



COMPARATIVE ANALYSIS AND SYNERGISTIC ACTIVITY OF SILVER NANOPARTICLES AGAINST HUMAN PATHOGENS

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ABSTRACT

In the present study Silver Nanoparticles (AgNPs) were synthesized from Silver nitrate (AgNO_3) through a simple and ecofriendly route using leaf broth of *Occimum sanctum* and its antimicrobial activity was determined against various test pathogens. , simultaneously to find out the efficacy of synthesized AgNPs and comparing it with commercial available antibiotics along with positive and negative control. In my research work AgNPs were 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-Bauer 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 to synergistic activity by using commercial available antibiotics such as Amoxicillin, tetracycline, oxacillin, ciprofloxacin along with 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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INTRODUCTION

Nanotechnology can be useful in diagnostic techniques, drug delivery, antimicrobial bandages, disinfectant, a friendly manufacturing process that reduce waste products¹. 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 materials². 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-friendliness³. In the earlier study *Occimum sanctum* silver nano particles was synthesized and characterized by various analytical method⁴ 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

RESULTS AND DISCUSSION

Characterization of green nano particles by FESEM

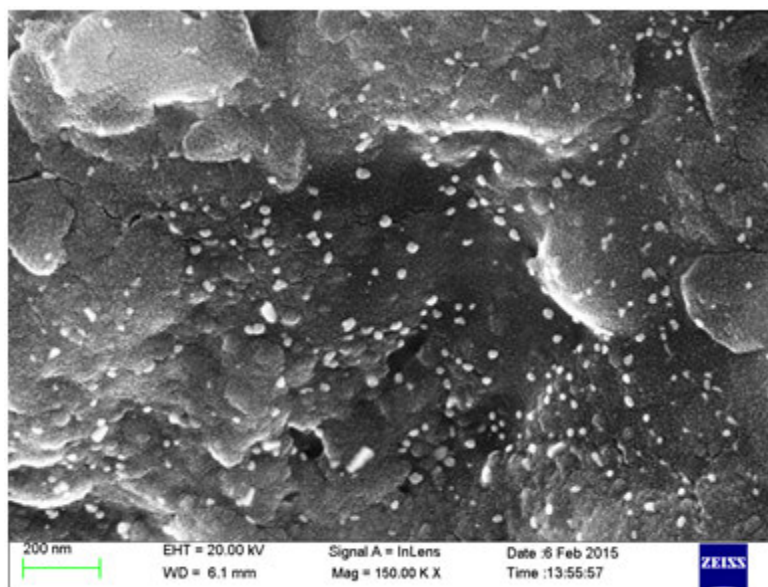


Figure 1
FE-SEM analysis showing nanoparticle size

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 monodispersed⁵.

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.

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 obtained⁵⁻⁶. (fig.2).

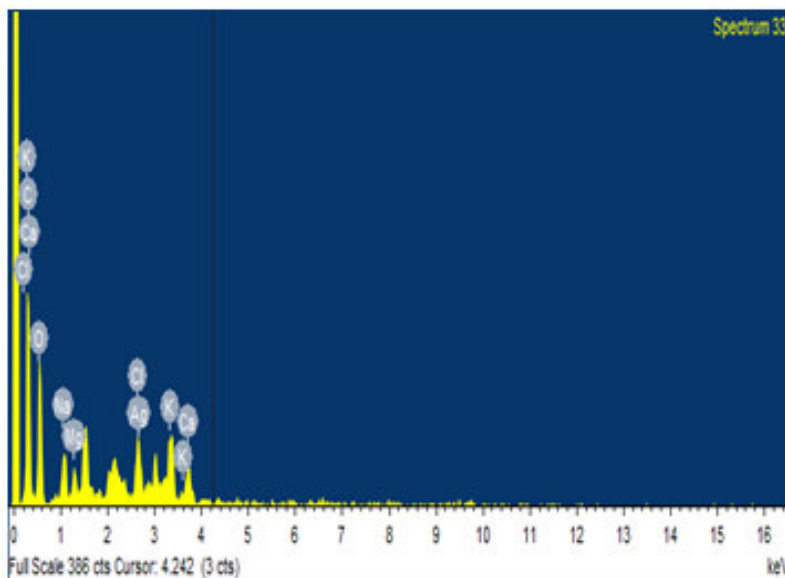


Figure 2
FE-SEM with EDAX mapping analysis

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

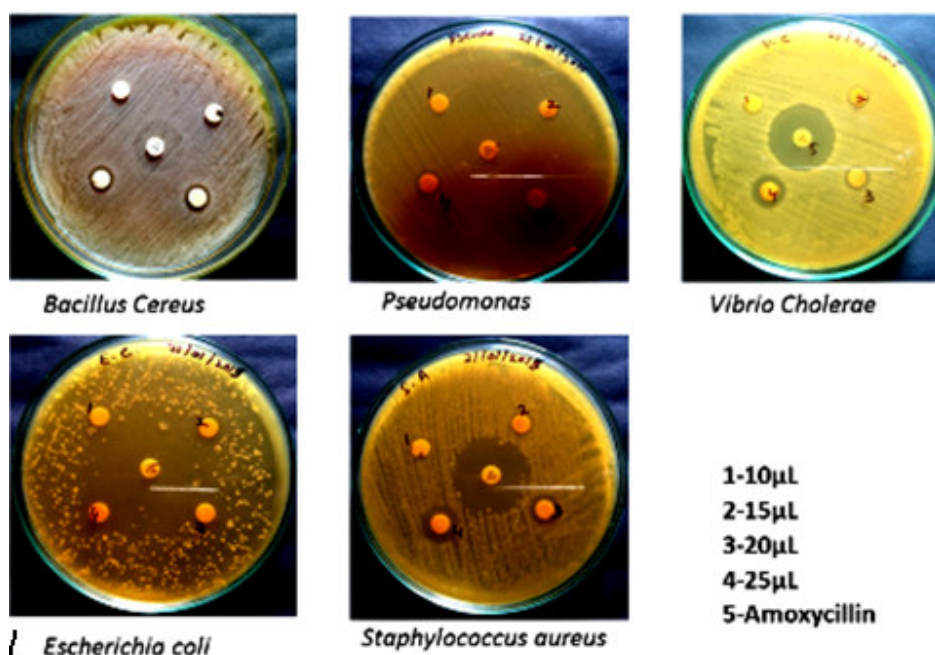


Figure 3
Antimicrobial activity of different concentrations of synthesised AgNPs

Table 1
Zone of Inhibition of Synthesized AgNps from Occimum sanctum

Pathogens	10µl	15µl	20µl	25µl	Antibiotics (Amoxicillin 10 mcg)
<i>Bacillus cereus</i>	—	—	10±1	10±1	19±1
<i>Pseudomonas sp.</i>	—	—	—	10±1	19±1
<i>Vibrio cholerae</i>	6±1	8±1	9±1	11±1	25±1
<i>Escherichia coli</i>	—	10±1	11 ±1	13±1	—
<i>Staphylococcus aureus</i>	—	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 used and their corresponding good zone of inhibition were recorded in Table 1, fig.3.

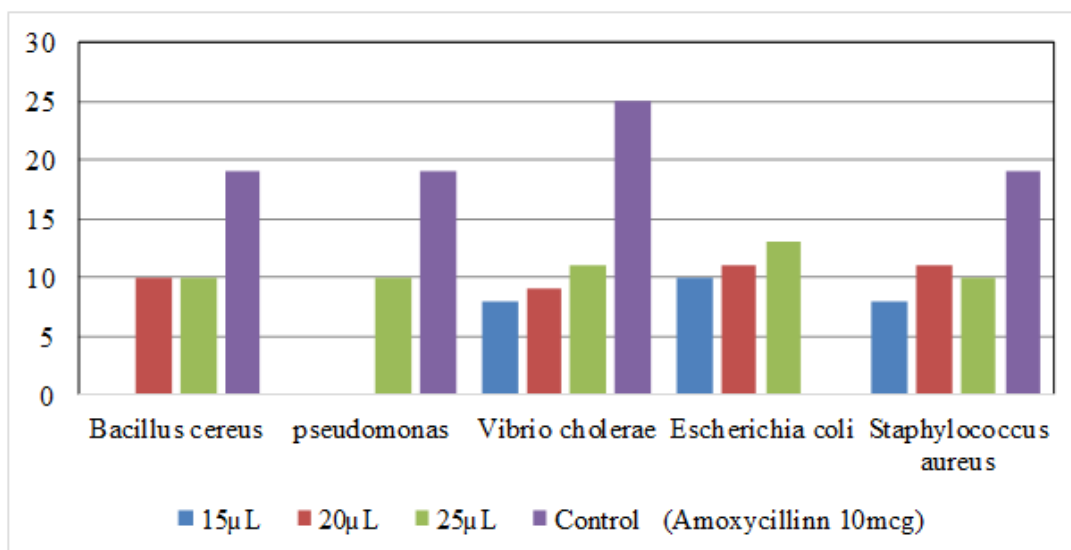


Figure 4
Graphical Representation of AgNps from Occimum sanctum

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 chosen 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.

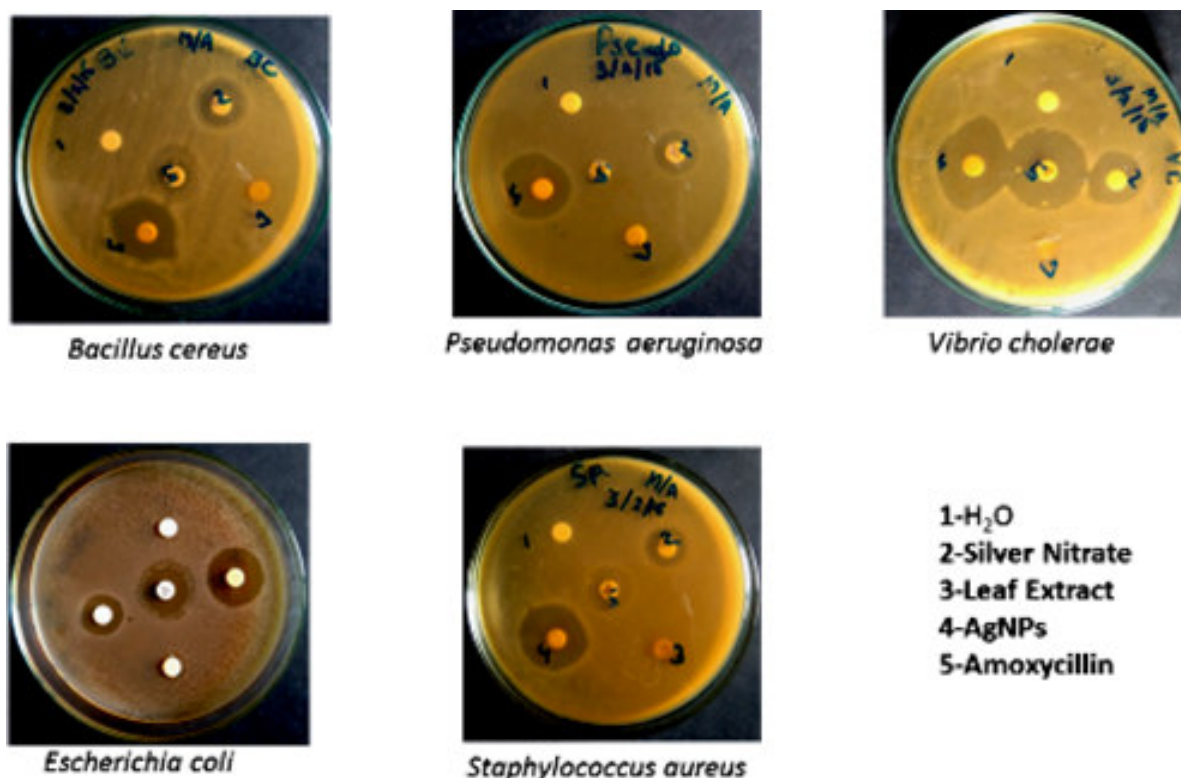


Figure 5
Comparative analysis of synthesised AgNPs from Occimum sanctum

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

Pathogens	H ₂ O	AgNO ₃	Leaf Extract	AgNPS	Antibiotics (Amoxycillin 10mcg)
(Zone of inhibition in mm)					
<i>Bacillus cereus</i>	—	16±0.8	—	20±0.7	9±0.6
<i>Pseudomonas sp.</i>	—	14±0.6	9±1.2	21±1.5	—
<i>Vibrio cholerae</i>	—	16±1.8	10±1	29±1.2	25±0.8
<i>Escherichia coli</i>	—	15±1	8±1	20±1	17±1
<i>Staphylococcus aureus</i>	—	14±1	11±1	24±1	16±1

[H₂O – water, AgNO₃ - Silver nitrate, AgNPS – Silver nanoparticles]

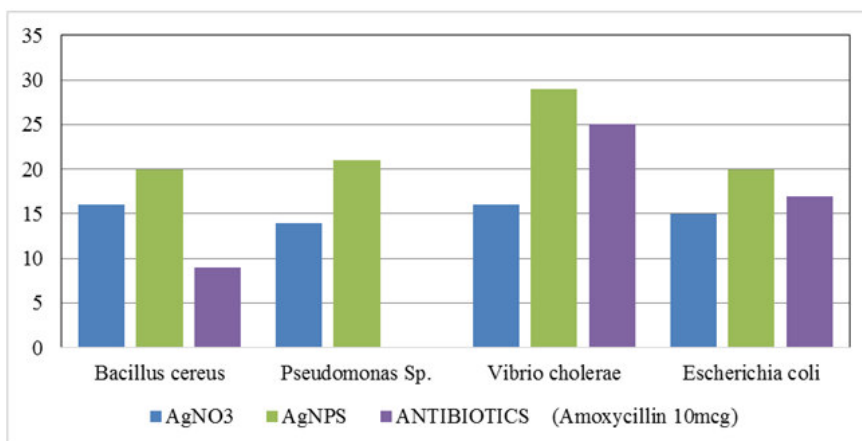


Figure 6
Graphical Representation AgNps from *Occimum sanctum*

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 AgNPs from *Occimum sanctum* along with commercial antibiotics

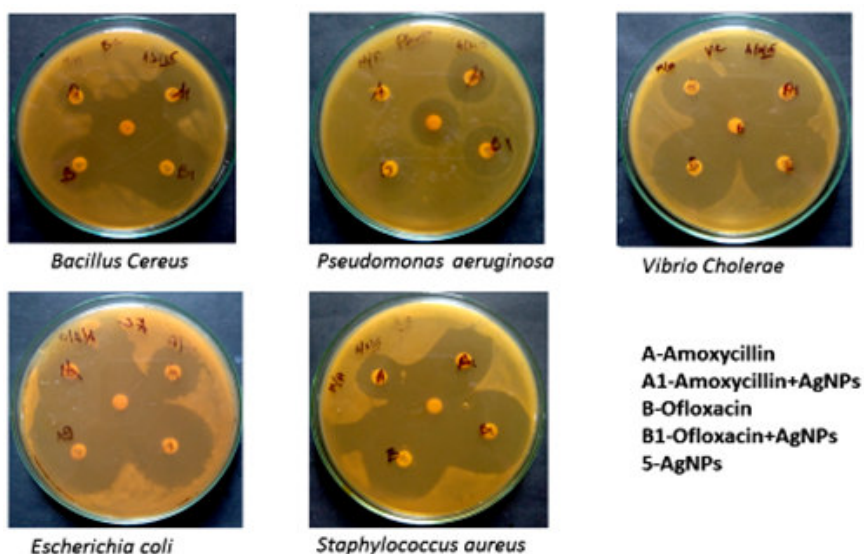


Figure 7a
Zone of inhibition in synergistic activity using Amoxycillin and Ofloxacin

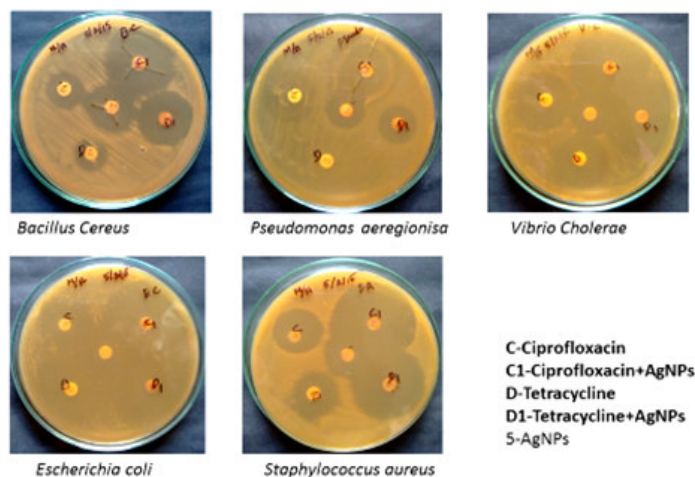


Figure 7b

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, Ofloxacin + 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 AgNPs incorporated 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 (zone of inhibition)	Amoxy +AgNPs (10mcg)	Ofloxacin + AGNPs	Cipro + AGNPs	Tetracycline + AgNPs	AgNPs			
<i>B. cereus</i>	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
<i>P. aeruginosa</i>	-	21±1.4	19±1.8	23±1.4	13±0.8	24±1.5	18±	19±1	19±1
<i>V. cholerae</i>	25±1	44±1	34±1	40±1	28±1	40±1	25±1	42±1	29±1
<i>E. coli</i>	20±1	45±1	33±1	44±1	20±1	32±0.9	32±1	42±1	38±1
<i>S. aureus</i>	18±1	26±1	33±1	33±1	24±1	40±1	22±1	37±1.4	33±1.2

[Amoxy – Amoxycillin, 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 work⁴. 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. aeruginosa*. 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 (H₂O, leaf extract) negative control (AgNO₃). 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, Ciprofloxacin, Tetracycline, Ofloxacin 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 results⁷⁻¹¹.

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 AgNO₃.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

- Shetty S, Udupa S, Udupa L, Somayaji N. Wound healing activity of *Occimum sanctum* Linn with supportive role of antioxidant enzymes. Indian J. Physiol. Pharmacol., 2006; 50(2): 163-8.
- Ahmed M, Ahamed RN, Aladakatti RH, Ghosesawar MG. Reversible anti-fertility effect of benzene extract of *Occimum sanctum* leaves on sperm parameters and fructose content in rats. J. Basic Clin. Physiol. Pharmacol. 2002; 13(1):51-9.
- Gupta S, Mediratta PK, Singh S, Sharma KK, Shukla R. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Occimum sanctum* (Linn) seed oil. Indian J. Exp. Biol. 2006; 44(4):300-4
- T.Manimaran,ThukkaramSudhakar, Anima Nanda, M. Amin Bhat, Abin Varghese. "Biosynthesis of Green Nanoparticles from *Occimum sanctum* and their Characterization", Res J Pharm Technol. 2016; 4(4): 397-400.
- Amin Bhat M, Nayak BK, Anima Nanda. Exploitation of filamentous fungi for biosynthesis of silver nanoparticle and its enhanced antibacterial activity. Int J Pharm Bio Sci, 2015; 6(1): 506 – 15.
- RavishankarBhat, RaghunandanDeshpande, Sharan abasava V, Ganachari, Do Sung Huh, and A. Venkataraman. Photo-Irradiated Biosynthesis of Silver Nanoparticles Using Edible Mushroom *Pleurotusflorida* and Their Antibacterial Activity Studies. Bio inorg Chem Appl. 2011;7 pages.
- K. Mallikarjuna, G. Narasimha, G. R.Dillip, B. Praveen, B. Shredhar, C. Sree Lakshmi, B. V. S. Reddy, Green synthesis of silver nanoparticles using *Occimum* leaf extract and their Characterization. Dig J Nanomater Biostruc . 2011; 6(1):181-6.
- Garima Singhal, RijuBhavesh, Kunal Kasariya, AshishRanjan, Sharma Rajendra Pal Singh. Biosynthesis of silver nanoparticles using *Occimum sanctum* (Tulasi) leaf extract and screening its antimicrobial activity. J Nanopart. Res. 2011; 13: 2981–8.
- Anima Nanda, Shahnaz Majeed. Improved bactericidal property of silver nanoparticles from *Penicillium pinophilum* (MTCC 2192) in a combined form with Carbenicillin and Moxifloxacin, Int J Pharm Pharm Sci. 2014; 6: 609- 12.
- Shivakumar Singh P, Vidyasagar G.M. Green synthesis, characterization and antimicrobial activity of Silver Nanoparticles by using *Sterculiafoetida* L. young leaves aqueous extract. Int J Green Chem Bioproc. 2014; 4(1): 1-5.
- Nithya R, Raghunathan R. Synthesis of silver nanoparticles using *Pleurotussajorcaju* and it antimicrobial study. Dig J Nanomater Biostruc. 2009; 4(4);623-9.

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