RESEARCH ARTICLE

An assay on the antibacterial performance of Co doped ZnO nanoparticles in dental microbes

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ABSTRACT

Green synthesis is a simple, cost-effective and environmentally friendly method for the synthesis of nanoparticles.The extraction of *Prosopis fracta* fruit prepared a beneficial material to achieve pure, 1 and 5% cobált doped zinc oxide nanoparticles (Co-Zn NP) via a green and uncomplicated approach. The characterizing features of the obtained product were configured by analyzing the data of XRD, FESEM, EDX, UV-Vis and FT-IR technics. As the existent of superb doped cobált within construction of zinc oxide was certified via the XRD and EDX, the FESEM análysis uncovered the altered morphology of pure ZnO subsequent to the addition of doped cobalt, while displaying the rod-looking framework of Co-ZnO. In coordination to the anti-bacterial results of produced NP towards *Streptococcus mutans* bacteria by employing the micro-dilution route, the doped NP exhibited a superior antibacterial functionality than pure NP, which signifies the potential role of Co doped ZnO nanoparticles as an economical choice to be applied for oral and dental infectious illnesses.

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INTRODUCTION

The lack of toxicity, as well as bio-safety and biocompatibility of Zinc oxide (ZnO) led to their implementation as biomedical products similar to drug cárriers, antibacteriál agents, and bioimáging probes for treating cancer[1]. The formation of reactive oxygen species (ROS) throughout the surface of ZnO NP is customarily acknowledged mechanism for materials antibacterial functionality, since the oxidative stress of bacterial cells transpires at light and ends with cells annihilation [2]. Additionally, the scientific focus of many was pulled towards the broad band gap (3.37 eV) wurtzite-pháse of ZnO as a semiconductor as a result of its essential stance in the fields of piezo-elèctronics, and high-power elèctronic devices[3] . Considering the contemporary theoretical prognoses, the ferromagnetic qualities of transitioned metal (TM) doped ZnO diluted mágnetic semiconductors (DMS), which can be observed, led to the extension of further material assessment. The enhanced designs of ambient temperature ferromagnetic semiconductors proved to be capable of causing fundamental improvements in spin-electronics tèchnology, while such spin-based electronic

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dèvices can offer certain benefits similar to facilitating the option of instant-on computer, accelerate the speed of data processing, intensify integration density, and demand for a very low consumption of electrical energy [4, 5].

The infectious-microbial illness of tooth decay is the reason behind the detected dissolute and destructed dental calcareous tissues, which implicates certain difficulties similar to the loss of teeth, induction of pain, and cosmetic defects[6, 7]. *Streptococci mutan* and lactobacilli are recognized as the fundamental etiological parameters of tooth decay. The manufacturing mechanics of nanomaterials gave rise to an immense innovation in antibacterial products to directly affect the progress of nano materials and facilitate numerous benefits over the chemical materials [8] . The importance of these outputs relies on their functionality as antibacteriál agents towards a broad range of antibiotic-resistant bacteriá. The accommodation of numerous antibacterial features by ZnO nanoparticles led to their configuration as antibacterials in varying laboratory activities[9-11] .

Considering the expanding demand for the design of environmentally-adaptable synthesizing mechanics, the focus of numerous scientists was pulled towards a novel biosynthesizing approach that proceeds through the interaction of nanotechnology and biotechnology [12-14] .This green project was designed under the objective of minimizing the occurrence of potential hazardous risks for humans and the environment. Among the varying benefits of biological routes when contrasted to that of chemical and physical technics, one can mention their adaptability with their surroundings, safety, shorter time of synthesizing process, production of trifling industrial waste, and the lack of requiring any of the applied toxic chemicals in biological methods [15, 16]. The superiority of these advantages next to the expensive, hazardous, and time-consuming procedures of the physical and chemical synthesizing routes is undeniable. In this regard, a higher rate of interest was invested in performing nanoparticles syntheses through the employment of organic resources, similar to plants and microbes, under the category of green synthesis [16]. Nowadays, the nanoparticles manufacturing with plant origins to produce green nanoparticles engrossed many researchers, which is attributable to their adaptability with the surrounding, implication of simple fabrication process, high speed of reaction, and exclusion of microbial growth in contrast to other approaches[17-19] . Hence, this work made an effort to arrange pure, and 1% and 5% Co doped ZnO nanoparticles through the design of a green route by the employment of extracted *P. fracta* fruit. We also examined the antibacterial performance of our nanoparticles towards *Streptococcus mutans* bacteria.

EXPERIMENTAL

Arrangement of pure and Co-ZnO NP

The arrangement of samples was initiated by procuring the extraction of *P. fracta* fruit. For this matter, we weighted and appended the fruit powder of *P. fracta* to distilled water (ratio 1:10) under a shaking condition for 12 hours at 150 rpm. Subsequent to the filtration of obtained mixture through a filter paper; the extract was exerted throughout the upcoming experiments. To continue the procedure, 10 mL of extract was appended to three individual Erlenmeyer's that were volumed up to 50 mL by distilled water and placed in a water bath at the temperature of 75 ˚C. The formula of $Co_{1-x}Zn_xO$ was applied to compose a 50 mL solution consisted of $Zn(NO_3)_2.9H_2O$ and $Co(NO_3)_2.6H_2O$ in the percentage ratios of 0, 1 and 5, respectively. Once the mixtures were stirred for 3 hours, the drying process was performed at 90 ˚C for the duration of 20 hours. As the last step, a furnace was utilized to calcine the dried samples at 600 ˚C for 2 hours.

Characterization

Several analytical methods were exerted to distinguish the characters of pure and doped ZnO NRs. The crystalline nature of our product was evaluated through the results of PXRD (Netherlands, PANalyticalX'Pert PRO MPD system, Cu Kα). Additionally, we assessed the surface morphology of samples through the performance of FESEM (MIRA3 TESCAN, Czech) analyses.

Antibacterial assay

Minimum Inhibitory Concentration

The exertion of Micro-broth dilution technic provided data on the MIC (minimum inhibitory concentrátion) of progressed nanoparticles in opposition to *Streptococcus mutans* bacteria, which was executed by the employment of sterile 96-well microplates. To begin the process, the sequential volumes of nanoparticles (1.95, 3.90, 7.81, 15.62, 31.25, 62.50, 125, 250, and 500 μg/mL) were availed to retrieve 100 μL of each concentration and have it added to the wells of microplate. Thereafter, we preceded by appending 90 μL of Muellèr Hinton Broth (MHB) medium as well as 10 μL (Merck) of microbiál suspension, which equivalent to 0.5 McFarland, to the micropláte wells that accommodated the nanoparticles. Subsequent to completing the cultivation, we set out the microplates on a shaker for three seconds up to the point of procuring an entirely uniformed mixture, which were then incubated by an incubator at the temperature of 37 ˚C for 24 hours. Once trial was redone in triplicate for every case of the nanoparticles volumes and succeeding to the duration of incubation, we configured the turbidity of wells through the utilization of ELISA Reader (ELX 800, BioTek USA) at the wavelèngth of 620 nm. The lowèst amount of nanoparticle that lacked any bacteriál growth (signified through the lack of any induced turbidity by bacteriál growth) was settled as the minimum inhibitory concentrátion (MIC).

Minimum Bactericidal Concentrátion

The values of MIC were studied to get a fix on the minimum bactericidal concentrátion (MBC). Initially, the culturing process of 10 μL of MIC dilution and various dilutions above this limit was completed on Muellèr Hinton Agar culture medium to be settled within an incubator for 24 hours at 37 ˚C. Once we checked the bacterial extension of plates, the lowest volume of nanoparticles that lacked the growth of 99.9% of bacteria was labeled as the minimum bactericidal concentrátion (MBC).

RESULTS AND DISCUSSION

XRD analysis

Figure 1 exhibits the XRD outcomes of pure, 1, and 5% Co-Zn NP. In conformity to PXRD analysis of pure ZnO NP, the diffraction peaks at 2θ= 31.91, 34.56, 36.38, 47.67, 56.73, 63.02, 66.52, 68.08, 69.21, and 77.11˚ were observed to be allocated to the (100), (002), (101), (102), (110), (103), (200), (112), (201), and (202) planes, which signifies the hexagonal wurtzite construction of pure ZnO (JCPDS card no. 36-1451) [9]. The data of Figure 1 display a litter alteration in the placement of ZnO (101) subsequent to increasing rate of doped cobalt into ZnO network; this observation can be related to the fragmentary replacement of Co ions throughout ZnO lattice. In following, we applied the Scherrer's equation to determine the crystallite

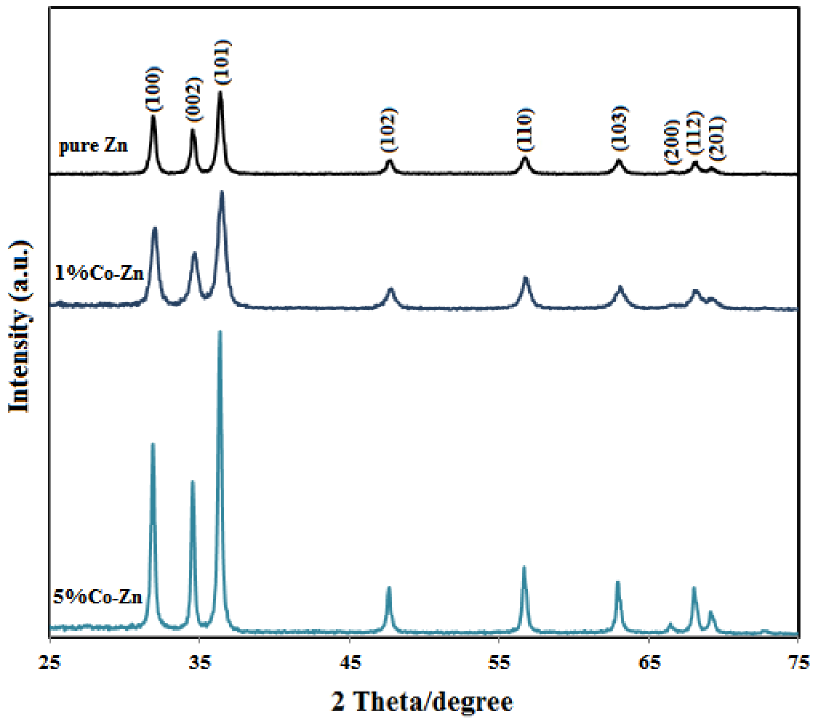

Fig. 1. XRD graphs of pure, 1, and 5% Co-Zn NP

size of synthesized products [20] and achieved the results of 17.76, 19.17, and 24.52 nm for pure, 1, and 5% for the case of Co-Zn NP, respectively. Accordingly, the crystal size of NP were enlarged due to extending the doped volume of cobalt, which is probably caused by the larger ionic radius of $Co^{2+/3+}$ is 0.75-0.9 Å cobalt when compared to that of zinc (0.74 Å).

FESEM and EDX analysis

The obtained FESEM images provided data on the morphology and particle size of the prepared products. The presented data in Figure 2 displays the estimated size of pùre ZnO to be around 40 nm, while an extension was observed in the size of doped particles subsequent to addition of Co to crystalline lattice of ZnO. According to this Figure, increased rate of doped cobalt over the volume of ZnO led to the inducement of a longitudinal growth in the particles, The XRD outcomes found a relation between this observation and the bigger ionic radius of cobalt atom than to zinc atom. EDX outcomes of NP approved the appropriate entry of cobalt into the construction of ZnO NP. As it is exhibited in Figure 3, the percentages of cobalt in pure, 1, and 5% Co-ZnO NP were 0, 0.86, and 4.35%, respectively. It signifies the absence of any impurity in this construction.

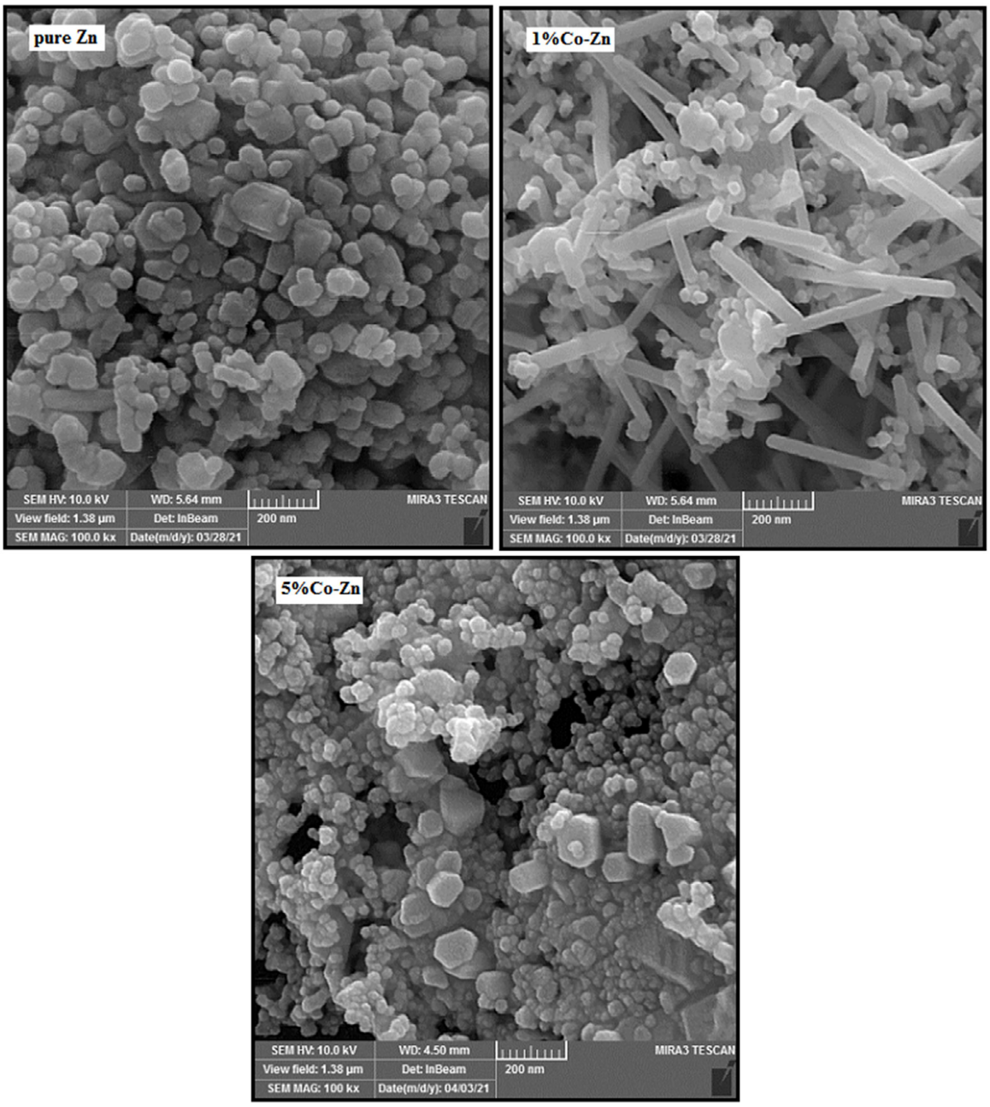

Fig. 2. FESEM images of pure, 1, and 5% Co-Zn NP

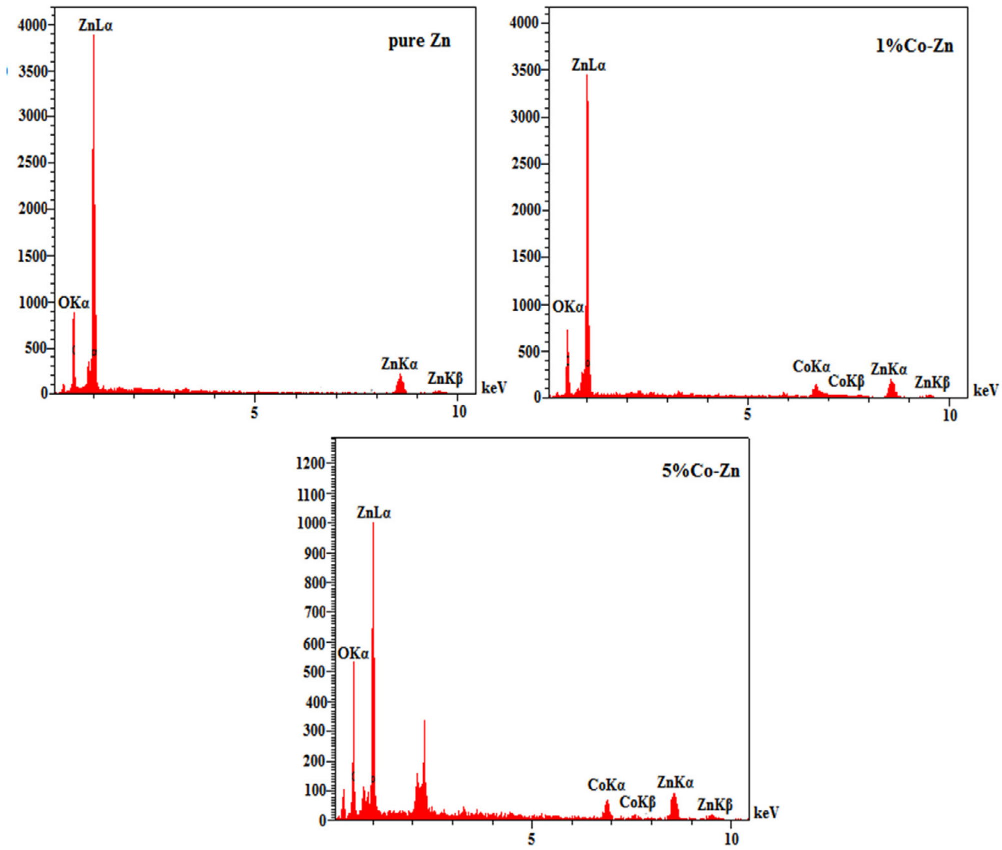

Fig. 3. EDX image of pure, 1, and 5% Co-Zn NP

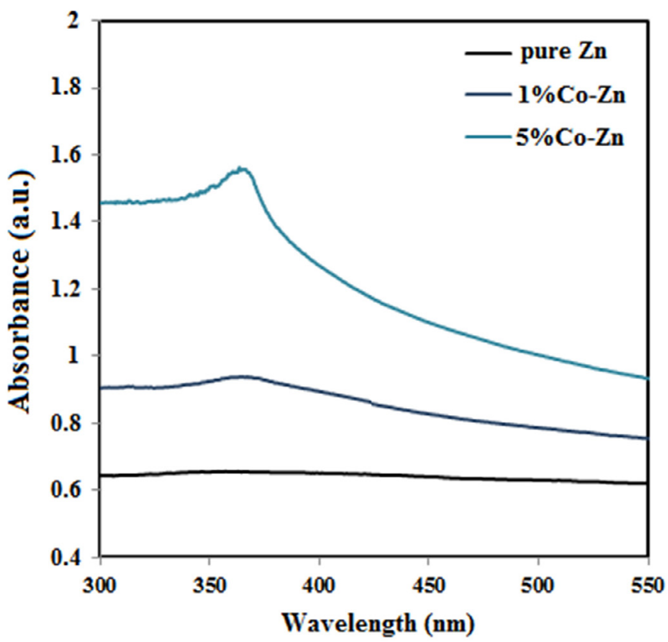

Fig. 4. UV-Vis spectra of pure, 1, and 5% Co-Zn NP

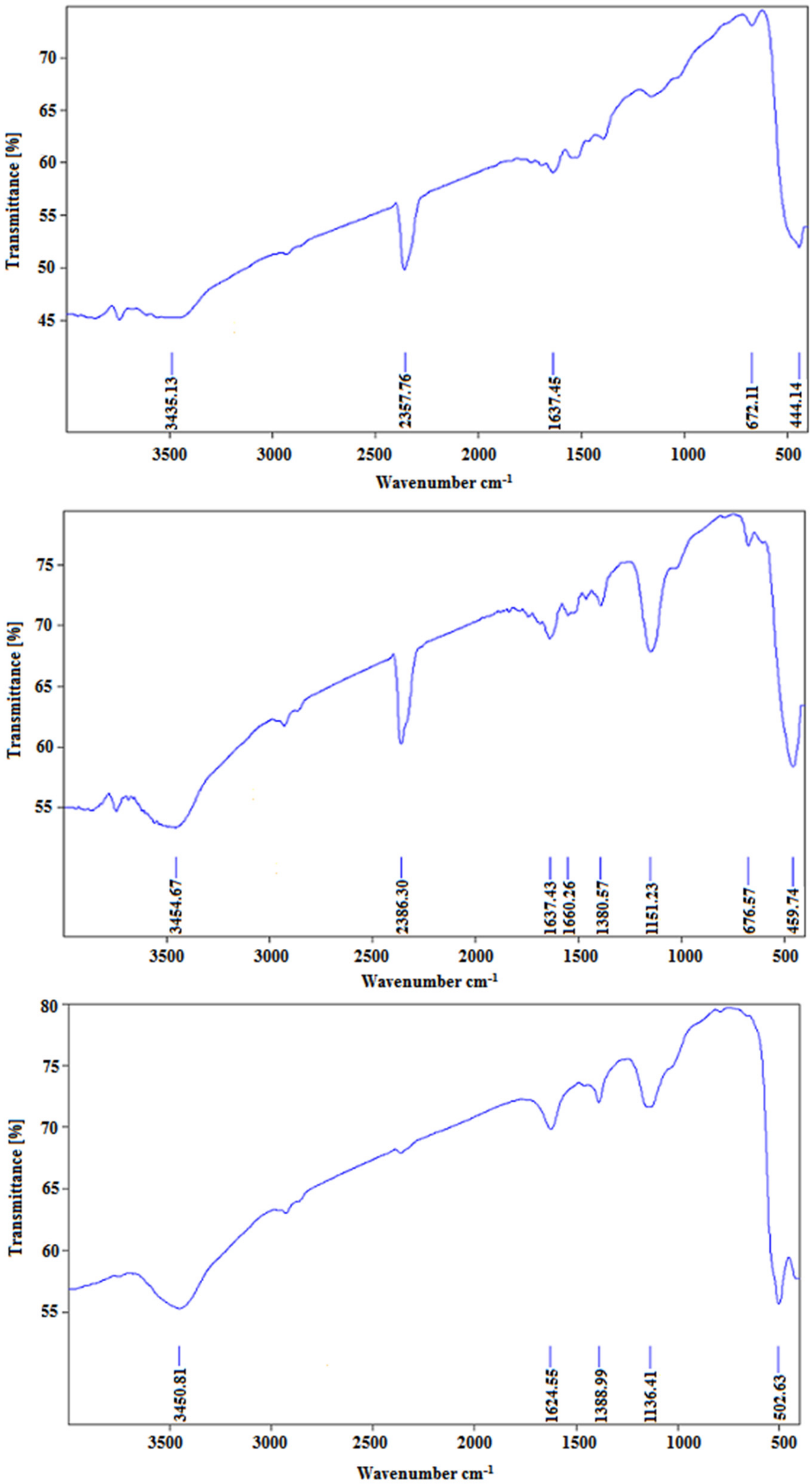

Fig. 5. FT-IR graphs of pure, 1, and 5% Co-Zn NP

Table 1. MIC and MBC results of pure, 1, and 5% Co-Zn NP on *S. mutans* bacteria

Nanoparticles	***Streptococcus mutans* bacteria**	
	MIC (μg/mL)	**MBC (μg/mL)**
pure Zn	**31.25**	**15.62**
1%Co-ZnO	**15.62**	**7.81**
5%Co-ZnO	**7.81**	**3.90**

UV-Vis analysis

The presented UV-Vis spectra of pure, 1, and 5% Co-Zn NP in Figure 4 pointed out the induced alterations in energy bánd construction of ZnO subsequent to Co doping. Exhibited optical absòrption peaks at the areas of 367, 360, and 341 nm were associated with pure, 1, and 5% Co-Zn NP, respectively. The repositioning of absorption edge was observed to be towards the lower wavelengths as a consequence of doped cobált into ZnO. The detected occurrence of a blue shift as a rèsult of enlarging the volume of cobált may be attributable to combined impact of optical transition to excitonic state of ZnO and electronic transitions that implicate the substitution of Zn^{+2}, as the responsible factor for the blue shift, by the crystal-field split in 3d levels of Co^{+2}. Our assert regarding the efficiènt replacement of Co into wurtzite framework of ZnO to replace the sites of Zn was further affirmed by the presented outcomes.

FT-IR analysis

In coordination's to the provided FT-IR graph of pure, 1 and 5% Co-Zn NP in Figure 5, the observance of a broad peak at the point of 3450 cm^{-1} is allocated to stretching vibration of OH bond, whereas peak at 1637 cm^{-1} assigned to the C=O group. Furthermore, C-O-C bonding is the reason behind the recorded peak at 1150 cm^{-1}, while the appearance of zinc oxide induced a peak at the point of 444 cm^{-1}. The altered position and intensity of the peak at 444 cm^{-1}, as well as its shifting towards higher regions, is attributable to the doped cobalt into the framework of zinc oxide.

Antibacterial assay

The performed assessment on MIC and MBC of the designed product towards responsible bacteria for tooth decay displayed the ability of pure and doped nanoparticles to exhibit bactericidal impacts and prevent the growth of *S. mutans* bacteria. The data of Table 1 denotes the stronger effect of doped NP on intensifying the inhibitory performance than pure NP.

The observations of previous assays signified the importance of particles size and shape in the level of nanoparticles antibacterial features; for on stance, a reduction in particles sizes can pave the way for an appropriate interaction with bacteria and empower this quality. Apparently, a decrease in particles sizes can extend ráte of released ion from surface and impart more antibacteriál features [20]. Antibacterial qualities of nanoparticles is fundamentally reliant on releáse of their ions, though some of major mechanisms consist of cell membrane damaging, as well as reactive oxygen spècies formation and cell attack via ions that proceeds by impairing the ATP products and preventing the replication of DNA[21-23] . Our procured pure and doped nanoparticles were composed of spherical and rod-looking frameworks, respectively. Due to the higher efficiency of doped NP surfaces with a rod-like construction towards bacteria than that of the pure NP, a stronger antibacterial impact was induced by cobalt doped nanoparticles on *S. mutans* bacteria.

CONCLUSION

The extracted product of *Prosopis fracta* fruit promoted and expedited the procuring of pure, 1, and 5% Co doped ZnO nanoparticles, which were also examined in terms of physicochemical qualities by the employment of laboratory devices and analytical technics. In coordination to the gathered outcomes, we succeeded in producing uniformed nanoparticles at nanoscale and observed an expansion in the lèngth and diameter of Co doped NP subsequent to increasing the applied amount of doped cobalt into ZnO. Furthermore, we assayed the antibacterial functionality of our NP in opposition to *Streptococcus mutans* bacteria by implementing the known micro-dilution route, which exhibited the superior impact of doped ZnO NP in contrast to the pure ZnO NP. As another noteworthy fact, the strength of ZnO antibacterial features was empowered by extending the applied percentage of cobalt into its construction. Hence, the appositeness of our synthesized product for biological implementations, as well as its potential and cost-effectiveness in Oral and dental infectious illnesses, was affirmed by the presented outcomes.

CONFLICT OF INTEREST

There is no conflict of interest.

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