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Biosynthesis and Characterization of Co_3O_4NPs Utilizing Prickly Pear Fruit Extract and its Biological Activities

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Abstract: In the current research, there is a low level of research and information about the interaction of cobalt oxide nanoparticles (Co_3O_4NPs) in biological systems. This research creates a very simple and cost-effective preparation of cobalt oxide nanoparticles by using prickly pear fruit extract as a reducing agent, which may be further used for biological applications like antimicrobial, antioxidant, DNA interaction and in-vitro anticancer activity. The use of prickly pear fruit extract acts as a good reducing agent and is responsible for easy preparation and reducing the toxicity of cobalt oxide nanoparticles. The fabricated biogenic nanoparticles were confirmed by microscopic and spectroscopic analytical techniques like Ultra Violet-Visible spectrometer, Fourier transforms infrared spectrometer (FTIR), X-ray Diffraction Method (XRD), Energy-dispersive X-ray spectroscopy (EDS), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM). The average size of the synthesized nanoparticles is 36.24 nm. In the MTT assay, the prepared cobalt oxide NPs haspotential mechanisms of cytotoxicity and in-vitro anticancer activity in Hepatocellular carcinoma cancer cells (HepG2). The microbial activities like antibacterial and antifungal studies of the biosynthesized nanoparticles were performed by the Disc method. The Co_3O_4NPs with DNA interaction were examined by UV-Visible and fluorescence spectroscopic methods. The binding constant value of biogenic Co_3O_4NPs with CT-DNA was observed by UV-Visible spectroscopy with a result of $2.57x10^5 mol^{-1}$. The binding parameters and quenching constants were observed by fluorescence spectroscopic methods having values of $Ksv=7.1x10^3$, $k_q=7.1x10^8$, $K_a=3.47.1x10^5$, n=0.9119. From the findings, Co_3O_4NPs may be utilized as a medicinal aid for their antibacterial, antifungal, antioxidant, DNA binding and in-vitro anticancer activities.

Keywords: Cobalt oxide nanoparticles, prickly pear, antibacterial, antifungal, antioxidant, DNA binding, in-vitro anticancer activity.

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INTRODUCTION

The development of nanomaterials has a huge impact on the growth of different fields like biomedicine, medicine, engineering, pharmacy, etc. Cobalt is a vital metal of research interest, most often utilized in the preparation of nanoparticles for its biomedical applications like antibacterial, antifungal, antioxidant, anti-inflammatory, in-vivo and in-vitro anticancer studies. The biosynthesis reactions are performed by suitable precursors and they depend on various parameters like temperature, pressure, pH and solvent (1, 2). The biogenic process of nanomaterials using various parts of plant extracts has increased their potential results because of their effective phytochemicals such as aldehydes, flavonoids, phenolic compounds, ketones, carboxylic acids, ascorbic acids, terpenoids and amides (3–5). In the previous studies, various plants like Aspalathuslinearis (6), Azadirachtaindica (7), Calotropisgiganta (8), Calotropisprocera (9), Euphorbia heterophylla (10), Ginkgo biloba (11), Hibiscusrasa (12), Helianthusannus (13), Manihotesculenta (14), Moringaoleifera (15),

Neriumindicum (16), Pipernigrum (17), Punicagrantum (18, 19), Sageretiathea (20), Sechiumedule (21), Tamarind (22), and Taraxacum officinal (23) were used for nanoparticle synthesis. Phytochemicals can condense metal salts into metal nanoparticles. Among the nanomaterials, the metallic nanoparticles, which have antibacterial and antitumor properties, open new avenues to combat and prevent different types of tumors and other infectious diseases. Hence, research on nanomaterials in bacteria has become important and there is a topical increase in the challenging strains of microorganisms to the potent antibiotics and the vital role of bactericidal nanomaterials as potential anticancer agents (24). Its inexpensive nature, environment friendliness, and its considerable role in the synthesis and production of cobalt nanoparticles are in wide use in various sectors.

Cobalt oxide nanoparticles are common industrial nanoparticles that are utilized in various applications due to their cost-effectiveness, eco-friendly, larger surface area per unit and weight than their bulk counterpart materials. Nano-cobalt oxide is an important magnetic material because of its innate role in electrochemical and biological applications (25).The biosynthesized nanoparticles with plant extract are biocompatible and have low toxicity in the physicochemical process. The antibacterial effect can be assessed by two methods like disc diffusion method and growth curve analysis. The antibacterial activities of Co3O4NPs were investigated using gram-positive and gram-negative bacteria in the agar disc diffusion method (26). Previous literature reports discussed a comparative study of gram-positive and gram-negative bacterial strains and the nanoparticles were higher against gram-positive strain than gram-negative strain (27). The nanocomposite of these nanoparticles increases the antibacterial activity against the pathogenic strains (28, 29). The biogenic Co3O4NPs were examined by the biocompatibility and the toxicity of Co3O4NPs toward the cancerous cells and normal human cells and points out the green synthesis of metal oxide nanoparticles with numerous biological applications (30). Hence, the study investigates the biosynthesis and biological applications of Co3O4NPs for their potential effect on the HepG2 tumour cell lines and their lower cytotoxicity in normal cells.

Compared with the other chemical methods, phytochemical mediated nanoparticles have beneficial efficiency, biocompatibility and useful biological applications. Medicinal plants are used as an alternative source to synthesize the nanoparticles to satisfy the limitations of the biological field (31). Opuntia ficus-indica is the botanical name for the prickly pear. The fruit is grown widely and is easy to obtain on the outskirts of the Virudhunagar city, Tamil Nadu, India. The prickly pear fruits have a good source of nutrients and antioxidants with excellent health benefits, such as protective effects of the hepatoprotector, cardiovascular system, chemopreventive, anticancer, antiproliferative, and neuroprotective [32, 33]. The objectives of this study are (i) to synthesize Co3O4NPs from the stabilizing and reducing agents present in the aqueous extract of the prickly pear fruit. (ii) to find the characterization of the biosynthesized Co3O4NPs, and (iii) to evaluate their therapeutic properties such as antibacterial, antioxidant, DNA interaction, and anticancer activities under in-vitro conditions. Recently, the synthesis of copper oxide and silver nanoparticles using prickly pear fruit extract has been reported. According to past literature surveys, the fruit extract of this plant has not been used for the synthesis of Co3O4-NPs so far (34–36).

MATERIALS AND METHODS

Materials

Cobalt Nitrate hexahydrate (Co(NO3)2.6H2O) as a precursor was purchased from Merck, National Scientific Company, Madurai. Methyl thiazolyl diphenyl tetrazolium bromide (MTT), diphenyl picrylhydrazyl (DPPH), methanol, Dimethylsulfoxide (DMSO) and ethanol were purchased from Alpha lab supplies, Thermo Fisher Brand, Madurai, Tamil Nadu, India.

Prickly Pear collection and aqueous extract preparation

The sample fruits of prickly pear were collected from the outskirts of Virudhunagar city, Tamil Nadu and the collected fresh fruits were washed three to four times and cut into tiny pieces. Twenty-five grams of fruits were mixed with 300mL of water: ethanol (2:1, v:v) and was refluxed at 70°C for 4-6 hrs, utilizing the soxhlet apparatus. After the reflux, the extract of the chemical mixture was collected.

Green synthesis of Co3O4NPs

The prepared cobalt oxide nanoparticles (Co3O4NPs) were prepared with 40mL of 0.1M cobalt precursor solution in the estimated ratio of ethanol: water (2:3, v:v) and were stirred for 15 min and were slowly added 20mL of fruits extracts with continuous stirring for half an hour. Additionally, adding five drops of ammonia solution drop wise with continuous stirring for the next 2h, then the potion was relocated to a 100mL autoclave at 120 °C for 6–7 hrs. After getting the mixture was subdued to the room temperature, the precipitate was collected and washed several times using water and ethanol and centrifuged for 15 min. Finally, the precipitate was dehydrated at 90 °C for two hours and then calcinated at 400–500 for three hours. The schematic diagram of the nanoparticle synthesis is given in Figure 1.

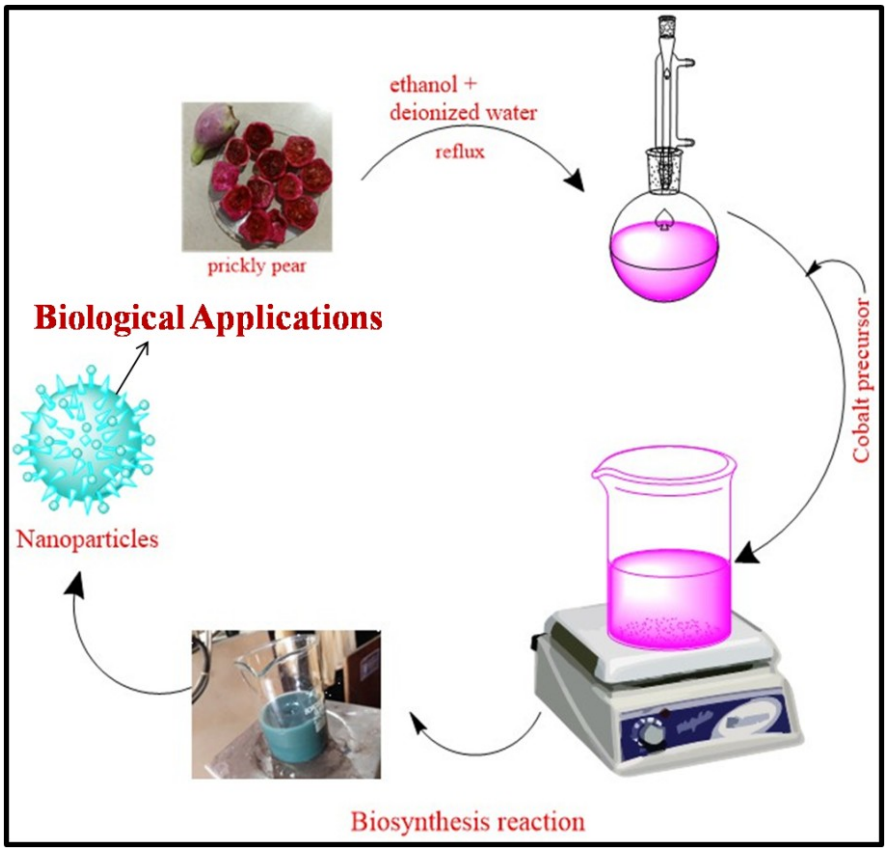

Figure 1: Preparation and mechanism of biogenic Cobalt oxide nanoparticles.

Physicochemical Characterization

The absorption properties of the biosynthesized Co3O4NPs and the fruit extract was examined by UV-Visible spectroscopic technique (UV-1601 Shimadzu spectrophotometer) using DMSO at a resolution of 1 nm, in the wavelength range of 400–800 nm. FT-IR measurement was done using FTIR8400S-spectrophotometer (Shimadzu, International, Co. Ltd, Tokyo, Japan) to determine the different types of chemical bonds between bioactive compounds of extract and cobalt oxide solution. Samples were scanned from 400–4000 cm-1 with potassium bromide pellets. The representative peaks of the cobalt oxide group with NP are expressed in a reciprocal wavelength (cm-1).The wide-angle X-ray diffraction (XRD, Bruker AXS D8) spectra were measured on a powder diffractometer with nickel-filtered Cu Ka, X-ray beam (l= 0.15418 nm).The morphology and the particle size of the nanoparticles were investigated by Scanning Electron microscope and Transmission Electron Microscope. The images were observed with the help of an electron microscope (VEGA3SB, TESCAN, and Czech). Energy Dispersive X-ray (EDS) spectroscopy (a part of SEM which was done using Quantax 200 with X Flash® 6130) was used for detecting the arrangement of elements in the sample. For the Transmission Electron Microscope (TEM) (Model: JEM-2010, JEOL, Japan) analysis, diluted and dispersed solutions of cobalt oxide nanoparticles were dropped onto a copper grid (~200 mesh), dried and observed at 200KV.

Biological Study

The antimicrobial activities of cobalt oxide nanoparticles were tested against bacterial species like three gram-negative (Staphylococcus aureus, Proteus vulgaris, Salmonella typhimurium) & two gram-positive (Escherichia coli, Bacillus subtilis) and fungal activity of these nanoparticles against fungi like Aspergillus flavus, Aspergillus niger, Candida albicans, Culvularia lunata and Rhizoctonia bataicolaby Well diffusion method. At room temperature, DNA interaction of nanoparticles in the presence or absence of DNA in Tris-HCl/NaCl (5 mM/50 mM) buffer solution at pH 7.4 was investigated by the use of the electronic absorption spectral titration method. The competitive binding of nanoparticles with ethidium bromide bound DNA in Tris-HCl buffer solution at pH 7.4 was investigated by fluorescence techniques (37, 38).The antioxidant activities of the nanoparticles were gauged by 2,2′-Diphenyl-1-picrylhydrazil (DPPH) method. The experiment was conducted using Visible spectrophotometer (39, 40). The Hepatocellular carcinoma cancer cells (HepG2) were purchased from National Centre for Cell Science, Pune, India. The procured HepG2 were incubated in an RPMI 1640 medium that was supplemented with L-glutamicin, 10% bovine serum, 100 U of penicillin, and 1 mg/mL of streptomycin. The cells were supplied with a fresh complete medium after four days of incubation in 5% CO2, 95% air, and 37°C environments. The HepG2 cell lines were also grown

in Dulbecco's Modified Eagle's Medium supplemented with sodium pyruvate and other components as mentioned earlier and incubated under similar growth conditions. All the reagents and growth media were purchased from Himedia, India. The cells were seeded at a density of 1x103 cells per well in 96-well plates and left overnight under similar growth conditions as mentioned previously. It was then treated at concentration levels of 50, and 100µg/mL of the tested compounds. The cells were treated with 10% MTT solution and incubated for 3 to 4 h at 37°C after 24 h of incubation. The spectrophotometric readingswere taken at wavelengths of 650 nm using a Multiskan EX instrument (Thermo Scientific, USA). The percentage of the viable cells was estimated using the following formula after subtracting the absorbance value at 650 nm (41).

Phytochemical Screening Tests

The phytochemicals of the fruit extract were analyzed with the help of available qualitative analysis. Wagner's test (alkaloids): 2 mL extract was added with some drops of Wagner's reagent. The alkaloid presence was calculated by the formation of the reddish-brown precipitate. Shinoda test (flavonoids): 2 mL extract was added to a pinch of magnesium, then added with 1–2 drops of concentrated HCl. The presence of flavonoids in the solution was confirmed by the formation of pink color. Lead acetate test (phenol and tannins): 2 mL extract was added with 0.5 mL of (1%) lead acetate solution and the presence of tannins and phenolic compounds was confirmed by the precipitate formation (42).

RESULTS AND DISCUSSION

Characterization of Co3O4NPs

UV –Vis Analysis

UV-Visible analysis is a common analytical technique employed to estimate the absorbance of nanoparticles. Figure 2 represents the UV spectrum of biosynthesizedcobalt oxide nanoparticles using prickly pear fruit extract. The peak at 513 nm is due to the inter-band transition of electrons in cobalt metalcores. The UV spectrum of the aqueous fruitextract did not show the type of excitation for these nanoparticlesin this region. The synthesis of aqueous fruit extracts mediatednanoparticles was validated by visual observation using prickly pear fruit extract and the reaction mixture from pink toblack showed the formation of cobalt oxide nanoparticles in Figure 2. Inthe UV spectrum, peaks at 536 nm and 513 nm are due to the absorptionof prickly pear fruit extract and cobalt oxide. The band of surface plasmon absorption at a maximum wavelength of 513 nm indicates the formation of CNPssimilar to the reports of previous literature. Cobalt oxide Nanoparticles formed by cobalt nitrate precursor with a mixture of prickly pear fruit extract act as reducing agents (43, 44). Figure 2 shows the clear UV Spectrum of prickly pear fruit extract-mediated cobalt oxide nanoparticles (45).

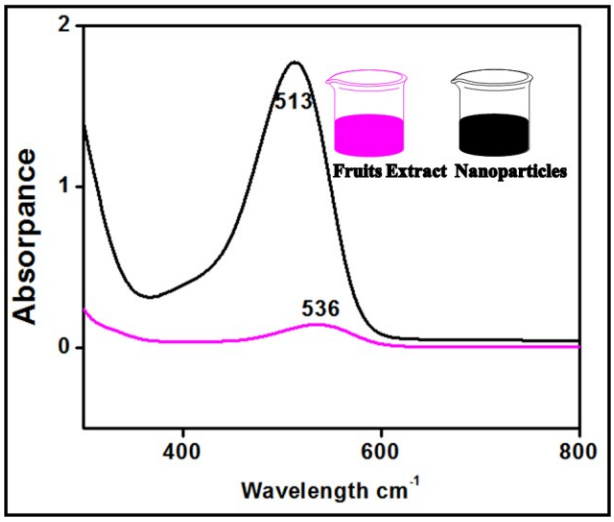

Figure 2: UV Spectrum of Prickly Pear fruit extract and Cobalt Oxide Nanoparticles.

Infrared Spectroscopic Analysis

The chemical composition and functional groups of these nanoparticles are determined by FTIR analysis. Figure 3 shows the FTIR spectrum of the prepared Co3O4NPs. The vibrational properties of Co3O4NPs were studied using Fourier Transform Infra-Red spectra and are in the range of 0 to 4000 cm-1 as pointed in Figure 3. The observed peaks are at 566cm-1 and 667cm1 and are represented with Co-O, O-Co-O stretching frequency that confirmed the configuration of Co3O4. Consequently, Co3+ located at the octahedral site is characterized by a peak at 566cm-1 and Co2+ located at the tetrahedral site is characterized by a peak at 667cm-1 respectively. The broadband at about 3450 cm−1 is assigned to the adsorbed water (46–48).

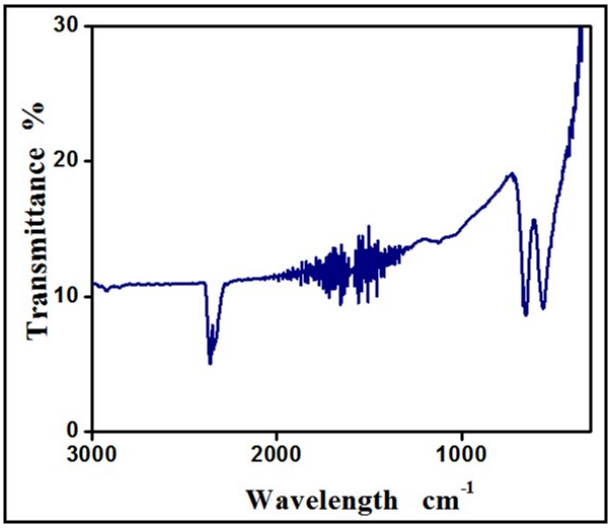

Figure 3: FTIR Spectrum of Co_3O_4NPs

XRD Analysis

The crystalline nature of the cobalt oxide nanoparticles was evaluated by X-ray diffraction analysis. In Figure 4, the XRD pattern of Co3O4

nanoparticles exhibits diffraction peaks with 2θ values of 30.84°, 36.31°, 37.99°, 44.16°, 54.89°, 58.53°, and 64.33° that are allocated to 220, 311, 222, 400, 422, 511, and 440 crystal planes of the crystalline Co3O4 phase correspondingly. These peaks are indexed to a pure cubic phase structure (JCPDS Card No. 80–1540). The average crystalline size of the nanoparticles was calculated using the Scherrer equation concerning the peaks(12).The average size of th synthesized Co3O4 nanoparticles was 36.24 nm.

Morphological Analysis

The size, shape, structure, morphology and microimaging of cobalt oxide nanoparticles were evaluated with the help of SEM and TEM analysis. Figure 5 shows SEM and TEM images of the prepared Co3O4NPs. Previous reports indicate that an increase in temperature up to 80°C forces the particles to agglomerate when nitrate was used as a counter ion of cobalt (49). The prepared Co3O4 nanoparticles showed a cubic spinel structure with a porous network. The pure nanosized crystal particles had an average size of 36.24 nm. Elemental analysis of cobalt oxide nanoparticles was performed using EDS techniques. Elements such as Co and O identified by EDS indicates the high purity of the prepared Co3O4 nanomaterial as shown in Figure 5. The chemical composition results of Co3O4 by XRD and EDS analysis are in excellent agreement. The atomic percentage of Co & O is 63.76 and 36.24 respectively and it is close to the abstract ratio (3:4) of cobalt oxide.

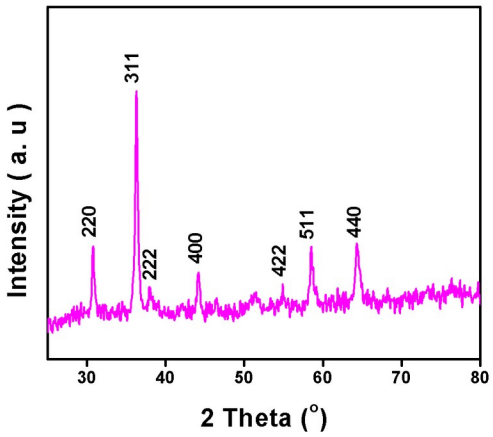

Figure 4: XRD Spectrum of Co3O4NPs

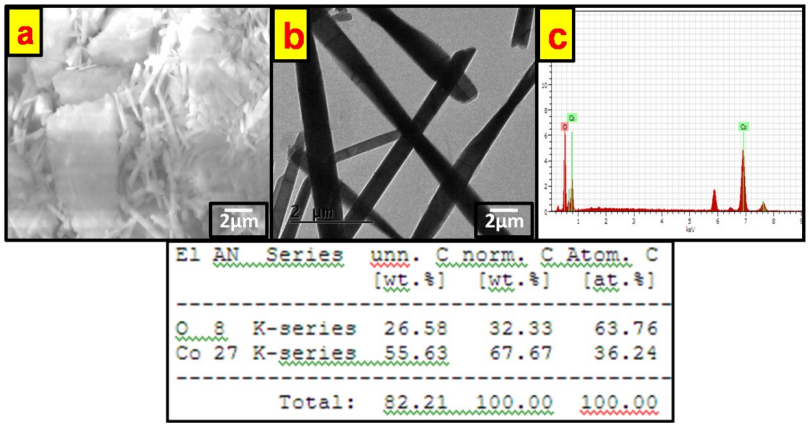

Figure 5: SEM, TEM& EDS Spectrum of Co3O4NPs.

Biological Applications of Co3O4NPs

Antimicrobial activity

The synthesized Co3O4NPs were estimated for their antimicrobial activity upon selected bacteria and fungi. The bacterial activity of these nanoparticles against three-gram negative (Staphylococcus aureus, Proteus vulgaris, Salmonella typhimurium) and two-gram positive (Escherichia coli, Bacillus subtilis) strains of bacteria was investigated. The minimum inhibition concentration (MIC) value of the synthesized cobalt oxide nanoparticles was summarized and compared to the control drug ciprofloxacin. The fungal activity of these nanoparticles against fungi like Aspergillus niger, Aspergillus flavus, Culvularia lunata, Rhizoctonia bataicola and Candida albicans was also investigated. In such treatments, fluconazol is the standard drug for treating fungi. The antimicrobial action of metal oxide nanoparticles (Co3O4NPs) shows potential antibacterial and antifungal activities at MIC=2.7–6.1µg/mL and MIC=3.1–6.5 µg/mL respectively as shown in Table 1. This analysis of antimicrobial activity data points out the biosynthesized nanoparticles that exhibit higher antibacterial and antifungal activities and their activities are very close to that of the control drugs.

Antioxidant activity

The antioxidant activity of the biosynthesized Co3O4NPs was analyzed and estimated using a radical scavenging assay (RSA) free from diphenylpicrylhydrazyl (DPPH) with spectrophotometric methods. The capacity of DPPH free radical scavenging was estimated using a free radical scavenging assay in different concentrations. The concentration of Co3O4NPs was mixed with 180 µL of diphenylpicrylhydrazyl substance and incubated for up to 30min in a dark place. After that, the absorbance peak at 518 nm was recorded and

the free radical scavenging of samples are calculated using the formula.

$$\text{Inhibition percentage of free radical scavenging} = \frac{\text{Absorbance of control}\,(A°) - \text{Absorbance of sample}\,(A)}{\text{Absorbance of control}\,(A°)}$$

When the Co3O4NPs concentration increases, it induces antioxidant activity. Figure 6 shows diphenylpicrylhydrazyl radical scavenging of a maximum of 59.9% at 450 mg/mL and minimum of 26.8% at 50 mg/mL. The results prove that are similar to the past research works (50–53).

Table 1: MIC value of the synthesized cobalt oxide nanoparticles against five bacterial growths (µg/mL).

Bacteria	*Staphylococcus aureus*	*Proteus vulgaris*	*Escherichia coli*	*Bacillus subtilis*	*Salmonella typhimurium*
cobalt oxide nanoparticles	2.7	3.5	4.1	5.8	5.6
ciprofloxacin	1.7	1.9	2.0	1.8	2.4

Fungi	*Aspergillus niger*	*Aspergillus flavus*	*Culvularia lunata*	*Rhizoctonia bataicola*	*Candida albicans*
cobalt oxide nanoparticles	3.1	4.2	4.8	5.8	6.5
Fluconazol	1.0	1.3	1.2	1.1	1.6

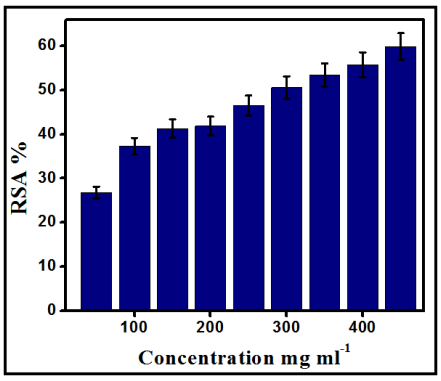

Figure 6: Antioxidant activity of cobalt oxide nanoparticles.

DNA Interaction / Binding Studies

The development of DNA technologies using functional nucleic acids is increasingly attractive with metal ions, proteins, small molecules, and cells (54, 55). The interfacing DNA with nanoparticle probes was based on the surface plasmon resonance, magnetic resonance signal, and fluorescence quenching (56, 57). DNA absorption in nanoparticles is robustly influenced by nucleotide properties. The first property is charge changeability where the nucleobases are uncharged when the pH value is 4–9. If the pH is lower than 4, cytosine and adenine become protonated and if the pH is higher than 9, thymine and guanine become deprotonated because of monophosphate nucleotides losing their protons (58). The second property is hydrophobicity, the rank of hydrophobicity in nucleotide unit is of nitrogenous base> deoxyribose> phosphate group. The third property is absorption ability, DNA can interact with metal ions via chemisorptions (59). Nucleic acids with nanoparticles have great potential for nanoelectronics and nanomedicine (60).

The binding mechanism of DNA with nanoparticles and the interaction studies is usually carried out by different methods like electronic absorption spectroscopy, spectrofluorescence, colorimetry, and viscometry (61). This research work has utilized the first two methods to find the interaction of DNA with the use of Co3O4 nanoparticles. In interaction studies, various types of interactions present in nanoparticles in DNA are more important. There are four types of interaction processes such as groove binding, electrostatic binding, hydrophobic interactions (intercalation binding), and hydrogen bonding interaction. The chemical interaction of nanoparticles with DNA is intercalation binding, major and minor grooves of DNA interaction is groove binding, and electrically charged species of DNA interaction is electrostatic binding (62, 63).

Electronic Absorption Spectroscopy

The electronic absorption spectroscopy method is the most common for finding nanoparticle interactions with DNA using the 260–300 nm range of tris HCl/NaCl buffer solution of CT-DNA. The absorption spectra were obtained and the constant

nanoparticle concentration with increased DNA concentration (64–66). Figure 7 a illustrates the hyperchromic shift of the absorption spectrum that was observed with the electrostatic intercalation binding present in DNA with Co3O4 nanoparticles. Table 2 lists the calculated details of the binding constant and percentage of chromism. The binding constant of DNA with Co3O4NPs is 2.57x103 and 21.42% of hyperchromism.

Spectrofluorometric Methods

The competitive binding or fluorescence studies of DNA with Co3O4NPs were analyzed with the use of a spectrofluorometer. Figure 7 b demonstrates the fluorescence behavior of ethidium bromide (EB) in DNA that increases the intercalative interaction of DNA. Then, EB fluorescence intensity is quenched by the addition of nanoparticles owing to the replacement of EB with NPs. The quenching constant is estimated by the utilization of the Stern-Volmer equation.

$$F_0 / F = 1 + K_{sv} [Q]$$

In the equation, F_0 is the absence of quencher fluorescence intensity, F is the presence of quencher fluorescence intensity, Stern-Volmer quenching constant is K_{sv} and [Q] represents the concentration of the quencher of Co3O4NPs (14). Ksv values of Co3O4NPs are $7.1x10^3$. The quenching constant values obtained from the Co3O4NPs indicated beneficial CT-DNA binding activity (67–69).

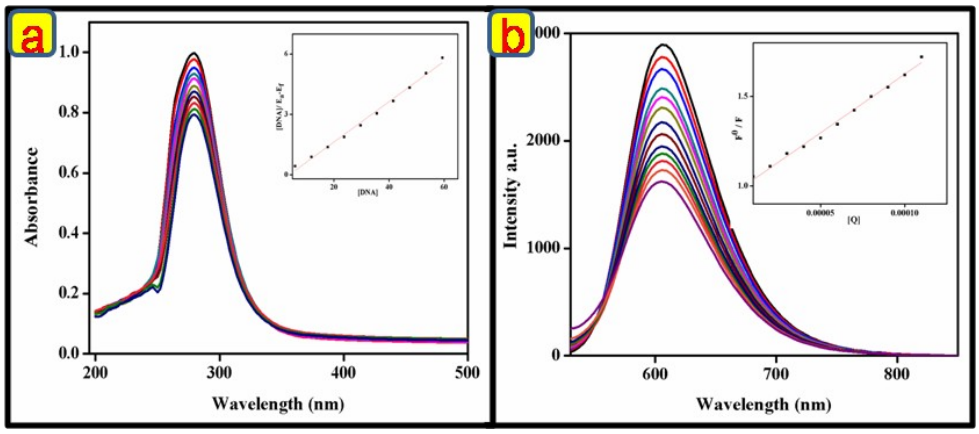

Figure 7: DNA interaction of cobalt oxide nanoparticles a) electronic absorption spectroscopy b) spectrofluorescence spectroscopy.

Table 2: Absorption spectral data and binding parameters of cobalt oxide nanoparticles.

λ_{max} (nm)		Δλ (nm)	% chromism	K_b (m^{-1})	K_{sv} (L mol^{-1})	K_Q (L mol^{-1} S^{-1})	K_a (L mol^{-1})	n
free	bound							
278	265	13.0	21.42	$2.57x10^3$	$7.1x10^3$	$7.1x10^8$	$3.47.1x10^5$	0.9119

Cytotoxicity Assay/Cell Viability

The cytotoxicity of the nanoparticles was calculated using colorimetric (MTT) assay using dimethylthiazol diphenyltetrazolium bromide with hepatocellular carcinoma cell lines, and the anticancer activity of nanoparticles was evaluated at IC50 against cancer cell lines as shown in figure 8. Drug concentration of IC50 value inhibits 50% cellular growth in 48 h of exposure to the drug. An average of three replicates from two determinations were acquired as result. The results specify that green synthesized Co3O4NPs have less toxicity and good in-vitro anticancer activity than the standard drug used for cancer applications (70). The cell viability value of the standard drug was 64.3%. The cell viability value of the Co3O4NPs at 50 µg mL-1 was 60.59% and the Co3O4NPs at 100 µg mL-1 was 48.41% respectively. This research work demonstrates the phenomenal anticancer activity of the Co3O4 nanoparticles with an IC50 value of (70.17%). The results-oriented to the past research works indicate that Co3O4NPs are biocompatible and have potential in-vitro anticancer activity (71).

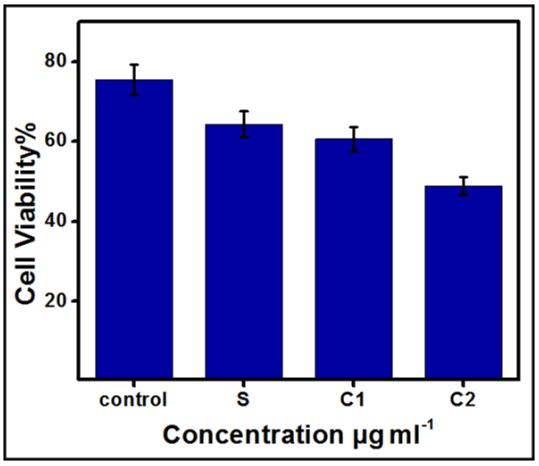

Figure 8: Cell Viability, S- standard drug (cisplatin), C1- cell treated Co3O4NPs at 50 µg mL-1, C2- cell treated Co3O4NPs at 100 µg mL-1

In-vitro Anticancer Activity

The in-vitro anticancer study of Co3O4NPs was evaluated by treating hepatocellular carcinoma cell staining method. The use of Co3O4NPs demonstrates early apoptotic presence with late apoptotic cells with fragment apoptotic bodies. The nuclear staining results indicate the stimulation of apoptosis continued by necrosis in hepatocellular carcinoma cells by cobalt oxide nanoparticles (72–75). The control group of untreated HepG2 cells showed full spherical shaped and homogeneous pink nuclear staining. While the cells treated with cobalt oxide nanoparticles (50 µg mL-1) indicated an irregular shape nucleus, cell shrinkage and scattering of nuclear granules. It suggested the indication of nuclear fragmentation (figure 9). Hence, the treatment of Co3O4NPs induces cell death in HepG2 cells. The observations indicate that synthesized Co3O4NPs have favorable in-vitro anti-cancer activity on Hepatocellular carcinoma cancer cells.

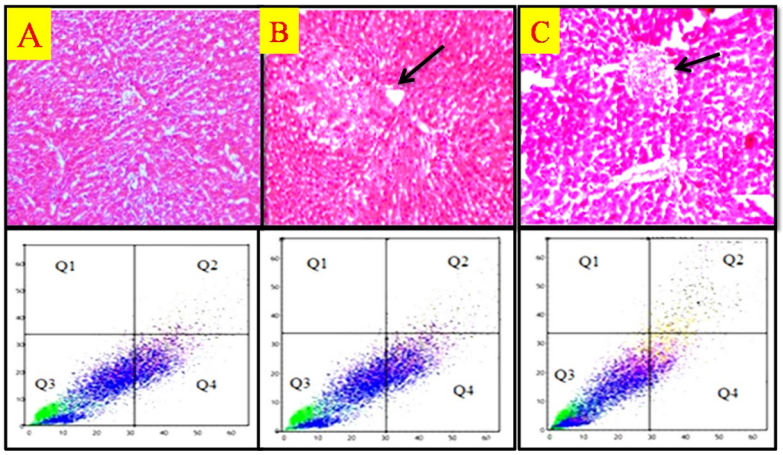

Figure 9: Detection of in-vitro anticancer activity in Hepatocellular carcinoma cells A. Control, B. treated with standard drug, C. cells treated with cobalt oxide nanoparticles (Co3O4NPs). Dot plots indicate increases in early and late apoptosis in hepatocellular carcinoma cell lines, respectively.

CONCLUSION

This study developed a green synthesis of Co3O4NPs utilizing prickly pear fruit extract to decrease the toxicity that arises while biological applications. This research successfully fabricated cobalt oxide nanoparticles Co3O4NPs and characterized them using various techniques and methods like UV, FTIR, XRD, SEM, EDX and TEM. The UV-Visible analysis shows the optical density and absorbance of Co3O4 NPs formed from cobalt nitrate precursor in addition to prickly pear fruit extract. FTIR analysis proves the functional group of cobalt oxide nanoparticles by the peak at 566cm-1 for the octahedral site and the peak at 667cm-1 for the tetrahedral site, which is confirmed by the formation of cobalt oxide nanoparticles. The crystal structure and crystalline nature of cobalt oxide (Co3O4) nanoparticles were confirmed by X-ray diffraction analysis and the major diffraction peak values of crystalline Co3O4 are30.84°, 36.31°, 44.16°, 64.33° assigned to (220), (311), (400), (440). These peaks were indexed to pure cubic phase structure and the average particle size (36.24nm), and lattice parameter values of cobalt oxide nanoparticles were also determined using XRD analysis. The SEM and TEM images showed the surface morphology and particle size of the Co3O4NPs. EDS indicates the purity and chemical composition of Co3O4 nanoparticles, exhibiting the atomic percentage of Co & O is 63.76% and 36.24%. Antimicrobial activity data indicated that the biosynthesized nanoparticles exhibit higher antifungal and antibacterial activities and are potential like the control drugs. The maximum and minimum mean bacterial activity of Co3O4 nanoparticles was found for A.niger (3.1) and S.aureus (2.7) (73).The maximum and minimum mean fungal activity of Co3O4 nanoparticles was found for B.subtilis (5.8) and C.albicans (6.5). In DPPH assay findings, Co3O4 NPs concentration

increased antioxidant activities also increased. The maximum and minimum radical scavenging activity of these nanoparticles is 59.9% at 450mg/mL and 26.8% at 50mg/mL. Anticancer activity and cytotoxicity assay affirm nuclear disintegration and cancerous cell death by the potential effect of the Co3O4 nanoparticles. The administration of Co3O4 NPs demonstrates the early presence of apoptosis and late apoptotic cells with fragment apoptotic bodies. In DNA binding, electronic absorption spectroscopy, and spectrofluorescence yielded positive results. The intrinsic binding constant of DNA with Co3O4 NPs is 2.57x103 and 21.42% of hyperchromism. Hence, the results show that Co3O4 NPs may be used as an excellent nanomaterial to administer HepG2 cancerous cell lines for its antimicrobial, antioxidant, DNA interaction properties.

CONFLICT OF INTEREST

There is no conflict of interest.

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