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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *GNIDIA GLAUCA* AND COMPUTATIONAL EVALUATION OF SYNERGISTIC POTENTIAL WITH ANTIMICROBIAL DRUGS

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ABSTRACT

Phytochemical diversity of medicinal plants makes them most suitable for synthesizing and stabilizing metal nanoparticles used for biomedical applications. Herein, we report an environmentally friendly, rapid and extremely efficient green synthesis of silver nanoparticles (AgNPs) using *Gnidia glauca* flower, leaf and stem extract. Rapid synthesis was monitored by UV–vis spectroscopy and characterized by high resolution transmission electron microscopy (HRTM), energy dispersive spectroscopy (EDS), dynamic light scattering (DLS) and X-ray diffraction (XRD) which revealed monodisperse AgNPs in a size range of 10 to 100 nm were predominant. Fourier transform infrared (FTIR) spectra indicated the mechanism of the synthesis and the role of the functional groups of the diverse phytochemicals involved in bioreduction and stabilization process. Further, the antibacterial synergy between AgNPs and diverse groups of antibiotics like *β*-lactam, chloramphenicols, aminoglycosides, tetracyclines, and glycopeptides showed greater bactericidal efficiency when combined with nanosilver. Streptomycin and chloramphenicol in combination with AgNPs showed marked synergism as

compared to others that supported our observed results *in-vitro* that was further studied by computational analysis.

KEYWORDS: *Gnidia glauca*, silver nanoparticles, high resolution transmission electron microscopy, X-ray diffraction, fourier transform infrared spectroscopy, antibacterial synergy.

INTRODUCTION

Medicinal plants are used as complementary and alternative medicine from ages owing to their diverse phytochemistry. Various polyphenols, flavonoides, terpenoids, alkaloids and saponins are isolated, identified and are being used as potential bioactive agents against cancer, diabetes, bacterial and viral infections.[1-13] Medicinal plants have got wide attention in the field of nanobiotechnology as well as it is treasure of reducing and stabilizing chemicals required for biofabrication of nanoparticles. Recently, we have reported the potential of *Dioscorea bulbifera*, *Dioscorea oppositifolia*, *Plumbago zeylanica*, *Gloriosa superba*, *Barleria prionitis* and *Litchi chinensis* for synthesis of gold, silver, copper, platinum and palladium nanoparticles.[14-20] Synthesis of nanoparticles using medicinal plant has always been advantageous as it is an environmentally benign process since toxic and hazardous chemicals are not used in such processes. Further, it is rapid, cost effective and one-pot synthesis where more biocompatible and less toxic nanoparticles are achieved which are ideal for biomedical applications. Among various metals, silver nanoparticles (AgNPs) have wide applications in the field of biology and medicine due to their strong inhibitory and bactericidal effects. It's broad spectrum activity includes anti-fungal, anti-inflammatory, anti-viral and anti-angiogenesis potential.[21]

Although many plants are reported to have nanobiotechnological potential, till date there are no reports on synthesis of AgNPs using *Gnidia glauca* which is one of the endemic medicinal plant with profound therapeutic significance. In African medicine it is used for treatment of abdominal pain, cancers, wounds, snake bites, sore throat and burns.[22,2] Spectacular success of our earlier reports on antidiabetic, antioxidant potential along with its detailed phytochemical analysis strongly supports its probable role in phytogenic nanoparticle synthesis. Although, recently we have reported its ability to synthesize gold nanoparticle (AuNPs) with catalytic potential, no reports are available on its role in synthesis of other metallic nanoparticles.[22,23] In view of the background, herein, we report for the first time the synthesis of AgNPs using *G. glauca* flower, leaf and stem which was monitored by UV-visible spectroscopy. Further, we also investigated the effects of reaction conditions such as

reaction temperature and $AgNO_3$ concentration on the rate of synthesis. The biogenic AgNPs were characterized employing high resolution transmission electron microscopy (HRTEM), energy dispersive spectroscopy (EDS), dynamic light scattering (DLS) and X-ray diffraction (XRD). Fourier transform infrared spectroscopy was used to explore the underlying mechanism of the biosynthesis. A detailed synergistic study was carried out between the AgNPs and twenty two antibiotics belonging to diverse groups against fourteen different bacteria and confirmed using computational tool.

MATERIALS AND METHODS

Plant material and preparation of extract

Gnidia glauca flowers, leaves and stem were collected and dried for 2 days at room temperature followed by grinding them into fine powder in an electric blender. Extracts of flower (GGFE), leaf (GGLE) and stem (GGSE) were prepared by taking 5g of respective plant powder in a 300 mL Erlenmeyer flask with 100 mL of distilled water and boiling for 5 min. The extract obtained was filtered through Whatman No.1 filter paper and stored at 4°C for further use.

Synthesis and characterization of AgNPs

AgNPs were synthesized by addition of 5 mL each of GGFE, GGLE and GGSE to 95 mL of 10^{-3} M aqueous $AgNO_3$ solution separately. The reduction of the Ag^+ ions was monitored by recording the UV-vis spectra of the solution at regular intervals. Reaction conditions were optimized by varying the temperatures between 4-50 °C and concentration of $AgNO_3$ solution between 0.3 - 5 mM. Bioreduced AgNPs were characterized by high resolution transmission electron microscopy (HRTEM), energy dispersive spectroscopy (EDS), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) as per our earlier reports.[19,20]

Bactericidal activity and evaluation of combined effect

Antimicrobial activity of AgNPs synthesized by GGFE, GGLE and GGSE were checked against 14 different bacteria as described earlier.[13] In short, AgNPs and standard antibiotics, individually and in combination were assessed by disk diffusion method on Muller-Hinton agar plates. Combined effects were analysed by impregnating each disc with 100 µg of different antibiotics and 50 µg of AgNPs. After overnight incubation at 37°C the zones of inhibition were measured. Computational analysis was carried out to confirm the synergistic response.

RESULTS

UV-visible spectroscopy

Synthesis of AgNPs by GGFE, GGLE and GGSE was confirmed by the visible color change from colorless to brown. Similarly, in UV-visible spectra the peak formation as a function of time at the signature absorbance maxima of AgNPs further confirmed the synthesis of AgNPs (Fig. 1). It is important to note that the synthesis was complete within 5 h as no increase in the peak intensity was observed even after further incubation. Rate of AgNPs synthesis with GGFE was found to be less till 2 h which increased significantly at 3h and almost completed by 4 h showing a very slight increase at 5 h (Fig. 1A). In case of GGLE, synthesis was at a lower rate till 3 h which increased slightly till 4 h that gradually increased significantly completing the synthesis by 5 h (Fig. 1B). GGSE showed a fairly uniform increase in the rate of synthesis of AgNPs beyond 1 h completing the reaction by 5 h similar to the other extracts. Optimization studies showed the best temperature and concentration of $AgNO_3$ at which maximum synthesis of AgNPs was achieved. The rate of synthesis was found to increase with the increase of concentration. However, 3 mM of $AgNO_3$ was found to be optimum for synthesis of AgNPs (Fig. 2). Similarly, rise in temperature enhanced the rate of synthesis of AgNPs. 50 °C was found to be most suitable temperature showing highest rate of synthesis (Fig. 3).

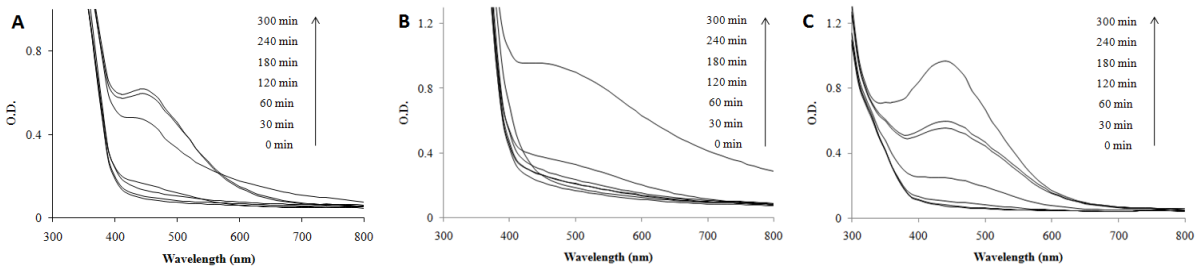

Figure 1: UV-vis spectra recorded as a function of reaction time for AgNPs formation using (A) GGFE; (B) GGLE and (C) GGSE at 40°C with 1mM $AgNO_3$ solution.

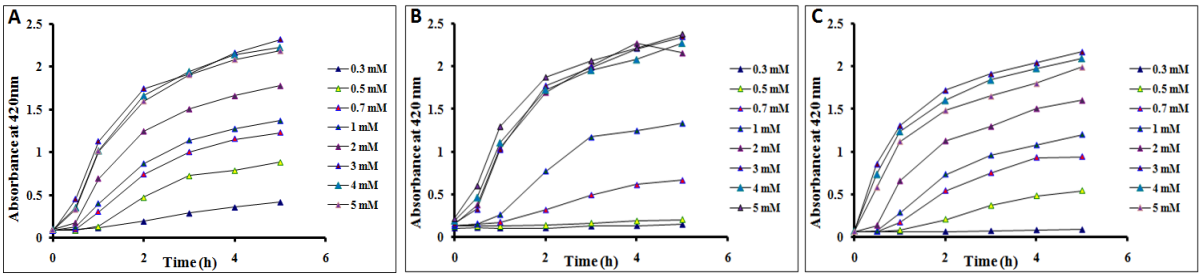

Figure 2: Time course of AgNPs synthesis at 40 °C with different concentrations of $AgNO_3$ by (A) GGFE; (B) GGLE and (C) GGSE.

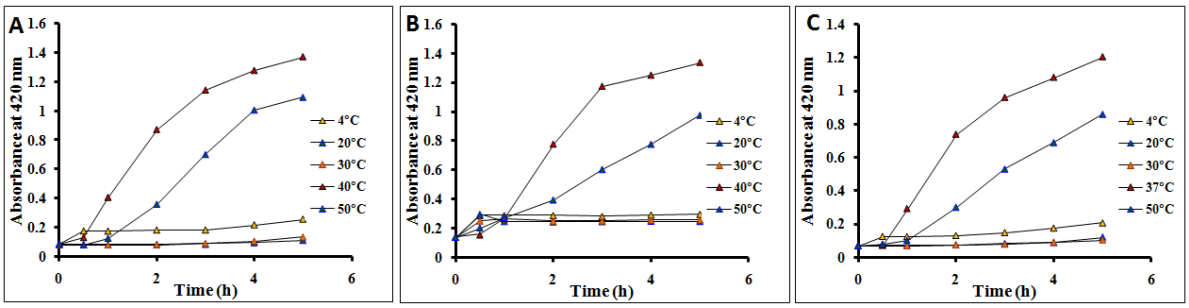

Figure 3: Time course of AgNPs synthesis at different reaction temperatures with 1 mM $AgNO_3$ by (A) GGFE; (B) GGLE and (C) GGSE.

HRTEM, EDS, DLS and XRD analysis

High resolution transmission electron micrographs revealed that the particles were monodispersed. Mostly the particles were found to be spherical and no agglomeration was observed. The particle size varied approximately between 10 to 100 nm in majority (Fig.4). EDS spectra confirmed the presence of elemental silver in the AgNPs synthesized by GGFE, GGLE and GGSE (Fig. 5). Particle size distributions recorded using dynamic light scattering for AgNPs were found to be well in agreement with the HRTEM results (Fig. 6). XRD data for AgNPs synthesized using GGFE, GGLE and GGSE were compared with the standard data released by Joint Committee on Powder Diffraction Standards-JCPSD (File no.04-0783). All the three AgNPs showed crystalline phase with face-centred cubic crystal structure (FCC). The diffraction peaks can be related to the 111, 200, 220 and 311 crystal lattice planes. All the three samples showed peak-broadening, indicating particles size restricted to few nanometers (Fig. 7).

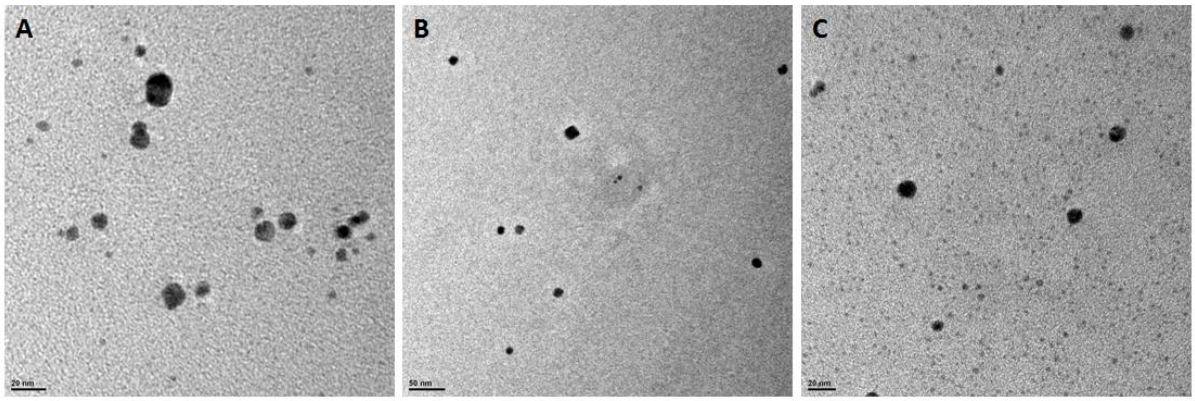

Figure 4: High-resolution transmission electron micrographs of AgNPs synthesized by (A) GGFE; (B) GGLE and (C) GGSE.

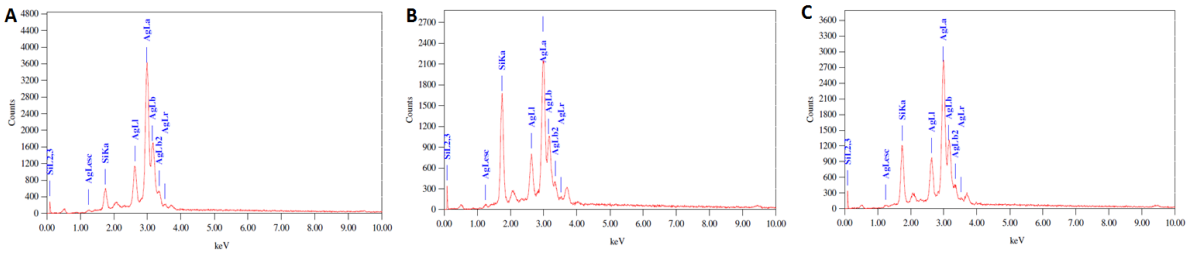

Figure 5: Representative spot EDS profile of AgNPs synthesized by (A) GGFE; (B) GGLE and (C) GGSE.

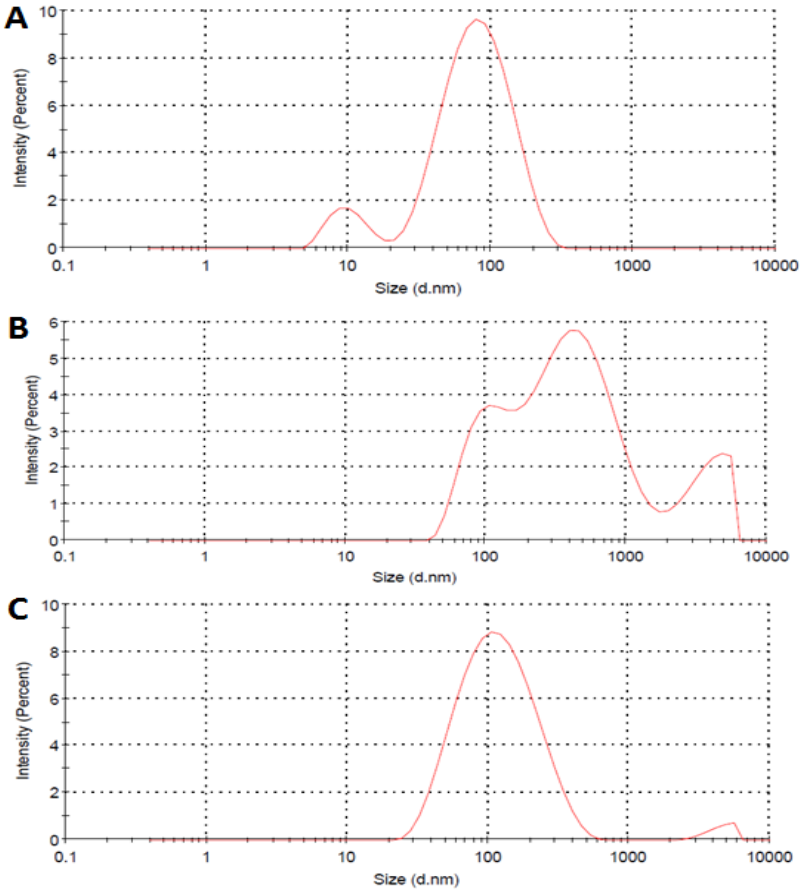

Figure 6: Histogram of size distribution of AgNPs synthesized by (A) GGFE; (B) GGLE and (C) GGSE.

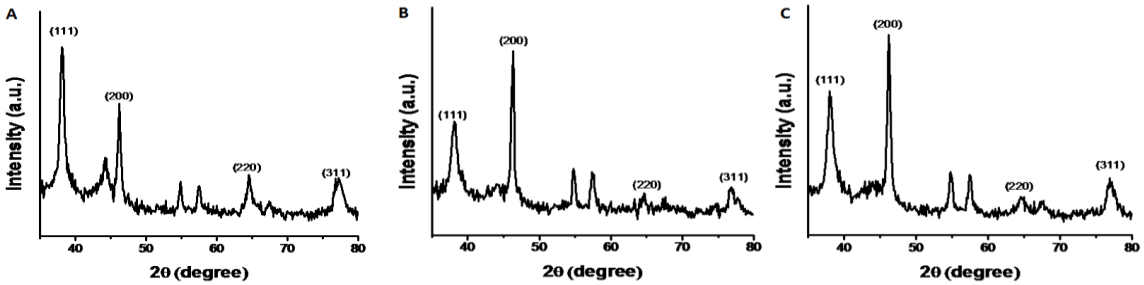

Figure 7: Representative X-ray diffraction profile of thin film AgNPs synthesized by (A) GGFE; (B) GGLE and (C) GGSE.

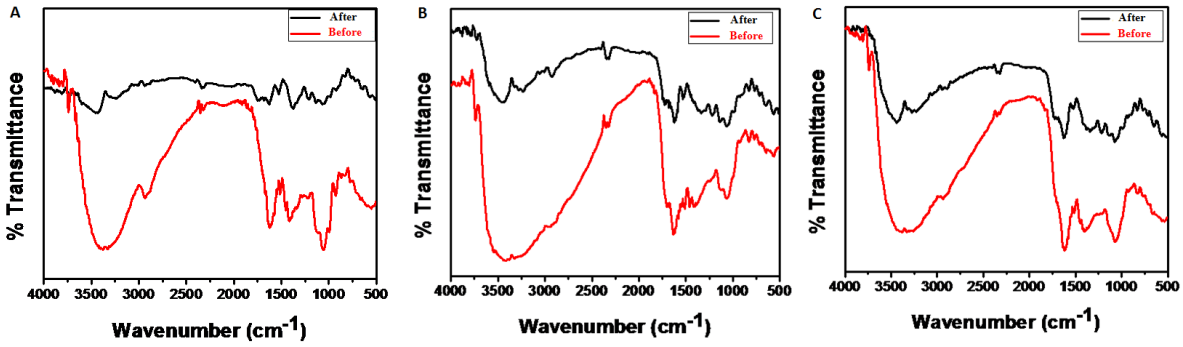

Figure 8: Fourier transform infrared absorption spectra before bioreduction and after complete synthesis AgNPs by (A) GGFE; (B) GGLE and (C) GGSE.

FTIR analysis

FTIR spectrum of GGFE, GGLE and GGSE were studied respectively, before and after bio-reduction of $AgNO_3$ to AgNPs (Fig.8). It is evident that all the three extracts contained similar functional groups with variation in their respective concentrations, as reflected through variation in their intensities. Strong peak at ~ 3330 is attributed to hydroxyl group in alcoholic and phenolic compounds. The characteristic peaks of the other important functional groups can be seen at 1611, 1402 and 1067.

Antimicrobial synergy

AgNPs synthesized by the plant extracts showed potent antimicrobial activity when combined with the antibiotics. This synergistic increase in the activity was estimated by evaluation of the fold increase in the zone diameter of the combination compared to the antibiotic alone. Among twenty two tested antibiotics streptomycin showed most superior synergy with AgNPs. Activity of streptomycin was increased upto 17.4, 21.2 and 15 folds with AgNPs from GGFE, GGLE and GGSE, respectively against *E. coli* (Table 1, 2 and 3). Similarly, chloramphenicol exhibited 17.4, 8.9 and 10.8 folds enhancement of antimicrobial activity in combination with AgNPs from GGFE, GGLE and GGSE, respectively against *P. aeruginosa*. Further the synergistic activity of the AgNPs in combination with antibiotics was confirmed by computational modeling (Fig. 9). The enhancement of zone diameter as a function of antimicrobial activity indicated that the synergy was dose dependent. In case of *S. typhi* a synergy was observed at a higher concentration of the AgNPs. Combination of AgNPs and chloramphenicol showed a steady increase in zone diameter against *P. aeruginosa*, thus confirming a potential synergy. In contrast, combination with AgNPs showed a steep rise in the zone diameter only at a higher dosage of streptomycin against *B. subtilis*. Although

individually both AgNPs and streptomycin were less effective at lower concentration, a combination of both increased the efficacy at higher concentration of the either of the nanoparticle or the antibiotic against *S. aureus*.

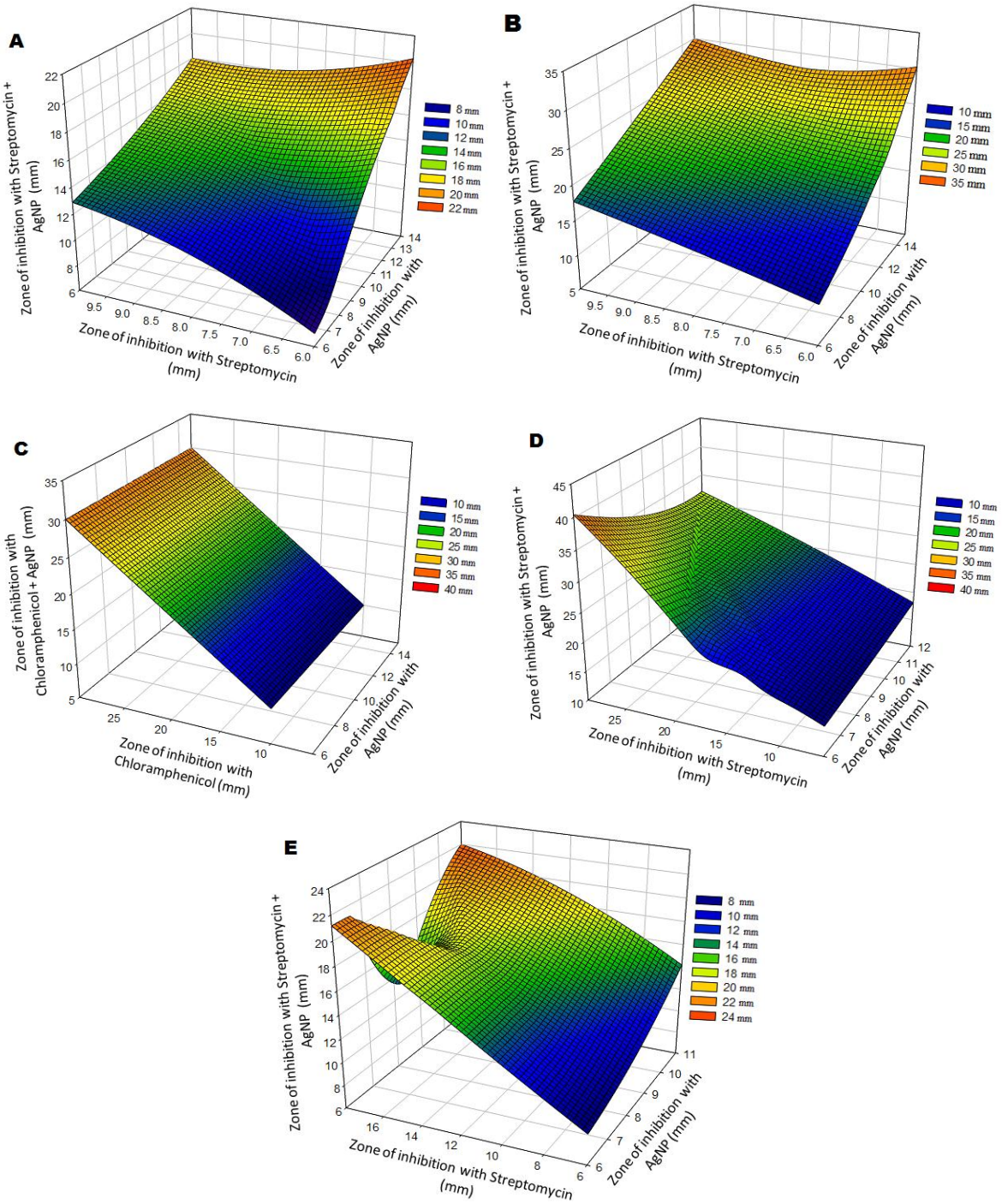

Figure 9: Computational evaluation of synergistic activity of AgNPs with antibiotics against A) *S.typhi* ; B) *E.coli* ; C) *P.aeruginosa* ; D) *B.subtilis* ; E) *S.aureus.*

Table 1: Antimicrobial synergy in terms of fold increase for different antibiotics in absence and in presence of AgNPs synthesized by GGFE.

Antibiotics	*Acinetobacter baumannii*			*Enterobacter cloacae*			*Escherichia coli*			*Haemophilus influenzae*			*Klebsiella pneumoniae*			*Neisseria mucosa*			*Proteus mirabilis*			*Pseudomonas aeruginosa*			*Salmonella typhi*			*Serratia odorifera*			*Vibrio parahaemolyticus*			*Bacillus subtilis*			*Paenibacillus koreensis*			*Staphylococcus aureus*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Aminoglycosides																																										
Amikacin	25.0	29.0	0.3	20.0	20.0	0.0	12.0	13.0	0.2	25.0	25.0	0.0	19.0	19.0	0.0	24.0	24.0	0.0	18.0	21.0	0.4	15.0	17.0	0.3	7.0	12.0	1.9	22.0	22.0	0.0	26.0	27.0	0.1	23.0	24.0	0.1	17.0	20.0	0.4	12.0	12.0	0.0
Gentamycin	16.0	16.0	0.0	20.0	21.0	0.1	19.0	19.0	0.0	26.0	26.0	0.0	21.0	21.0	0.0	32.0	36.0	0.3	22.0	22.0	0.0	30.0	31.0	0.1	7.0	8.0	0.3	28.0	30.0	0.1	33.0	33.0	0.0	22.0	23.0	0.1	23.0	23.0	0.0	19.0	20.0	0.1
Kanamycin	7.0	8.0	0.3	20.0	20.0	0.0	15.0	15.0	0.0	23.0	23.0	0.0	7.0	11.0	1.5	29.0	29.0	0.0	18.0	18.0	0.0	7.0	11.0	1.5	7.0	8.0	0.3	28.0	31.0	0.2	24.0	24.0	0.0	25.0	25.0	0.0	21.0	24.0	0.3	18.0	25.0	0.9
Streptomycin	18.0	18.0	0.0	24.0	27.0	0.3	7.0	30.0	17.4	28.0	28.0	0.0	23.0	24.0	0.1	29.0	30.0	0.1	22.0	22.0	0.0	26.0	28.0	0.2	10.0	18.0	2.2	25.0	27.0	0.2	31.0	34.0	0.2	29.0	32.0	0.2	26.0	27.0	0.1	18.0	22.0	0.5
β-Lactams																																										
Amoxicillin	7.0	8.0	0.3	7.0	11.0	1.5	14.0	14.0	0.0	11.0	11.0	0.0	7.0	11.0	1.5	41.0	42.0	0.0	18.0	18.0	0.0	7.0	8.0	0.3	7.0	10.0	1.0	30.0	30.0	0.0	12.0	15.0	0.6	12.0	12.0	0.0	7.0	8.0	0.3	7.0	18.0	5.6
Ampicillin	7.0	16.0	4.2	16.0	17.0	0.1	30.0	30.0	0.0	12.0	12.0	0.0	7.0	12.0	1.9	23.0	47.0	3.2	7.0	8.0	0.3	12.0	30.0	5.3	22.0	25.0	0.3	30.0	30.0	0.0	15.0	20.0	0.8	22.0	23.0	0.1	12.0	14.0	0.4	24.0	24.0	0.0
Penicillin	7.0	14.0	3.0	7.0	8.0	0.3	11.0	11.0	0.0	15.0	17.0	0.3	7.0	11.0	1.5	48.0	48.0	0.0	16.0	16.0	0.0	7.0	9.0	0.7	20.0	20.0	0.0	25.0	25.0	0.0	16.0	19.0	0.4	7.0	12.0	1.9	8.0	18.0	4.1	16.0	17.0	0.1
Piperacillin	12.0	12.0	0.0	20.0	20.0	0.0	16.0	17.0	0.1	19.0	20.0	0.1	21.0	21.0	0.0	41.0	44.0	0.2	20.0	20.0	0.0	28.0	29.0	0.1	18.0	23.0	0.6	25.0	27.0	0.2	22.0	22.0	0.0	12.0	12.0	0.0	11.0	13.0	0.4	17.0	18.0	0.1
Carbapenem																																										
Feropenem	20.0	20.0	0.0	15.0	15.0	0.0	20.0	27.0	0.8	16.0	27.0	1.8	7.0	11.0	1.5	49.0	50.0	0.0	20.0	20.0	0.0	15.0	15.0	0.0	16.0	18.0	0.3	27.0	27.0	0.0	32.0	32.0	0.0	38.0	38.0	0.0	25.0	25.0	0.0	20.0	20.0	0.0
Cephalosporin																																										
Ceftazidime	10.0	15.0	1.3	23.0	23.0	0.0	10.0	25.0	5.3	21.0	21.0	0.0	12.0	13.0	0.2	34.0	35.0	0.1	22.0	22.0	0.0	30.0	30.0	0.0	18.0	21.0	0.4	25.0	27.0	0.2	22.0	22.0	0.0	23.0	24.0	0.1	20.0	20.0	0.0	13.0	14.0	0.2
Ceftriaxone	13.0	13.0	0.0	23.0	23.0	0.0	7.0	8.0	0.3	17.0	17.0	0.0	19.0	19.0	0.0	48.0	49.0	0.0	20.0	20.0	0.0	25.0	25.0	0.0	17.0	17.0	0.0	25.0	28.0	0.3	17.0	22.0	0.7	16.0	16.0	0.0	18.0	18.0	0.0	18.0	19.0	0.1
Cefotaxime	12.0	12.0	0.0	26.0	26.0	0.0	7.0	13.0	2.4	16.0	16.0	0.0	18.0	19.0	0.1	32.0	32.0	0.0	16.0	17.0	0.1	25.0	25.0	0.0	20.0	21.0	0.1	26.0	29.0	0.2	18.0	18.0	0.0	17.0	20.0	0.4	20.0	20.0	0.0	21.0	22.0	0.1
Cyclic peptide																																										
Polymyxin	17.0	21.0	0.5	15.0	15.0	0.0	7.0	9.0	0.7	12.0	12.0	0.0	17.0	17.0	0.0	17.0	27.0	1.5	7.0	8.0	0.3	16.0	16.0	0.0	7.0	11.0	1.5	16.0	17.0	0.1	13.0	25.0	2.7	7.0	8.0	0.3	13.0	13.0	0.0	7.0	13.0	2.4
Glycopeptides																																										
Vancomycin	15.0	15.0	0.0	7.0	8.0	0.3	20.0	20.0	0.0	25.0	26.0	0.1	7.0	15.0	3.6	27.0	27.0	0.0	7.0	9.0	0.7	7.0	12.0	1.9	20.0	21.0	0.1	7.0	17.0	4.9	25.0	25.0	0.0	25.0	25.0	0.0	19.0	20.0	0.1	19.0	19.0	0.0
Macrolide																																										
Erythromycin	7.0	11.0	1.5	7.0	8.0	0.3	7.0	8.0	0.3	20.0	20.0	0.0	7.0	13.0	2.4	27.0	27.0	0.0	7.0	11.0	1.5	7.0	8.0	0.3	7.0	10.0	1.0	7.0	11.0	1.5	22.0	30.0	0.9	21.0	27.0	0.7	18.0	27.0	1.3	7.0	9.0	0.7
Quinolones																																										
Nalidixic acid	7.0	14.0	3.0	15.0	15.0	0.0	10.0	10.0	0.0	9.0	11.0	0.5	7.0	15.0	3.6	17.0	22.0	0.7	7.0	8.0	0.3	7.0	23.0	9.8	10.0	10.0	0.0	10.0	15.0	1.3	13.0	13.0	0.0	10.0	11.0	0.2	8.0	9.0	0.3	7.0	8.0	0.3
Rifamycin																																										
Rifampicin	22.0	22.0	0.0	14.0	21.0	1.3	16.0	17.0	0.1	19.0	19.0	0.0	11.0	18.0	1.7	46.0	46.0	0.0	18.0	18.0	0.0	17.0	18.0	0.1	23.0	23.0	0.0	14.0	14.0	0.0	20.0	20.0	0.0	19.0	19.0	0.0	19.0	19.0	0.0	7.0	8.0	0.3
Tetracycline																																										
Tetracycline	21.0	21.0	0.0	23.0	23.0	0.0	30.0	35.0	0.4	27.0	27.0	0.0	21.0	21.0	0.0	38.0	38.0	0.0	21.0	21.0	0.0	23.0	23.0	0.0	18.0	19.0	0.1	26.0	29.0	0.2	27.0	27.0	0.0	32.0	32.0	0.0	23.0	24.0	0.1	26.0	30.0	0.3
Doxycycline	22.0	25.0	0.3	20.0	20.0	0.0	13.0	18.0	0.9	26.0	26.0	0.0	15.0	16.0	0.1	36.0	39.0	0.2	11.0	16.0	1.1	13.0	14.0	0.2	23.0	23.0	0.0	22.0	22.0	0.0	32.0	32.0	0.0	20.0	24.0	0.4	22.0	26.0	0.4	13.0	21.0	1.6
Others																																										
Chloramphenicol	13.0	13.0	0.0	25.0	25.0	0.0	27.0	27.0	0.0	20.0	20.0	0.0	7.0	8.0	0.3	34.0	34.0	0.0	21.0	21.0	0.0	7.0	30.0	17.4	25.0	25.0	0.0	25.0	27.0	0.2	22.0	29.0	0.7	7.0	26.0	12.8	24.0	27.0	0.3	7.0	10.0	1.0
Nitrofurantoin	7.0	10.0	1.0	10.0	18.0	2.2	9.0	9.0	0.0	11.0	11.0	0.0	7.0	13.0	2.4	29.0	29.0	0.0	10.0	10.0	0.0	7.0	11.0	1.5	7.0	8.0	0.3	7.0	12.0	1.9	14.0	16.0	0.3	12.0	29.0	4.8	7.0	10.0	1.0	7.0	13.0	2.4
Trimethoprim	7.0	8.0	0.3	17.0	17.0	0.0	19.0	23.0	0.5	7.0	11.0	1.5	7.0	15.0	3.6	26.0	26.0	0.0	7.0	13.0	2.4	7.0	12.0	1.9	7.0	18.0	5.6	7.0	8.0	0.3	7.0	11.0	1.5	7.0	14.0	3.0	7.0	11.0	1.5	7.0	8.0	0.3

All experiments were performed in triplicates, and standard deviations were negligible. Fold increases 'C' for different antibiotics against five bacterial pathogens were calculated as $(B^2-A^2)/A^2$, where A and B are the inhibition zones in mm for only antibiotic and antibiotic in combination with Ag-NPs respectively. In the absence of bacterial growth inhibition zones, the disks' diameters (6 mm) were used to calculate the fold increase C. NI-no inhibition.

Table 2: Antimicrobial synergy in terms of fold increase for different antibiotics in absence and in presence of AgNPs synthesized by GGLE.

Antibiotics	*Acinetobacter baumannii*			*Enterobacter cloacae*			*Escherichia coli*			*Haemophilus influenzae*			*Klebsiella pneumoniae*			*Neisseria mucosa*			*Proteus mirabilis*			*Pseudomonas aeruginosa*			*Salmonella typhi*			*Serratia odorifera*			*Vibrio parahaemolyticus*			*Bacillus subtilis*			*Paenibacillus koreensis*			*Staphylococcus aureus*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Aminoglycosides																																										
Amikacin	21	23.0	0.2	17	24.0	1.0	9	9.0	0.0	21	21.0	0.0	14	23.0	1.7	17.0	30.0	2.1	16.0	16.0	0.0	9	22.0	5.0	7	11.0	1.5	19	19.0	0.0	22.0	22.0	0.0	18	20.0	0.2	14	17.0	0.5	10	11.0	0.2
Gentamycin	12	15.0	0.6	16	16.0	0.0	15	17.0	0.3	22	22.0	0.0	16	17.0	0.1	28.0	28.0	0.0	19.0	19.0	0.0	23	26.0	0.3	7	12.0	1.9	22	24.0	0.2	27.0	27.0	0.0	18	19.0	0.1	19	22.0	0.3	14	24.0	1.9
Kanamycin	7	10.0	1.0	16	17.0	0.1	13	19.0	1.1	20	20.0	0.0	7	7.0	0.0	24.0	24.0	0.0	16.0	16.0	0.0	7	7.0	0.0	7	8.0	0.3	23	25.0	0.2	20.0	20.0	0.0	22	22.0	0.0	18	19.0	0.1	15	18.0	0.4
Streptomycin	13	13.0	0.0	22	22.0	0.0	7	33.0	21.2	22	22.0	0.0	21	21.0	0.0	26.0	26.0	0.0	19.0	27.0	1.0	23	23.0	0.0	7	18.0	5.6	22	22.0	0.0	29.0	29.0	0.0	25	25.0	0.0	22	22.0	0.0	14	15.0	0.1
β-Lactams																																										
Amoxicillin	7	8.0	0.3	7	8.0	0.3	11	11.0	0.0	9	9.0	0.0	7	7.0	0.0	36.0	37.0	0.1	14.0	15.0	0.1	7	7.0	0.0	7	9.0	0.7	22	22.0	0.0	10.0	11.0	0.2	9	9.0	0.0	7	9.0	0.7	7	7.0	0.0
Ampicillin	7	13.0	2.4	13	13.0	0.0	27	27.0	0.0	9	9.0	0.0	7	7.0	0.0	21.0	26.0	0.5	7.0	7.0	0.0	7	20.0	7.2	19	19.0	0.0	26	26.0	0.0	12.0	12.0	0.0	17	18.0	0.1	10	10.0	0.0	16	17.0	0.1
Penicillin	7	10.0	1.0	7	7.0	0.0	9	9.0	0.0	12	14.0	0.4	7	11.0	1.5	45.0	45.0	0.0	12.0	13.0	0.2	7	9.0	0.7	16	22.0	0.9	18	24.0	0.8	14.0	18.0	0.7	7	8.0	0.3	7	18.0	5.6	13	13.0	0.0
Piperacillin	9	16.0	2.2	16	17.0	0.1	13	17.0	0.7	14	14.0	0.0	16	17.0	0.1	39.0	40.0	0.1	15.0	17.0	0.3	25	25.0	0.0	13	23.0	2.1	22	23.0	0.1	18.0	21.0	0.4	9	12.0	0.8	8	11.0	0.9	13	14.0	0.2
Carbapenem																																										
Feropenem	14	15.0	0.1	12	12.0	0.0	16	17.0	0.1	23	23.0	0.0	7	13.0	2.4	46.0	46.0	0.0	14.0	14.0	0.0	8	10.0	0.6	12	13.0	0.2	23	24.0	0.1	28.0	29.0	0.1	30	32.0	0.1	20	20.0	0.0	17	17.0	0.0
Cephalosporin																																										
Ceftazidime	7	7.0	0.0	18	22.0	0.5	7	12.0	1.9	16	17.0	0.1	8	13.0	1.6	30.0	31.0	0.1	19.0	19.0	0.0	22	22.0	0.0	15	15.0	0.0	23	23.0	0.0	17.0	20.0	0.4	18	18.0	0.0	15	15.0	0.0	10	12.0	0.4
Ceftriaxone	7	9.0	0.7	19	19.0	0.0	7	7.0	0.0	12	12.0	0.0	15	15.0	0.0	45.0	45.0	0.0	16.0	16.0	0.0	21	21.0	0.0	13	13.0	0.0	22	28.0	0.6	13.0	18.0	0.9	12	13.0	0.2	13	13.0	0.0	16	16.0	0.0
Cefotaxime	8	9.0	0.3	21	21.0	0.0	7	11.0	1.5	12	24.0	3.0	14	15.0	0.1	28.0	28.0	0.0	13.0	14.0	0.2	21	21.0	0.0	17	19.0	0.2	23	23.0	0.0	15.0	15.0	0.0	13	14.0	0.2	15	17.0	0.3	19	24.0	0.6
Cyclic peptide																																										
Polymyxin	12	14.0	0.4	13	13.0	0.0	7	7.0	0.0	9	12.0	0.8	14	17.0	0.5	15.0	15.0	0.0	7.0	8.0	0.3	13	13.0	0.0	7	9.0	0.7	13	13.0	0.0	10.0	12.0	0.4	7	7.0	0.0	9	10.0	0.2	7	7.0	0.0
Glycopeptides																																										
Vancomycin	10	10.0	0.0	7	8.0	0.3	17	17.0	0.0	21	22.0	0.1	7	16.0	4.2	23.0	23.0	0.0	7.0	7.0	0.0	7	17.0	4.9	16	16.0	0.0	7	7.0	0.0	22.0	22.0	0.0	21	22.0	0.1	17	17.0	0.0	13	14.0	0.2
Macrolide																																										
Erythromycin	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0	16	23.0	1.1	7	10.0	1.0	24.0	34.0	1.0	7.0	7.0	0.0	7	8.0	0.3	7	7.0	0.0	7	8.0	0.3	18.0	20.0	0.2	18	18.0	0.0	14	22.0	1.5	7	7.0	0.0
Quinolones																																										
Nalidixic acid	7	7.0	0.0	10	10.0	0.0	7	8.0	0.3	7	7.0	0.0	7	7.0	0.0	12.0	15.0	0.6	7.0	18.0	5.6	7	15.0	3.6	7	7.0	0.0	8	9.0	0.3	10.0	10.0	0.0	7	7.0	0.0	7	9.0	0.7	7	9.0	0.7
Rifamycin																																										
Rifampicin	19	19.0	0.0	11	12.0	0.2	13	14.0	0.2	15	15.0	0.0	10	10.0	0.0	42.0	42.0	0.0	14.0	14.0	0.0	12	12.0	0.0	20	25.0	0.6	12	13.0	0.2	17.0	17.0	0.0	15	15.0	0.0	16	16.0	0.0	7	8.0	0.3
Tetracycline																																										
Tetracycline	14	15.0	0.1	19	19.0	0.0	25	30.0	0.4	23	23.0	0.0	17	17.0	0.0	31.0	33.0	0.1	16.0	16.0	0.0	17	18.0	0.1	14	14.0	0.0	20	22.0	0.2	23.0	27.0	0.4	28	28.0	0.0	20	21.0	0.1	20	22.0	0.2
Doxycycline	18	19.0	0.1	16	20.0	0.6	10	11.0	0.2	23	23.0	0.0	12	12.0	0.0	32.0	32.0	0.0	9.0	13.0	1.1	10	10.0	0.0	19	19.0	0.0	19	19.0	0.0	25.0	26.0	0.1	18	18.0	0.0	17	17.0	0.0	9	10.0	0.2
Others																																										
Chloramphenicol	8	9.0	0.3	17	19.0	0.2	25	25.0	0.0	16	16.0	0.0	7	7.0	0.0	30.0	33.0	0.2	12.0	13.0	0.2	7	22.0	8.9	20	20.0	0.0	17	19.0	0.2	17.0	19.0	0.2	7	21.0	8.0	21	24.0	0.3	7	7.0	0.0
Nitrofurantoin	7	9.0	0.7	7	14.0	3.0	7	9.0	0.7	9	9.0	0.0	7	8.0	0.3	17.0	18.0	0.1	8.0	12.0	1.3	7	11.0	1.5	7	7.0	0.0	7	9.0	0.7	11.0	17.0	1.4	10	14.0	1.0	7	9.0	0.7	7	8.0	0.3
Trimethoprim	7	10.0	1.0	15	17.0	0.3	13	13.0	0.0	7.0	7.0	0.0	7	7.0	0.0	22.0	23.0	0.1	7.0	7.0	0.0	7	9.0	0.7	7	7.0	0.0	7	7.0	0.0	7.0	16.0	4.2	7	20.0	7.2	7	8.0	0.3	7	7.0	0.0

All experiments were performed in triplicates, and standard deviations were negligible. Fold increases 'C' for different antibiotics against five bacterial pathogens were calculated as $(B^2-A^2)/A^2$, where A and B are the inhibition zones in mm for only antibiotic and antibiotic in combination with Ag-NPs respectively. In the absence of bacterial growth inhibition zones, the disks' diameters (6 mm) were used to calculate the fold increase C. NI-no inhibition.

Table 3: Antimicrobial synergy in terms of fold increase for different antibiotics in absence and in presence of AgNPs synthesized by GGSE.

Antibiotics	*Acinetobacter baumannii*			*Enterobacter cloacae*			*Escherichia coli*			*Haemophilus influenzae*			*Klebsiella pneumoniae*			*Neisseria mucosa*			*Proteus mirabilis*			*Pseudomonas aeruginosa*			*Salmonella typhi*			*Serratia odorifera*			*Vibrio parahaemolyticus*			*Bacillus subtilis*			*Paenibacillus koreensis*			*Staphylococcus aureus*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Aminoglycosides																																										
Amikacin	20	20.0	0.0	16	25.0	1.4	7	7.0	0.0	20	20.0	0.0	13	16.0	0.5	16.0	16.0	0.0	13.0	20.0	1.4	8	22.0	6.6	7	7.0	0.0	18	18.0	0.0	19.0	19.0	0.0	16	23.0	1.1	12	13.0	0.2	9	10.0	0.2
Gentamycin	8	9.0	0.3	15	15.0	0.0	14	15.0	0.1	20	20.0	0.0	14	15.0	0.1	27.0	31.0	0.3	18.0	18.0	0.0	20	22.0	0.2	7	7.0	0.0	20	22.0	0.2	24.0	25.0	0.1	17	18.0	0.1	18	18.0	0.0	11	14.0	0.6
Kanamycin	7	7.0	0.0	15	16.0	0.1	12	15.0	0.6	19	19.0	0.0	7	7.0	0.0	20.0	20.0	0.0	16.0	18.0	0.3	7	7.0	0.0	7	12.0	1.9	22	22.0	0.0	18.0	24.0	0.8	21	21.0	0.0	17	17.0	0.0	13	13.0	0.0
Streptomycin	12	15.0	0.6	21	21.0	0.0	7	28.0	15.0	20	21.0	0.1	19	19.0	0.0	25.0	25.0	0.0	18.0	18.0	0.0	21	34.0	1.6	7	16.0	4.2	20	20.0	0.0	27.0	27.0	0.0	24	27.0	0.3	21	21.0	0.0	12	14.0	0.4
β-Lactams																																										
Amoxicillin	7	7.0	0.0	7	8.0	0.3	10	10.0	0.0	8	8.0	0.0	7	7.0	0.0	32.0	32.0	0.0	12.0	16.0	0.8	7	11.0	1.5	7	9.0	0.7	15	17.0	0.3	9.0	12.0	0.8	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0
Ampicillin	7	7.0	0.0	12	24.0	3.0	25	26.0	0.1	8	8.0	0.0	7	14.0	3.0	20.0	21.0	0.1	7.0	7.0	0.0	7	18.0	5.6	18	18.0	0.0	25	25.0	0.0	11.0	13.0	0.4	16	16.0	0.0	9	12.0	0.8	15	15.0	0.0
Penicillin	7	10.0	1.0	7	7.0	0.0	8	9.0	0.3	11	11.0	0.0	7	10.0	1.0	44.0	44.0	0.0	12.0	12.0	0.0	7	7.0	0.0	15	15.0	0.0	16	17.0	0.1	13.0	14.0	0.2	7	15.0	3.6	7	7.0	0.0	12	12.0	0.0
Piperacillin	8	10.0	0.6	15	16.0	0.1	10	12.0	0.4	13	17.0	0.7	15	15.0	0.0	38.0	38.0	0.0	14.0	15.0	0.1	24	24.0	0.0	12	18.0	1.3	21	21.0	0.0	16.0	18.0	0.3	8	11.0	0.9	8	9.0	0.3	12	13.0	0.2
Carbapenem																																										
Feropenem	10	13.0	0.7	12	12.0	0.0	15	15.0	0.0	21	21.0	0.0	7	7.0	0.0	45.0	45.0	0.0	10.0	13.0	0.7	7	7.0	0.0	11	12.0	0.2	23	23.0	0.0	25.0	25.0	0.0	27	32.0	0.4	18	19.0	0.1	16	16.0	0.0
Cephalosporin																																										
Ceftazidime	7	7.0	0.0	17	18.0	0.1	7	16.0	4.2	14	15.0	0.1	7	7.0	0.0	26.0	26.0	0.0	7.0	19.0	6.4	21	21.0	0.0	14	14.0	0.0	20	24.0	0.4	13.0	15.0	0.3	16	23.0	1.1	14	17.0	0.5	9	10.0	0.2
Ceftriaxone	7	7.0	0.0	17	19.0	0.2	7	7.0	0.0	10	11.0	0.2	12	12.0	0.0	44.0	44.0	0.0	14.0	16.0	0.3	19	20.0	0.1	12	16.0	0.8	20	20.0	0.0	12.0	16.0	0.8	10	10.0	0.0	12	12.0	0.0	15	20.0	0.8
Cefotaxime	7	10.0	1.0	19	21.0	0.2	7	7.0	0.0	10	12.0	0.4	13	15.0	0.3	27.0	27.0	0.0	12.0	13.0	0.2	20	21.0	0.1	15	16.0	0.1	21	21.0	0.0	13.0	13.0	0.0	12	12.0	0.0	14	20.0	1.0	17	17.0	0.0
Cyclic peptide																																										
Polymyxin	11	13.0	0.4	12	22.0	2.4	7	7.0	0.0	8	8.0	0.0	13	14.0	0.2	14.0	14.0	0.0	7.0	7.0	0.0	12	13.0	0.2	7	7.0	0.0	11	12.0	0.2	8.0	12.0	1.3	7	7.0	0.0	7	8.0	0.3	7	7.0	0.0
Glycopeptides																																										
Vancomycin	9	10.0	0.2	7	7.0	0.0	16	16.0	0.0	20	21.0	0.1	7	7.0	0.0	21.0	21.0	0.0	7.0	7.0	0.0	7	10.0	1.0	14	14.0	0.0	7	7.0	0.0	20.0	20.0	0.0	20	20.0	0.0	15	16.0	0.1	7	7.0	0.0
Macrolide																																										
Erythromycin	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0	15	18.0	0.4	7	12.0	1.9	22.0	22.0	0.0	7.0	7.0	0.0	7	13.0	2.4	7	10.0	1.0	7	7.0	0.0	17.0	20.0	0.4	17	17.0	0.0	13	15.0	0.3	7	7.0	0.0
Quinolones																																										
Nalidixic acid	7	9.0	0.7	9	9.0	0.0	7	11.0	1.5	7	7.0	0.0	7	7.0	0.0	10.0	10.0	0.0	7.0	7.0	0.0	7	7.0	0.0	7	10.0	1.0	7	7.0	0.0	9.0	9.0	0.0	7	7.0	0.0	7	7.0	0.0	7	12.0	1.9
Rifamycin																																										
Rifampicin	18	18.0	0.0	9	10.0	0.2	12	15.0	0.6	14	16.0	0.3	9	9.0	0.0	38.0	38.0	0.0	13.0	13.0	0.0	10	10.0	0.0	19	20.0	0.1	10	13.0	0.7	16.0	16.0	0.0	14	15.0	0.1	15	15.0	0.0	7	7.0	0.0
Tetracycline																																										
Tetracycline	12	15.0	0.6	18	19.0	0.1	23	29.0	0.6	22	22.0	0.0	15	16.0	0.1	28.0	30.0	0.1	14.0	15.0	0.1	16	16.0	0.0	13	13.0	0.0	18	18.0	0.0	21.0	21.0	0.0	26	29.0	0.2	17	19.0	0.2	19	20.0	0.1
Doxycycline	17	19.0	0.2	14	15.0	0.1	9	13.0	1.1	21	22.0	0.1	11	12.0	0.2	30.0	30.0	0.0	8.0	12.0	1.3	7	13.0	2.4	18	20.0	0.2	18	21.0	0.4	23.0	24.0	0.1	17	17.0	0.0	16	18.0	0.3	8	9.0	0.3
Others																																										
Chloramphenicol	7	7.0	0.0	15	18.0	0.4	22	23.0	0.1	14	15.0	0.1	7	7.0	0.0	29.0	29.0	0.0	10.0	11.0	0.2	7	24.0	10.8	16	18.0	0.3	15	17.0	0.3	15.0	17.0	0.3	7	19.0	6.4	17	20.0	0.4	7	19.0	6.4
Nitrofurantoin	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0	8	9.0	0.3	7	7.0	0.0	14.0	18.0	0.7	7.0	7.0	0.0	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0	10.0	18.0	2.2	9	12.0	0.8	7	7.0	0.0	7	7.0	0.0
Trimethoprim	7	7.0	0.0	13	14.0	0.2	10	12.0	0.4	7.0	7.0	0.0	7	9.0	0.7	19.0	21.0	0.2	7.0	7.0	0.0	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0	7.0	9.0	0.7	7	7.0	0.0	7	7.0	0.0	7	7.0	0.0

All experiments were performed in triplicates, and standard deviations were negligible. Fold increases 'C' for different antibiotics against five bacterial pathogens were calculated as $(B^2-A^2)/A^2$, where A and B are the inhibition zones in mm for only antibiotic and antibiotic in combination with Ag-NPs respectively. In the absence of bacterial growth inhibition zones, the disks' diameters (6 mm) were used to calculate the fold increase C. NI-no inhibition.

DISCUSSION

Growing multidrug resistance among bacterial pathogens has rendered most of the commercially available antimicrobial agents ineffective. Hereby, there is a continuous growing need to explore more effective bactericidal therapeutic agents that might check the bacterial growth and the spread of drug resistance as well.[13] Silver and its compounds are well known for their bactericidal effects and thus it has got wide spread applications in medicines for treatment of burnt wound infections, dental materials, stainless steel coating materials, textile fabrics, water treatment, sunscreen lotions owing to their low toxicity to human cells, high thermal stability and low volatility.[24] Hence in this report we have designed a green fabrication of AgNPs using the medicinal plant *G. glauca.* The synthesis was found to be rapid and completed within 5 h. It was found that higher $AgNO_3$ concentrations and temperature facilitate the rapid synthesis. Our results are in well agreement with the previous reports on synthesis of AgNPs using *Plumbago zeylanica, Litchi chinensis* and *Barleria prionitis.*[20,18,15] The biogenic nanoparticles were monodispersed within a size range of 10 to 100 nm and free from aggregation indicating the stability. As indicated by XRD analysis, although all crystal planes specific to AgNPs are present in AgNPs synthesized by GGFE, GGLE and GGSE, the highest intensity peaks varied depending upon the source of the extract with which the synthesis was carried out. For example, AgNPs synthesized using GGFE showed the highest growth of 111 crystal planes while rest two showed the highest growth of 200 crystal planes. This could be attributed to the variation in the chemical contents hence their role in restricting or promoting the growth of certain crystal planes. The other peaks, which have not been assigned any crystal plane details might be attributed to the crystalline phase of the organic impurities of the extract.[25-28] As mentioned in our previous reports of synthesis of AuNPs with GGFE, the water-soluble flavonoids are responsible for synthesis and stabilization of the nanoparticles.[23,22] The trend of characteristic peak intensity reduction or peak shift was different for all the three extracts. This indicates that every extract has different functional group, which is strongly responsible for bio-reduction of the $AgNO_3$ salt. This in turn also supports the variation in the peak intensities, hence different crystal plane growth, as seen in the XRD data. The bioreduced AgNPs showed a pronounced synergy with selective antibiotics against specific bacteria which might be facilitated by adsorption onto the bacterial surface. Nanosilver carrying antibiotics can approach the hydrophobic cell membrane that is composed of phospholipids and glycoprotein and binds to the sulfur containing proteins increasing the permeability of the membrane leading to the enhanced infiltration of the antibiotics within the cell. It might also

interfere with DNA from unwinding which add up to the bactericidal effect of the antibiotic synergistically.[13]

CONCLUSIONS

In an attempt to develop an eco-friendly novel route for synthesis of AgNPs we have used *G. glauca* flower, leaf and stem which was found to be very rapid and highly efficient. The synthesized nanoparticles were stable, monodispersed and showed no signs of agglomeration. Compositional characterization was done using various physical techniques. Combination of nanosilver and antibiotic showing a selective synergistic enhancement of antibacterial efficiency provides a strong scientific rationale towards the promises of drug conjugated AgNPs as emerging bactericidal agents. This research, will provide impetus to the development of novel broad spectrum antimicrobial nanomedicine.

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REFERENCES

1. Ghosh S, Parihar VS, More P, Dhavale DD, Chopade BA. Phytochemistry and therapeutic potential of medicinal plant: *Dioscorea bulbifera*. Med Chem, 2015; 5(4): 154-159.
2. Ghosh S, Parihar VS, Dhavale DD, Chopade BA. Commentary on therapeutic potential of *Gnidia glauca*: A novel medicinal plant. Med Chem, 2015; 5(8): 351-353.
3. Kitture R, Ghosh S, More PA, Date K, Gaware S, Datar S, Chopade BA, Kale SN. Curcumin-loaded, self-assembled *Aloe vera* template for superior antioxidant activity and trans-membrane drug release. J Nanosci Nanotechnol, 2015; 15(6): 4039-4045.
4. Kitture R, Chordiya K, Gaware S, Ghosh S, More PA, Kulkarni P, Chopade BA, Kale SN. ZnO nanoparticles-red sandalwood conjugate: A promising anti-diabetic agent. J Nanosci Nanotechnol, 2015; 15(6): 4046-4051.
5. Kitture R, Ghosh S, Kulkarni P, Liu XL, Maity D, Patil SI, Jun D, Dushing Y, Laware SL, Chopade BA, Kale SN. Fe_3O_4-citrate-curcumin: Promising conjugates for superoxide

scavenging, tumor suppression and cancer hyperthermia. J Appl Phys, 2012; 111: 064702-064707.

6. Ghosh S, Harke AN, Chacko MJ, Gurav SP, Joshi KA, Dhepe A, Dewle A, Tomar GB, Kitture R, Parihar VS, Banerjee K, Kamble N, Bellare J, Chopade BA. *Gloriosa superba* mediated synthesis of silver and gold nanoparticles for anticancer applications. J Nanomed Nanotechnol, 2016; 7: 390.
7. Ghosh S, Ahire M, Patil S, Jabgunde A, Dusane MB, Joshi BN, Pardesi K, Jachak S, Dhavale DD, Chopade BA. Antidiabetic activity of *Gnidia glauca* and *Dioscorea bulbifera*: potent amylase and glucosidase inhibitors. Evid Based Complement Alternat Med, 2012; 2012: 929051.
8. Ghosh S, More P, Derle A, Patil AB, Markad P, Asok A, Kumbhar N, Shaikh ML, Ramanamurthy B, Shinde VS, Dhavale DD, Chopade BA. Diosgenin from *Dioscorea bulbifera*: Novel Hit for treatment of Type II Diabetes Mellitus with inhibitory activity against α-Amylase and α-Glucosidase. PLoS One, 2014; 9(9): e106039.
9. Ghosh S, Derle A, Ahire M, More P, Jagtap S, Phadatare SD, Patil AB, Jabgunde AM, Sharma GK, Shinde VS, Pardesi K, Dhavale DD, Chopade BA. Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*. PLoS One, 2013; 8(12): e82529.
10. Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, Kitture R, Asok A, Kale S, Singh S, Shaikh ML, Ramanamurthy B, Bellare J, Chopade BA. Antidiabetic and antioxidant properties of copper nanoparticles synthesized by medicinal plant *Dioscorea bulbifera.* J Nanomed Nanotechnol, 2015; S6: 007.
11. Ghosh S, Nitnavare R, Dewle A, Tomar GB, Chippalkatti R, More P, Kitture R, Kale S, Bellare J, Chopade BA. Novel platinum–palladium bimetallic nanoparticles synthesized by *Dioscorea bulbifera*: Anticancer and antioxidant activities. Int J Nanomedicine, 2015; 10: 7477–7490.
12. Ghosh S, Jagtap S, More P, Shete UJ, Maheshwari NO, Rao SJ, Kitture R, Kale S, Bellare J, Patil S, Pal JK, Chopade BA.. *Dioscorea bulbifera* mediated synthesis of novel $Au_{core}Ag_{shell}$ nanoparticles with potent antibiofilm and antileishmanial activity. J Nanomater, 2015; 2015: 562938.
13. Ghosh S, Patil S, Ahire M, Kitture R, Kale S, Pardesi K, Cameotra SS, Bellare J, Dhavale DD, Jabgunde A, Chopade BA. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. Int J Nanomed, 2012; 7: 483–496.

14. Ghosh S, Patil S, Ahire M, Kitture R, Jabgunde A, Kale S, Pardesi K, Bellare J, Dhavale DD, Chopade BA. Synthesis of gold nanoanisotrops using *Dioscorea bulbifera* tuber extract. J Nanomat, 2011; 2011: 354793-354800.
15. Ghosh S, Chacko MJ, Harke AN, Gurav SP, Joshi KA, Dhepe A, Kulkarni AS, Shinde VS, Parihar VS, Asok A, Banerjee K, Kamble N, Bellare J, Chopade BA. *Barleria prionitis* leaf mediated synthesis of silver and gold nanocatalysts. J Nanomed Nanotechnol, 2016; 7: 394.
16. Sant DG, Gujarathi TR, Harne SR, Ghosh S, Kitture R, Kale S, Chopade BA, Pardesi K R.. *Adiantum philippense* L. frond assisted rapid green synthesis of gold and silver nanoparticles. J Nanopart, 2013; 2013: 1-9.
17. Ghosh S, Gurav SP, Harke AN, Chacko MJ, Joshi KA, Dhepe A, Charolkar C, Shinde VS, Kitture R, Parihar VS, Banerjee K, Kamble N, Bellare J, Chopade BA. *Dioscorea oppositifolia* mediated synthesis of gold and silver nanoparticles with catalytic activity. J Nanomed Nanotechnol, 2016; 7: 398.
18. Salunke GR, Ghosh S, Santosh Kumar RJ, Khade S, Vashisth P, Kale T, Chopade S, Pruthi V, Kundu G, Bellare JR, Chopade BA. Rapid efficient synthesis and characterization of silver, gold, and bimetallic nanoparticles from the medicinal plant *Plumbago zeylanica* and their application in biofilm control. Int J Nanomedicine, 2014; 9: 2635–2653.
19. Rokade SS, Joshi KA, Mahajan K, Tomar G, Dubal DS, Parihar VS, Kitture R, Bellare J, Ghosh S. Novel anticancer platinum and palladium nanoparticles from *Barleria prionitis*. Glob J Nano, 2017; 2(5): 555600.
20. Shende S, Joshi KA, Kulkarni AS, Shinde VS, Parihar VS, Kitture R, Banerjee K, Kamble N, Bellare J, Ghosh S. *Litchi chinensis* peel: A novel source for synthesis of gold and silver nanocatalysts. Glob J Nano, 2017; 3(1): 555603.
21. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv, 2009; 27: 76–83.
22. Ghosh S, Patil S, Chopade NB, Luikham S, Kitture R, Gurav DD, Patil AB, Phadatare SD, Sontakke V, Kale S, Shinde V, Bellare J, Chopade BA. *Gnidia glauca* leaf and stem extract mediated synthesis of gold nanocatalysts with free radical scavenging potential. J Nanomed Nanotechnol, 2016; 7: 2.
23. Ghosh S, Patil S, Ahire M, Kitture R, Gurav DD, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare J, Dhavale DD, Chopade BA. *Gnidia glauca* flower extract mediated

synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. J Nanobiotechnol, 2012; 10: 17.

24. Duran N, Marcarto PD, De Souza GIH, Alves OL, Esposito E. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. J Biomed Nanotechnol, 2007; 3: 203–208.
25. Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C. Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res, 2011; 108(3): 693-702.
26. Philip D, Unni C, Aromal SA, Vidhu VK. *Murraya Koenigii* leaf-assisted rapid green synthesis of silver and gold nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc, 2011; 78(2): 899-904.
27. Dwivedi AD, Gopa K. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. Colloids Surf A Physicochem Eng Asp, 2010; 369(1–3): 27-33.
28. Kumar R, Roopan SM, Prabhakarn A, Khanna VG, Chakroborty S. Agricultural waste *Annona squamosa* peel extract: biosynthesis of silver nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc, 2012; 90: 173-176.