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Natural Antimicrobial Agents in Wound Care: Comparative Analysis of Honey, Turmeric, and Neem Oil Against *Staphylococcus aureus*

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***Abstract*— *Staphylococcus aureus* is a Gram-positive bacterium commonly implicated in the infection of wounds. Often, the bacterium forms biofilms, complicating treatment and leading to chronic and drug-resistant infections. This research paper evaluates the antibacterial activity of natural efficacy honey, turmeric, and neem oil at different concentrations: 25%, 50%, 75%, and 100% against *S. aureus* by using the well diffusion method. For the purpose of determining the efficacy of each agent, the diameters of the inhibition zones were measured. Honey, pure, proved the most effective against bacteria, showing 1.5 centimetres of zone of inhibition. Neem oil exhibited dose-dependent activity with a maximum of 1.1 cm at 100%. Turmeric oil demonstrated similar antibacterial effectiveness in concentrations of 25% and 50% at 1.0 cm. These findings indicate the potential role of natural agents as alternative antimicrobial therapies in the management of wounds, as they may potentially reduce dependence on synthetic antibiotics and diminish the rate of antibiotic resistance development. Determination of optimal concentrations and combinations needs further studies, as that will help enhance their clinical applicability.**

***Index Terms*— *Staphylococcus aureus*, wound infection, honey, turmeric, neem oil, antibiotic resistance, biofilm, natural antimicrobial.**

I. Introduction

Staphylococcus aureus is a Gram-positive bacterium frequently isolated from wound infections owing to its ability to attach itself to tissues, produce toxins, and make evasion from host immune responses possible (1). As such, this bacterium is one of the most common causes of skin and soft tissue infections both in hospital and community environments, with high risks of dissemination into deeper tissues to cause complications such as cellulitis, abscesses, or even systemic infections (2). Rapid application of antimicrobial agents to wounds is very necessary to inhibit bacterial colonization and biofilm formation, which aggravate the infection and delay the healing process (3). A few natural antimicrobials, such as turmeric, neem, and honey, have gained recognition because they show potential in wound healing due to their broad-spectrum antibacterial effects by disturbing microbial growth, reducing inflammation, and accelerating tissue repair (4). The integration of such agents into primary wound care limits the chance for infection, enhances the healing process, and reduces reliance on synthetic antibiotics, thus helping to decrease the development of antibiotic resistance (5). *S. aureus* infections frequently give rise to difficulties in treatment because of the ability of the pathogenic agent to form biofilms, especially in chronic wounds, where afterwards it establishes stubborn infections that are hardly effectively treated by conventional antibiotics (6). Biofilms protect bacteria against immune responses and increase tolerance to antimicrobial agents. This makes consideration of adjunct therapies an important issue in wound management (7). The role of natural antimicrobials like turmeric, neem, and honey has been widely researched in the context of action against *S. aureus* biofilms through inhibition of quorum sensing, disruption of the biofilm matrix, and immune responses (8). For instance, curcumin in turmeric exerts anti-inflammatory and antibacterial activity, whereas neem contains constituents such as nimbidin, which inhibit the bacterial membrane (9). Honey, at high osmotic effect, remains viscous and releases hydrogen peroxide, allowing bacteria hardly to survive (5). Since there is a gradual increase in *S. aureus* strains, early intervention by using natural antimicrobial agents in first-aid wound care is a promising approach in the control of infection and support of tissue repair with the added potential benefit of reducing resistant pathogens spread.

A. Honey:

Natural honey has derived much interest due to its considerable antibacterial activities, especially against *Staphylococcus aureus*, one of the most common etiologic agents found in infected wounds. Its antimicrobial activity may be due to several components and factors that include hydrogen peroxide production, acidity or low pH, high osmolarity, and the presence of bioactive compounds such as flavonoids and phenolic acids (10) (11). Reports have shown that honey reveals considerable inhibitive activities against both methicillin-sensitive and methicillin-resistant *S. aureus*, with certain varieties, like Manuka honey, proving particularly effective due to their unique chemical composition (12) (13). The potential

clinical application of honey has highlighted not only its role as an alternative treatment against resistant bacteria but also its wound-healing properties, supporting further integration into conventional medical practice (14).

B. Turmeric:

Turmeric powder, derived from the rhizome of *Curcuma longa*, has well-documented antimicrobial effects, largely attributed to its active compound, curcumin. Numerous studies have shown that turmeric exhibits strong antimicrobial activity against bacterial strains like *Staphylococcus aureus*, a primary pathogen in wound infections. Curcumin interacts with bacterial cell membranes, inhibits biofilm formation, and regulates inflammation, which enhances its therapeutic effect (15) (16). In vitro studies confirm turmeric powder's effectiveness against both methicillin-sensitive and MRSA strains of *S. aureus*, suggesting its potential as an alternative treatment for antibiotic-resistant infections (17) (18). Research supports turmeric's relevance as a natural antimicrobial agent in clinical applications, particularly in wound management (19).

C. Neem Oil:

Neem oil is extracted from the seed of the *Azadirachta indica* tree and is widely recognized for its broad-spectrum antibacterial effects, primarily due to active compounds like azadirachtin and nimbidin. Studies report that neem oil has significant antimicrobial effects against various pathogens, including *Staphylococcus aureus*, a common isolate in wound infections (20) (21). Research suggests that neem oil disrupts bacterial cell membranes, inhibiting biofilm formation and reducing bacterial viability (22). In vitro studies confirm its effectiveness against both methicillin-sensitive and methicillin-resistant *S. aureus*, positioning neem oil as a promising alternative for wound management (23) (24). These findings suggest neem oil may be beneficial as an adjunct treatment, especially in the face of rising antibiotic resistance (25).

The well diffusion method on a petri dish is commonly used to test the antimicrobial potency of agents against bacterial and fungal pathogens. The antimicrobial agent is added to wells made on the nutrient agar plates that were inoculated with the microorganism. As the agent diffuses, a gradient forms, creating a zone of inhibition if the agent is effective (26), which is measured in millimetres to reflect potency. This straightforward, cost-effective, and reproducible method is widely used in research settings (27) (28).

At MGM Hospital, medical personnel documented the isolation of *Staphylococcus aureus* from a wound sample from a 24-year-old male patient with thyroid nodules and swelling. The specimen, collected as pus on 29/9/24 at 4 pm, followed an intramuscular 1.2 g Augmentin injection, with no operation or catheterization performed. Routine culture and sensitivity tests were requested, with AFB and anaerobic culture test were not indicated.

The antimicrobial efficacy of honey, turmeric, and neem oil against *S. aureus* was evaluated using the well diffusion method, testing honey at 25%, 50%, 75%, and 100%; turmeric powder at 10%, 25%, and 50%; and neem oil at 25%, 50%, 75%, and 100%, with isopropanol dilution. Inhibition zones were measured and averaged to assess each concentration's effectiveness.

II. METHODOLOGY

The primary objective of this study is to evaluate the antibacterial efficacy of natural agents, namely turmeric, honey, and neem oil, against a common wound pathogen, *Staphylococcus aureus*, using three different concentrations by the well diffusion method. The study will measure and compare the inhibition zones for each agent to determine the relative efficacy of each and assess their potential as alternative antimicrobial therapies for wound care, especially considering the rise in antibiotic resistance. This research aims to deepen the understanding of clinical applications for natural compounds, exploring how they can prevent biofilm formation, reduce inflammation, and promote wound healing. Specifically, it will assess the antimicrobial activity of honey, turmeric, and neem oil using well diffusion against *Staphylococcus aureus*, a prevalent wound pathogen. By measuring and comparing the inhibition zones, the study will determine each agent's relative potency and potential as alternative wound treatments amid increasing antibiotic resistance. Additionally, the study will contribute to knowledge of these natural compounds in clinical applications, focusing on biofilm inhibition, inflammation reduction, and wound healing support.

A. Materials Required:

Mueller-Hinton Agar: Prepared according to manufacturer instructions for bacterial cultures.
Petri Dishes: Sterile Petri dishes (90-100 mm Diameter).
Test Microorganism: *Staphylococcus aureus* culture standardized to 0.5 McFarland turbidity standard (approximately 1.5 × 10^8 CFU/mL for bacteria).
Sterile Cotton Swabs: To spread the inoculum evenly on the agar surface.
Sterile Cork Borer or Pipette Tips: Used to create wells in the agar.
Antimicrobial Agents: Test solutions of natural honey, turmeric and neem oil.
Micropipette: For adding specific volumes of antimicrobial agents.
Caliper: For measuring the diameter of inhibition zones.
Incubator: Set to 37°C.

B. Method:

S. aureus was isolated from a wound specimen received from a 24-year-old male patient with thyroid nodules and swelling, collected as pus by medical staff at MGM Hospital, Chhatrapati Sambhaji Nagar, on 29/9/24 at 4 pm. The patient had already received a 1.2 g dose of Augmentin and had no history of surgical procedures or catheterization. Routine culture and sensitivity tests were requested, excluding AFB and anaerobic cultures.

Mueller-Hinton Agar was autoclaved at 121°C for 20 minutes and cooled to 45-50°C to prevent condensation in petri dishes. Afterward, 20-25 mL of agar medium was poured into each sterile petri dish to form a uniform layer about 4 mm thick. The agar was left to solidify at room temperature.

A bacterial suspension was prepared, with its turbidity matched to the 0.5 McFarland standard to ensure consistent bacterial concentration. Using a sterile cotton swab, the bacterial inoculum was streaked across the solidified agar plate for even coverage. Plates were left undisturbed for 5-10 minutes to absorb excess moisture and secure the inoculum to the agar.

Wells in the agar were created with a sterile 3.0 cm diameter cork borer by gently pressing down and removing agar plugs, keeping the wells far enough apart to prevent overlapping inhibition zones. Residual agar was carefully removed to avoid contamination.

Using a micropipette, 100 µL of the antimicrobial solution was added to each well without overflow. Each well was labeled with the name and concentration of the antimicrobial agent.

The plates were incubated at 37°C for 24-48 hours for optimal bacterial growth.

After incubation, clear zones around each well indicated microbial inhibition. Each inhibition zone's radius, including the well, was measured with a caliper across two perpendicular angles for accuracy, and the average was calculated.

Inhibition zone diameters were recorded in a table, with each tested antimicrobial agent labeled. The relative efficacy of each antimicrobial agent against the tested pathogen was determined by comparing inhibition zone diameters, with larger zones indicating greater antibacterial activity.

III. RESULTS AND DISCUSSIONS

The antimicrobial efficacy of honey, turmeric, and neem oil at different concentrations against Staphylococcus aureus: one of the most common pathogens responsible for wound infection was studied using the well diffusion technique. In light of the urgent need for alternatives due to rising antibiotic resistance, this research explores the feasibility of these natural agents as substitutes for conventional treatments in wound management. By measuring and comparing inhibition zones, the study provides insights into their clinical use in preventing biofilm formation, reducing inflammation, and enhancing wound healing.

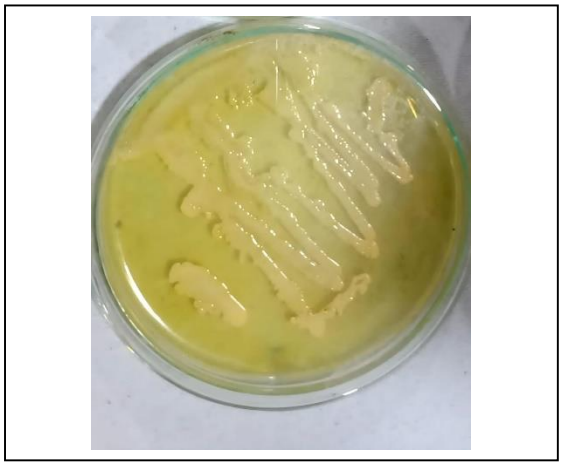


Fig 1: Growth of isolated *S. aureus* observed on petri plate.

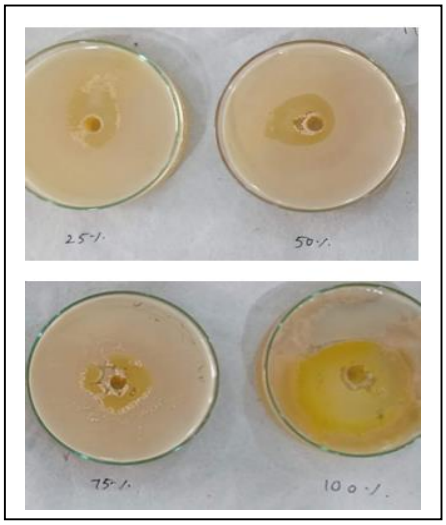


Fig 2: Antibacterial activity of natural honey was assessed at 25%, 50%, 75%, and 100% concentrations against *Staphylococcus aureus* using the well diffusion method

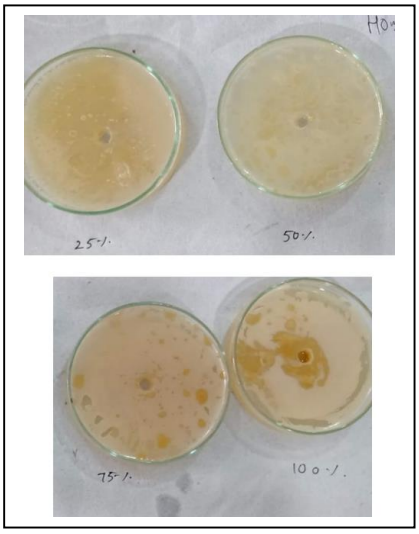


Fig 3: Antibacterial activity of neem oil was assessed at 25%, 50%, 75%, and 100% concentrations against *Staphylococcus aureus* using the well diffusion method.

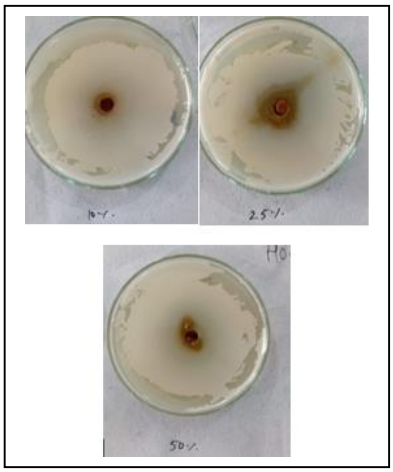


Fig 4: Antibacterial activity of turmeric was assessed at 10%, 25%, and 50% concentrations against *Staphylococcus aureus* using the well diffusion method.

Natural honey and neem oil were tested against Staphylococcus aureus using the well diffusion method for their antimicrobial effectiveness at concentrations of 25%, 50%, 75%, and 100%, while turmeric was tested at 10%, 25%, and 50%. Neem oil was diluted at different concentrations using isopropanol. The diameter of inhibition zones around each well was measured to determine the antibacterial effect at each concentration.

A. Collection and Spoilage of Biodegradable Waste:

TABLE I. INHIBITION ZONE MEASUREMENT AND CONCENTRATION.

Concentration (%)	**Honey Inhibition Zone (cm)**	**Turmeric Inhibition Zone (cm)**	**Neem Oil Inhibition Zone (cm)**
25	1.0	1.0	0.0
50	1.5	1.0	0.8
75	1.1	-	0.9
100	1.0	-	1.1
Concentration (%)	Honey Inhibition Zone (cm)	Turmeric Inhibition Zone (cm)	Neem Oil Inhibition Zone (cm)

B. Analysis and Interpretation:

Natural Honey:

The largest zone of inhibition was observed at a 50% concentration, measuring 1.5 cm, indicating peak antibacterial activity against *Staphylococcus aureus*.

A 75% concentration produced a slightly smaller inhibition zone of 1.1 cm, suggesting a slight decline in activity at this higher concentration.

Concentrations of 25% and 100% resulted in inhibition zones of 1.0 cm, demonstrating moderate and consistent antibacterial action.

Overall, the data suggests a trend where honey's antimicrobial compounds exhibit stable efficacy across various concentrations, with peak effectiveness at 50%.

Turmeric:

At both 25% and 50% concentrations, turmeric showed an inhibition zone of 1.0 cm. This consistent result suggests that turmeric's active compounds, such as curcumin, exert a stable antibacterial effect at these levels.

Higher concentrations are not tested since the turmeric powder cannot properly incorporate into the agar medium within the Petri dish itself; therefore, it is likely that results obtained at higher concentrations may have inaccuracies.

Neem Oil:

A 100% concentration exhibited the largest inhibition zone of 1.1 cm, indicating that neem oil is most effective in its pure form.

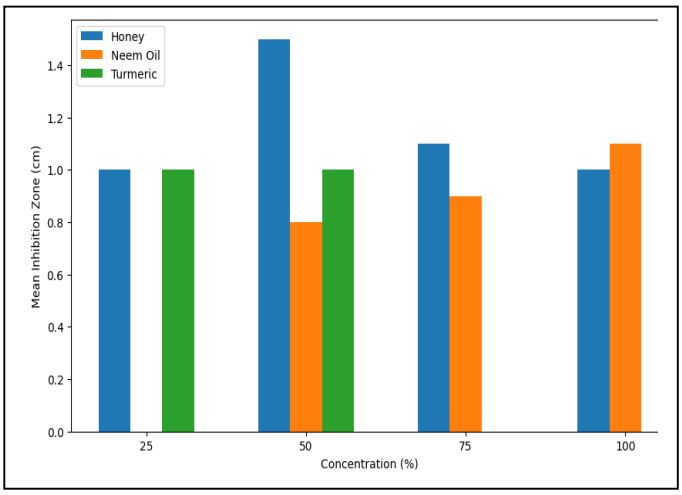
Concentrations of 75% and 50% showed moderate inhibition zones of 0.9 cm and 0.8 cm, respectively, reflecting reduced antibacterial activity at these lower levels.

A 25% concentration showed no inhibition zone, indicating that neem oil requires higher concentrations for effectiveness.

These findings suggest that neem oil's antibacterial efficacy is concentration-dependent, achieving significant activity only at higher concentrations.

C. Graphical Representation:

GRAPH II. BAR GRAPH REPRESENTING ANTIBACTERIAL INHIBITION ZONES FOR HONEY, TURMERIC, AND NEEM OIL.



The highest inhibition zone for honey was observed at 50% concentration, measuring 1.5 cm. This peak suggests that at this moderate concentration, honey creates an optimized antimicrobial environment, likely due to a combination of hydrogen peroxide release, low pH, high osmolarity, and bioactive compounds like flavonoids. Honey demonstrated consistent activity across concentrations, consistent with previous findings that highlight its broad-spectrum antibacterial properties and wound-healing capabilities. These qualities make honey a suitable adjunct or alternative therapy in clinical wound management, especially against antibiotic-resistant strains of *S. aureus*.

Turmeric showed stable antibacterial activity across the tested concentrations, with inhibition zones of 1.0 cm at both 25% and 50%. This stability suggests that its active compound, curcumin, remains effective within this concentration range, disrupting bacterial cell membranes and inhibiting biofilm formation. Turmeric's reliable antibacterial effect highlights its potential for inclusion in topical formulations, offering an antimicrobial action that supports wound healing and reduces inflammation.

Neem oil demonstrated concentration-dependent antibacterial activity, with the highest inhibition zone of 1.1 cm at 100% concentration. Lower concentrations of 75% and 50% yielded inhibition zones of 0.9 cm and 0.8 cm, respectively. The absence of inhibitory effects at 25% concentration suggests neem oil's efficacy is reliant on higher concentrations. Neem's active constituents, such as azadirachtin and nimbidin, disrupt bacterial membranes and inhibit biofilm formation, making undiluted neem oil particularly effective. These findings suggest that neem oil could be a potent antimicrobial agent for wound management when applied at high concentrations, although further research is necessary to optimize its clinical dosages.

IV. CONCLUSION

The results obtained in this research indicated that honey, turmeric, and neem oil were indeed effective against *Staphylococcus aureus*, each with characteristic antimicrobial activity with regard to concentration. Maximum inhibition for honey was at 50%, while turmeric provided consistent action, and neem oil showed dose-dependent efficacy, with considerable inhibition at full strength. These findings underpin the potential of these natural agents as alternatives to conventional therapies for wound infections, particularly in the era of antibiotic resistance. Further studies should be conducted to explore combination therapies and investigate clinical effectiveness in complex real-world environments, focusing on optimizing their use for infection prevention, tissue repair, and the potential to minimize reliance on synthetic antibiotics.

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