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RESEARCH ARTICLE

Green synthesis of Cobalt Oxide nanoparticles with *in-vitro* cytotoxicity assessment using pomegranate (*Punica granatum* L.) seed oil: A promising approach for antimicrobial and anticancer applications

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Abstract

Green synthesis of nanoparticles and their pharmacological implementation have gained importance in the field of nanotechnology. This study primarily aims to explore the use of *Punica granatum* L. seed oil as a reducing agent for the synthesis of cobalt nanoparticles, making it both economically and pharmacologically valuable. Gas chromatography-mass spectroscopy analysis was carried out to study the active metabolites present in *P. granatum* seed oil. The green synthesis of cobalt nanoparticles was established based on the color change of the reaction mixture from dark green to light green. These particles showed a λ_{max} at 279.88 nm for UV-visible spectrometry analysis. Furthermore, X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Field Emission Scanning Electron Microscope (FE SEM) and Dynamic Light Scattering (DLS) were performed to confirm the nature of these nanoparticles. The pharmacological potential of these cobalt oxide nanoparticles was tested against microbial pathogens. The results suggest that these nanoparticles exhibited significant activity against various human bacterial and fungal pathogens. Additionally, in *in vitro* cytotoxicity analysis, demonstrated that CoONPs selectively targeted MCF-7 cancer cells with a significant IC_{50} value compared to non-cancerous cells (L929). In conclusion, this study demonstrated that green synthesized CoONPs using *P. granatum* show significant potential against eukaryotic cancer cells and microbial pathogens. Furthermore, this study has implications for medical research centers and pharmaceutical industries in addressing modern challenges such as increasing antibiotic resistance in communities.

Keywords

green synthesis; *Punica granatum* seed oil; cobalt oxide nanoparticles; antimicrobial; anticancer cytotoxicity

Introduction

In 1959, American physicist Phillips Feynman established the nanotechnology in his lecture known as 'There's plenty of room at the bottom'. Professor Taniguchi from Tokyo University of Science introduced the term 'Nanotechnology' (1). Nanoparticles differ from other particles due to their unique physicochemical characteristics, such as their surface area to mass ratio, small size and high reactivity (2). Nanoparticles have a wide range of applications in fields such as chemical and biomedical industries, drug delivery, health care, food and feed and environmental sectors (3, 4).

The green synthesis method for nanoparticles play a crucial role in controlling the enzyme-reducing ability and biological molecules. This method has been proven by many researchers to be more effective, cost efficient, eco-friendly and easily to characterize (5, 6). Green synthesized nanoparticles are widely used in agriculture, such as nano-pesticides, nano-fertilizers, nano-herbicides and nano-coating. In drug delivery, metal nanoparticles like AuNPs exhibit a wide spectrum of optical and physicochemical properties, biocompatibility and flexibility making them excellent nano-carriers (7).

Cobalt-based nanoparticles have gained importance in synthetic biology due to their unique and promising nature. CoNPs are commonly used in energy storage systems, gas sensors, solar selective absorbers, lithium-ion batteries, field emission materials, capacitors, magnetic fluids, magnetic composites and electro-chromic thin films (8). Recently, an extract of *P. granatum* peel was utilized to synthesize cobalt nanoparticles, using cobalt nitrate hexahydrate as a precursor salt (9). *Aspalathus linearis* leaves have shown potential as a bio-reducing agent in the synthesis of Cobaltosic oxide (Co3O4) nanoparticle. CoNPs synthesized from *Ziziphora clinopodioides* and *Cardiospermum halicacabum* leaves extracts have exhibited antimicrobial and anticancer activity respectively (10, 11). With these applications, CoNPs have gained importance in the biomedical field for combating bacterial and fungal growth. CoNPs are considered bio-safe due to their minimal hemolytic activity (7, 12).

Punica granatum is a deciduous plant belonging to the family Lythraceae. It is well-known in traditional medicinal systems like Ayurveda for its medicinal properties. Researchers have proven its antioxidant, anticancer, anti-diabetic, anti-inflammatory and antimicrobial activities, making it suitable for pharmaceutical applications (13, 14). Pomegranate seeds exhibit nephroprotective activities and help prevent prostate cancer by inducing apoptosis and altering cell growth pathway (15, 16). The liquid extract of pomegranates seeds has shown antibacterial and antifungal properties against *Salmonella enteric* and *Penicillium* sps respectively (16). Pomegranate seed oil acts as an effective anticancer agent in the treatment of colon and breast cancer (17). The abundance of conjugated linoleic acid in pomegranate seed oil helps activate of TNF-α and suppress NF-κB making it effective against diabetes (18). Silver nanoparticles were synthesized from various parts of *P. granatum* such as leaves, peel, seed, juice and fruit extracts, exhibited antimicrobial, antioxidant and anticancer activity against different pathogens (19, 20). Similarly, iron oxide nanoparticles synthesized from leaf and peel extract of pomegranate have shown beneficial effects in wastewater treatment and solar hydrogen production respectively (21, 22).

Since there is no report available on the green synthesis of *P. granatum* seed oil, this study aims to fill the gap and promote green synthesis with different oil samples in the future. This study will help to enhance the economic and pharmacological significance of various oils in the realm of nanomedicine. The primary objective of this study is to highlight the applications of *P. granatum* seed oil in synthesizing CoNPs through the green synthesis approach, while assessing its pharmacological efficiency in terms of antimicrobial and anticancer studies.

Materials and Methods

1. Sample collection:

Pure pomegranate seed oil was purchased from ICAR-NRCP (Indian Council of Agricultural Research- National Research Centre on Pomegranate), Solapur, Maharashtra, India. The cold-pressed extraction method was used to extract the pomegranate seed oil.

2. Sample preparation for Gas Chromatography-Mass Spectrometry:

Samples for GC-MS analysis were prepared using pure pomegranate seed oil and modified methylation method. To prepare the sample, 0.5 mL of pure pomegranate seed oil was taken in a test tube. Then, 1 mL of methylation solution (acetyl chloride and methanol in a 1:20 ratio) was added. The test tube was covered with paraffin and aluminum foil and placed in a boiling bath for 5-10 min. After the solution cooled down, 2 mL of double distilled water and 1 mL of hexane were added. The solution was mixed using a vortex and the top layer of the sample was collected and sent for GC-MS analysis. The prepared sample was subjected to GC-MS using the SHIMADZU, JapanGCMS-QP2010SE instrument. Helium gas was used as the carrier gas. The received molecular spectrum band was compared with the NIST library database to identify active metabolites (23).

3. Synthesis of cobalt nanoparticle:

Pomegranate seed oil (5% *v/v*) was used as a reducing agent while a cobaltous chloride (concentration- 0.1 M) solution was used as a precursor agent in the green synthesis. Polyethylene glycol (PEG) was used as a steadying agent. The complete mixture was stirred constantly at a temperature of 45 °C for 1 h. After 1 h of reaction, the solution was centrifuged at 10000 rpm for 10 min. One wash with acetone and 2 washes with ethanol were performed to remove unwanted remnants from the solution. After washing, the remaining particles were collected in a glass petri-plate and dried at 100 °C in a hot air oven to obtain cobalt particles. The dried particles were dissolved in 10-15 mL double distilled water and filtered using a 0.5 µm pore size syringe filter. The filtrate was washed with ethanol 2 times and then centrifuged at 10000 rpm for 8 min. The final particles were dried at 100 ° C overnight in a hot air oven, resulting in purified cobalt particles.

4. Characterization of cobalt nanoparticle:

The synthesized particles were characterized using UV-spectroscopy, X-ray diffraction (XRD), Field Emission Scanning Electron Microscopy (FE SEM), Dynamic Light Scattering (DLS) and Fourier Transform Infrared Spectroscopy (FTIR). UV spectroscopy was performed

using the SHIMADZU UV-1800 model with a range of 190 nm to 1100 nm. X-ray diffraction patterns were obtained using the RIGAKU miniflex 600 XRD instrument (power-600W, Japan). Dynamic Light Scattering measurements were performed using the Zetasizer ZEN 3600 instrument from Malvern, Germany. This instrument can measure particles suspended in a liquid medium ranging from 0.3 nm to 10.0 µm. Fourier-Transform Infrared Spectroscopy analysis of the nanoparticles was conducted using the SHIMADZU FTIR spectrometer (Japan) and spectra band were measured from 4000 to 500 cm^{-1}. For Scanning Electron Microscopy was performed using the Apreo 2S model from Thermo Scientific.

4.1. Antimicrobial activity of cobalt nanoparticles:

Antibacterial activity: The agar-well diffusion technique was used to test the antibacterial activity of the green synthesized nanoparticles. *In vitro,* the antibacterial activity of CoONPs was tested using 5 different bacterial strains: *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 2146-01060P), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633). Chloramphenicol was used as a standard antibiotic to determine the antibacterial activity. A stock solution of chloroamphenicol with the concentration of 10 mg/mL was prepared and a working solution of 1 mg/0.1 mL was used. Different concentrations of green synthesized nanoparticles (20, 40, 60, 80 and 100 µg/mL) were used. The antibacterial activity was measured by determining the zone of inhibition in millimeters (mm) (24, 25). The results obtained from antibacterial studies were analyzed and standard deviation and standard error were calculated. The variation in the values is mentioned in Table 2.

Antifungal activity: The agar-well diffusion technique was used to perform the antifungal activity of the green synthesized nanoparticle. Five different fungal strains belonging to the genus *Candida* were used: *Candida tropicalis* (ATCC 10231), *Candida glabrata* (MTCC3019), *Candida parapsilosis* (ATCC 22019), *Candida albicans* (ATCC90028) and *Candida krusei* (ATCC 14243). Different concentrations of green synthesized nanoparticles (20, 40, 60, 80 and 100 µg/mL) were used. Fluconazole was used as a working solution with a concentration of 1 mg/0.1 mL prepared from a stock solution of 10 mg/mL. The inhibition zone was measured in millimeters (mm) to determine the antifungal activity (25, 26). The results obtained from antifungal studies were analyzed and standard deviation and standard error were calculated. The variation in the values is mentioned in Table 3.

4.2. *In vitro* toxicity of cobalt nanoparticles:

The cytotoxicity of CoNPs was determine using the *in vitro* MTT assay. The L929 cell line (skin epithelial cells/ non-cancer cells) and the MCF-7 cell line (breast cells/ cancer cells) were utilized for the MTT assay. Non-cancerous cells and cancerous cells were used as references for anticancer studies respectively. Standard culture techniques were used at 37 °C, in a CO_2 environment and removed when 80% confluence was obtained for the growth and culture of cell lines. The cells were then treated with various concentrations (0-100 µM serial two-fold dilutions) of green synthesized CoNPs and incubated again. After 24 h of incubation, cells were treated with 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide stain (MTT). These plates were incubated for 5 h at 37 °C in a 5% CO_2 atmosphere. The supernatant was removed and 100 µg/mL of dimethyl sulfoxide was added. UV- Visible spectrometry was used to measure the color change absorbance at wavelength of 590 nm. The viability of control and treated cells was calculated using absorbance value (27, 28).

Results and Discussion

1. Analysis of active metabolites in seed oil:

GC-MS was done to analyze the active metabolites present in *P. granatum* seed oil. The gas chromatography pattern of *P. granatum* seed oil is shown in Fig. 1. Known metabolites were identified by analyzing the separate peaks. Fatty acids were the major group of active metabolites analyzed in abundance. Palmitic acid, stearic acid, pentadecanoic acid, linoleic acid, azelaic acid, squalene, 2,4-Di-tert-butylphenol and conjugated linoleic acids were obtained in pomegranate seed oil. These active metabolites work as antimicrobial and antioxidant agents in the oil. The list of active metabolites is given in Table 1.

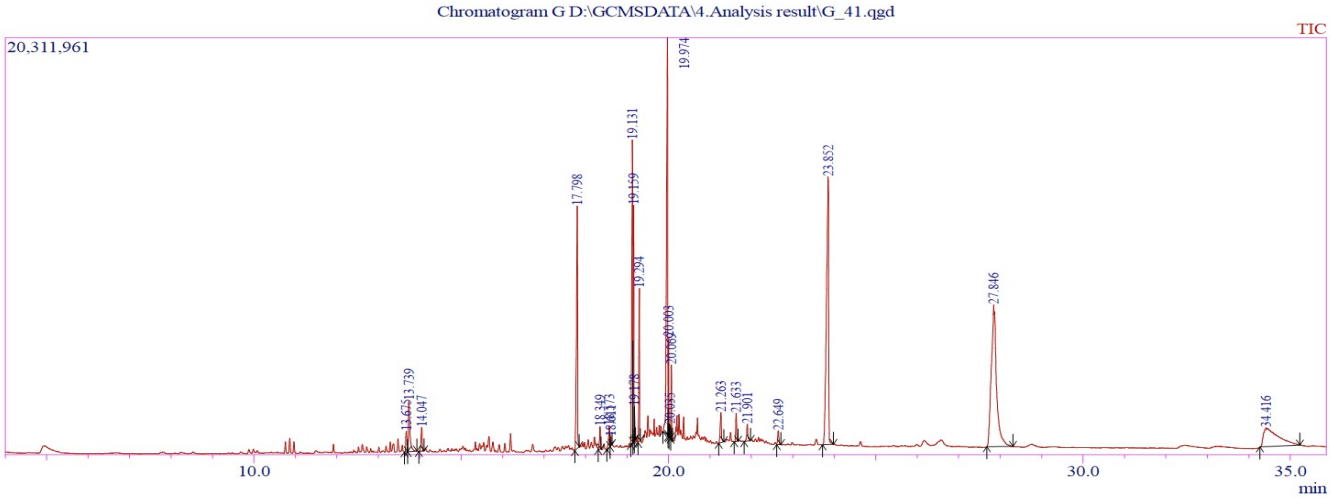
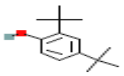
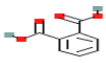
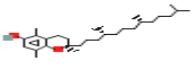


Fig. 1. GC-MS analysis of *Punica granatum* seed oil.

Table 1. List of active metabolites present *in Punica granatum* L. seed oil

Sl. No.	Name of Active Metabolites	Retention time	Area %	Molecular weight (g/mol)	Molecular structure
1	Nonanedioic acid	13.675	0.70	188.22	
2	2,4-Di-tert-butylphenol	13.739	2.43	206.32	
3	Hexadecane	20.035	0.79	226.44	
4	Palmitic acid (Hexadecanoic acid)	17.798	7.91	256.42	
5	Pentadecanoic acid	18.611	0.23	242.4	
6	Heptadecanoic acid (Margaric acid)	18.611	0.23	270.5	
7	9,11-Octadecadienoic acid (Conjugated Linoleic acid)	19.131	9.35	280.4	
8	Stearic acid	19.294	3.14	284.5	
9	Heneicosane	22.649	0.51	296.6	
10	1,2-Benzenedicarboxylic acid	21.901	0.63	390	
11	Squalene	23.852	17.32	410.7	
12	Eicosane	18.349	0.79	282.5	
13	gamma-Sitosterol	34.416	8.40	414.7	
14	Pentacosane	22.649	0.51	352.7	
15	beta-Tocopherol	27.846	22.33	416.7	

2. Synthesis of cobalt nanoparticle:

The reaction mixture was prepared with 5% (5 mL) pomegranate seed oil, 2% PEG (2 mL of Polyethylene glycol), and 93 mL of Cobalt Chloride ($CoCl_2$) 0.1 M solution. The mixture was kept at 600 rpm in a magnetic stirrer for 1 h at a temperature of 45 °C. The completion of the reaction mixture was indicated by a color change in the mixture. As shown in Fig. 2, the initial color of the reaction mixture was dark bluish green while the end color was light green. The green mixture was centrifuged at 10000 rpm for 10 min. The resulting pellets were washed with acetone and ethanol twice. The completely dried cobalt particles were in a green powdery form yielding 30%. These cobalt particles were dissolved in distilled water to perform UV-visible spectrophotometry analysis. The absorbance of the UV-visible spectrum of cobalt particles and precursor salt cobalt chloride is shown in the given Fig. 2. The observed lambda max (λ_{max}) value for cobalt particles and precursor salt were 279.88 nm and 510 nm respectively. This confirms that cobalt chloride particles were reduced during the synthesis of CoNPs. The resultant product showed a different surface plasmon resonance than the precursor salt, indicating successful synthesis of cobalt nanoparticles through the green synthesis method with the reduction of cobalt salt.

3. Characterization of cobalt nanoparticle:

3.1. Dynamic Light Scattering:

The average particle size of the synthesized cobalt particles was analyzed using dynamic light scattering to analyze the individual particle size. Different trial and error methods were performed to optimize the reaction conditions, including the concentration of oil (3%, 5%, 7%) and temperature (30, 35 and 45 °C). A reaction at 30 °C with different concentrations of pomegranate seed oil showed more agglutination whereas increase in temperature to 35 °C reduced the agglutination with an average particle size of 189 nm. At a concentration of 5% oil and a temperature of 45 °C, the average particle size of cobalt particles was 129.6 nm, as shown in Fig. 3. As this is a green synthesis, optimization of reaction conditions cannot be performed above 50 °C. The particle size in various conditions was in the nano-scale range, as analyzed by DLS, confirming that the synthesized cobalt particles were nanoparticles.

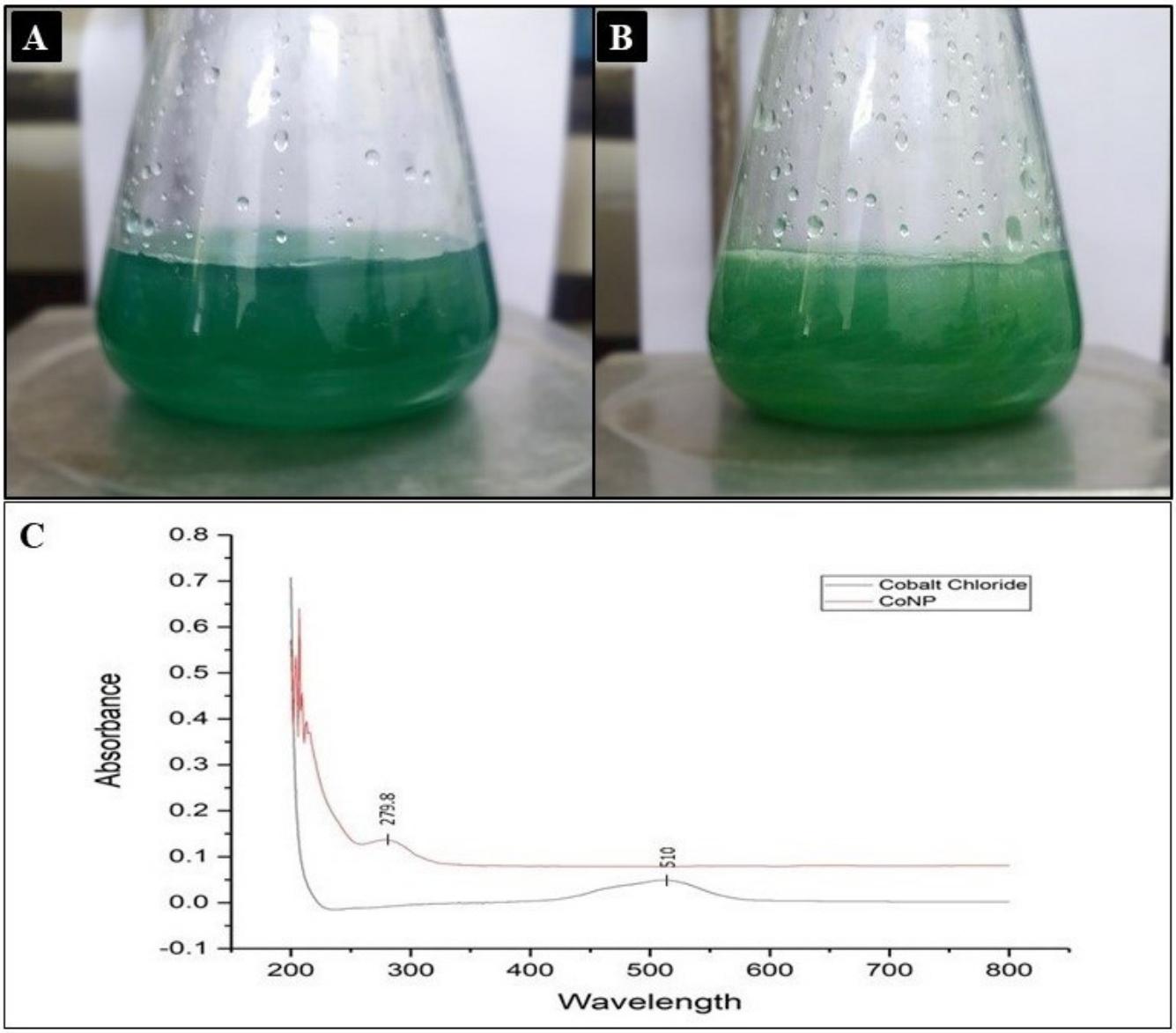


Fig. 2. A) Initial colour of reaction mixture B) End colour of reaction mixture C) UV-visible spectrum for CoNPs shows peak 279.8 nm and 510 nm for precursor salt solution.

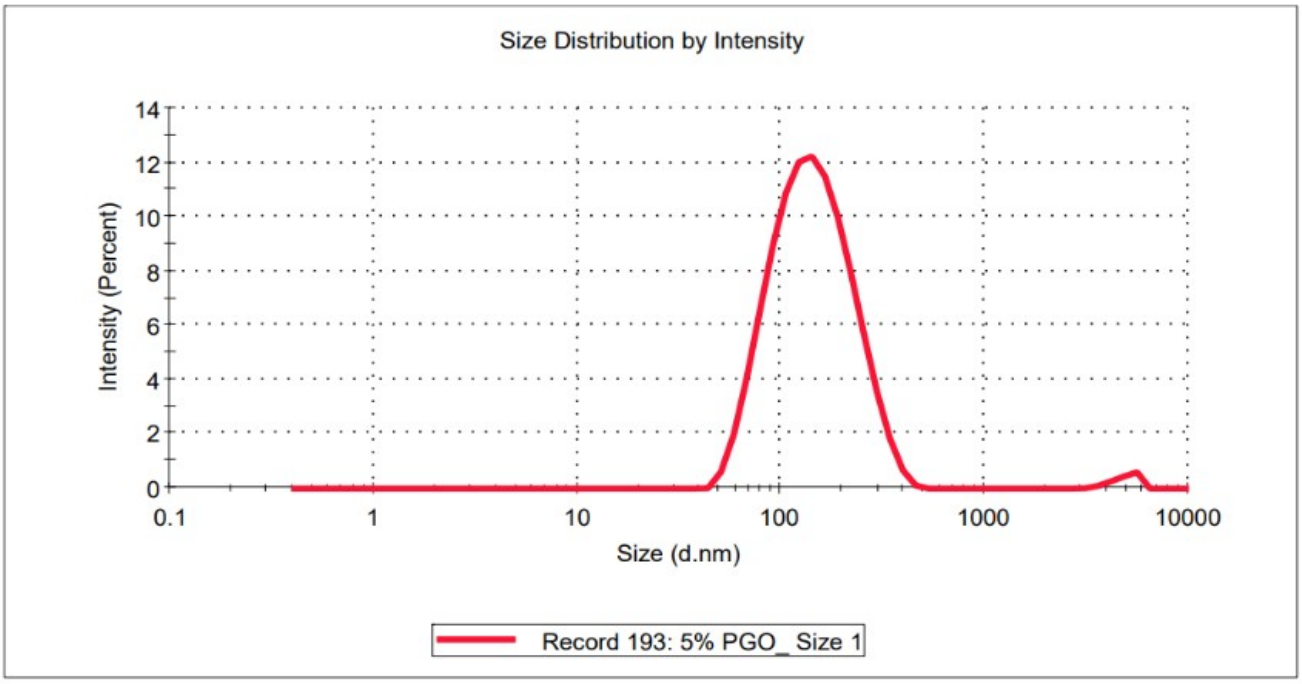


Fig. 3. Dynamic light scattering analysis of CoONPs.

3.2. X-ray diffraction analysis:

X-ray diffraction analysis was performed on the green synthesized cobalt nanoparticles (CoNPs) to observe the different XRD patterns. The graphical pattern of synthesized CoNPs is shown in Fig. 4. The significant 2 theta peak values; 20.0, 37.08, 62.8 74.2 were compared with previously studied cobalt particle patterns, which matched with cobalt oxide peaks as reported by (29). XRD patterns confirmed that the green synthesized CoNPs are cobalt oxide nanoparticles (CoONPs).

3.3. Field emission scanning electron microscopy:

CoONPs were further analyzed using of a field emission scanning electron microscope to study their texture and morphology. The synthesized CoONPs were found to have a spheroidal shape with a smooth texture and uneven distribution, as shown in Fig. 5. The size of each nanoparticle was below 100 nm.

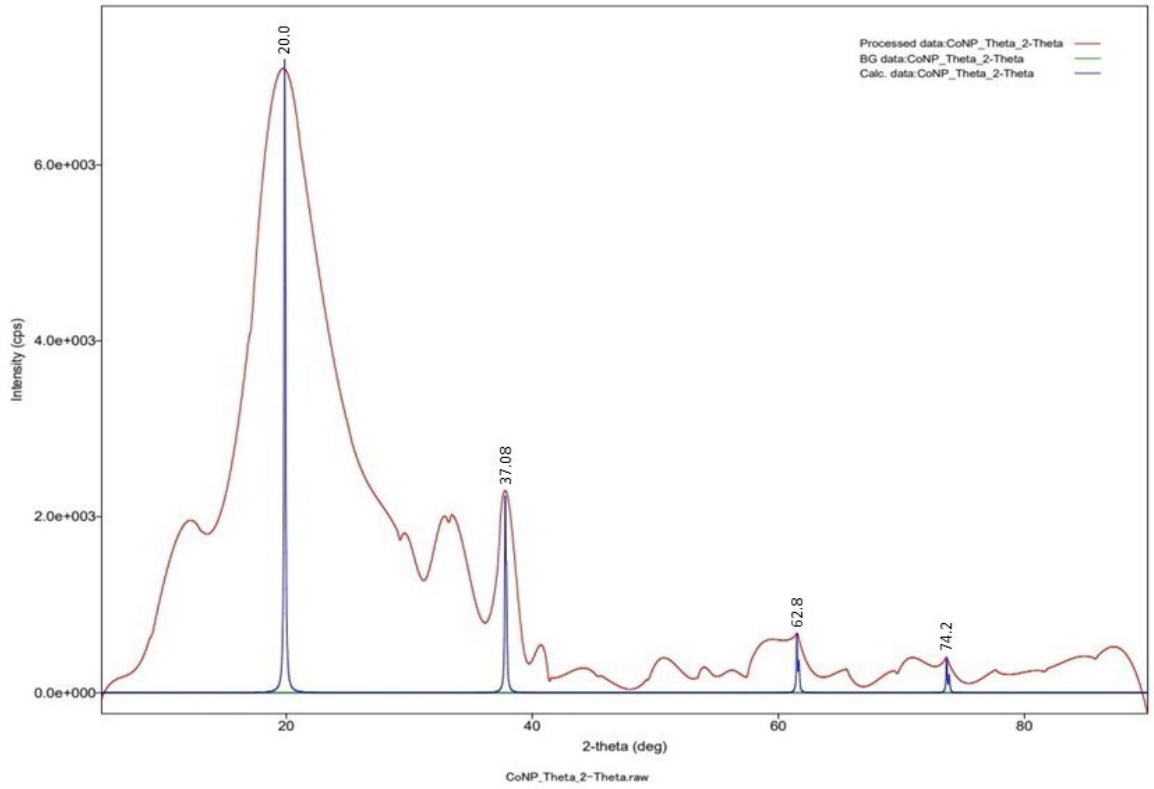


Fig. 4. XRD analysis of cobalt oxide nanoparticles.

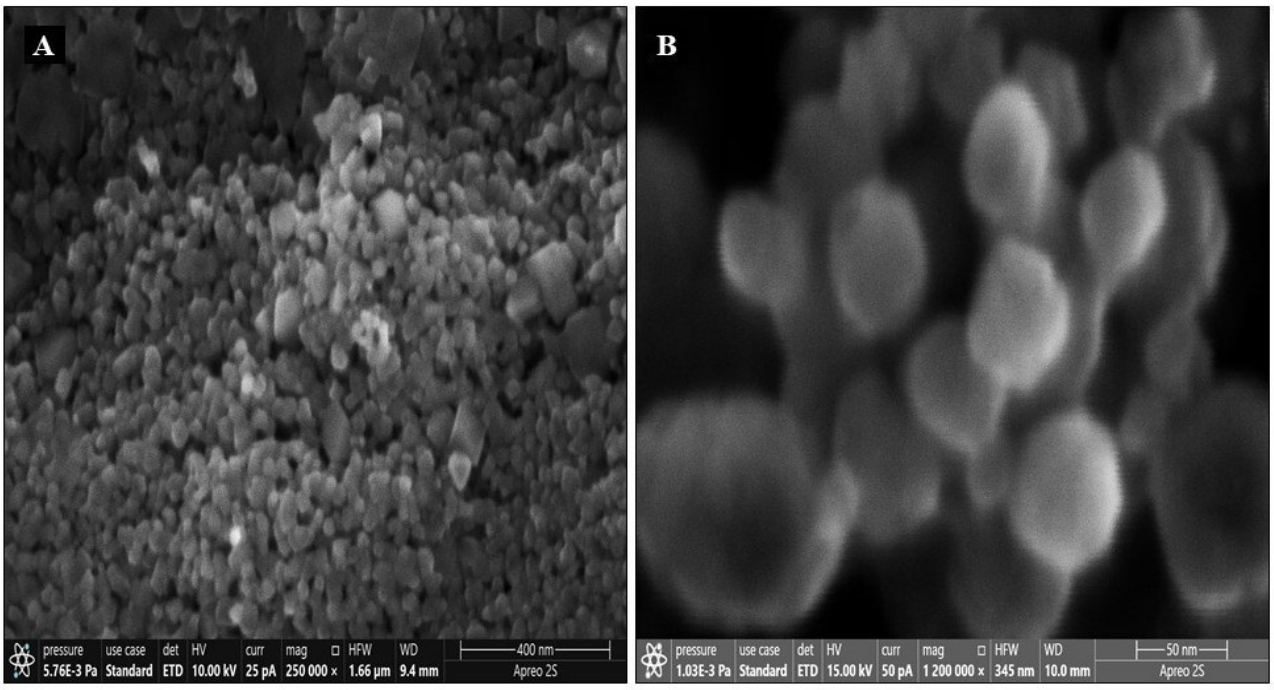


Fig. 5. Field emission scanning electron microscope analysis of CoNPs A) 400 nm B) 50 nm.

3.4. Fourier Transform Infrared Spectroscopy:

FTIR was performed to analyze the different functional groups present in the green synthesized CoONPs. Functional groups such as metal carbonyl and alkyl were found at wavelengths of 2100-1930 cm^{-1} and 2975-2855 cm^{-1} respectively. The graph for different functional groups present in the CoONPs is shown in Fig. 6.

4.Pharmacological applications of cobalt oxide nanoparticles:

4.1. Antimicrobial activity of cobalt oxide nanoparticle:

The antibacterial and antifungal activities of CoONPs were studied *in vitro* using the agar well diffusion technique against different pathogenic bacteria and fungi. Green synthesized CoONPs showed significant activity against

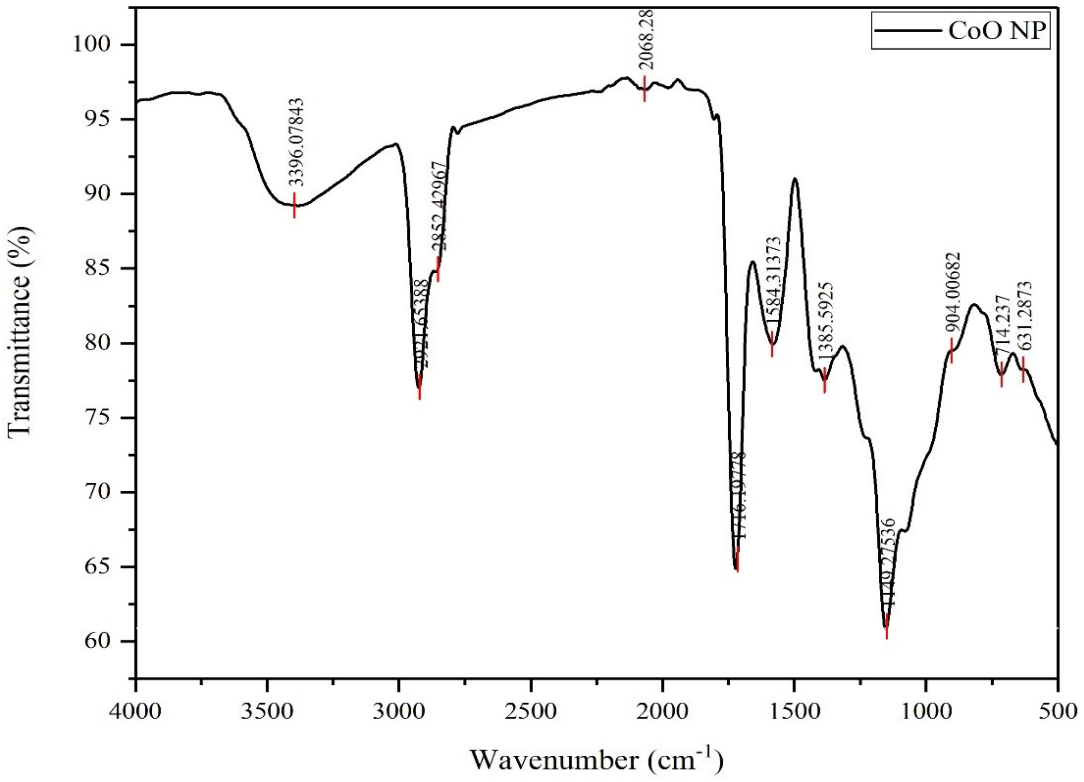


Fig. 6. Graphical representation of different functional groups present in CoONPs.

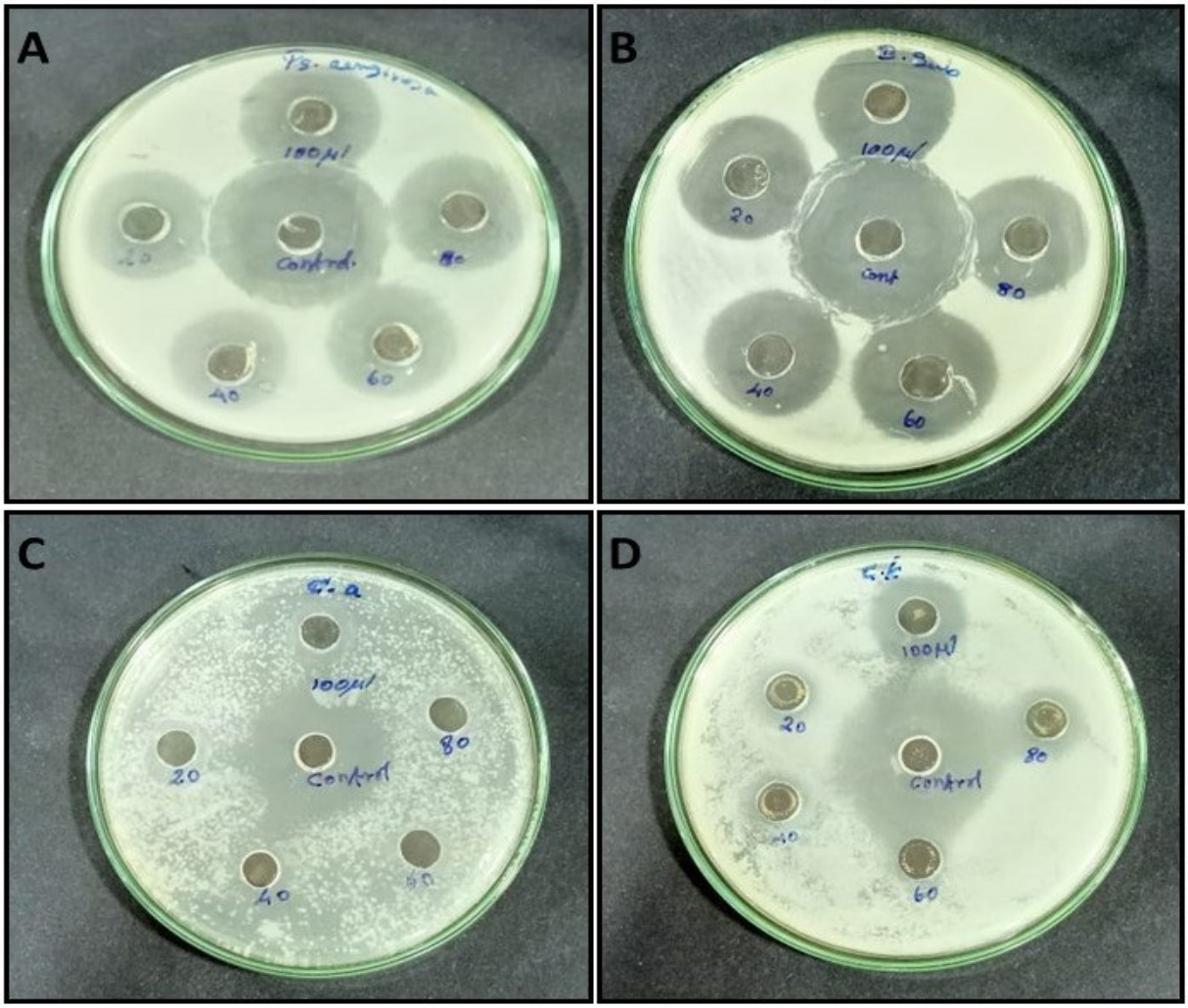


Fig. 7. Antimicrobial activity of CoONPs using the well-diffusion method against A) *Pseudomonas aeruginosa* B) *Bacillus subtilis* C) *Candida albicans* D) *Candida krusei*.

Pseudomonas aeruginosa and *Bacillus subtilis* with the highest inhibition zone of 27 mm at concentration of 100 µg/mL. They also showed activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* shown in Table 2 and Fig. 7. Against 5 different pathogenic fungi belonging to the genus *Candida*. CoONPs showed the highest antagonism against *Candida albicans* and *Candida krusei* with zones of inhibition of 16 mm and 18 mm at a concentration of 100 µg/mL. They also showed activity against *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis*as shown in Table 3 and Fig. 7.

Table 2. Antibacterial activity of CoONPs against different bacteria

Sl. No.	Bacterial Strains	Control	Zone of clearance for different concentrations (mm)				
		Chloramphenicol Zone of clearance in (mm)	Concentration level (µg/ml)				
			20	40	60	80	100
1	***Escherichia coli***	32 ± 0.8	14 ± 1.2	15 ± 0.86	16 ± 1.45	18 ± 1.20	21 ± 1.45
2	***Staphylococcus aureus***	26 ± 0.6	18 ± 0.89	19 ± 1.93	20 ± 0.88	22 ± 0.88	24 ± 1.97
3	***Klebsiella pneumoniae***	35 ± 0.8	17 ±1.15	18 ± 1.15	20 ± 1.20	21 ± 1.45	22 ± 1.20
4	***Pseudomonas aeruginosa***	33 ± 0.3	22 ± 1.52	23 ± 1.44	24 ± 0.88	25 ± 1.76	27 ± 0.88
5	***Bacillus subtilis***	34 ± 0.8	22 ± 1.45	23 ± 1.73	24 ± 0.57	25 ± 1.52	27 ± 1.15

The above mentioned data is the mean of triplicate values and the standard deviation is mentioned with ± symbol

Table 3. Antifungal activity of CoONPs against different fungi

Sl. No.	Fungal Strains	Control	Zone of clearance for different concentrations (mm)				
			Concentration level (µg/ml)				
		Flucanozole Zone of clearance in (mm)	20	40	60	80	100
1	*Candida tropicalis*	32 ± 1.15	12 ± 0.88	12 ± 1.15	15 ± 1.2	14 ± 1.20	16 ± 1.2
2	*Candida glabrata*	31 ± 1.85	10 ± 1.2	10 ± 1.45	12 ± 1.73	13 ± 1.52	14 ± 1.83
3	*Candida parapsilosis*	34 ± 1.52	0	0	0	13 ± 1.20	14 ± 1.76
4	*Candida albicans*	26 ± 1.45	12 ± 1.45	12 ± 1.08	14 ± 1.45	15 ± 1.45	16 ± 1.45
5	*Candida krusei*	32 ± 1.15	13 ± 1.20	13 ± 1.34	15 ± 1.52	16 ± 1.13	18 ± 1.17

The above mentioned data is the mean of triplicate values and the standard deviation is mentioned with ± symbol.

4.2. In vitro toxicity of CoONPs:

MTT assay was performed at different concentrations (10-100 µg/mL) for both cancerous and non-cancerous cells. The morphological results of control and treated cells for both cells types are shown in Fig. 8. The IC_{50} value of L929 non-cancerous cell line was found to be more than 100 µg/mL while the cancerous cell MCF-7 showed an IC_{50} value of 57.85 ± 0.10 µg/mL. In L929, at the highest concentration of 100 µg/mL, cell viability was 96% while in MCF-7 (cancerous cell line), cell viability was only 4%. This comparative study strongly concludes that green synthesized CoONPs show high cytotoxicity against MCF-7 cancerous cell lines and can act as a potential anti-cancer agent in pharmacology (Fig. 9).

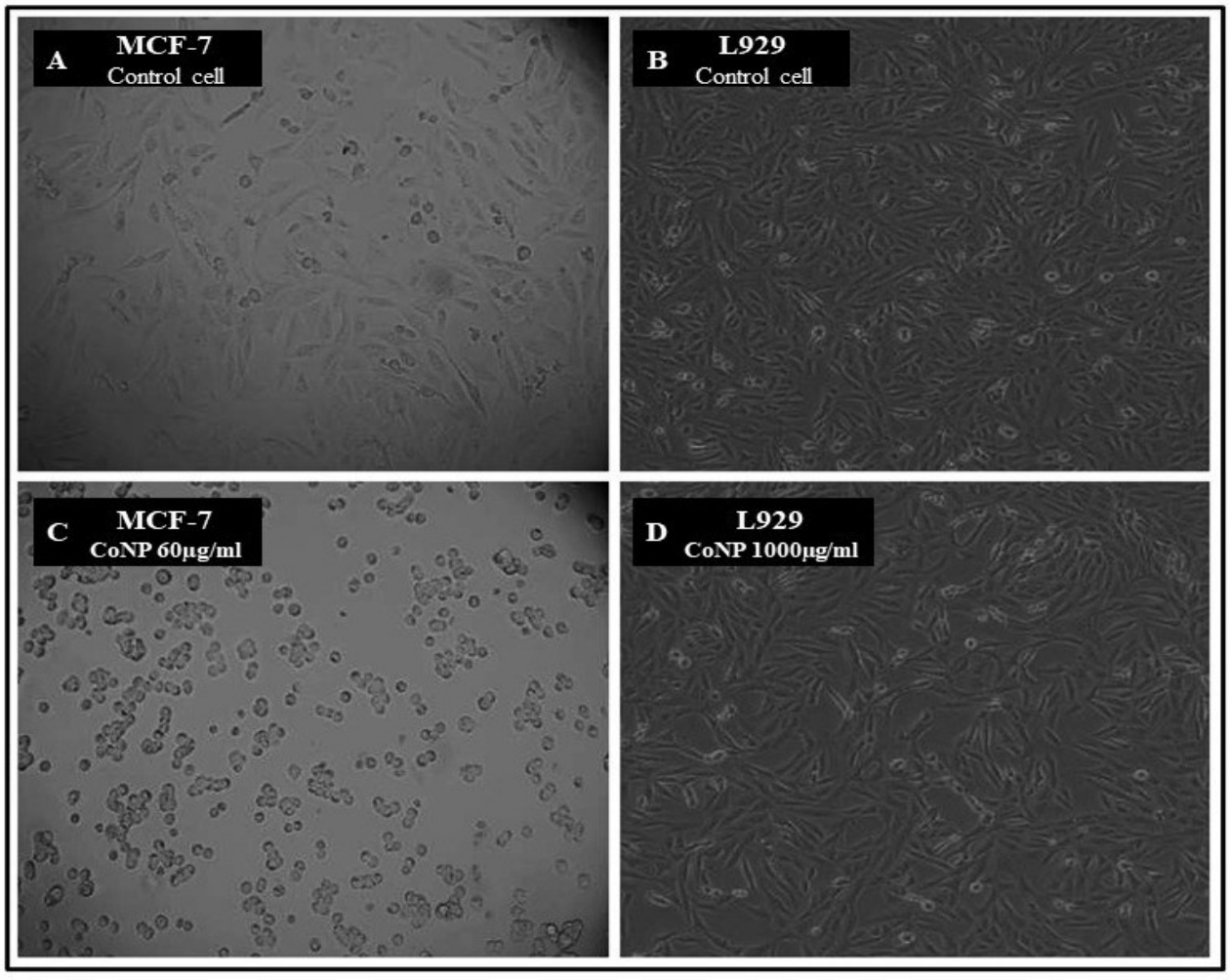


Fig. 8. Morphology of MCF-7 and L929 cells treated with CoONPs.

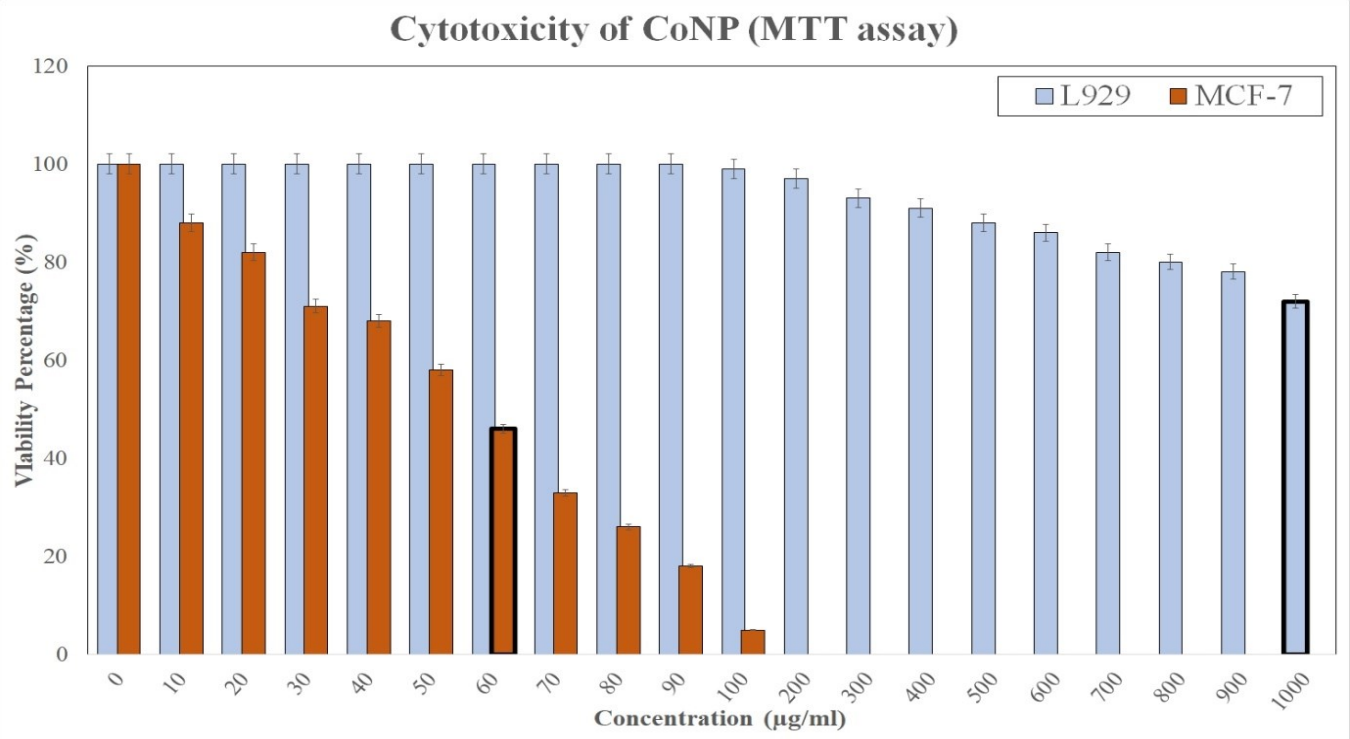


Fig. 9. Comparative analysis of *in vitro* toxicity of CoONPs against MCF-7 and L929 cell line.

Discussion

This study reports the green synthesis of cobalt oxide nanoparticles (CoONPs) from *P. granatum* seed oil. The oil played an important role as a reducing agent in this green synthesis. As discussed earlier, pomegranate seed extract is an adequate source of antioxidant and antimicrobial agents (16). This study aims to assess the efficiency of pomegranate seed oil in pharmacological properties such as antimicrobial and anticancer effects and to provide commercial and pharmacological value beyond its edible purpose. Similarly, this study explores the opportunities for the synthesis of various bioactive nanoparticles like iron, gold, silver, zinc and copper from oil. The green synthesized CoONPs exhibited smooth texture, uneven in number and spheroidal-shaped morphology under scanning electron microscope (SEM). The average size of the green synthesized CoONPs is 129.6 nm which falls within the range of commercially available CoNPs 90 nm to 200 nm (30). Cobalt is not a widely explored element in green synthesis due to its size-reducing nature. It is difficult to reduce the size of green synthesized cobalt elements on the nano-scale. However, a few experimental studies have shown that cobalt nanoparticles can help reduce hydroxyl proline, wound contracture, hexuronic acid, fibrocyte and hexosamine in rats and can be utilized as a cutaneous wound healing agent (31). The antimicrobial well-diffusion study proved that green synthesized CoONPs are potent antibacterial and antifungal agents against different pathogenic micro-organisms and can prevent common infections and candidiasis in humans. Additionally, these CoONPs showed beneficial effects on *in vitro* toxicity in cancer studies against breast cancer (MCF-7) cells without any harmful effects.

Previously reported studies have strongly proven that cobalt nanoparticles have a beneficial prospect in pharmaceutical industries due to their various pharmacological applications. Synthesis studies of different nanoparticles like iron, silver, gold and copper have been reported from different parts of pomegranate (leaves, fruit peel, juice and seed) which are both pharmacologically and economically important. For example, zirconium nanoparticles synthesized by pomegranate peel extract have shown potential as antimicrobial and antioxidant agents (32). The fleshy pericarp of the pomegranate fruit has been used for the synthesis of silver nanoparticles which have shown fine pharmacological and biocompatibility potential (28). Additionally, silver nanoparticles synthesized using pomegranate peel extract have demonstrated good antimicrobial activity against different micro-organisms (33). Zinc nanoparticles synthesized using pomegranate peel extract have shown good cytotoxicity and antibacterial activity antagonist to colon cancer (HCT116) cells and various gram-negative and gram-positive bacteria respectively (34). However, there are not much findings related to seed oil nanoparticle synthesis. Selenium nanoparticles synthesized using pomegranate peel extract have shown good anticancer activity against Mg63 and MCF7 cell lines (35). Gold nanoparticles (AuNPs) synthesized using *P. granatum* seed oil where laser attrition was used for stabilization, have shown good source of antioxidant and anti-inflammatory activities (36). According to various literature surveys, the synthesis of CoONPs using the oil-water method is the first successful attempt with its pharmacological applications. The current study results provide additional evidence for the green synthesis of CoONPs and their antibacterial, antifungal and anticancer properties. In the future, *in vivo*, studies can be conducted to further to justify these results.

Conclusion

This study demonstrates that, the synthesis of CoONPs from *P. granatum* seed oil has potential in the pharmaceutical industry as an antibiotic. It also fills the gap in green synthesis with oil samples. These CoONPs can be used to control various bacterial and fungal pathogens responsible for infections such as candidiasis. Furthermore, CoONPs can be used as an anti-cancer agent as they have shown positive results with MCF-7 cell lines without much effect on L929 cell lines, indicating that these CoONPs would not have negative impact on healthy human cells. It is suggested that these CoONPs can be used in the form of ointments, plasters, first-aid creams, bandages and emulsions form for external use. Moving forward, this study can be very helpful for medical research centers and pharmaceutical industries in dealing with modern problems such as increasing antibiotic resistance in the community.

Abbreviations

CoNPs- Cobalt nanoparticles, AuNPs- Gold nanoparticles, PEG- Polyethylene glycol, GC-MS - Gas Chromatography-Mass Spectrometry, CoONPs - Cobalt oxide nanoparticles, MTT - 3-(4, 5-Dimethylthiazol2-yl)-2,5-diphenyl-2H-tetrazolium bromide; ZOI - Zone of inhibition MCF-7-Breast cell line, L929 - Skin epithelial cell line

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Authors' contributions

PBC provided basic idea of work and complete research work was done under the guidance of MBT. PBC and MBT contributed for the preparation of manuscript. Both authors read and approved the final manuscript.

Compliance with ethical standards

Declaration : Authors do not have any conflict of interest to declare.

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