Assessment of antibacterial and antifungal activity of cream incorporating silver and zinc oxide nanoparticles synthesized via ginger and garlic extract

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Abstract

Green nanotechnology is a part of green innovation that incorporates green chemistry and green engineering principles. Green chemistry aims to reconsider chemical use in order to make it safe, secure and much more effective. In this study, Silver and zinc oxide nanoparticles were prepared using ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extract. TSC (Trisodium citrate) solution and metal salts (Silver nitrate and Zinc sulfate) were also used in combination with green extract for preparation of nanoparticles. TSC is green chemical and no serious or toxic effects have been reported yet. Different characterization techniques including UV-Vis spectroscopy, FTIR, SEM and XRD confirmed the formulation of silver and zinc oxide nanoparticles. Confirmed nanoparticles were used for production of antibacterial and antifungal cream. Nanoparticles were incorporated in formulated cream separately at different concentrations (2%, 4%, and 6%) and antibacterial activity of cream was tested against *E.coli* and *S.aureus*. Cream without nanoparticles was used as control in antibacterial testing. Control did not show any antibacterial activity. While nanoparticle's cream exhibited good activity against both strains. But inhibition zone were larger for *S.aureus*. 6% concentration of formulated cream samples incorporating silver and zinc oxide nanoparticles were used for antifungal testing against *Candida albicans.* In comparison to both nanoparticles containing cream, Zinc oxide NPs containing cream exhibited better antibacterial and antifungal activity than nanosilver cream. Nanocream was effective against skin pathogens.

Key words: Green nanotechnology; green chemistry; nanoparticles; nanocosmetics; nanocream; antibacterial; antifungal

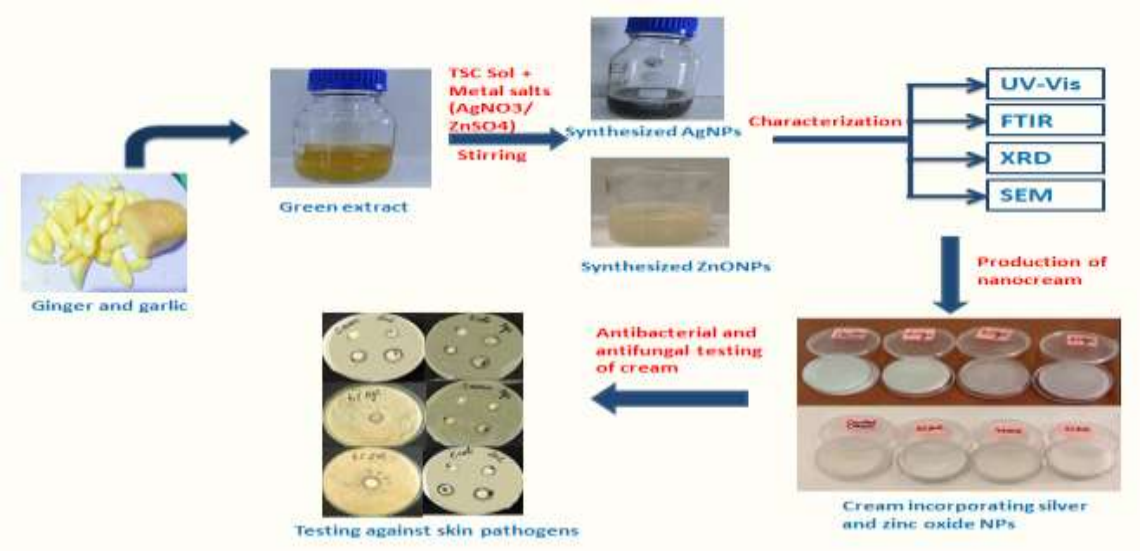

Figure 1 Graphical representation of abstract

Introduction

Nanotechnology is the preparation, evaluation, identification, and application of nanoscale (1-100nm) objects for scientific innovation. Sustainable nanotechnology has proven to be effective in science and engineering in fixing problems in a variety of sectors (Mata, Palmer, Tejeda-Montes, & Stupp, 2012). Nanotechnology is an emerging idea in the food, cosmetics, hygiene products, biomedical, agricultural, and environmental sectors for the production and regulation of nanosized properties in materials and structures, enabling the creation of modern consumer product (Prasad, Bhattacharyya, & Nguyen, 2017).

Green nanotechnology has developed environmentally sustainable nanomaterials that can be employed for a broad range of applications, such as treating toxic waste, removal of salts and minerals from water, and handling or controlling contaminants (Vijay Kumar, Pammi, Kollu, Satyanarayana, & Shameem, 2014). Green technology includes the utilization of environmental technology for monitoring and evaluation, pollution control and prevention as well as the manufacture and processing of materials (Ayaz Ahmed, Subramanian, Sivasubramanian, Veerappan, & Veerappan, 2014). The main objective of utilizing green sources to make nanomaterials is to address environmental hazards (Makarov et al., 2014).

Green chemistry aims to reconsider chemical use in order to make it safe, secure and much more effective. Green chemistry aims to redesign chemical production and use in our society, making them naturally safer and efficient (Anastas & Eghbali, 2010). Green chemistry principles can be used to develop nanoparticles and manufacturing techniques that are simpler, secure, and more sustainable (Jahangirian, Lemraski, Webster, Rafiee-Moghaddam, & Abdollahi, 2017).

During the biosynthesis of nanoparticles, non-toxic byproducts, such as biomolecules, are bonded to the produced nanoparticles. Since the metabolites of plants and microorganisms serve as stabilizers, the biogenic approach does

not demand for use of stabilizing agents (Singh, Kim, Zhang, & Yang, 2016). Biosynthesis of metal oxide nanoparticle is a safer, non-toxic, and ecofriendly alternative to physical and chemical methods (Keerawelle, Chamara, & Thiripuranathar, 2019).

Gingerols, shogaols and paradols are the phytochemicals which are found in ginger and they exhibit good antioxidant and antimicrobial potential (Kota, Panpatil, Kaleb, Varanasi, & Polasa, 2012). Garlic extracts include allicin, which has been demonstrated to be a powerful antimicrobial agent (Baljeet, Gupta, Yadav, & Yadav, 2015). Garlic extract stimulates antibacterial potential and defends against bacterial infection. (Fufa, 2019).

The key benefits of utilizing nanoparticles in cosmeceuticals are improved cosmetic ingredient stability, targeted delivery of ingredients to the desired position (Mu & Sprando, 2010). Nano cosmetics can be defined as a formulation of cosmetics by using nanotechnology as a delivery method to improve the performance of bioactive ingredients (Kaul, Gulati, Verma, Mukherjee, & Nagaich, 2018). L'Oreal, the world's largest cosmetics corporation, is investing $600 million of its $17 billion in Nano patents, and has trademarked the utilization of nanosome particles. It is the 6th largest of nanotechnology patents holder in the United States (Raj, Jose, Sumod, & Sabitha, 2012).

ZnONPs when used at concentrations up to 25%, their potential risk to humans in sunscreens or cosmetics is generally considered insignificant, and the toxicity profiles of ZnONps in vitro have no impact on health (Sonia S, Linda Jeeva Kumari H, Ruckmani K, & Sivakumar M, 2017). Zinc oxide is utilized in cosmetic, pharmaceutical, and medicinal applications and is acknowledged as a nutritional ingredient (Rajalakshmi et al., 2012). These are affordable and less toxic than that of other metal oxide NPs, have a variety of applications in medical field (Kołodziejczak-Radzimska & Jesionowski, 2014). FDA has designated ZnO as GRAS compounds which stands for "Generally Recognized as Safe." The usage of ZnONPs is safe for public health. (Bondarenko et al., 2013). They are widely used as UV blockers in sunscreens and a variety of medical applications (Mallikarjunaswamy, Ranganatha, Ramu, Gowda, & Ganganagappa, 2020). There is no conclusive proof that metal oxide NPs used in consumer goods are harmful to human health instead, their specific physicochemical properties offer apparent health benefits (Adamcakova-Dodd et al., 2014).

Silver nanoparticles show potential antibacterial, antifungal and healing properties. It can be used as a preservative in cosmetics products due to its antibacterial properties. Silver nanoparticles can be utilized in topical cosmetic formulation. At concentration of 0.2-2% nanoparticles can penetrate skin and show no toxicity at this level (Campbell, Contreras-Rojas, Delgado-Charro, & Guy, 2012).

AgNPs adherence to the cell wall and membrane of microbes, is dependent on electrostatic attraction between the negatively charged cell membrane of microbes and positively or less negatively charged AgNPs, which begins by the interaction between AgNPs and microbes (Farshad, Abbaszadegan, Ghahramani, & Jamshidzadeh, 2017). The nanoparticle causes morphological alterations in structure of membrane , which leads to membrane permeation and respiratory functions being disrupted by depolarization of membrane and ultimately cell structure is disrupted and

death of cells happen. Denaturation of DNA as result of treatment with silver nanoparticle may be regulated by oxidative stress generated by AgNPs as shown in figure 2 (Roy, Bulut, Some, Mandal, & Yilmaz, 2018).

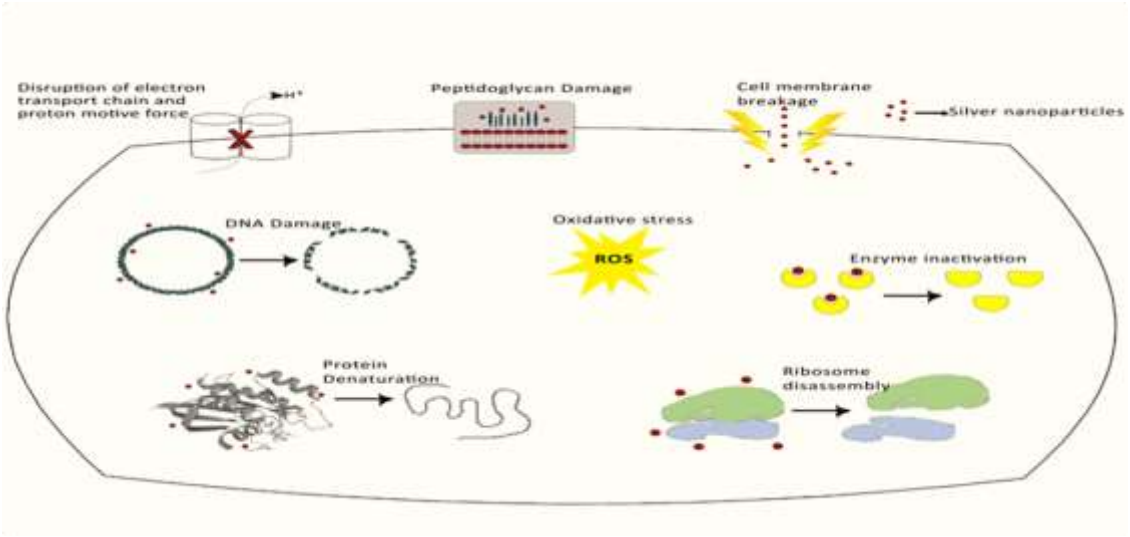

Figure 2 Antimicrobial action of silver nanoparticles

In this study, silver and zinc oxide nanoparticles were synthesized using ginger and garlic extract. Synthesized nanoparticles were characterized. Cream was formulated and synthesized nanoparticles were incorporated in cream at different concentrations. Antibacterial and antifungal activity of cream incorporating nanoparticles was investigated. Graphical representation of work is shown in Fig 1.

Material and methods

Collection of samples

For formulation of silver and zinc oxide nanoparticles, ginger (*Zingiber officinale*) and garlic (*Allium sativa*) were bought from a local store in Multan, Pakistan. For the production of silver nanoparticles, silver nitrate (AgNO3) was utilized, while zinc sulphate (ZnSO4) was utilized for the production of zinc oxide nanoparticles.

Preparation of green extract

Ginger and garlic were used for preparation of green extract. Both were properly washed off using sterile distilled water in order to remove dust particles and then allowed to air dry for few minutes. Exactly 50g of fresh unpeeled ginger and 50g of peeled garlic cloves were blended with morter and pastle and then boiled blended mixture in 1 liter of distilled water for the time until it reduced half volume. Prepared extract solution was filtered using whatman filter paper no 1. Extract was stored at 4°C until it was required for the preparation of Ag and ZnO nanoparticles.

Formulation of silver nanoparticles

For biosynthesis of silver nanoparticles, 25ml of prepared TSC solution (prepared using 2:1 of citric acid and NaOH in distilled water) was added in 125ml of green extract in a beaker and then 1.25g of silver nitrate was dissolved in that solution. Solution was allowed to stir for 2 hours. Colour of solution was turned from milky white to dark brown. The solution was centrifuged at 9000rpm at 25 ^{0}C for 25 mins. Supernatant was discarded and pellet was washed off with distilled water and then dried in hot air oven at 70^{0}C overnight to obtain powder. Obtained powder of silver nanoparticles was stored in epindroff tube and further used for characterization.

Formulation of Zinc oxide nanoparticles

For biosynthesis of zinc oxide nanoparticles, 25ml of prepared TSC solution was added in 125ml of green extract in a beaker and then 15g of zinc sulphate was dissolved in that solution. Solution was allowed to stir for 2 hours. Colour of solution was turned to light yellow. The solution was centrifuged at 9000rpm at 25 ^{0}C for 25 mins. Supernatant was discarded and pellet was washed off with distilled water and then dried in hot air oven at 70 ^{0}C overnight to obtain powder. Obtained powder of zinc oxide nanoparticles was stored in epindroff tube and further used for characterization.

Techniques used for characterization of nanoparticles

The size of synthesized nanoparticles was investigated as part of the characterization. Techniques including UV-visible spectroscopy, Fourier Transmission Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and SEM were used for characterization of nanoparticles.

UV-vis spectroscopy

The characterization of silver and zinc oxide nanoparticles was done using double beam UV-visible spectrophotometer (C-7200S –PEAK Instruments Inc., USA). This technique was used to determine reduction of silver and zinc oxide ions to nanoparticles in solution. 10% of green extract solution was taken as blank. After dilution of a small volume of the sample into distilled water in glass cuvette, the UV-Vis spectrum was examined after 24 hours. The principal of UV-vis spectroscopy is excitation of electrons, When UV or visible light falls on molecule it cause transition of electrons from lower energy orbit toward higher energy orbit.

Fourier Transmission Infrared spectroscopy (FTIR)

FTIR spectroscopy was done to analyze the functional groups of biomolecules using Bruker ALPHA FTIR spectrophotometer. The spectra were examined in the 4000–400 cm^{-1} range. Dried powdered samples of silver and zinc oxide nanoparticles were used for analysis. Much information regarding the nature of the bond present in the sample can be obtained by analyzing at the frequency of infrared radiations that are absorbed.

X-Ray Diffraction

X-ray diffraction analysis (XRD) technique is used for determining a crystalline phase and chemical composition of material. The distinctive X-ray diffraction pattern employed for crystal structure analysis is produced by reinforced diffracted X-rays. XRD analysis was done at Central research Lab, Lahore College for women university, Lahore (Lahore, Pakistan). The Ag and ZnO samples were scanned in 2θ range of 10 to 80 ^{0}C at scan rate of 0.04^0 per second. 2θ peak position, FWHM and particle size were measured using Origin lab (2019) software.

Scanning electron microscopy

SEM analysis was done to check the morphology of samples using SEM model HitachiS2380 N, Japan. Dried powdered samples of silver and zinc oxide nanoparticles were used for analysis.

Production of nanocream

For preparation of nanocream, stock solution of synthesized nanoparticles was prepared at concentration of 200µg/ml in autoclaved distilled water. Solutions were ultrasonicated to get homogenous solution. Cream was consisted of two phases. Firstly the oil phase was prepared. For preparation of oil phase, 2 g of bees wax, 0.6g shea butter, 0.5 g emulsifying wax and 4ml of liquid paraffin were melted in water bath at 70 ^{0}C in a beaker. For preparation of aqueous phase, 0.1g of borax was heated in 2ml of autoclaved distilled water. Aqueous phase was transferred in oil phase and continuously stirred to homogenize cream. With the same volume of aqueous and oil phase ingredients, cream was prepared in 7 disposable cream jars. Out of 7, one sample was used as control because it was without nanoparticles. In other samples, silver and zinc oxide nanoparticles were incorporated separately with 2%, 4% and 6% concentration of total weight of cream in aqueous phase during preparation of cream as shown in figure 3(a) and 3(b). In each jar, 5g of cream was prepared. For 2%, 4% and 6% concentrations, respectively 100 µL, 200 µL and 300 µL of nanoparticles were incorporated in cream. PH of cream was checked with pH paper which was 5.5. Antibacterial activity of all cream preparations was tested while 6% concentration of cream was used for antifungal testing.

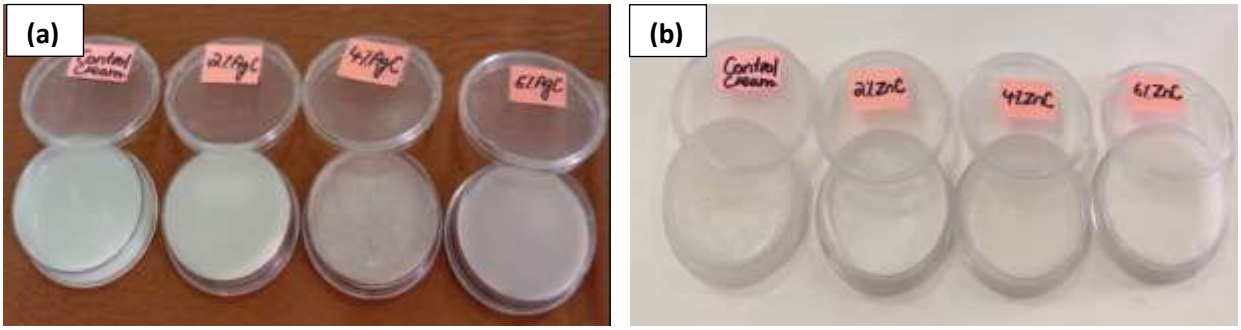

Figure 3 (a) AgNPs formulated cream of different concentration (b) ZnO formulated cream of different concentration

Antibacterial testing of cream

Antibacterial testing of cream was done against *Escherichia coli* and *Staphylococcus aureus* bacteria. Bacterial strains were obtained in pure form from Department of Microbiology and Molecular Genetics, The Women University Multan (Multan, Pakistan).

For preparation of nutrient agar, peptone, yeast extract, Nacl and agar were dissolved in distilled water. For preparation of nutrient broth, peptone, yeast extract, and Nacl were dissolved in distilled water. Nutrient both was poured in 4 conical flasks, each containing 20ml of broth. With the help of sterilized loop single colony of bacterial strain was picked up and dropped in conical flasks containing broth media. Conical flasks were put in orbital shaker for 24 hours because it maintains optimal condition for growth of microbes as shown in Fig 4. After 24 hours flasks were removed from shaker and stored in refrigerator. Agar well diffusion method was used for antibacterial testing. Different volumes of extract solution and nanoparticles incorporating cream of different concentrations were injected in wells. Agar plates were incubated overnight at 37 ^{0}C. Plates were removed from incubator after 24 hours to check zone of inhibitions of nanoparticles against bacterial strains.

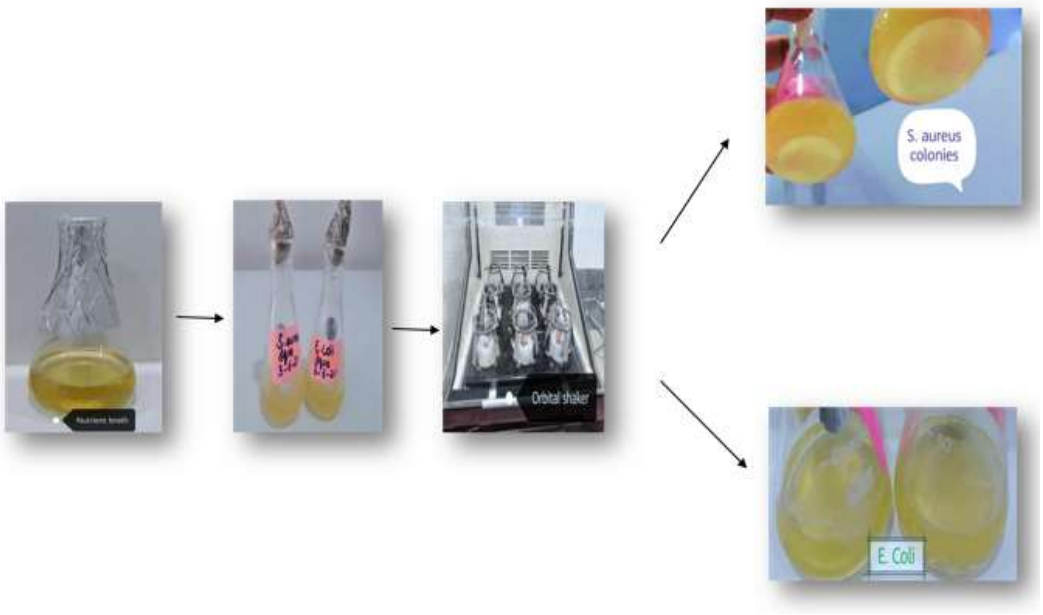

Figure 4 Nutrient broth cultures

Antifungal testing of cream

For antifungal testing of cream incorporating silver and zinc oxide nanoparticles at concentration of 6% against *Candida albicans*, samples were sent to Department of Biotechnology, IBBB, The Islamia University, Bahawalpur (Bahawalpur, Pakistan).

Statistical Analysis

The experiment was carried out in triplicate. Microsoft Excel was used to manage the data. The statistical analysis was done with Instat 3 software. The presented data was expressed as mean ± SD. Using p values, the significance level was determined.

Results and Discussion

Visual detection of silver and zinc oxide nanoparticles

Ginger and garlic extract was light yellow in colour after addition of TSC solution and silver metal salt, colour changed to milky white. After an hour of stirring it changed to yellowish brown colour. Change in colour was visually observed from milky white to dark brown within 24 hours as shown in Figure 5(a). Change in colour is indication of formation of silver nanoparticles. Also reported by other studies (Yang et al., 2017). Green extract organic molecules undergoes chemical reaction with AgNO3. Electron transfer occurs during chemical reduction and converts silver ions (Ag^+ ions) to silver atoms (Ag). Agglomeration of silver atoms causes the production of silver nanoparticles.

Colour of zinc oxide nanoparticles was changed from medium yellow to colourless as shown in Figure 5(b). Bioactive compounds in green extract serve as reducing, stabilizing, and capping agent, causing the zinc ions to be reduced to metallic ZnONPs efficiently (Sonia S et al., 2017)

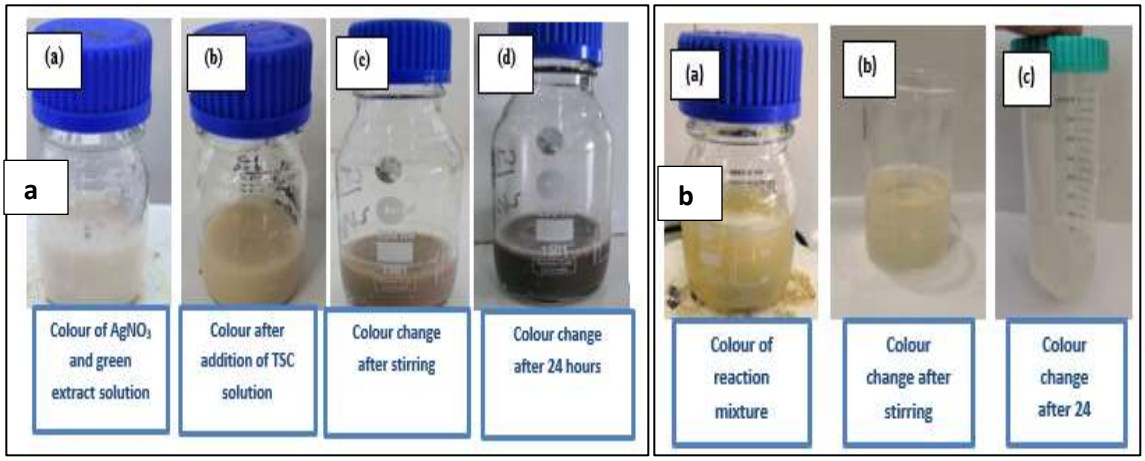

Figure 5 Visual detection of color change of (a) Silver nanoparticles (b) ZnONPs

Characterization of nanoparticles

Confirmation of production of silver and zinc oxide nanoparticles was also done through different techniques including UV-Vis spectroscopy, FTIR and XRD.

UV-Vis spectroscopy analysis

UV-vis analysis of both silver nanoparticles and zinc oxide nanoparticles was done using glass cuvettes. Sample was scanned at range of 200-700nm. Silver nanoparticles exhibit maximum absorption peak of UV at range of 400 to 450 nm (Ashraf, Ansari, Khan, Alzohairy, & Choi, 2016). Silver nanoparticle exhibited maximum absorbance at 437nm that indicated the formation of silver nanoparticle as shown in figure 6. The peak at 437nm wavelength is created by phenomena of surface Plasmon resonance, which is due to excitation of electrons on the outer surface of silver nanoparticles that are activated by the applied electromagnetic field. The distinctive peak of 437nm was seen in the 400–500 nm wavelength region, which was the maximum range of normal AgNPs (Sadeghi & Gholamhoseinpoor, 2015).

Due to the nanosize of ZnO particles and the limited particle size distribution, a sharp absorption peak develops. Zinc oxide nanoparticles exhibit strong UV absorption at range of 200-400 nm (Al-Asady, Al-Hamdani, & Hussein, 2020). Synthesized zinc oxide nanoparticles exhibited maximum absorbance at 327nm as shown in Figure 7. Maximum absorption peak at 327nm wavelength confirmed reduction of zinc ions to metallic zinc oxide nanoparticles.

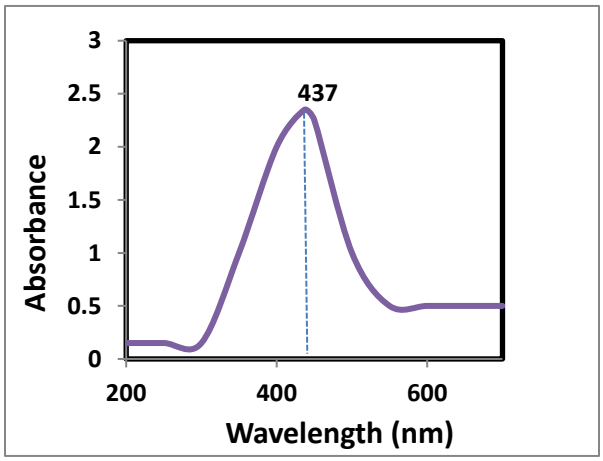

Figure 6 UV-Vis spectrum of silver nanoparticles

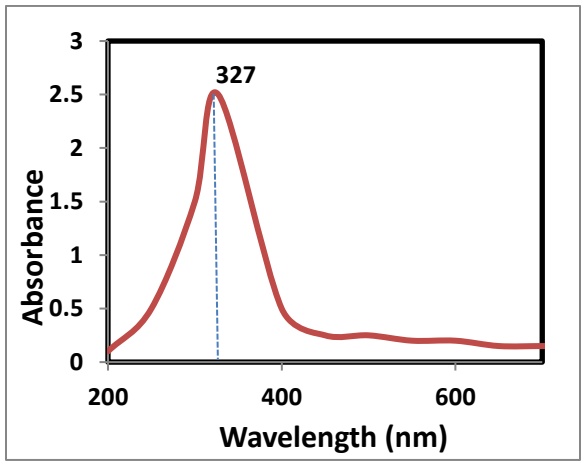

Figure 7 UV-Vis spectrum of zinc oxide nanoparticles

X-ray Diffraction analysis

XRD analysis was done to investigate crystallinity of silver and zinc oxide nanoparticles. For silver nanoparticles, XRD spectrum was recorded at 2θ angle range of 10-85^0 in continuous scan mode. XRD spectra of silver nanoparticles is shown in Figure 8. Diffraction peaks were observed at 2θ angle of 12.59^0, 21.48^0, 31.30^0, 32.13^0, 38.01^0 and 38.98^0 having intensity of 151, 56, 102, 110, 74 and 79 counts per second, respectively. Distinct diffraction peaks confirmed crystalline nature of silver nanoparticles.

XRD spectrum of zinc oxide nanoparticles was recorded at 2θ angle range of 10-850 in continuous scan mode. XRD spectra of zinc oxide nanoparticles is shown in Figure 9. Diffraction peaks were observed at 2θ angle of 12.27^0,

19.95^0, 31.91^0, 33.99^0, 46.13^0 and 47.93^0 having intensity of 259, 709, 270, 145, 132 and 110 counts per second, respectively. Presence of strong diffraction peaks confirmed crystalline nature of zinc oxide nanoparticles.

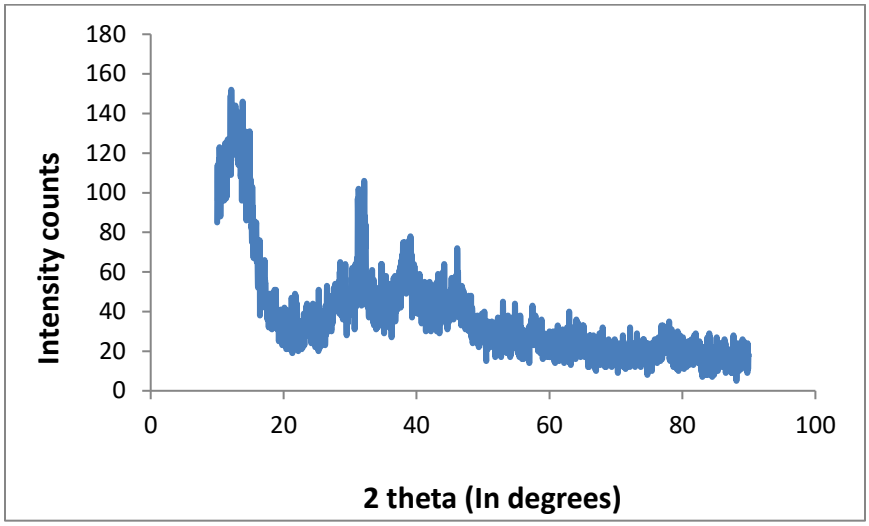

Figure 8 XRD spectrum of synthesized silver nanoparticles

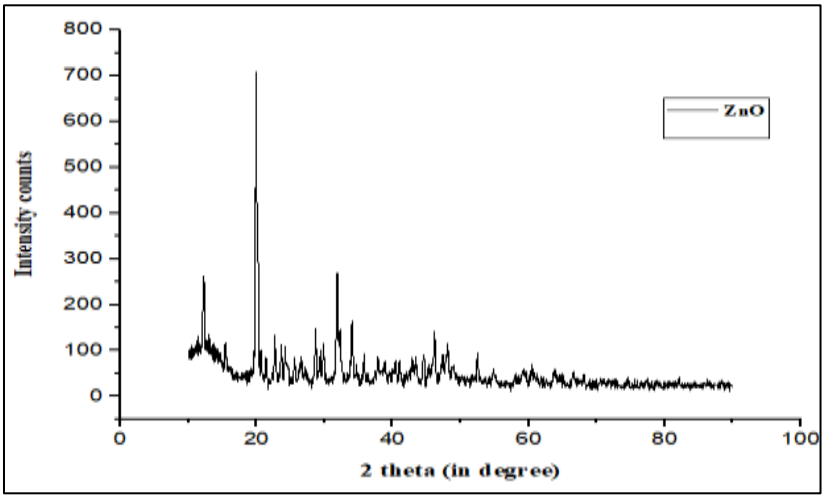

Figure 9 XRD spectrum of synthesized zinc oxide nanoparticles

Scherer's equation was used to determine the size of the NPs which is:

$$D = K\lambda / \beta \cos \theta$$

Where , **D** is Crystallites size (nm) , **K** is Scherrer coefficient having value of 0.9, **λ** is wavelength of x-ray source having value of 0.15406 nm, **β** is FWHM (e full width at half maximum) in radians and **θ** is Bragg's angle (2θ) which is value of peak position. The values of peak position (2 theta), FWHM and grain size of both nanoparticles were calculated for silver nanoparticles and for zinc oxide nanoparticles. Average particle size of silver nanoparticle

was 11.52 nm. Average particle size of zinc oxide nanoparticles was 14.87 nm. XRD results indicated the synthesis of crystalline nanoparticles.

FTIR analysis

FTIR estimations were done to distinguish the conceivable biomolecules answerable for covering and productive adjustment of the metal nanoparticles synthesized by ginger and garlic extract. FTIR spectra for silver nanoparticles was taken from 4000-400cm^{-1}. Peak found at 3269.12cm^{-1} which is associated with O-H stretching. Strong peak was present at 2924.92cm^{-1} as shown in Figure 10 which is associated with N-H stretching that is indication of synthesis of silver nanoparticle. Sharp and strong absorption bands were found at 1561.55cm^{-1}, 1381.89cm^{-1}, 1019.82cm^{-1} that were assigned to N-O stretching, C-H stretching, C-N stretching . Other peaks were present at 1718.63cm^{-1}, 1260.50cm^{-1}, 1125.45cm^{-1} and 827.59cm^{-1} that found to be associated with C=O stretching, C-O stretching in alkyl aryl ether, C-O stretching in tertiary alcohol and C=C stretching. Peaks found below 1000cm^{-1} were indication of heterocyclic compounds in extract. Peaks at 402.84cm^{-1}, 413.03cm^{-1}, 471cm^{-1} are representatives of alkaloids, flavonoids present in green extract. Sharp peak found at 446.21cm^{-1} was due to presence of silver metal (Uddin, Siddique, Rahman, Ullah, & Khan, 2020).

FTIR spectra of zinc oxide nanoparticles shown in figure 11 were taken in range of 4000-400 cm^{-1}. Peaks found at 2922.89cm^{-1} and 2853.52cm^{-1} were assigned to C-H stretching of alkanes. Peaks found at 1722.94cm^{-1}, 1560.23cm^{-1} and 1404.27cm^{-1} are associated with C=O stretching, C=C stretching in cyclic alkanes and S=O stretching in sulfate, respectively. While peaks observed at 1260.68cm^{-1} was found to be associated with C=O stretching and 1068.11cm^{-1} with S=O stretching in sulfoxide. Metal oxides usually display absorption bands in the fingerprint region below 1000 cm^{-1} because of interatomic vibrations. Peaks found in region between 415–480 cm^{-1} belong to ZnO and indicate the stretching vibration of Zn-O in the infrared range (Mahamuni et al., 2018). FTIR spectrum of zinc oxide nanoparticles represented strong and sharp peak at 442.31cm^{-1} which is characteristic peak that confirmed the presence of zinc oxide nanoparticles (Modi & Fulekar, 2020).

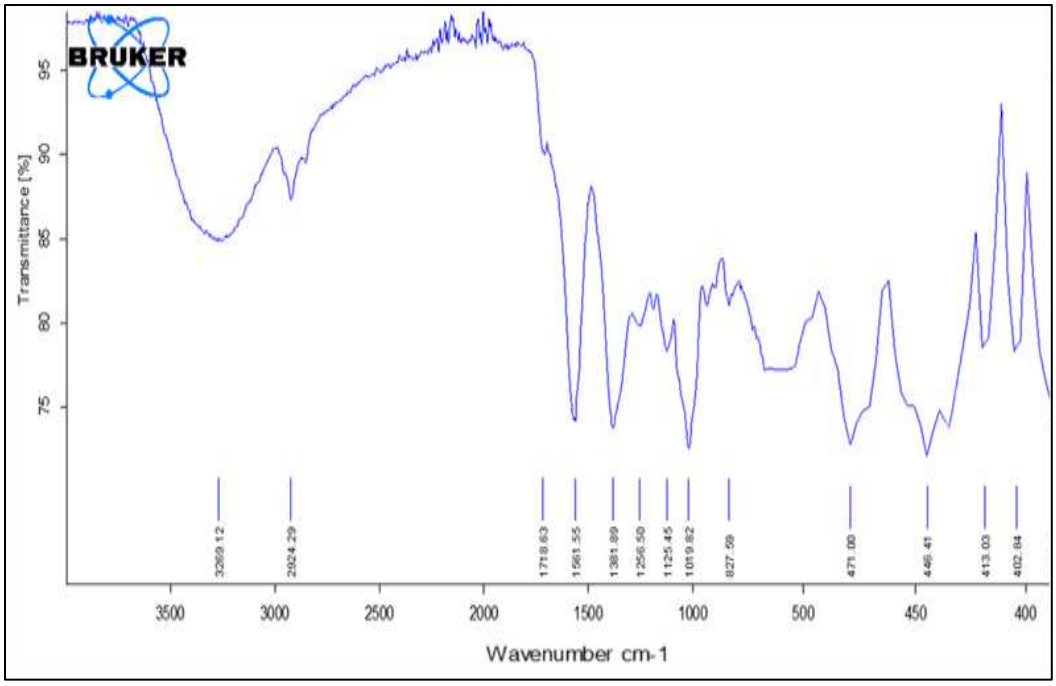

Figure 10 FTIR spectrum of silver nanoparticles

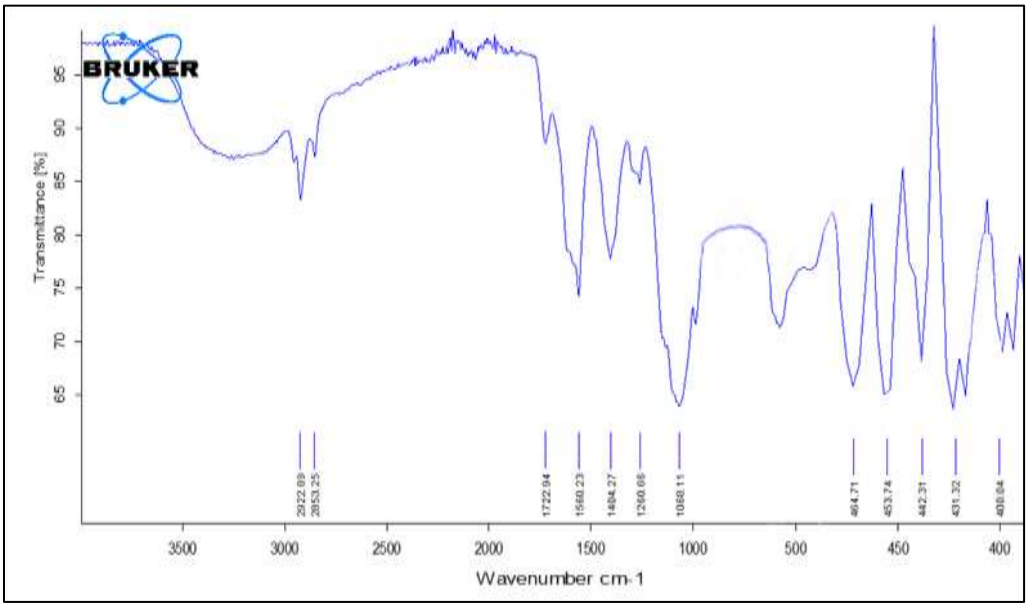

Figure 11 FTIR spectrum of Zinc oxide nanoparticles

Scanning electron microscopy

For morphological analysis of nanoparticles samples were uniformly distributed on surface of slide. Silver nanoparticles were agglomerated and some were seen irregular in shape as shown in figure 12(a, b). Zinc oxide nanoparticles were agglomerated and some were cubic spherical in shape as shown in figure 12(c, d).

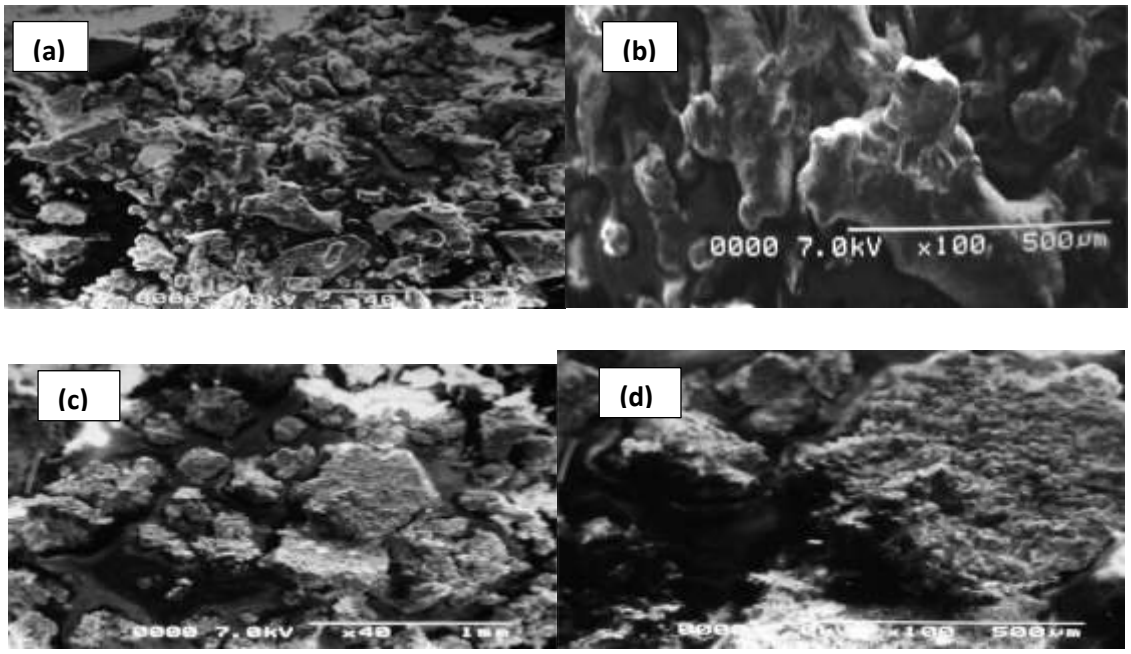

Figure 12: (a) SEM image of AgNPs at low magnification (b) SEM image of AgNPs at high magnification (c) SEM image of ZnONPs at low magnification (d) SEM image of ZnONPs at high magnification

Antibacterial potential of synthesized silver and zinc oxide nanoparticles

Agar well diffusion assay of nanoparticles

Antibacterial potential of synthesized silver and zinc oxide nanoparticles was evaluated by agar well diffusion assay against *E.coli* (gram negative) and *Staphylococus aureus* (gram positive) bacterial strains. Antibacterial activity of both nanoparticles was evaluated at different concentrations (5, 10, 15 and 20µl). 20µl of green extract was taken as control. When concentration of nanoparticles was increased, antibacterial activity was also increased as sown in Figure 15(a). No activity was noticed by green extract in both strains. All assays were performed in triplicates. All results were expressed as Mean ± SD and P-value was also measured. Statistical analysis was done using Instat 3 software.

Antibacterial potential of Ag nanoparticles was estimated by measuring the diameter of zone of inhibition. Results are mentioned in Table 1. Zones were clearer for *S.aureus* than *E.coli*. But silver also exhibited good antibacterial activity against *E.coli* as shown in figure 13.

Table 1: Inhibition zone of silver nanoparticles at different concentration

Bioactive agent	Concentration (µl/ml)	Zone of inhibition (Diameter, mm) Mean±SD		P-value
		E.coli (Gram –ve)	*Staphylococcus aureus* (Gram +ve)	
Ag Nanoparticles	5	15.43±0.40	15.33±0.35	<0.0001
	10	16.23±0.25	24.36±0.47	
	15	17.33±0.35	20.06±0.30	
	20	23.46±0.45	26±0.36	
Green extract	20	Nil	Nil	Nil

Antibacterial potential of ZnO nanoparticles was also estimated by measuring the diameter of zone of inhibition. Results are mentioned in table 2. ZnO nanoparticles showed comparatively good antibacterial activity to gram positive (*S.aureus*) bacteria than gram negative (*E.coli*) bacteria as shown in Figure 14. These results were consistent with previous reports (Lingaraju et al., 2016).

Table 2: Inhibition zone of zinc oxide nanoparticles at different concentration

Bioactive agents	Concentration (µl/ml)	Zone of inhibition (Diameter, mm) Mean±SD		P-value
		E.coli (Gram –ve)	*Staphylococcus aureus* (Gram +ve)	
ZnO nanoparticles	5	14.4±0.45	16.36±0.35	0.0003
	10	21.33±0.30	19.23±0.25	
	15	23.26±0.35	17.36±0.40	
	20	24.36±0.36	26.36±0.32	
Green extract	20	Nil	Nil	Nil

This happens because outer membrane is absent in gram positive bacteria while they have a thick peptidoglycan layer which makes them vulnerable to ZnONps. While an outer membrane with a thin peptidoglycan layer rich in lipids, lipopolysaccharide and lipoprotein is present in gram negative bacteria which make them more resistant to ZnONps (S. S, L. J. K. H, R. K, & S. M, 2017). Our analysis indicated that nanoparticles' antibacterial potential is proportional to their concentration, increase in concentrations results in a larger zone of inhibition as shown in Figure 15(b).

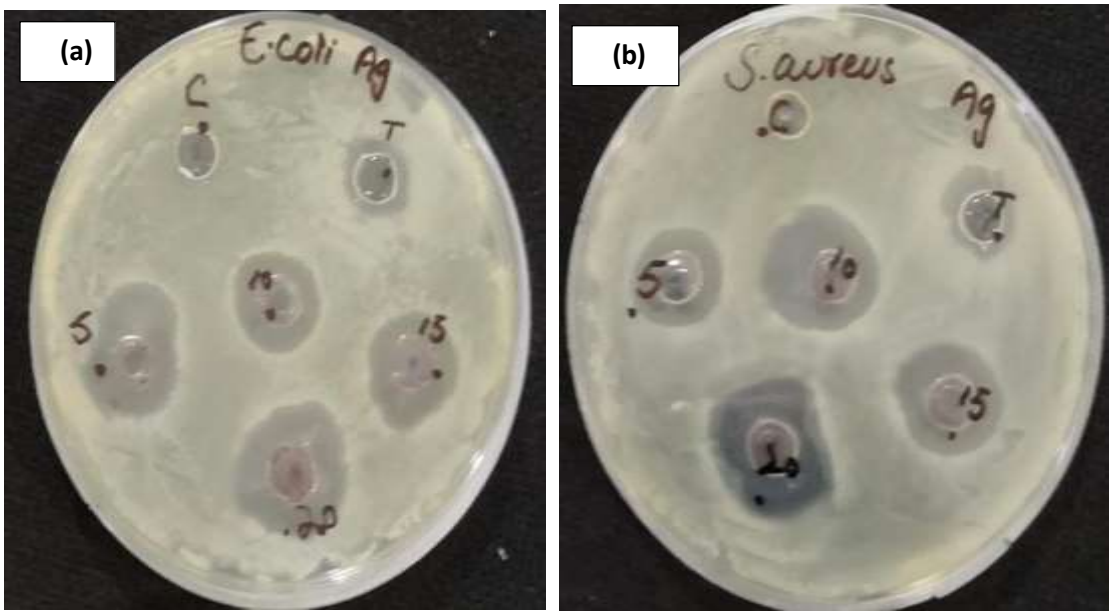

Figure 13: Antibacterial action of silver nanoparticles (a) E.coli (b) S.aureus

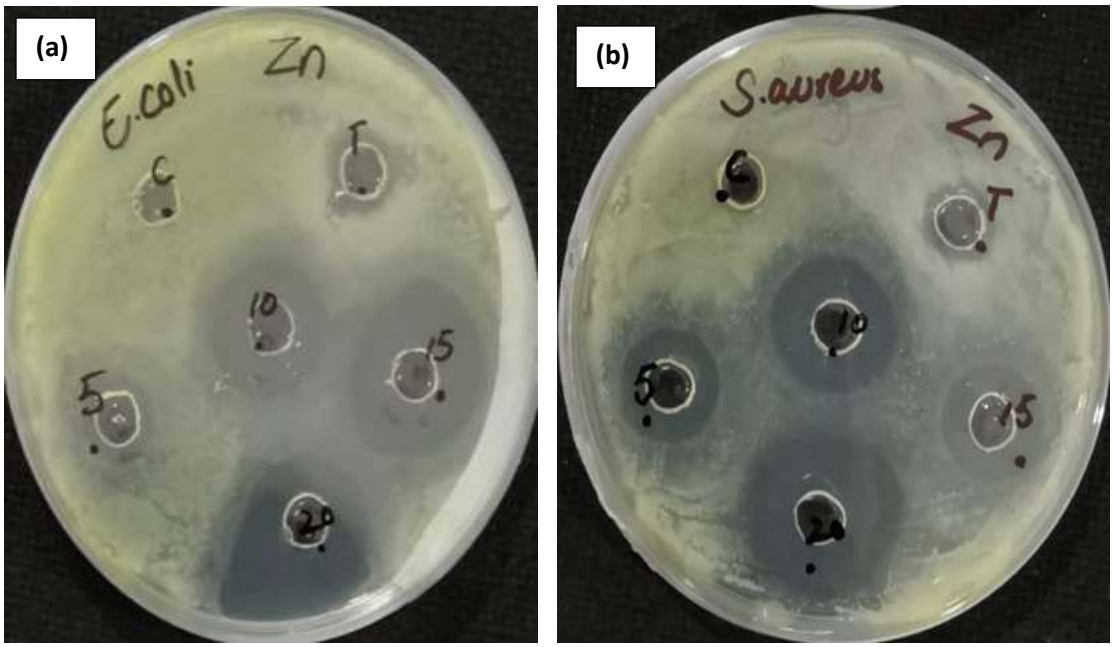

Figure 14: Antibacterial action of ZnONPs (a) E.coli (b) S.aureus

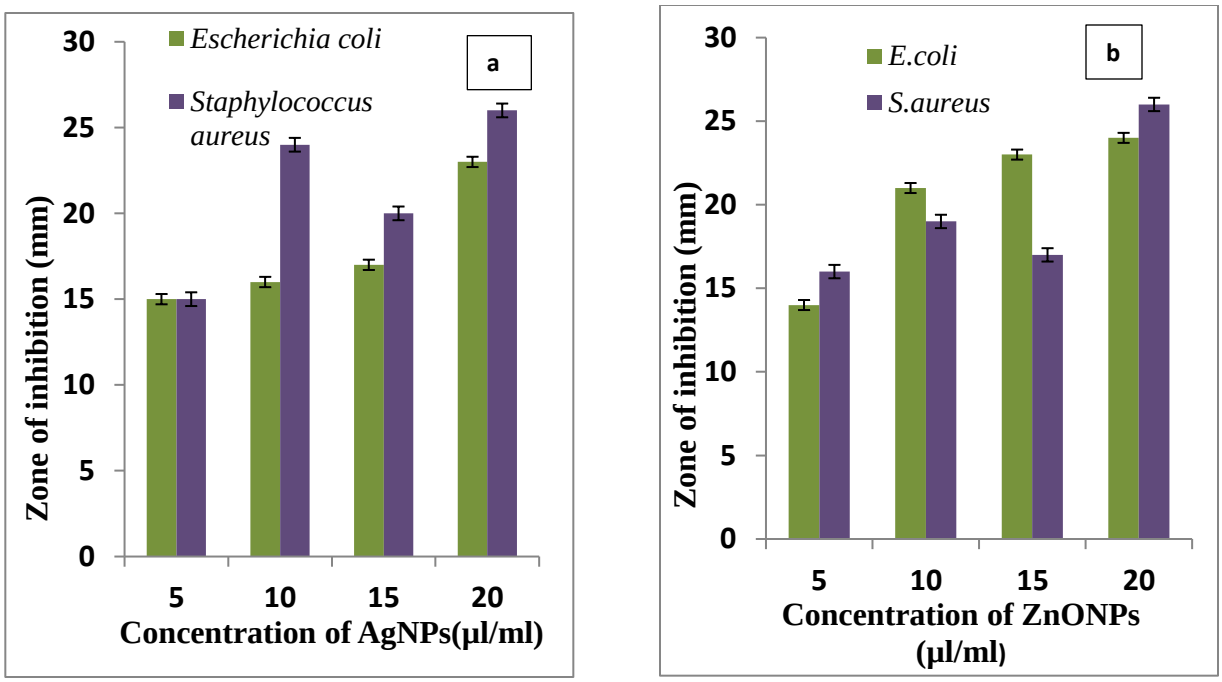

Figure 15 (a) Results of Zone of inhibition of silver nanoparticles (b) Results of zone of inhibition of ZnONPs nanoparticles

Antibacterial activity of nano-cream formulations

Cream formulations incorporating silver nanoparticles at concentration of (2%, 4% and 6%) and control cream (without nanoparticles) were loaded in agar wells to check antibacterial potential against *E.coli* and *S.aureus*. Antibacterial potential of formulations was estimated by measuring diameter of zone of inhibition. Control cream did not show any activity against both strains. Cream formulations of different concentrations exhibited good activity against both strains. Clear zones are shown in figure 16.

At different concentrations, the difference between measured zones for both strains was minimum. Results of measured zones of inhibition for each sample are mentioned in Table 3.

Table 3: Inhibition zone of nano silver cream

Formulated cream		Zone of inhibition (Diameter, mm) Mean±SD			
		E.coli (Gram –ve)	P-value	*Staphylococcus aureus* (Gram +ve)	P-value
Nanosilver cream concentration	2%	8.5±0.5	0.0015	13.13±0.32	0.0002
	4%	12.43±0.45		12.5±0.45	
	6%	14.13±0.32		14.33±0.35	
Control (without NPs)		Nil	-	Nil	-

By increasing the concentration of silver nanoparticles in cream, the antibacterial activity of cream was increased as shown in Figure 18. Silver nanoparticles incorporating cream exhibited same activity against both strains but exhibited highest (average) zone of inhibition for *S.aureus*.

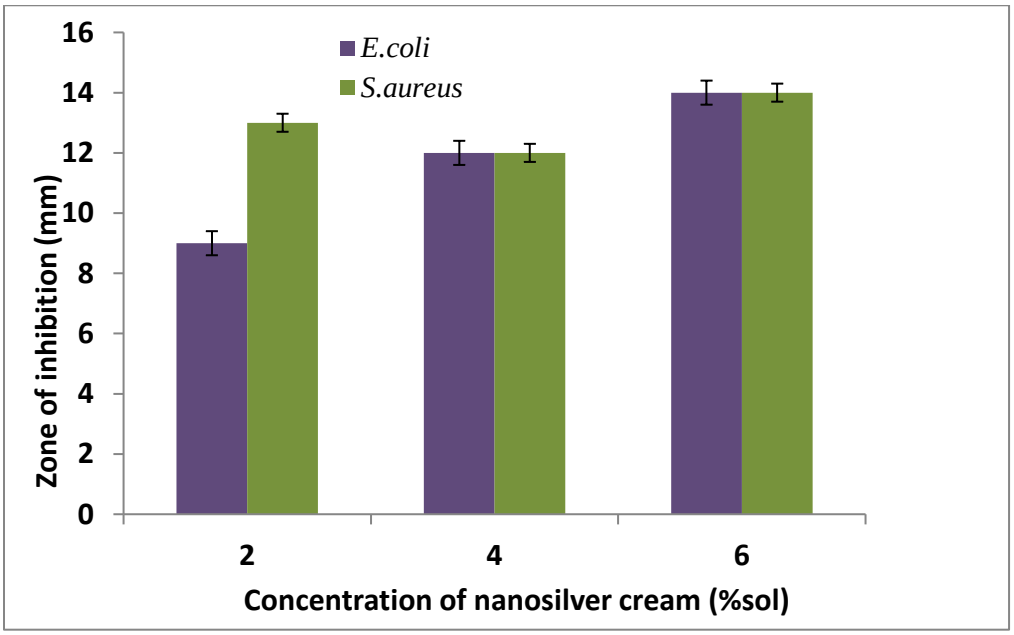

Figure 18 Results of zone of inhibition of nanosilver cream

Antibacterial activity of cream incorporating ZnO nanoparticles was also observed against *E.coli* and *S.aureus*. Clear zones were observed against both bacterial strains as shown in Figure 17.

It was noticed that zones of inhibition were dependent on concentration of nanoparticles as shown in figure 19. Diameter of zones of inhibition for each sample is mentioned in Table 4. Zinc oxide nanoparticles incorporating cream exhibited a little bit highest (average) zone of inhibition for S.aureus. In previous study, cream was prepared

using AgNPs and tested against skin pathogenic bacteria. In comparison to control, cream consisting AgNPs retarded the growth of all tested bacteria (Niraimathi, Sudha, Lavanya, & Brindha, 2013).

Table 4: Inhibition zone of ZnONPs incorporating cream

Formulated cream		Zone of inhibition (Diameter, mm) Mean±SD			
		E.coli (Gram –ve)	P-value	*Staphylococcus aureus* (Gram +ve)	P-value
ZnONPs incorporating cream	2%	13.4±0.45	0.0004	12.4±0.3	0.0003
	4%	15.5±0.43		16.43±0.45	
	6%	18.33±0.35		21.43±0.37	
Control (without NPs)		Nil	-	Nil	-

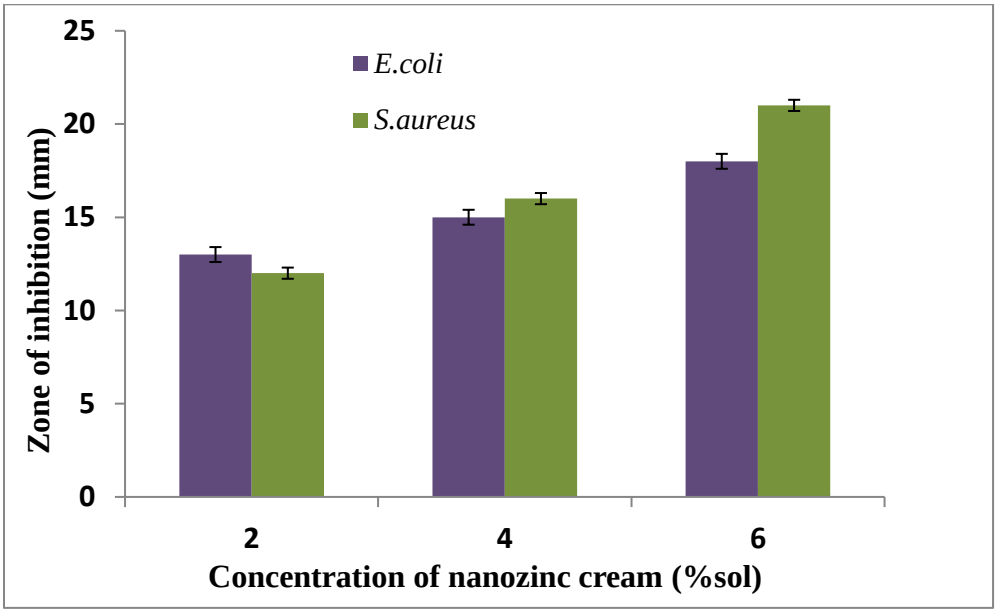

Figure 19 Results of inhibition zone of nanozinc cream

It was concluded that Zinc oxide nanoparticles incorporating cream exhibited better growth inhibiting effect against *E.coli* and *S.aureus* in comparison to silver nanoparticles incorporating cream. Both silver and zinc oxide nanoparticles exhibited better antibacterial action against gram +ve bacteria (*S.aureus*). Because they don't have outer membrane. As zinc oxide nanoparticles were more crystalline in nature.

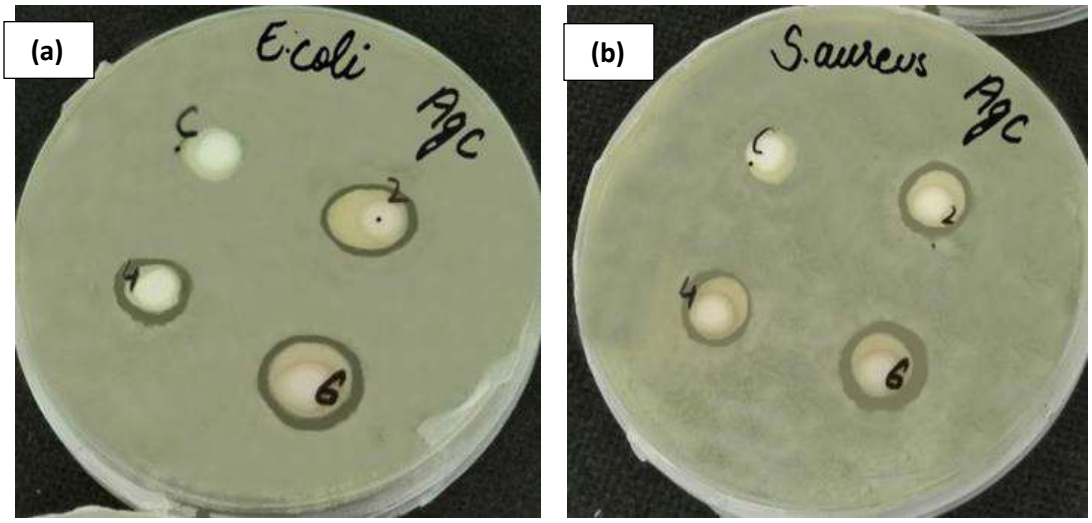

Figure 16: Antibacterial action of nanosilver cream (a) E.coli (b) S.aureus

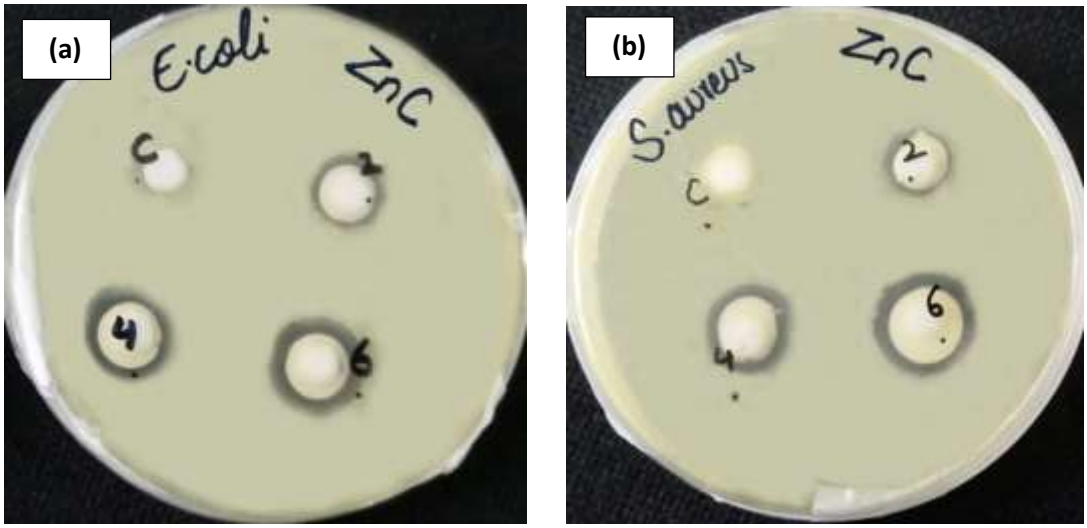

Figure 17: Antibacterial action of nanozinc cream (a) *E.coli* (b) *S.aureus*

Antifungal activity of nano-cream formulations

6% concentration of formulated cream samples incorporating silver and zinc oxide nanoparticles were used for antifungal testing against *Candida albicans*. Cream sample without nanoparticles was used as control. Antifungal potential of formulations was estimated by measuring diameter of zone of inhibition. Control did not show any activity. While cream samples of silver and zinc oxide nanoparticles exhibited good antifungal activity. Clear zones are shown in figure 20. In comparison to both cream samples, Zinc oxide nanoparticles incorporating cream exhibited better antifungal activity than nano silver cream.

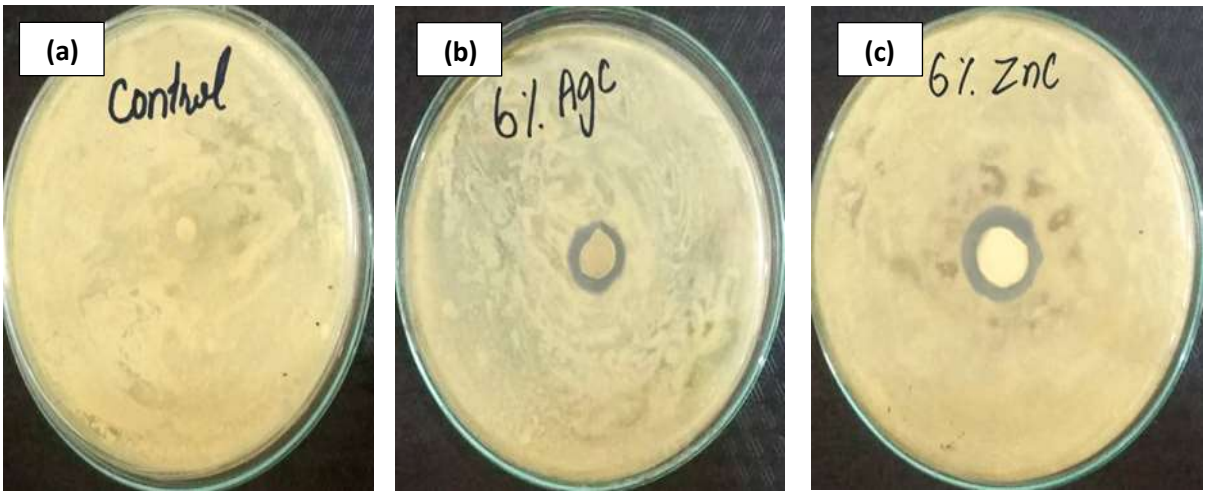

Figure 20 Antifungal activity of cream against *C.albicans* (a) Control (b) Nano silver cream (c) Nanozinc cream

Mean zone of inhibition for nanosilver cream was 7.33mm while for nanozinc cream was 11.53mm. Values of mean± SD of triplicates and p-value are shown in table 5.

Table 5: Inhibition zones of nanoparticles incorporating cream against fungus

Formulated cream	**Zone of inhibition (mm) Mean± SD**	**P-value**
	Candida albicans	
Nanosilver cream	7.33±0.25	0.0004
Nanozinc cream	11.53±0.30	0.0002
Control (without NPs)	Nil	Nil

ZnO's antifungal activity is caused by the distortion of fungal hyphae and retardation of conidiophore growth and results to the death of fungal hyphae. Cream was tested against skin *Candida* which is skin pathogen. Both silver and zinc oxide nanoparticles incorporated cream exhibited good antifungal activity against skin pathogens. Nanozinc cream exhibited better activity than nanosilver cream as shown in figure 21. It was concluded that nanozinc cream has more antifungal potential towards *C.albicans* than that of nanosilver cream.

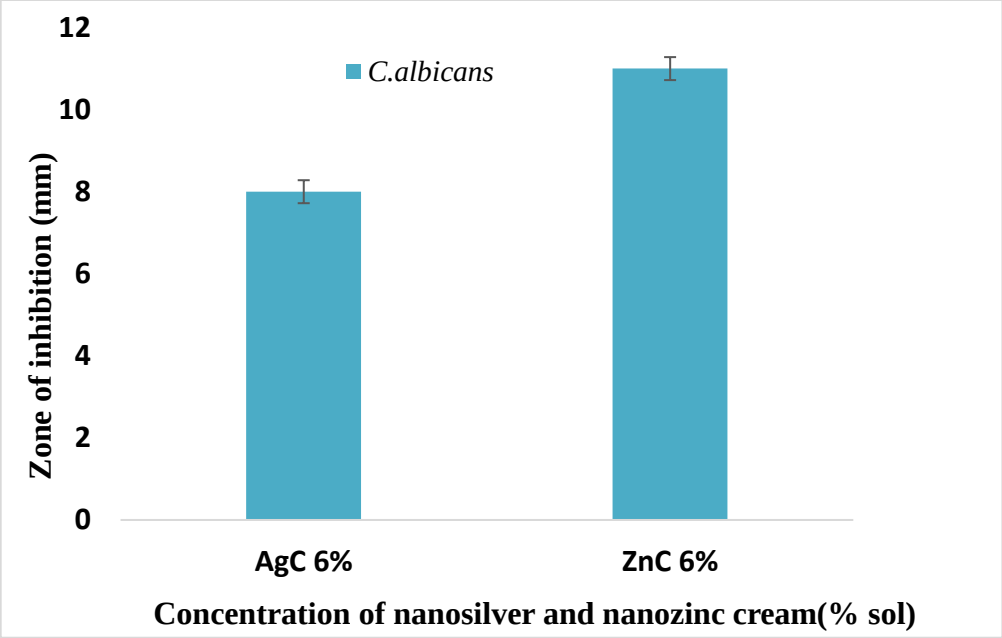

Figure 21 Results of antifungal activity of nanosilver and nanozinc cream

In previous study, cold cream was prepared using zinc oxide nanoparticles which represented exceptional antibacterial properties against *E.coli* and *S.aureus*. It also exhibited good antifungal properties towards *C.albicans*. ZnONps' antimicrobial action towards all skin pathogens that were used for test was increased in a dose-dependent way (S. S et al., 2017).

Conclusion

In present study silver and zinc oxide nanoparticles were green synthesized using ginger (*Zingiber officinale*) and garlic (*Allium sativum*) extract. TSC (Trisodium citrate) solution and metal salts (Silver nitrate and Zinc sulfate) were also used in combination with green extract for preparation of nanoparticles. TSC is green chemical as no serious or toxic effects have been reported yet. Silver nanoparticle exhibited maximum UV absorbance at 437nm that indicated the formation of silver nanoparticle. Synthesized zinc oxide nanoparticles exhibited maximum absorbance at 327nm that confirmed the formation of zinc oxide nanoparticles. Average particle size of silver nanoparticle was 11.52 nm. Average particle size of zinc oxide nanoparticles was 14.87 nm. XRD results indicated the synthesis of crystalline nanoparticles. Sharp peak was observed in FTIR spectrum at 446.21cm^{-1} which confirmed the presence of silver nanoparticles. While FTIR spectrum of zinc oxide nanoparticles represented strong and sharp peak at 442.31cm^{-1} which is characteristic peak that confirmed the presence of zinc oxide nanoparticles. Confirmed nanoparticles were used for production of antibacterial and antifungal cream. Silver and zinc oxide nanoparticles were incorporated in formulated cream separately at different concentrations (2%, 4% and 6%) and antibacterial activity was tested against *E.coli* and *S.aureus.* 6% concentration of formulated cream samples incorporating silver and zinc oxide nanoparticles were used for antifungal testing against *Candida albicans*. In comparison to both nanoparticles cream, Zinc oxide containing cream exhibited better antibacterial and antifungal

activity than nanosilver cream. It was concluded that synthesized nanoparticles were stable and crystalline and zinc oxide nanoparticles were more antibacterial and antifungal than silver. Better antibacterial activity was noticed by increasing the concentration of silver or zinc oxide nanoparticles in cream.

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