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NANOSILVER SYNTHESIS USING A GRAM NEGATIVE *PSEUDOMONAS AERUGINOSA* (MTCC 2453) AND ITS POTENTIAL APPLICATION ON URINARY TRACT INFECTING BACTERIA

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ABSTRACT
The use of gram negative bacteria like *Pseudomonas aeruginosa* in the synthesis of nanosilver emerges as an ecofriendly approach and an alternative to the chemical method. In the present attempt, the microbial mediated synthesis of silver nanoparticle (AgNPs) using a gram negative bacteria, *Pseudomonas aeruginosa*. Silver nanoparticles were synthesized Ag^{+} ion using the bacterial culture supernatants at room temperature. Synthesized nanosilver was observed visually by color change from pale yellow to brown which was confirmed by UV-vis spectroscopy. The AgNps were further characterized by FTIR, SEM, XRD and AFM analyses. The AgNps were found to be spherical in shape with a size in the range of 10-20nm. The synthesized nanosilver were found to have an antibacterial activity against four selected urinary tract infecting pathogenic bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Proteus vulgaris*. Thus, the biosynthesis of silver nanoparticles using a gram negative *Pseudomonas aeruginosa* deserves to be a good candidate as an antibacterial agent for UTI pathogens.

KEYWORDS: AgNPs, FTIR, XRD, SEM, AFM and UTI pathogens.

INTRODUCTION
There is an emerging strategy to develop more and more strains turn resistance to antibiotics and bactericides is an alarming concern in medical field. The pharmaceutical companies and the researchers are searching for developing novel antibacterial formulations. Development of new, cost effective antimicrobial agents has been an objective of the present research. Several researchers are investigating various alternative strategies to combat this problem. Gram negative pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus vulgaris* have been associated with increasingly resistant diseases of the urinary tract and cases of aggravated infection in burn and wound affected patients. Nanomedicine has been routed as the solution against the progressive increase in drug resistance. Nanoparticles exhibit a crucial role in inhibiting bacterial growth because of their high reactivity and the large surface to volume ratio.[1] Antibacterial activity of silver containing materials used in the medicine to diminish infections in burn treatments and arthroplasty, as well as to prevent bacterial colonization on prostheses, catheters, vascular grafts, dental materials, stainless steel materials and human skin. Silver containing materials can be employed to abolish microorganisms on textile fabrics or they can be used for water treatment. Due to the extensive progress and application of nanoparticles in miscellaneous field, a problem arises as to the relation between the environmental safety and useful properties of nanosystem. The synthesis of nano range metallic particles using biosystem has been explored for the production of silver nanoparticles. The antibacterial activity of silver is well documented and has been known to be non toxic to humans in low concentrations.[2] Extracellular synthesis is preferred due to the ease of control over the process, the possibility of large scale synthesis and easy downstream processing.[3] Biologically synthesized nanoparticles are naturally protein capped which prevents aggregation and avoids the use of external toxic capping agents. Therefore, biological approaches to nanoparticle synthesis have been suggested as valuable alternatives to physical and chemical methods. The synthesis method reported in this work might be helpful for providing an economic route for the large scale production of highly stable and biologically compatible silver nanoparticles. In the present attempt, bacterial mediated fabrication of SNPs by the reduction of silver nitrate salt using *Pseudomonas aeruginosa* (MTCC 2453). The fabricated SNPs were characterized via UV, XRD, SEM, EDS, FTIR and AFM. Finally, the fabricated SNPs were applied on

urinary tract infecting pathogens and its synergistic effect with broad spectrum antibiotics.

MATERIALS AND METHODS

MATERIALS

Silver nitrate ($AgNO_3$), nutrient broth and nutrient agar, purchased from Himedia (P) Ltd., Mumbai were used as starting materials without further purification. Sterile milli Q water was used throughout the experiment.

Source of microorganisms

Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris were procured from the Microbial Type Culture Collection, Chandigarh, India. All the bacterial cultures were subcultured and maintained in nutrient agar medium at monthly intervals.

METHODS

Synthesis of silver nanoparticles

The extracellular biosynthesis of silver nanoparticles using *Pseudomonas aeruginosa* was followed according to the method.[4] Nutrient broth was prepared, sterilized and inoculated with a fresh culture of *Pseudomonas aeruginosa*. The culture flask was incubated at 37^0C for 72 hrs in an orbital shaker at 150 rpm. After the incubation period, the culture was centrifuged at 12,000 rpm for 5 min and the supernatant was used for the synthesis of silver nanoparticles. 150ml of *Pseudomonas aeruginosa* culture supernatant was added separately to the reaction vessels containing silver nitrate at a concentration of 0.1 g/l. The reaction between these supernatant and silver ions was carried out in bright conditions for 72 hrs.

Characterization of nanoparticles

Visual observation

After 72 hrs of incubation, the preliminary detection of silver nanoparticles was carried out by visual observation of color change of culture filtrate.

UV –Vis spectrophotometer

The resultant sample was further subjected to optical measurement, which was carried out by UV-Vis spectroscopy of the solution following the method.[5] 3ml of sample was taken and the spectral analysis was recorded at room temperature using a 1cm quarts cuvette. The silver nanoparticles were measured in a wavelength ranging from 200-1100nm. UV-Vis spectroscopy measurements of silver nanoparticles were recorded on Shimadzu dual beam spectrophotometer (model UV-1650 PC) operated at a resolution of 1nm.

FTIR

FTIR spectral analysis of the synthesized silver nanoparticle was carried out by the following method.[6] The bio-transformed product present in cell-filtrate were freeze-dried and diluted with potassium bromide in the ratio of 1:100. FTIR spectrum of samples was recorded in Nicolet Impact 400FT-IR spectrophotometer instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurement was carried out in the range of 400-4000 cm^{-1} at a resolution of $4cm^{-1}$.

XRD (X-ray diffraction)

The synthesized silver nanoparticles solutions were air dried, powdered and used for XRD analysis.[7] X-ray diffraction pattern was measured in the scanning mode on an X' PERT-PRO analytical instrument operated at 40KV and a current of 30 mA with cu Kα radiation in Alagappa University, Karaikudi. The diffraction intensities were recorded from 100 to 790 in 2θ angles. The diffraction intensities were compared with the standard JCPDS files. The software gave the information about the crystal structure of the particles. The average size of the particle can be estimated using the Debye Scherrer equation.

The size of the silver nanoparticles was made from the line broading of the (111) reflection using the following Debye-Scherrer's formula.

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

Where D = thickness of the nanocrystals,

K = Constant,

λ = Wave length of x-rays

β = Width of half maxima of reflection at Bragg's angle 2θ,

θ =Bragg's angle.

Scanning electron microscopy

Morphology of the biosynthesized AgNPs was examined using scanning electron microscopy method.[8] SEM analysis was carried out using JEOL-6390 SEM instrument at Bharathiar University Coimbatore. The sample was prepared on a carbon coated grid by dropping a small amount of the sample and then allowed to dry prior measurements. The synthesized silver nanoparticles can be calculated by using the scale provided in the micrograph.

Atomic Force Microscopy

After an incubation of about 72 hours, the filtrate containing silver nanoparticles were characterized using atomic force microscopy. AFM of silver nanoparticles was measured according to the method.[9] To know the exact particle size and nano size effect the samples were characterized using Atomic Force Microscopy (Nanonics imaging MN1000) which measures the atomic range of particles using tapping mode.

Antibacterial activity of silver nanoparticle against UTI pathogens

Antibacterial assay

The antibacterial assay of silver nanoparticles was evaluated using agar diffusion method.[10] Pure cultures were subcultured in nutrient broth for 12h at 37^oC. A 20 ml volume of Mueller Hinton agar medium was poured into each petriplate on horizontally levelled surface. The test strains such as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli,* and *Proteus vulgaris* were swabbed uniformly into the individual plates using sterile

cotton swabs. Wells of 6mm diameter were made onto each bacterium inoculated agar plates using sterile gel puncture. 100µl of silver nanoparticles suspensions was introduced into the corresponding wells. The bactericidal activity was determined by a clear inhibition zone around the sample loaded wells after incubation of plates overnight at 37°C.

Synergistic effect of silver nanoparticle

Synergistic effect of silver nanoparticles was studied by the method.[11] To assay the synergistic effect of silver nanoparticles with commonly used antibiotics was adopted to test the bactericidal efficacy of these nanoparticles alone and in combination with antibiotics. To determine the synergistic effects, each standard antibiotic discs namely amikacin, streptomycin, vancomycin and ampicillin, were impregnated with 100µl of freshly prepared silver nanoparticles and was placed onto the MHA medium inoculated with test organisms. Standard antibiotic discs were used as positive control. These plates were incubated overnight at 37°C. After incubation, results were recorded by measuring the inhibitory zone diameter (mm).

Activity Index

The inhibition zones were measured and compared with the standard reference antibiotics. The activity index for each sample was calculated by using the formula[12],

$$\text{Activity Index (AI)} = \frac{\text{Inhibition zone of the samples}}{\text{Inhibition of the standard}}$$

Assessment of increase in fold area

The increase in fold area was assessed by calculating the mean surface area of the inhibition zone generated by an antibiotic alone and in combination with AgNPs. The fold increase area was calculated by the following equation[13],

$$\text{Fold area increase} = b^2 - a^2/a^2$$

Where a and b refers to the zone of inhibition for antibiotic alone and antibiotic with AgNPs respectively.

Statistical analysis

The obtained data in the present experiment were presented as mean ± standard deviation. A computer program (excel 2010) was used for statistical analysis. The Two way-ANOVA test was performed to examine the difference among the groups. A P value of <0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

In the present experiment, the gram negative bacterium *Pseudomonas aeruginosa* was found successful in synthesizing silver nanoparticles. The yellowish brown colour formed at 72 hours of incubation time which confirm the reduction of silver nitrate into silver nanoparticles. Several physical and chemical methods exist for this nanoparticle synthesis.[14] However recently there has been an increasing interest in developing and promoting an eco-friendly route for their synthesis. This involves utilizing biological system like bacteria, fungi, which show the ability to reduce metal ions to metallic nanoparticles. Fungi were widely used as tools for this purpose and are more advantageous in both processing as well as handling the bio-mass.[15]

Earlier studies[6] reported that an aqueous silver nitrate ion was reduced during exposure to the *Fusarium oxysporum* cell filtrate. The colour of the reaction mixture changed from pale yellow to brown, which indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in water due to excitation of surface plasmon vibration in metal nanoparticles. The reduction of silver ions into silver nanoparticles using bacterial cultures was evidenced by the visual change of colour from yellow to reddish brown.[16] In the present experiment, the appearance of yellow to brown colour precipitate in the reaction mixture indicates the formation of silver nanoparticles (Fg.1). Brown precipitate was appeared after 72 h. NADPH-dependent nitrate reductase enzyme function as a reducing agent to reduce the silver nitrate solution to produce the AgNPs. Biologically synthesized silver nanoparticles in the reaction solution is taken and air dried to get a powder form silver nanoparticles.

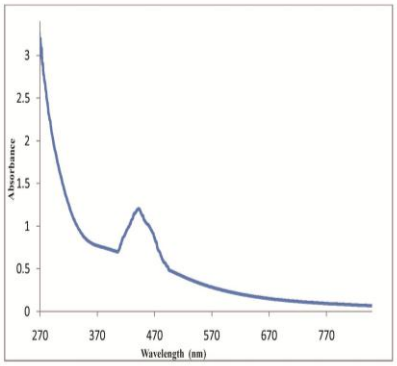
Fig.1: Visual inspection of silver nanoparticles synthesized by *Pseudomonas aeruginosa*

Characterization of nanoparticles

UV-Vis spectroscopy of silver nanoparticles

The UV-Vis spectrum of the silver nanoparticles showed at 440nm (Fig.2) which indicated the formation of silver nanoparticles. The observed band was corresponding to the surface plasmon resonance at 410 to 420 nm.[16]

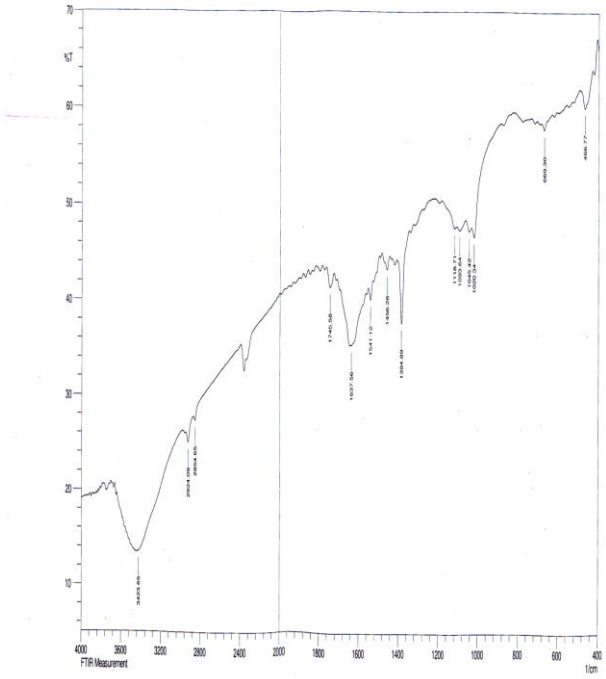
Fig. 2: UV-Vis Spectra of Silver Nanoparticles synthesized by *Pseudomonas aeruginosa*



The strong peak observed in the present attempt was at 420 nm. Similarly, in earlier reports[17] there was a strong peak at 420nm. Corresponding to the surface plasmon resonance of the synthesized silver nanoparticles the UV-Vis spectral band at 410 to 430 nm was reported earlier.[18]

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR aids in identifying the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles. The dried samples of silver nanoparticles were recorded on Perklin Elmer instrument. The FTIR spectra of silver nanoparticles with absorption peaks 3444.87, 2964, 1649 and 1558.48 cm^{-1} for *Pseudomonas aeruginosa* are seen in Fig 3. FTIR spectrum of silver nanoparticles band at 3444.87 and 2964.59cm^{-1} were assigned to the stretching vibrations of primary and secondary amines. The peak at 1649.14 cm^{-1} was identified as the C-C alkene stretching. The bands at 1558.48 cm^{-1} is characteristic of N-H amine salt and β diketone. Earlier reports[19] suggested that the band observed at 3338.5cm^{-1} were attributed to stretching vibrations of the primary and secondary amines respectively while corresponding band vibrations were observed at 1635$cm^{-1}$and 1186cm^{-1}. Earlier studies[20] revealed that the representative spectra of nanoparticles obtained manifest absorption peaks located at 3843.68cm^{-1} (-NH group of amines), 3597.73cm-^{1} (-OH group of phenols), 2080.65cm^{-1} (aromatic – CH stretching), 1631.66 cm^{-1} (- NHCO of amide) and 767.16cm^{-1} (C – CI). The FTIR spectrum reveals the bands at 1633 (3) and 1554 cm^{-1} are identified as the amides I and II which arises due to carbonyl stretch and –N-H stretch vibrations in the amide linkages of the proteins, respectively.[21] The band at 1423(5) cm^{-1} is assigned to methylene scissoring vibration from the proteins in the solution. In the present experiment FTIR spectrum of silver nanoparticles band at 3444.87 and 2964.59cm^{-1} were assigned to the stretching vibrations of primary and secondary amines. The band at 1649.14cm-1 is identified as the C-C alkene stretching. The band at 1558.48 cm^{-1} is characteristic of N-H, amine salt and β diketone.



Wave number (cm^{-1})

Fig.3: FTIR Spectra of silver nanoparticles synthesized by *Pseudomonas aeruginosa*

XRD (X-ray Diffraction)

An XRD pattern obtained for the silver nanoparticles revealed that a number of Bragg's reflections corresponding to (111), (200), (220), (311) sets of lattice planes was observed (Fig.4). The XRD pattern thus clearly indicates that the silver nanoparticles are crystalline in nature. The diffraction peaks at 2θ value of 31.70^0. From the lattice plane and three additional broad bands are observed at 45.43^0 (2θ), 66^0 (2θ) and 75.25^0 (2θ) they corresponds to the (200), (220), and (311) planes of biologically synthesized silver nanoparticles. In the obtained spectrum, the Bragg's peak position and their intensities were compared with the standard JCPDS files. The crystalline size of the nanoparticles was calculated using Scherrer's formula and the size of the synthesized nanoparticles was found to be 27 nm.

Debye Scherrer's formula (D) = Kλ / β cosθ

Diffraction Bragg's angle 2θ = 38.20

θ = 13.7

D = 0.94 X 1.5406 X 10-^{10} X 180 /0.33 X COS13.7 X 3.4

D = 27.56 X 10-^{10}

Earlier reports[6] said that the XRD analysis showed three distinct diffraction peaks at 38.28^0, 44.38^0, 64.54^0, and 77.64^0 and can be indexed 2θ values of (111), (200), (220), (311) crystalline planes of cubic Ag. Earlier studies[8] reported that the absorption peak of SNPs was observed at 2θ values of 38.28^0, 44.27^0, 64.64^0, and 77.66^0. The XRD pattern of biosynthesized AgNPs revealed that the silver peaks noted at 2θ values of 37.8^0,

44.1^0, 62.9^0, 75.9^0.[22] In the present study, the diffraction peak at 31.70^0. From the lattice plane and three additional broad bands are observed at $45.43^0(2\theta)$, $66^0(2\theta)$ and $75.25^0(2\theta)$ they corresponds to (200), (220) and (311) planes.

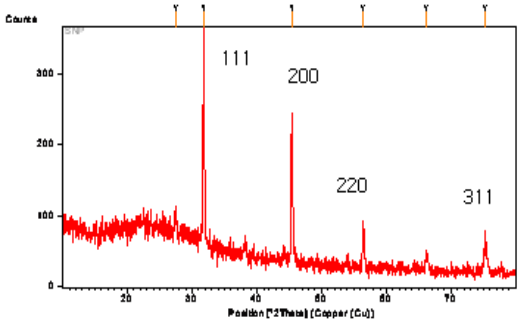


Fig. 4: X- ray Diffraction pattern of silver nanoparticles synthesized by *Pseudomonas aeruginosa*

Scanning Electron Microscope

SEM images observed in the present study were spherical, pseudo spherical and some undefined morphology with traces of agglomeration (Fig.5). The particle size ranges from 20-100nm and possesses an average size of 50 nm. Earlier studies[18] reported that the morphology of the SEM images having spherical, pseudo spherical and some undefined morphology with traces of agglomeration because of biological molecules bind with nanoparticles present in the bacteria. The SEM micrograph showed that the nanoparticles were in aggregate form.[6] Earlier reports[16] said that the biosynthesized SNPs are small and spherical in shape. In the present experiment, biosynthesized silver nanoparticles are spherical in nature and some undefined morphology with traces of agglomeration.

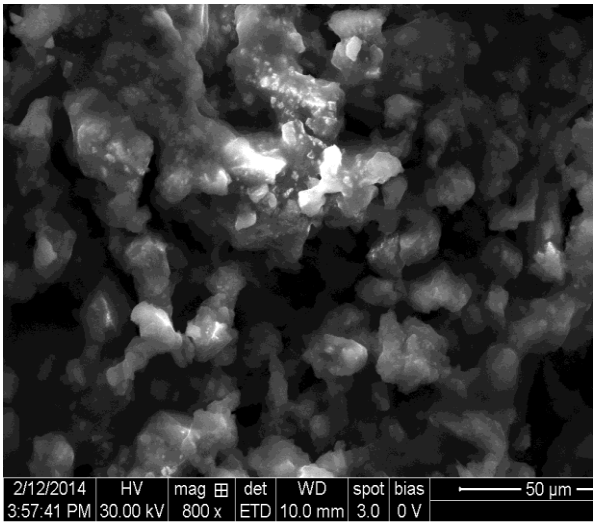


Fig.5: Scanning Eelectron Micrograph of silver nanoparticles synthesized by *Pseudomonas aeruginosa*

Atomic Force Microscopy

The surface morphology and size of the synthesized silver nanoparticles was exhibited spherical in shape, smooth surface and the three dimensional view reveals uniform size and shape of the silver nanoparticles (Fig. 6). AFM data obtained in the present research revealed that the three dimensional image of silver nanoparticles size was 25 nm. In contrary to this, the earlier studies[23] observed that 75 nm sized nanoparticles. The observed particles were spherical in shape, smooth surface and monodispersity in nature under optimized condition for the production of silver nanoparticles.[21]

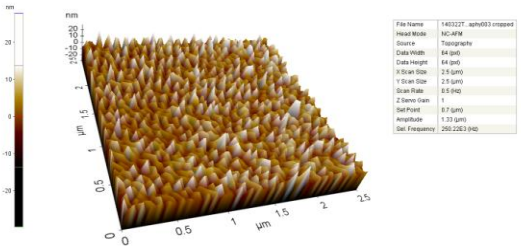


Fig. 6: Three dimensional AFM image of silver nanoparticles synthesized by *Pseudomonas aeruginosa*

Antibacterial activity of biologically synthesized silver nanoparticles against UTI pathogens

The antagonistic activity of biologically synthesized silver nanoparticles has been investigated against UTI pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Proteus vulgaris*. The zone of inhibition in diameter (mm) of silver nanoparticles against UTI pathogens is represented in Table 1. *Escherichia coli* have the highest zone of inhibition 28 nm compared to the other UTI pathogens. Earlier studies[24] reported that the antimicrobial activity of silver nanoparticles on gram-negative bacteria was dependent on the concentration of Ag nanoparticles and was closely associated with the formation of pits in the cell wall of bacteria. Then Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. The efficacy of mycogenic metal nanosilver to kill MDR strains which is difficult through the conventional chemotherapy.[21] It is reasonable to state that the binding of particles to the bacteria depends on the surface area available for interaction. Smaller particles having the larger surface area available for interaction will give more bactericidal effect than the larger particles. Nanosilver may also penetrate inside the bacteria and cause damage by interacting with phosphorous and sulfur-containing compounds such as DNA.[25] In the present study, the AgNPs were effectively inhibited the bacterial growth. Biosynthesized silver nanoparticles showed high inhibitory effect (23.62

± 1.82mm) on the growth of *E.coli* and the moderate inhibitory effect (20.16 ± 1.12mm) on the growth of *Klebsiella pneumoniae.*

Table 1: Zone of Inhibition in (mm) of silver nanoparticles synthesized by *Pseudomonas aeruginosa* against UTI pathogens

S.No.	UTI Pathogens	Zone of inhibition (mean± SD)
1	*Staphylococcus aureus*	14.60 ± 0.98
2	*Proteus vulgaris*	10.03 ± 0.78
3	*Escherichia coli*	23.62 ± 1.82
4	*Klebsiella pneumoniae*	20.16 ± 1.12

Synergistic effect of silver nanoparticles

The synergistic effect of silver nanoparticles synthesized by *Pseudomonas aeruginosa* is given in Table 2. It revealed that the distinct difference was observed between the inhibitory zones by antibiotics with and without silver nanoparticles. The zone of inhibition was increased from 6.18 ± 0.08 to 22.20 ± 1.18 and 6.46 ± 0.05 to 23.42 ± 1.94 mm when the silver nanoparticles are incorporated with streptomycin antibiotic against *Staphylococcus aureus* and *Proteus vulgaris* respectively. An extended zone of inhibition recorded in the study depends on the concentration of silver nanoparticles as well as on the initial bacterial load. The highest fold increase (16.36 ± 1.02) was observed against *Klebsiella pneumoniae*. Followed by this, 12.14 ± 1.02 fold increases was observed while using silver nanoparticles and streptomycin antibiotic together against *Proteus vulgaris.* The two way ANOVA for the data on fold increase of silver nanoparticles against selected bacterial pathogens revealed that the obtained data are statistically significant between antibiotics with silver nanoparticles and UTI pathogens (F= 2.31 and 0.41; table value 3.86 and 3.86; P > 0.05). Zone of inhibition showed an increased activity index for all the cases with a range of 1.150 ± 0.019 to 3.630 ± 0.029. The highest activity index (3.630 ± 0.029) was observed against *Proteus vulgaris.* Followed by this, 3.590 ± 0.028 activity index was observed while among silver nanoparticles streptomycin antibiotics together against *Staphylococcus aureus* (Table 3).

Table 2: Synergistic effect of selected broad spectrum antibiotics with and without silver nanoparticles against UTI pathogens

Pathogens	Antibiotics (µg/disc)	Zone of inhibition (mm)		Increased zone size(mm)	Fold Increase
		Antibiotics alone	Antibiotics +AgNPs		
E.coli	Amikacin (30 µg)	10.07±0.94	25.90±1.67	15.83±1.46	4.860±0.340
	Streptomycin (10 µg)	15.33±1.37	19.17±1.18	03.84±0.02	0.560±0.004
	Vancomycin (30 µg)	10.00±0.50	11.48±0.68	01.48±0.01	0.320±0.002
	Ampicillin (10 µg)	8.90±0.09	28.65±1.82	19.75±1.14	9.360±0.834
Staphylococcus aureus	Amikacin (30 µg)	16.10±1.12	23.67±1.24	07.57±0.05	1.160±0.008
	Streptomycin (10 µg)	06.18±0.08	22.20±1.18	16.02±1.12	11.90±0.082
	Vancomycin (30 µg)	10.67±0.94	24.17±1.82	13.50±1.22	04.13±0.026
	Ampicillin (10 µg)	12.00±1.00	25.90±1.68	13.90±1.24	03.66±0.021
Proteus vulgaris	Amikacin (30 µg)	16.67±1.48	24.93±1.64	08.26±0.62	01.24±0.002
	Streptomycin (10 µg)	06.46±0.05	23.42±1.94	16.96±0.96	12.14±1.02
	Vancomycin (30 µg)	10.24±0.52	25.24±1.86	15.00±0.84	05.08±0.02
	Ampicillin (10 µg)	12.48±1.20	24.98±1.92	12.50±1.12	03.01±0.022
Klebsiella pneumoniae	Amikacin (30 µg)	07.78±0.06	16.26±1.06	08.48±0.68	03.37±0.024
	Streptomycin (10 µg)	11.48±0.68	26.12±1.86	14.64±0.86	04.18±0.028
	Vancomycin (30 µg)	15.67± 0.47	19.87±1.14	04.20±0.24	00.61±0.004
	Ampicillin (10 µg)	05.53±0.05	23.04±1.84	17.51±0.94	16.36±1.48

Table 3: Activity index of synthesized SNPs in combination with and without different antibiotics against UTI pathogens

Antibiotics	Activity Index/Pathogens			
	E.coli	*Proteus vulgaris*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Amikacin (30 µg)	2.570 ± 0.015	1.490 ± 0.024	2.090 ± 0.015	1.470 ± 0.018
Streptomycin (10 µg)	1.250 ± 0.007	3.630 ± 0.029	2.280 ± 0.018	3.590 ± 0.028
Vancomycin (30 µg)	1.150 ± 0.019	2.460 ± 0.017	1.270 ±0.052	2.270 ± 0.014
Ampicillin (10 µg)	3.220 ± 0.020	2.000± 0.014	4.170 ± 0.022	2.160 ± 0.026

The two way ANOVA for the data on fold area increase of silver nanoparticles against UTI pathogens revealed that the obtained data are statistically significant between antibiotics with silver nanoparticles and bacterial pathogens (F= 15.1 and 11.1; table value 3.84 and 3.46; P < 0.005). The zone of inhibition of these antibiotics against UTI pathogens were measured separately and along with nanoparticles showed significant increase in fold area in all the cases with amikacin, streptomycin, vancomycin and ampicillin. Similarly, earlier study[26] reported that an increase in synergistic effect may be caused by the bonding reaction between antibiotic and nanosilver. The antibiotic contains many active groups such as hydroxyl and amido groups, which react easily with nanosilver by chelation. Earlier studies[27] reported that the Silver is known to have broad spectrum of antimicrobial activity against bacteria, vruses and eukaryotic microorganisms. Earlier reports[28] said an effective antimicrobial activity of AgNPs against *E.coli* and *Staphylococcus aureus* and its synergistic effect showed corroboration with the earlier reports[29] who reported the increase in antibacterial activities of PenicillinG, amoxicillin, erythromycin, clindamycin and vancomycin in combination with the mycosynthesized AgNPs against *E.coli, Pseudomonas aeruginosa* and Staphylococcus *aureus.* Early studies[30] also reported that an increase in the antibacterial activities of ampicillin, erythromycin and chloromphenicol, incombination with AgNPs against *S.typhi, E.coli, S.aureus* and *Micrococcus luteus.*

CONCLUSION

The present results clearly emphasize the biobased approach towards the synthesis of SNPs has many advantages such as ease with which the process can be scaled up and economic viability. Applications of nanoparticles in medical and other fields make this method potentially use for the large-scale synthesis of other inorganic nanomaterials. Narrow size distribution and small nanosize silver particles also offer advantages for self-assembled monolayer formation and enhanced surface area. Silver colloidal solution is biologically well suited and has the potential to be used in medical and pharmaceutical applications due to their homologous size distribution.

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