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GREEN SYNTHESIS OF ZnO NANOPARTICLES USING LEAVES EXTRACT CASSIA FISTULA AND DOPED WITH COPPER (Cu: ZnO NPs) TO ENHANCE THE ACTIVITY OF ANTIBIOTICS AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

In the present study green synthesis of ZnO nanoparticles (70 nm) carried out from leaves of Cassia fistula and doped with copper (Cu:ZnO NPs). These Cu:ZnO NPs further used to study the effect on antibacterial activity of different antibiotics by disk diffusion method. Staphylococcus Aureus was used as the test strain. The combination of antibiotics with Cu:ZnO NPs increases there activity against Staphylococcus Aureus. Surface modified copper doped Cu:ZnO NPs have significantly enhance the activity of antibiotics. The Cu:ZnO NPs was quite stable at different temperature. According to the SEM image the nanoparticles synthesized have different size and heterogeneous morphology.

KEYWORD: Copper doped zinc oxide nanoparticles, Green Synthesis, Antibacterial properties, Staphylococcus Aureus, Cassia Fistula.

INTRODUCTION

Nanotechnology is science of formulating materials at the smallest possible scale. Nanotechnology can be utilized in drug delivery designs, diagnostic techniques, sunscreens, disinfectant, a shape, composition, crystalline and morphology determines the intrinsic properties of metal nanoparticles.[1] Nanotechnology is being envisioned as a hurriedly developing field, it has potential to revolutionize pharmaceuticals and cosmetics.

Nanotechnology or the use of materials with constituent dimensions on the atomic or molecular scale has become increasingly applied to pharmaceuticals & cosmetics and is of great interest as an approach to killing or reducing the activity of numerous microorganisms. Some natural antibacterial materials, such as zinc and silver, are being claimed to possess good antibacterial properties.[2-5] In recent years, green synthesis of metal nanoparticles is an interesting issue of the nanoscience and nanobiotechnology. There is a growing attention to biosynthesis the metal nanoparticles using organisms. The use of plant systems has been considered a green route and a reliable method for the biosynthesis of nanoparticles owing to its environmental friendly nature.[6] The antibacterial agents are used to kill or prevent the growth of bacteria. Zinc oxide nano particles are better antibacterial agent. The antibacterial activity of zinc oxide nano particles were probed by many researchers. Thus, zinc oxide (ZnO) and copper oxide nanomaterials are incorporated into a variety of medical and skin coatings because of their antimicrobial and / or antifungal properties; and indeed they are generally regarded as safe materials for human beings and animals.[7-8]

MATERIALS AND METHODS

Preparation of the leaf extract

Fresh leaves were collected from Cassia fistula plants. The leaves were washed several times with water to remove the dust particles and then sun dried to remove the residual moisture. The extract used for the reduction of zinc ions (Zn^{2+}) to zinc nanoparticles (ZnO) was prepared by placing 50g of washed dried fine cut leaves in 250 mL glass beaker along with 100 mL of sterile distilled water. The mixture was then boiled for 60 minutes until the colour of the aqueous solution changes from watery to light yellow. The extract was cooled to room temperature and filtered using filter paper. The extract was stored in a refrigerator in order to be used for further experiments.

Preparation of zinc nanoparticles

For the synthesis nanoparticles 50 ml of Cassia fistula leaves extract was taken and boiled to 60-80 degree Celsius using a stirrer heater. 5 grams of Zinc Nitrate was added to the solution as the temperatures reached 60 degree Celsius. This mixture is then boiled until it reduced to a deep yellow coloured paste. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400 degree Celsius for 2 hours. A white coloured powder was obtained and this was carefully collected and packed for characterization purposes. The material was washed in a mortar so as to get a finer nature for characterization

Preparation of in situ surface-modified copper doped ZnO nanoparticles (Cu:ZnO NPs)

Preparation of *in situ* surface-modified copper doped ZnO nanoparticles (Cu:ZnO NPs) was done according to standard procedure.[11] Cu:ZnO NPs were fabricated under mild hydrothermal conditions (T = 100°C, P = autogenous, t = 12 hr). 2 mole of ZnO was taken as starting material and the dopant, copper oxide at 0.5, 1, 1.5, 2, and 2.5 mol% was added into it. A certain amount of 1 M NaOH was added as mineralize to the precursors. At the same time, 1 ml of *n*-butylamine was added to the above-mentioned mixture and it was stirred vigorously for a few minutes. The final compound was then transferred to the Teflon liner (Vfill = 10 ml), which was later placed inside a General Purpose autoclave. Then the assembled autoclave was kept in an oven with a temperature programmer controller for 12 h. The temperature was kept at 100°C. After the experimental run, the autoclave was quenched to the room temperature. The product in the Teflon liner was then transferred to a clean beaker, washed with double distilled water several times, and then allowed to settle down. The surplus solution was removed using a syringe. Finally, the remnants were allowed to dry naturally at room temperature. The dried nanoparticles were subjected to systematic characterization and antimicrobial studies.

Thermal stability of antibacterial activity of Cu:ZnO NPs

To determine the effect of temperature on stability of Cu:ZnO NPs, screw ampoules containing 100 µl of ZnO:Cu were kept at 40, 50, 60, and 70 mg/l for one hour in water bath and then residual activity was determined against the target cultures.

ANTIBACTERIAL STUDY

Staphylococcus Aureus characterized and maintained in this laboratory was used as a test organism. The disk diffusion method was used to test the activity of 20 different antibiotics against test strain on Mueller-Hinton agar plates according to NCCLS.[9] The standard antibiotics disks impregnated with sub-inhibitory concentration of Cu:ZnO NPs (100µg/disc). A single colony of test strain was grown overnight in Mueller-Hinton broth on a rotary shaker at 35^0C. The inoculums were prepared by diluting the overnight culture with 0.9% NaCl to a 0.5 McFarland standard. A lawn of the test organisms was made on the agar plates using a sterile cotton swab and the antibiotic disks (with and without Cu:ZnO NPs, 100µg/disc) were placed on the bacterial lawn. After incubation at 37^0C for 24 hrs, the zones of inhibition were measured.

RESULT AND DISCUSSION

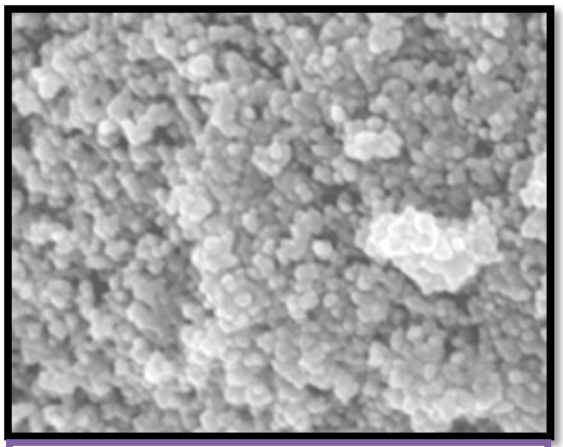

Fig. 1: SEM Image of ZnO Nanoparticles

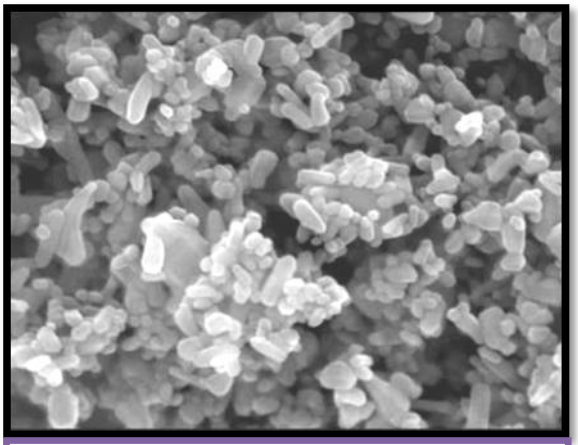

Fig. 2: SEM Image of Cu:ZnO Nanoparticles

Table 1: The comparative activities of various antibiotics with and without Cu:ZnO NPs against Staphylococcus Aureus (inhibition zone in mm)

Sr. No.	Antibiotic	Inhibition	Antibiotic With Cu:ZnO NPs (70 nm)	Increased
1	Penicillin G	25	39	14
2	Methicillin	10	22	12
3	Oxacilin	15	29	14
4	Cloxacillin	20	33	13
5	Ampicillin	28	40	12
6	Amoxicillin	21	35	14
7	Cephalexin	21	34	13
8	Cefotoxime	25	37	12
9	Ceftazidime	15	29	14
10	Amikacin	14	29	14
11	Gentamycin	16	30	14
12	Streptomycin	15	28	13
13	Ciprofloxacin	21	28	07
14	Norfloxacin	16	22	06
15	Clarithromycin	18	24	06
16	Clindamycin	21	34	13
17	Cotrimoxazole	16	27	11
18	Nalidixic acid	14	17	03
19	Tetracyclin	22	35	13
20	Chloramphenicol	19	26	07

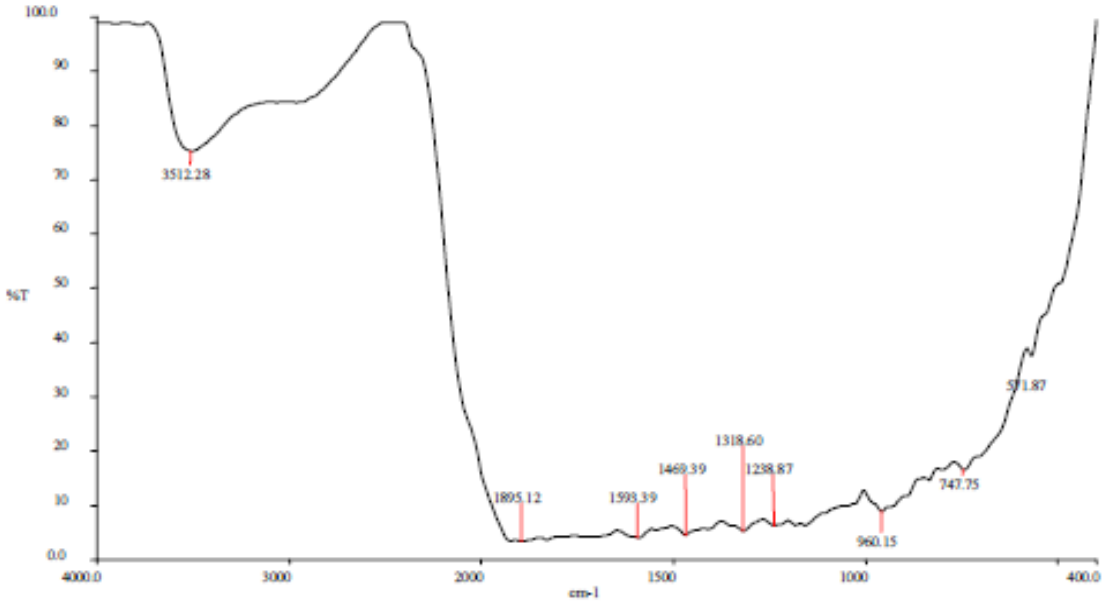

Fig. 3: FTIR Spectra of ZnO Nanoparticles

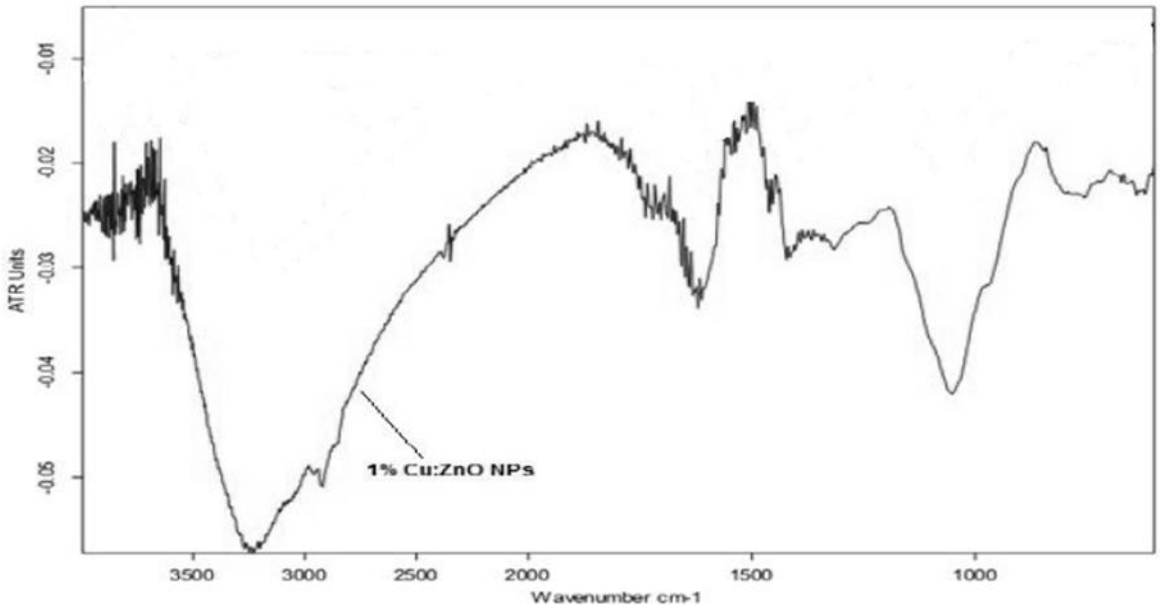

Fig.4: FTIR Spectra of Cu:ZnO Nanoparticles

Our results with a simple approach clearly showed to have antimicrobial effects, proved the inhibition of human pathogens as shown in Table 1. FTIR spectra indicate that the nanomaterials synthesized have higher peak intensity compared with ZnO. However, no new peaks were observed. According to the SEM image indicates that the nanoparticles synthesized have different size and heterogeneous morphology; among them nanorods and spherical nanoparticles are more obvious. Moreover, the change in the morphology could be contributed to the effect of dopant and surface modifier applied. Since the activity of the

Cu:ZnO NPs was quite stable at RT, experiments were also conducted to see the effect of elevated temperature on stability of Cu:ZnO NPs.

Zinc oxide has a very good potential to move into clinic. In this investigation the effect of copper doped zinc oxide nanoparticles (Cu:ZnO NPs) on the antibacterial properties of different antibiotics was investigated against Staphylococcus Aureus by the disk diffusion method. The effect of Cu:ZnO NPs was observed by increase in diameter of inhibition zone (mm) around the different antibiotic disk. The activities of all the antibiotics have increased in the presence of Cu:ZnO NPs against the test strain. So the effect observed in this condition could be due to the antibiotic-Cu:ZnO NPs combination. Cu:ZnO NPs particles significantly increased the efficiency of antibiotics against the Staphylococcus Aureus.

CONCLUSION

The synthesis of nano size zinc oxide nanoparticles (ZnO NPs) 70 nm was carried out successfully using leaves of Cassia Fistula and doped with copper. The small nanometer scale doped ZnO nanoparticles (Cu:ZnO NPs) enhance the activity of several antibiotics successfully. The antibacterial activity was quite stable at room temperature as well as to various temperatures.

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