Int J Pharm Bio Sci 2017Apr; 8(2): (P) 30-36

Original Research Article Microbiology

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

COMPARATIVE ANALYSIS AND SYNERGISTIC ACTIVITY OF SILVER NANOPARTICLES AGAINST HUMAN PATHOGENS

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ABSTRACT

In the present study Silver Nanoparticles (AgNPs) were synthezised from Silver nitrate ($AgNO_3$)through a simple and ecofriendly route using leaf broth of Occimum sanctum and its antimicrobial activity of was determined against various test pathogens. , simultaneously to find out the efficacy of synthesized AgNPs and comparing it which commercial available antibiotics along with positive and negative control. In my research work AgNPs was synthesized from *Occimum sanctum* efficient plant extract using a specific solvent. Further Analytical characterization of the test sample was done to determine the size of nanoparticles and their chemical constituents by XRD and FE-SEM. The main objective was to find out the potency of the AgNPs from *Occimum sanctum*. This work was carried out by usual Kirby bauers method by using three different parameters. Initially concentration of the zone of inhibition of AgNPs was observed which was about 25 µl, further it was subjected synergistic activity by using commercial available antibiotics such as Amoxycillin, tetracycline, oxacillin, ciproploxacin along which was incorporated with AgNPs at predetermined concentration. The potency of antimicrobial activity was more in AgNPs incorporated with commercial available antibiotics when compared with antibiotics alone. This antimicrobial agent can be given to the microbes, which shows resistance to the most of the fourth generation drugs after proper structural modifications and toxicity studies.

KEYWORDS: *Characterisation, synergistic activity, AgNPs, commercial available antibiotics.*

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Received on : 21.11.2016
Revised and Accepted on : 16-02-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.p30-36

INTRODUCTION

Nanotechnology can be useful in diagnostic techniques, drug delivery, antimicrobial bandages, disinfectant, a friendly manufacturing process that reduce waste products[1.] Nanotechnology is the production and use of materials at the smallest possible scale. leading to atomically precise molecular manufacturing, as catalyst for greater efficiency in current manufacturing process by minimizing or eliminating the use of toxic materials[2.] Nano scale particles have emerged as novel antimicrobial agents owing to the high surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistances against metal ions, antibiotics and the development of resistant strains. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness[3.] In the earlier study *Occimum sanctum* silver nano particles was synthesized and characterized by various analytical method[4] in the present study antimicrobial activity of nano particles synthesized from *Occimum sanctum* was determined against test pathogens.

MATERIALS AND METHODS

Collection of plant materials, synthesis and characterisation of silver nanomaterials from Occimum sanctum

Occimum sanctum leaves were collected from the local garden. Then the leaves were air dried and kept in hot air oven at 80 for 2 hours. The leaves were ground in to a fine powder and mixed in 100 ml of distilled water and kept in dark place for 24 hours. Further the plant extract was collected , and the filtrate were further subjected for the synthesis of silver nanoparticles. To confirm the presence of silver nano particles, various characterization studies were carried out such as UV, spectroscopic analysis, FTIR, FE-SEM, EDAX, XRD. All these above studies confirmed and the presence of silver nanoparticles from *Occimum sanctum*[4.] The synthesised nanoparticles were analysed for various analytical studies for the confirmation of green nanoparticles.

Antimicrobial activity of silver nanoparticles synthesised from Occimum sanctum

Antimicrobial activity of silver nanoparticles was determined by Kirby bauer's disc diffusion method by comparing it with Mac Farlands 0.5N standard along with positive and negative control against the test pathogens such as *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Vibrio cholerae, Bacillus cereus*. These cultures were procured from IBMS, Taramani,Chennai.

RESULTS AND DISCUSSION

Characterization of green nano particles by FESEM

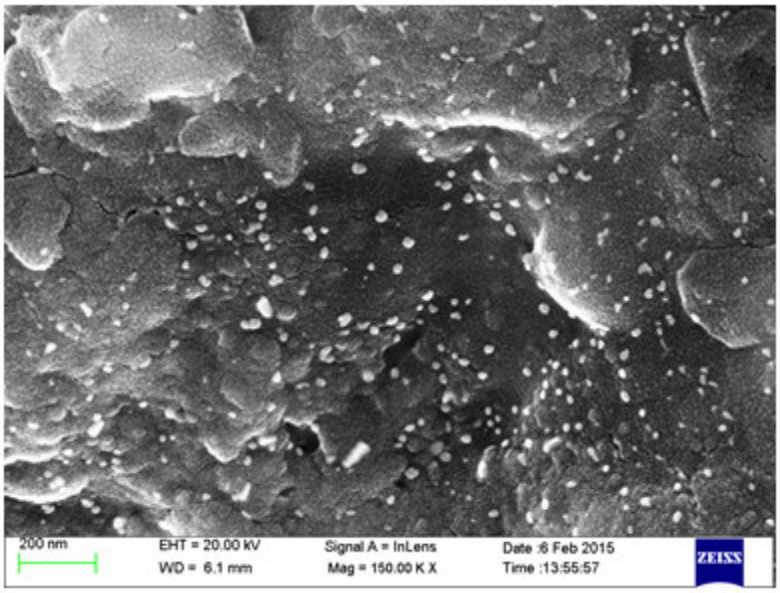

Figure 1
FE-SEM analysis showing nanoparticle size

FE-SEM analysis was done to determine the size and shape of nanoparticles. The particles size was uniform found around 22 to 30 nm (fig. 1) and shape mostly were spherical and monodispersed[5.]

Energy Dispersive X-Ray Analysis (EDAX)

EDAX is widely used technique to analyse the chemical components in a material under SEM. This method detects the X-Rays produced as the electron beam intersections with the sample. Mapping of the distribution of the different chemical elements constituting the specimen can be obtained[5-6.] (fig.2).

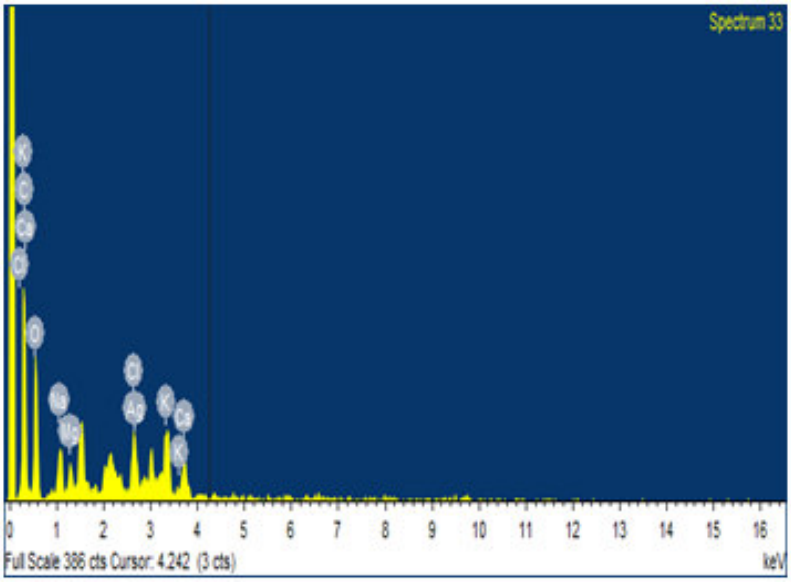

Figure 2
FE-SEM with EDAX mapping analysis

Anti-Bacterial Activity of Different Concentration of Silver Nanoparticles from Occimum sanctum against test pathogens

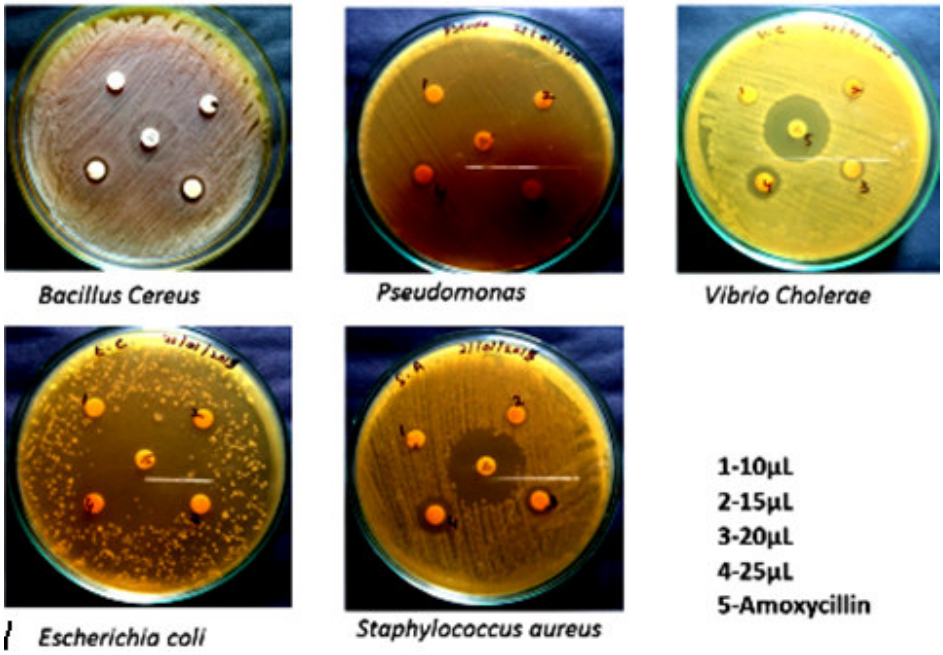

Figure 3
Antimicroboial activity of different concentrations of synthesised AgNPs

Table 1
Zone of Inhibition of Synthesized AgNps from Occimum sanctum

Pathogens	10µl	15µl	20µl	25µl	Antibiotics (Amoxicillin 10 mcg)
Bacillus cereus	_	_	10±1	10±1	19±1
Pseudomonas sp.	_	_	_	10±1	19±1
Vibrio cholerae	6±1	8±1	9±1	11±1	25±1
Escherichia coli	_	10±1	11 ±1	13±1	_
Staphylococcus aureus	_	8±1	11 ±1	10±1	19±1

A wide research reports were available as antimicrobial agent against gram positive and gram negative bacteria. In this study five pathogens were selected such as *Bacillus cereus, Pseudomonas sp,Vibrio cholerae, Escherichia coli, Staphylococcus aureus* are usedand their corresponding good zone of inhibition were recorded in Table 1, fig.3.

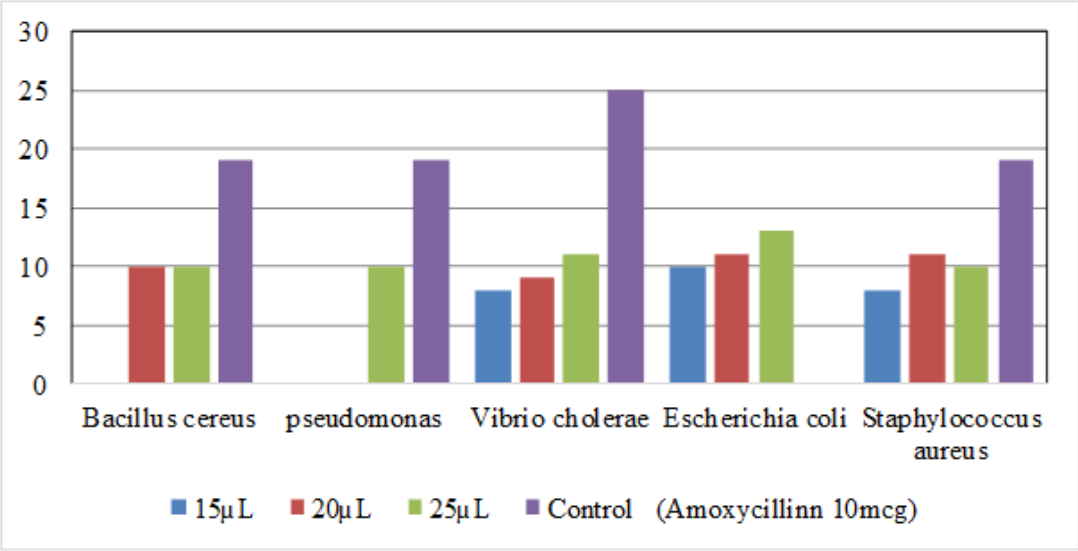

Figure 4
Graphical Representation of AgNpsfromOccimum sanctum

The different concentration of synthesis AgNPs 10, 15, 20, 25 µl were added in the sterile disc along with the control against the test pathogens. Among the various concentration of 25µl showed a zone of inhibition around 13 mm which was found to be maximum when compared with other concentration that is 10 – 20µl. This 25 µl concentration had been choosen for further antimicrobial activity that is synergistic activity and comparative analysis. Simultaneously 25 µl concentration has inhibited the growth of both gram positive and gram negative pathogens and it shows that synthesized green nanoparticles was very effective (fig. 4) it can be used for further studies.

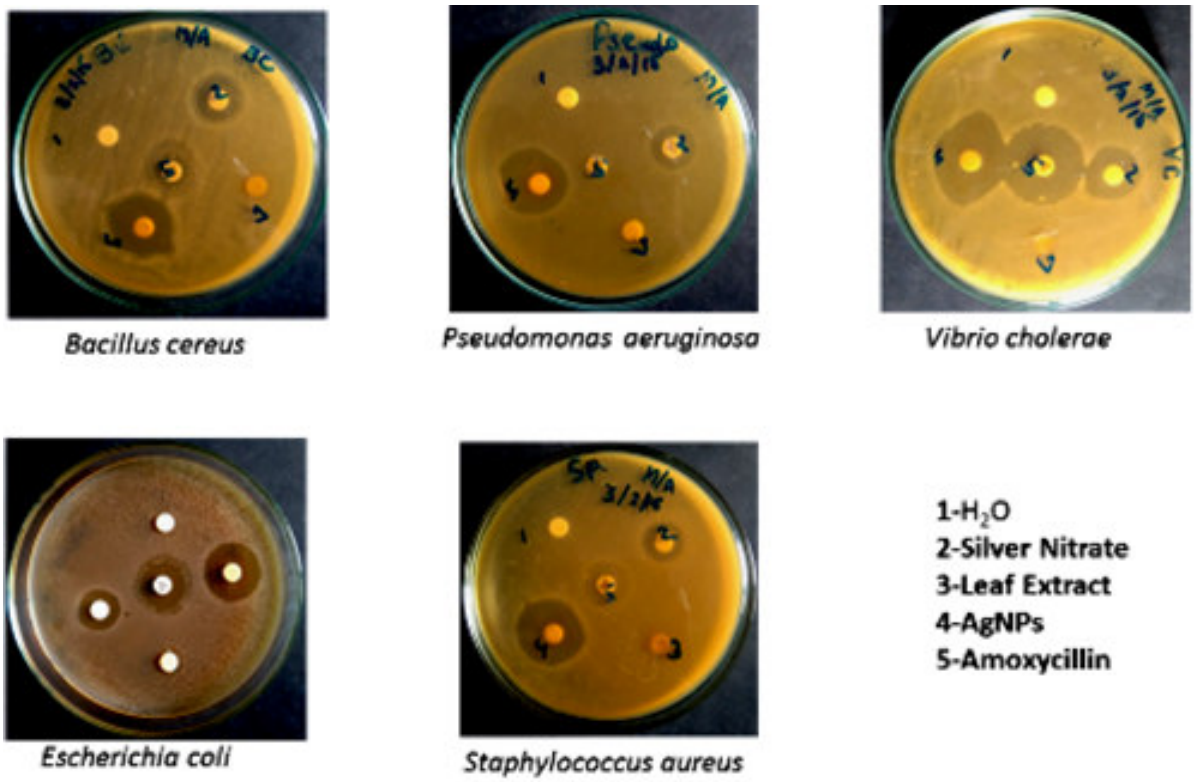

Figure 5
Comparative analysis of synthesised AgNPs from Occimum sanctum

Table 2
Comparative analysis of antimicrobial activity of silver nanoparticles with positive and negative control

Pathogens	H_2O	$AgNO_3$	Leaf Extract	AgNPS	Antibiotics (Amoxycillin 10mcg)
	(Zone of inhibition in mm)				
Bacillus cereus	_	16±0.8	_	20±0.7	9±0.6
Pseudomonas sp.	_	14±0.6	9±1.2	21±1.5	_
Vibrio cholerae	_	16±1.8	10±1	29±1.2	25±0.8
Escherichia coli	_	15±1	8±1	20±1	17±1
Staphylococcus aureus	_	14±1	11±1	24±1	16±1

[H_2O – water, $AgNO_3$ - Silver nitrate, AgNPS – Silver nanoparticles]

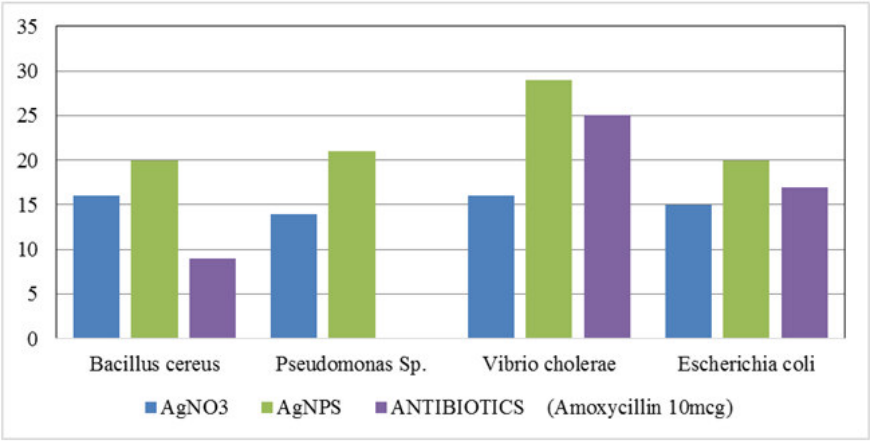

Figure 6
Graphical Representation AgNps from Occimum sanctum

Comparative analysis of antimicrobial activity of silver nanoparticles was determined against test pathogens along with the positive, negative control and commercial available antibiotics. Water and leaf extract act as positive control and silver nitrate alone used as a negative control along with synthesized AgNPs from *Occimum sanctum* and commercial available antibiotics Amoxicillin. The maximum zone of inhibition was observed in AgNP's which was around 20 – 29 mm. When compared with positive, negative and commercial available antibiotic Amoxicillin. The results of the antimicrobial activity of positive, negative and antibiotics have been tabulated in Table 2 and shown in Figures 5 and 6. In comparative analysis biosynthesized AgNPs shown better zone of inhibition than leaf extract, silver nitrate and commercially available drugs.

Synergistic activity of synthesised AgNPsfrom Occimum sanctum along with commercial antibiotics

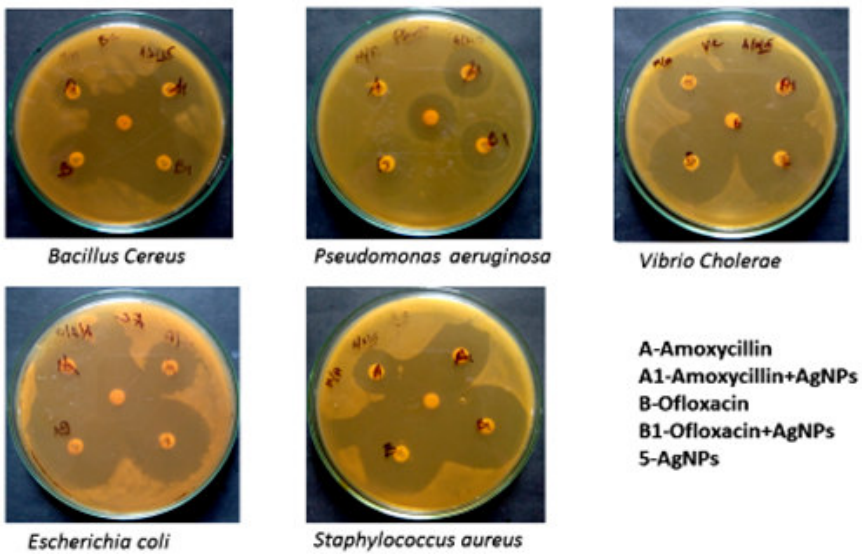

Figure 7a
Zone of inhibition in synergistic activity using Amoxycillin and Ofloxacin

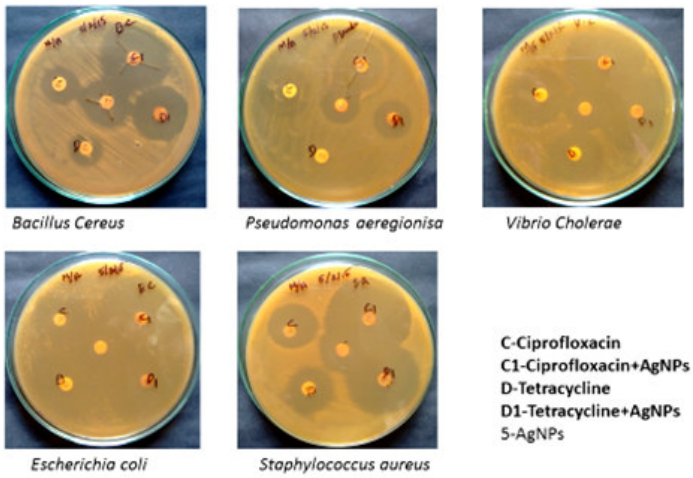

Figure 7b
Zone of inhibition in synergistic activity using Ciprofloxacin and Tetracycline

Synergistic antimicrobial activity AgNPs from *Occimum sanctum* along with commercial antibiotics such as Amoxycillin with AgNPs,Olfoxacin + AgNPs, Ciprofloxacin + AgNPs, Tetracycline+ AgNPs. The antibiotic incorporated with AgNPs shows efficient antimicrobial activity when compared with commercial available antibiotics and silver nanoparticles. The results of the antimicrobial activity have been tabulated in Table 3 and shown in Figures 7a and 7b. In comparative analysis, synergistic activity of biosynthetic AgNPsincorporated with commercial available drugs shows more efficacy than the drug alone .

Table 3
Synergistic activity of synthesised AgNPs from *Occimum sanctum along with commercial antibiotics*

Pathogens	Amoxy	Amoxy +AgNPs	Ofloxacin (10mcg)	Ofloxacin + AGNPs	Cipro	Cipro + AGNPs	Tetracycline	Tetracycline + AgNPs	AgNPs
	(zone of inhibition)								
B. cereus	15±1.2	26±0.8	23±1.1	34±0.8	16±1.4	30±1.3	14±0.5	27±1.1	30±1.7
P. aeruginosa	-	21±1.4	19±1.8	23±1.4	13±0.8	24±1.5	_	18±	19±1
V. cholerae	25±1	44±1	34±1	40±1	28±1	40±1	25±1	42±1	29±1
E. coli	20±1	45±1	33±1	44±1	20±1	32±0-9	32±1	42±1	38±1
S. aureus	18±1	26±1	33±1	33±1	24±1	40±1	22±1	37±1.4	33±1.2

[Amoxy – Amoxycilliin, Cipro – Ciprofloxin, AgNPS – Silver nanoparticles. – no activity]

DISCUSSION

Green nanoparticles were synthesized from *Occimum sanctum* and in earlier studies the synthesized nanoparticles were subjected to various analytical studies those reports revealed the presence of nanoparticles based upon the results of UV absorbance, FTIR, XRD, FE-SEM, EDAX which were correlating with the work[4]. In present research study antimicrobial activity of silver nanoparticles was determined by Kirby bauer disc diffusion method by three parameters. Initially the concentration of silver nanoparticles was found out to determine at which concentration the AgNPs inhibited the growth of test pathogens. A concentration of about 10 – 25 µl of silver nanoparticles was added in sterile disc. The concentration of about 25 µl inhibited the growth of various test pathogens such as *E. coli*, *B. cereus*, *S. aureus, V. cholerae, P. aeruguinosa.* This concentration was used for the other two parameters for determined the antimicrobial activity. That is comparative analysis and synergistic activity. In comparative analysis, test sample (AgNPs) positive control (H_2O, leaf extract) negative control ($AgNO_3$). Antibiotics Amoxycillin (standard) was used to determine the efficacy of antimicrobial activity against test pathogens. Among all the three samples test sample AgNP's showed better antimicrobial activity when compared with positive and negative control against test pathogens.A zone of inhibition was maximum in AgNPs around 20 - 29 mm whereas in other three samples the range of about 9 – 17 mm was analysed which was found to be less efficient than AgNPs. Synergistic antimicrobial activity of AgNPs along with commercially available antibiotics such as Amoxycillin, Ciprofloaxin, Tetracycline, Olfoxacin was done against test pathogens. The results revealed that AgNPs and antibiotics showed zone of inhibition around (15 – 38 mm) where AgNPs incorporated with antibiotics tabulated in Table 3 showed maximum zone of inhibition around (21 - 45 mm) which proved that antibiotics along with AgNPs incorporated on a sterile disc possess efficient antimicrobial activity and potency when compared with other samples against test pathogens. This product can be used as antimicrobial agent in future to inhibit the growth of MDR strain after proper toxicity test and chemical structural modifications. All these results were in accordance with the results[7-11].

CONCLUSION

In our research work AgNPs synthesized from *Occimum sanctum*Tulasi leaf possessed efficient antimicrobial activity against the various test pathogens. This antimicrobial compound can be used as a medicine in

future against the MDR strains after proper toxicity testing and structural modification of the compound. This research work revealed that the compound possessed efficient bactericidal and bacteriostatic activity when compared with standard antibiotics, leaf extract and $AgNO_3$.

CONFLICT OF INTEREST

Conflict of interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript