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NANO SILVER INFUSED ANTIFUNGAL UNDERGARMENT: DEVELOPMENT, CHARACTERIZATION, AND CLINICAL EVALUATION

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ABSTRACT: Background: Fungal infections, particularly dermatophytosis, affect nearly one-fourth of the global population, with *Tinea cruris* being prevalent in humid climates. Silver nanoparticles (AgNPs) exhibit strong antimicrobial particularly antifungal properties and may offer an effective textile-based solution. This study aimed to develop, characterize, and clinically evaluate nanosilver-infused antifungal undergarments to combat recurrent *Tinea cruris*. **Methods:** A 95% cotton and 5% elastane fabric blend was treated with a 0.3% nanosilver suspension using the exhaust method. Characterization involved scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) to assess nanoparticle deposition. Wash durability was evaluated over 30 cycles. Antimicrobial activity was assessed using AATCC 100:2019 for antibacterial efficacy against *Staphylococcus aureus* and *Klebsiella pneumoniae* and AATCC Part 3:2017 for antifungal activity against *Candida albicans*. A prospective, real-world evidence, experimental clinical study was conducted on 90 patients with recurrent *tinea cruris* who wore nanosilver-infused innerwear for twelve weeks alongside standard antifungal treatment. Clinical outcomes were assessed at four and twelve weeks. **Results:** SEM-EDX confirmed the successful deposition of AgNPs, while XRD analysis indicated crystalline stability. Wash durability testing demonstrated consistent nanoparticle retention. Antimicrobial assays showed significant bacterial and fungal growth inhibition. Clinically, patients exhibited improved symptom resolution and reduced recurrence rates. **Conclusion:** Nanosilver-infused fabric exhibits strong antifungal properties, offering a promising adjunct to conventional antifungal therapy for managing recurrent *Tinea cruris*. Their durability through multiple washes and patient benefits highlights broader dermatological applications. This approach may enhance treatment efficacy and reduce reinfection rates. Further large-scale studies are needed.

INTRODUCTION: Fungal infections affect nearly one-fourth of the global population, with dermatophytosis being among the most common superficial mycoses¹. Despite the widespread use of antifungal agents, rising resistance to terbinafine and azoles has led to frequent therapeutic failures, imposing a significant public health burden²⁻⁵. *Tinea cruris* ("jock itch"), a dermatophytosis affecting the groin, is particularly prevalent in

humid climates where heat and moisture facilitate fungal growth. It is primarily caused by *Trichophyton rubrum*, and its management is complicated by recurrent infections due to poor treatment adherence, fungal biofilm formation, and the persistence of fungal elements in clothing^{6,7}.

Alarming, recent studies indicate that fungal spores can remain in undergarments even after laundering, leading to reinfection cycles⁸. Given these challenges, antimicrobial fabrics are becoming a more desirable goal for textile makers due to growing interest in personal health and hygiene⁹. Nanotechnology-based interventions, particularly silver nanoparticle (AgNP)-infused textiles, offer a promising alternative. AgNPs

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exhibit broad-spectrum antimicrobial properties, disrupting microbial cell walls, inhibiting enzymatic activity, and generating reactive oxygen species (ROS), which collectively prevent fungal proliferation^{10,11}. AgNP-treated cotton fabrics have been shown to reduce bacterial loads by over 99%, demonstrating strong antimicrobial efficacy against pathogens like *Staphylococcus aureus* and *Escherichia coli*^{9,12}. Furthermore, the breathable and moisture-wicking properties of AgNP-infused textiles create an environment unfavorable for fungal growth, making them ideal for antifungal applications¹³. In this background, our study aims to develop and characterize nanosilver-infused antifungal fabric and design functional antifungal undergarments. By incorporating nanotechnology into textile-based interventions, this approach offers a proactive, adjunctive solution to standard antifungal therapies, addressing the limitations of conventional treatments. If successful, this strategy could significantly improve clinical outcomes and reduce recurrence rates in patients with drug-resistant *Tinea cruris*.

MATERIAL & METHODS:

Fabric Preparation: A fabric blend of 95% cotton and 5% elastane was selected for the study. Prior to nanosilver application, the fabric underwent a pre-treatment process, including thorough washing to remove impurities and neutralization to pH 5–6 using acetic acid. The fabric was then dried at 100–120°C to ensure optimal readiness for nanosilver deposition.

Nanosilver Solution Preparation: Silver nanoparticles (AgNPs) in powder form (size: 100–200 nm) were procured for the study. AgNPs are not soluble in water, a stable suspension was prepared using acetic acid as a dispersing agent. To achieve a stable dispersion, silver nanoparticles were gradually added to deionized water under constant stirring. This process helped break up aggregates and ensured uniform dispersion. A final nanosilver concentration of 0.3% per kg of fabric was maintained, resulting in a consistent particle size distribution (100–200 nm) that remained stable without visible precipitation or color change.

Application of Nanosilver onto Fabric: The nanosilver coating was applied using the exhaust method, where the fabric was immersed in a

solution of nanosilver (diluted in warm water at 35–45°C) and processed at 40–50°C for 30 minutes with a liquor ratio of 1:6, and leave the garment in the solution for 10 minutes. 70% of the moisture is initially removed and dried at 100–120°C to fix the nanosilver particles onto the fibers.

Characterization of Nanosilver-Coated Fabric:

Scanning Electron Microscopy (SEM) & Energy Dispersive X-ray Spectroscopy (EDX): For SEM analysis, fabric samples (~1 cm²) were mounted on aluminum stubs with carbon tape for proper adhesion. Nanosilver deposition was evaluated using the Phenom Pro G6 Desktop SEM (Thermo Fisher Scientific) at 5 kV. Secondary electron (SE) imaging assessed surface morphology, while backscattered electron (BSE) imaging provided compositional contrast. Nanoparticle distribution, size, and shape were analyzed, and elemental composition was confirmed through an integrated EDX detector.

X-ray Diffraction (XRD) Analysis: XRD characterization was performed using an XPERT-3 diffractometer with a PW3050/60 (Theta/Theta) goniometer. The analysis was conducted in continuous mode over a 2 θ range of 10.015° to 79.975° with a step size of 0.030° and a scan time of 0.8 seconds per step. A Cu anode (K α 1 = 1.54060 Å, K α 2 = 1.54443 Å) was used, operating at 30 mA and 40 kV. This analysis quantified the crystal structure of nanosilver deposited on the fabric fibers.

Wash Durability Testing: To evaluate the durability of the nanosilver coating, the fabric underwent 30 standardized wash cycles. Each cycle consisted of washing nanosilver-treated fabric swatches in a detergent solution at 45°C for 40 minutes using a domestic washing machine, followed by rinsing with distilled water and air drying. This process was repeated for each wash cycle. SEM, EDX, and XRD analyses were performed after 0, 10, 20, and 30 washes to assess nanoparticle retention. The samples were labelled as follows:

Sl. no.	Sample Label	Particulars
1	S1	Without wash Sample
2	S2	Sample after 10 washes
3	S3	Sample after 20 washes
4	S4	Sample after 30 washes

Microbiological Assays:

Antibacterial Activity (AATCC 100: 2019): The antibacterial activity of the nanosilver-coated fabric samples (S1, S2, S3, and S4) was assessed using the AATCC 100: 2019 test method against *Staphylococcus aureus* (ATCC 6538, Gram-positive bacteria) and *Klebsiella pneumoniae* (ATCC 4352, Gram-negative bacteria). A total of 48 discs were cut from the larger fabric samples, with 1 g of each sample used per 100 ml flask. The fabric samples were exposed to bacterial cultures for a contact time of 24 hours, and a control sample was maintained at zero contact time. The neutralizing agent used in this study was Dey-Engley neutralizing broth, ensuring accurate assessment of bacterial viability post-treatment.

Antifungal Assay (AATCC Part 3: 2017): It was performed following the AATCC Part 3-2017 test method using *Candida albicans* (ATCC 90028) as the test inoculum. Fabric swatches measuring 3.8 cm were cut from the larger submitted samples and incubated in a humidity chamber (>90% RH) at 28°C for six days. The fungal growth on the specimen was rated on a scale from 0 to 4, where 0 indicated no growth, 1 represented trace growth (<10%), 2 indicated light growth (10–30%), 3 denoted medium growth (30–60%) and 4 represented heavy growth (60% to complete coverage). The evaluation criteria were based on the percentage of *Aspergillus niger* growth observed on the textile material, providing a quantitative measure of its antifungal resistance.

Clinical Evaluation:

Study Design and Patient Recruitment: A prospective, experimental, real-world clinical evidence study was conducted at the dermatology outpatient department of Cutis Hospital, Bengaluru, to assess the antifungal efficacy of S4 material. The trial was conducted over a twelve-week period and institutional ethics committee approval was taken before the initiation of the study. Inclusion criteria comprised adult patients (>18 years of age) of any gender diagnosed with recurrent or persistent *tinea cruris* who were willing to participate in the study. Exclusion criteria included patients with a history of allergy or sensitivity to silver-based textiles, those with active secondary bacterial infections, newly diagnosed cases responding adequately to standard treatment (lesions showing complete

clinical remission), and individuals with comorbid conditions such as diabetes mellitus, tuberculosis, HIV, or solid organ transplants. Additionally, patients on long-term systemic or topical corticosteroids, other immunosuppressive therapies, or those with congenital or acquired immunosuppression were excluded from the study.

Intervention and Follow-up: 90 patients were recruited and given a pair of innerwear garment made from nanosilver-infused fabric (S4 sample) and were instructed to wear it daily for twelve weeks alongside prescribed topical and oral antifungal medications. They were monitored regularly, with a physician evaluation at four weeks to assess lesion resolution and symptom improvement. A follow-up telephonic interview was conducted at twelve weeks to evaluate long-term efficacy, patient satisfaction, and compliance using a structured questionnaire **Fig. 1**.

Nano-silver infused anti-fungal undergarments Fungal Infection Treatment Follow-Up Survey

Personal Information

1. Age:

☐ Below 18 ☐ 18-25 ☐ 26-35 ☐ 36-45 ☐ 46-55 ☐ Above 55

2. Gender:

☐ Male ☐ Female ☐ Other

3. Date of First Treatment: (MM/DD/YYYY)

4. Duration of the Disease Before Treatment:

☐ Less than 1 month ☐ 1-3 months ☐ 3-6 months ☐ More than 6 months

Treatment Follow-Up

5. After 6 weeks of treatment, have you taken your follow-up consultation?

☐ Yes ☐ No

6. Was the infection in multiple areas or a single spot?

☐ Multiple areas ☐ Single spot

7. If single spot, specify the body part:

☐ Legs ☐ Thighs ☐ Groin ☐ Buttocks ☐ Hands ☐ Axilla ☐ Neck ☐ Cheeks ☐ Chin ☐ Forehead ☐ Foot ☐ Fingers ☐ Other (Specify) _____

Effectiveness of Treatment

9. Is the fungal infection cured?

☐ Yes ☐ No

10. If yes, specify the progress in percentage:

☐ 0-20% ☐ 21-40% ☐ 41-60% ☐ 61-80% ☐ 81-100%

11. Rate the level of cure on a scale of 1-10 (1 = not cured, 10 = completely cured):
(Scale: 1 2 3 4 5 6 7 8 9 10)

12. Have you noticed a decrease in infection after using Nano silver-infused anti-fungal undergarments?

☐ Yes ☐ No

13. Have you regularly used Nano silver-infused anti-fungal undergarments during the treatment?

☐ Yes ☐ No

14. Are you still using Nano silver-infused anti-fungal undergarments?

☐ Yes ☐ No

Product Experience & Feedback

15. How is the sweat absorption capacity of Nano silver-infused anti-fungal undergarments?

☐ Good ☐ Very good ☐ bad ☐ very bad

16. How is the breathability of Nano silver-infused anti-fungal undergarments?

☐ Good ☐ Very good ☐ bad ☐ very bad

17. Rate the overall comfort of Nano silver-infused anti-fungal undergarments on a scale of 1-10 (1 = very low, 10 = very high):
(Scale: 1 2 3 4 5 6 7 8 9 10)

18. How do you feel about Nano silver-infused anti-fungal undergarments overall? (1 = very low satisfaction, 10 = very high satisfaction)
(Scale: 1 2 3 4 5 6 7 8 9 10)

FIG. 1: TREATMENT FOLLOW-UP SURVEY QUESTIONNAIRES

RESULTS:

Characterization of Nanosilver-Coated Fabric-SEM-EDX & XRD: SEM analysis revealed that Ag nanoparticles were not uniformly distributed on

the fabric surface across all samples (S1–S4), with particle size ranging from 80–200 nm. S1 and S2 exhibited smaller nanoparticles (80–150 nm) with minimal agglomeration and smooth coating. S3 showed slightly larger particles (120–180 nm) with some clustering, while S4 had the largest particles (150–200 nm) and higher agglomeration. EDX confirmed the presence of Ag nanoparticles, with

silver composition ranging from 9 to 77% by weight. S3 and S4 displayed higher Ag content (~77% and ~67%, respectively), indicating decreased coating density over the washes. Oxygen and carbon peaks were also detected, consistent with the fabric substrate. Increased oxygen levels in S1 and S2 suggested possible oxidation Fig. 2&3.

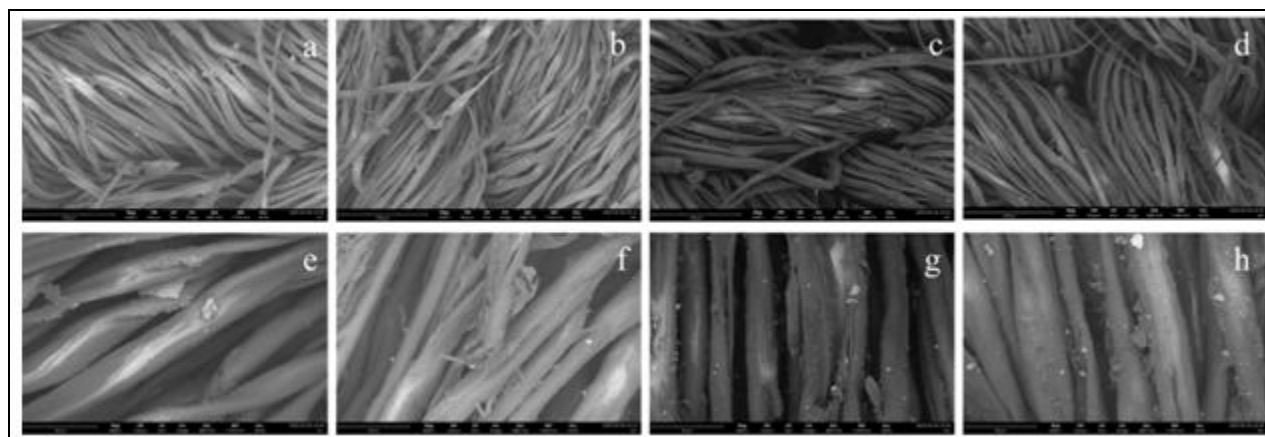


FIG. 2: SEM IMAGES OF COTTON FABRIC COATED WITH SILVER NANOPARTICLE AT MAGNIFICATION 1000X A) S1 B) S2 C) S3 D) S4 AND 4000X OF SAMPLES E) S1 F) S2 G) S3 H) S4

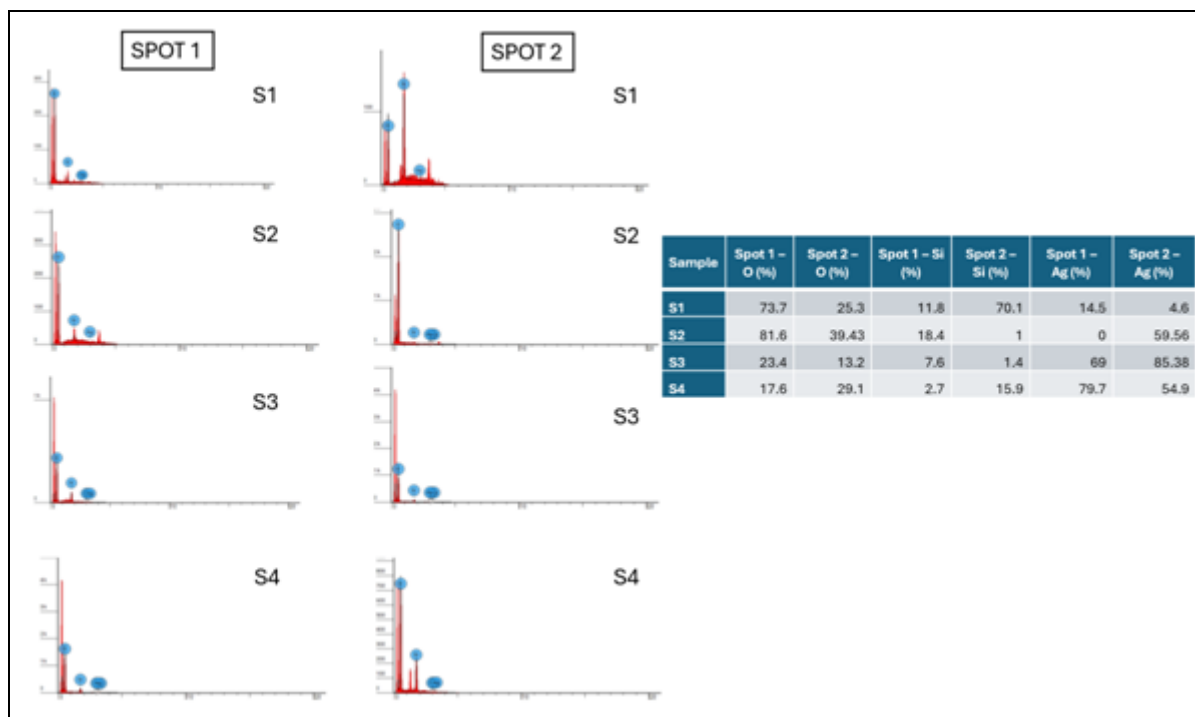


FIG. 3: EDX ELEMENTAL DISTRIBUTION OF ALL SAMPLES (S1, S2, S3, S4)

XRD analysis confirmed the crystalline nature of Ag nanoparticles in all samples, showing distinct peaks at $2\theta = 38.1^\circ$, 44.2° , 64.5° , and 77.5° corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (FCC) silver. S2 and S4 showed sharper and more intense peaks,

indicating better crystalline formation and larger particle size. Broader peaks in S3 suggested smaller crystal size or partial amorphous nature. Overall, the consistent diffraction pattern across samples confirmed the structural integrity of the Ag nanoparticles Fig. 4.

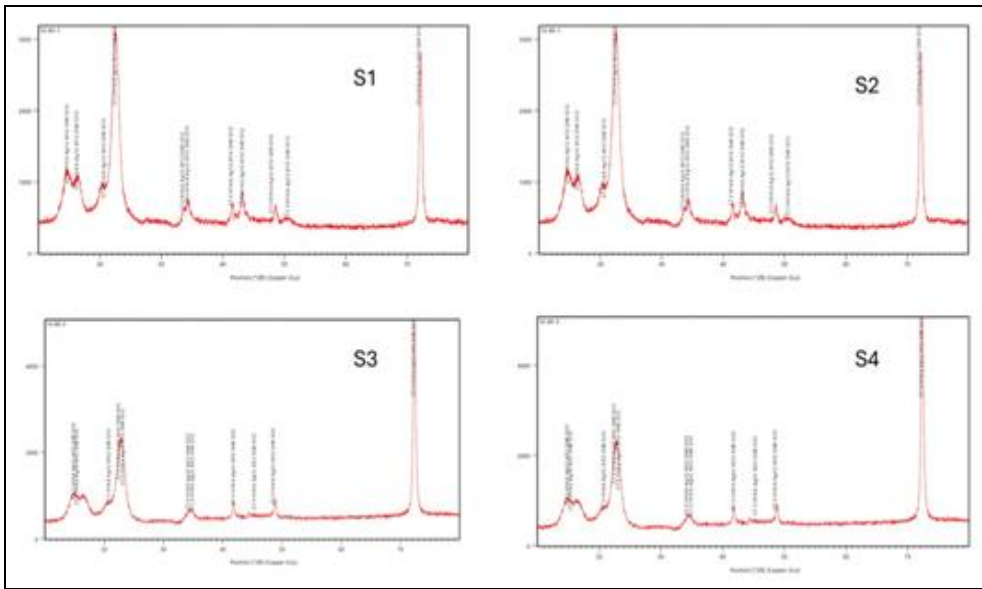


FIG. 4: DIFFRACTION PATTERN OF SAMPLES (S1, S2, S3, S4)

Antifungal and Antibacterial Activity: The antifungal and antimicrobial activity of the treated fabrics was evaluated against *C. albicans*, *K. pneumoniae*, and *S. aureus*. The control sample showed heavy fungal growth (60% to complete coverage), indicating no antifungal effect. The untreated sample (UN) exhibited medium fungal growth (30% to 60%) with moderate antimicrobial activity of 76.98% and 76.63% against *K. pneumoniae* and *S. aureus*, respectively. In contrast, treated samples (S1–S4) demonstrated

complete inhibition of *C. albicans* (no growth) and significantly higher antimicrobial activity. S1 showed the highest antimicrobial efficacy (99.75% against *K. pneumoniae* and 99.81% against *S. aureus*), followed by S2 (99.69%, 99.72%), S3 (99.02%, 99.13%), and S4 (96.63%, 97.5%). These results confirm the potent antifungal and antimicrobial properties of the treated fabrics, highlighting their effectiveness in preventing fungal and bacterial growth **Table 1**.

TABLE 1: ANTIFUNGAL AND ANTIMICROBIAL ASSAY

Treatment	Antifungal	Antimicrobial (%)	
	<i>Candida albicans</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>
Control	Heavy Growth (60% to complete coverage)		
UN	Medium Growth (30 to 60%)	76.98	76.63
S1	No growth	99.75	99.81
S2	No growth	99.69	99.72
S3	No growth	99.02	99.13
S4	No growth	96.63	97.5

Control- Whatmann filter paper viability; UN- Nano untreated fabric.

Clinical Evaluation: The clinical evaluation of the S4 sample nanoAg undergarment involved 90 participants (69 males and 21 females) across various age groups, with the majority being between 26–35 years. Most participants had a disease duration exceeding six months (37 males and 13 females), indicating chronic or recurrent tinea infections. Among participants, multiple infection sites were more common (43 males and 17 females) than single-site involvement, with the groin being the most frequently affected area **Table 2**. In terms of treatment outcomes, 29 males and 16

females reported complete cure of the fungal infection. Regular use was reported by 34 males and 15 females, and a notable proportion (29 males and 15 females) continued using the undergarment after the study. Regarding user experience, most participants rated sweat absorption and breathability as “good” or “very good,” with only a few reporting negative feedback. Comfort and product feel were highly rated, with the majority scoring between 8–10 **Fig. 5 & 6**. These results indicate that the S4 demonstrated both clinical efficacy and high user satisfaction, contributing to

improved outcomes in patients with chronic or recurrent tinea infections **Fig. 7 & 8**. Patients reported no adverse effects in the clinical evaluation, such as skin irritation or allergic reactions.

TABLE 2: DETAILS OF CLINICAL EVALUATION & FOLLOW-UP

	Male(n)	Female (n)
Gender	69	21
Age		
18-25	15	9
26 to 35	20	8
36 to 45	15	3
46 to 55	10	1
Above 55	9	0
Disease Duration		
Less than 1 month	2	0
1 to 3 months	22	4
4 to 6 months	8	4
More than 6 months	37	13
Call answered		
Yes	36	16
No	33	5
Follow-up		
Yes	19	1
No	50	20
Spot		
Multiple	43	17
Single	26	4
Body part- Single spot		
legs	2	1
thighs	2	0
groin	17	2
buttocks	4	1
cheeks	1	0
Cured		
Yes	29	16
No	7	0
Regular use		
Yes	34	15
No	2	1
Still using		
Yes	29	15
No	7	1

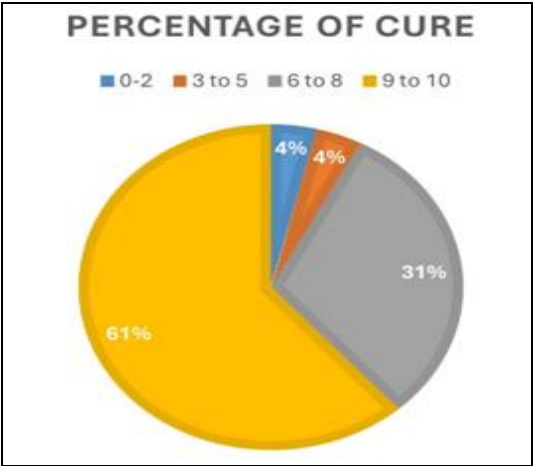


FIG. 5: PERCENTAGE OF CURE ON THE SCALE 0- 10 (0= NOT CURED, 10= COMPLETELY CURED)

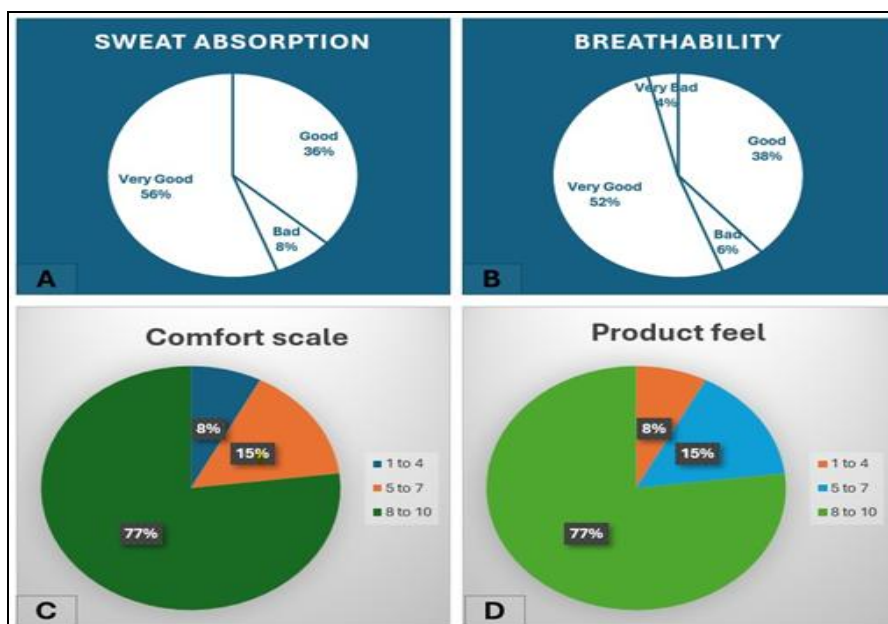


FIG. 6: PRODUCT EXPERIENCE AND FEEDBACK OF PATIENTS USED NANO-SILVER INFUSED UNDERGARMENT

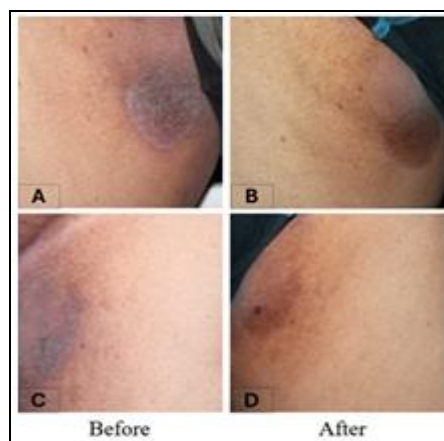


FIG. 7: *TINEA CRURIS*. A & C) BASELINE LESION ON THE GROIN AREA; B & D) RESOLUTION OF LESION AND NO RECURRENCE OBSERVED AFTER THE USE OF AGNP INFUSED ANTIFUNGAL UNDERGARMENT



FIG. 8: *TINEA CRURIS ET FACIEI*. A) BASELINE LESIONS ON THE FACE; B) RECURRENCE OF THE LESION AFTER THE MEDICAL TREATMENT; C & E) BASELINE LESION ON THE GROIN AREA; D&F) NO RECURRENCE OF LESION AFTER THE USE OF AGNP INFUSED ANTIFUNGAL UNDERGARMENT

DISCUSSION:

Nanoparticle Coating and Distribution: SEM analysis revealed non-uniform AgNP deposition, with aggregation affecting coating uniformity. Such variability may influence antimicrobial efficacy, as denser nanoparticle clusters could release silver ions inconsistently. XRD findings suggest that crystalline differences among samples impact AgNP dissolution and microbial inhibition.

The study utilized the exhaust method for nanosilver coating, but achieving a uniform, durable coating remains a challenge. Literature suggests that *in situ* deposition methods such as ultrasonic irradiation, sonochemical deposition, layer-by-layer assembly, and electrochemical deposition may improve nanoparticle adhesion and long-term antimicrobial performance^{14, 15}.

Antifungal and Antibacterial Activity: Our study demonstrates that AgNP-coated fabrics exhibit potent antifungal activity, supporting their potential application in functional textiles for managing dermatophytosis even after multiple laundering cycles. *C. albicans* was used instead of dermatophytes due to its faster growth, standardized antifungal testing protocols, and ability to form biofilms, which mimic fungal colonization on textiles. This *in-vitro* model allows for reliable antifungal assessment, while clinical evaluation in dermatophytosis patients provides real-world validation of the AgNP infused fabric efficacy. Notably, S4 sample demonstrated slightly lower antimicrobial activity (96.63%) compared to S1 (99.75%), suggesting that factors such as AgNP distribution, coating uniformity, and silver release kinetics may influence performance. Previous studies support the broad-spectrum antimicrobial action of AgNP against bacterial and fungal pathogens such as *Staphylococcus aureus* and *Escherichia coli*¹⁶. It functions by slow release of silver ion, disrupting microbial cellular functions by binding to thiol groups in proteins, impairing enzymatic activity and inhibiting replication^{17, 18}.

Clinical Implications and Real-World Assessment: From a clinical perspective, dermatophytosis is often complicated by bacterial co-infections and biofilm formation, which contribute to antimicrobial resistance and recurrent infections. Studies indicate that *Trichophyton*

rubrum can form biofilms with *S. aureus* and *S. epidermidis*, where bacterial colonization precedes fungal overgrowth, influencing infection persistence and therapeutic challenges.¹⁹ Treatment failure in dermatophytosis is further exacerbated by resistance mechanisms, including *ERG1* and *ERG11* gene mutations, heat shock proteins, efflux pumps, and biofilm-mediated drug resistance^{20, 21}.

Our real-world clinical evaluation revealed that patients using AgNP-coated undergarments reported improvements in comfort, reduced odor, and fewer instances of fungal recurrence. These findings align with *in-vitro* results, suggesting that AgNP-coated fabrics can serve as an adjunctive strategy for preventing and managing dermatophytosis. A recent real-world study by Du *et al.*²² on antifungal socks demonstrated significant *in vitro* antifungal activity and a 72.9% effectiveness rate in alleviating *Tinea pedis* symptoms, highlighting the potential of functional textiles in dermatological care. Our results similarly suggest that AgNP-infused fabrics could play a role in daily infection management by reducing microbial load and minimizing reinfection risks. However, further controlled clinical trials with larger sample sizes are necessary to validate long-term dermatological benefits.

Durability and Practical Applications: A key advantage of AgNP-coated textiles is their resistance to laundering, which determines their long-term effectiveness. Our study demonstrated that the antimicrobial efficacy of AgNP-coated fabrics remained intact even after 30 wash cycles, indicating strong nanoparticle adhesion to textile fibers. Qu *et al.*¹⁸ reported similar findings, where AgNPs incorporated into catechol formaldehyde resin microspheres exhibited high antimicrobial activity against *S. aureus*, *E. coli*, and *Candida albicans*, maintaining a 99.99% bacteriostatic and fungistatic rate even after 50 laundering cycles. This durability underscores the commercial potential of AgNP-coated textiles for applications in consumer and healthcare applications, including antifungal undergarments, facemasks, wound dressings, and hospital linens, where microbial control is critical^{15, 16}.

Challenges and Future Considerations: Despite the promising antimicrobial properties of AgNP-

coated fabrics, several challenges must be addressed. The potential cytotoxic effects of prolonged AgNP exposure on human skin cells require further investigation, particularly in intimate apparel. Long-term safety studies are essential to evaluate risks such as skin irritation, allergic reactions, and possible disruptions to the skin microbiome.

Environmental concerns also arise from AgNPs' slow degradation, which may contribute to ecological toxicity. Developing sustainable alternatives, such as biodegradable nanoparticles or controlled-release formulations, could mitigate these risks.

Another critical issue is the potential for microbial resistance development. While AgNPs exhibit broad-spectrum antimicrobial activity, prolonged use could drive adaptive resistance in bacterial and fungal strains, similar to antibiotic resistance mechanisms. Future research should assess whether AgNP textiles influence resistance patterns and compare their efficacy with conventional antifungal treatments.

Additionally, optimizing nanocoating techniques is crucial to balancing antimicrobial efficacy, biocompatibility, and cost-effectiveness. Comparative studies evaluating durability, user safety, and economic feasibility will help determine the large-scale applicability of AgNP-infused textiles in medical and consumer markets.

CONCLUSION: Our study provides experimental and real-world clinical evidence supporting the antifungal efficacy of AgNP-coated fabrics, marking an important advancement in functional textiles for dermatophytosis management. This is the first study to evaluate AgNP-coated undergarments in real-world clinical settings, demonstrating their potential as an adjunctive strategy in antifungal therapy. The durability of antimicrobial activity through multiple washes and the reported patient benefits highlight their promise for broader dermatological applications. However, concerns regarding cytotoxicity, environmental impact, and microbial resistance necessitate further research. With continued advancements in nanotechnology and material science, AgNP-coated textiles could become a valuable innovation in both

medical and consumer applications, offering an effective and practical approach to fungal infection prevention.

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