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Research Article

WET CHEMICAL SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF MOLYBDENUM OXIDE NANOPARTICLES AGAINST *STAPHYLOCOCCUS EPIDERMIDIS* AND *ENTEROBACTER AEROGENES*

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ABSTRACT

Objective: The objective of this study was to synthesize the molybdenum oxide nanoparticles (NPs) by employing wet chemical method and investigation of their antibacterial properties against pathogenic bacteria.

Methods: Molybdenum trioxide (MoO_3) NPs were synthesized using an eco-friendly wet chemical sol–gel technique. The synthesized MoO_3 NPs were characterized using X-ray diffraction (XRD), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy, and ultraviolet-visible spectroscopic techniques to confirm the obtained product, size shape, morphology, functional groups, and absorption spectra, respectively. The size of the MoO_3 NPs was found to be 41 nm. The antibacterial activity of these metal NPs was investigated on *Staphylococcus epidermidis* and *Enterobacter aerogenes* by measuring the zone of inhibition and colony-forming units on solid medium and by measuring the optical density of the culture solution. Antibacterial activity of MoO_3 NPs was also compared with well-known standard antibiotics.

Results: The antibacterial activities of molybdenum oxide NPs possessing size 41 nm were compared with standard antibiotics such as oxacillin, cotrimoxazole, erythromycin, clindamycin, chloramphenicol, and tetracycline. It was found that all of these antibiotics were effective against *Staphylococcus epidermidis* while *Enterobacter aerogenes* was resistant to oxacillin, co-trimoxazole and clindamycin, whereas the MoO_3 nanoparticles were found to be effective against both of these bacterial pathogens.

Conclusion: Inorganic antimicrobial agents have advantages over organic antimicrobial agents due to their stability, preparation methods, and their ability to prevent bacteria to develop multidrug resistant. It was observed that MoO_3 nanoparticles (NPs) possess good antibacterial properties; therefore, these can be used in pharmaceutical industries and provide a path for further research regarding the toxicity study for its use in human being.

Keywords: X-ray diffraction, Transmission electron microscopy, *Staphylococcus epidermidis*, *Enterobacter aerogenes,* antibacterial activity.

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INTRODUCTION

In recent years due to eruption of infectious diseases caused by different pathogenic bacteria and the development of antibiotic-resistant species due to the repeated use of the antibiotics, the researchers are searching for new antibacterial agents. The number of organic substances shows the antibacterial activity, but most of them are toxic in nature. Therefore, the ultimate target of nanoscience and nanotechnology is to present opportunities for exploring the bactericidal effect of metal oxide NPs due to their high stability and non-toxic nature [1]. NPs, especially metallic ones, tend to offer an effective solution for overcoming bacterial resistance [2-7]. The transition metal oxides are attracting a great deal of attention in the field of material science due to its variety of crystal phases and properties. Among the transition metal oxide, molybdenum trioxide (MoO_3) exhibits better intercalation chemistry with unique chemical, electrochemical, electronic, and catalytic properties. MoO_3 is renowned model compound for selective oxidation catalysis [8,9], photocatalysis [10], electrochromism [11], lithium-ion batteries [12], light-emitting diodes, etc. [13]. MoO_3 shows polymorphism and can be synthesized in different morphologies such as nanorods [14], nanobelts [15], and nanowires [16] for various applications. The nanomaterials, based on the metal ions, exhibit broad-spectrum biocidal activity toward diverse bacteria, fungi, and viruses [17]. *Zollfrank et al.* [18] reported that antimicrobial activity of transition metal acid MoO_3 is related to their surface acidity involving the intermediate formation of molybdic acid. The modification of various materials (e.g., polymers and metals) with MoO_3 particles showed that the modified material surfaces were practically free of microorganisms 6 h after contamination with infectious agents. Krishnamoorthy *et al*. [19] studied the toxicity of MoO_3 toward pathogenic bacteria. According to this report, the prepared MoO_3 nanoplates exhibit good antibacterial activity against four tested bacterial species *Escherichia coli*, Salmonella typhimurium, Bacillus subtilis, *and* Enterococcus faecalis. *Ruben et al. reported that* MoO_3 NPs act as potential therapeutic for sulfite oxidase deficiency [20]. Neha *et al*. [21] reported the synthesis of nanocrystalline h-MoO_3 through chemical bath deposition technique studies on its phase transition and investigated the antibacterial properties of h-MoO_3 and α-MoO_3 against Gram-positive *Bacillus megaterium* and *Streptococcus aureus* and Gram-negative *E. coli*.

METHODS

Reagents and test organisms

Staphylococcus epidermidis (Microbial Type Culture Collection [MTCC] 3382) and *Enterobacter aerogenes* (MTCC 2823) were obtained from MTCC, Institute of Microbial Technology, Chandigarh. All other chemicals used in experiment were of analytical grade and from standard chemical sources.

Synthesis of MoO_3 NPs

In the present study, the MoO_3 NPs were synthesized using environment-friendly sol–gel technique [22]. The MoO_3 solution was prepared by dissolving it in double distilled water, and in another beaker, oxalic acid solution was prepared. The MoO_3 solution was then stirred for 1 h,

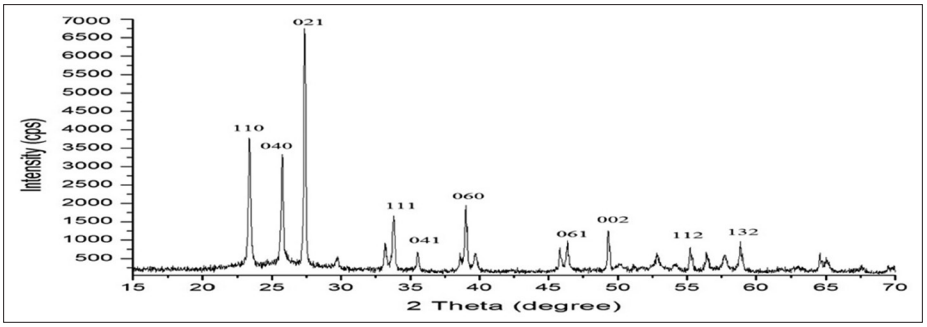

Fig. 1: X-ray diffraction pattern of molybdenum oxide nanoparticles

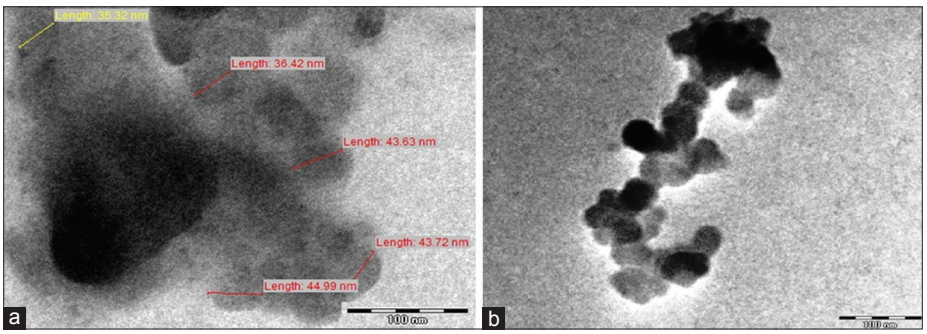

Fig. 2: (a and b) Transmission electron microscopy images of molybdenum oxide nanoparticles

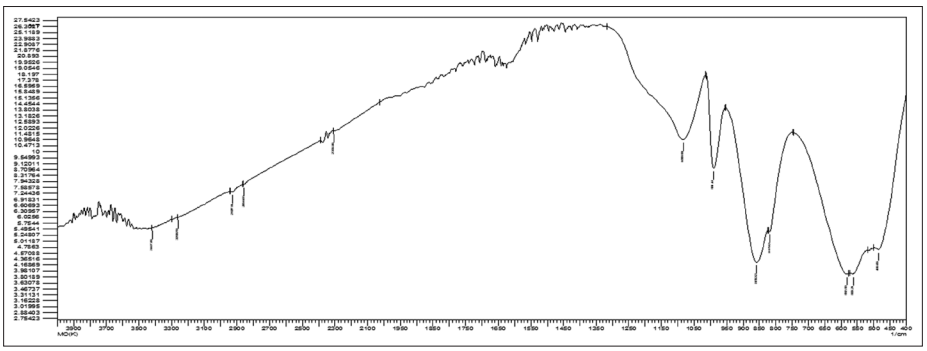

Fig. 3: Fourier transform infrared spectra of molybdenum oxide nanoparticles

and simultaneously, the oxalic acid solution was stirred for ½ h. The oxalic acid solution was then mixed in MoO_3 solution dropwise with continuous stirring for 3.0 h. The resultant solution was then filtered and washed with double distilled water several times. The resulting gelatinous precipitates were then heated at 100°C into an oven for 5.0 h, and finally, the precipitates were put in a muffle furnace at 500°C for 2.0 h.

Characterization techniques

The size, structure, and morphology of as-prepared metal NPs were characterized by X-ray diffraction (XRD) (Rigaku mini-2 using Cuα1, λ=0.15406 nm radiations), transmission electron microscopy (TEM) (FEI-Philips, Morgagni 286D with magnification up to ×280,000, Acc. Voltage: 100 Kv), Fourier-transform infrared spectroscopy (FTIR) (Shimadzu Corp-02014) in the wavelength range of 400–4000 cm^{-1}, and ultraviolet (UV)-visible spectroscopy (Shimadzu 1800) in the wavelength range of 200–1000 cm^{-1}.

Antibacterial study

Antibacterial activity of the MoO_3 NPs against a Gram-positive [23] *S. epidermidis* and a Gram-negative *E. aerogenes* bacterial pathogens was investigated by measuring zone of inhibition (ZOI), colony-forming unit (CFU), and optical density (OD). Antibacterial activity of MoO_3 NPs was also compared with standard antibiotics. The zone of inhibition was measured in mm by agar well diffusion method. The bacterial

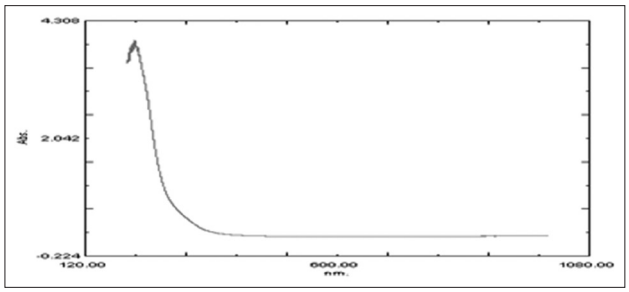

Fig. 4: Ultraviolet-visible spectra of molybdenum oxide nanoparticles

Table 1: MIC of molybdenum oxide and titanium dioxide metal NPs against *S. epidermidis* and *E. aerogenes*

Sr. No.	Bacteria	MIC in mg/ml
		Molybdenum oxide nanoparticles
1	*S. epidermidis*	30.0
2	*E. aerogenes*	30.0

E. aerogenes: Enterobacter aerogenes, MIC: Minimum inhibition concentration, *S. epidermidis: Staphylococcus epidermidis*, NPs: Nanoparticles

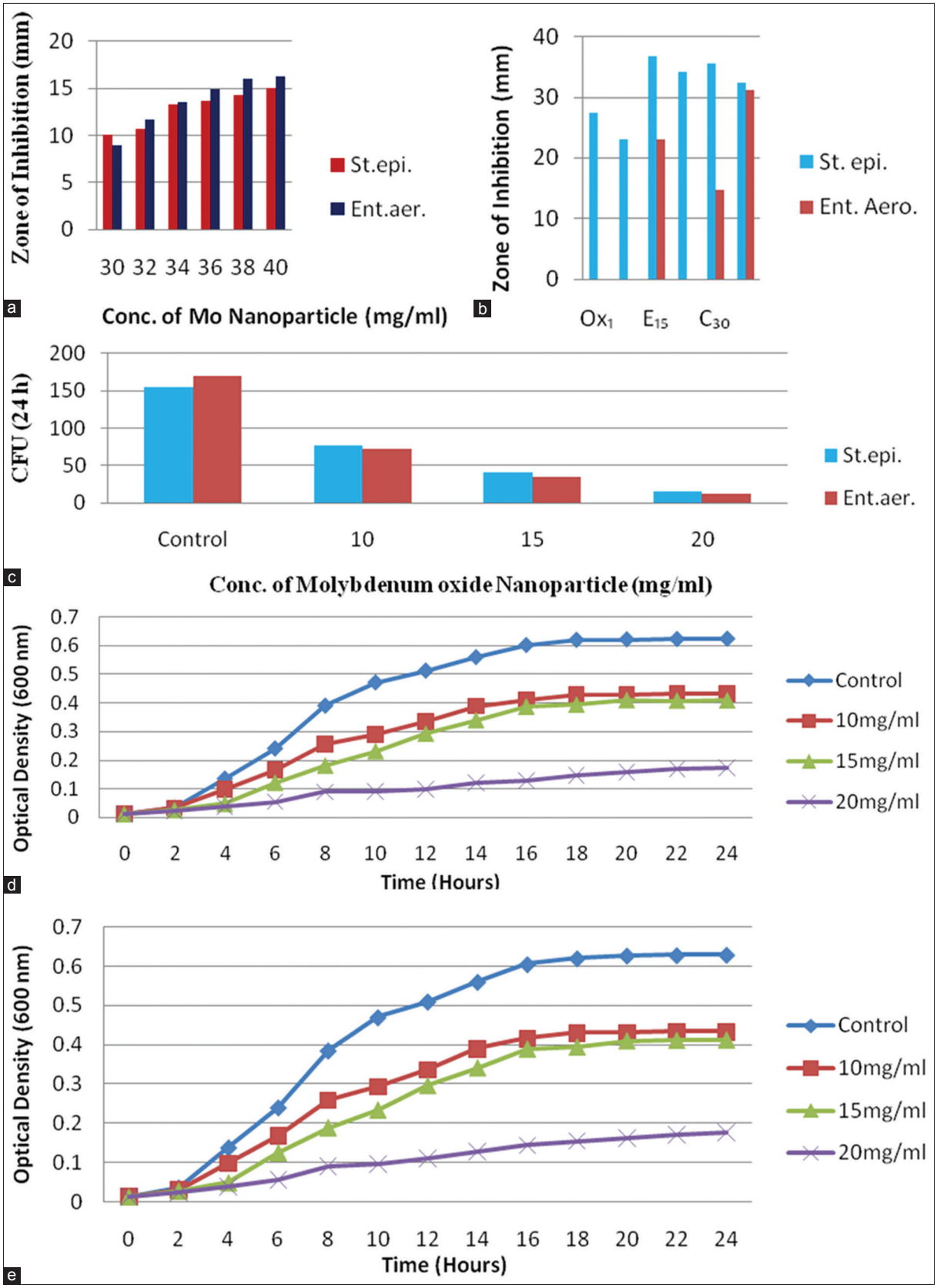

Fig. 5: (a) Zone of inhibition for Mo oxide nanoparticle (NP), (b) zone inhibition with different antibiotics, (c) colony-forming unit characterization of molybdenum oxide NPs against *Staphylococcus epidermidis* and *Enterobacter aerogenes*, (d) effect of molybdenum oxide NPS on the growth of *S. epidermidis*, (e) effect of molybdenum oxide NPS on the growth of *E. aerogenes*

test organisms *S. epidermidis* and *E. aerogenes* were grown in nutrient broth at 37°C and 30°C for 24 h. A100 µl of the bacterial culture was spread onto the solidified agar plates. The wells were prepared in the agar medium with the help of a cork borer. These wells were then

loaded with different concentrations of MoO_3 NPs suspension in double distilled water. The CFU of the bacterial culture was evaluated with different concentrations (10, 15, and 20 mg/ml) of MoO_3 NPs using the standard broth dilution method. The growth behavior of *S. epidermidis* and *E. aerogenes* was investigated by measuring the OD through the administration of different concentrations of MoO_3 NPs into the dilute solution of the broth.

RESULTS AND DISCUSSION

Characterization of molybdenum oxide NPs

Fig. 1 shows XRD pattern of the chemically synthesized molybdenum oxide NPs. The XRD pattern revealed major peaks at 2q values of 23.36 (110), 25.74 (040), 27.36 (021), 33.78 (111), 35.52 (041), 39.02 (060), 46.34 (061), 49.3 (002), 55.24 (112), 58.84 (132), and 64.56, respectively [24,25]. The experimental XRD pattern agrees with the JCPDS card no. 35-0609, *a*=3.9630 Å, *b*=13.8560 Å, and *c*=3.6966 Å. The XRD peaks correspond to the orthorhombic crystal structure of MoO_3. The XRD analysis of the sample synthesized by the sol–gel technique confirms the formation of α-MoO_3 NPs. Average particle size of the molybdenum oxide NPs corresponding to the highest intensity peak was found to be 41.0 nm using Scherrer's formula ($d = K\lambda/\beta \cos\theta$).

Fig. 2 shows the TEM images of molybdenum oxide nanoparticles. The particles were observed to be almost spherical. It can be seen from Fig. 2 that there is a uniform distribution of particle with mean particle size 40.81 nm which is in close agreement with the XRD result.

Figure 3 shows the FTIR spectra of molybdenum oxide nanoparticles. Broadband at 582 and 563 cm^{-1} is may be due to O-Mo-O bending. The band at 991and 1083 cm^{-1} is due to Mo=O stretching and two bands at 858 and 817 cm^{-1} came from Mo-O-Mo stretching. These bands almost correspond to α-MoO_3 data [25]. A band at 3417 is due to O-H stretching vibration of adsorbed water molecule on the surface of MoO_3 nanoparticles.

Fig. 4 shows the ultraviolet (UV)-visible spectra of molybdenum oxide nanoparticles (NPs) synthesized by sol–gel method. The absorption maxima observed at 212.2 nm, which falls under the UV-C region. The UV-C region is called the short wave UV region which ranges from 200 to 280 nm. Hence, this indicates that MoO_3 NPs absorb the UV rays effectively. The band energy gap was found to be about 5.84 eV.

Antibacterial activity of molybdenum oxide nanoparticles

Fig. 5 (a) shows the ZOI produced by different concentrations (30, 32, 34, 36, 38, and 40 mg/ml) of the MoO_3 NPs against *S. epidermidis* and *E. aerogenes*. It was found that the ZOI increases with increase in the concentration of the MoO_3 NPs. Fig. 5 (b) shows the ZOI produced by standard antibiotics such as oxacillin, cotrimoxazole, erythromycin, clindamycin, chloramphenicol, and tetracycline. It was found that all of these antibiotics were effective against *Staphylococcus epidermidis* while *Enterobacter aerogenes* was resistant to oxacillin, co-trimoxazole and clindamycin, whereas the MoO_3 nanoparticles were found to be effective against both of these bacterial pathogens. Fig. 5 (c) shows the CFU/ml of the dilute bacterial broth supplemented with different concentrations (0, 10, 15, and 20 mg/ml) of MoO_3 NPs. It was found that the CFU has been reduced significantly with increase in the conc. Of the MoO_3 NPs. Fig. 5 (d) and (e) shows the time-dependent changes in the bacterial growth monitored at a regular interval of 2 h (up to 24 h) by measuring OD of the control and bacterial solutions supplemented with different concentrations (10, 15, and 20 mg/ml) of MoO_3 NPs. It is clear that slope of the bacterial growth curve is continuously decreased with increasing concentration of the NPs. Table 1 shows the minimum inhibitory concentration (MIC) of the MoO_3 NPs against both of the investigated bacterial strains.

CONCLUSION

In this study, molybdenum oxide NPs of mean size 41 nm were synthesized using eco-friendly, rapid, and inexpensive sol–gel technique. Antibacterial activity of these MoO_3 NPs against a Gram-positive *S. epidermidis* and Gram-negative *Enterobacter aerogenes* bacterial pathogens was investigated by measuring ZOI, CFU, and OD. Antibacterial activity of MoO_3 NPs was also compared with standard antibiotics. The MIC of these NPs was found to be 30 mg/ml for both of the investigated bacteria. The CFUs decrease with increase in the conc. of the metal NPs. The results obtained from the ZOI, CFU, and OD measurements were in close agreement with each other. IIt was found that all of these antibiotics were effective against *Staphylococcus epidermidis* while *Enterobacter aerogenes* was resistant to oxacillin, co-trimoxazole and clindamycin, whereas the MoO_3 nanoparticles were found to be effective against both of these bacterial pathogen. This study shows that MoO_3 NPs hold good antibacterial properties; consequently, these can be used in pharmaceutical industries and provide a path for further research regarding the toxicity study for its use in human being.

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AUTHORS' CONTRIBUTIONS

Poonam Sangwan was involved the synthesis, characterization, and antibacterial study of molybdenum oxide NPs.

Harish Kumar guided this research at each and every step.

Renu Rani helps in antibacterial study.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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