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Green Synthesized Nano-Silver Transparent Cosmeceutical Product and its Antimicrobial, Antioxidant Activity and In-vitro cytotoxicity studies against A-431 skin cancer cell line

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ABSTRACT
Silver Nanoparticles (AgNPs) are well received in the cosmeceutical industry due to their broad spectrum of pharmaceutical applications. Green-synthesized AgNPs from Turmeric Curcuma longa (TAgNPs) aqueous extract was integrated into formulated cosmetic product green nano silver transparent soap bar. The formation of TAgNPs was validated by the spectroscopic techniques, UV-Visible showed single broad peak at 420-430 nm; FT-IR peaks at 523,770,1022,1400,1565,3315 common for all reveals the stability of TAgNPs; X-Ray Diffraction analysis evaluates crystallite size of 29.85 nm of synthesised TAgNPs; SEM depicts the spherical shape and this high-end microscopy reveals the destruction of the bacterial cells of E. coli. TEM microscopy confirms the nanoparticle size 20-55 nm. and energy dispersive X-ray confirm the presence of Ag. Five bacterial strains namely Rhizobium Tropici, Xanthomonas, E. coli shigella Klebsiella, Pseudomonas Putida, Bacillus Tequilensis and two fungal strains Aspergillus Parasiticus, Aspergillus Niger concrete the antimicrobial activity of the transparent nano silver The IC_{50} for formulated Soap Bar was found to be 25.59 ± 1.03 mg/mL by DPPH Assay and the % cytotoxicity by MTT assay on Human Skin Carcinoma (A431) cell line was found to be 17.42 ± 2.15%, 42.82 ± 1.78%, 54.76 ±4.33% at 1000µg/mL for TAgNPs, Soap Base (SB), SB+TAgNPs provide scope for anticancer activity. Formulations of Transparent nano silver soap from biogenic silver nanoparticles may be considered as excellent green synthesised, sustainable pharmaceutical product for the treatment of pimples, acne, and other skin-related problems.

INTRODUCTION:

Nanotechnology has crop up as a transformative field, providing innovative solutions to longstanding challenges in vibrant sectors, involving medicine, agriculture, and environmental sciences. Among the numerous nanomaterials developed, silver nanoparticles (AgNPs) have elicited substantial scientific interest owing to their pronounced and broad-spectrum antimicrobial efficacy. The synthesis of AgNPs using environmentally friendly or "green" methods is particularly noteworthy as it conforms to the core tenets of sustainable development by minimizing the use of toxic chemicals and reducing hazards[1]. Conventional silver nanoparticle synthesis employs toxic reductants posing environmental and health hazards[2]. In contrast, green synthesis leverages natural reducing agents derived from plant extracts, microorganisms, or other biological systems,

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making the process safer, more cost-effective, and environmentally benign[3,4]. Phytogenic synthesis, in particular, has been widely reported due to their rich phytochemical content, which can effectively mediate (Ag+) to (Ag^0) transformation while also capping the nanoparticles to enhance their stability[5,6,7]. The characterization of AgNPs is a critical step in understanding their properties in potential applications. A range of sophisticated analytical methodologies is utilized to elucidate the morphological attributes of the nanoparticles, and their crystalline architecture. 'X-ray diffraction XRD, HR-Transmission electron microscopy HR-TEM, FE-SEM scanning electron microscopy are commonly used methods for characterizing the morphology and size distribution of AgNPs[8]. The optical traits of nanoparticles are discerned via UV-Vis spectroscopy, which are directly linked to their size and shape[9]. These characterizations are essential for optimizing the synthesis process and tailoring the nanoparticles for specific applications. Silver nanoparticles have demonstrated a broad spectrum of pharmacological activities, with their antimicrobial properties being the most extensively studied[10]. AgNPs demonstrate robust and large-spectrum antimicrobial efficacy against diverse pathogenic microorganisms, encompassing bacteria and fungi making them valuable in the development of antimicrobial agents[11,12,13]. The mechanism of action of AgNPs is multifaceted, involving the microbial cell membranes disruption, Elicitation of reactive oxygen intermediates, and interaction with intercellular targets such as DNA and proteins leading to cell death[14]. Beyond antimicrobial activity, AgNPs have also revealed encouraging outcomes in anticancer therapy, wound healing, and as anti-inflammatory agents, expanding their potential therapeutic applications[15]. The incorporation of silver nanoparticles into consumer products, such as antimicrobial soapfilm, represents a practical application of their properties. The formulation of a nano silver antimicrobial soapfilm combines the benefits of conventional hygiene products with the enhanced antimicrobial efficacy of TAgNPs[16]. The antioxidant potential was evaluated using DPPH scavenging assays, where the green-synthesized TAgNPs demonstrated exceptional performance compared to the standard antioxidant, ascorbic acid[17]. The thorough evaluation of their properties is necessary for optimizing their applications in various fields. The development of nano silver antimicrobial soap bar illustrates a practical and innovative application of TAgNPs, with the potential to significantly impact public health by providing enhanced protection against microbial infections. This work not only elucidate the importance of green chemistry in nanomaterial synthesis but also highlights the broad pharmacological potential of TAgNPs in the trend of green organic synthesis of nanocosmeceutical products.

EXPERIMENITAL

Material and methods

Chemical required for formulating Soap Bar and Turmeric Silver Nanoparticles TAgNPs

The study used pure silver nitrate from Merck, turmeric extract from crushed rhizomes, virgin coconut oil from local markets and sodium hydroxide, sorbitol, stearic acid, Isopropyl alcohol, glycerin from BRM chemicals, New Delhi and distilled water from the Chemistry laboratory at ITM University Gwalior for the preparation. Procurement of Plant specimens of dried turmeric procured from the Bada herbal area zone of Gwalior, during January 2023. Botanical identification was verified by a subject expert and a docket specimen sample (ITM/5/T/23) archived in the Chemistry department, ITM University.

Extraction of botanical bioactives and Turmeric Silver nanoparticles (TAgNPs)

Five grams of turmeric powder extract (T-ext), mixed with 100mL of distilled water and soaked for 24 hours. Solution was boiled for 30 min with continuous stirring and cooled down, subsequently passed through Whatman filter paper for filtration before it was used to synthesize Turmeric Silver nanoparticles (TAgNPs). This extract was mixed with 1,3,6,9,12 mM silver nitrate solution, stirred overnight at room temperature to enable the eco-friendly synthesis of (1,3,6,9,12-TAgNPs) as represented in Figure 1, change in color from dirty yellow to reddish-brown. Lower to Higher concentrations (1 mM to 12 mM) of $AgNO_3$ were similarly prepared for comparative analysis.

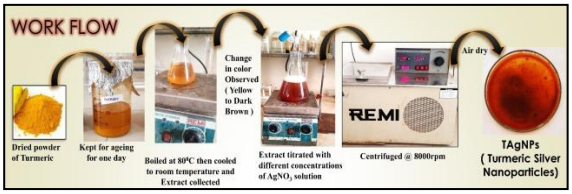

Figure 1: Represents the Preparation of Plant extract and Turmeric Silver nanoparticles (TAgNPs)

Phytochemical investigation

Turmeric an ayurvedic medicine used in various skin application ointments gels as anti-inflammation and anti-oxidant as well as an analgesic material. Prepared plant extract was evaluated for phytochemicals screening by performing a thorough qualitative phytochemical analysis to determine whether or not various phytoconstituents were present.

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Procedure for the preparation of Herbal Nano Silver soap

Preparation of Transparent Soap

The saponification process utilized coconut oil as the neutral fatty acid and sodium hydroxide (lye solution) as the alkaline reagent. Prepared the lye solution by adding 12.25 g of NaOH to 20.29g of distilled water and left for 12 hours to cool at room temperature. In another beaker 10.25g of stearic acid and 56.4 g of coconut oil were mixed gently so that the crystals of stearic acid dissolved completely in the oil. Maintaining the 80°C temperature, gently added drop wise sodium hydroxide solution to the coconut oil and stearic acid mixture. A white, cloudy mixture was formed while stirring continuously as the saponification reaction occurred efficiently. Then, added 16.08g of sorbitol and 4.2g glycerol while stirring continuously, at last added the 40.3g of Iso-propyl alcohol and closed the lid immediately due to readily evaporation of alcohol. A clear transparent mixture was formed [18] as represented in Figure 2.

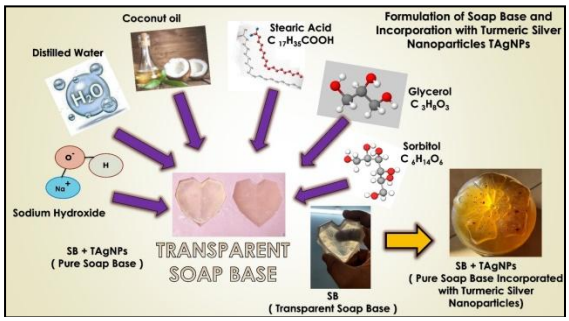

Figure 2: Represents the preparation of Transparent Soap base and Formulation of Turmeric silver nanoparticle Transparent Soap Bar

Evaluation Criteria

a) pH of the Turmeric Silver Nano soap: A 10% soap formulation was tested by mixing 5 g of soap in 500 ml of deionized water by digital pH meter. b) Color and Transparency: Visual inspection was done against a white background to ascertain its color and evaluate the transparency of the manufactured Turmeric Silver Nano soap[18]. c) Foaming ability test: Approximately 0.5 grams of Turmeric Silver Nano soap were dissolved in about 25 milliliters of distilled water within a 100-millilitre graduated measuring cylinder to evaluate the soap's foaming capacity. Following a 2–3-minute agitation of the measuring cylinder, and remain undisturbed for and Foam height was measured after 10 minutes. Following three successive experiments, the findings were documented, and the mean was reported. Foam retention time defined as the duration of foam persistence generated by the soap was measured by repeating the procedure over a 5–10 minute interval.

d) Saponification value: The quantity of KOH in milligrams necessary for the total saponification of 1 gram of lipid compound. It is defined as the average molar weight of lipid-derived fatty acid constituents within oils or fats [19] . Approximately 5 grams of the soap sample were weighed into a conical flask, proceeded by the addition of 0.5 M potassium hydroxide solution to determine the saponification value. The mixture was heated to around 600°C while being continually stirred in a hot water bath. The temperature subsequently raised by 1000°Celsius, and boiling maintained approximately for one hour. Titration was conducted using 0.5 molar HCl, with phenolphthalein as the indicator. The endpoint identified is the cessation of the pink hue. The saponification value is determined using the formula provided below in KOH/gm.

$$Saponification\ value = \frac{(\text{Blanck} - \text{Titrate}) * 28.05}{\text{weight of oil(gm)}}$$

Preparation of Different concentrations of Turmeric Silver Nanoparticles

With certain changes, the standard method was followed to produce the green synthesis of AgNPs. In order to reduce Ag+ ions, 100 mL of T-extract (Turmeric extract) combined with 100 mL of dropwise 1 mM $AgNO_3$ while swirling gently at room temperature. Until the TAgNPs (Turmeric Silver Nanoparticles) were fully synthesized, the solution was stirred continuously throughout the night. When the extract was first added, the mixture had a pale-yellow tint. After stirring all night, the solution's color turned dark reddish-brown color. Same process was repeated for 1, 3, 6, 9 and 12 mM of $AgNO_3$ for the synthesis of 1 TAgNPs,3 TAgNPs, 6TAgNPs, 9TAgNPs, and 12TAgNPs. After 24 hours all the different concentrations were centrifuged at 9000 rpm and kept for drying at room temperature.

Formulation of Turmeric silver nanoparticle Soap Bar

While cooling down the mixture, add the 1 mg of (1mM, 3mM, 6mM, 9mM, and 12mM TAgNPs) concentrations for different batches per 100 g soap base, which also imparts its natural color and transfer the mixture to the mould for the desired shape as represented in Figure 2. The soap mixture was thereafter let to harden and maintained at room temperature.

Characterization of formulated Turmeric Silver Nanoparticle Soap Bar

Green synthesized TAgNPs were elucidated by UV-Vis spectrophotometer using a Perkin Elmer model Lambda 25. FTIR was performed on Perkin

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Elmer using KBr pellet method and in liquid phase, spectra recorded at 400-4000 cm^{-1}. XRD patterns were recorded to determine crystallinity using a Bruker D8 instrument. FESEM and FESEM EDX (Hitachi, Japan, SU 8010 Series) and HRTEM Hitachi (H-7500) analyses were used to assess morphology and size. EDX confirmed elemental composition of 3TAgNPs as well as SB+3TAgNPs.

Assessment of Antioxidant Activity by DPPH Assay

Evaluation of Formulated Soap Bar for Antioxidant potential was done by adding 50mg soap in 1 mL of water in separate containers and 50 mg/mL to 10mg /mL concentration was used for experiment. DPPH (100 µM) was prepared by diluting 18 mL of a 22 mg/100 mL methanolic stock to 100 mL. Antioxidant activity was assessed in a 96-well format by incubating test compounds with DPPH at 37 °C for 30 min, followed by absorbance measurement at 517 nm. Standard was prepared with 1g of Ascorbic acid was dissolved in 1mL of water in separate containers and 1000mg/mL to 62.5mg/mL concentration was used for experiment[17].

Antimicrobial studies

Method outline

The microbicidal activity was investigated by Disc-Diffusion assay using Nutrient Agar media in case of bacteria and Potato Dextrose Agar in case of fungal strain with measuring inhibition zones in millimeter (mm). Five Bacteria culture *Rhizobium Tropici, Xanthomonas, E. coli, Pseudomonas putida, Bacillus tequilensis* and fungal strain *Aspergillus parasiticus, Aspergillus Niger* were used for antimicrobial assay.

Sample Preparation for antimicrobial assay

To make 1000 µg/ml of T-extract, 100 mg T-extract were diluted in 100 ml of deionizedwater. Similarly, for TAgNPs 100 mg was thoroughly mixed in 100 ml of double-deionized water to produce a 1000 µg/ml for 1, 3, 6, 9 and 12mM concentrations respectively. For Soap Base,100 mg SB is normally dissolved in100 ml of distilled water intending to formulate conc. 1000 µg/ml. In Silver nano Soap product sample, 10 mg of 3TAgNPs biosynthesized silver nanoparticles were combined with 100g of soap while preparation, therefore 10 µg/100 mg is the concentration of 3TAgNPs in our product sample. Now that 100 mg of this final product had been dissolved in 10 ml of deionized water, therefore, in SB+ 3TAgNPs stock sample only 10 µg/ml of biosynthesized silver nanoparticles remains. For Control in anti-bacterial studies, amoxicillin (AMOX 500), 500 mg of the commercially available amoxicillin was dissolved in 50 milliliters of water to produce 10000 µg/ml for antibacterial activity. For Control in anti-fungal studies, Itraconazole (ITRA 200), 200 mg of the commercially available itraconazole was dissolved in 20 milliliters of water to produce 10000 µg/ml for anti-fungal activity.

Culture media preparation:

Nutrient broth was prepared by 5gm peptone powder, 3gm beef extract; 5 gm NaCl in 1litre of distilled water adjusting pH to 7.O-7.2 by the help of digital pH meter. Fungus strains were directly inoculated with the help of sterile cotton swab from pure culture after 2 times sub culturing. 37g Nutrient Agar media solubilize in 1litre ml of deionized water autoclaved and were used in case of bacteria culture and 39 gm $potat_0$ $dextr_0$se Agar was dissolved in 1 litre of Deionisedwater autoclaved and used in case of fungus culture.

Determination of Antimicrobial Activity:

The sterile disc made up of Whatman filter paper 6mm in diameter were taken dipped in prepared stock for 24 hrs. All the glassware including media was autoclaved oven dry and placed in laminar and treated with UV light for 2 hr. 25 ml nutrient media in case of bacteria and potato dextrose media in case of fungus was poured on petri plates and left for 3 hrs. To solidify under UV light for sterile condition and then 200 µl of bacteria inoculated on plates with the help of spreader from stock bacteria culture broth media left for drying. In case of fungus culture, the inoculation was done with the help of sterile cotton swab from the pure culture plates. The sterile disc 6mm in diameter were dried and placed on different bacteria and fungus inoculated plates containing different concentrations of Biosynthesized turmeric Silver Nanoparticles along with pure soap base, soap base containing 3TAgNPs, T-Extract and Antibiotics amoxicillin 500mg and Itraconazole 200mg and zone of inhibition was measured with HI media Zone Scale[20,21,22]. All the experiments were done in triplicate (n=3) and average of the zone of inhibition were presented in mm in table 2,3.

Cytotoxicity Activity against epidermoid Skin Cancer A-431Cell line MTT Assay

Outline of the method: The in vitro MTT method performed for the 3TAgNPs, SB, SB + 3TAgNPs on Human Skin Carcinoma (A431) cell line to determine the cytotoxicity level.

Preparation for cytotoxicity evaluation

10 mg of 3TAgNPs, SB, SB + 3TAgNPs was reconstituted in DMEM-HG supplemented with 2% fetal bovine serum to achieve a stock soln. of 10 mg/mL. Furthermore, serial dilutions were prepared to lower the concentrations for cytotoxicity testing. For cell line and its culture

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medium epidermoid Human Skin Carcinoma (A431) cell line procured from Pune NCCS, India was cultured in DMEM-HG. The culture medium was enriched with heat-inactivated fetal bovine serum 10%FBS. Cells were initially grown in high glucose medium DMEM added with deactivated FBS10% along with antibiotics—penicillin concentration at (100 IU/mL) and streptomycin(100µg/mL), also with antifungal compound amphotericin B (5 µg/mL). Cultures at 37 °C in a precisely humidified chamber incubated to simulate in vivo-like conditions for optimal growth with 5% CO2 until a confluent monolayer was formed.

Evaluation of A-431 cell cytotoxicity by MTT Assay:

96 Wells were washed with DPBS, and varying concentrations of test compounds were added. Untreated wells served as controls. After 24 h incubation, morphological changes were observed microscopically. Absorbance at 570 nm taken to evaluate cell viability After media removal, 100 µL of formazan dye (diluted in DPBS) was added to each well, followed by a 3 h incubation under the same conditions. The supernatant discarded and MTT crystals were dissolved in 100 µL DMSO [23,24].

RESULTS AND DISCUSSION:

Qualitative phytochemical analysis of the turmeric extract was conducted to detect flavonoids, volatile oils, glycosides, steroids, and anthraquinones. Table 1 represents the list of phytoconstituents present in the T-extract. These constituents play a vital role in the reduction and stabilization of silver ions into Silver-nanoparticles. The golden yellow colour of turmeric is due to curcumin [31].

Table 1: Phytochemical identification of aqueous T-extract:

Phytochemical constituent		Results
	Flavonoids	+
	Volatile oils	+
	Anthraquinone	+
	Steroids	+
	Glycosides	+
	Tannin	-
	Reducing Sugar	-
	Resin	-

Evaluation of Turmeric Nano Silver Soap Bar

pH of the Turmeric Silver Nano soap measured at 7.8 ± 0.15, which is more in line with pH of the skin. Clear transparent soap base was formed and when incorporated with curcumin silver nanoparticles imparts light yellow to the finished soap bar. After 3 consecutive repetition of the experiment foaming ability was found to be 28±3 ml in 100 ml measuring cylinder. Retention time was found to be 10±2 minutes. Saponification value for oil sample found 258±0.2 KOH/mg.

UV Spectroscopic Analysis

This technique serves as a critical and dependable method for the preliminary identification of TAgNPs because the metallic peak position and the shapes are very sensitive to the particle size of metallic nanoparticles. [25] In this electronic spectroscopy analysis, a sharp peak at 400–420 nm wavelength corresponds to the formation of AgNPs. Tint yellow colour of the turmeric extract was observed to dark brown within 24 hr. of continuous stirring indicating the reduction of silver nitrate ($AgNO_3$) solution with 1,3,6,9,12 millimolar concentrations. The prominent peaks were observed at 397,420,422, 426,424,423 nm for Turmeric (T extract), 1, 3, 6, 9, and 12-TAgNPs in Figure 3, and when 3mM TAgNPs were incorporated into soap base, peaks were observed between 424-428 nm for soap base + 3mMT AgNPs and soap base, in Figure 4, which indicate the presence of 3 mM TAgNPs and their stability in their impregnated form. Single SPR band between 200-700 nm suggest that the TAgNPs are spherical in shape also confirmed by the TEM.

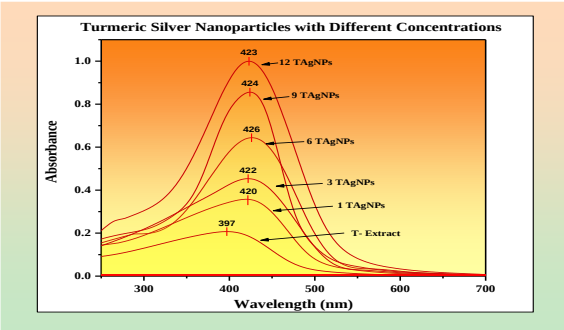

Figure 3: UV- Vis Spectroscopic Analysis of turmeric silver nanoparticles prepared with different concentrations of $AgNO_3$.

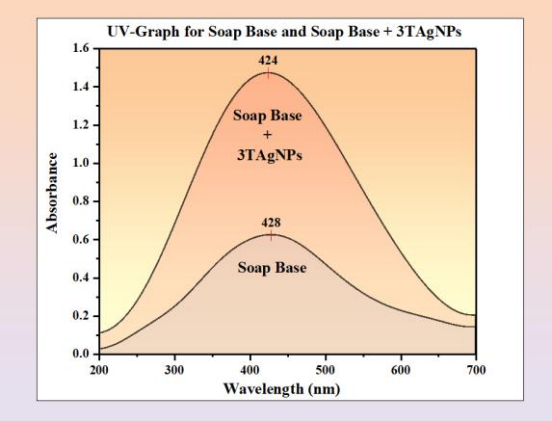

Figure 4: UV- Vis Spectroscopic Analysis of Soap Base and Soap Base+ 3mM TAgNPs.

Fourier Transformed-Infrared screening

FTIR analysis of aqueous extract of turmeric solution was executed to ascertain the manifestation of phytochemicals mediating the reduction, encapsulation, and stabilization of biosynthesized metallic nanoparticles. Figure 5,

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show the major IR peaks of biosynthesized AgNPs. The absorption band of aqueous extract of turmeric were observed at 3288, 1633, 1050, 1030, 518 cm-1 due to existence of functional groups (O-H, C–H, C=N, C-O) respectively.

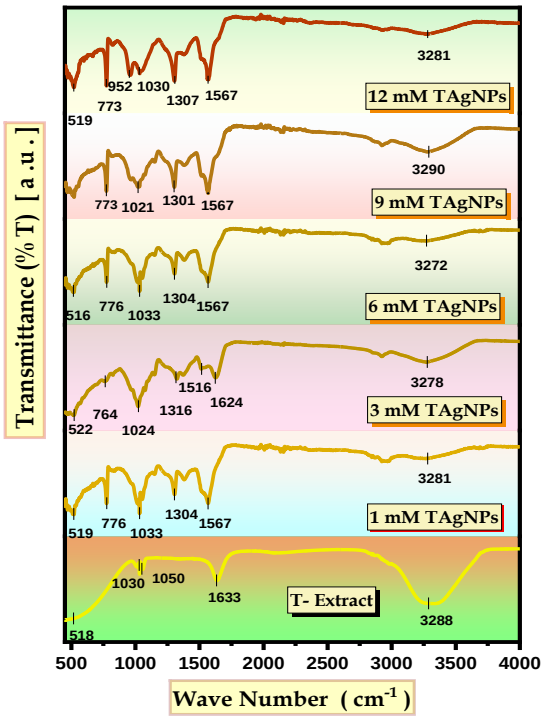

Figure 5: FTIR studies of Turmeric extract and different concentration of TAgNPs

Similarly, absorption band appeared at 522, 764, 1024, 1316, 1516, 1624, 3278 and 3278 cm-1 in 3mM TAgNPs corresponds to the formation of TAgNPs from aqueous extract of turmeric solution. Major peaks found were 523,770, 1022, 1400,1565,2918,3315 were also found in 3TAgNPs as well as SB + 3TAgNPs Figure 6.

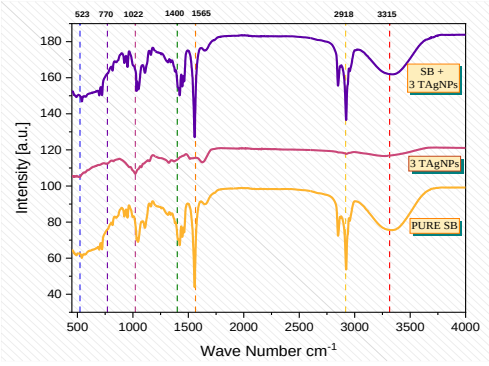

Figure 6. FTIR studies of Soap Base (SB), 3mM TAgNPs and Soap Base with silver nanoparticles SB + 3mM TAgNPs

The structural analysis of 1, 3, 6, 9and 12mM TAgNPs was confirmed by powdered X-ray Diffraction. Synthesized crystalline TAgNPs by green synthesis and observed XRD diffraction peaks at 2θ = 32.16, 46.15, 54.71, and 76.61 in Figure 7, which correspond to the AgNPs 311, 220, 200, and 111 planes reflect the patterns of the (FCC)face-centered cubic crystalline structure of the TAgNPs, and similar patterns were observed in 1, 3, 6, 9and 12mM TAgNPs, i.e., in different concentrations of TAgNPs aligning with the values of JCPDS card 893722. Further the average size of TAgNPs was calculated by Debye Scherrer equation (n = Kλ/β•cos θ) and the average size (n) of 1 mM, 3 mM, 6 mM, 9 mM, and 12 mM TAgNPs was 24.9, 28.85, 10.88, 10.36, and 8.14 respectively. Considering the largest in size among the different concentrations, further analysis and development was taken with the 3 mM TAgNPs.

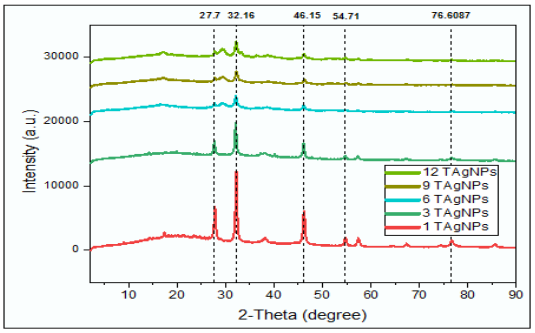

Figure 7: Represents the Crystallographic Analysis of 1,3,6,9,12 mM TAgNPs by Powder X-ray Diffraction

SEM, SEM-EDX and TEM Analysis

The SEM analysis performed in between 500 nm to 1 µm. The synthesized turmeric silver nanoparticles have a spherical form and nearly identical sizes, demonstrating no notable morphological changes in Figure 8. In Figure 9, the soap base containing the turmeric silver nanoparticles are clearly visible and stable after incorporation.

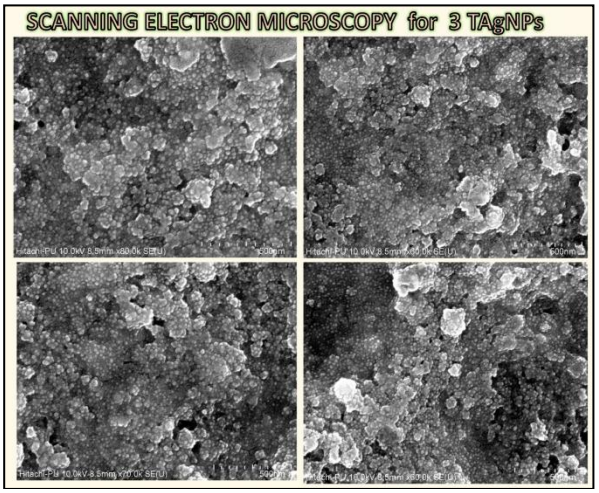

Figure 8: SEM images of Turmeric Silver nanoparticles 3TAgNPs at 500 nm

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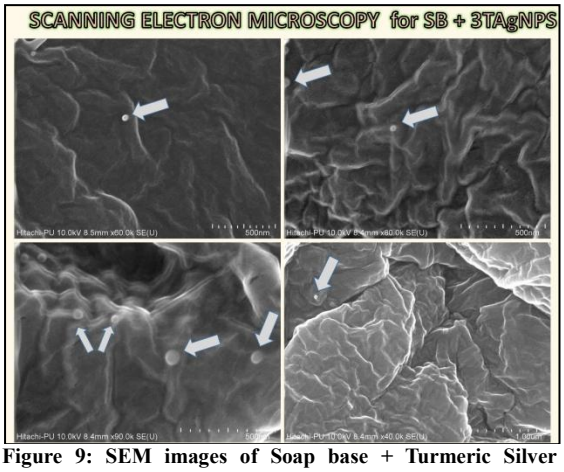

Figure 9: SEM images of Soap base + Turmeric Silver nanoparticles (SB+3TAgNPs) at 500 nm

According to a study, AgNPs made with curcumin extracts had a spherical form and ranged in size from 30 to 80 nm and were found to have a homogeneous crystalline structure. Scanning electron microscopy analysis of nanoparticles (NPs) made with C. longa revealed that the metals in the colloidal solution were uniformly spherical in shape. Figure 10, (A, B) shows the control images of the E. coli bacterial cells, (C, D, E, F) the TAgNPs embedded on the bacterial cell wall and (G, H) the destruction of the cell wall causing the death of the bacterial cells.

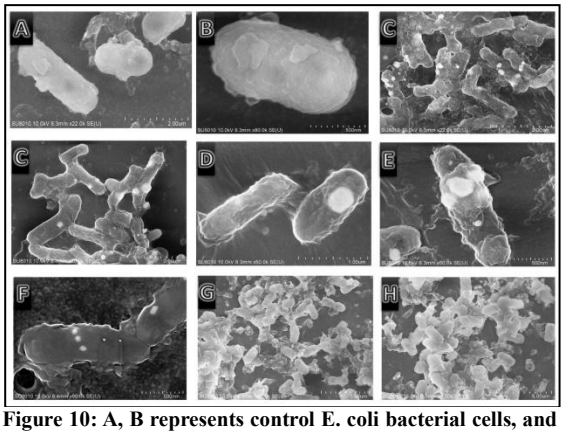

Figure 10: A, B represents control E. coli bacterial cells, and C, D, E, F represents TAgNPs embedded on bacterial cell wall, G and H represents the destruction of bacterial cells.

Figure 11, Represents FESEM-Edx elemental spectra for the Turmeric Silver nanoparticles 3TAgNPs and Formulated Soap Bar (SB+3TAgNPs) the peaks around 2.1 and 9.8 keV are related to the binding energies of Turmeric Silver nanoparticles. In figure 11, the peaks around 2.1 and 9.8 keV relates to the 3TAgNPs silver element in the Turmeric Silver nanoparticle. Additionally, the FESEM-EDX spectra for the SB+3TAgNPs confirmed the presence of TAgNPs. Moreover, signals of Sodium, oxygen, and carbon elements were also observed, which were due to the organic compounds in the soap base and Turmeric Silver nanoparticles. The results indicate that the synthesized TAgNPs are of high purity.

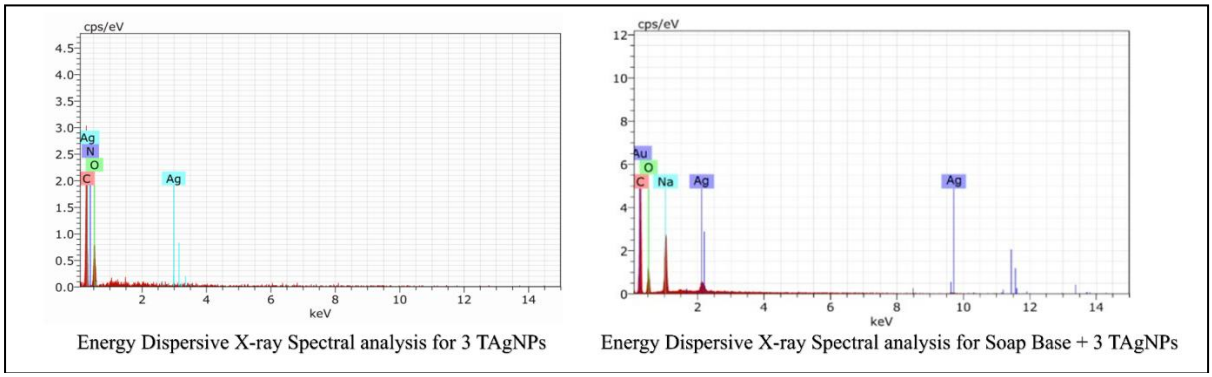

Figure 11: FESEM-Energy Dispersive X-ray Spectra of 3TAgNPs and SB+3TAgNPs.

The High-Resolution Transmission Electron microscopic analysis was used to determine the morphology and size distribution of biosynthesized 3 TAgNPs Figure 12. Consuming the turmeric extracts and in course of time decreasing the reducing agents lead to different size of curcumin silver nanoparticles (Venkatadri et al., 2020) · In our study, HR-TEM analysis reveals the size of 3 TAgNPs were spherical and in range 20 to 55 nm Figure 13 reveals size of 3 TAgNPs at 5,10,20,50,100 and 200 nm.

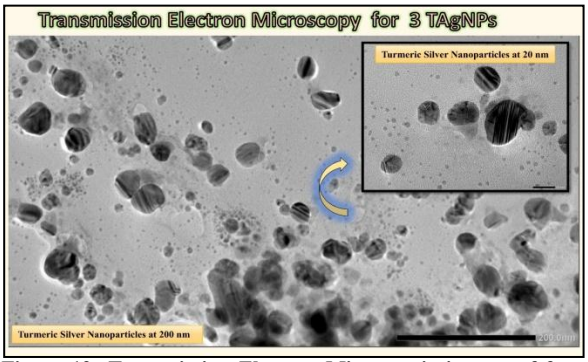

Figure 12: Transmission Electron Microscopic images of 3 TAgNPs at 200 nm.

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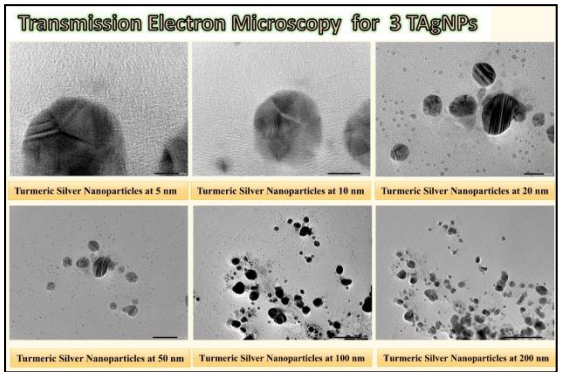

Figure 13: High Resolution Transmission Electron Microscopic images of 3 TAgNPs at 5,10,20,50,100 and 200 nm.

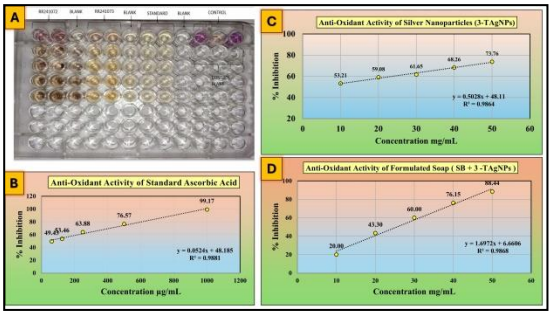

Figure 14: (A) 96 well microplate reader employed to find the absorbance simultaneously of control, standard, 3-TAgNPs (RR241072) and SB+3TAgNPs (RR241073) for antioxidant activity by DPPH assay. (B, C, D) IC_{50} values were calculated using the graph plotted between % inhibition vs concentration.

Anti-Oxidant activity DPPH Assay:

The Formulated Soap Bar and 3TAgNPs were examined for anti-oxidant potential by DPPH Inhibitory activity Figure 14 (A). The IC_{50} value was found to be 38.80 ± 0.83 mg/mL of standard Ascorbic acid as control, for formulated Soap Bar was found to be 25.59 ± 1.03 mg/mL and 4.11 ± 1.51 mg/mL for 3TAgNPs by DPPH Inhibition Assay. Hence, it was found that both 3TAgNPs and Formulated Soap Bar the exhibits potential Antioxidant Property. Graph were plotted between % inhibition vs concentrations used to determine the IC_{50} values Figure 14 (B, C, D). These results suggest that both 3-TAgNPs and Formulated Soap Bar possesses substantial free radical scavenging potential.

Antimicrobial Studies:

The antimicrobial activity results demonstrate that turmeric silver nanoparticles (TAgNPs) when incorporated with soap base exhibit significant antibacterial and antifungal properties compared to pure turmeric extract, soap base (SB), AMOX 500 mg and ITRA 200 mg. When 3-TAgNPs incorporated into Soap Base, SB+3 mM TAgNPs at 1000 µg/ml concentration demonstrated notable antimicrobial effects, particularly against E. coli shigella Klebsiella (R-25) with an inhibition zone of 40 mm, comparable to Amoxicillin 48 mm. E. coli Shigella Klebsiella (R-25) were also used for SEM analysis as it is more pathogenic to humans. Table 3, represents the observed antimicrobial activity by measuring inhibition zone in (mm). Minimum inhibitory concentration (MIC) in Figure 15.

Table: 2 Antimicrobial activity of T-extract, TAgNPs and marketed drugs against R-60 – Rhizobium Tropici, R-20 - Xanthomonas, R-25- E. coli shigella Klebsiella, R – 71 – Pseudomonas Putida, R - 40 – Bacillus Tequilensis, A-1 – Aspergillus Parasiticus, A – 2 – Aspergillus Niger.

Antimicrobial Activity (Inhibition Zone mm) Against							
Treatment	Bacteria					Fungus	
	R - 20	R - 25	R - 40	R – 60	R – 71	A – 1	A – 2
T-EXTRACT	12 ± 0.5	12 ± 0.4	11 ± 0.4	10 ± 0.5	16 ± 0.4	12 ± 0.2	13 ± 0.3
SB	12 ± 0.3	13 ± 0. 1	12 ± 0.1	12 ± 0.2	12 ± 0.3	16 ± 0.1	13 ± 0.4
1 TAgNPs	13 ± 0.4	13 ± 0.3	20 ± 0.3	14 ± 0.3	10 ± 0.1	13 ± 0.2	13 ± 0.1
3 TAgNPs	15 ± 0.2	16 ± 0.5	20 ± 0.2	16 ± 0.4	12 ± 0.5	11 ± 0.3	12 ± 0.3
SB + 3 TAgNPs	20 ± 0.3	24 ± 0.2	23 ± 0.2	19 ± 0.4	13 ± 0.2	17 ± 0.3	18 ± 0.5
6 TAgNPs	18 ± 0.5	15 ± 0.5	20 ± 0.4	17 ± 0.3	16 ± 0.5	14 ± 0.1	13 ± 0.4
9 TAgNPs	22 ± 0.4	15 ± 0.1	27 ± 0.1	17 ± 0.4	15 ± 0.3	14 ± 0.2	11 ± 0.2
12 TAgNPs	17 ± 0.1	16 ± 0.4	22 ± 0.3	18 ± 0.1	15 ± 0.1	14 ± 0.4	19 ± 0.3
AMOX 500mg	14 ± 0.2	48 ± 0.3	16 ± 0.5	35 ± 0.3	32 ± 0.2	-	-
ITRA 200mg	-	-	-	-	-	22 ± 0.2	13 ± 0.1

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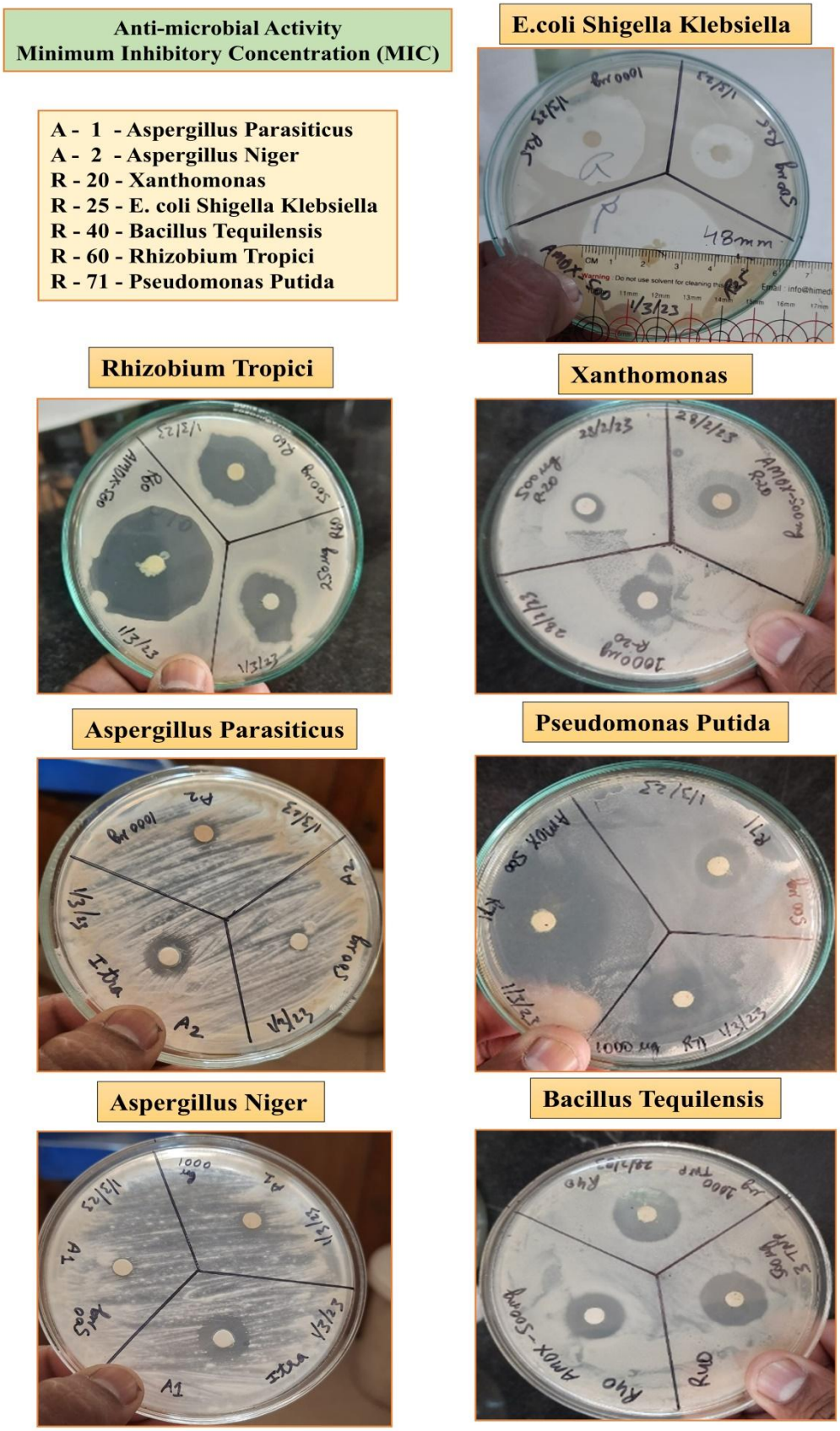

Figure 15: Anti-microbial studies by measuring the zone of inhibition of (R-60 – Rhizobium Tropici, R-20 - Xanthomonas, R-25- E. coli shigella Klebsiella, R – 71 – Pseudomonas Putida, R - 40 – Bacillus Tequilensis, A-1 – Aspergillus Parasiticus, A – 2 – Aspergillus Niger) using the Nano-Formulated Soap Bar (SB+3TAgNPs) with the control Amoxicillin (AMOX-500) for anti-bacteria studies and for anti-fungal Itraconazole (ITRA-200) was used.

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Table: 3 Minimum inhibitory concentration for 3TAgNPs and 3 TAgNPs incorporated in soap base and the comparison of serial diluted (1000, 500,250 µg/ml) 3TAgNPs v/s Amoxicillin 500 mg and Itraconazole 200 mg market drugs.

Antimicrobial Activity (Inhibition Zone mm) Against							
Treatment	Bacteria					Fungus	
	R-20	R-25	R-40	R-60	R-71	A – 1	A – 2
AMOX-500, (10000µg/ml)	13 ± 0.2	48 ± 0.3	34 ± 0.1	38 ± 0.2	36 ± 0.2	-	-
SB+3TAgNPs1000µg/ml	15 ± 0.3	40 ± 0.2	21 ± 0.4	16 ± 0.1	24 ± 0.4	16 ± 0.3	18 ± 0.1
SB+3TAgNPs 500µg/ml	11 ± 0.1	19 ± 0.2	17 ± 0.1	11 ± 0.4	16 ± 0.1	6 ± 0.2	6 ± 0.4
ITRA 200, (10000µg/ml)	-	-	-	-	-	22 ± 0.1	12 ± 0.2

Cytotoxicity Activity Against Skin Cancer Cell Line (A431) MTT Assay:

Compounds (3 TAgNPs, SB, SB + 3 TAgNPs) were assayed for in vitro cytotoxicity study against Human Skin Carcinoma (A431) cell line by exposing the cells to decreasing concentrations, ranging from 1000 to 7.8µg/mL. MTT assay was employed to test the cytotoxic effect of selected concentrations 3 TAgNPs, SB, SB + 3 TAgNPs on the cell viability of Human Skin Carcinoma (A431) cell line by calculating the metabolic activity through chromogenic assessment. Cell viability is the indicative of metabolically active cells is quantitatively assessed through a colorimetric determination using the MTT assay wherein mitochondrial dehydrogenase activity correlates directly with the viable cell population. In our study, the % cytotoxicity on Human Skin Carcinoma (A431) cell line was found to be 17.42 ± 2.15%, 42.82 ± 1.78%, 54.76 ±4.33% at 1000µg/mL for 3 TAgNPs, SB, SB + 3 TAgNPs respectively Figure 7 (A). The CTC_{50} values by MTT assay for 3 TAgNPs, SB, SB + 3 TAgNPs on A431 cells were found to be >1000µg/mL for both 3 TAgNPs, SB, and 756.862µg/mL SB + 3 TAgNPs depicted the greater potential apoptosis activity with the incorporation of soap base with turmeric silver nanoparticles Figure 7 (B, C, D).

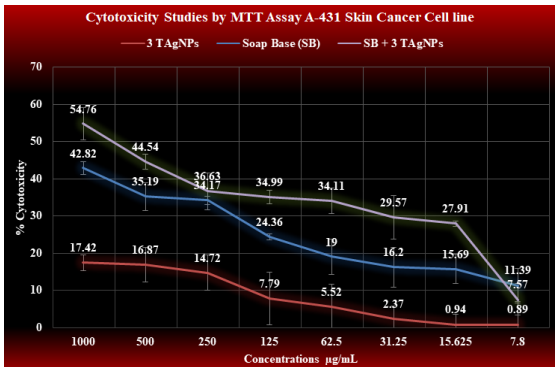

Figure 16: Graphs represent the cytotoxic studies of the Soap Base (SB), Turmeric silver nano particle (3TAgNPs) and the Nano-Formulated Soap Bar (SB+3TAgNPs)

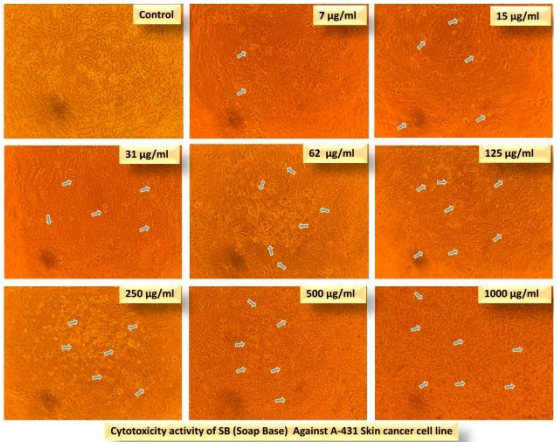

Figure 17: Cytotoxicity MTT assay of Soap Base (SB) against A-431 Skin cancer Cell Line.

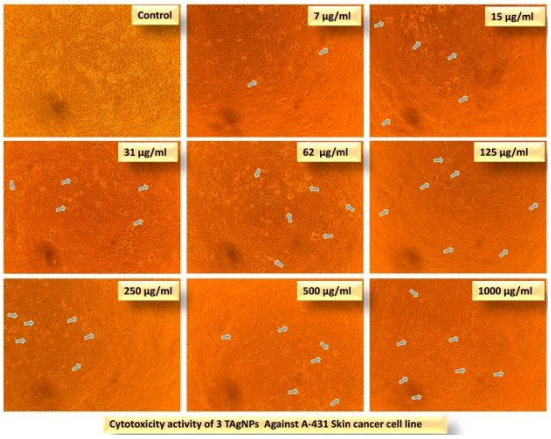

Figure 18: Cytotoxicity MTT assay of Turmeric Silver nanoparticles 3TAgNPs against A-431 Skin cancer Cell Line.

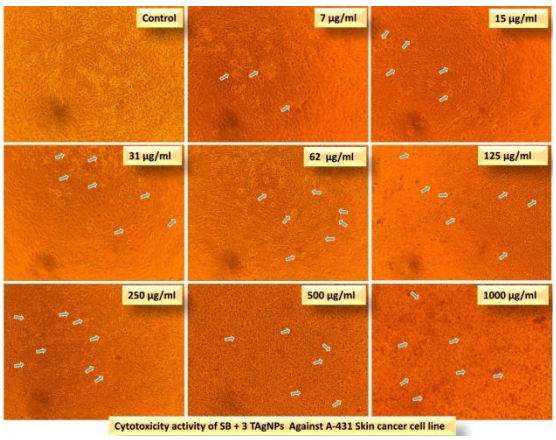

Figure 19: Cytotoxicity MTT assay of Turmeric Silver nanoparticles 3TAgNPs against A-431 Skin cancer Cell Line.

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CONCLUSION:

Strategic Phytogenic Fabrication, Multimodal Characterization, and Therapeutic Profiling of Bioactive Silver Nanoparticles (TAgNPs) in a nano-silver antimicrobial soap bar highlights the growing potential of eco-friendly nanotechnology in biomedical and consumer products. Pronounced pan-microbial inhibitory efficacy was evidenced against taxonomically diverse pathogens, including wide range of bacteria, as well as opportunistic fungal species. The green synthesis of TAgNPs by plant extracts offers a sustainable and environmentally friendly approach, minimizing the use of hazardous chemicals and reducing ecological impact. UV-Vis, FESEM, Edx, XRD, FTIR and TEM analyses corroborated nanoparticle formation, stability, morphology, and dimensional integrity, affirming their applicative potential. The incorporation of these nanoparticles into a soap bar formulation further demonstrated their ability to enhance the antimicrobial properties of a conventional cleansing agent, providing an effective alternative to traditional antimicrobial soaps with high anti-oxidant properties. In-vivo research could explore optimizing the concentration of AgNPs in different cosmetics formulation, ensuring long-term stability, and assessing the product's safety profile for widespread use. The successful green synthesis approach to other nanomaterials opens new avenues for developing environmentally responsible, high-performance antimicrobial products for both medical and commercial applications.

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CONFLICT OF INTEREST:

The authors report no conflicts of interest.

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