledge and thank the following for their generous support of this research: Sri Ramakrishna College of Arts and Science for Women, Coimbatore, Tamil Nadu. Genolites Research and Development Laboratory, Coimbatore, Tamil Nadu.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

SEM: Scanning Electron Microscopy; **XRD:** X-ray Diffraction; **FTIR:** Fourier-Transform Infrared Spectroscopy; **OD:** Optical Density; **ROS:** Reactive Oxygen Species; **MDR:** Multi Drug Resistant; **MIC:** Minimum Inhibitory Concentration.

SUMMARY

The objective of the current work was to formulate the hydrogel against the multidrug-resistant skin infection-causing bacteria using carbon nanoparticles made from the burned ash of *Piper longum*. UV-vis spectroscopy, FTIR, XRD and SEM were used to analyze the carbon nanoparticles and the nanoparticles showed bactericidal activity against multidrug-resistant *Escherichia coli*,

Staphylococcus aureus and *Klebsiella pneumoniae*. The new wound healing hydrogel displayed considerable wound healing and antimicrobial activity.

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Cite this article: Janaranjani S, Madhanasundareswari K, Raja K. Formulation of Wound Healing Hydrogel Using Carbon Nanoparticles Synthesized from Piper longum. Asian J Biol Life Sci. 2024;13(1):109-19.