International Journal of Scientific Research and Engineering Development-– Volume 5 Issue 5, Sep- Oct 2022

Available at www.ijsred.com

RESEARCH ARTICLE OPEN ACCESS

COMPARATIVE STUDY OF ANTIMICROBIAL SUSCEPTIBILITY ANALYSIS OF SYNTHESIZED NANOPARTICLES

Dr.E.Bharathi [*1], S.Ravikumar[*2], Dr.S.Senthilvelan[*3]

[*1]Assistant professor, Chemistry, Joseph Arts and Science College, Thirunavallur, cuddalore, Tamilnadu, India

[*2] Research Scholar, Physics, Anna University, Chennai, India

[*3] Associate Professor, Chemistry, Annamalai University, Annamalai nagar, Chidambaram, India

I. ABSTRACT

In this study converse anti bacterial (gram positive, gram negative) and anti fungal activity of all the synthesized nanomaterials were Ag/CuO, Ag/ZrO_2, Ac/Ag-In_2O_3, Ac/Ag-ZrO_2, Ag/In_2O_3, ZrO_2, In_2O_3, CuO, and AC-Ag@CuO against Gram positive Bacillus subtilis, S. aureus, and Gram negative E.coil, K. pneumoniae, S. Paratyphi A, S. Paratyphi B, Shigella sp., P. Vulgaris, P. Mirabilis, Pseudomonas.SP, are showed in the success manner. The zone of inhibition values are discussed successfully. The comparative microbial studies were suggested that silver containing nanoparticles were showing better activity against bacterias and fungals. The enhanced superior antimicrobial activity of obtained Ac/Ag@CuO and Ag@CuO were study suggests the significance activity achieved against Gram positive namely (Bacillus subtilis, Staphylococcus aureus) and Gram negative (E.Coli, S. Paratyphi A,B).The microbial DSSC results were compared with ciprofloxacin,as the positive control.

Keywords: Antibacterial, Antifungal, Analysis, Gram positive, Gram Negative

I. INTRODUCTION

The synthesized silver containing nanoparticles were applied in various medical field, water painting, and wound dressing application. Antibacterial activity of synthesized metal oxides nanoparticles were performed against Gram + (ve) and gram (-ve) Bacteria. The Antibacterial activity was done by Modified Kirby -Bauer well diffusion Method. In brief, the pure cultures of organisms were inoculated in Nutrient broth and kept it on the rotatory shaker 160 RPM/35^{0}C/24 hrs. For bacterial growth a lawn of culture was prepared by spreading the 100 µl fresh culture having 10^6 colony – forming units (CFM)/ml of each test organism on Muller Hinton agar plates with the help of a sterile buds. Plates were left standing for 10 minutes to let the culture get absorbed.

Then 8nm wells were punched into the Muller Hinton agar plates for testing nano material antimicrobial activity. Using a micropipette, 30 µl(10mg/ml) of the sample of nanoparticle suspension was poured onto each of the five wells on all plates. After overnight incubation at 35℃ ± 2℃, the different levels of Zone of inhibition were measured. Solvent blank was used as negative control. Antibiotic Amoxicillin 30µl (1mg/ml) was used as positive control.

The synthesized nanomaterials were screened for the antibacterial activity against two bacillus subtills (gram-positive) and (b) vibrio cholerae (gram-negative).The synthesized nanomaterials were screened for the antibacterial activity against two the bacterial strains viz., (a) Escherichia coli (e.coli) that is a gram negative and (b) staphylococcus aureus (s.aureus) that is a gram positive.

The synthesized nanomaterials were screened for the antibacterial activity against two gram-positive bacteria staphylococcus aureus and gram-negative bacteria Escherichia coli. The synthesized nanomaterials were screened for the antibacterial activity against two (a) bacillus subtills (positive) (b) vibrio cholerae (negative) by using the disc diffusion method ciprofloxacin was used as reference standard for comparing results.The inhibition zones (mm) around of prepared nps are showed .Where the zone of inhibition of Ac/Ag@CuO, silver doped CuO, Ac/Ag-In_2O_3 was significantly higher activity as compared to and undoped CuO, In_2O_3, ZrO_2.On the other hand, Zone of inhibition Ac/Ag@CuO Ag@CuO was lesser activity against gram negative bacteria (Pseudomonas.SP, K. pneumoniae) compared to the undoped CuO. **[1]**

II. METHODOLOGY

Experimental Procedure

Culture Medium

Nutrient broth was used for the preparation of inoculums of the bacteria and nutrient agar was used for the screening method.

Composition of Nutrient Agar Medium

Peptone (5.0 gm) Sodium chloride (5.0gm) beef extract (1.5 gm), yeast extracts (1.5 gm), agar (15.0 gm),distilled water (1000 ML) and pH (7.4 ± 0.2)

Determination of Antibacterial Activity By Disk-Diffusion Method

Nutrient agar plates prepared under sterile condition's and incubated overnight to identify contamination. About 0.2 ml of working stock culture was shifted into separate nutrient agar plates and spread thoroughly using a glass spreader. whattmann No.1 discs (6 mm diameter) were impregnated in the testing compounds dissolved in DMSO (200 mg/mL) for about half an hour. Commercially available drug disc, Ciprofloxacin (10 mg/disc) was used as positive reference standard. All the compounds were tested at dose levels of 1000 µg and DMSO used as control. The solutions of each test compound, control and reference standard were added separately in the cups and the plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37 ± 1^0C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

Antifungal activity

The synthesized nanomaterials were screened for the antifungal activity against Aspergillus niger. The synthesized nanomaterials were screened for the antifungal activity against *Trigoderma viride* by using the filter paper method. Amphoterin B was used as reference standard for the results.

Determination of Antifungal Activity by filter paper disc method

All those compounds screened earlier for antibacterial activity were tested for their antifungal activity. The fungi employed for the screening was Aspergilus niger and Trichoderma viride Amphotericin B was employed as standard to compare the results. The test organisms were sub-cultured using potato-dextrose-agar (PDA). The tubes containing sterilized medium were inoculated with test fungi and kept at room temperature for obtaining growth. After that they were stored at 4^0C in a refrigerator.

Composition of potato-dextrose-agar medium

Peeled potato-=50.0gm

Dextrose-5.0 gm

Agar-4.0 gm

Distilled water- up to 200 ml

The test organisms were sub cultured using PDA medium. The tubes containing sterilized medium were inoculative with respective fungal strain and kept aside at room temperature for growing the organism. After confirming the growth, they were stored in refrigerator. The inoculums were prepared by aseptically transferring 10 ml of sterile water into freshly sub-cultured slants of the test fungi and making a suspension by scraping the growth with an inoculation medium.

The PDA medium was sterilized by autoclaving at 121^0C for 15 min. The Petri plates, tubes and flasks plugged with cotton, were sterilized in hot-air oven at 160^oC, for an hour. Into each sterilized Petri plate (20 cm diameter), poured about 125ml of molten PDA medium which was already inoculated with respective strain of fungi (5 ml of inoculums to 250 ml of nutrient agar medium).The plates were left at room temperature aseptically to allow the solidification. After solidification, the measuring 10.0 mm in diameter were published from whattmann No.1 filter paper. Batches of 100 discs were dispersed to each

screw capped bottles and sterilized by dry heat at 50^0C for 3hrs. The discs of each compound were placed individually on sterilized PDA medium with fresh fungal respectively using ampicillin as the standard.

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml, Analar grade) to give concentration of 1000 g/mL. Ampotericin-B solution was also prepared at a concentration of1000 g/mL in sterilized distilled water. The PH of all the test solutions and control was maintained at 2 to 3 by using conc.HCl. All the compounds were tested at dose levels of 200 g/ (0.2mL) and DMSO used as a control. The solutions of each test compound, control and reference standards were added separately in the cups and the plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the PDA medium.petri dishes were subsequently kept at room temperature for 48 hours. After that diameter of zone of inhibition in mm surrounding each of the dishes were measured with the help of an antibiotic zone reader**[2]**

III. RESULTS AND DISCUSSION

Anti bacterial Activity

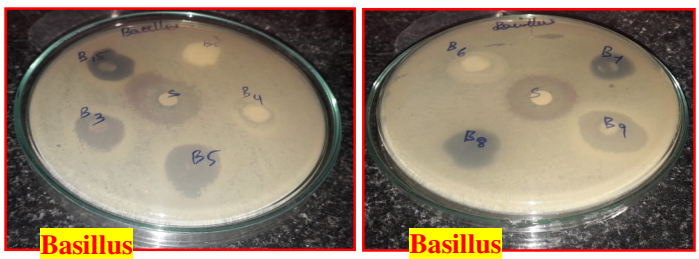
Determination of antibacterial activity by Disc - diffusion Method

Fig.1.1(1-9) explains the antibacterial activity of 1.Ag/ CuO, 2.Ag/ZrO_2 3. Ac/Ag -In_2O_3, 4. Ac/Ag- ZrO_2, 5. Ag/ In_2O_3 6.ZrO_2, 7.In_2O_3, 8.CuO and 9.AC-Ag@CuO nanomaterial were proposed against the experienced (10 human pathogens) bacterial strains. The antibacterial activity of the synthesized materials was evaluated through zone of inhibition (mm). The undoped synthesized nano material of CuO, ZrO_2, In_2O_3 material showed extremely poor activity against Bacillus subtilis, (Gram positive) and (Gram negative) pathogens and the CuO nanomaterial proved that significantly higher activity against Bacillus subtilis (Gram positive) and (Gram negative), as shown in **Fig.1.1.(1.9)**

The ZrO_2 material exhibited very poor activity against among all of the tested set of bacterial strains.AC-Ag@CuO was found to have better antibacterial activity and superior photocatalytic activity, and the comparison of its antibacterial activity with that of the Ag/ CuO, Ag/ZrO_2, Ac/Ag- ZrO_2, Ag/ In_2O_3, In_2O_3 and CuO material is shown in **Table.1.1.**

The sequence of antibacterial activity against Gram positive bacteria (Bacillus subtilis, Staphylococcus aureus) was following order CuO, > ZrO_2 > In_2O_3, Results exhibited that the proportional microbial studies were recommended that silver containing nanoparticles were performance enhanced activity against bacterias. The order of synthesized silver containing nanoparticles **Ag/ CuO** > **Ag/ZrO_2** > **Ag/ In_2O_3** as shown in clustered coloumn **chart.1.1.A**.The improved antimicrobial activity of Ac/Ag@CuO were study suggests the importance activity achieved in opposition to Gram negative specifically (Gram negative Shigella sp. strain [3]

The microbial DSSC consequences were compared with ciprofloxacin, the same as the positive control. The inhibition zones (mm) approximately of equipped nps are shown in **Fig.1.1**. and **Table..1.** On the other hand, Zone of inhibition order showed as Ac/Ag@CuO > Ag@CuO > CuO > Ac-Ag-In_2O_3 > Ag-In_2O_3 >Ag-ZrO_2 > Ac-Ag-In_2O_3 > ZrO_2 was minor activity adjacent to gram positive bacteria (B.subtils.) respectively. Where the zone of inhibition of silver doped nanomaterial was extensively superior activity as compared to other bare synthesized nanomaterial with Gram positive and negative bacteria.**[4-5]** Ac/Ag@CuO and undoped CuO with Gram positive bacteria (B.subtils and E.coli strains) were practical in a variety of medical field, water painting, and injury dressing appliance. Thus the HR-SEM and HR-TEM particle size plays a very important role in communicating enhanced antibacterial activity to the synthesized nanomaterials. The selection results are indicating Ac/Ag@CuO has superior antibacterial activity than that of other nanomatreial.



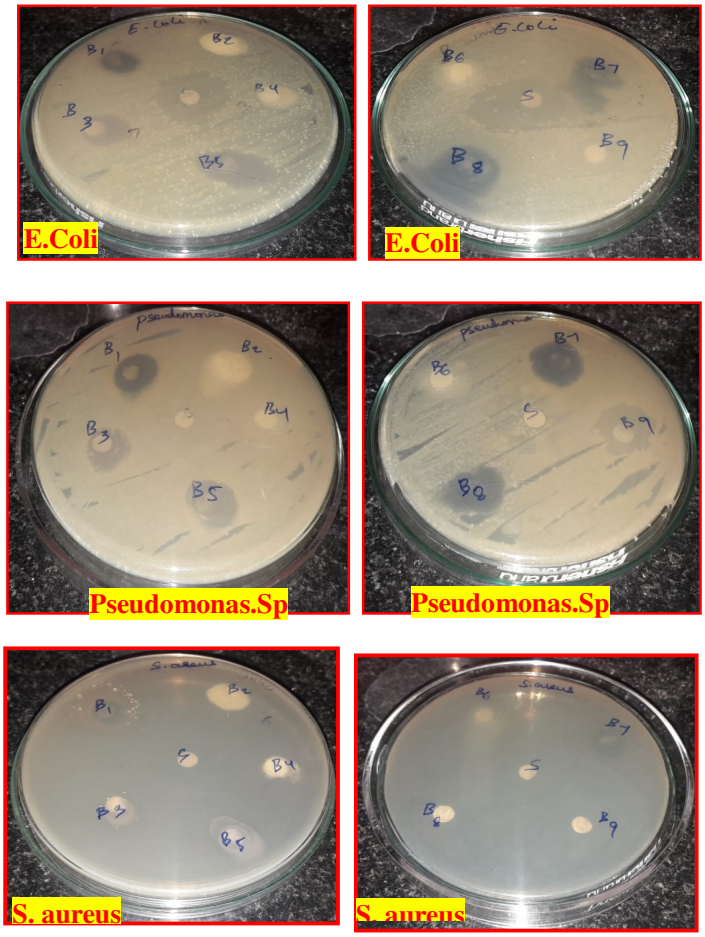
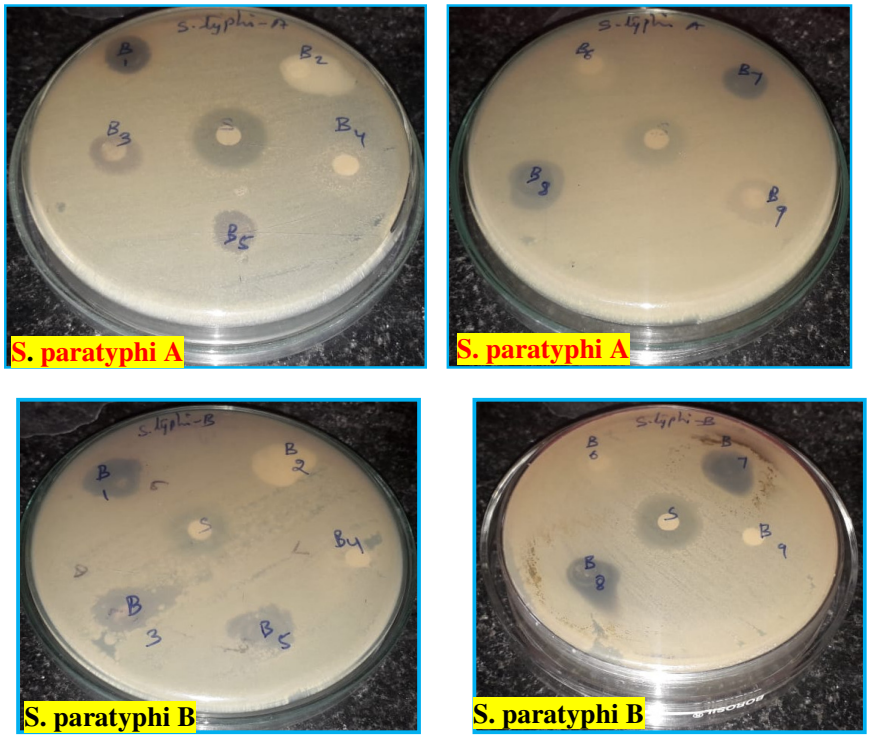


Figure..1. Antibacterial activity Plate photos of synthesized nanomaterial (1-9)

-



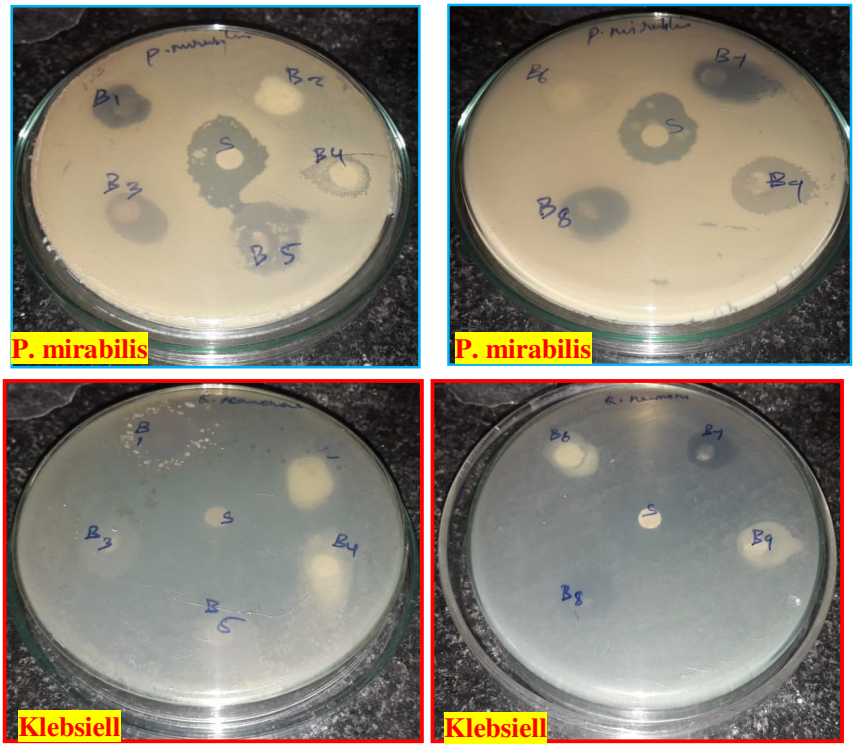
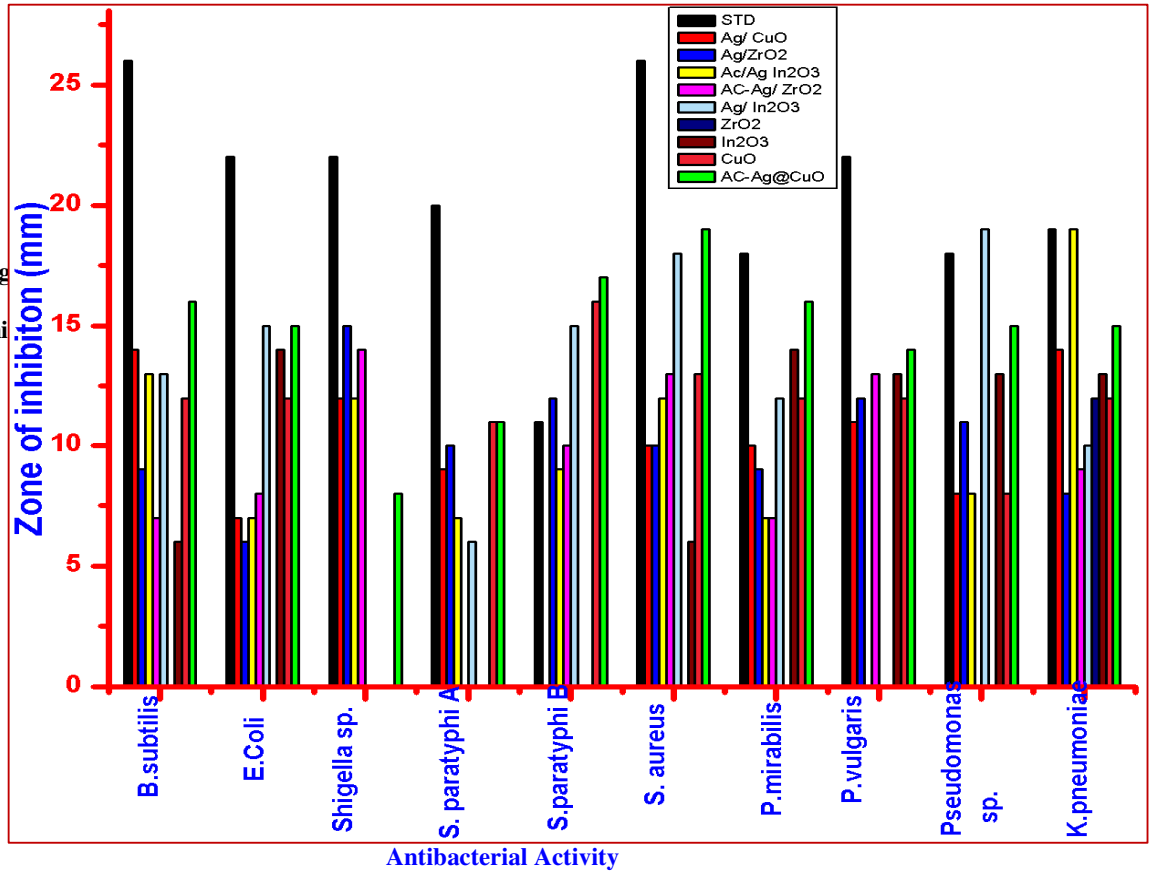


Figure.8.1. Antibacterial activity Plate photos of synthesized nanomaterial (1-9)

Table. 1.2. Antifungal activity [disc diffusion method]

Antifungal Assay Report for synthesized doped nanomaterials against three strains (Fungal) (Zone of inhibition (mm))										
Tested fungal strains	**Std**	**Ag/ CuO**	**Ag/ZrO_2**	**Ac/Ag-In_2O_3**	**Ac-Ag/ ZrO_2**	**Ag/ In_2O_3**	**ZrO_2**	**In_2O_3**	**CuO**	**AC-Ag@CuO**
Rhizopus sp.	25	20	30	25	30	24	-	30	25	35
Mucor sp.	30	22	26	24	23	27	-	26	22	40
Aspergillus fumigatus	15	16	30	24	30	25	20	20	20	27

Chart .1.A. The antibacterial effect of synthesized nanoparticles



Antifung

Determi

The anti fungal activity of all the 9 nanomaterials were studied against fungal strains are Rhizopus.sp., Aspergillus fumigates, Mucor sp.shown in **Figure .1.2** The zone of inhibition values are given in **Table .1.2**. The Clustered column **Chart.1.B.** was obtained. Antifungal activities of synthesized NPs were also evaluated against set of 3 fungal strains (Figs. 8.1.2). Results indicate that the Ac/Ag@CuO NPs illustrate superior antifungal activity (%) against Rhizopus.sp., Aspergillus fumigates, Mucor. sp strains, respectively. The ZrO_2NPs showed the poor antifungal activity, alongside fungal respectively. The arrangement of of antifungal activity against as in Chart-B was described in following order Ac/Ag@CuO > Ag@CuO > CuO > Ac-Ag-In_2O_3 > Ag-In_2O_3 >Ag-ZrO_2 > Ac-Ag-In_2O_3 > ZrO_2.Results displayed that the silver doped metal oxide reserved was extensively superior activity as compared to other bare synthesized nanomaterial with fungal strains. These finding are in line with previous studies related to the antibacterial activity of NPs **[6, 7, 8].** Thus the HR-SEM and HR-TEM particle size plays a very important role in communicating enhanced antibacterial activity to the synthesized nanomaterials. Author suggested the use of Ac/Ag@ CuO nanostructures as an proficient antifungal agent when compared to other synthesizd nanomaterials.**[9]**

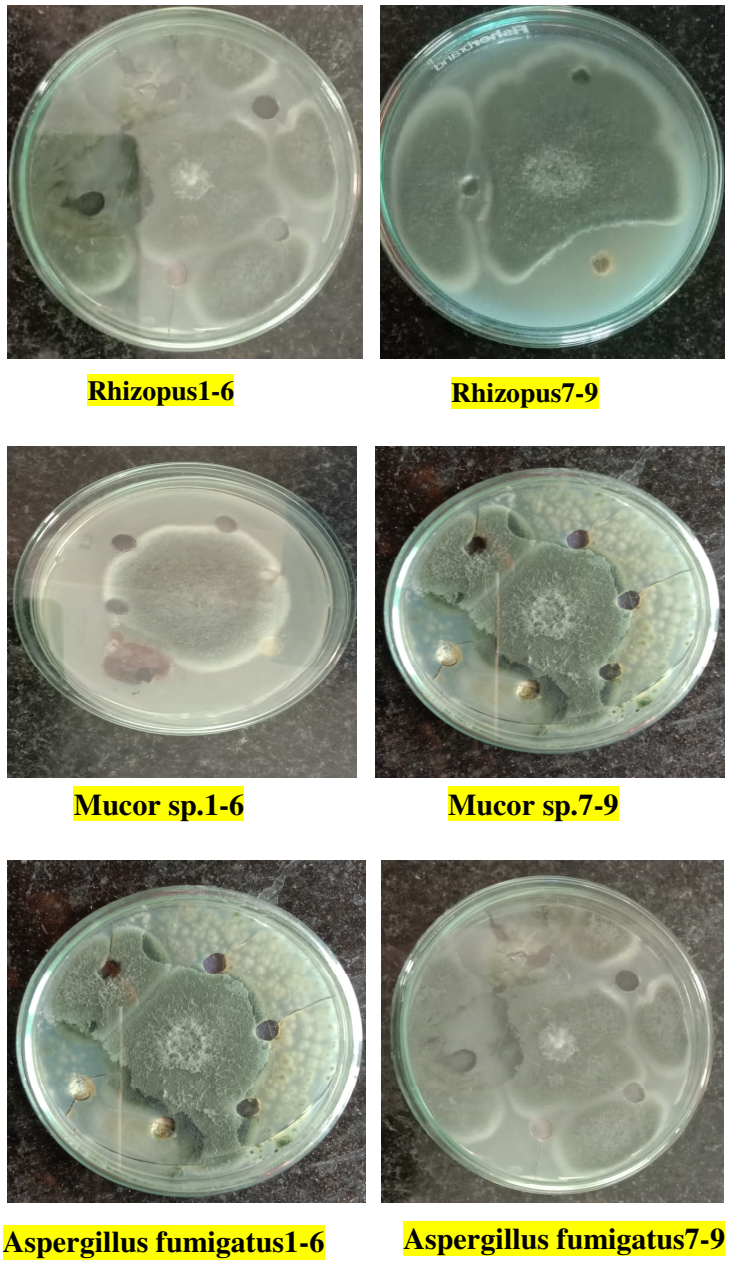
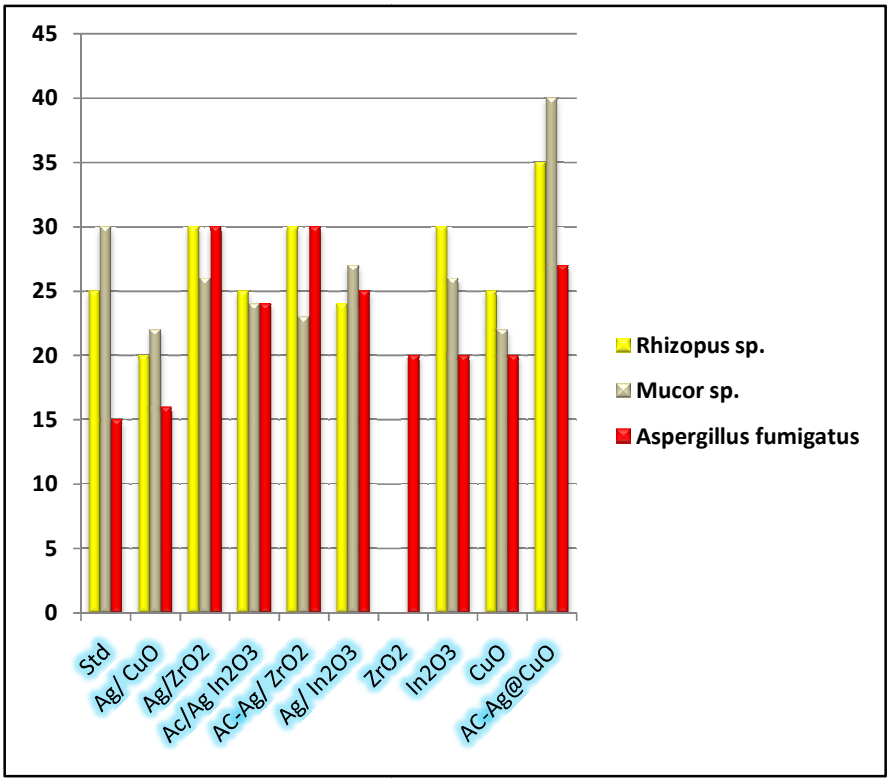


Figure.8.1.2. Antifungal activity Plate photos of synthesized nanomaterial (1-9)

Table.1.2. Antifungal activity [disc diffusion method]

Antifungal Assay Report for synthesized doped nanomaterials agnist three strains (Fungals) (Zone of inhibition (mm))										
Tested fungal strains	**Std**	**Ag/ CuO**	**Ag/ZrO_2**	**Ac/Ag In_2O_3**	**AC-Ag/ ZrO_2**	**Ag/ In_2O_3**	**ZrO_2**	**In_2O_3**	**CuO**	**AC-Ag@CuO**
Rhizopus sp.	**25**	**20**	**30**	**25**	**30**	**24**	**-**	**30**	**25**	**35**
Mucor sp.	**30**	**22**	**26**	**24**	**23**	**27**	**-**	**26**	**22**	**40**
Aspergillus fumigatus	**15**	**16**	**30**	**24**	**30**	**25**	**20**	**20**	**20**	**27**

Chart.1.B.Antifungal effect of synthesized nanoparticles



Mechanism for Antibacterial activity of Ac/Ag@CuO nps.

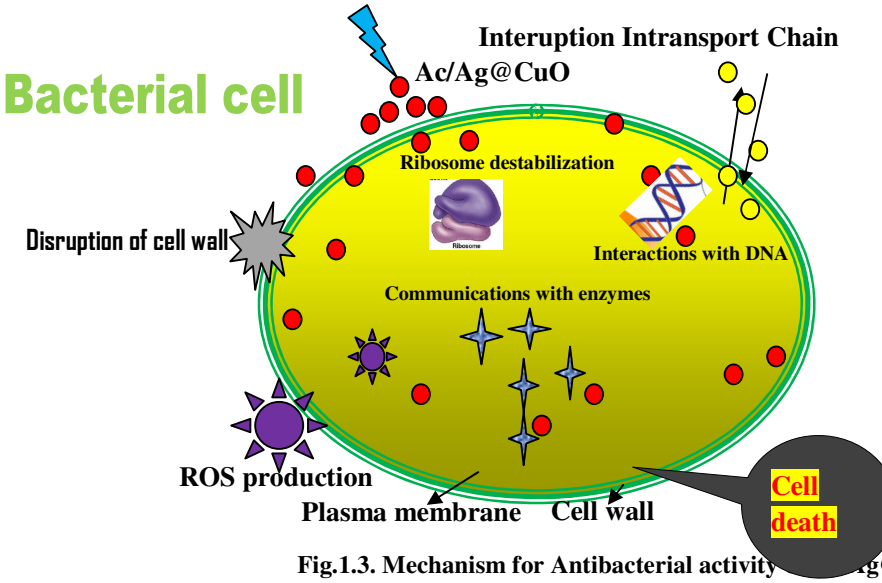


Fig.1.3. Mechanism for Antibacterial activity [illegible]g@CuO nps

Photolytic fabrication of reactive oxygen species (ROS) on the surface of Ac/Ag@cuO was appropriate to the formation of hydroxyl, superoxide radicals, and H_2O_2 (ROS) through the Fenton reaction important to lipid peroxidation, DNA injure and protein decompose can kill bacteria lacking disparaging nonbacterial cells.More over supplementary possible ladder engaged in the antibacterial activity. Ac/Ag@CuO holds up the bacteria cell covering and attached with mesosome (DNA-reproduction, cellular inhalation, cell separation[9]. This kind of intracellular practical alteration is commencing with the oxidative stress influenced by ROS leading to cell killing as demonstrated in **Fig.1.3.** A modest work of literature explains the antibacterial mechanism.

IV. CONCLUSION

Results are concluded that converse anti bacterial (grampositive, gramnegative) and anti fungal activity of all the synthesized nanomaterials were 1.Ag/ CuO, 2.Ag/ZrO_2 3.Ac/Ag-In_2O_3, 4. Ac/Ag-ZrO_2, 5. Ag/ In_2O_3 6.ZrO_2, 7.In_2O_3, 8.CuO, 9.AC-Ag@CuO against Gram positive Bacillus subtilis, S. aureus, and Gram negative E.coil, K. pneumoniae, S. Paratyphi A, S. Paratyphi B, Shigella sp., P. Vulgaris, P. Mirabilis, Pseudomonas.SP, are shown in **Figure.1.1. and Chart.1.A.**

The zone of inhibition values are discussed successfully. The comparative microbial studies were suggested that silver containing nanoparticles were showing better activity against bacterias and fungal. The enhanced superior antimicrobial and fungal activity of obtained Ac/Ag@CuO and Ag@CuO were study suggests the significance activity achieved against Gram positive namely (Bacillus subtilis, Staphylococcus aureus) and Gram negative (E.Coli, S. Paratyphi A,B) and Mucor sp.

The microbial DSSC results were compared with ciprofloxacin, as the positive control. The inhibition zones (mm) around of prepared nps are shown in **Fig.8.1.2.**Where the zone of inhibition of Ac/Ag@CuO, silver doped CuO, Ac/Ag-In_2O_3 was significantly higher activity as compared to and undoped CuO, In_2O_3, ZrO_2.

On the other hand, Zone of inhibition Ac/Ag@CuO Ag@CuO was CuO, In_2O_3, ZrO_2. On the other hand, Zone of inhibition Ac/Ag@CuO Ag@CuO was lesser activity against gram negative bacteria (Pseudomonas.SP, K. pneumoniae) compared to the undoped CuO. The mechanism of antibacterial activity has been discussed effectively.

The synthesized silver containing nanoparticles were applied in various medical field, water painting, and wound dressing application.

V. REFERENCES

1. Jaffri, S. B., & Ahmad, K. S. (2017). Augmented photocatalytic, antibacterial and antifungal activity of prunosynthetic silver nanoparticles. Artificial Cells, *Nanomedicine, and Biotechnology, 11.* doi:10.1080/21691401.2017.1414826

2. De la Rosa-García, S. C., Martínez-Torres, P., Gómez-Cornelio, S., Corral-Aguado, M. A., Quintana, P., & Gómez-Ortíz, N. M. (2018). Antifungal Activity of ZnO and MgO Nanomaterials and Their Mixtures against Colletotrichum gloeosporioides Strains from Tropical Fruit. *Journal of Nanomaterials, 2018, 1–9.* doi:10.1155/2018/3498527

3. Paszkiewicz, M., Gołąbiewska, A., Rajski, Ł., Kowal, E., Sajdak, A., & Zaleska-Medynska, A. (2016). The Antibacterial and Antifungal Textile Properties Functionalized by Bimetallic Nanoparticles of Ag/Cu with Different Structures. *Journal of Nanomaterials, 2016, 1–13.*

4. Devi, J. S., & Bhimba, B. V. (2014). Antibacterial and Antifungal Activity of Silver Nanoparticles Synthesized using Hypnea muciformis. *Biosciences Biotechnology Research Asia, 11(1), 235–238.* doi:10.13005/bbra/1260

5. Arshad, M., Qayyum, A., Shar, G. A., Soomro, G. A., Nazir, A., Munir, B., & Iqbal, M. (2018). Zn-doped SiO 2 nanoparticles preparation and characterization under the effect of various solvents: Antibacterial, antifungal and photocatlytic performance evaluation. *Journal of Photochemistry and Photobiology B: Biology, 185, 176–183.* doi:10.1016/j.jphotobiol.2018.04.043

6. K. Kannan, D. Radhika, A.S Nesaraj, K. Kumar Sadasivuni, K. Raghava Reddy, D. Kasai, A.V. Raghu, (2020) Photocatalytic, antibacterial and electrochemical properties of novel rare-earth-based nanohybrids, *Materials Science for Energy Technologies,* doi: https://doi.org/10.1016/j.mset.2020.10.008

7. Hassan Alsaud, Ahmed Abibat, Roger Painter, Lonnie Sharpe, Samuel Hargrove, *Chem Engineering* 2 (3) (2018) 2030-33. Doi: 10.3390/Chemengineering2030033.

8. E. Albiter, M.A.Valenzuela, S.Alfaro , G. Valverde-Aguilar, F.M. Martinez-Pallares, *Journal Of Saudi Chemical Society* 19 (2015) 563-573.
9. G. Manjari, S. Saran, T. Arun, A. Vijaya Bhaskara Rao, Suja P. Devipriya, *Journal Of Saudi Chemical Society* 21 (2017) 610-618.

