Journal of Engineering Sciences
Vol 10, Issue 10, Oct /2019
ISSN NO:0377-9254

# CHEMICALLY SYNTHESIZED SILICA-ZINC NANOCOMPOSITE ACTS AS POTENTIAL ANTIBACTERIAL AGENT AGAINST URINARY PATHOGEN

D. SWARNA BHARATHI[1] , A. BOOPATHY RAJA[2]
[1,2]PG and Research Department of Zoology, Nehru Memorial College (Autonomous),
Puthanampatti – 621007, Tiruchirappalli, Tamilnadu, India.
E.mail:boopathyraja07@gmail.com

## ABSTRACT

The innovative drug necessary for treating urinary infections which might be efficient than other commercial drugs that emerging the idea to evaluate the antibacterial activity of Silica-Zinc nanocomposites against urinary pathogens. There are 18 various bacterial strains of klebsiella pneumoniae, Escherichia coli, Enterobacter faecalis, Flavobacterium species, Klebsiella oxytoca, Proteus mirabilis, Providentia rettigeri, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Serratia marcescens, Shigella flexineri, Shigella sonnei, Staphylococcus aureus, Methicillin Resistant S. aureus, Staphylococcus epidermidis, Vibrio cholera and pseudomonas aeruginosa inoculated in culture plate along with 100µl of Silica-Zinc nanocomposites through Disk diffusion method. After that, zone of the inhibition in each culture plate was examined the antibacterial activity of Silica-Zinc nanocomposites which proved that, higher degree of inhibition against Klebsiella oxytoca when compared to rest of others. The synthesized Silica-Zinc nanocomposites exhibited significant antibacterial activity against Gram negative microorganisms.

Keywords : Nanocomposite, XRD image, SEM analysis, Antibacterial activity, Urinary pathogens.

## INTRODUCTION

The shape and size of the nanoparticle have been play important roles in properties and functions of their own. The synthesis of nanomaterials involved in various methods such as arc discharge, hydrogen plasma metal reaction, laser pyrolysis, microemulsion, sol-gel, hydrothermal. Apart from that, novel antibacterial drugs can be synthesized through nanoparticles. Sewage water mostly contaminates with large amount of microorganisms. It is essential to separate organic compounds as well as microorganisms from the water to stimulate the biodegradation with the help of silica nanoparticles used as immobilizers (1,2,3)

Bacterial strains are classified into two types based on their staining. First one gram positive bacteria and another one is gram negative bacteria. Gram positive and gram negative bacterial growth could be inhibited by biocidal action of nanoparticles (4). Human beings are constantly exposed into various types of bacteria such as cocci, bacilli, vibrio, spirillium. These bacteria were causes numerous infections in both plants and animals. In America there are 48million cases belong to bacterial related diseases which indicates bacteria dispersed throughout the world.

The resistance strains of some bacteria have potentiality to act against antibiotics which can suppress the action of bactericides. Recently novel drugs must be cheapest and Safety components for host when compared to antimicrobial agents of other. Best novel drugs exhibit selective toxicity and that kills or inhibits the pathogenic microbes, without harmful effect or having least harm to the host. The efficient bactericidal material would be prepared from nanoparticle. The nanoparticles have greater capacity to invade the bacteria (5,6).

Oxygen is a basic requirement for bacteria to metabolizing their enzymes. A bacterial membrane wrap with cell wall. It may be formed into various thicknesses. Gram positive strains surround with thick peptidoglycan layer whereas gram negative strains are inhabited within thin

layer of peptidoglycan. This layer facilitates the uptake of oxygen into the cell. Nanoparticle prevents the oxygen carrying capacity of bacterial enzymes (7) Bacterial membrane easily binds with nanoparticle which enhances bactericidal capacity of nanoparticles (8).

The surface modification of inert nanoparticles shows the best antibacterial activity in very quick manner (14).The silica nanoparticles have induces cellular toxicity in bacterial cell whereas it does not toxic to mammalian cell line. It was interesting to know that silica nanoparticle have much of antibacterial activity in various spectrums. Biofilm formation is a main characteristics feature of bacteria. It could be completely inhibited by silica nanoparticle against pseudomonus aeruginosa and escherichia coli(15). The two various strains of Gram negative and Gram positive growth could be completely inhibited by functionalities silica nanoparticle (16).

Table 1. Literature Survey

| S.No | Nanoparticles | Functions | References |
|---|---|---|---|
| 1 | Gold nanorods | Gold nanoparticles may helps to synthesis new drug because of their antioxidant activity as well as antibacterial activity. | (9) |
| 2 | Aramide | Chiral N-Phthaloyl of aramide nanoparticles inhibits the carcinogenic effect on the liver of male rat.. | (10) |
| 3 | Silver, carbon, silica | Water borne e.coli growth could be completely inhibited using nanoparticles. | (11) |
| 4 | Copper | Copper nanoparticle acts as water purification and suppress the e.coli culture in sewage water. | (12) |
| 5 | Zinc oxide | Green synthesis of zinc oxide induces both antioxidant activity and excellent anti inflammatory activity. | (13) |

Zinc nanoparticle also shows moderate antibacterial properties against various microorganisms especially Gram negative bacteria. The moderate inhibitory concentration of zinc nanoparticle towards microorganisms would enhanced by hybridization with silica nanoparticle. The resulting silica-zinc nanocomposites showed unique kind of antibacterial capacity against harmful bacteria such as Proteus vulgaris, Pseudomonus aeruginosa, Klebsiella pneumoniae.

In the previous studies, the disc diffusion method reveals the antibacterial capacity of copper silica nanocomposites (17). Based on this, we demonstrate that silica-zinc nanocomposites have the ability to invade the bacteria in a culture media.

## MATERIALS AND METHODS

### Experimental Procedure

### Materials

Tetraethyl orthosilicate (TEOS) (99.99%, Aldrich), ethanol (99.99%, Aldrich), and ammonium hydroxide (28%, Wako), Zinc acetate dehydrate $Zn(CH_3COO)_2$. $2H_2O$(99.99%, Aldrich),Copper acetate monohydrate Cu $(CH_3COO)_2$ $H_2O$ (>98%, Merk) and acetic acid glacial (Merck) were used without any further purification. Sodium hydroxide NaOH (pellets) and Polyvinylpyrrolidone (PVP) were purchased from Aldrich.All the glass wares used in this work were acid washed. Ultrapure water was used for dilution and sample preparation.

### $SiO_2$-ZnO nanoparticle preparation

First, 0.5g of zinc acetate solid was taken in 10 ml DI with addition of 0.2g PVP under stirring at 80°C. Then, 0.2g of NaOH pellet in 10 ml DI was added into the above solution drop by drop. The final solution was stirred continuously for 2h. The solution was added drop by drop into the silica solution which prepared in separately. Finally, the solution was washed several time by DI water and annealed at 600°C for 4h in air atmosphere.

### Characterization of silica-zinc nanocomposite

UV-Vis spectral analysis

The silica zinc nanocomposite was monitored periodically by UV-Vis spectroscopy after the dilution of the samples with distilled water. A UV –Vis spectroscopy of the silica zinc nanocomposite was recorded by using quartz cuvette with water as standard. Diffuse reflectance absorption spectra were recorded using a UV-VIS-NIR double beam spectrophotometer (VARIAN, Cary 5000).

### SEM Analysis

The silica-zinc nanocomposites were synthesized by chemical method which characterized using a scanning electron microscope. The image showed spherical shaped nanocomposite, observed at a magnification 450.00 kx. From this confirms that the presence of silica-zinc nanocomposite in synthesized material.

### Determination of antimicrobial activity

Strains of klebsiella pneumoniae, Escherichia coli, Enterobacter faecalis, Flavobacterium species, Klebsiella oxytoca, Proteus mirabilis, Providentia rettigeri, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Serratia marcescens, Shigella flexineri, Shigella sonnei, Staphylococcus aureus, Methicillin Resistant S. aureus, Staphylococcus epidermidis, Vibrio cholera and pseudomonas aeruginosa were obtained from stock cultures preserved at -4ºC at research laboratory of SRM university , Trichy, Tamil nadu. All the bacteria tested were both gram negative and gram positive. All bacteria were allowed to grow on nutritive medium.

### Particle size measurement

Particle sizing experiments were carried by laser diffractometry using Zeta sizer nano.

**X Ray Diffraction analysis**

X ray diffractograms of silica-zinc nanocomposites were obtained with an X ray diffractometer (shimadzu XRD 7000 maxima, Japan) using standard radiation.

**MEASUREMENTS OF ANTIBACTERIAL ACTIVITY**

All bacteria need nutrients for their survival. Nutrient agar commonly used as simple solid medium for their inducing ability of bacterial population.

DISC DIFFUSION METHOD

I) Agar of known concentration added along with distilled water in a flask and boiled

ii) The boiling media immediately pour into culture plate before solidification

iii) Three various strains of inoculated into culture vessels.

vi) Each sample of klebsiella pneumoniae, Escherichia coli, Enterobacter faecalis, Flavobacterium species, Klebsiella oxytoca, Proteus mirabilis, Providentia rettigeri, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Serratia marcescens, Shigella flexineri, Shigella sonnei, Staphylococcus aureus, Methicillin Resistant S. aureus, Staphylococcus epidermidis, Vibrio cholera and pseudomonas aeruginosa inoculated in culture plate.

v) The agar plate incubated in incubator for 37 degree Celsius

vii) Labelling of concentration helps to obtaining the results.

viii) Zone of inhibition measured after 24 hours.

**RESULTS**

**Characterization of Silica - Zinc nanocomposite**

UV-Vis spectroscopy is an essential equipment for determining the formation, stability and shape of nanocomposite.

Figure A: shows that broad peak was observed at 300-400 nm due to the surface Plasmon absorption of silica-zinc nanocomposite.

Figure B: Chemically synthesized silica-zinc nanocomposite which was confirmed by the characteristics peaks observed in the XRD image. The structure of as prepared samples was characterized by X-Ray powder diffraction (Bruker 1.54 Å) with Cu $k_\alpha$ radiation at a voltage of 40 kV and a current of 40 mA in the 2θ range 10-80°.

An SEM image of chemical synthesized silica-zinc nanocomposite showed the presence of spherical nanocomposites. The surface morphology of the nanoparticles was investigated using a FE-SEM instrument (Oxford) with a separate EDS detector connected to that instrument. The scanning electron microscopy was used to characterize the particle size and assume the image.

Figure C. It shows that the particles size is 200 nm. The particles in nanocomposite were found with almost spherical morphology.

The FTIR Band Were observed at 3431.82cm$^{-1}$, 1628.25cm$^{-1}$, 1099.55cm$^{-1}$, 802.53cm$^{-1}$, and 479.88cm$^{-1}$ which were assigned to bands at 3431cm$^{-1}$ due to the stretching vibrations of water molecules and N-H stretches of amide groups which is strong in the observed range. The absorption peak at 1628cm$^{-1}$ are indicates the C-O stretching group. The broad band absorption peak at 1099cm$^{-1}$ is the Si-O-Si asymmentric which indicates the binding of ZnO and $SiO_2$ (18). Next the peak absorption at 802cm$^{-1}$ is to be Si-OH bands (19). Finally the peaks at 479 cm$^{-1}$ are stretching vibrations of $ZnO_4$ units (20).

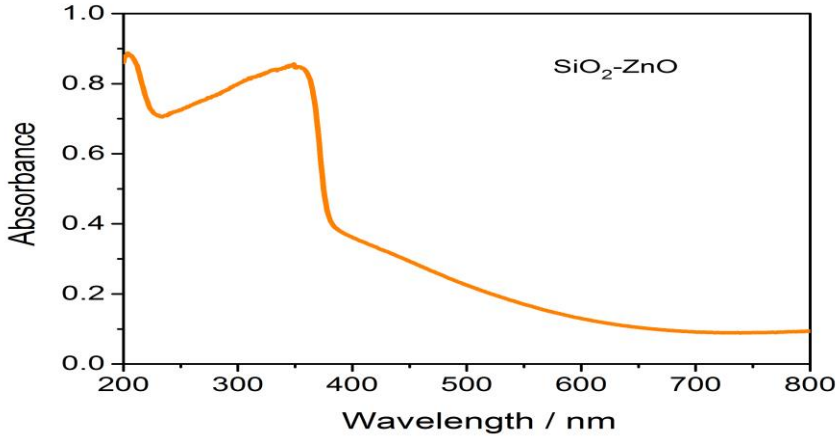


Figure A: UV Vis spectral analysis

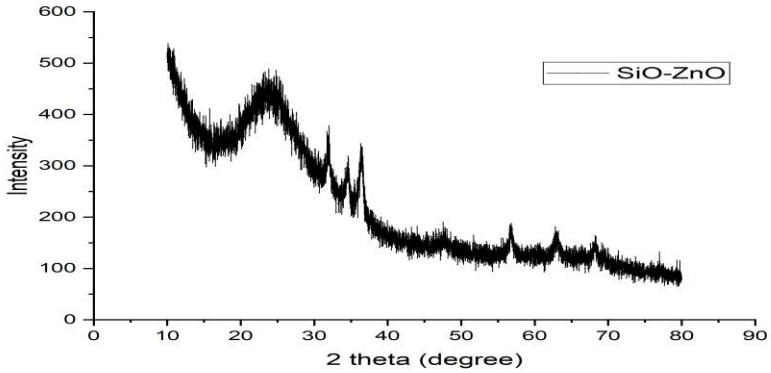


Figure B: XRD characteristic peak of chemically synthesized Silica-Zinc nanocomposite

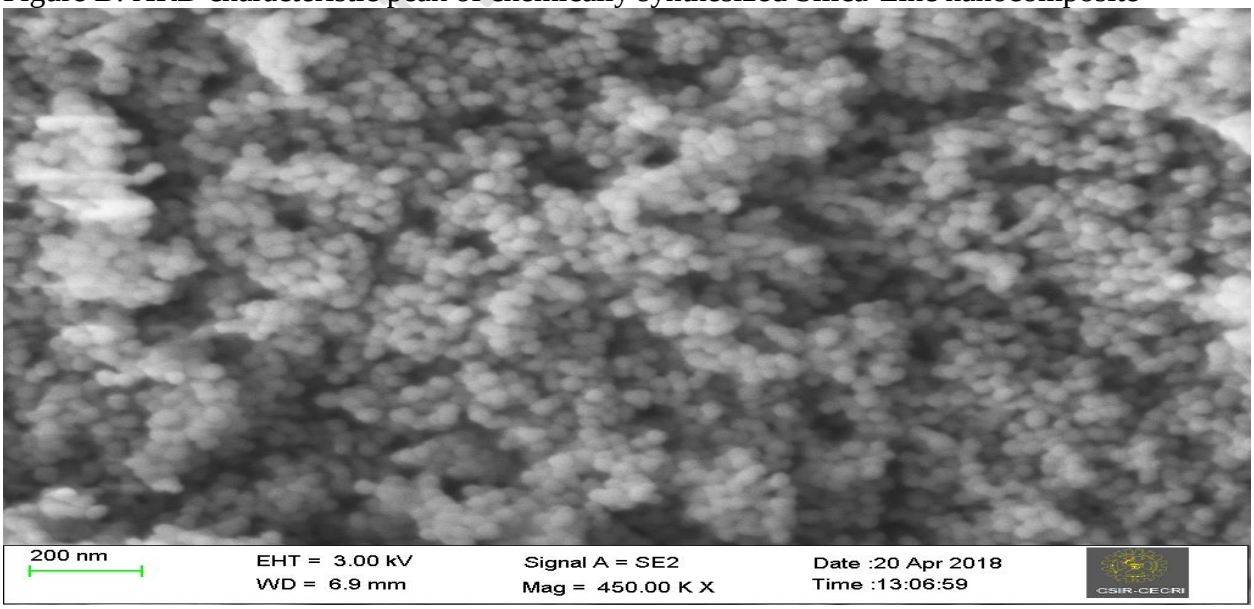


Figure C: SEM micrograph of Silica-Zinc nanoparticles

The Table.2 shows that silica-zinc nanocomposite have antibacterial active against pathogenic microorganisms such as klebsiella oxytoca, proteus rettiger. The silica-zinc nanocomposite exhibited highest zone of inhibition against K. oxytoca (8mm for 2%, 10mm for 3% and 12mm for 4%) and P. rettigeri (7mm for 3% and 11mm for 4%) but unfortunately other bacteria developing resistant against these drugs.

Table.2. Zone of inhibition against Gram positive and Gram negative bacteria[Except K. oxytoca and P. rettigeri; others showed resistant]

| Bacterial pathogens | Bacterial pathogens verses antimicrobial susceptibility (zone of inhibition) in mm (Column define concentration of the sample) | | | |
|---|---|---|---|---|
| | 1% | 2% | 3% | 4% |
| Escherichia coli | - | - | - | - |
| Enterobacter faecalis | - | - | - | - |
| Flavobacterium species | - | - | - | - |
| Klebsiella oxytoca | - | 8 | 10 | 12 |
| Klebsiella pneumonia | - | - | - | - |
| Proteus mirabilis | - | - | - | - |
| Providentia rettigeri | - | - | 7 | 11 |
| Pseudomonas aeruginosa | - | - | - | - |
| Salmonella typhi | - | - | - | - |
| Salmonella paratyphi A | - | - | - | - |
| Salmonella paratyphi B | - | - | - | - |
| Serratia marcescens | - | - | - | - |
| Shigella flexineri | - | - | - | - |
| Shigella sonnei | - | - | - | - |
| Staphylococcus aureus | - | - | - | - |
| Methicillin Resistant S. aureus | - | - | - | - |
| Staphylococcus epidermidis | - | - | - | - |
| Vibrio cholera | - | - | - | - |

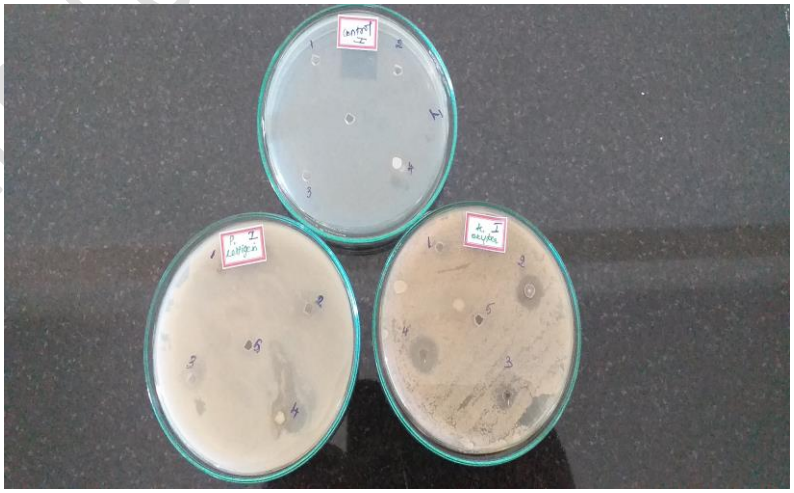

Figure D: antibacterial activity of two potential urinary pathogens

This resulting Figure D depicted antibacterial activity of silica-zinc nanocomposite was examined by disc diffusion method.

**DISCUSSION**

Enterococcus faecalis is a gram positive bacteria which is responsible for damaging the teeth and causes dental related problems and that belongs to facultatively anaerobic with non sporing bacteria. Recently some oral infections, root canal infection caused by enterococcus faecalis and affects marginal part of the teeth also that named as marginal peridonitis (21,22)

Flavobacterium species, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Serratia marcescens are a kind of Gram negative bacteria which causes various diseases in respiratory tract and urinary tract. The five strains of rod shaped are found everywhere in the world especially P.mirabilis bacteria generally found in human intestine but excess amount of colonies disturb the intestine as well as urinary tract. It does not require oxygen for their survival and they can thrive oxygen free environment, those species commonly known as facultative anaerobes. Mostly the people affected with diabetes, may easily susceptible to k.pneumonia infection. Among the four bacteria E.coli have reach the first place of urinary tract infection. Nosocomial disease are also commonly outcome of k.pneumoniae infections(23,24).

Nowadays, k.pneumoniae resistance greatly increased against certain antimicrobial agents (25,26). P. mirabilis are main case of forming stones in urinary bladder, kidney and causes septicemia. Flavobacterium species are highly prevalent fish pathogen.

Pseudomonas aeruginosa mainly resides in the lungs and kidney. These types of Gram negative bacteria causes' urinary infection in a huge range. Patients with cystic fibrosis mostly susceptible to P.aeruginosa infections. Apart from that, infections could not completely exterminate if once enters into patient with cystic fibrosis (27).The person without cystic fibrosis would affects P.aeruginosa infection as sudden onset, whereas cystic fibrosis patients fight against P.aeruginosa in a prolonged interval (28). This bacterial strains does not have a sensitivity to antibiotics which reveal that mutations occurred in P.aeruginosa too fastly, when compared to past decades. Intracellular enzymes of P.aeruginosa may changes for each corresponding mutations (29).

Salmonella typhi, salmonella paratyphi A, salmonella paratyphi B are a gram negative, motile and facultative anaerobic bacteria. This bacterium is mainly invading the portions of bone marrow, liver, spleen, kidney, gall bladder and small intestine. These flagellated organelles are helps to attach the epithelial cells of host. S.typhi is a prime reason for causing typhoid fever, ulceration, inflammation of spleen. S.paratyphi B is a bacterium which well grow and cause disease only in human beings. Salmonella infections are generally spreading through contamination of food and water.

Shigella fexneri and Shigella sonnei are also belongs to Gram-negative and under the group of facultative intracellular pathogen which highly targets rectal part of the humans and producing toxic called shigellosis. The contamination food and water have with fecal exposure of patients to outside environment causing infections.

Staphylococcus aureus and Staphylococcus epidermidis are Gram-positive bacterium that causes number of illnesses such as infections in skin, damages the joining between muscle and bones, skin infections, food poisoning, pneumonia, inflammation of cardiac muscles and sepsis (30). Methicillin-resistant S. aureus strains have resistant over to methicillin thus named as methicillin resistant s.aureus species.

Vibrio cholerae is a gram negative bacteria which produces enterotoxins that ruptures mucosal membrane of small intestine and penetrates deeper into each and every muscle. The predominant symptoms of cholera is bloody stool diarrhoea, increases blood pH, dehydration and untreated persons are die within 48 hours.

Providencia rettgeri is a facultative anaerobe able to survive oxygen free environment and classified under the group of gram negative stains which mainly belongs to rare variety (31). P.rettgeri was found in bloody fluids like urine, blood, wound infections, fecal stools and vital regions of axilla, throat, perineum. This species commonly have a resistance against antibiotic drugs and in future most efficient drug needed for treating the infections. invaded area Human isolates of Providencia species have been recovered from urine, throat, perineum, axilla, stool, blood and wound specimens. The organisms are mainly found in gastrointestinal tract and gastrourinary tract which responsible for causing diarrhoea with highest degree of resistance. There are few or more members easily susceptible to this infection by low nutrition and ruptures of skin (32) other than that urinary tract infections, ICD and brain fever may cause by p.rettigeri. There are currently no reported cases of P. rettgeri causing cellulitis but lost of previous report indicates that urinary tract infections, sepsis, implantable cardioverter defibrillator (ICD) pocket infection and meningitis infection and meningitis (33,34,35,36).

Klebsiella belongs to gram negative bacterium, rod shaped with non motile which plays vital role in economy of most countries. Dairy industries mainly depends on cattle health and earn the profit which important for raising national economy. But in presence of klebsiella in cattle causes severe mastitis that familiarly known as inflammation of mammary gland and shows significance signs such as low amount of milk, infections, wound in mammilary glands, low amount of calcium in milk etc.(37,38,39) But commonly klebsiella present as normal flora of urinary tract, mucosa, kidney and skin but they are considered as opportunistic bacterial disease such as sudden arthiritis, pneumonia, urinary tract infections (40). The chemically synthesized silica-zinc nanocomposite having a increasing inhibition zone against k.oxytoca.

Among the silica-zinc nanocomposite showed excellent zone of inhibition for controlling bacterial growth. Similary, this infection may treat with phytochemical compound with smaller size drugs gives efficient killing action than allopathic drugs.

## CONCLUSION

The present study revealed that silica-zinc nanocomposites are exhibit potential antibacterial activity against Gram negative bacteria especially urinary pathogens. In this, silica-zinc nanocomposites were preparing from physical method and size measured using SEM analysis in a specific nanoscale. In future, the research will be further extended to synthesizing of silica-zinc nanocomposites through plant materials and design drugs for treating urinary infections.

## REFERENCES

1. Kangari, A., Tavakoil, A., SohrabI, M .:Chem. Pap. 61, 151 (2007)
2. Hsiao, M.S., Chen, D., Shieh and Yeh, C.: One-pot synthesis of hollow Auzcul spherical-like biomineral botallackite Cu2(OH)3Cl flower like architecture exhibiting antimicrobial activity.J.Phys,Chem.B.110;205-210(2006)
3. Pedrazzani, R.G., Bertanza, C., Maffezzoni, M., Gelmi, N., Manca and Depero,L.:Bacteria enclosure between Silica-Coated membranes for the degradation of organic compounds in contaminated water. Water. Res. 39; 2056 – 2064 (2004)

4. Stoimenov, P. K., Klinger, R.L., Marchin, G.L., Klabunde, K.J.:Metaloxide nanoparticles as Bactericidal Agents, Langmuir.18:6679-6686(2002)
5. Fresta, M., Puglisi,G., Giammona,G., Cavallaro, G., Micali, N.:Pefloxacine mesilate-loaded and ofloxacin-loaded polyethylcyanoacrylate nanoparticles characterization of the colloidal drug carrier formulation.J.Pharm.Sci.84;895-902(1995)
6. James,G.V.: Water treatment.4th ed.Cleveland: CRC press; (1971),P.38.
7.Theivasanthi,T.,Alagar,M. Anti-bacterial studies of silver nanoparticles(2012)
8.Gordon,O.,Slenters,T.V.,Brunetto,P.S.,Vilaruz,A.E.,Sturdevant,D.E.,Otto,M.,Landmann,R., Fromm,K.M.: Antimicrobial.Agents chemother.54,4208(2010)
9.Shyamalima sharma, Ajay Kumar Manhattan, Proton Jyoti Bora, Swapan kumal dolui, Manabendra Mandal.: Adv. Mater. Lett. 6 (3), 235- 241 (2015)
10.Hammed, H., Hassan, A.M., Sabah, G., El- Bama., Amelia, F., Elhusseiny and Sayed, E.I., .Mansour, M.E.: Antioxidant activity of new aramide nanoparticles containing Redox - Active N- Phthaloyl valine moieties in the hepatic cytochrome $P_{450}$ in male rats, Molecules, 17, 8255-8275(2012)
11.Karnib, M., Holail, H., Olama, Z., Kabbani, A., Hines, M.: International journal of current microbiology and Applied sciences, ISSN:2319-7706, volume 2 number 4pp.20-30(2013)
12.Harikumar, P.S., Anisha Aravind.: International journal of sciences, volume-5 (2016)

13.Nagajyothi, P.C., Sang Ju cha., In Jun Yang., Sreekanth,T.V.M., Kwang joong Kim., Heung Mook Shin.: Journal of photochemistry and photobiology B; Biology 146,10-17(2015)
14.Hajipour, M.J., Fromm, K.M., Akbar Ashkarran, A., Jimenez de Abserasturi, D., Larramendi IRD., Rojo, T., Serpooshan, V., Parak, W.J., Mahmoudi, M.; Antibacterial properties of nanoparticles,Trends in Biotechnology,30:499-51(2012)
15.Camparotondi, D.E., Foglia, M.L., Anarez, G.S., Mebert, A.M., Diaz, L. E., Coradin, T. and Desimone, M. F.:Antimicrobial silica modified nanoparticles. University of Argentina;(2013)(Formatex)
16.Song, J., Kong, H., Jang, J.: Bacterial adhesion inhibition of the quaternary ammonium functionalized Silica nanoparticles. Colloids and Surfaces B Biointerfaces ,82:651-656(2011)
17. Kim, Y.H., Lee, D.K., Cha, H.G., Kim, C.W., Kang, Y.S.: Preparation and Characterization of the antibacterial Cu nanoparticle formed on the surface of $SiO_2$ nanoparticles. Journal of Physical Chemistry B,110:24923-24928 (2006)
18.Gao,G., Da, N., Reibstein, S., Wondraczek,L.: Enhanced photoluminescence from mixed-valence Eu-doped nanocrystalline silicate glass ceramics. Opt. Express 18,575–583 (2010)

19.OH, T., Choi, C.J.: Comparison between SiOC thin films fabricated by using plasma enhance chemical vapor deposition and SiO2 thin films by using fourier transform infrared spectroscopy, J. Korean Phys. Soc. 56, 1150–1155 (2010)
20.Tarafder, A., Molla, A.R., Dey, C., Karmakar, B.: Thermal structural, and enhanced photoluminescence properties of Eu3+ doped transparent willemite glass ceramic nanocomposites, J. Eur. Ceram. Soc. 96,2424–2431 (2013)
21.Guven Kayaoglu, Dag Orstavik.: Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Crit. Rev. Oral. Biol. Med; 15 (5): 308 – 320 (2004)
22.Isabela, N.R., Jose, F.S., Katia, R.N.S.: Association of Enterococcus faecalis with different forms of periradicular diseases. J Endodon; 30(5): 315 -320 (2004)

23.Grimont, F., Grimont, A.D., and Richard,C.: The genus klebsiella , P.2775-2796 (1992)
24.Podschun, R., Ullmann,U.: klebsiella sp. as nosocomial pathogens; epidemiology, taxonomy, typing methods and pathogenicity factors. Clinical.Microbiol.Rev.11:589-603(1998)
25.Brisse, S., Milatovic, D., Fluit, A. C., Verhoef, J., And Schmitz.: Epidemiology of quinoline resistance of klebsiella pneumoniae and klebsiella oxytoca in Europe. Eur.J.Clin. Microbiol. Infect. Dis.19;64-68(2000)
26.Paterson, D.L., Mulazimoglu, J.M., Casellas, W.C., ko, H., Goosens, A., Von Gottberg, S., Mohanpatra, G.M., Trenholme, K.P., Klugman, J.G., Mccormact, and Yu. V.L.:Epidemiology of ciprofloxacin resistance and it's relationship to extended-spectrum beta-lactamase production in klebsiella pneumonia isoltes causing bacteremia. Clin.Infect.Dis.30:473-478(2000)
27.Birgitte Frederiksen, Christian Koch, Niels Hoiby.: Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. pediatric pulmonology 23(5), 330-335, (1997)
28.Oliver, A., Canton, R., Campo, P., Vaquero, F., Blazquez, J.: High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection, science, Vol 288(2000)
29.Kochler,T., Epp, S.F., Curry, L. K.& Pechere, J.C.: Characterization Mex T, the regulator of the Mex E- Mex F - Opr N multi drug efflux system of Pseudomonas aeruginosa. J Bacteriol 181, 6300-6305(1999)
30.Kakai, R., Wamola, I.A.: Minimising antibiotic resistance to Staphylococcus aureus in developing countries East Afr Med J 79: 574-579 (2002)
31.Marull, J.M., De Benedetti, M.E.: Automatic implantable cardioverter defibrillator pocket infection due to Providencia rettgeri: a case report. Cases J; 2: 8607 (2009)
32.Wilson Kwong., Mohammad Shafiee., Maan Hasso., Umar Sharif.: Providencia rettgeri: an unexpected case of Gram-negative cellulitis. Wounds International.Vol 6 Issue 4 (2015)
33.Lee, G., Hong, J.: Xanthogranulomatous pyelonephritis with nephrocutaneous fistula due to Providencia rettgeri infection. J Med Microbiol; 60(Pt 7): 1050–2 (2011)
34.Yoh, M., Matsuyama, J., Ohnishi, M.: Importance of Providencia species as a major cause of travellers' diarrhoea. J Med Microbiol; 54(Pt 11): 1077–82(2005)
35.Pinto, V., Telenti, M., Bernaldo de Quirós, J.F., Palomo, C.: Two cases of fatal transfusion-associated bacterial sepsis provoked by Providencia rettgeri. Haematologica; 84(11): 1051–2 (1999)
36.Picard, D., Klein, A., Grigioni, S., Joly, P.: Risk factors for abscess formation in patients with superficial cellulitis (erysipelas) of the leg. Br J Dermatol; 168(4): 859–63(2013)
37.Högenauer, C., Hammer, H.F., Krejs, G.J., Reisinger, C.: Mechanisms and management of antibiotic-associated diarrhea. Clin infect Dis.;27(4):702-710 (1998)
38.Barbut, F., Beaugerie, L., Delas, N., FossatiMarchal, S., Aygalenq, P., Petit, J.C.: Comparative value of colonic biopsy and intraluminal fluid culture for diagnosis of bacterial acute colitis in immunocompetent patients. Clin Infect Dis.29(2):356-360 (1999)
39.Joainig, M.M., Gorkiewicz, G., Leitner, E., Weberhofer, P., Zollner-Schwetz, I., Lippe, I.: Cytotoxic effects of Klebsiella oxytoca strains isolated from patients with antibioticassociated hemorrhagic colitis or other Dis caused by infections and from healthy subjects. J Clin Microbiol.48(3):817-824 (2010)
40.Ménard, A., Harambat, J., Pereyre, S., Pontailler, J.R., Mégraud, F., Richer, O.: First report of septic arthritis caused by Klebsiella oxytoca. J Clin Microbiol.48(8):3021-3023 (2010)