

Medicinal application of Cobalt Oxide Nanoparticles (Co₃O₄NPs) derived from *Pergularia daemia* L, leave extract

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ABSTRACT

The synthesis of cobalt oxide nanoparticles was consummate by *Pergularia daemia* L leaves extract; the extract was prepared from double distilled water. During these synthesis without the addition of any external chemical reducing agent. The reduced cobalt oxide nanoparticles were characterized by UV and FTIR spectroscopy, the synthesized cobalt oxide nanoparticles analysed by antibacterial activity against four antibacterial agent along this bacterial strain contain two gram positive and two gram negative bacterial strains, antifungal active measured by two different fungal strains, antioxidant activity evaluated by DPPH method finally screened anticancer activity by MCF-7 cell line, IC50 values of antioxidant and anticancer activity measured by graphical method. Really, this study provides new possibilities of using *Pergularia daemia* L leaves extract act as a capping and reducing agent for the synthesis of cobalt oxide nanoparticles, which may be applicable for the future medicinal industries.

Keywords: Eco-friendly Synthesis, *Pergularia daemia* L, Co₃O₄NPs, characterization, Medicinal applications

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1. 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], *Helianthusannuus* [13], *Manihotesculenta* [14], *Moringaoleifera* [15], *Neriumindicum* [16], *Pipernigrum* [17], *Punicagranatum*

[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 MCF-7 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]. *Pergularia daemia* leaves (veliparuthi) possess significant medicinal value, traditionally used for respiratory issues (asthma, bronchitis), anti-inflammatory effects (rheumatism), and skin, digestive, or menstrual disorders. Rich in bioactive compounds like flavonoids and alkaloids; they offer antimicrobial, wound-healing, and hepatoprotective benefits.

In this connection, a simple, less toxic and cost-effective process using the plant and microorganisms as a resource is reported for the synthesis of desired cobalt or cobalt oxide nanomaterials. During the preparation, the use of natural sources as a reducing and stabilizing agent, the constituents of plant extracts such as alkaloids, flavonoids and so on may enhance the properties of the nanoparticles. Nanoparticles can be produced by using various biological resources, including fungi, plants, microorganisms etc. In comparison to chemical and physical approaches, biological approaches are more environmentally friendly as reduce pollution and waste

production, synthesize materials more efficiently, save time and energy, and above all nanoparticles produce at a lower cost.

2. MATERIAL AND METHODS

2.1. Collection of Sample

Fresh *Pergularia daemia* leaves were collected from botanical garden, Department of Botany, Government arts college, Chidambaram. Leaves were washed in three times thoroughly by running ordinary tap water (OTW), then washed two times with double distilled water (DDW) to remove any dust particles on the leaves, washed leaves were allowed to dry in air at room temperature. The dried leaves were grained and powdered by using electric mixer. This powder was used to prepare the leaves extracts

2.2. Chemicals, Solvents and Starting Materials

De-ionized water, whatmann 1 μ and whatmann 41 μ filter papers, cobalt nitrate hexa hydrate (Co(NO₃)₂·6H₂O), Sodium hydroxide pellets, hydrochloric acids, sulphuric acid, vitamin C, Acrobosc, Diclofenac sodium, Muller Hinton agar(MHA), Sabouraud Dextrose agar(SDA), sodium phosphate, ammonium molybdate, Amylase, DNSA reagent, Dimethyl formamide, Bovine serum albumin solution, 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide, tetrazolium, phenol red, DMSO, and other chemicals were purchased from Merck (India) Ltd. All chemicals were used without further purification.

2.3 Instruments and equipment

Electric oven, Magnetic stirrer (REMI 2 MLH), E-1 portable TDS & EC meter, pH-009(I)A pen type pH meter, petri plate, ordinary incubator, CO₂ incubator, Micro Plate reader, Inverted microscope, Refrigerated centrifuge, sterilized 250ml separating funnels, sterilized conical flasks, sterilized 400ml beakers, watch glasses, 7" funnels, glass rods, and 10ml measuring cylinders,

2.4. Plant material processing

5 grams of *Pergularia daemia* leaves powder with 50 mL of double-distilled water (DDW) taken in the 250 mL round bottomed flask, water condenser fitted and fix the running tap water then heated for 20 min at 80°C. Then the extract was filtered with Whatman 1 μ filter paper. The filtrate was used to the further green synthesis of process.

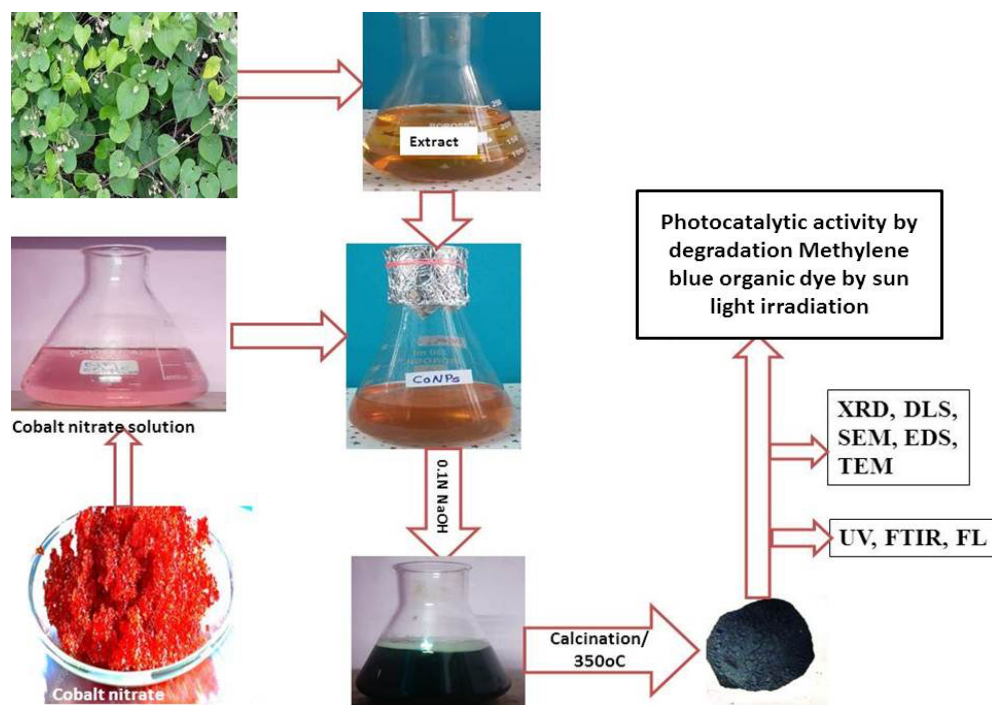


Figure:1 Green Synthesis of cobalt oxide nanoparticles by *Pergularia daemia* leaves extract

2.5. Biosynthesis procedure

For the synthesis of Cobalt oxide nanoparticles by reducing cobalt nitrate hexahydrate (M.F:Co(NO₃)₂.6H₂O, MW: 291.04 g/mol), 180 mL of homogenous solution of cobalt nitrate is steadily mixed with 10mL of *Pergularia daemia* leaves extract followed by continuous heating (70 °C) and stirring at 500 rpm for 3 hr at magnetic stirrer with heating instrument, to achieve reddish pink solution [32], The obtained brownish red colour solution was added with 0.1M NaOH solution maintain by pH 10, the solution was changed to blue colour precipitate. The obtained precipitate was filtered by whatmann 1# filter paper. The precipitate was dried and powdered then calcinated at 350°C through muffle furnace. After calcination to obtained gray colour

fine crystalline cobalt oxide nanoparticles. Finally, Co₃O₄NPs were steadily characterized. Figure.1, have shown in scheme of green synthesis of cobalt oxide nanoparticles.

3. RESULT AND DISCUSSION

3.1 UV spectral analysis

The collection of excitation present in the surface of the nanoparticles known as surface plasmon resonance was used for the confirmation of synthesis, size and shape of the nanoparticles. The UV-vis spectra was (Fig.2) recorded (20-21) from the *Pergularia daemia* L leaves extract with cobalt ion. The spectra show a well-defined surface plasmon band centered at around 273 nm and 398.15 nm for cobalt oxide nanoparticles, which is the characteristic absorbance of cobalt oxide nanoparticles.

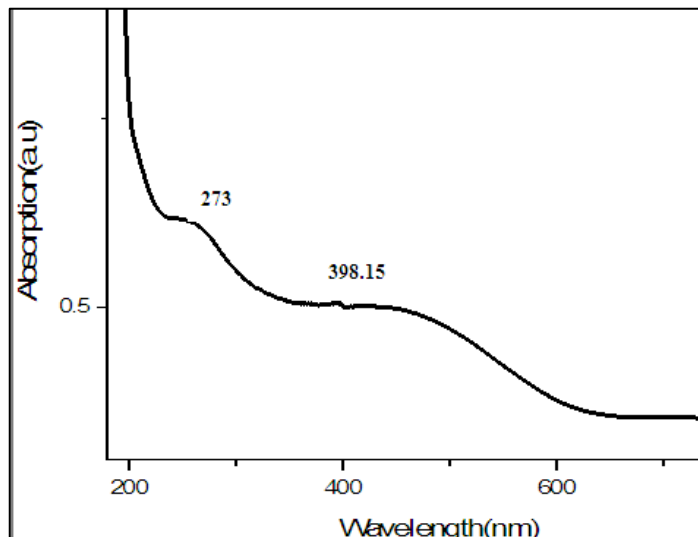


Figure:2 UV spectrum of Co₃O₄NPs by *Pergularia daemia* leave extract

The color intensity of cobalt is raised while increasing the incubation time and it reveals the formation of an increased number of nanoparticles. Observation of this peak, assigned to a surface plasmon is well-documented for various metal nanoparticles with sizes ranging from 2 nm to 100 nm. The stability of nanoparticles is mainly based on the capping agents like enzymes and proteins present in the leaves extract.

3.2 FT-IR Spectral analysis

FTIR analysis for the biogenically synthesized, cobalt oxide nanoparticles and the leaf extract of *Pergularia*

daemia L were carried out in order to explore the formation of cobalt oxide nanoparticles and confirm the functional groups present in the aqueous leaf extract. This in turn enables to identify the bioactive molecules which were actively involved during the synthesis process as stabilizing and capping agents to prevent the overall growth of cobalt oxide nanoparticles. Fig:3 and 4 shows the FTIR spectra of aqueous leaf extract of *Pergularia daemia* L and Co₃O₄NPs by *Pergularia daemia* L and were recorded in the range of 4000–400 cm⁻¹.

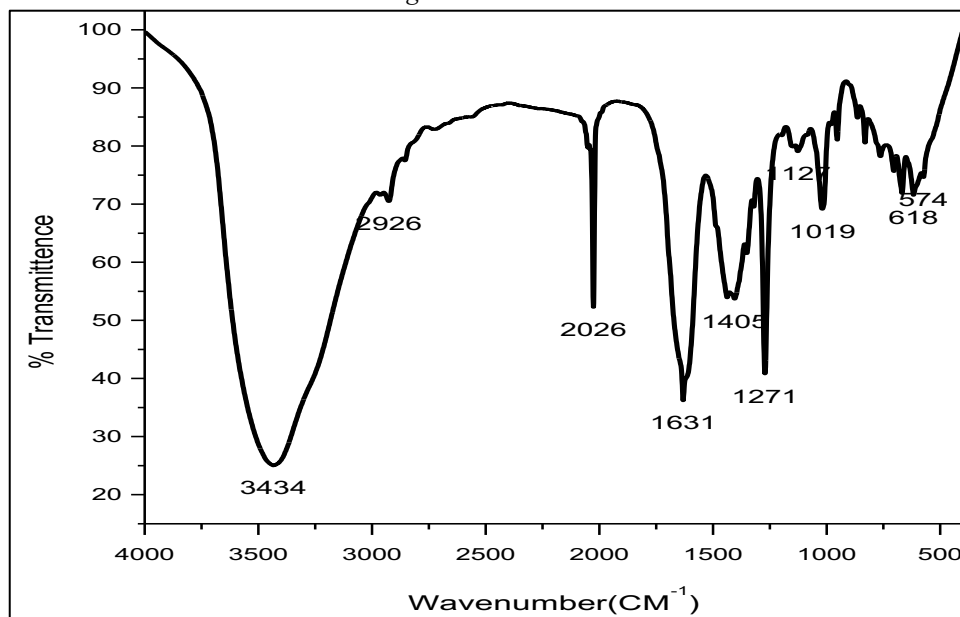


Figure:3 FT-IR Spectrum of *Pergularia daemia* leave extract

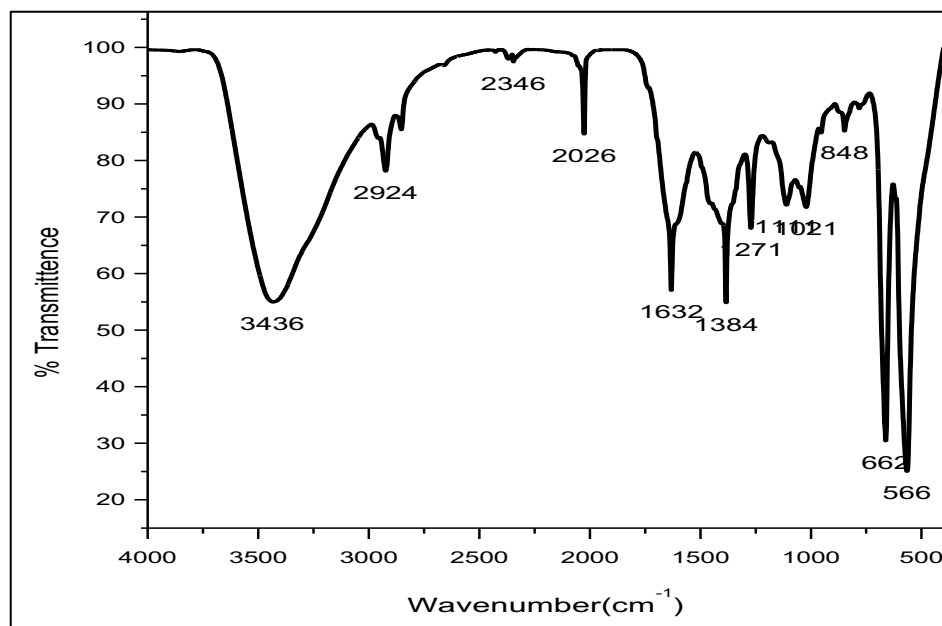


Figure:4 FT-IR Spectrum of Co₃O₄NPs by *Pergularia daemia* leave extract

The FTIR spectrum of leaf extract have shown in (Figure:7.2), broad peak observed in the spectrum at around 3434 cm⁻¹ represents the O-H stretching vibration. The peak at 2926 cm⁻¹ have represent CH₃, CH₂ & CH stretching vibration, While, the prominent levels of absorption peak located at around 2026 cm⁻¹ reveal the presence of C-H stretching vibrations of an alkane and alkene. The medium absorption peak observed at 1631 cm⁻¹ corresponds to C=O peak, which indicates the ketone functional group and also the ammine functional group. The band observed at 1405 cm⁻¹ corresponds to α-CH₂ bending vibrations, An absorption peak found at 1271 cm⁻¹ corresponds to the saturated primary alcohol containing the O-C bond, the sharp peak at 1019 cm⁻¹ reveals that O-H in plane, the small peak observed in 618 cm⁻¹ report C-H deformation and carbon-carbon chain linkage. The FTIR spectrum of cobalt oxide nanoparticles by *Pergularia daemia* L leaf extract have shown in (Fig:7.3), broad peak observed in the spectrum at around 3436 cm⁻¹ represents the O-H stretching vibration. The peak at 2926 cm⁻¹ have represent CH₃, CH₂ & CH stretching vibration, While, the prominent levels of absorption peak located at around 2026 cm⁻¹ reveal the presence of C-H stretching vibrations of an alkane and alkene.

The medium absorption peak observed at 1632 cm⁻¹ corresponds to carbonyl stretching vibrations which indicates the ketone functional group and also the ammine functional group. The band observed at 1384 cm⁻¹ corresponds to C-C stretching vibrations, an absorption peak found at 1271 cm⁻¹ corresponds to the saturated primary alcohol containing the O-C bond, the sharp peak at 1102 cm⁻¹ reveals that hydroxy in plane, The IR spectrum of cobalt oxide exhibits two major bands at 662 cm⁻¹ and 566 cm⁻¹. The first band is associated with the Co³⁺ vibration in the octahedral hole and the second band (ν) is attributed to the Co²⁺ vibration in the tetrahedral hole, which confirms the formation of the Co₃O₄ spinel.

3.3 FL Spectral analysis

In this study, we have investigated the fluorescence emission spectra of cobalt oxide nanoparticles. The spectral characteristics of fluorophores must match the wavelengths of the excitation light, Dichroic and emission filters of the fluorescence microscope on which the experiments are to be done. Moreover, it is advised that their spectra are well separated from the spectra of other fluorophores that will be used simultaneously in the experiments to minimize cross-talk and bleed through, which can give rise to false co-localization and misinterpretations if not appropriately corrected.

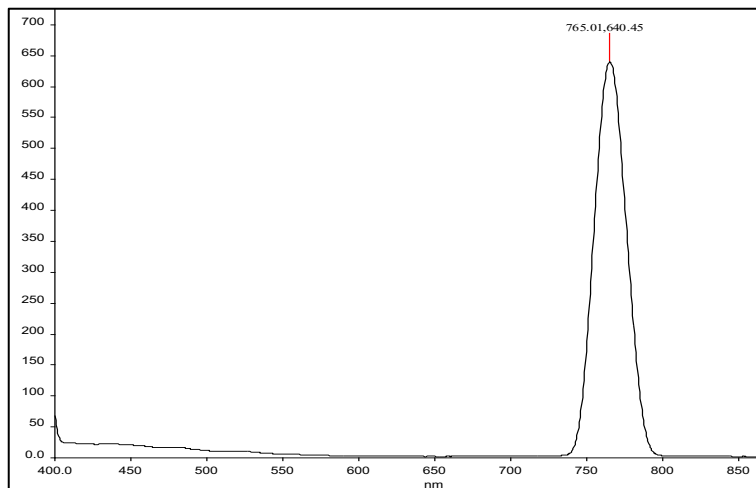


Figure:5 FL Spectrum of Co₃O₄NPs by *Pergularia daemia* leave extract

For use with advanced optical techniques (such as higher resolution microscopies, two-photon microscopy, fluorescence lifetime imaging, spectral imaging or correlation spectroscopy), further photo-physical aspects (e.g., lifetime, blinking, and environmental sensitivity) must also be taken into account. **Fig:5** shows the fluorescence emission of cobalt nanoparticles by aqueous leaves extracts of *Pergularia daemia* L, It shows that the plasmonic resonance in the range close to 765.01 nm, fluorescence intensity 640.45

3.4 XRD analysis

X-ray diffraction (XRD) technique was used to determine the purity and phase of the powdered cobalt oxide nanoparticles from aqueous leaves extract of *Pergularia daemia* L. **Fig.6** represented the typical diffraction pattern in which the peaks at 2θ were the diffraction angles of 31° (220), 37° (311), 45° (400), 49° (101), 61° (511) and

65° (440) in the XRD curve corresponds to the standard diffraction peak of Co₃O₄ nanoparticles by *Pergularia daemia* L, respectively corresponds to Co₃O₄ having spinel structure and cubic close packed phase [JCPDS card no.-01-073-1701]. Insignificant peaks observed could be attributed to organic substances⁽²⁴⁾. A shift in some peaks was due to the presence of impurities owing to the biomass residue. The presence of broad peaks suggests the synthesized particles to be very small in size in the nano dimensional state and amorphous in nature. The average crystallite size determined by the Scherrer formula, $D = 0.9\lambda/\beta \cos \theta$ using the half-width of the intense peak in the powder pattern. Where D is the crystallite size, λ is X-Ray wavelength which is 1.54 Å, β is full width at half maxima (FWHM) and θ is Bragg's angle. The crystallite size of biologically synthesized Co₃O₄ NPs corresponding to the highest peak observed in XRD pattern was approximately 55.8 nm.

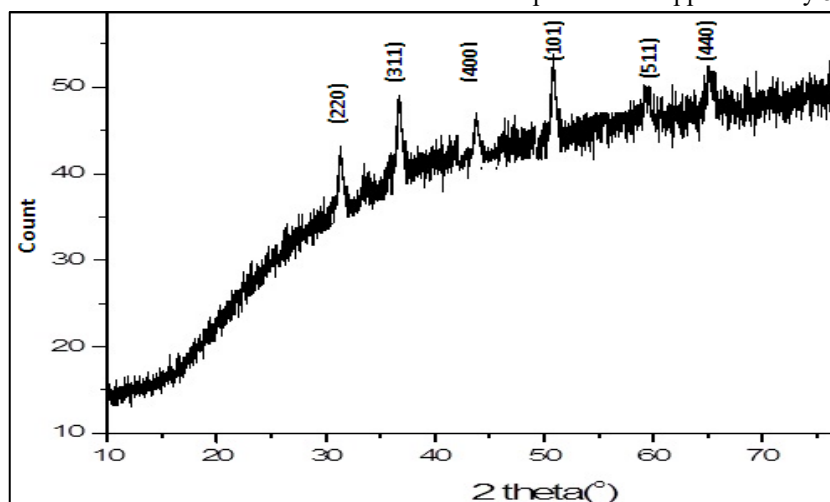


Figure:6 XRD pattern of Co₃O₄NPs by *Pergularia daemia* leave extract

3.5 DLS analysis

DLS is the most often applied technique to determine the distribution of Cobalt oxide nanoparticles size in its condensed state. The evaluated result of nano particle size shows the size variation range mendacities amongst 8to

80 nm with highest size variation around 22.48 nm as shown in **Fig:7**. Comparatively the size variation diagrams in DLS analysis have good symmetry and reveal the homogeneity of resultant cobalt oxide nanoparticles.

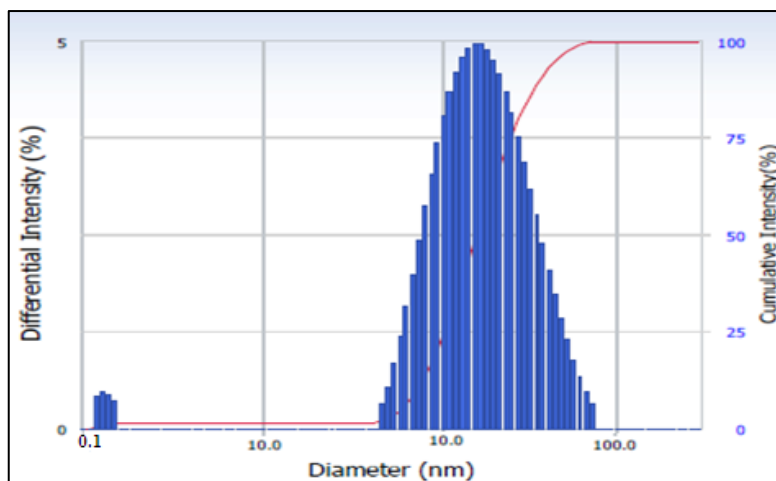
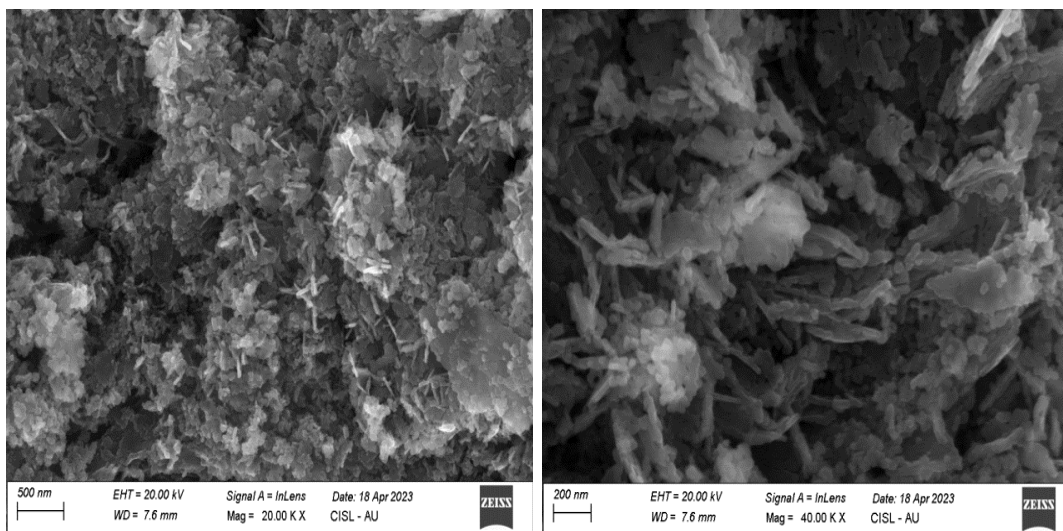


Figure:7 DLS spectrum of Co₃O₄NPs by *Pergularia daemia* leave extract

3.6 SEM analysis

SEM images in **Fig.8 (a-d)** showed irregular, needle, rod, cubic and spherical shapes of various sizes that are agglomerated. Further observations with higher magnifications reveal that these images possess smooth surfaces. Surface morphology of Co₃O₄ NPs. Biomolecules from leaf extract of *Pergularia daemia* L acts as capping and stabilizing agent which form coating

on the individual nanoparticles and contains hydroxyl group which causes intermolecular hydrogen bonding resulting in agglomeration. This agglomeration depends upon the nature and compounds present in the extract. Eco toxic properties of transition metal oxide are due to shape, small size, high chemical reactivity, biological activity and agglomeration tendency which cause threat to the environment and human beings.



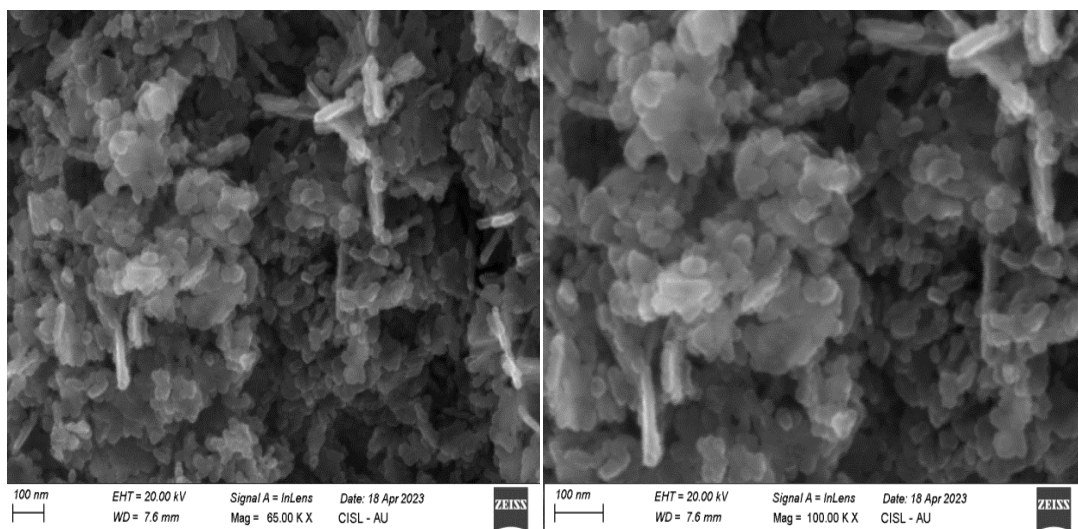


Figure:8 SEM images of Co_3O_4 NPs by *Pergularia daemia* L leave extract

3.7 EDS analysis

The structural characterization of Co_3O_4 NPs was implemented utilizing an analysis of dispersive energy X-ray spectroscopy (EDX) as indicated in between 0 to 10 keV. **Figure:9 (a-b)** shows the element quantitative and qualitative analysis may involve the formation of Co_3O_4 NPs. The obtained results shows strong signals at 6.8 keV and were for Co and intense signal between 0.0-0.5 keV for O suggesting that Co and O were the major elements and formation of synthesis of cobalt oxide arise from the sample and other unexpected weak signals at 0.3 keV, 1

keV 1.9 keV, 2.0 keV, 2.4 keV, 3.6 keV and 3.9 keV were from bio-compounds present in the leaf extract. The analysis of the EDX spectrum also shown in the composition atom percentage of oxygen is 49.87 %, mass percentage of oxygen is 36.33 other impurities are slightly presented. The trace amounts existence of cobalt demonstrated that plant phytochemical groups are involved in reducing and capping of synthesized Cobalt nanoparticles.

3.8 TEM analysis

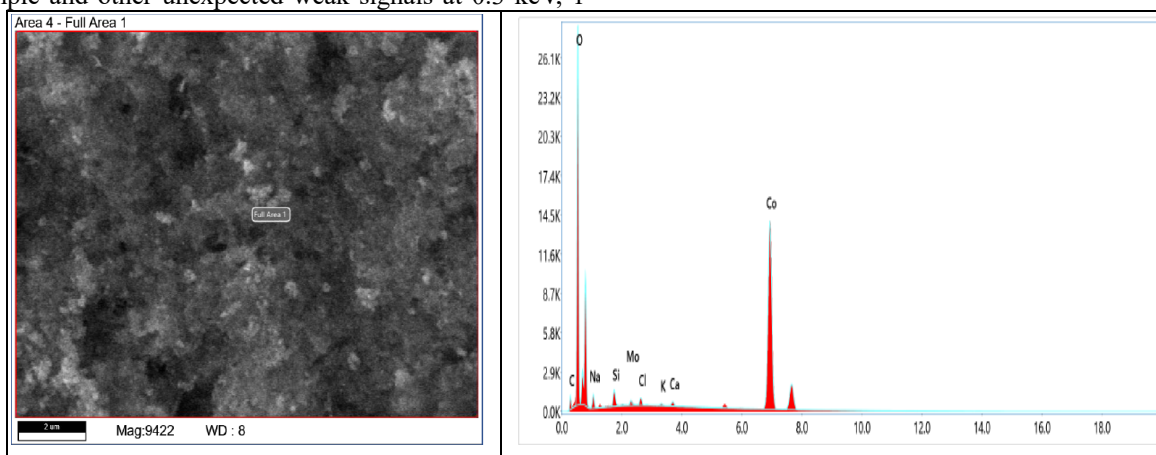


Figure:9 EDS Micrography and spectrum of Co_3O_4 NPs by *Pergularia daemia* leave extract

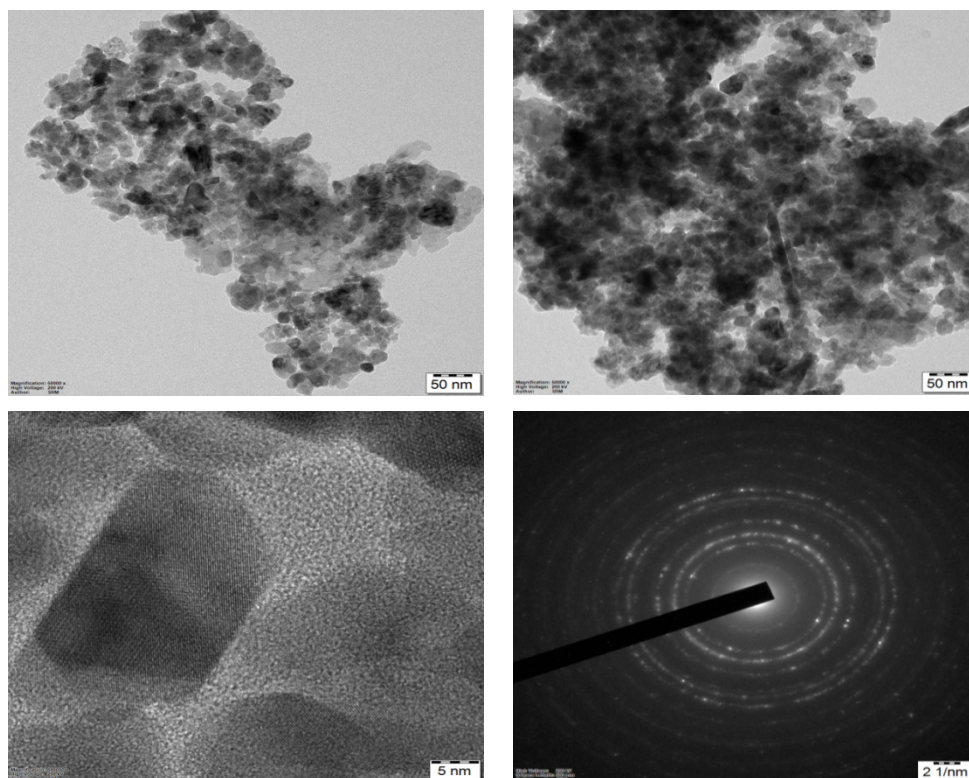


Figure:10 TEM images of $\text{Co}_3\text{O}_4\text{NPs}$ by *Pergularia daemia* leave extract

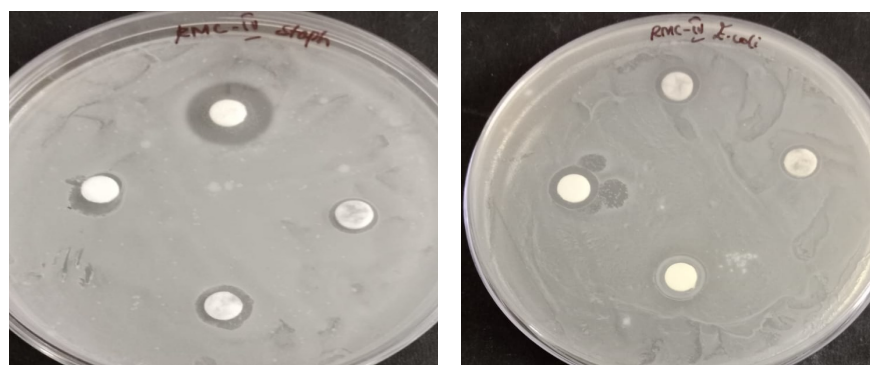
TEM images of the obtained samples (Fig.10) reveal a polycrystalline nature. The TEM images show that there is not a well-defined and uniform shape. However, a tendency toward polycrystalline shapes is observed. A closer look at the crystal edges also reveals trigonal, needles, pentagonal and hexagonal shapes. The crystallite sizes revealed by TEM are in the figure 10.a and 10.b have shown in 100 nm sized cobalt oxide nanoparticles, figure 10.c have shown in $1\mu\text{m}$. Figure;10.d have shown in SAED pattern of cobalt oxide nanoparticles by *Pergularia daemia* L. This roughly corroborates the

estimates from XRD, DLS and SEM analysis which, for instance, provided values of 100 nm for above three analyses.

3.9 Medicinal analysis

3.9.1 Antibacterial activity

Antibacterial activities of synthesized $\text{Co}_3\text{O}_4\text{-NPs}$ by *Pergularia daemia* L have shown in Figure:11 (petri plates), Figure 12 have shown in cluster column chart of antibacterial activity of $\text{Co}_3\text{O}_4\text{-NPs}$ by *Pergularia daemia* leave extract.



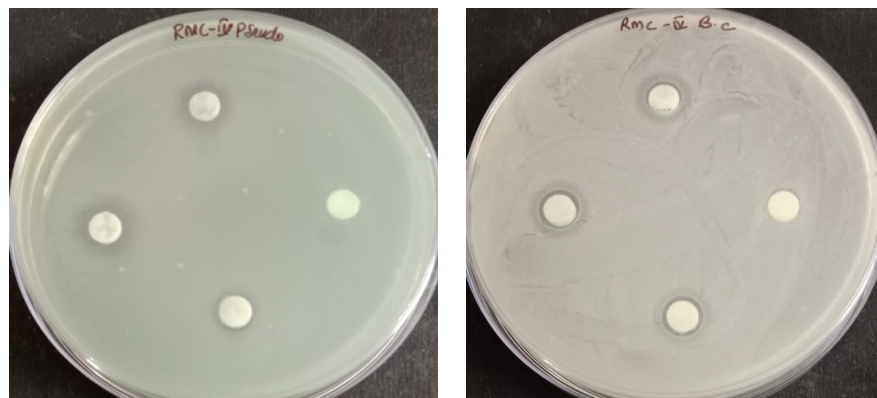


Figure:11 Antibacterial activity of Co₃O₄NPs by *Pergularia daemia* leave extract

The antibacterial activities[35-37] were studied amongst both (Gram positive and Gram negative) pathogenic bacteria. Co₃O₄NPs showed good antibacterial activity against *Pseudomonas aeruginosa* (12 mm) at 1000

mg/ml, moderate antibacterial activity have shown in *Escherichia coli* (10 mm at 1000 mg/ml and 9 mm 750 mg/ml), the worthy antibacterial activity have shown in Co₃O₄-NPS against *Staphylococcus aureus*,

Table:7.4 Antibacterial activity of Zone of inhibition values Co₃O₄NPs by *Pergularia daemia* L leave extract

Organisms	Zone of Inhibition (mm)			
	Sample (µg/ml)			Standard
	1000	750	500	
<i>Staphylococcus aureus</i>	11	11	9	15
<i>Escherichia coli</i>	10	9	7	8
<i>Pseudomonas aeruginosa</i>	12	9	7	11
<i>Bacillus cereus</i>	12	12	7	12

Antibacterial activity of Co₃O₄NPs against *Bacillus cereus* have shown unchanged activity (12 mm at 1000 and 750 mg/ml), 7 mm at 500 mg/ml of the test solutions. The zone of inhibition values have shown in Table:7.4.

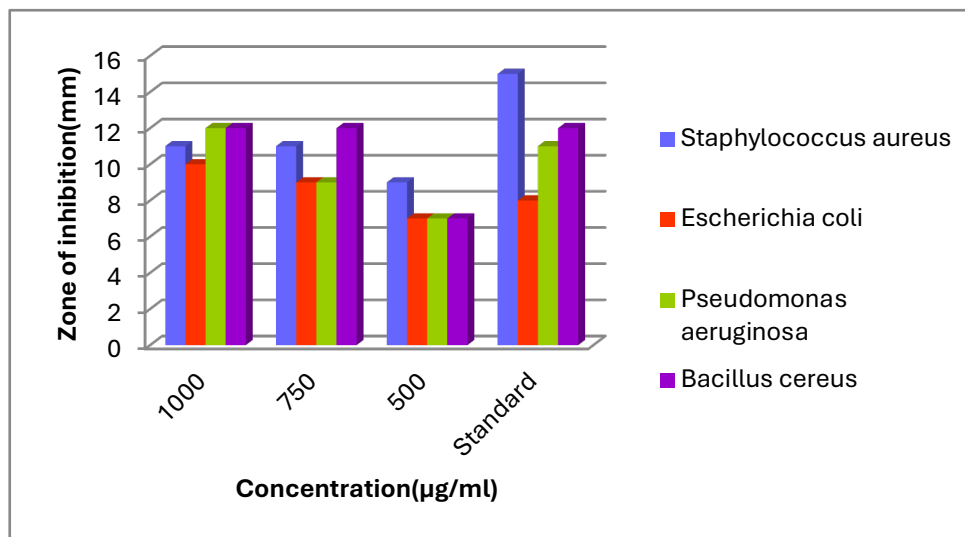


Figure:12 Cluster column chart of antibacterial activity of Co₃O₄NPs by *Pergularia daemia* leave extract

3.3.2 Antifungal activity

The antifungal activities of Co₃O₄-NPs by *Pergularia daemia* L have shown poor antifungal activity (**Figure:13 and 14**) against both the *Candida albicans* and

Trichoderma viride fungal strains in all the three concentrations solutions comparing Amphotericin-B (20µl/disc) as standard antifungal medicine. The zone of inhibition values have shown in Table.7.5

Table:7.5 Antifungal activity of Zone of inhibition values Co₃O₄NPs by *Pergularia daemia* L leave extract

Organisms	Zone of Inhibition (mm)			
	Sample (µg/ml)			Standard
	1000	750	500	
<i>Candida albicans</i>	8	8	8	20
<i>Trichoderma viride</i>	9	8	6	18

3.3.3 Antioxidant activity

The DPPH assay is widely used for screening antioxidant activity because it is sensitive enough to detect active compounds at low concentrations.

Table:7.6 Antioxidant activity of Co₃O₄NPs by *Pergularia daemia* L leave extract

S.No	Concentration (µg/ml)	AVERAGE OD	DPPH %
1	200	0.373	37.52
2	400	0.31	48.24
3	600	0.246	58.79
4	800	0.177	70.35
5	1000	0.113	81.07

DPPH is a nitrogen-centered free radical, and hence any compound that scavenges significant amounts of DPPH could reduce levels of other reactive nitrogen species in living cells. From our results, the biologically synthesized

Co₃O₄-NPs by *Pergularia daemia* L leaves extract quenched DPPH free radicals appreciably and dose-dependently (200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000 µg/ml) (**Figure.15**).



Figure:13 Plates of antifungal activity of Co₃O₄NPs by *Pergularia daemia* leave extract

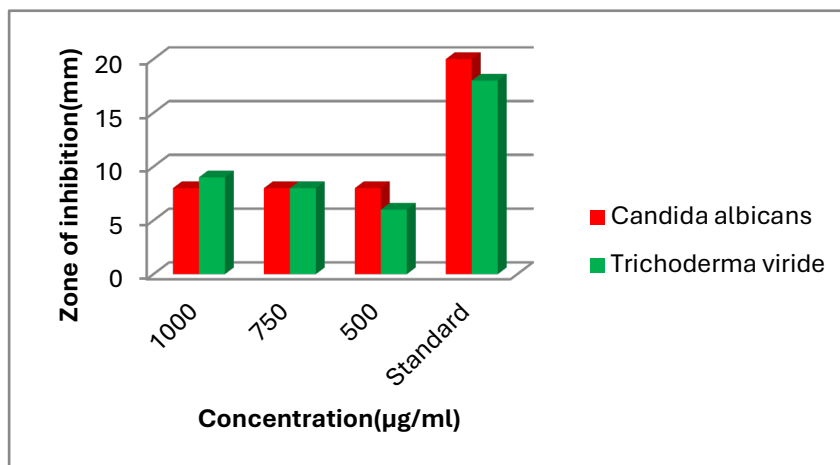


Figure:14 Cluster column chart of antifungal activity of $\text{Co}_3\text{O}_4\text{NPs}$ by *Pergularia daemia* leave extract

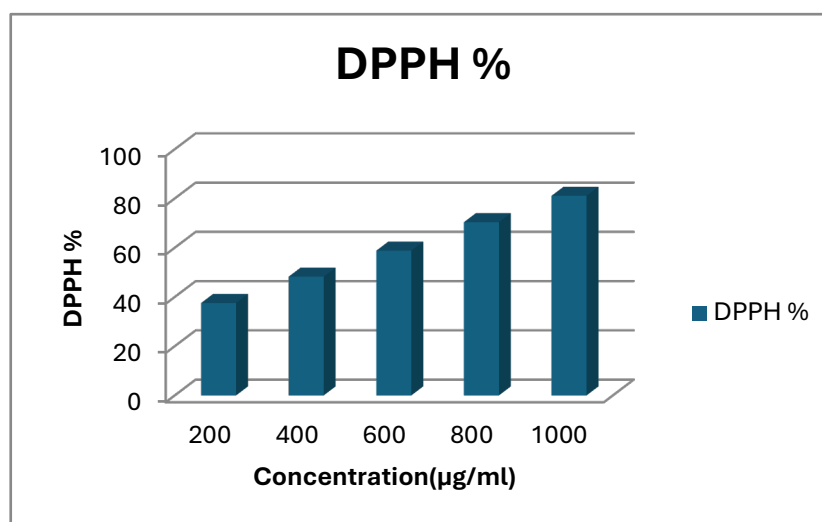


Figure:15 Cluster column chart of antioxidant activity of $\text{Co}_3\text{O}_4\text{NPs}$ by *Pergularia daemia* leave extract

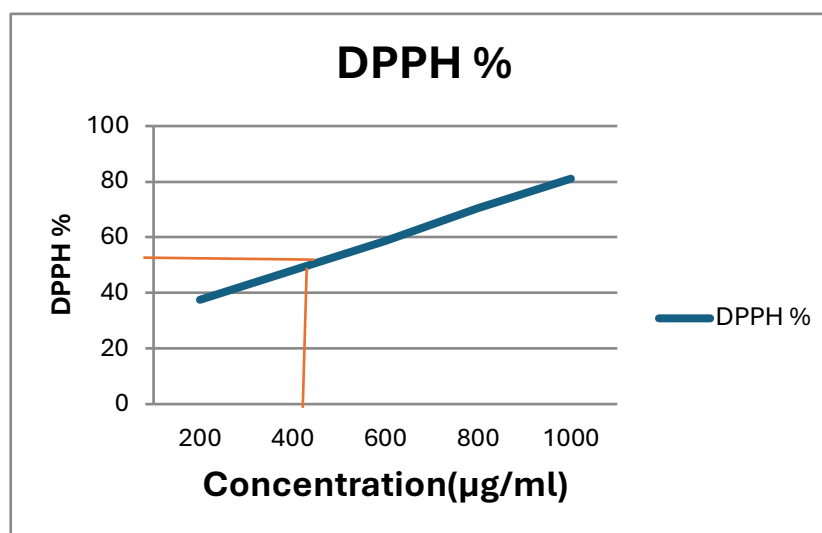


Figure:16 IC_{50} values of antioxidant activity of $\text{Co}_3\text{O}_4\text{NPs}$ by *Pergularia daemia* leave extract

However, this activity was much lower than that of the BHA standard. BHA is a pure antioxidant standard whose mechanism of action is accurately known[33]. The half of the inhibitory concentration have shown in 480 µg/ml, The IC₅₀ graph have shown in **Figure:16**. Co₃O₄-NPs by *Pergularia daemia* L however have no known mechanism of action as an antioxidant as this is one of the earliest reports on its antioxidant effect on DPPH free radicals.

with the standard drug cyclophosphamide. The anticancer activities of the cobalt oxide nanoparticles were performed with different concentrations such as 7.8, 15.6, 31.6, 62.5, 125, 250, 500 and 1000 µg/ml. The anticancer activity of cobalt oxide nanoparticles against MCF-7 increased while in the concentration of cobalt oxide nanoparticles (**Fig: 17 and 18**).

3.3.4 Anticancer activity

In vitro cytotoxic activity against MCF-7 cell line at different concentrations was evaluated and compared

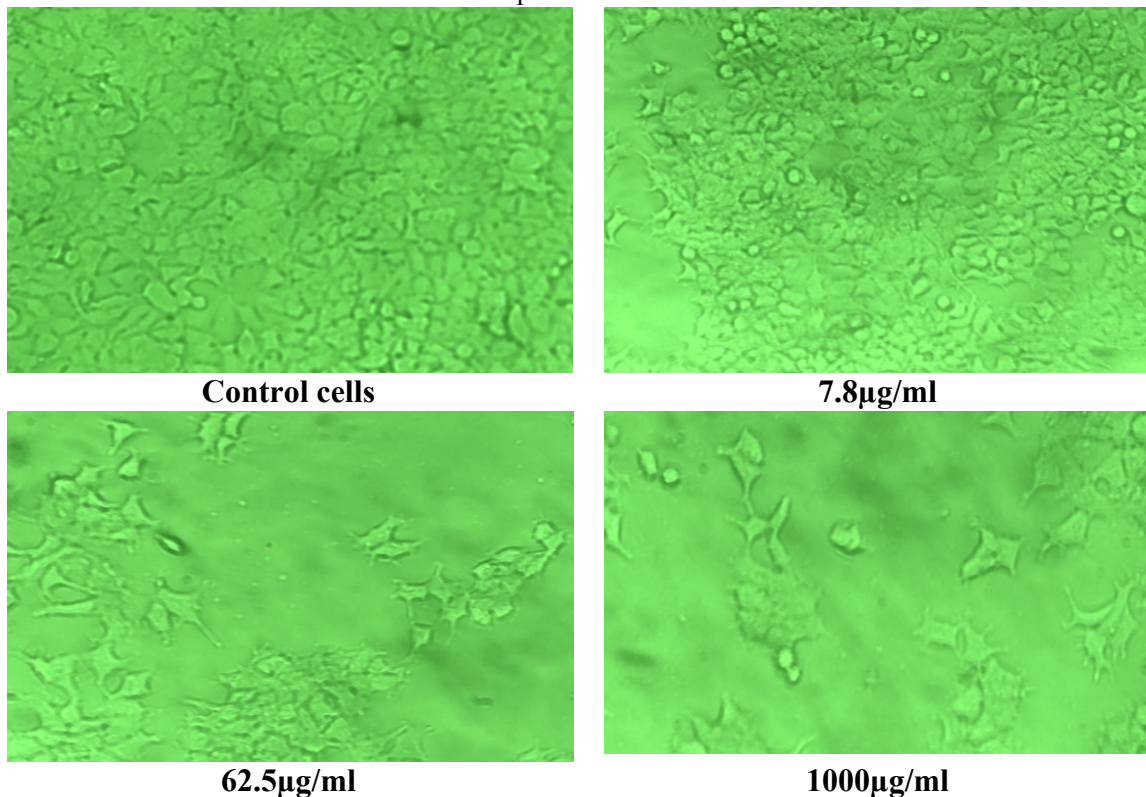


Figure:17 Anticancer activity of Co₃O₄NPs by *Pergularia daemia* leave extract

Cobalt oxide nanoparticles exhibit good results when compare with the standard cyclophosphamide. The anticancer effect of cobalt oxide nanoparticles against MCF-7 lines was performed.

Table:7.7 Anticancer activity of Co₃O₄NPs by *Pergularia daemia* leave extract

S.No	Concentration (µg/ml)	Average	Cell Viability (%)
1	1000	0.155	25.00
2	500	0.194	31.29
3	250	0.233	37.58
4	125	0.272	43.87
5	62.5	0.311	50.16
6	31.2	0.350	56.45

7	15.6	0.389	62.74
8	7.8	0.428	69.03
9	Cell control	0.620	100

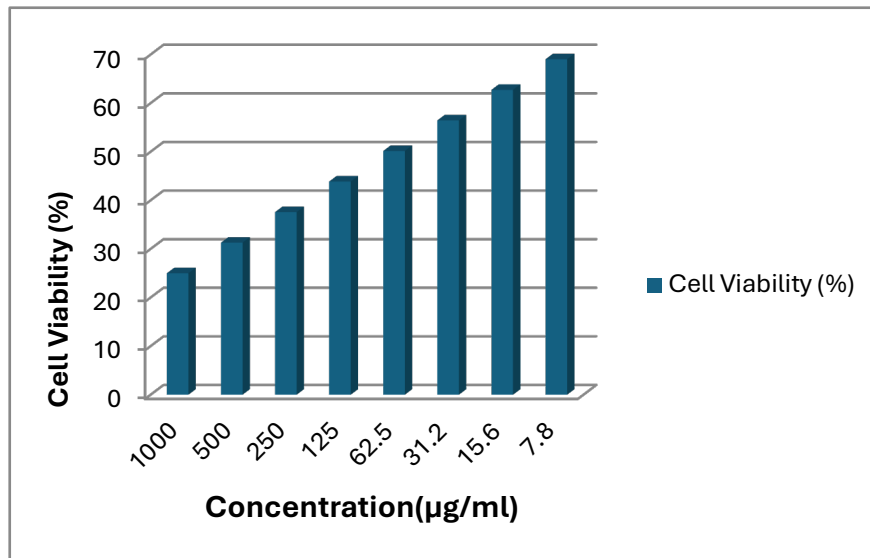


Figure:18 Cluster column chart of anticancer activity of Co₃O₄NPs by *Pergularia daemia* leave extract

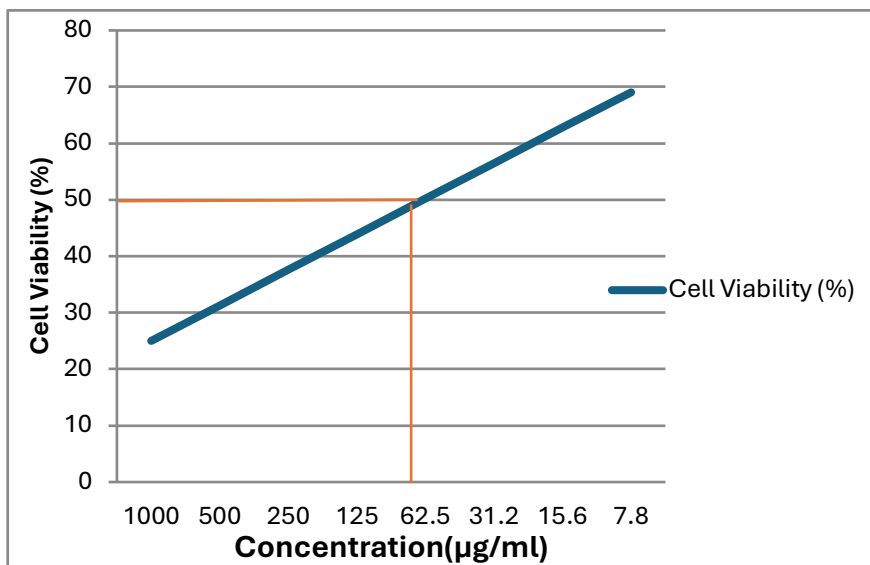


Figure:19 IC₅₀ values of anticancer activity of Co₃O₄NPs by *Pergularia daemia* L leave extract

The results show the good cytotoxic activity against the cancer cells. The concentration of cobalt oxide nanoparticles plays an important role in the anticancer activity. The cobalt nanoparticles are having the good results against MCF-7 lines in that 62.5 µg/ml show fine results followed by 125, 250, 500 and 1000 µg/ml. The lowest inhibitory action was observed from the concentration of 250 µg/ml. The IC₅₀ values (62.3 µg/ml)

of anticancer activity of cobalt oxide nanoparticles by *Pergularia daemia* L have shown in **Figure:19**.

CONCLUSION

The cobalt oxide Nanoparticles were synthesized by the green method utilizing the leaf extract of *Pergularia daemia* L. The synthesized cobalt oxide Nanoparticles were initially recognized by the production of a vivid blue precipitate during ocular examination. The UV-vis

spectrophotometer examination reveals surface Plasmon resonance bands at 273 nm and 398.15 nm. The fluorescence spectra of green produced “cobalt,-oxide” Nanoparticles indicate a fluorescence intensity of 765.01 nm and a fluorescence intensity of 640.45 au. Numerous organic phyto-constituents present in *Pergularia daemia* L. are evident in the FTIR-spectrum of leaf extract. Additionally, the FTIR-spectrum of cobalt,-oxide Nanoparticles combined with leaf extract demonstrates a diminished transmittance percentage of various organic functional groups. The principal peaks observed in the cobalt-oxide Nanoparticles are at 662 and 566 cm,- indicating the spinel structure of the cobalt oxide Nanoparticles. The crystalline structure of cobalt oxide Nanoparticles was verified using X-ray, diffraction’ (XRD), dynamic light scattering (DLS), scanning electron microscopy (SEM) with energy-dispersive X-ray’ spectroscopy (EDS), transmission electron microscopy (TEM), and Selected Area Electron Diffraction (SAED) pattern imaging, which illustrates the size, shape, and distribution of the Nanoparticles. Antibacterial, antifungal, and antioxidant properties were also evaluated. The anticancer efficacy of cobalt oxide Nanoparticles was assessed using the MTT test on the MCF-7 cell line. This study revealed that enhanced anticancer activity occurs at elevated concentrations of cobalt oxide Nanoparticles, with IC₅₀ values recorded at 62.3 µg/ml. This biosynthesis method was straightforward, scalable, and environmentally sustainable. The produced Nanoparticles exhibited enhanced efficacy in biomedical applications for cancer treatment due to their elevated anticancer activity.

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