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IR & UV Irradiation Methods to Improve the Efficacy of Silver Nanoparticles in the Eradication of Biofilm-Forming Microbes Isolated from Surfaces of Severe Accidental Wounds

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***Abstract*—** Bacterial biofilms are a major concern towards human health. Nanoparticles work against bacteria through a unique mechanism, making them a promising alternative for biofilm eradication. Silver nanoparticles are widely known and used for their efficient antibacterial property. In the current study, a swab sample was collected from the surfaces of 10-day-old wounds resulting from a road accident and cultured. Four biofilm-forming bacterial strains were isolated by studying the colony morphology and staining properties. Genomic characterization of the isolated strains was carried out by 16s rRNA sequencing using 27F and 149R universal primer. The fasta sequences were subjected to phylogenetic analysis following the maximum likelihood method using Mega software (Version 10.2.5). The biofilm-forming strains were identified as *Escherichia coli* (CMPD1), *Klebsiella pneumonia* (CMPD2), *Pseudomonas aeruginosa* (CMPD3), and *Staphylococcus aureus* (CMPD4). Silver nanoparticles were synthesized following the green synthesis approach for the anti-biofilm study using *Cinnamomum tamala* leaf extract as a natural reductant. 10 ml of different concentrations of silver nitrate (2 mM, 4 mM, 6 mM, 8mM, and 10mM) and plant extracts (1ml to 5 ml) were taken for the synthesis. The change in colour of the solution to dark brown confirmed the synthesis of silver nanoparticles. The absorption maxima of the nanoparticles obtained by using 4mM silver nitrate and 5 ml of leaf extract were found to be 430 nm using a UV-Vis spectrophotometer. Anti-microbial activity of the same sample showed higher inhibition efficiency as compared to others thereby confirming high concentrations of silver nanoparticles. The selected nanoparticle sample was divided into two parts. One part was irradiated with a 39W UV lamp (254 nm) and the other with a 100W IR lamp (1000 nm) for 10 minutes each. Post irradiation, the anti-biofilm assay was conducted in a 96-well microtiter plate. Irradiated nanoparticles showed higher anti-biofilm activity as compared to the non-irradiated ones. The activity of nanoparticles followed the sequence – IR>UV>Non-irradiated.

Keywords— Antibacterial, Biofilms, Human health, Silver nanoparticles, *Cinnamomum tamala* , Wounds.

I. INTRODUCTION

Wounds are the injuries or breaks in the skin surface caused due to an external action such as an accident or surgery, which include scratches, cuts, and punctured skin. Infection of the wound is a major medical concern as continuous pathogen colonization delays healing and may lead to sepsis. Chronic wounds develop an ideal habitat for the growth of mature biofilms, that defends bacteria from antibiotics and the host's immune system [1]. Biofilms are exopolymeric substances secreted by polymicrobial communities that help them to resist environmental stress by providing protection against natural immune responses and antibiotics [2]. The body provides the biofilm with nutrition in the form of exudates while fighting an immune response that damages the healing tissue and delays the wound healing process [3]. *E. faecalis*, *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *S. aureus*, *S. viridians*, and *S. epidermidis* are some of the common biofilm-producing microorganisms [4]. *Staphylococcus aureus* with the ability to develop multi-antibiotic resistance has been recognized as a prominent source of wound infection [5].

Bacterial biofilm causes nosocomial infections and is mainly responsible for device and non-device-related infections. They also provide a suitable environment for the development of a syntrophic association. Biofilm infections related to non-devices are periodontitis, native valve endocarditis, and osteomyelitis [6]. Biofilm occurs within medical devices such as pacemakers, voice prostheses, contact lenses, mechanical heart valves, central venous catheters, prosthetic joints, and peritoneal dialysis catheters [7].

Antimicrobial peptides [8, 9], quorum quenchers [10], plant-derived components [11], photodynamic therapy [12], chitosan [13], and probiotics [14] are some of the conventional treatment methods used for the eradication of biofilms [15]. These conventional methods have many limitations such as chemical instability, high volatility [11],

and high production cost. Photodynamic therapy has a limited effect against biofilm whereas chitosan is poorly soluble in water [13], with limited survival of viable probiotic cells [14]. Polymicrobial biofilms are more resistant with respect to their single species and thereby limit the use of conventional treatment methods. Greater dosages of therapeutic drugs are necessary for the complete eradication of pathogens, as biofilms develop metabolic barriers leading to poor antimicrobial penetration, and high resistance. Furthermore, biofilm-associated chronic wounds are difficult to cure, culminating in tissue dysfunction and scarring [16]. The ability of microbes to produce antibiotic resistance has significantly enhanced in the past few years, posing a major challenge to the health economy due to the rampant and inappropriate usage of antibiotics [17]. Therefore, treatment options targeted at the appeasement of bacterial biofilms while promoting healing have allured significant attention. However, the success of silver nanoparticles in treating wounds has been limited, owing to their toxicity due to the uncontrolled release of silver ions, which necessitates higher concentration for effective results [18, 1].

Nanotechnology is a fast-expanding scientific discipline with numerous applications ranging from medical to aeronautics, and agriculture to bio-pharming. Metal nanoparticles are desirable for applications in catalysis, optics, sensing, and antibacterial compounds because of their specific sizes and shapes [19]. Silver nanoparticles (AgNPs) possess strong antioxidant, photocatalytic, and antibacterial properties against both Gram-positive as well as Gram-negative bacteria. Antibiotic resistance genes are produced by bacterial pathogens as a consequence of antibiotic administration, and nanoparticles have been proposed as an alternative to conventional antibiotics due to their exceptional antibacterial properties [20].

Synthesis of AgNPs through various physical and chemical processes is not cost-effective. Chemical methods of nanoparticle synthesis including lithography, laser ablation, and ultrasonic fields can produce toxic byproducts and necessitate high temperature and pressure conditions. They may also cause toxic substances to adsorb on the nanoparticle's surface, which could have negative consequences in medical and pharmaceutical applications [21]. Furthermore, the chemical processes used to make AgNPs frequently yield a variety of hazardous chemicals that are harmful to the environment. As a result, alternative environmentally friendly and sustainable processes must be developed [22]. The synthesis of silver nanoparticles through green chemistry to limit the usage and manufacture of harmful compounds have gained a lot of attention in the past few years. Green synthesis of silver nanoparticles is now carried out using plants such as *Trifolium resupinatum*, *Laminaria japonica*, *Fritillaria flower*, *Aloe vera*, *Taxus baccata*, and *Solanum melongena* [23]. Biological synthesis is a green alternative to conventional processes of metal nanoparticle synthesis, usually involving toxic reagents and harsh reaction conditions [24]. Silver nanoparticles prevent surface colonization and have prolonged antimicrobial activity hence can act as a better alternative for the eradication of bacterial biofilm [25, 26]. Nanoparticles with all dimensions confined are particularly effective in dealing with microorganisms in both plants as well as humans. Silver (Ag) is the only noble metal that has no harmful effect, competent in surface contact, and is reasonable [27]. The effectiveness of synthesized silver nanoparticles (AgNP) in limiting the growth and metabolism of bacteria has already been demonstrated in several research [28].

Cinnamomum tamala, a member of the Lauraceae family, is exploited in the food industry for its aroma. *C. tamala* leaves contain alkaloids, flavonoids, terpenoids, tannins, sterols, and saponins. Ayurvedic medicine has employed leaf extracts of *C. tamala* to treat diarrhea, rheumatism, colic, bladder problems, anorexia, coryza, and dryness of the mouth. *C. tamala* leaves have been demonstrated to be antibacterial, antifungal, anti-dermatophyte, antihelminthic, diuretic, hypolipidemic, and anti hypoglycemic and good for the liver and spleen. They have been primarily used to treat inflammation as well as heart problems. The essential oil of *C. tamala* has the inhibitory potential against quorum-sensing [29]. It contains significant levels of sesquiterpenoids and eugenol and is utilized as a stimulant as well as having astringent, carminative, anti-biofilm, anti-flatulent qualities, and therefore it is considered for pharmaceutical applications [30].

In this research, *Cinnamomum tamala* leaf extracts with exceptional therapeutic properties were employed as surface stabilizing agents for the 'green synthesis' of AgNPs. The purpose is to create nano-sized silver particles utilizing silver nitrate as a precursor molecule and *Cinnamomum tamala* leaf extract as a reducing agent. The objective of the current research was to enhance the antibacterial activity of green synthesized silver nanoparticles by UV and IR irradiation, thereby making them a potent option for the eradication of biofilm-forming microbes on wound surfaces.

II. RELATED WORK

The WHO (World Health Organization) has figured out antimicrobial resistance as one of the major issues that must be encountered in order to improve human health. The most significant cause behind the emergence of this global issue is the widespread and indiscriminate use of antibiotics. Biofilm-associated infections continue to pose a serious challenge and represent ineffectiveness of the modern medicine. There has been an earnest need for the development of alternative treatments for multidrug-resistant microbial infections besides antibiotics [31]. Since the eradication of biofilm-forming bacteria has become a challenging issue, therefore, it is necessary to look for a novel therapeutic approach. Silver nanoparticles (AgNPs) are known to have an antimicrobial effect against antibiotic-resistant bacteria as well as pathogenic bacteria. The significantly rising number of multidrug-resistant bacteria is threatening the worth of antibiotics, annihilating

its golden era for combating infections [32]. Since plant leaf extracts have the potential to reduce antibiotic resistance and inhibit biofilm development. According to research by Anju et al. (2019) [33], the biosynthesized nanoparticles efficiently disrupted the Pseudomonas biofilm, where EPS formation was reduced by 44%. Similar research was conducted by Supriya et al. (2019) [34], who found that green synthesized silver nanoparticles (AgNPs) at low concentrations have broader antibacterial activity than antifungals and could potentially be used as an alternative therapeutic strategy in the medical and pharmaceutical industries.

III. METHODOLOGY

Condition of wounds and standard treatment

Swab sample was collected from the surfaces of wounds resulting from a severe road accident, 10 days after the incident. Medication and treatment strategies followed were documented.

Microbial isolation and cultivation

The swab samples were cultured in the laboratory on nutrient agar (HIMEDIA) and incubated for 24 hours at 37°C. Colonies were selected based on their morphology and staining properties. Streak plate cultures of the isolated colonies were made in the Luria-Bertani (HIMEDIA) agar plate. These pure cultures were then preserved at 4°C for further studies.

Molecular identification and characterization of isolated bacteria by 16s rRNA sequencing

Genomic DNA was isolated from selected bacterial species by rapid one-step extraction (ROSE) method followed by PCR amplification [35]. Quantification of the DNA was carried out in a nanodrop spectrophotometer by taking Abs 260/280 ratio. Amplification of 16S rRNA gene in isolated genomic DNA was carried out using universal primers i.e. forward primer 27F(5'-AGAGTTTGATCCTGGCTCAG-3') and reverse primer 149R (5'-GGTTACCTTGTTACGACT-3'). The amplification result was analyzed using 1.5% agarose gel electrophoresis followed by UV visualization. Further, it was sequenced commercially using the Sanger sequencing method. The 16S rRNA sequence data was analyzed to confirm the taxonomical identification of the isolated bacterial strains. Alignment of the forward and reverse 16S rRNA sequences were carried out using the ClustalW program in the GeneStudio software (GeneStudio Professional Edition version 2.2.0.0) and noise editing was performed. Chromas software (version 2.6.6) was used for analyzing the peaks of the sequencing data. The presence of chimera in the sequence was checked using the DECIPHER software version 2.0 [36]. The sequence data were compared against the 16S ribosomal RNA sequences (Bacteria and Archaea) existing in the GenBank database of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using BLASTn (https://blast.ncbi.nlm.nih.gov/). The sequence having a high percentage query cover and lowest E-value was selected and categorized under the same group of genus and species as per the homology.

Phylogenetic analysis and Sequence submission

The construction of the phylogenetic tree was carried out based on the 16S rRNA sequence with the reference sequence from GenBank. The divergence analysis was carried out by the Maximum Likelihood Method using MEGA Version 10.2.5 (Molecular Evolutionary Genetics Analysis). Finally, the edited gene sequence was submitted to the NCBI GenBank.

Preparation of leaf extract

Fresh *Cinnamomum tamala* (Figure 1) leaves were identified and collected from indigenous plants in Bhubaneswar, India. The leaves were thoroughly cleaned to eliminate dirt and then dried in shade to remove moisture content. Half of the dried leaves were powdered, while the other half was cut into small pieces and stored in zip lock bags for further extractions. In 2 separate flasks, 10gm powdered and cut leaves were mixed with 100 ml deionized or Milli Q water and incubated in a water bath at 100 °C for 30 minutes. The aqueous leaf extract was filtered through Whatman no. 1 filter paper and stored at 4 ºC for further analysis.

Figure 1. Fresh leaves of *Cinnamomum tamala*

Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized following the green synthesis approach [37]. The nanoparticles were synthesized by taking $AgNO_3$ as a precursor and *Cinnamomum tamala* leaf extract as a natural reductant. The optimum parameters for the synthesis were selected based on the variation in leaf extract concentration, plant extract ratio to $AgNO_3$ solution, and $AgNO_3$ concentration on Ag-NPs formation for the efficient synthesis of Ag-NPs. Plant extracts (1-5 ml) along with different concentrations of silver nitrate (2 mM, 4 mM, 6 mM, 8mM, and 10mM) and 10 ml each were taken for nanoparticle synthesis. The CT-AgNPs (*Cinnamomum tamala* silver nanoparticles) were synthesized by adding 5 ml of CT leaf extract with 10 ml of 4 mM $AgNO_3$ solution (Sigma Aldrich) after optimization. The solution was agitated with a magnetic stirrer at room temperature, leading to a change in colouration from colourless to light yellow, and incubated

in the dark for 24 hours. The colour change confirms the synthesis of CT-AgNPs. Then it was centrifuged to separate silver nanoparticles from the dispersion, after which the CT-AgNPs were washed with deionized water to eliminate water-soluble contaminants and stored in an Eppendorf tube for further analysis.

Characterization of silver nanoparticles

The characterization of the synthesized *Cinnamomum tamala* silver nanoparticles (CT-AgNPs) was performed using UV-Vis Spectrophotometer. 1ml of CT-AgNP sample was scanned between 200-800 nm for determining the standard peak (350 - 450nm) against a blank [38].

Analysis of anti-bacterial property of silver nanoparticles

The anti-bacterial activity of synthesized CT-AgNPs against the isolated bacterial strain was determined by Kirby-Bauer's disc diffusion method using standard reference antibiotic streptomycin (5mm diameter) as the positive control [39]. The plates were incubated at 37°C for 48 hours. The inhibition zone diameter (IZD) was measured and the antibacterial activity against the isolated bacteria was evaluated. All the studies were done in triplicate, and the results were expressed as mean ± standard deviation.

UV & IR assisted enhancement of anti-bacterial property

The silver nanoparticles were subjected to UV & IR irradiation separately. A part of the synthesized nanoparticle was irradiated with a 39W UV lamp (254 nm) and the other with a 100W IR lamp (1000 nm) for 10 minutes each. The anti-biofilm activity of the irradiated silver nanoparticle samples was subsequently evaluated in a 96-well microtiter plate using triphenyl tetrazolium tri-chloride (TTC) as an indicator [40].

IV. Results and Discussion

Condition of wounds and standard treatment

Initially, the severe injury included a laceration wound with bleeding and scarring along with raised skin tissue, ecchymosed, and edematous knee. The medications used during the treatment were Zerodol SP, Calcical 400, Amoxicillin, Betadiene Lotion, and Mega heal tropical Gel with silver colloid (32ppm). The wounds have partially recovered even after 10 days of treatment; the phase comprises diffused wound edges with slough and eschar (Figure 2).

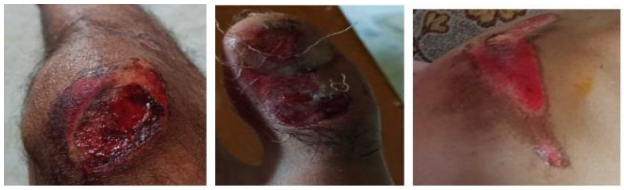

Figure 2. Severe accidental wounds after 10 days of treatment

Microbial isolation and cultivation

Microbial growth was identified following 24 hours of the incubation period. Biofilm-producing bacteria were isolated from the wound using LB agar as the nutrient medium. The pure culture was obtained by streaking the morphologically distinct colonies. Streak plate cultures of the isolated colonies were stored at 4°C for further research.

Molecular identification and characterization of isolated bacteria by 16s rRNA sequencing

A consensus sequence was obtained by aligning the forward and reverse sequences from 16S rRNA sequencing reports. The sequence having a high percentage query cover and lowest E-value was selected and categorized under the same group of genus and species as per the homology. All four isolates were identified up to the species level as strain **CMPD1** (*Escherichia coli*), strain **CMPD2** (*Klebsiella pneumonia*), strain **CMPD3** (*Pseudomonas aeruginosa*), and strain **CMPD4** (*Staphylococcus aureus*) (Table 1).

Ancestry analysis and Sequence submission

The ancestry analysis aids in deciphering the connection between the isolated species and closely related species. The evolutionary distance between the species was determined using the maximum likelihood technique. After phylogenetic analysis, all the species were confirmed and the partial sequences were submitted to the GenBank database of NCBI. The sequences of biofilm-producing bacteria isolated from wound surfaces submitted to the atabase were: *Escherichia coli* strain CMPD1 with accession number **MZ224445**, *Klebsiella pneumonia* strain CMPD2 with accession number **MZ224451**, *Pseudomonas aeruginosa* strain CMPD3 with accession number **MZ224454**, and *Staphylococcus aureus* strain CMPD4 with accession number **MZ224456**.

Table 1. Identification of bacterial strains isolated from the wound

Isolate	Description	Query coverage (%)	E-value	Identity (%)	Sequence length	Characteristics
CMPD1	*Escherichia coli*	100	0.0	99.58	1421	Gram-negative
CMPD2	*Klebsiella pneumonia*	100	0.0	99.73	1497	Gram-negative
CMPD3	*Pseudomonas aeruginosa*	100	0.0	99.67	1498	Gram-negative
CMPD4	*Staphylococcus aureus*	100	0.0	99.59	1463	Gram-positive

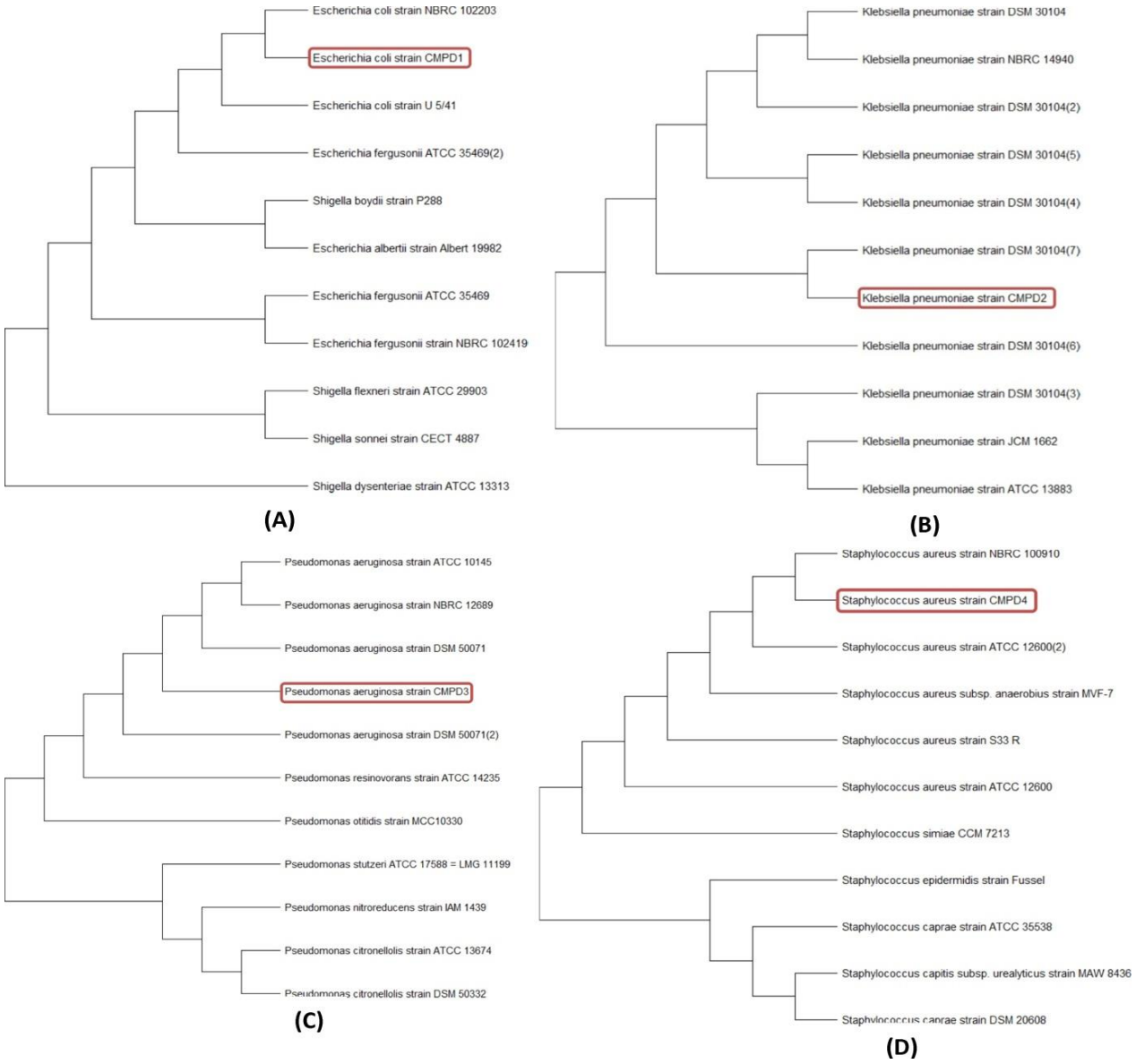

Figure 3. Ancestry analysis from cladogram of (A) CMPD1, (B) CMPD2, (C) CMPD3, (D), CMPD4

The *E. coli* species is a facultative anaerobe that accounts for about 0.1% of gut microbiota, sometimes acts opportunistically, and produces biofilm. The cladogram of the *Escherichia coli* strain CMPD1 reveals that it descended from an unidentified ancestor. All of the related species descended from the same unidentified ancestor and *Escherichia coli* strain CMPD1 has a sister strain *Escherichia coli* strain NBRC 102203(Figure 2A). *Klebsiella pneumonia* is a biofilm-producing pathogen. The cladogram of *Klebsiella pneumonia* strain CMPD2 reveals that all the related species share an unknown common ancestor. *Klebsiella pneumonia* strain CMPD2 and *Klebsiella pneumonia* strain DSM 30104(7) are sister strains and have a common ancestor (Figure 2B). *Pseudomonas aeruginosa* strain CMPD3 is a multidrug-resistant pathogen responsible for causing infection. The cladogram of bacterial strain *Pseudomonas aeruginosa* strain CMPD3 indicates that this particular strain has originated from an unidentified ancestor. Other related strains have originated from this unknown common ancestor through several genomic variations (Figure 2C). *Staphylococcus aureus* strain CMPD4 is a common member of the microbiota of the body but it is often responsible for causing skin infections and pneumonia. The cladogram signifies that this strain has originated from an unknown common ancestor. Many related strains can be traced back to the unknown ancestor through genomic alterations along various generations. *Staphylococcus aureus* strain CMPD4 has a sister strain named *Staphylococcus aureus* strain NBRC 100910 and they share a common ancestor (Figure 2D).

Green synthesis of silver nanoparticles

The colour change of the resultant solution from pale yellow to dark brown during the reduction process confirms the biosynthesis of the nanoparticle. Plant extracts have

been extensively used as phytochemicals present in plants exhibit better reduction and stabilization. Plant secondary metabolites such as steroids, alkanoids, flavonoids, saponins, and the colour of plant extract are responsible for the change of colour as they act as reductants that reduce Ag^+ (silver ions) to Ag^0 (silver atom) [41]. 5 ml of CT leaf extract with 10 ml of 4 mM $AgNO_3$ aqueous solution and 24 hours of incubation in the dark were found to be the best conditions for the biosynthesis of CT-AgNPs. The maximum CT-AgNPs biosynthesis occurred in the ratio 1:2 ratio (the ratio of *C. tamala* leaf extract to silver nitrate) demonstrated by the highest peak in the spectral analysis. The absorbance band formation between 400nm and 450nm along with the colour shift in the resultant mixture corresponds to the excitation of surface plasmon resonance (SPR) and reduction of silver (Ag^+) ions [42].

Characterization of silver nanoparticles

Absorption spectroscopy is being used to examine the optical characteristics of silver nanoparticles (AgNPs). The UV-Vis absorption spectrum is mostly used to study the alteration in the structure of the particles, which helps to understand the complex formation. It's the most common way to indicate that silver is being reduced from aqueous $AgNO_3$ solution to silver nanoparticles. Morphology, shape, and size depend on the surface plasmon resonance bands [43]. The absorbance level rises with the increase in the concentration of leaf extract. The sample containing 4mM $AgNO_3$ and 5 ml plant extract exhibit maximum absorption with a sharp, narrow intense peak at 430 nm, indicating the formation of CT-AgNPs (Figure 4). The SPR tail grew from 350 nm and centered at 430 nm indicating the characteristic surface plasmon resonance peak of synthesized AgNPs. Furthermore, the CT-AgNPs solution's surface plasmon resonance band stuck around 430 nm, indicating that silver nanoparticles (AgNPs) were monodispersed in the aqueous phase. The sharpness of the peak varies according to the size of the nanoparticle synthesized [44]; this indicates that smaller-sized nanoparticles are formed. The formation and stabilization of AgNPs (silver nanoparticles) can be attributed to the biomolecules present in the plant extract i.e. anthocyanins, flavonoids, and polyphenols. Chelating sites of biomolecules chelate the AgNPs (silver nanoparticles) thus preventing them from further aggregation during the fabrication of CT-AgNPs[42].

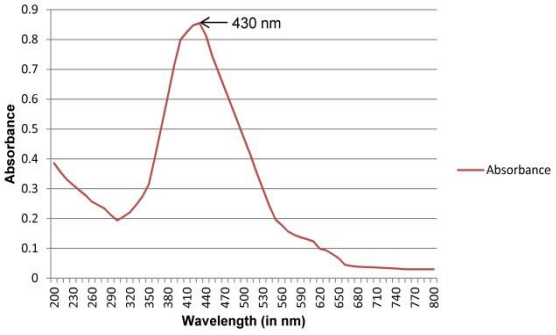

Figure 4. UV absorption spectrum of green synthesized nanoparticle (4mM $AgNO_3$ + 5 Ml plant extract)

Analysis of anti-bacterial property of silver nanoparticles

Silver nanoparticles are widely being used in industries, inclusive of pharmaceutical, food storage, paint, and water treatment. In this work, the invitro anti-bacterial potential of the synthesized nanoparticle was evaluated against isolated strains of bacteria i.e. *S. aureus, P. aeruginosa, K. pneumoniae, and E. coli*. The CT-AgNPs showed a zone of inhibition against the isolated bacterial strain (Table 2). The isolated strains were found to be more susceptible to CT-AgNPs than the positive control antibiotic streptomycin. The efficacy of CT-AgNPs against pathogens is dose-dependent and varies according to their size. The synthesized CT-AgNPs show the highest antibacterial activity against E. coli (Table 2). The CT-AgNPs were found equally effective against all the strains but exerted the highest antibacterial activity against gram-negative bacteria, due to variation in cell wall structure. Specifically, *Escherichia coli* strain CMPD1, *Klebsiella pneumonia* strain CMPD2, showed a better inhibition zone than gram-positive organism *Staphylococcus aureus* strain CMPD4. This antimicrobial activity was higher than that reported for *Cinnamomum tamala* leaf extract mediated AgNPs [45] against strains of gram-negative and gram-positive bacteria [42].

Ag+ ions can lead to oxidative stress through the generation of free radicals in the cytoplasm and disruption of the cell membrane. Silver exposure results in DNA replication loss and inactivation of the cellular proteins. Additionally, it was demonstrated that protein denaturation results from the binding of Ag+ to functional protein groups. However, the Ag+ ions have only limited usage as antibacterial agents due to the interference of salts and the irregular release of inadequate concentrations of Ag+ ions. These constraints can be circumvented due to the large surface area and highly reactive species of AgNPs. AgNPs yield reactive oxygen species that function as oxidative stressors. One putative mode of action for plant-mediated Ag-NPS is to bind to the cell surface and rupture the cell membrane, allowing them to interact AgNPs released silver ions after penetrating. These ions interacted with DNA, proteins, and sulfur-containing cell constituents, resulting in growth inhibition; ultimately leading to cell death [41].

Table 2. Anti-bacterial activity of selected silver nanoparticle against wound microbial isolate

Sample	**Zone of inhibition (in mm ± S.D) on wound isolates**			
	***E.coli* CMPD1**	***K. pneumonia* CMPD2**	***P. aeruginosa* CMPD3**	***S. aureus* CMPD4**
Streptomycin (Control)	22 ± 0.57	21 ± 0.25	18 ± 0.04	15 ± 0.11
AgNP (4 mM $AgNO_3$ + 5 Ml PE)	28 ± 0.15	26 ± 0.47	24 ± 0.20	22 ± 0.23

UV & IR assisted enhancement of anti-bacterial property

The irradiated silver nanoparticles were checked for their anti-biofilm activity and compared with the non-irradiated ones. Maximum eradication was observed in the case of IR irradiated AgNPs followed by UV irradiated and non-irradiated ones respectively. Maximum reduction efficiency was observed in the case of IR-AgNPs on E.coli CMPD1. The activity of AgNPs was found to be the least on *S.aureus* CMPD4 with a maximum reduction percentage of 70% observed in the case of IR-AgNPs.

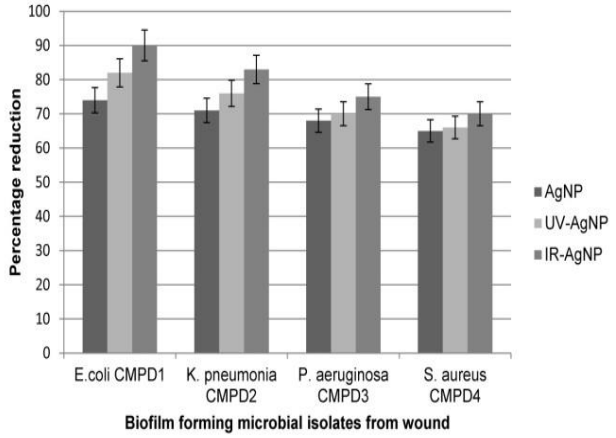

Figure 5. Reduction efficiency of Non-irradiated and irradiated AgNPs against biofilm-forming microbial isolates from wound

A maximum 82% of reduction in the growth of E.coli CMPD1 was found in UV-exposed CT-AgNPs, the growth reduction decreased by 7% in S. aureus CMPD4 (Figure 5). The growth of E. coli colonies was substantially lower in the case of the UV irradiated CT-AgNPs than that of the non-irradiated CT-AgNP. These findings imply that UV irradiation accelerates the production of hydroxyl radicals from AgNPs, thereby enhancing their bactericidal activity. This bactericidal activity can be attributed to the enhanced production of highly reactive hydroxyl radicals under UV exposure [46]. The UV radiation employed in the research was UVA, which has a stronger bactericidal impact than UVC and is safer for humans.

The findings of this research clearly demonstrate the pharmacological significance of *Cinnamomum tamala* by highlighting its potential pharmaceutical perspective as a medicinal source against biofilm-forming bacteria. However, further research is necessary to comprehend the medicinal and curative properties of this plant completely [47].

V. Conclusion and Future Scope

Bacterial biofilm is a major concern for human health and must be dealt with seriously keeping in mind its pathogenicity. The green synthesis of CT-AgNPs is an environmentally friendly technique. Our findings showed that *Cinnamomum tamala* silver nanoparticles have strong anti-bacterial activity against both gram-negative and gram-positive microorganisms. The findings infer that this plant could be a potential alternative for the development of effective and efficient drugs from natural resources for the treatment of bacterial infections. IR irradiated silver nanoparticles can be a promising candidate for the eradication of these biofilms and hence a healthy human life. The AgNPs were shown to have moderate to excellent bactericidal activities; however, a stronger inhibitory action was found at higher doses. Future research will presumably focus on the development of nanoparticles with maximum antibacterial activity and minimum toxicity.

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