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Effect of Metal Nanoparticles on Pseudomonas Bacteria

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Abstract:- *Pseudomonas is the Gram negative bacteria which causes many diseases to human beings and livestock's. Nanotechnology is the current emerging area in medical fields to cure many bacterial diseases. $Cu(MnO_2)_2$, MnO_2 and $Ag(MnO_2)$ nanoparticles were synthesized by hydrothermal method. The structural and morphology of nanoparticles were confirmed by XRD and SEM. The effect of these $Cu(MnO_2)_2$ and $Ag(MnO_2)$ nanoparticles against the pesudomonas bacteria were studied using zone of inhibition method. $Cu(MnO_2)_2$, and $Ag(MnO_2)$ nanoparticles were found to be highly effective against pseudomonas, but the MnO_2 nanoparticles did not show any notable effect against pesudomonas genus. The diameter of zone of inhibition of $Cu(MnO_2)_2$, and $Ag(MnO_2)$ nanoparticles were compared with reference to MnO_2 nanoparticles..*

Key words; *Pesudomonas, $Cu(MnO_2)_2$, MnO_2, and Ag(MnO2), XRD,SEM*

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I. Introduction

Nanomaterials having a length scale less than 100nm have received increasing interest, owing not only their fundamental scientific areas [1]. Which has broad applications in many diverse field, including energy conversion and storage, chemical manufacturing, biological applications and environmental technology [2-3]. Various method are available to synthesis metal nanoparticles, Wet chemical method, Microwave assisted method ,Ultrasonic agitation method, Ball milling , Sol-gel method, Electrochemical synthesis and Hydro thermal method etc,,. Among these methods we have to choose hydro thermal method because without wasting solvent which give constant energy given to the reaction we get purity of the material and also very easiest method.

Pseudomonas infections are diseases caused by a bacterium from the genus Pseudomonas. The bacteria are found widely in the environment, such as in soil, water, and plants. They usually do not cause infections in healthy people. If an infection does occur in a healthy person, it is generally mild. (Brain Wu 2015)[4].An external ear canal infection may sometimes be caused by pseudomonas and result in "swimmer's ear." Symptoms may include: swelling, ear pain, itching inside the ear, discharge from the ear, difficulty hearing. Symptoms of an eye infection may include: inflammation pus pain swelling, redness impaired vision.(Euzeby J.P. 1997.,Jacquelyn Cafasso 2016[5].

Nanotechnology represents a modern and innovative approach to develop new formulations based upon metallic nanoparticles with antimicrobial properties. A probe of the interactions of antibiotics with silver (Ag) nanoparticles is the most common among these studies dedicated to the testing of combined action of nanoparticles with antibiotics. Few studies have reported that the efficiency of antimicrobial agents can be improved by combining them with nanoparticles against different pathogens, including Staphylococcus, aureus, Pseudomonas aeruginosa, Escherichia coli, etc. recently, some metal nanoparticles have been valued for increasing the antibacterial actions of different antibiotics. Therapeutic roles for zinc in different diseases have been established in recent years. Zinc oxide has a very good potential to get into the clinic (Shopsin et al. 1999).[6] Studies have revealed improved activity of nano ZnO when used in combination with cephalosporins, beta lactums and amino glycosides against different pathogenic microorganisms (Gaddadet al. 2010, Solomon et al. 2007).[7].

One of the most promising strategies for overcoming microbial resistance is the use of nanoparticles. Development of resistance to these nanoparticles is, again, unlikely (Friedman et al. 2013),[8] possibly because it would require multiple simultaneous gene mutations in the same microbial cell. Therefore, the interaction of nanoparticles with commonly used antimicrobials was studied by checkerboard method.

The purpose of the present investigations is to evaluate the antimicrobial activity of the metal nanoparticles [$Cu(MnO_2)_2$, MnO_2 and $Ag(MnO_2)$] on Pseudomonas Gram negative bacteria.

II. Material And Methods

All the chemicals were used in this experiment were AR grade. $CuCl_2$ (96%), $MnCl_2$, $KMnO_4$ (99%), $AgNO_3$, and ethanol were Purchased from Merck. All the chemicals and regents were used as without further purification.

2.1 Instumentation

The sample were characterized using analytical method such as the Structural of

MnO_2 and Ag (MnO_2) nanoparticles was taken out XRD (Expert pro).The morphology of the nanoparticles were carried out by SEM (VEGA3 TESCAN).

2.2 SYNTHESIS OF Cu (MnO_2)$_2$, MnO_2, and Ag(MnO_2) NANOPARTICLES

Cu(MnO_2)$_2$, MnO_2 and Ag(MnO_2) nanoparticles were synthesized by hydrothermal method using precursor materials such as $CuCl_2$, $MnCl_2$ and $AgNO_3$ were used. Ethanol was used as solvating reagent and $KMnO_4$ as an oxidizing agent. A (50ml) of ethanol and $CuCl_2$ (0.2g), were added to the round-bottom flask (RB) and heated at 78^0C to boil under slow evaporation method. In a flask, 10 ml of $KMnO_4$ solution was poured. The reaction was carried out under heating for 7 hr. The mixture was cooled to room temperature and centrifuged by which a precipitate was obtained. The precipitate was filtered and washed with distilled water and purified with absolute ethanol for several times. The resulting product was dried at hot- air oven for 8 hrs to obtain the Cu(MnO_2)$_2$ nanoparticles.. MnO_2 and Ag (MnO_2) nanoparticles were prepared by similar method.
chemical reactions:

$3CuCl_2 + 6KMnO_4 + 2C_2H_5OH \rightarrow 3Cu(MnO_2)_2 + 6KCl + 6H_2O + 4CO_2\uparrow$ $MnCl_2$
$+ 6KMnO_4 + 2C_2H_5OH \rightarrow 9MnO_2 + 6KCl + 3H_2O + 3CO_2\uparrow$
$3AgNO_3 + 3KMnO_4 + C_2H_5OH \rightarrow 3Ag(MnO_2) + 3KNO_3 + 3H_2O + 2CO_2\uparrow$

2.3 PREPARATION OF MOTHER CULTURE
2.3.1 MEDIUM PREPARATION

Chemicals required 1)
Agar agar
2) Nutrient agar

0.7 grams of nutrient agar and 0.375grams of agar agar are dissolved in 25 ml of distilled water. Prepared medium is now kept for sterilization at 121^0C and are poured into the petriplates (100×15), allowed to solidify .now the inoculation loop. After inoculating the plates was incubated at 37^0C for 24 hours.

2.3.2 BROTH PREPARATION

0.13 gram of nutrient broth is dissolved in 10ml of distilled water. Prepared medium was kept for sterilization, after sterilization it is cooled and the culture from the mother culture was inoculated for 24 hours.

2.4 ANTIMICROBIAL TESTING
2.4.1 MEDIUM PREPARATION

2.8grams of Nutrient Agar and 1.5 grams of Agar agar are dissolved in 100ml of distilled water, this preparation was transferred to a screw capped bottles are were kept for sterilization at 121^0C and are poured in to the petriplates(100×15) evenly. All the plates were allowed to solidify.

2.4.2 Anti-microbial testing with Ag (MnO_2) nanoparticles:

Sterilized cotton swab were taken and sample from the broth prepared is swabbed in the plates completely over the surface of semi solid agar medium evenly, Ag(MnO_2) nanoparticles are taken carefully with the help of the filter paper and are placed at the centre of the culture swabbed semi solid agar medium.

2.4.3 Anti-microbial testing with Cu(MnO_2)$_2$ **nanoparticles:**

Sterilized cotton swab were taken and sample from the broth prepared is swabbed in the plates completely over the surface of semi solid agar medium evenly, Cu(MnO_2)$_2$ nanoparticles are taken carefully with the help of the filter paper and are placed at the centre of the culture swabbed semi solid agar medium.

2.4.4 Anti-microbial testing with MnO_2 **nanoparticles:**

Sterilized cotton swab were taken and sample from the broth prepared is swabbed in the plates completely over the surface of semi solid agar medium evenly, MnO_2 nanoparticles are taken carefully with the help of the filter paper and are placed at the centre of the culture swabbed semi solid agar medium.

2.5 EFFECT OF Ag(MnO_2) NANOPARTICLES ON PSEUDOMONAS

A zone of inhibition was observed after 24 hours of incubation. **Ag(MnO_2)** nanoparticles was effective in pseudomonas. Effects on pseudomonas were observed at 48 hours and 72 hours also. The diameter of inhibition zone of Ag(MnO_2) nanoparticles in pseudomonas was 13mm after 24 hours. Likewise more or less same extent of inhibition was observed in the pseudomonas at 48 hours and 72 hours.

2.5.1 EFFECT OF Cu(MnO_2)$_2$ **NANOPARTICLES ON PSEUDOMONAS**

A zone of inhibition was observed after 24 hours of incubation Cu(MnO_2)$_2$ nanoparticles was effective in pseudomonas. Effects on pseudomonas were observed at 48 hours and 72 hours also. The diameter of inhibition zone of Cu(MnO_2)$_2$ nanoparticles in pseudomonas was 14mm after 24 hours. Likewise more or less same extent of inhibition was

observed in the pseudomonas at 48 hours and 72 hours.

2.5.2 EFFECT OF MnO_2 NANOPARTICLES ON PSEUDOMONAS

MnO_2 nanoparticles did not show any notable effect against bacterial culture. No zone of inhibition was found in the culture after 24 hours of incubation. Likewise no effects were found at 48 and 72 hours also.

III. Result and discussion

3.1 Surface Morphological study of $Cu(MnO_2)_2$, MnO_2 and $Ag(MnO_2)$by XRD and SEM

3.1.(a)$Cu(MnO_2)_2$ nanoparticles:

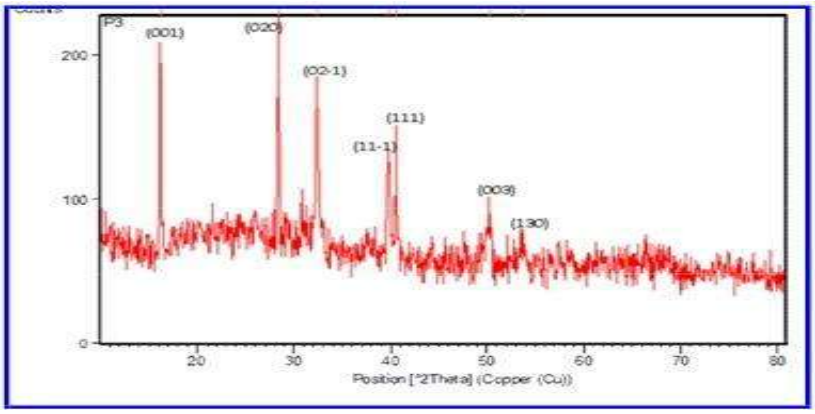
X-ray diffraction study of $Cu(MnO_2)_2$ nanoparticles synthesized by hydrothermal method was observed to be in purely crystalline in nature . Average particle size of copper oxide nanoparticles was found to be in the range of 28.41nm.

The XRD spectra showing the intense peak at 28.41˚ is having plane (020) which is the crystal plane of $Cu(MnO_2)_2$. The low intensity peak at 16.27˚, 32.41˚, 39.82˚, 40.5˚, 50.29˚ and 53.69˚which match well with the plane (001), (02-1), (11-1), (111), (003) and (130) indicates that the prepared $Cu(MnO_2)_2$ composite is highly crystalline characteristic of pure monoclinic crystals and well arrange in specific orientation. The size of the $Cu(MnO_2)_2$ crystals were estimated from the Debye-Scherer equation.

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

Where, K is the Scherer constant, which is related to the crystallite shape and are the radiation wavelength and Bragg's angle, respectively and is the full width at half maximum of the diffraction peak.

The crystal size of the products as calculated by Scherer formula was 28.41nm.



Fig; 1 XRD pattern of $Cu(MnO_2)_2$

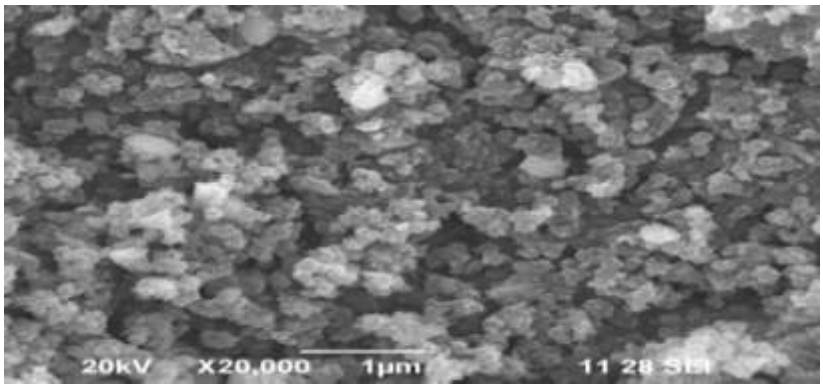


Fig . 2 SEM image of $Cu(MnO_2)_2$

SEM image of $Cu(MnO_2)_2$ is shown in Fig.2 from the SEM image it is observed that the shape of the particles have cubic morphology .High magnification image show that the small cubic structures are grown on the surface of the big cubic structures. The resultant morphology is looking a cluster of graphs. The morphology of the prepared $Cu(MnO_2)_2$ is very interesting and looks like cubic structures .The formation of small structures on bigger one may increase its. surface area and the indirectly the catalytic activity.

3.1.(b)MnO_2 nanoparticles

X-ray diffraction study of manganese oxide nanocomposite synthesized by hydrothermal method was

observed to be amorphous in nature. Powder X-ray diffraction pattern was recorded using XPERT PROX-ray diffractometer with CuK$_\alpha$ radiation by crushing the MnO_2 nanoparticles into fine powder. From the XRD pattern analysis grin size of the MnO_2 nanoparticles range 10.21nm .

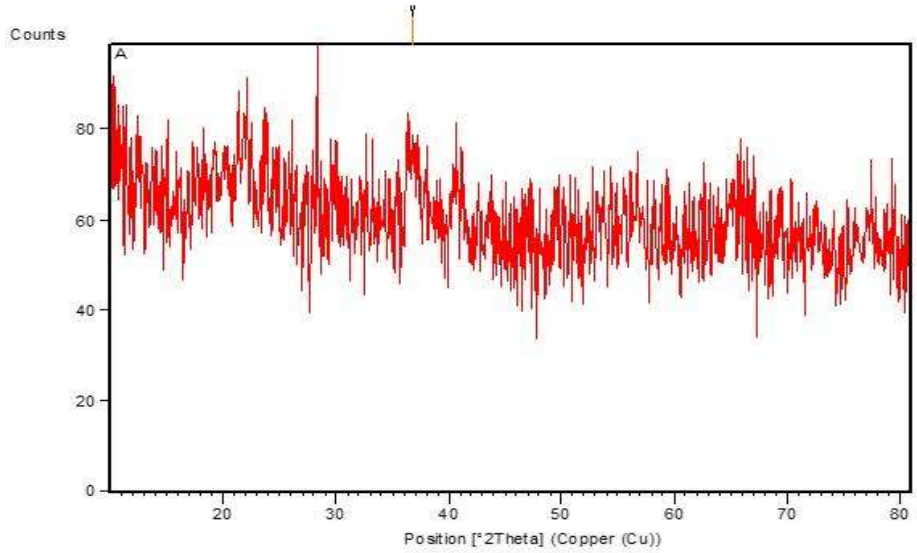


Fig 3 Powder XRD pattern of MnO_2

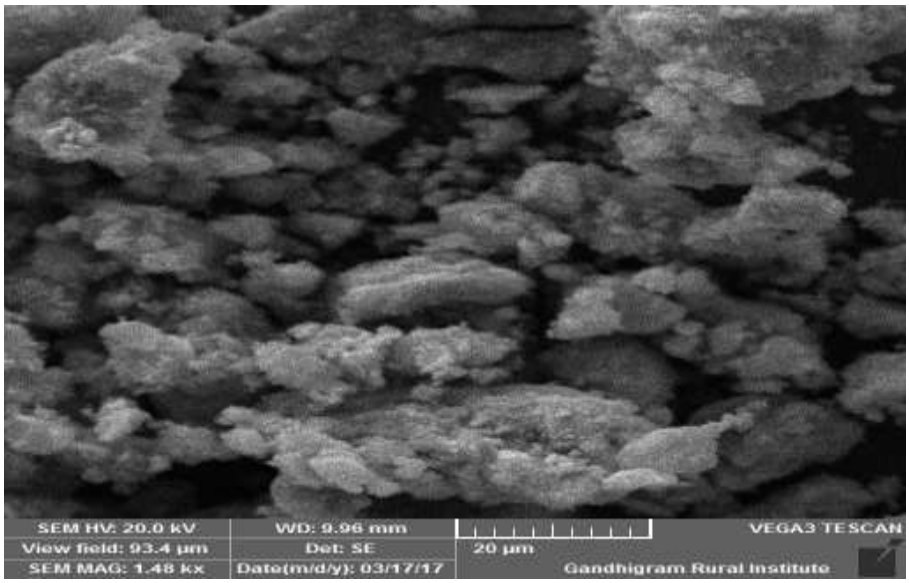


Fig 4 SEM image of MnO_2

The SEM studies give information about surface morphology and also can be used to check presence of imperfections in the MnO_2 nanoparticles. From the image it is clear that the MnO_2 posses almost smooth surface with cracks and transparent amorphous nature. However, many nanoparticles are seen on the surface.

3.1.(c) Ag(MnO_2) (Silver manganese dioxide)

X-ray diffraction study of silver manganese oxide synthesized by hydro thermal method was observed to be in purely crystalline cubic in nature. Average particle size of silver oxide nanoparticles was found to be in the range of 62.9nm. X-ray diffraction pattern of **Ag(MnO_2)** nanoparticles four peaks at 2θ values of 31.3º, 35.7º, 37.2º and 70.5º corresponding respectively to (100), (101), (102) ,(110) (103) and (112) planes of **Ag(MnO_2)**, this indicates that the prepared Ag(Mno_2) is highly crystalline characteristic of and well arrange in specific orientation. The XRD spectra showing the intense peak at 32.3 ˚ is having plane

(202) which is the crystal plane of Ag(MnO_2) The size of the silver crystals were estimated from the Debye-Scherer equation.

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

Where, K is the Scherer constant, which is related to the crystallite shape and are the radiation wavelength and Bragg's angle, respectively .and is the full width at half maximum of the diffraction.

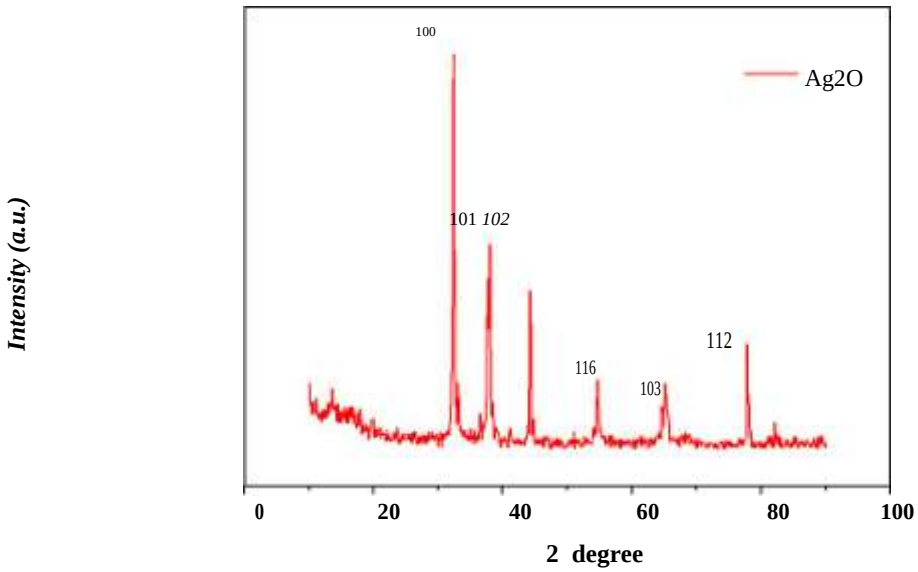


Fig.5 XRD patttern of Ag(MnO_2)

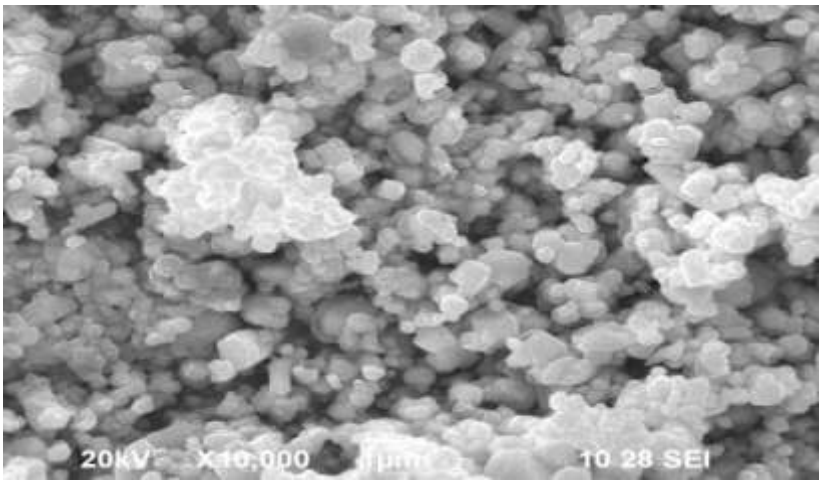


Fig.6 SEM image of Ag(MnO_2)

The morphology of **Ag(MnO_2)** nanoparticles are looks like cubic nature from the SEM of the above Fig.6

TABLE.1 Details of Diameter of inhibition zones observed in bacterial cultures

COMPONENTS	**ZONE OF INHIBTION**		
	24 Hrs (mm)	48 Hrs (mm)	72Hrs(mm)

Ag(MnO_2) nanoparticles	13	13	13
$Cu(MnO_2)_2$ nanoparticles	14	14	14
MnO_2 nanoparticles	-	-	-

4. DIAMETER OF INHIBITION

Fig: 7 inhibition in the Ag(MnO_2)

Fig: 8 inhibition in the Cu (Mno_2)$_2$

Fig: 9 inhibitions in the MnO_2

V. CONCLUSION

The $Cu(MnO_2)_2$, MnO_2, and $Ag(MnO_2)$ nanoparticles were synthesized by hydrothermal method and the morphological behavior of these nanoparticles were confirmed by XRD and SEM analysis. Antibacterial activity of these nanoparticles was studied against the pseudomonas gram negative bacteria by zone inhibition method. From the above study it is confirmed that both $Cu(MnO_2)_2$ and $Ag(MnO_2)$ nanoparticles were very effective against pseudomonas bacteria at 24 hrs, 48hrs and 72hrs with 14mm and 13mm inhibition respectively. The bacterial inhibition of these nanoparticles are size dependent, the one with smaller size has effective inhibition and hence the $Cu(MnO_2)_2$ nanoparticles with size 28.41nm was observed to be better inhibition compared to $Ag(MnO_2)$ nanoparticles with size 62.9 nm. The mechanism of these inhibition of nanoparticles on pseudomonas gram negative bacteria was the toxicity of nanoparticles towards killing of cell walls of bacteria.

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