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Biogenic synthesis a green approach to synthesize and study antimicrobial activity of CuO Nanoparticles by using Bos Taurus urine.

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Abstract: *Now- a- days green synthesis have received crucial attention as a reliable, developing and ecofriendly protocol for synthesizing nanomaterials. In recent years biogenic synthesis of nanoparticles is in more demand as this method is ecofriedly, biocompatible and easy to synthesize nanomaterials. According to ayurvedic literature the cow urine has a medicinal properties used to cure various diseases like diabetes, blood pressure, anti-asthma, anticancer, ulcer, leprosy, anemia etc. Hence our research group used biowaste and easily available Indian cow urine to synthesize copper oxide (CuO) nanoparticles as it acts both as green reducing and stabilizing agent. The synthesized CuO NPs were characterized by various advanced technique like XRD, FTIR, Zeta potential etc. From XRD data it is clear that CuO NPs have monoclinic structure and its crystallite size as calculated by Debye-Scherrer equation is 22.27nm. These synthesized nanomaterials shows excellent biological activity against pathogenic micro-organisms and also shows effective catalytic efficacy. We studied antibacterial and antifungal activity by using various microorganism. We synthesized CuO NPs in monoclinic form with average size 22.27nm. No such morphological formation was observed from synthetic urea.*
Keywords- Cow urine, Biosynthesis, Biomedical.

I. Introduction:

The use of nanomaterials being worldwide expanded because nanomaterials shows unique properties than their bulk particles. In textile industry,, aerospace, food , agriculture and medicines etc nanoparticles show more applied impact. Hence researchers started synthesizing nanoparticles since last three decades by using different methods physical, chemical, and biological . Now a days biological method being environment friendly, cost effective, green and non toxic is used for the synthesis of nanomaterials.

Hence the synthesis MO NPs is largely carried out by biological method.. Among metal oxide nanomaterials, CuO and ZnO are used specially in bio medicinal field. ZnO and CuO NP's are very sensitive to human pathogenic bacteria such as Escherichia Coli and Staphylococcus aureus. Our research group synthesized CuO by using biological waste Bos- Tarus urine. In Hinduism cow urine is called as Gomutra. In Indian scriptures it is mentioned that Gomutra can be used for the cure and the treatment of many diseases. Ancient ayurvedic scriptures such as Charaka Samhita, Sushruta Samhita mentions various medicinal properties of cow urine. Some research groups reported ZnO and CuO shows good antimicrobial activity. This indicates that ZnO act as excellent antibacterial agent. Padvi et.al first synthesized CuO NPs by using cow urine. They concluded that cow urine can act as a novel reducing agent.

II. Materials and Method

2.1 Chemicals.

Analytical grade copper sulphate ($CuSO_4 . 5H_2O$), Cetyltrimethyl Ammonium Bromide (CTAB),Double distilled water.

2.2 Collection of cow urine

We collected a fresh sample of cow urine of Khilar Cow which is filtered by using Whatmann filter paper 41. The slight yellow colored cow urine with pH 8 was used for the experimental procedure.

2.3 Preparation of CuO nanoparticles

100 ml 0.1M copper sulphate solution was prepared by dissolving 2.4968g copper sulphate in double distilled water. Then pinch of CTAB was dissolved in 10ml double distilled water and added to above solution. 50 ml of cow urine was taken in burette and dropwise added at about 40^0c to 50^0c with constant stirring by using magnetic stirrer in the above reaction mixture. The colour changed of reaction mixture blue to faint green which was indication of completion of reaction. The precipitate was centrifuged with repeated washings with distilled water followed by alcohol till it is free from impurities. The resultant precipitate was dried in a hot air oven at 90^0c for 6 hours and the annealed into muffle furnace at 400^0c for 2 hours to obtain black colourd CuO NPs.

III. Result and Discussion:

3.1 XRD Analysis:

The morphology and crystallite size was determineed by XRD analysis. The XRD patterns of CuO is shown in Fig. 1. The XRD pattern of CuO is assigned to the miller indices of (110) , (-111), (111), (100), (-202), (020), (202), (-113), (-311), (220), (311) and (004) which located at 2θ values of 32.53^0 , 35.52^0 , 38.70^0 , 42.52^0 , 48.70^0 , 53.43^0 , 58.28^0, 61.57^0 , 66.30^0, 68.06^0, 72.38^0, and 75.06^0 respectively.(JCPDS card no-01-073-6023 CuO Tenorite). The calculated average crystallite size of CuO by using Debye-Scherrer's formula is 22.27nm ($D=0.9\lambda/\beta\cos\theta$)

The table of average crystallite size calculated is as cited below.

Table No.1-Calculation of crystalline size of CuO NPs

2 θ	FWHM	Crystallite Size	Average Size in nm
35.54977	0.33423	24.95074	
38.75893	0.46128	18.24955	
48.84044	0.39294	22.19533	
53.49165	0.42162	21.0915	
58.27987	0.40071	22.68956	**22.27093**
61.59137	0.38466	24.03347	
68.0571	0.42237	22.68635	

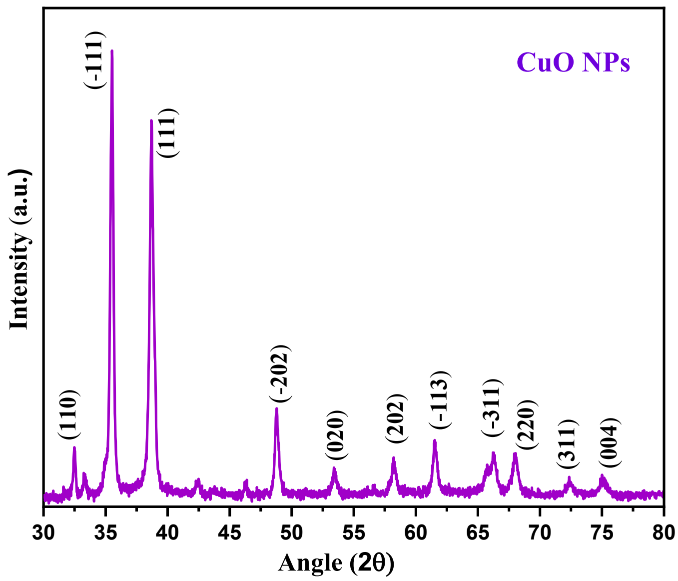

Fig: 1-XRD diffraction pattern of CuO nanoparticles.

3.2. FTIR Analysis:

The FT-IR analysis was used to determines functional groups in the synthesis MO NPs. CuO NPS peaks were located at 525, 881, 1104, 2920, 3483, 3573 cm^{-1}.The broad peak 400 to 600 cm-1confirms the formation of CuO NPs shown in Fig-2. The appearance of Broad peak centered at 3483 and 3573 cm^{-1} indicates O-H stretching vibrations. The less intense peaks at 2920 cm^{-1} indicates the C-H stretching vibration in the aldehyde group. The peak at 1104 cm^{-1} and 881cm^{-1} shows the presence of C-O bond stretching vibrations and O-H bend stretching respectively. FTIR validates the existence of Cu and O bonds.

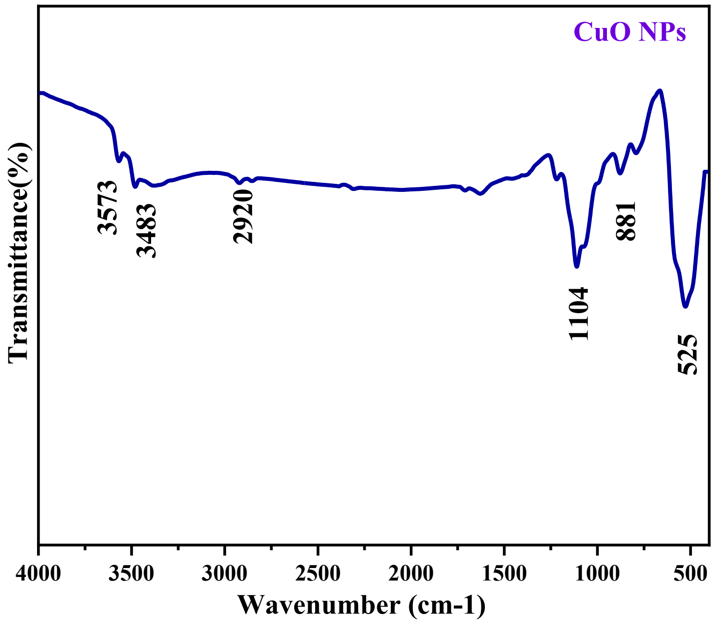

Fig:-2 FTIR spectra of CuO NPs

3.3 Zeta Potential:

The zeta potential of nanoparticles indicate the potential difference between the surface charge and colloidal stability of nanoparticles in a suspension. The zeta potential of biologically synthesized CuO NPs was found -11.8mV Shown in Fig:-3, indicating a negatively charged surface with low colloidal stability. The single narrow peak in the zeta potential distribution suggest a uniform surface charge distribution.

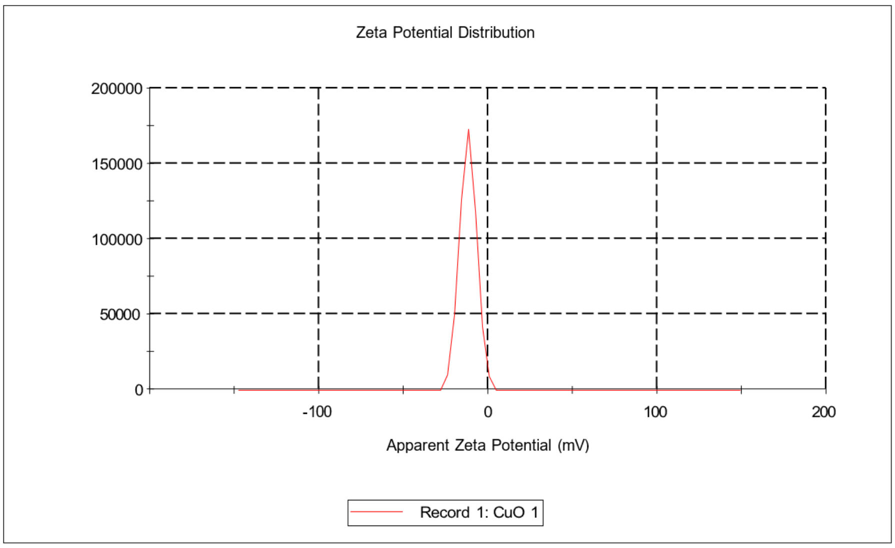

Fig:-3 Zeta Potential of Synthesized CuO NPs

IV. Applications:

The Stock solutions of the synthesized MO NPs were prepared at a concentration of 1 mg/ml in Sterile HPLC grade water (Sigma Aldrich). Then,100 ug/ml concentrations of sample was examined for the antimicrobial and antifungal activity by well diffusion assay. Each sample was loaded in well for the antimicrobial activity and antifungal activity in the respective test organism plate. The plates were incubated at 37°C for 24 h for bacterial pathogens and at 27 °C for 48-72 h fungal pathogens.

The antimicrobial activities of the Nanoparticles was assessed against Gram negative Escherichia coli [NCIM 2832] and Gram-positive bacteria Bacillus Cereus [NCIM 2703] by agar well diffusion assay method. All the above strains were collected from NCL pune, India. 24 hrs Old fresh bacterial cultures were spreaded over the surface of nutrient agar plate using sterilized glass spreader to obtain uniform growth. Meanwhile solutions of all the Nanoparticles were prepared by dissolving in sterilized distilled water. On the Nutrient agar plate 3 wells were made using a cork borer aseptically, one for the respective nanoparticle, one for the standard (S) and one for the water (C). Then 100µL of each preparation was added to the agar wells, further all plates kept in refrigerator 10 min for diffusion purpose. Then all plate were incubated for 24 h at 38 °C. After 24 h incubation antibacterial activities of the preparations observed were calculated as diameter zone of inhibition in mm.

Then all the wells were filled with the nanoparticle's solutions (100µl in each well). After that all the plates were kept inside refrigerator for proper diffusion of the nanoparticles inside the agar plate. Then all plates were incubated at 37 °C for 24 h. after 24 Hrs incubation plates were observed for antimicrobial activity.

Antifungal activities of Nanoparticles (NPs) was assessed against Aspergillus niger [NCIM 679] and candida albicance [NCIM 3471] by agar well diffusion assay method. [2] both the fungal cultures were collected from NCL, Pune, India. Potato Dextrose agar plates were prepared and same procedure followed as antimicrobial assay for antifungal activity. Then all plates were incubated at 37 °C for 48 hrs. after incubation plates were observed for antifungal activity calculated as diameter zone of inhibition in mm. After proper incubation results were interpreted in the form of zone of inhibition in the form of mm by using scale meter. The antimicrobial activity increases against both Gram positive and Gram-negative pathogens while nanoparticles show antifungal activity against only C. albicans , not against A.niger shown in Fig-4.

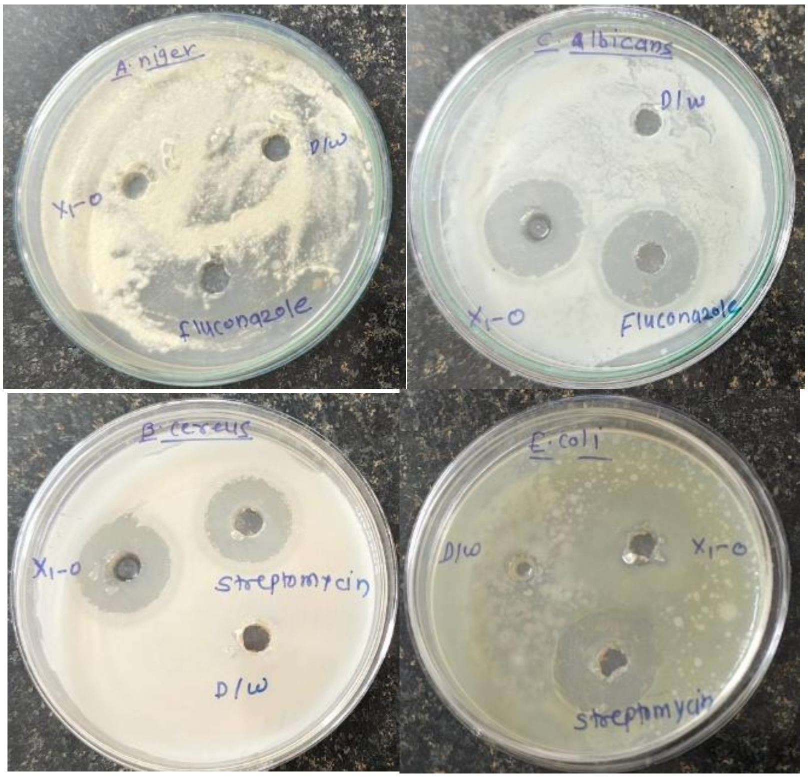

Fig:-4 Antifungal activity of CuO NPs(X_1-O) against C.albicans and A.nigar (a,b) and antimicrobial activity against B.cereus and E.coli (c,d).

Table No:2-Zone of inhibition in mm

Nanomaterial	Zone of Inhibition in (mm)			
	Bacteria		Fungi	
	B. cereus	***E.coli***	***A.niger***	***C.albicans***
CuO	24	21	nil	19
Streptomycin [Antibiotic Standard]	31	28	nil	nil
Fluconazole [Antifungal standard]	nil	nil	24	25
H_2O (Control)	nil	nil	nil	nil

IV. Conclusion:

The synthesized CuO MO NPs shows efficancy against bacterial pathogens B.cereus and E.coli and antifungal C albicans. The present research completely and successfully validates a sustainable ,cost effective and non-toxic biosynthesis of biologically active CuO NPs using Gomutra, a traditional components of Vedic medicine.

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