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Development of Nano-Silver Functionalized Wound Dressings Using *Wrightia tinctoria* Extract for Enhanced Antibacterial Activity Against MRSA and *Staphylococcus aureus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Wound infections remain a major source of postoperative morbidity, accounting for about a quarter of the total number of nosocomial infections. This leads to errors in establishing the true incidence of their occurrence but undoubtedly decreases the overall real cost and length of hospital stay. The

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healing of defected skin tissue is a complex process, especially for chronic wounds. Poor healing of these wounds may cause extensive suffering and high cost for patients. Traditional wound dressings are typically designed for a single function and they cannot satisfy all requirements for the whole process of wound healing. So it is essential to develop new types of wound dressings with many functions for wound healing. In specific, adding an antibacterial function to the dressings has been shown to be of great benefit during healing of any kind of wounds. Nano-silver is widely used in wound treatment as of various advantages, it has wide antibacterial spectrum and lower drug resistance. Therefore, wound dressings loaded with nano-silver have attracted widespread attention in wound healing. In this work we focused on nano-silver functionalized wound dressings including their preparation methods, antibacterial performances and classification of nano-silver wound dressings. The toxicity of nano-silver based wound dressings is discussed and the prospective research direction is elaborated. This work aims to provide a little guidance for the research of nano-silver functionalized polysaccharide-based wound dressings. The study showed the ability to synthesize environmentally friendly silver nanoparticles (AgNPs) using extracts from *Wrightia tinctoria.*

Keywords: Wound dressings; silver nanoparticles; Wrightia tinctoria; anti-bacterial; Staphylococcus aureus; MRSA.

1. INTRODUCTION

Over the past decade, numerous studies have highlighted the individual risk factors that increase the likelihood of postoperative infections across different types of surgeries. Understanding these risk factors is crucial for improving preventive and treatment measures, especially in high-risk patients. Effective infection monitoring in both hospital and outpatient settings is essential, and leveraging computer technology can significantly enhance the tracking, analysis, and management of infections in surgical patients.

Wound healing, particularly for chronic wounds, is a complex process. Traditional wound dressings often serve only one purpose and may not meet all needs throughout the healing process. Therefore, there is a need to develop new multifunctional wound dressings. Incorporating antibacterial properties into these dressings, such as using nano-silver, has shown great promise for improving tissue repair. Nanosilver is favored in wound care because of its broad antibacterial effects and reduced likelihood of resistance. Polysaccharides derived from natural sources also hold great potential for wound dressings because of their availability, cost-effectiveness, and compatibility with the body. This review explores the preparation, antibacterial efficacy, and categorization of nano-silver-enhanced polysaccharide wound dressings, and discusses their potential toxicity and future research directions. The goal was to provide a comprehensive overview of the advancements in silver nanotechnology and guide future research in this area.

Wound infection leads to errors in establishing the true incidence of their occurrence but undoubtedly decreases the overall real cost and length of hospital stay. The pathogens implicated in the development of wound infections are largely human microorganisms from the exogenous environment and endogenous organ microflora. Many perioperative factors have been identified to increase the incidence of postoperative wound infections. Avoiding these factors, as well as the appropriate use of perioperative antibiotic prophylaxis, has decreased the incidence of wound infection.

"S. aureus is a versatile pathogen able to infect humans with a broad spectrum of illnesses causing both infection and soft tissue infection". (Turner et al., 2019) "Skin and soft tissue infections caused by *S. aureus,* as life-threatening systemic illnesses, are a significant hospital and community-acquired infections". (Sharaf et al., 2021) "Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) signify a global public health hazard because of their risk and spread". (Stryjewski 2008)

Wrightia tinctoria, a medium-sized evergreen tree, is notable for its milky-white latex, which has been recognized for its therapeutic properties. Traditionally, various parts of this plant have been used in folk medicine for various purposes. Latex and other plant components have been used as galactagogues to enhance milk production in nursing mothers, as antipyretics to lower fever, and as remedies for dysentery and diarrhea. Additionally, it has been utilized to control bleeding, serve as an antidote

for snake venom, and treat various skin conditions and disorders (Srivastava, 2014). *Wrightia tinctoria* also demonstrated significant antimicrobial activity, contributing to its therapeutic value. Research has demonstrated its effectiveness against various microbial pathogens (Vedhanarayanan et al*.,* 2013). Despite these promising findings, many phytochemicals, such as terpenoids, steroids, flavonoids, and alkaloids, have been identified and characterized; however, their pharmacological effects remain largely unexplored. This gap in research presents an opportunity for further investigation of the full range of its therapeutic potential (Oviya et al., 2015; Selvakumar et al., 2016).

This study explored the integration of AgNPs with *Wrightia tinctoria,* to enhance wound healing and infection management in wound dressings. This combination leverages the antimicrobial effects of AgNPs with the traditional therapeutic properties of *Wrightia tinctoria*. This synergy aims to create advanced wound dressings and therapeutic products that not only promote healing but also effectively combat microbial infections, including those caused by *Staphylococcus aureus*

2. MATERIALS AND METHODS

2.1 Sample Collection and Primary Screening

Clinical sample: Pus samples were obtained from Micro tech Diagnostic centre Coimbatore, Tamil nadu India.

Organism: Methicillin resistant *Staphylococcus aureus* (MRSA)

2.2 Isolation of Microorganism from Pus Sample

Staphylococcus aureus was identified on the basis of its microscopy appearance and morphological characteristics followed by Gram Staining, catalase and coagulase test. (Duguid et al., 1996)

2.3 Congo Red Agar Method

The Congo red agar method is based on phenotypic characteristics such as colony morphology and colour of biofilm-forming bacteria compared to that of non bio film formers in the presence of Congo red agar. The Congo red agar are used widely for slime-producing or biofilm-producing *Staphylococcus aureus*. The Congo red plates were inoculated with pus samples isolates and incubated at 37c for 24 hours (Mariana, N. S et al., (2018).

2.4 Plant Collection and Identification

The leaves of *Wrightia tinctoria* were collected near Saravanampatti, Coimbatore, Tamil Nadu, India. The plant was authenticated and its confirmed as *Wrightia tinctoria* belonging to the family Apocynaceae by Botanical survey of India, Tamil Nadu Agricultural University Campus (TNAU), Coimbatore, Tamil Nadu, India.

2.5 Sample Extraction

"The collected fresh leaves of *Wrightia tinctoria* were washed well using running water. This helped to remove all impurities present in the collected leaves. They were dried under shade for a period of 7 days to remove the excess moisture present in them and this process helps to avoid destruction of active compounds. After drying, they were ground completely and stored. The extraction of *Wrightia tinctoria* leaf powder (20 g) was executed with the help of Soxhlet extractor based on continuous hot extraction method using ethanol as solvent. The solvent from extraction was distilled off after it becomes concentrated, evaporated".Saxena, D., & Jain, M. (2024).

2.6 Synthesis of Silver Nanoparticles

To convert the Ag^{+} ions Dhayalan et al. (2017), 25 ml of the plant extract sample is combined with 100 ml of an aqueous solution that contains 1 nM of $AgNO_3$. After 24 hrs, the reaction mixture was settled to its final state. Silver nanoparticle production was inferred from the change in reaction mixture colour. To further separate the reaction mixture, it was centrifuged at 2,000 rpm for 10 min. After that, the AgNPs were separated from the sample by centrifuging it for 15 min at a speed of 16,000 rpm. The AgNPs were collected and dispersed in acetone before being stored.

For synthesis of silver nanoparticles about known concentration 1ml of plant extract was interacted with 10 ml of 1mM of silver nanoparticles AgNO3 solution. In the ratio of 1:10. Another bottle Control is also kept without leaf extract. Both the bottle are incubated in room temperature (dark) for 48 hours. After 48 hours the bottle were observed for the colour change from green to brown.

2.7 Antimicrobial Activity of Leaf Extract

The disc diffusion method is classified as the agar diffusion method (ADM) because the plant extract to be tested diffused from the reservoir through the agar medium seeded with test sample (*Staphylococcus aureus*, MRSA) in the concentration of 20,40 and 60 µl. The diameter of the inhibition zone properly describes the antimicrobial potency of the plant extract. (Das et al., 2020) (Bassey et al., 2020).

2.8 Antibacterial Activity of Gauze Piece

Cotton gauze piece finished with herbal extracts were subjected to antibacterial assay. The assay used for measuring antibacterial properties was based on the AATCC Test Method 147-2012. Briefly, finished cotton gauze piece were cut into pieces (25mm x 50mm) and hygienically transferred to testing conditions. The 50mm length of the cotton strips permits the specimen to lay across 5 parallel inoculums streaks each of diminishing width from both 8mm to 4mm wide. Sterile AATCC bacteriostatic agar plates were prepared. Using sterile 4mm inoculating loop, one loop full of culture was loaded and transferred to the surface of the agar plate by making five parallel inoculum streaks spaced 10mm covering the central area of the petridish without refilling the loop. The test specimen was gently pressed transversely, across the five inoculums of streaks to ensure intimate contact with agar surface. The plates were incubated at 37°C for 18-24 hours.

2.9 Confirmation of Nanoparticle Impregnation Using SEM

The surface morphology of silver nano particles with *W. tinctoria* impregnated cotton bandages were analyzed using a scanning electron microscope (Carl Zeiss SEM) at an accelerating voltage of 5 kV. The normal cotton bandage was used as a control. Accelerating Voltage was 5.00 kV with the Working Distance ranged from 8.3 mm to 8.7 mm, ensuring controlled sample-to-detector distance. Different magnification was used ranging from 100X to 1000X, enabling visualization of the sample structure at different scales. Signal Mode is SE2, indicating the use of a secondary electron detector for capturing topographical surface information. Mirza et al., (2022).

2.10 The Gas Chromatography-Mass Spectrometry Analysis (GC-MS)

"The GC-MS analysis of various organic crude extracts isolated from leaves of *W.tinctoria* was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 500, USA) equipped with a VF-5 MS fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). GC-MS spectroscopic detection, an electron ionization system with ionization energy of 70 eV was used. Pure helium gas (99.999%) was used as a carrier gas at a constant flow rate of ±1 mL/min. Mass transfer line and injector temperature were set at 220°C and 290°C, respectively. The oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal for 10 min and finally raised to 300°C at 10 °C/min. Diluted samples (1/100, v/v, in methanol) of 1 µL was injected in the split mode with a split ration 120:1. The relative percentage of the chemical constituents in crude extracts from leaves of *T. vulgaris* was expressed as percentage by peak area normalization" (Balushi et al., 2017).

2.11 Identification of Chemical Constituents

The chemical compounds in extracts isolated from *Wrightia tinctoria* were identified based on GC retention time on VF-5 capillary column, computer matching of mass spectra with those of standards (Mainlab, Replib and Tutorial data of GC-MS systems). Whenever it is possible by co-injection with authentic or standard compounds.

3. RESULTS AND DISCUSSION

3.1 Preliminary Screening Test for the Microorganism

3.1.1 Microscopic examination of the organisms – Gram Staining

Gram staining was performed to identify the isolated culture from the pus sample. It stained in purple colour and in Cocci. Further biochemical characteristics showed gram positive Cocci and appeared as purple colour.

3.1.2 Biochemical analysis

Further the given bacterial culture is analysed for biochemical parameters catalase, coagulase and Mannitol tests. Catalase Coagulase and Mannitol shows positive result in biochemical analysis.

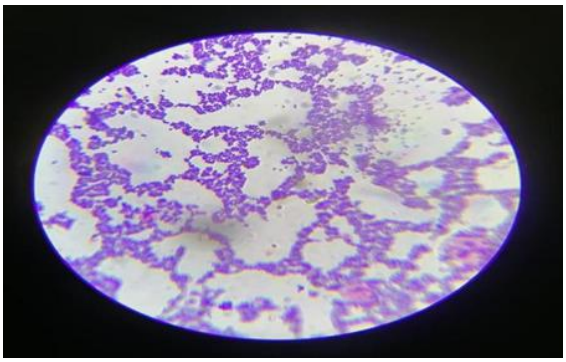


Fig. 1. *Staphylococcus aureus* in gram staining

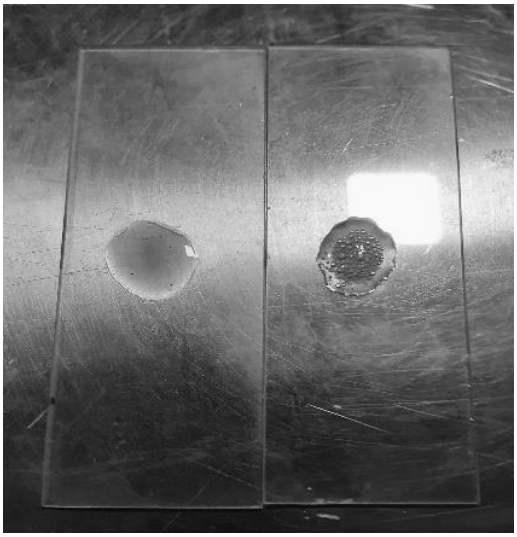


Fig. 2. Catalase and coagulase positive results

Fig. 3. Mannitol positive result

3.1.3 Cultural characteristics

The Isolated colonies were sub cultured on blood agar, nutrient agar and MRSA in Congo Red Agar.

Fig. 4. Blood agar plate

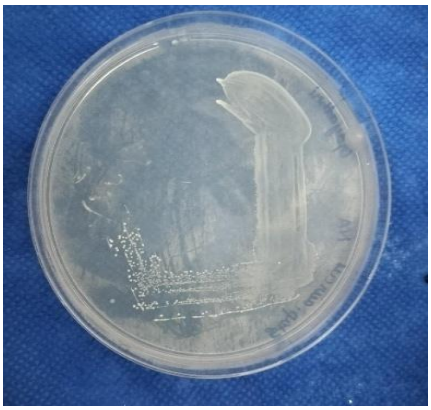


Fig. 5. Nutrient agar plate

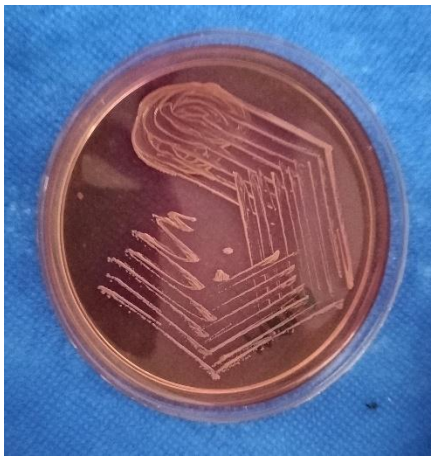


Fig. 6. MRSA

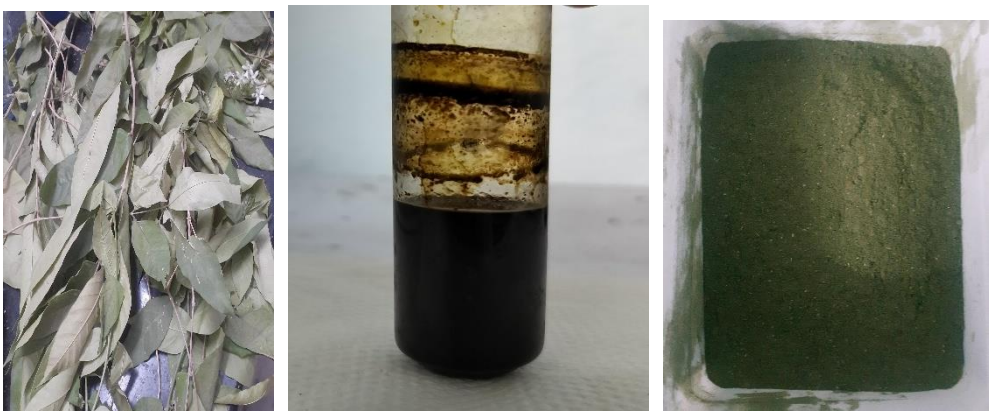


Fig. 7. Leaf, powdered form of the sample and soxhlet extractraction sample

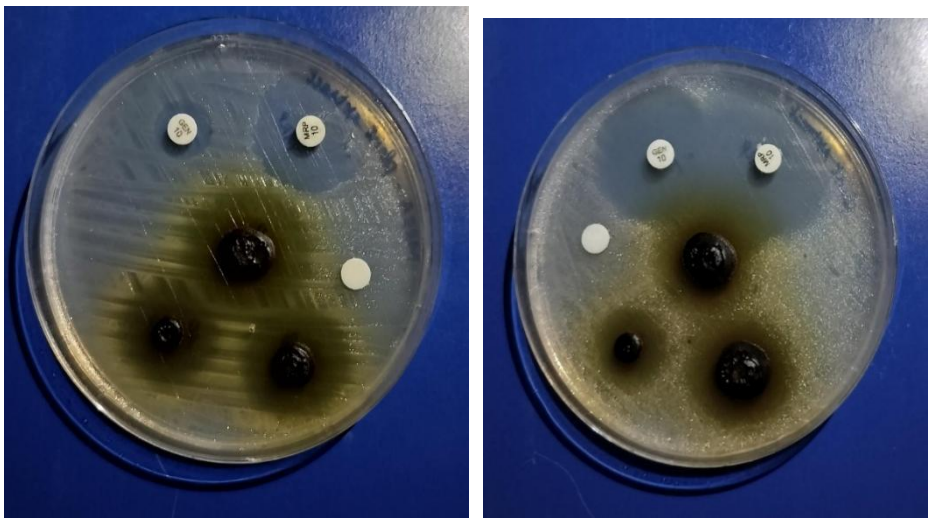


Fig. 8. Antimicrobial activity of leaf extract (Befeorw treated with silver nana particles)

Table 1. Agar diffusion method

Test organisms	20 µl	40 µl	60µl
Staphylococcus aureus	15mm	19mm	20mm
MRSA	19mm	20 mm	25mm

3.2 Plant Extraction *Wrightia tinctoria* – Collection and Extraction Using Soxhlet Extractor

3.2.1 Green synthesis

Table 1 and Fig. 8 shows the result of antimicrobial activity of *Wrightia tinctoria* before treating it with silver nano particle.

3.2.2 Synthesis of silver nanoparticles

The bottles which were added with known concentration of 1ml of the *Wrightia tinctoria* leaf extract was interacted with 10 ml of 1mM Silver nanoparticles AgNO3 solution.In the ratio of 1: 10 was served as a test shows colour changes from green to brown colour after 48 Hrs incubation.

3.3 Confirmative Test - In Congo Red Agar

The Congo red agar plates are inoculated with *Staphylococcal* isolates and incubated for 24 hours for confirmative analysis. Black colour colonies were observed after the incubation period which is the indicate the confirmative test for *Staphylococcus aureus.*

Table 2. Measurment of zone of inhibition with silver nano particle treated plant sample

Test organisms	20 µl	40µl	60µl
Staphylococcus aureus	15mm	18mm	25mm
MRSA	19mm	21mm	27mm

Fig. 9. Test and the control bottles

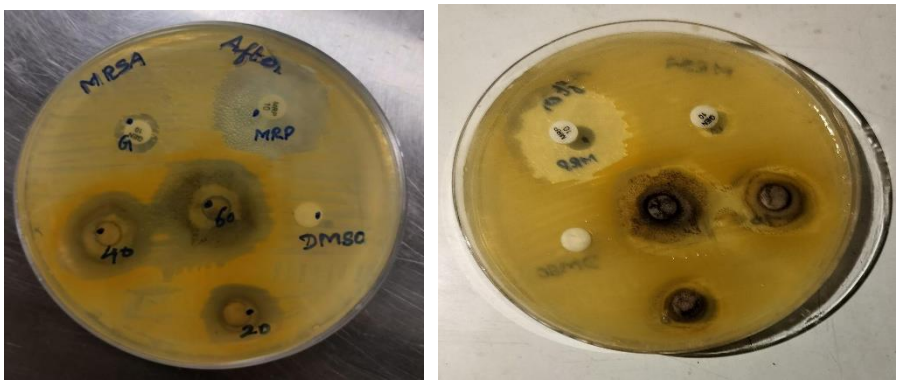
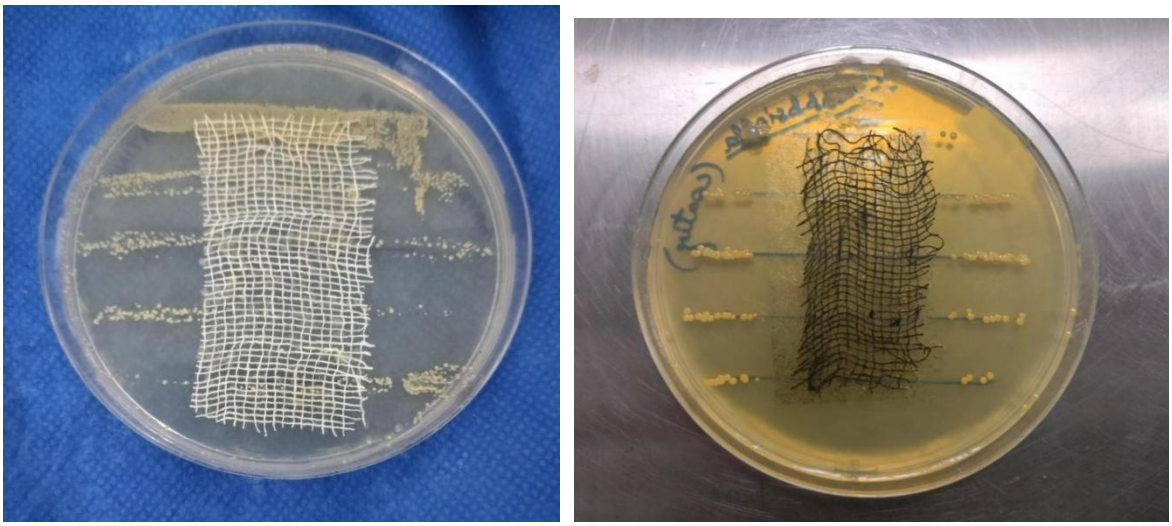


Fig. 10. Plate shows the agar diffusion method – silver nanoparticles with different concentration of the plant sample

Fig. 11. Black colour colonies on Congo red plates



12a Control **12b Treated Gauze**

Fig. 12. Antibacterial activity of treated gauze Piece and Conterol

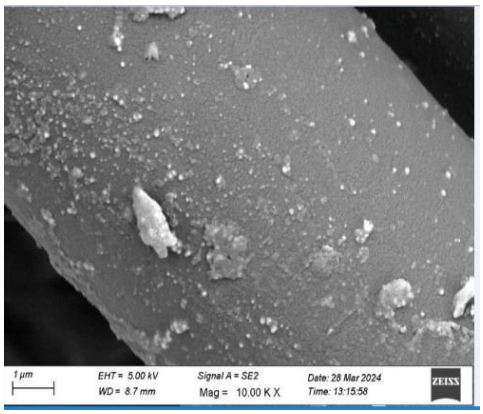


Fig. 13.

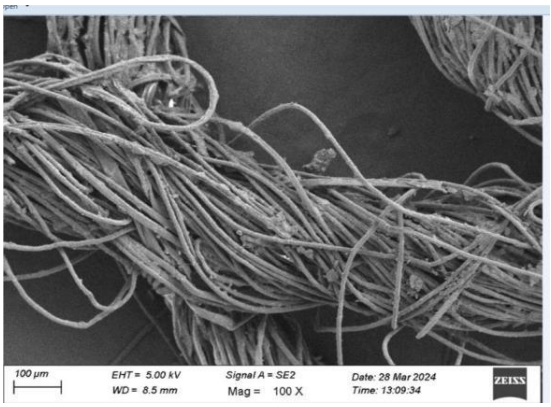


Fig. 14.

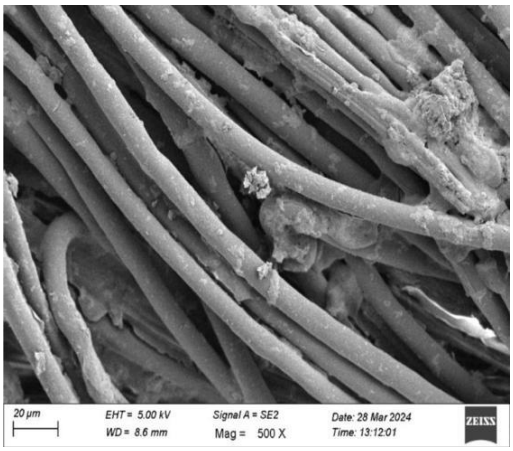


Fig. 15.

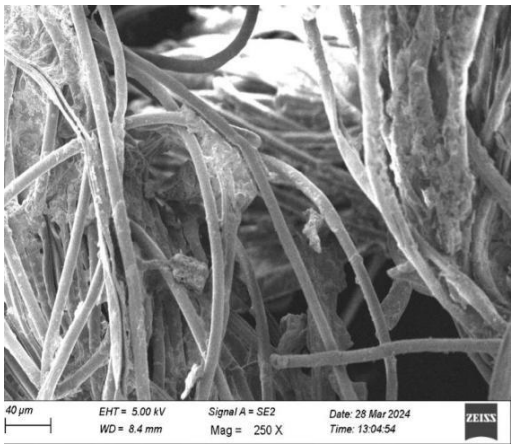
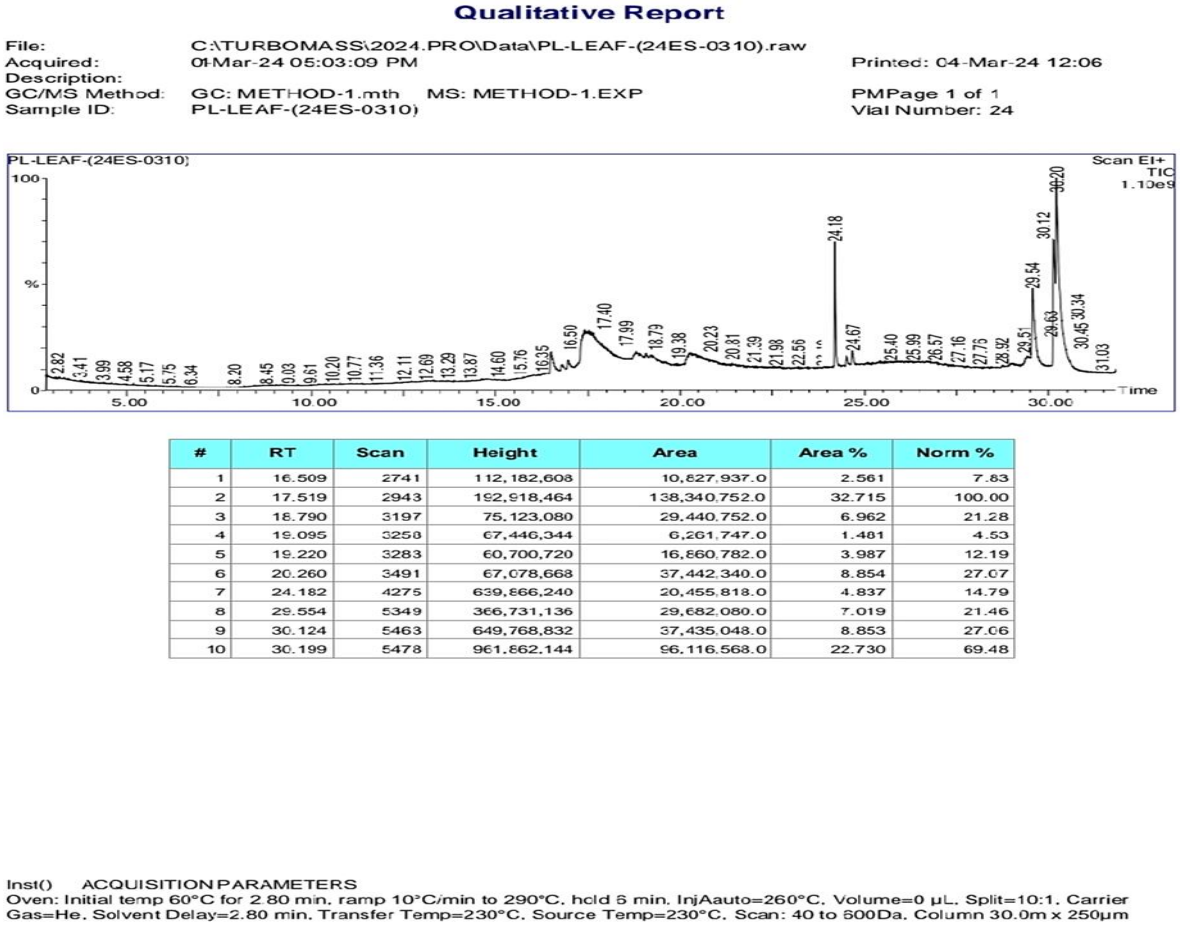


Fig. 16.

Figs. 13 – 16. SEM analysis, with different magnification



#	RT	Scan	Height	Area	Area %	Norm %
1	16.509	2741	112,182,608	10,827,937.0	2.561	7.83
2	17.519	2943	192,918,464	138,340,752.0	32.715	100.00
3	18.790	3197	75,123,080	29,440,752.0	6.962	21.28
4	19.095	3258	67,446,344	6,261,747.0	1.481	4.53
5	19.220	3283	60,700,720	16,860,782.0	3.987	12.19
6	20.260	3491	67,078,668	37,442,340.0	8.854	27.07
7	24.182	4275	639,866,240	20,455,818.0	4.837	14.79
8	29.554	5349	366,731,136	29,682,080.0	7.019	21.46
9	30.124	5463	649,768,832	37,435,048.0	8.853	27.06
10	30.199	5478	961,862,144	96,116,568.0	22.730	69.48

Fig. 17. The chromatogram shows peaks representing different compounds detected over time. The major peaks suggest a high concentration of triterpenoids and indole alkaloids, which are known bioactive compounds in this plant species

3.4 Antibacterial Activity of Silver Nanoparticle Coated Leaf Extract

Qualitative antibacterial activity was determined based on the zone of inhibition around the finished fabrics against the test organisms. Maximum zone of inhibition was influenced by the greater action of finished herbal particles.

3.5 Scanning Electron Microscopy

SEM image showing the synergistic coating of silver nanoparticles with *Wrightia tinctoria* on the gauze cloth. The bright spots represent a hybrid coating of AgNPs and *Wrightia tinctoria* Particle size distribution appears to be in the range of ~50-200 nm The coating shows excellent adherence to the gauze fiber surface. Distribution Analysis shows Dense and uniform distribution of the hybrid coating Coverage appears to be approximately 70-80% of the visible surface area Some larger aggregates (200-300 nm) are visible, likely due to the interaction between AgNPs and *Wrightia tinctoria* compounds .The hybrid coating shows excellent surface coverage at 10.00K X magnification The morphology suggests enhanced stability of AgNPs, likely due to the bioactive compounds from *Wrightia tinctoria* .Technical Significance shows as the uniform distribution suggests synergistic antimicrobial activity The hybrid coating appears well-integrated with the gauze fiber The particle size range is optimal for both silver ion release and plant compound interaction with bacterial cells.Secondary electron detector (SE2) used for imaging.

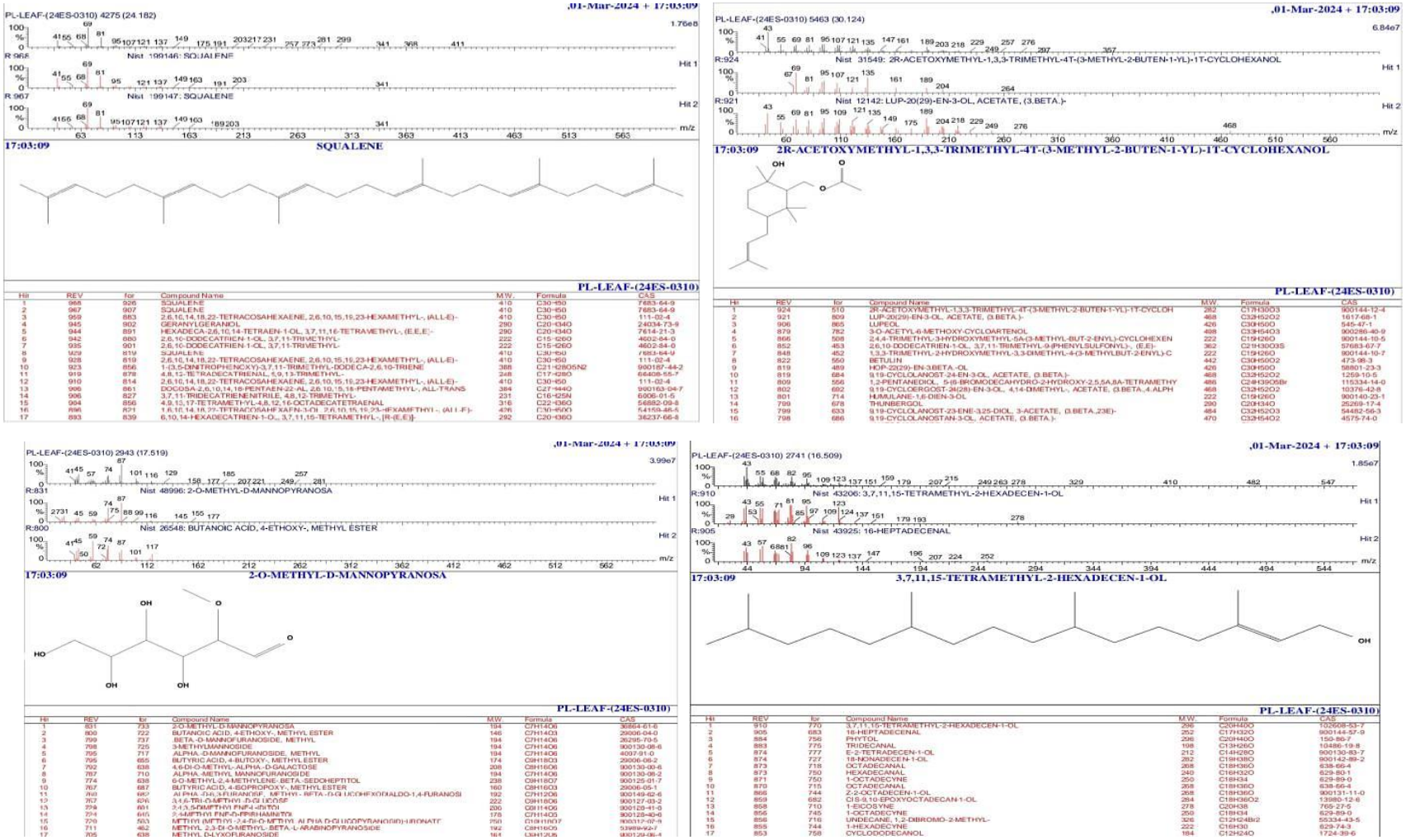


Fig. 18. Identified compounds are: Squalene; 2R-Acetoxymethyl-1,3,3-Trimethyl-4T-(3-Methyl-2-Buten-1-Yl)-1T-Cyclohexanol; 2-O-Methyl-D-Mannopyranosa; 3,7,11,15-Tetramethyl-2-Hexadecen-1-OL

3.6 Gas Chromatography Mass Spectrometry

The chromatographic separation was performed under optimized conditions with an initial oven temperature of 60°C (2.80 min hold), ramped at 10°C/min to 290°C with a final hold time of 5 minutes. The injection was automated at 260°C using a split ratio of 10:1 with helium as the carrier gas. The mass spectrometric analysis was conducted with both transfer and source temperatures maintained at 230°C, scanning masses from 40 to 600 Da after a solvent delay of 2.80 minutes. The chromatogram revealed several significant peaks, with the most prominent one at retention time 17.519 minutes, contributing 32.715% of the total area (normalized to 100%). Among the identified compounds, 3,7,11,15-Tetramethyl-2-Hexadecen-1-OL (MW 296, Formula C20H40O) was a notable component. Another identified compound was 2-O-Methyl-D-Mannopyranosa (MW 194, Formula C7H14O6). So to Conclude, The GC-MS analysis successfully separated and identified multiple compounds in the leaf sample, with terpenoid and carbohydrate derivatives being prominent components. The presence of 3,7,11,15-Tetramethyl-2-Hexadecen-1-OL as a major compound suggests this could be a biologically active constituent, while the identification of 2-O-Methyl-D-Mannopyranosa indicates the presence of glycosidic compounds. The analysis demonstrates that the leaf sample contains a complex mixture of organic compounds, primarily consisting of high molecular weight alcohols and sugar derivatives, which could be relevant for further phytochemical or pharmaceutical studies.

4. SUMMARY AND CONCLUSION

The gauze fabrics treated with *Wrightia tinctoria* extracts exhibited remarkable antibacterial properties, demonstrating both bactericidal and bacteriostatic effects against pathogens such as Methicillin-Resistant Staphylococcus aureus (MRSA) and *Staphylococcus aureus*. This efficacy is attributed to the bioactive compounds inherent in the herbal extracts. These treated materials hold immense potential for applications in healthcare settings, including hospital bed linens, surgical gowns, patient attire, and other medical textiles. Given the abundant availability of *Wrightia tinctoria* in tropical and subtropical regions, its utilization for developing antibacterial finishes presents a sustainable and promising avenue for enhancing infection control measures in healthcare environments.

In the present study, cotton gauze piece finished with silver nanoparticles showed good qualitative antibacterial activity. Qualitative antibacterial activity revealed the antimicrobial effectiveness against standard test cultures *Staphylococcus aureus* (gram positive). The zone of bacterial inhibition was indicated by a halo around the fabric samples. It was apparent that the activity of herbal extract finished fabrics was excellent for 1:10 concentration. The qualitative antibacterial analysis clearly reveals the potential of *Wrightia tinctoria* extract gets increased with increase in concentration of extract. It emphasizes that, the plant comprising the natural ingredients have potential to reduce bacterial growth; and its mechanism of action is effective, safe to human and environment.

The effective mechanism of the herbal finished Cotton gauze piece was reported earlier. According to Ai et al., (2024) bacterial inhibition was due to the slow release of active substances from the fabric surface. Amino groups of the herbal extract were responsible for its excellent antimicrobial activity. reported that in presence of slight acidity the amino groups will be converted to positive amino group ions. Thus converted ions will react with the negatively charged protoplasm of microorganisms and breaks the cell wall to destroy the microorganisms.

Recent studies corroborate the effectiveness of plant-mediated AgNPs. For instance, biosynthesized AgNPs using Skimmia laureola and Clitoria ternatea leaf extracts exhibited potent antibacterial properties, particularly against *Staphylococcus aureus (*Vanlalveni et al., 2021). The nanoparticles impair bacterial adhesion and cell wall stability, a mechanism influenced by their size and the phytochemical composition of the plant extract. Research highlights that these nanoparticles offer scalable and sustainable solutions for combating Gram-positive bacteria while maintaining eco-friendliness

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input

prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Paper pal for checking grammer

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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