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Full Length Research Article

Aero Gel Method for the Preparation and Characterizations of Cobalt oxide Nanoparticles with its Antibacterial Studies

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ABSTRACT

One of the transition metal oxide so-called cobalt oxides is successfully synthesized into nanophase powder by aerogel method which has benefits to make the low-size range nanoparticles (NPs) and analyzed at ambient as well as annealed temperature at 200º C. The Cobalt oxide nanoparticles (Co_3O_4 NPs) samples are studies by the following techniques that are powder X-ray diffraction (PXRD) crystallographic for essential crystal parameters, transmission electron microscope (TEM) for particles information, ultraviolet visible (UV-Vis) spectroscopy for band gap energy with optical properties and Fourier transform infrared (FTIR) spectrum for molecular vibrational analysis. The impact of the applied samples on inhibition activities at the bacteria's zone against Pseudomonas aeruginosa and Escherichia coli (E. coli) respectively were interpreted.

***Key words*:** Aero gel method, *Pseudomonas aeruginosa, Escherichia coli*, Cobalt oxide, Unstable NPs

Now a day the nanoscience and technology are represented as icon of revolution in electrical and electronics industry as well as medical field. For that reason, it is acting as active factors in current trends of the modern world particularly in increasing the economic background of individual countries and its novel perspective on future technology using an optimized artificial intelligence [1-4]. The preparation of nanoparticles with fixed surface morphology, high crystalline structure and prominent peak orientations with reproducibility on crystallographic parameters, shape and size is a primary entry towards the applications in the field of nanoscience & technology [5-6]. The low-cost synthesis process is the attentive key for the initiative the ideology into the researchers to come across the optimized characteristics materials for innovative application apart from the physical and chemical properties lead towards the potential applications in various field such as photo conductivity, sensing ability of gas, digital bases, energy productions photocatalytic activity for analysis and removal of complex molecules and antibacterial activity [7-9]. This simple technique can able to produce the size of nanograins which are closely packed with average size more below 80 nm. Because of the novel properties and applications in effective manner based on the sizes effect of nanoparticles, the synthesis method should be compatible with the process involved in condensation of solid structures. So, the mechanically strong products which are participating in evolving the nanotechnological related problems & its solutions on facing the various challenges to overcome the complex into the simple and the best working novel products [10].

Nanotechnology is one of the alternative key of usage of antibiotics mainly in the oscillating pandemic situations from 2021. The functional groups associated with derived nanoparticles which will be act against the growth of microbe without decreasing the body immunity as well as increasing the drug resistance whereas one of the unique parameters namely surface to volume ratio (S/V) which will increase the ability of penetration of ROS species and making perturbation with cell wall and other parts of microbes effectively [11].

Cobultus oxide NPs have unique properties and various applications in super capacitor, gas sensors, thermal sensors,

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battery, electrode for energy storage system and widely used in factories due to their bio-adjustability, biocompatibility, biodegradability, cost effective and ecofriendly disposable in nature. In drug industry, Co_3O_4 NPs is used as external agency against bacterial, viral, fungal, cancer, cholinergic and healing property on injured skin. Co_3O_4 NPs can be used as effective antibacterial agent. The preparations of Co oxide NPs interaction in reduction of Co ions (Co^+) in an aero-aqueous medium, deriving the colloidal Co with size of particle in various ranges in nm. At first stage, the size reduction of Co oxide with sodium hydroxide solution at various pH values lead to formation of nanoparticles in large diameter due to the effect of oligomeric clusters formation [12]. In this paper, optimized aerogel synthesis of Cobalt oxide NPs as antibacterial agents have been applied. The optimum conditions for synthesis of Cobalt oxide NPs were determined using soft chemical route method. Then, the properties of nanoparticles were determined using powder X-ray diffraction technique, TEM with SAED, UV-Vis and Fourier transform infrared (FTIR). The antibacterial activity of different concentrations of synthesized nanoparticles against two gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa were studied [13].

MATERIALS AND METHODS

Sigma Aldrich 99.9% purity of Cobalt (III) chloride ($CoCl_3$) salt is used to prepare precursor solution with MilliQ water. Aloe Vera gel was used as supplementary minerals to enhance the bioactivity. The samples were prepared by Aerogel method. Aerogels are among the lightest solid materials are familiar one. They are created by combining a polymer with a solvent to form a gel, and then removing the liquid from the gel and replacing it with air. These derived Aerogels are extremely porous and very low in density. The natural proteins of egg white act as Aero gels here, it has a more significant impact on the purifications of air [14].

Preparation of Co_3O_4 NPs by Aerogel method

For synthesis of Co_3O_4 NPs, 99 % of Cobalt (III) chloride ($CoCl_3$) salt was added into natural egg white of 2 ml with distilled water (25 ml Milli Q) stirred vigorously for 2 hours. In the next step, 5 ml of Glycine was mixed with the solution plus 25 ml of Milli Q water was added into the precursor solution. Within a few minutes, the solution developed a distinct characteristic colour (Red-Brown) by keeping the Cobalt chloride concentration volume constant with constant stirring till the reaction got over (15 min), was obtained and placed on a hot plate at 200^o C. It becomes powder after 30 min then the powder was transferred into a mortar and made it as fine powder after mortaring for 16 hours. This fine powder was annealed to 200°C for further studies [15].

Characterization of Co_3O_4 NPs

Synthesis of Co_3O_4 NPs was examined by a powder X-ray diffraction (PXRD) using a Panalytical X-pert Pro instrument; FTIR spectrum of the entitled samples were examined in the range of 400 – 4000 cm^{-1} using an Invenio FTIR spectrometer. The optical properties such as absorption, transmission and energy band gap of the sample were recorded in the UV-1280 Multipurpose UV-Visible Spectrophotometer. Their TEM and SAED pattern was derived using FEI Tecnai G220 S-TWIN TEM instrument [16].

Antibacterial activity

The gram-negative bacteria pathogens were grown by well diffusion method under the appropriate ambient condition to study the inhibition activity by streaming of species for the two different pathogens of as prepared ASP sample. An active zone of colony was formed for the antibacterial activity and optimum condition maintained for experiment and it was collected at the nutritional values added agar plate. Wells which are occupied by two different pathogens of as prepared ASP were incubated for 24 hours at room temperature. Finally, the diameter of geometrically almost circled shape of zone of inhibition is noted in mm [17].

RESULTS AND DISCUSSION

On considering the enhanced opposition against the pathogens to every numerable known antibiotic, it is crucial approach to investigate and inhibit the antimicrobials by utilizing the new bio-compatible promising NPs candidates. Because of that reason only, this work focused at Aero gel technique to synthesis of Co_3O_4 NPs for the effective actions towards nil percentage of pathogens [18]. For the purpose of selected gram-negative bacteria, the reason that among many trials this both samples as prepared (ASP) and annealed at 200°C were provided the appreciable activity on comparing the others. The obtained ZOI of these bacteria represent that that the Co_3O_4 NPs having the good growth dumping capability and decreases the number of bacterial counts. Even though the result was congruent with this kind of past studies of individual, the combination of phytochemicals of egg white plus nanoparticles provide considerable action against the bacterial growth. So, for as concerned with the obtained result, the researchers encouraged the Co_3O_4 NPs having better inhibiting species against the particular pathogens [19].

XRD analysis

The (Fig 1-2) showed the XRD patterns of Co_3O_4 nanoparticles without annealed and with annealed at 200°C. The figures show a sharp narrow peak with the diffraction pattern of (220), (311), (222), (400), (422), (511), (440) and (533) at 2θ angle of 31.23, 36.8, 38.52, 44.79, 55.65, 59.34, 65.21 and 77.22 without annealed [20]. The corresponding angles of 2θ for annealed sample are observed as 31.28, 36.83, 38.53, 44.82, 55.67, 59.38, 65.27 and 77.39. The diffraction angle is slightly increased which reveals that there was a dislocation in the lattice points due to annealing. In both of the samples the same planes are identified and they are well matched with JCPDS file no. 42-1467 which indicates the crystalline nature of the material with face-centered cubic (FCC). The average crystallite sizes were calculated using Debye-Scherrer's equation and shown in (Tables 1-2) [21].

$$D = \frac{0.94\,\lambda}{\beta cos\theta} - - - - - - - - (1)$$

where λ is the wavelength of Cu-kα radiation, β is the full width half-maximum (FWHM) in radians, and θ is the angle of diffraction (in radians). The average crystallite size of Co_3O_4 and Co_3O_4 (200^{o}C) were found to be 29.47 nm and 33.97 nm respectively. Its lattice parameters a = b = c= 8.08417 Å calculated from the relation:

$$a = d_{hkl}\sqrt{h^2 + k^2 + l^2} \quad - - - - - (2)$$

From the calculated crystallite size, dislocation density (δ) is used to identify the amount of crystal defects in the synthesized sample [22]. It is defined as the length of crystal dislocation lines per unit volume of the crystal and was estimated using the following relation:

δ= $1/D^2$ where, D denotes the crystallite size. The dislocation density decreases with crystallite size as shown in (Fig 3). The dislocation density of annealed sample at 200°C is minimized [23].

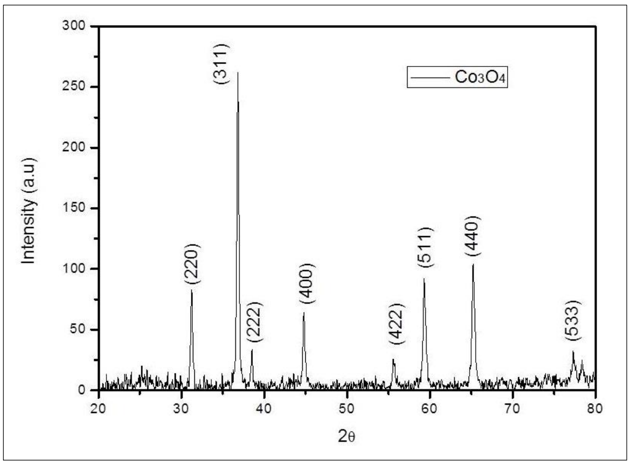

Fig 1 XRD Pattern of green synthesized Co_3O_4 Nanoparticles for Asprepared sample

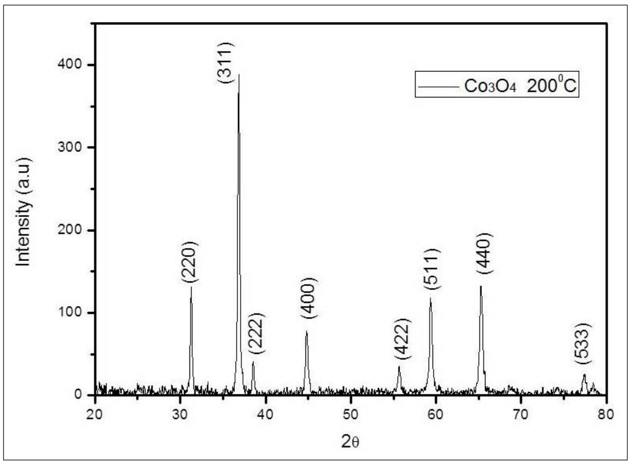

Fig 2 XRD Pattern of green synthesized Co_3O_4 Nanoparticles annealed at 200°C

Table 1 XRD analysis of CO_3O_4 Nanoparticles for ASP

2θ	θ	Cos θ	FWHM β	D = (0.94*0.15406) / βCosθ(nm)	hkl	δ $1/D^2$ $\times10^{-3}(nm^{-2})$
31.2364	15.6182	0.963077096	0.3346	25.74858623	220	1.5083190
36.8137	18.40685	0.948838267	0.2007	43.57132984	311	0.5267425
38.5205	19.26025	0.944030025	0.2676	32.84493927	222	0.9269644
44.7975	22.39875	0.924554347	0.2676	33.5368158	400	0.8891117
55.6526	27.8263	0.8843668	0.3346	28.04025845	422	1.2718502
59.3413	29.67065	0.868885201	0.3346	28.53987341	511	1.2277103
65.2147	32.60735	0.842383276	0.3011	32.71296342	440	0.9344589
77.2282	38.6141	0.781366917	0.9792	10.84461042	533	8.5029982
		Average		29.47992211		

Table 2 XRD Analysis of CO_3O_4 Nanoparticles at 200°C

2θ	θ	Cos θ	FWHM β	D = (0.94*0.15406) / βCosθ(nm)	hkl	δ $1/D^2$ $\times10^{-3}(nm^{-2})$
31.2869	15.64345	0.962958356	0.184	47.4308708	220	0.444506179
36.8387	18.41935	0.948769356	0.2175	40.7255086	311	0.6029301
38.5396	19.2698	0.943975031	0.2676	33.2690049	222	0.903483815
44.8239	22.41195	0.924466535	0.2676	33.971062	400	0.866526307
55.6791	27.83955	0.884258828	0.3346	28.4041035	422	1.239475157
59.3808	29.6904	0.868714517	0.2676	36.1512434	511	0.765162225
65.2741	32.63705	0.842103828	0.3346	29.8259887	440	1.124113855
77.3958	38.6979	0.780453323	0.4896	21.9936892	533	2.067301555
		Average		33.9714339		

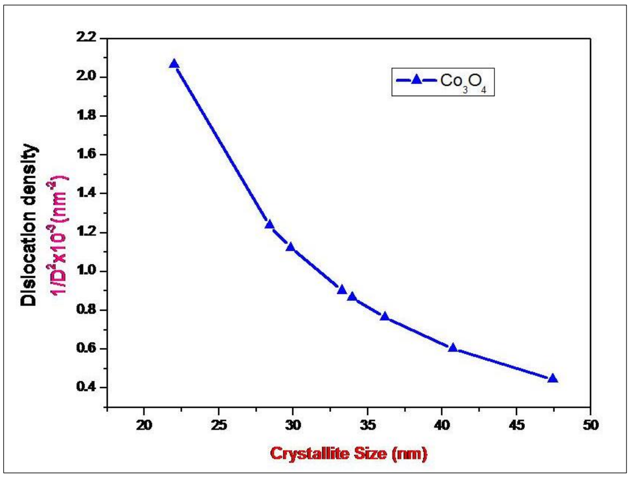

Fig 3 Dislocation density of Co_3O_4 Nanoparticles for the ASP sample

TEM and SAED analysis

(Fig 4a-b) show the TEM images of green synthesized Co_3O_4 NPs annealed at 200°C. TEM with SAED characterization can be used to give the inter relation between the crystalline characteristics and size and shape of the synthesized nanoparticles [24]. TEM picture as shown in the (Fig 4a) explains that the green synthesized Co_3O_4 NPs were agglomerated due to zener pinning effect for that reason they are closely to each other as well as nonuniform in shape and the particles size which is also in agreement with the result obtained from PXRD data. The crystalline orientations with prominent peaks for both samples are is interpreted and revealed by the Selected area electron diffraction SAED analysis and shown in the figure is represented by (Fig 4b). This pattern indicates the polycrystalline nature of the prepared Co_3O_4 NPs. Hence the particle nature and polycrystallinity which refers to the degree of structural order in different $2\boldsymbol{\theta}$ position of the samples in naocrystals confirmed that the Aerogel method entitles samples using Aero gel are interconnected and nonuniform random in shape which is resemble to the earlier stated articles [25].

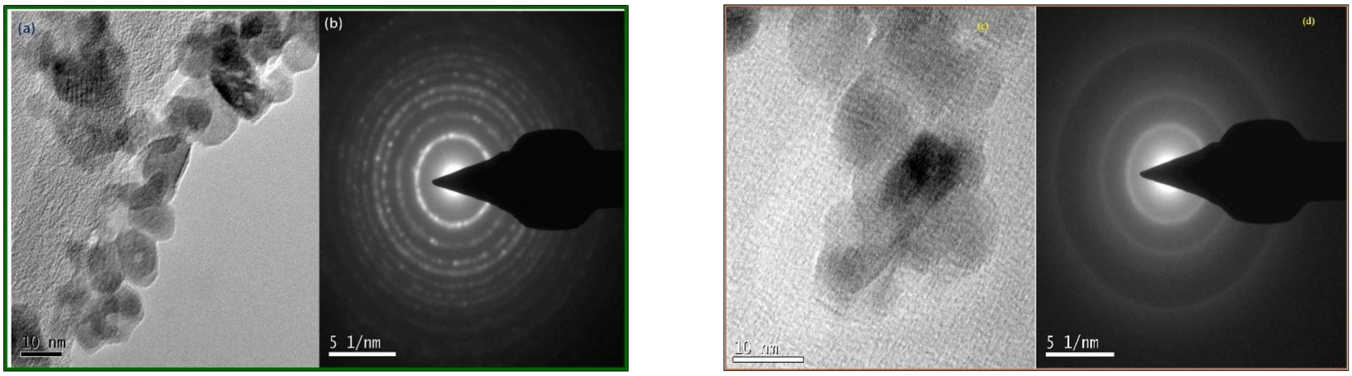

Fig 4(a) TEM image and (b) SAED pattern of synthesized Co_3O_4 Nanoparticles at ASP and (c) TEM image annealed at 200°C (d) SAED pattern annealed at 200°C

UV-Vis analysis

The response of UV-Vis interaction of nanoparticles is sensitive to surface morphology, grain size, surface to volume ratio and absorption coefficient. UV-Vis spectroscopy is an effective technique used for analyzing these materials optically [26]. (Fig 5a-b) represent the transmittance, (Fig 6a-b) absorbance spectra and (Fig 7a-b) energy band gap curve of the samples respectively. As per the plotted graph of the both spectrum, in the visible portions, where the sample does not absorb strongly, the transmittance is close to 100%. But in the case of the UV region, where the sample absorbs strongly, the transmittance drops to around 10% or less. The band gap of $Co_3O_4$the samples are expected to be 2.811 eV and 3.09 eV from the plots of the $(\alpha h\nu)^2$ versus photon energy ($h\nu$). As per the Tauc plot theory, Co_3O_4 NPs have strong absorption in the region 300 nm which involves the charge transfer transitions such that $O_2^{2-} \rightarrow Co^{2+}$ and $O_2^{2-} \rightarrow Co^{3+}$ [4]. According to the theory of energy band gap in semiconductors associated with nano-compounds, the absorbance in the vicinity of the onset due to the electronic transition is given by the following relationship:

$$\alpha = \frac{A(h\nu - E_g)^n}{h\nu} \quad -------- \quad (1)$$

Where, α is the absorption coefficient, A is a constant, Eg is the band gap, and ν is a value that depends on the transition (1/2 for a direct allowed transition or 2 for an indirect allowed transition). The band gap can be estimated in our case from a plot of $(\alpha h\nu)^2$ versus photon energy where the transition is direct allowed [27].

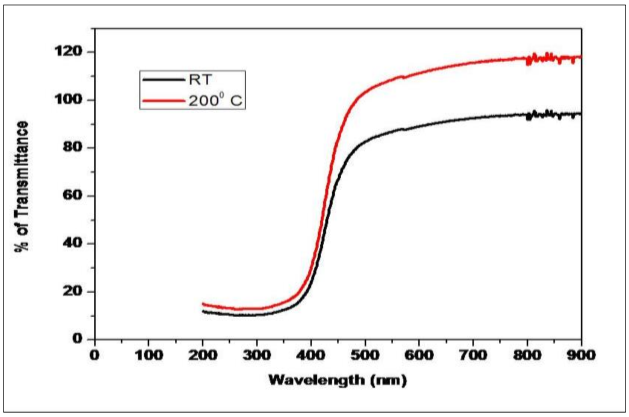

Fig 5(a-b) UV-Vis Transmittance Spectrum Co_3O_4 Nanoparticles at ASP and 200º C

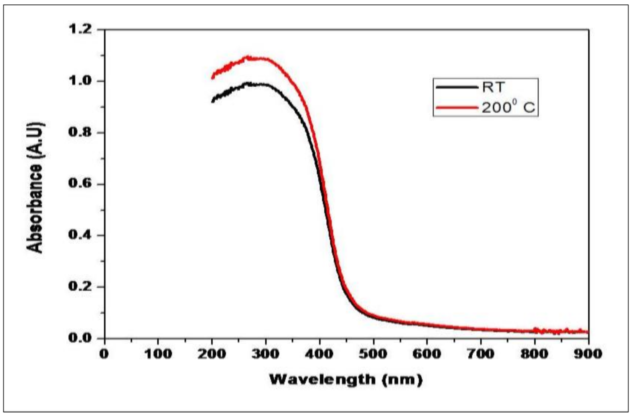

Fig 6(a-b) UV-Vis Absorbance Spectrum Co_3O_4 Nanoparticles at ASP and 200º C

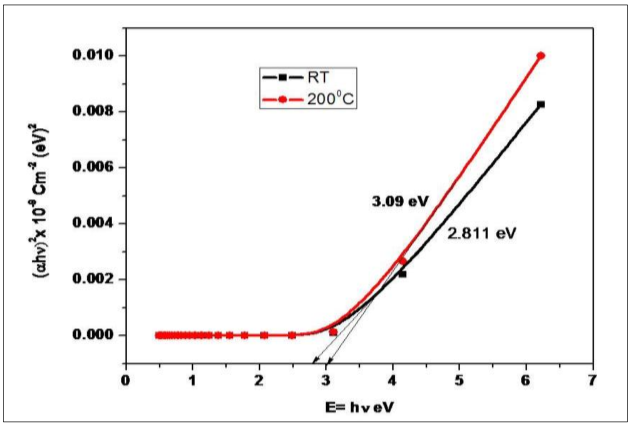

Fig 7(a-b) Energy band gaps of Co_3O_4 Nanoparticles at ASP and at 200º C

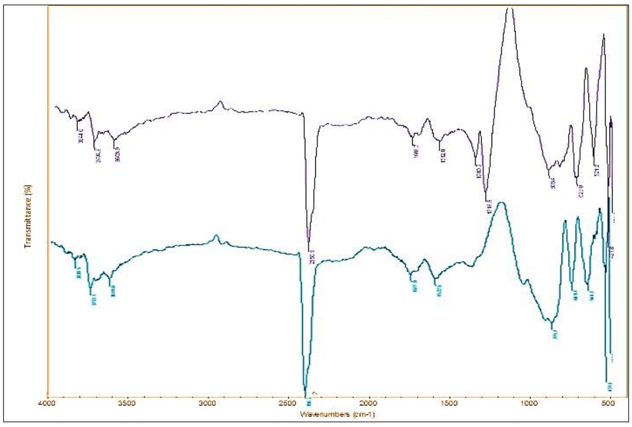

Fig 8(a-b) FTIR spectrum of Co_3O_4Nanoparticles of as prepared (ASP) and annealed at 200°C

FIIR analysis

FTIR spectra of CO_3O_4samples are shown in Figure 8 in the wave number range of 4000–400 cm^{-1}. The peaks at about 3844.6, 3734.9 and 3609.9 cm^{-1} are attributed to the stretching vibration of the O-H group of molecular water and hydrogen-bound O-H groups, n(O-H) [28]. The presence of CO_3^{2-} in the samples is evidenced by its vibration bands from middle to lower wavenumbers. The bands observed at 1512.8, 1216.3, 806.4, 628.8 and 521.2 cm^{-1} are assigned to the stretching vibration ν (OCO_2), ν (C O), $\delta(CO_3)$, δ(OCO), and ρ(OCO) [7]. The band at 512 cm^{-1} is ascribed to ρ_w(CoOH) vibration. In the FTIR spectrum of the sample (200° C), Figure 12 shows two distinctive bands at 649 and 544 are the characteristics of the Co-O stretching vibrations in Co_3O_4. In addition, the weak adsorptions at 3830.5, 3733.1, 3610.0 and 1677.9 cm^{-1} correspond to the bending and stretching vibration modes of absorbed water molecules on the surface [29].

Antibacterial activity

Cobalt oxide (CO_3O_4) nanoparticles were tested *In vitro* for their anti-bacterial activities against bacterial strains by the agar diffusion technique. Results of Co_3O_4 NPs' antibacterial activities are represented in (Table 5). Cobalt oxide nanoparticles produced good antibacterial activities against gram negative bacteria. The zone inhibition was observed with increasing the quantity of the sample such as 15mg to 25 mg shown in (Table 5) [30]. The maximum inhibition zone observed at 25mg of Co_3O_4 nanoparticles, (12mm) was found against *Escherichia coli* and (18mm) against *Pseudomonas aeruginosa* bacterium. So based upon the structural characteristics of cell walls of bacteria, it is assumed that an enhanced permeability and hence greater activity of Co_3O_4 for gram negative bacteria. Highly reactive oxygen species (ROS) such as hydrogen peroxide formed in the presence of metallic ions, result in a substantial damage to bacterial DNA, cell membrane and cause protein disinfection. The cobalt ions interact with these groups of bacterial enzymes, causes such results indicate the potential of these NPs against human pathogens inactivation and lead to death [31]. For control zone, streptomycin is used.

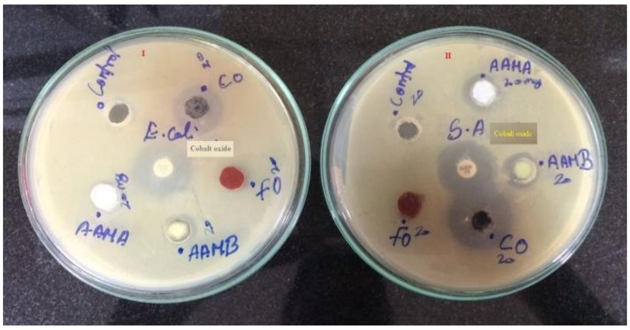

Fig 9 Screening of antibacterial activity by agar well diffusion method for the ASP sample

Table 5 Zone of inhibition of synthesized Co_3O_4 Nanoparticles ASP sample

Bacteria	Zone of Inhibition (mm)					
	5mg	10mg	15mg	20 mg	25mg	Control
Escherichia coli (E. coli) (G-)	4	11	10	11	12	16
Pseudomonas aeruginosa (G-)	12	16	15	17	18	17

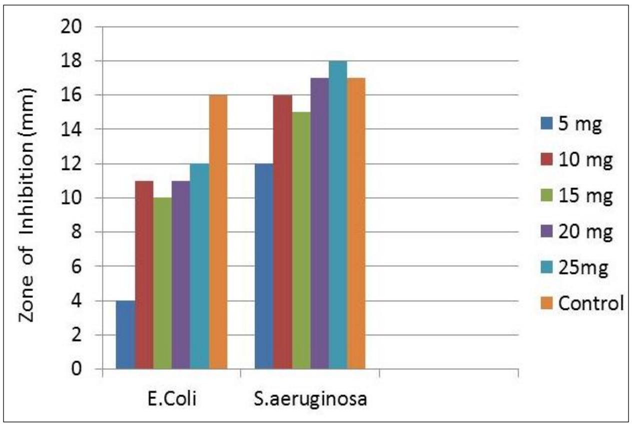

Fig 10 Zone of inhibition of green synthesized Co_3O_4 nanoparticles

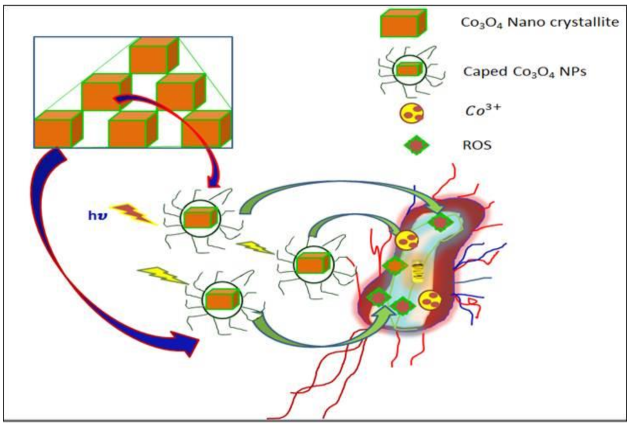

Fig 11 Antibacterial mechanisms of synthesized Co_3O_4 nanoparticles ASP sample

Gram-negative *Escherichia coli* (G-) *Pseudomonas aeruginosa* (G-) bacterial pathogens were used for antimicrobial activity. The inhibition functions of prepared samples towards G- were inferred by well diffusion method. (Fig 10) shows the photographic images which depict the inhibition zones formed around the well. As per the study, it is revealed that the size of the boundary of zone of inhibition is most considerable for green synthesized Co_3O_4 NPs samples particularly at high concentration 25mg/ml compared with other concentration were high zone of inhibition as observed in the tabulated list [32]. G- ZOI variation stated that the samples at higher concentration exhibits higher antibacterial efficiency towards reduction of number of bacteria. As shown in the described picture of mechanism of antibacterial activity (Fig 11), having 4 crucial stages to cross over to complete inhibitions is proposed clearly as follows 1. Production of ROS reactive oxygen species 2. Generations of super ion entities 3. Delivering of Co^{3+} ions from the nano crystallites 4. Grain shape and size. Whenever the photos interact with sample, optimum energy to be falls during the culture development where electron hole pair formation caused and these excitons are predecessors of reactive oxygen species ejection themselves [33]. The involvement of external photon with samples offers the considerable oxidative rigidity strain than critical strain in the cell walls since the interaction of super ROS ions can be damaged the walls of external surface, nucleus, membrane, proteins and DNA successively. Some SR (super-radicals) are also trying to make sticking on the cell walls and produce the large oxidative stress on it for making aperture in micrometer. Both mechanisms ROS is essential key in the function of inhibitions.

CONCLUSION

Cobalt oxide nanoparticles were synthesized by Aerogel method by Cobalt (III) chloride using Egg white natural aerogel. The prepared nanoparticles, were analyzed by various techniques such as PXRD, TEM with SAED, UV-Vis, FTIR and applied for antibacterial activities. This technique revealed the successful synthesis of cobalt oxide nanoparticles. The structure of the nanoparticles is well matched with JCPDS card 42-1467. The UV analysis reveals that this particle most suitable for some applications such as photocatalytic activity. FTIR analysis confirms the presence of Co-O stretching at finger print region and O-H groups at higher wave numbers with weak absorption. Antibacterial activities of synthesized cobalt oxide nanoparticles were analyzed against gram negative bacteria and it was found that by increasing concentration of cobalt oxide nanoparticles, antibacterial activity was increased. Therefore, the green synthesis of the nanoparticles is very useful in biological potential applications such as antibacterial activity and it can be expanded this into anticancer activity. The present finding concludes that antibiotic studies will helpful for developing a drug in future.

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