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Impact of Silver Nanoparticles on Human Skin Normal Flora

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KEYWORDS

Silver nanoparticles, AgNPs, Skin flora, Disc diffusion, Zone of inhibition, Antibacterial activity

ABSTRACT

Silver nanoparticles (AgNPs), ranging from 1–100 nm, are widely used for their strong antimicrobial properties in medical and healthcare applications. Human skin hosts normal flora such as Staphylococcus aureus, Staphylococcus epidermidis, and Escherichia coli, which protect against pathogens. AgNPs act by disrupting cell membranes, generating reactive oxygen species, and damaging proteins and DNA, leading to microbial death. This study evaluated their effect on normal skin flora using the disc diffusion method. Skin swabs (2×2 cm) from individuals aged 20–23 were collected and cultured on Nutrient Agar, MacConkey Agar, and Rose Bengal Agar. Isolated organisms included S. aureus, S. epidermidis, E. coli, and fungi. AgNP discs with concentrations from 0.1 to 0.7 were applied. Zones of inhibition were measured after incubation. Results showed a concentration-dependent antimicrobial effect. Higher concentrations produced larger inhibition zones. S. aureus showed maximum sensitivity, followed by E. coli and S. epidermidis. The study confirms strong antibacterial activity of AgNPs. However, it also indicates possible disruption of beneficial skin flora. Thus, careful use of AgNPs is recommended in healthcare products.

I. INTRODUCTION

Human skin hosts a diverse group of microorganisms known as normal flora, including bacteria and fungi. Common bacteria include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*. These organisms are usually harmless but can become pathogenic under certain conditions.

Silver nanoparticles (Ag NPs) are widely studied for their strong antimicrobial properties. They act by damaging microbial cell membranes, generating reactive oxygen species, and interfering with DNA replication.

Biological way of silver nano particles synthesis is cost effective and eco-friendly. Also biosynthesis is very simplest way to produce nano particles.

This study aims to evaluate the effect of silver nanoparticles on normal skin flora using the disc diffusion method and to analyze their effectiveness at different concentrations.

Silver nanoparticles (AgNPs) have gained significant attention in recent years due to their unique physicochemical properties and potent antimicrobial activity. With sizes ranging between 1–100 nm, these nanoparticles possess a high surface area-to-volume ratio, which enhances their interaction with microbial cells. As a result, AgNPs are extensively incorporated into a wide range of products, including wound dressings, coatings, cosmetics, textiles, and personal care items. Their effectiveness against a broad spectrum of microorganisms has made them an important tool in preventing infections and improving hygiene standards.

Human skin is a complex ecosystem inhabited by diverse microorganisms collectively known as normal skin flora. These include bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Escherichia coli*, along with various fungal species. This microbial community plays a crucial role in maintaining skin health by preventing colonization of pathogenic organisms, modulating immune responses, and contributing to the skin barrier function. Any disturbance in this balance may lead to skin disorders, infections, or reduced immunity.

Silver nanoparticles exert their antimicrobial effects through multiple mechanisms, including disruption of cell membranes, generation of reactive oxygen species (ROS), and interference with cellular proteins and genetic material. While these properties are beneficial for eliminating harmful pathogens, there is growing concern that excessive or uncontrolled use of AgNPs may also harm beneficial microorganisms present on the skin. Such alterations in the natural microbiota may lead to microbial imbalance, known as dysbiosis, which can have adverse effects on skin health.

Therefore, it is essential to evaluate the impact of silver nanoparticles on normal skin flora. Understanding their effects will help in balancing their antimicrobial benefits with potential risks, ensuring their safe and effective use in medical and consumer products. This study aims to assess the antimicrobial activity of AgNPs against human skin flora using standard microbiological techniques, thereby providing insights into their influence on the natural microbial ecosystem of the skin.

II LITERATURE REVIEW

Table: Impact of Silver Nanoparticles on Human Skin Normal Flora

Author/ Year	Study Focus	Methodology	Key Findings	Limitations
Rai et al., 2009	Antimicrobial properties of AgNPs	Review of experimental studies	AgNPs show broad-spectrum activity against bacteria, fungi, and viruses	Limited focus on skin microbiota
Morones et al., 2005	Mechanism of action of AgNPs	Electron microscopy & bacterial studies	AgNPs damage cell membranes, penetrate cells, and disrupt DNA and proteins	In vitro study only
Kim et al., 2007	Antibacterial activity of AgNPs	Disc diffusion & MIC methods	Strong activity against *Staphylococcus aureus* and *E. coli*; concentration-dependent effect	Limited organism diversity
Franci et al., 2015	Review on AgNP applications	Literature analysis	Smaller-sized AgNPs show higher antimicrobial activity due to larger surface area	Generalized review
Choi et al., 2018	Cytotoxicity of AgNPs	Cell culture studies	AgNPs may cause oxidative stress, inflammation, and DNA damage in human cells	Focus on cells, not skin flora
Brandwein et al., 2023	Effect on human skin microbiome	Skin microbiome analysis (short-term)	Minimal change in microbial diversity but temporary shifts in composition	Short-term study

Author/ Year	Study Focus	Methodology	Key Findings	Limitations
		exposure)	were observed	
Dakal et al., 2016	Mechanism and toxicity of AgNPs	Review study	ROS generation and protein/DNA damage are key antimicrobial mechanisms	Lack of clinical data
Present Study	Impact on normal skin flora	Disc diffusion method using AgNPs (0.1–0.7)	Concentration-dependent inhibition; *S. aureus* is most sensitive, followed by *E. coli* and *S. epidermidis*	Small sample size, limited age group

Rai et al. (2009) studied the antimicrobial properties of silver nanoparticles (AgNPs), focusing on their broad-spectrum activity. The study was based on a review of experimental research. The key findings showed that AgNPs are effective against bacteria, fungi, and viruses. However, a limitation of this study is that it did not specifically address their impact on human skin microbiota.

Morones et al. (2005) investigated the mechanism of action of AgNPs using electron microscopy and bacterial studies. Their findings revealed that AgNPs damage cell membranes, penetrate microbial cells, and disrupt DNA **and proteins,** leading to cell death. The limitation of this study is that it was conducted only under in vitro conditions.

Kim et al. (2007) focused on the antibacterial activity of AgNPs using disc diffusion and minimum inhibitory concentration (MIC) methods. The results showed strong antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, with a concentration-dependent effect. However, the study was limited by the use of a small number of microbial species.

Franci et al. (2015) conducted a review on the applications of AgNPs and their antimicrobial efficiency. The methodology involved analysis of multiple studies. The findings indicated that smaller nanoparticles exhibit higher antimicrobial activity due to their larger surface area. The limitation is that it was a generalized review without experimental validation.

Choi et al. (2018) examined the cytotoxic effects of AgNPs using cell culture studies. The study found that AgNPs can induce oxidative stress, inflammation, and DNA damage in human cells. However, the limitation is that the study focused on human cells rather than normal skin flora.

Brandwein et al. (2023) studied the effect of AgNPs on the human skin microbiome using microbiome analysis under short-term exposure conditions. The findings showed minimal changes in overall microbial diversity, although temporary shifts in microbial composition were observed. The limitation of this study is its short duration, which does not reflect long-term effects.

Dakal et al. (2016) reviewed the mechanisms and toxicity of AgNPs through literature analysis. The study concluded that AgNPs act mainly through reactive oxygen species (ROS) generation and damage to proteins and DNA. The limitation is the lack of clinical or in vivo data.

The present study focuses on the impact of AgNPs on normal skin flora using the disc diffusion method with concentrations ranging from 0.1 to 0.7. The results demonstrated a concentration-dependent antimicrobial effect, with *Staphylococcus aureus* showing the highest sensitivity, followed by *Escherichia coli* and *Staphylococcus epidermidis*. The limitation of this study includes a small sample size and a restricted age group of participants.

III. MATERIALS REQUIRED

Sterile cotton swabs, Nutrient Agar plates, MacConkey Agar plates, Rose Bengal Agar plates, Silver nanoparticle solution, Sterile distilled water, Micropipettes, Laminar air flow, Sterile filter paper discs, Incubator (37°C), Petri plates, Forceps, Gram staining kit, Microscope.

IV. METHODS

Sample Collection

A 2×2 cm skin area was swabbed using sterile swabs from individuals aged 20–23 years.

Culture

Swabs were inoculated on Nutrient Agar (general bacteria), MacConkey Agar (Gram-negative bacteria), and Rose Bengal Agar (fungi). The Plates were incubated at 37°C for 24–48 hours.

Identification- Gram staining was performed.

Organisms identified: *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*

PREPARATION OF SILVER NANOPARTICLE DILUTIONS

Stock: Silver nanoparticles in water

Prepared 7 concentrations: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7

preparation:

- 0.1 = 1 ml stock + 9 ml distilled water
- 0.2 = 2 ml stock + 8 ml water
- Continue similarly up to 0.7

PROCEDURE (DISC DIFFUSION METHOD)

1. Prepare agar plates with cultured bacteria.
2. Spread the inoculum evenly using a sterile swab.
3. Place sterile discs on the agar surface.
4. Add different concentrations of silver nanoparticles to each disc.
5. Incubate at 37°C for 24 hours.
6. Measure the **zone of inhibition (mm)** around each disc.

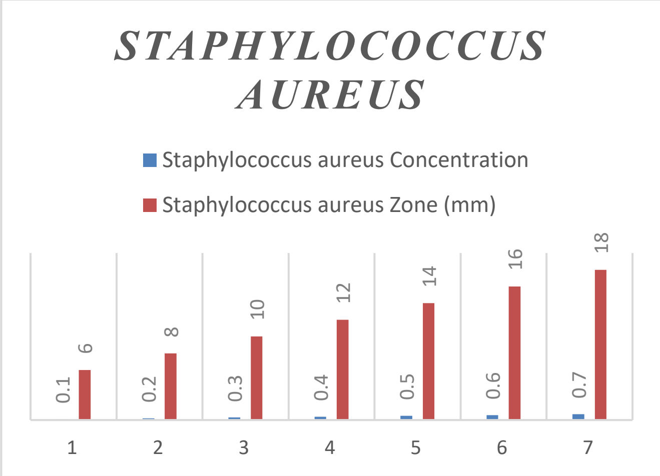
V Results

OBSERVATION TABLE (ZONE OF INHIBITION in mm)

Staphylococcus aureus

Concentration	Zone (mm)
0.1	6
0.2	8
0.3	10
0.4	12
0.5	14
0.6	16
0.7	18

Table: 1 Zone of Inhibition In mm Observation table of staphylococcus aureus.

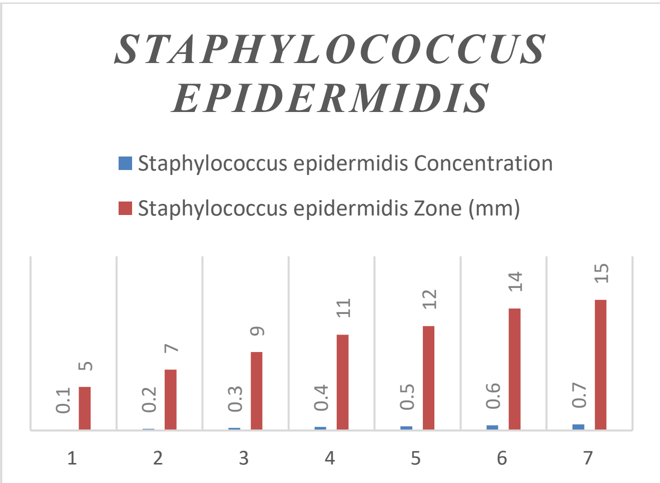


Graph :1 staphylococcus aureus concentration and zone.

Staphylococcus epidermidis

Concentration	Zone (mm)
0.1	5
0.2	7
0.3	9
0.4	11
0.5	12
0.6	14
0.7	15

Table: 2 Zone of Inhibition In mm Observation table of *Staphylococcus epidermidis*.

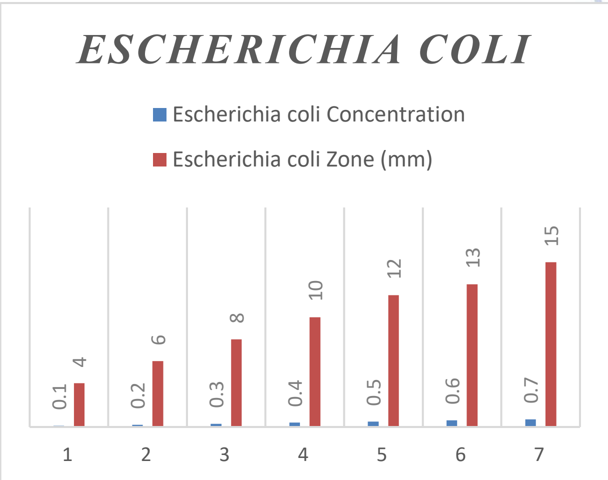


Graph:2 staphylococcus epidermidis concentration and zone.

Escherichia coli

Concentration	Zone (mm)
0.1	4
0.2	6
0.3	8
0.4	10
0.5	12
0.6	13
0.7	15

Table: 3 Zone of Inhibition In mm Observation table of Escherichia coli.



Graph:3 Escherichia coli concentration and zone.

Fig: 1 Sample collection with sterile swab.

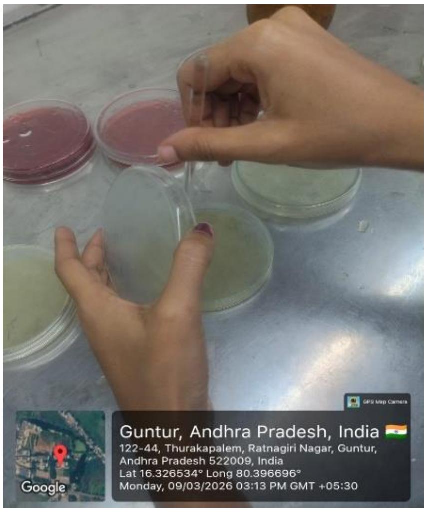


Fig 2 spread plate.

Fig:3 Zone of Inhibition (mm)

Silver nanoparticles showed significant antibacterial activity against all tested organisms. The zone of inhibition increased with increasing concentration. *Staphylococcus aureus* showed the highest sensitivity, followed by *Escherichia coli* and *Staphylococcus epidermidis*.

VI DISCUSSION

The study demonstrates that silver nanoparticles are effective antimicrobial agents. Their activity increases with concentration due to enhanced interaction with microbial cells. Gram-positive bacteria like *S. aureus* showed higher sensitivity compared to Gram-negative *E. coli*, possibly due to differences in cell wall structure. The presence of inhibition zones confirms that AgNPs disrupt microbial growth. These findings are consistent with previous studies showing strong antibacterial effects of nanoparticles.

VII LIMITATIONS

◎ The study was conducted on a **small sample size**, which may not represent the entire population.

◎ Participants were limited to a **specific age group (20–23 years)**, which reduced the generalizability of the results to other age groups.

◎ Only a **few microbial species** (*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*) were analyzed, whereas normal skin flora is highly diverse.

◎ The study used only the **disc diffusion method**, which provides qualitative or semi-quantitative results and may not accurately reflect minimum inhibitory concentrations (MIC).

◎ The research was performed under **in vitro conditions**, which may not fully replicate the natural skin environment.

◎ **Short-term exposure** to silver nanoparticles was studied; long-term effects on skin microbiota were not evaluated.

◎ The study did not assess the **impact on fungal diversity in detail**, despite isolating fungal species.

◎ Variability in **skin conditions (e.g., moisture, pH, hygiene practices)** among individuals was not controlled.

◎ The study did not evaluate the **cytotoxic effects of AgNPs on human skin cells**.

◎ Only limited concentrations of AgNPs (0.1–0.7) were tested, which may not cover the full range used in real-life applications.

VIII FUTURE WORK

Research can be extended to include **different age groups and skin types** to understand variability in response to silver nanoparticles.Advanced techniques such as **metagenomic or microbiome analysis** can be used to study the **complete diversity of skin flora** instead of limited species. Further studies should evaluate the **long-term effects of AgNP exposure** on normal skin microbiota and overall skin health. Quantitative methods such as **minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)** assays can be performed for more precise results.

IX CONCLUSION

Silver nanoparticles exhibit strong antimicrobial activity against normal skin flora. Their effectiveness increases with concentration. This suggests their potential use in medical applications such as wound healing, antibacterial creams, and infection control.

X . AKNOWNLEGMENT

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Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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