



Green Synthesis Of *Laminaria Japonica* (Brown Algae) Seaweed Extract By Using Cerium Oxide Nanoparticles And Its Antibacterial & Antioxidant Activities

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Abstract :

An eco-friendly synthesis of cerium oxide nanoparticles (CeO_2 NPs) was achieved using *Laminaria japonica* seaweed ethanolic extract as a natural reducing and stabilizing agent. The colour change from dark brown to light greenish-grey indicated nanoparticle formation, confirmed by UV-Vis absorption at 410 nm. FTIR analysis revealed O-H, C=O, N-H, and C-O groups, indicating the role of phenolic and proteins in reduction and stabilization. XRD confirmed a cubic fluorite CeO_2 phase with 20–40 nm crystallite size, and SEM showed spherical, well-dispersed particles. Biological studies revealed that L.J- CeO_2 NPs exhibit concentration-dependent antioxidant and antibacterial activities. In the DPPH assay, %RSA increased with concentration; notably, CeO_2 NPs outperformed ascorbic acid at low doses due to $\text{Ce}^{3+}/\text{Ce}^{4+}$ redox activity. Antibacterial results showed broad-spectrum inhibition against *E. coli*, *Pseudomonas*, *Staphylococcus*, and *Bacillus*, with Gram-negative strains being more sensitive. These findings highlight *L. japonica*-derived CeO_2 NPs as stable, biocompatible, and multifunctional nanomaterial for potential biomedical and catalytic applications.

Key words: *Laminaria japonica*, *Cerium dioxide nanoparticles*, *Antibacterial activity*, *Antioxidant activity*

Introduction

Nanotechnology has emerged as one of the most dynamic and rapidly advancing fields of modern science, focusing on the design, synthesis, and application of materials with dimensions typically below 100 nm [1]. At this Nano scale, materials exhibit unique optical, catalytic, electronic, and biological properties that differ significantly from their bulk counterparts, enabling a wide range of applications in medicine, catalysis, energy, and environmental remediation [2]. Among various nanomaterials, metal oxide nanoparticles have attracted substantial interest due to their remarkable physicochemical stability, tunable surface properties, and multifunctional behaviour in biomedical and technological fields [3]. Cerium oxide (**CeO₂**), commonly known as Nano ceria, is a rare-earth metal oxide that has received extensive attention because of its excellent redox behaviour, oxygen storage capacity, and catalytic activity. The reversible conversion between Ce³⁺ and Ce⁴⁺ oxidation states allows CeO₂ to act as an effective antioxidant and antibacterial agent, which makes it highly suitable for biomedical and environmental applications [4]. However, traditional physical and chemical synthesis methods often involve toxic chemicals, high temperatures, and energy-intensive processes, which limit their sustainability and biocompatibility [5]. To address these concerns, green synthesis approaches utilizing biological sources have emerged as an eco-friendly, cost-effective, and sustainable alternative [6]. Marine seaweeds (macro algae) have gained considerable attention in green nanotechnology due to their richness in natural biomolecules that can act as reducing, capping, and stabilizing agents [7]. Seaweeds contain various secondary metabolites such as phenolics, flavonoids, proteins, polysaccharides (alginates, laminarin, fucoidan), and terpenoids, which play a vital role in reducing metal ions to nanoparticles and stabilizing them to prevent agglomeration [8]. Among them, *Laminaria japonica*—a brown seaweed widely distributed in cold marine waters—is particularly rich in fucoidan, laminarin, and alginic acid, compounds known for their antioxidant, *Antibacterial*, and reducing activities [9]. These phytoconstituents make *L. japonica* an ideal natural source for the biosynthesis of metal oxide nanoparticles. In the present study, cerium oxide nanoparticles (CeO₂ NPs) were synthesized using *Laminaria japonica* extract as a biogenic reducing and stabilizing agent. This approach offers a green, non-toxic, and sustainable synthesis route that aligns with environmental safety and enhances nanoparticle biocompatibility. The synthesized CeO₂ NPs were characterized using UV–Visible spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) analyses to confirm their structural, optical, and morphological properties. Furthermore, the antibacterial activity of the biosynthesized CeO₂ nanoparticles was evaluated against both Gram-positive and Gram-negative bacteria to explore their potential biomedical applications. The antibacterial mechanism is mainly attributed to the generation of reactive oxygen species (ROS), disruption of bacterial cell walls, and interference with enzymatic and DNA functions, ultimately leading to microbial cell death [10]. The presence of bioactive compounds from *L. japonica* on the nanoparticle surface may further enhance antibacterial performance through synergistic effects. Thus, this study demonstrates an eco-friendly and biologically driven approach for

synthesizing CeO_2 nanoparticles with promising antibacterial efficacy, suitable for use in pharmaceutical, biomedical, and environmental disinfection applications.

2. MATERIALS AND METHODS

2.1. . MATERIALS :

2.1.A) Chemicals :

Cerium Oxide Nanoparticles(APS:<100nm, Purity 99%) (EDAYUKT INDIA PVT. LTD) is used in the synthesis of Ceria nanoparticles and without any further cleaning , and Ethyl alcohol(99.99% pure), Con.Hydrochloric acid.

2.1.B) Seaweed Collection :

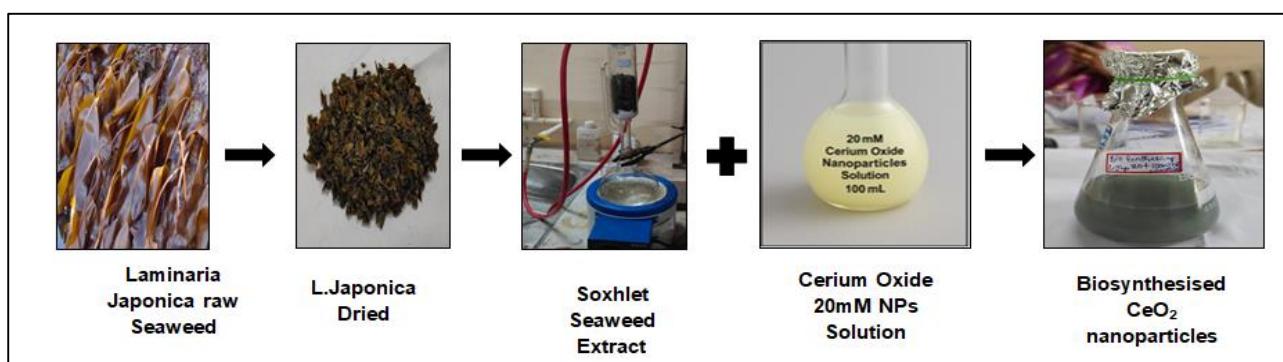
Laminaria japonica (Saccharina Japonica) (Brown Algae) was purchased from a local seaweed industry HVG Industries (GST No: 33AAIFH1476K1Z9) , Bharathi Nagar, Maduravoyal, Chennai – 600095, Tamil Nadu, India.

2.2.A) Preparation of the *Laminaria japonica* alga extract:

The collected *Laminaria japonica* seaweed samples were thoroughly washed with tap water followed by double-distilled water to remove adhering impurities such as salts, sand, and epiphytes. The cleaned samples were then shade-dried at room temperature for 4–6 days until complete dehydration. The dried biomass was cut into small fragments and ground into a fine powder using an electric grinder[23]. For the preparation of the ethanolic extract, 50 g of the powdered seaweed was placed in a Soxhlet apparatus (Fig. 1) and extracted with 95% ethanol for approximately 8 hours at 35 °C. The obtained extract was subjected to simple distillation to remove excess solvent and concentrated to obtain the crude extract. The resulting

Laminaria japonica extract was stored at 4 °C in a refrigerator for subsequent use in nanoparticle synthesis. The *Laminaria japonica* extract colour was dark brown.

Figure.1



2.2.B) Preparation of 20mM CeO₂ Nanoparticles

An accurately weighed amount of 0.34422 g of cerium oxide Nano powder was transferred into a clean 100 mL volumetric flask. Before use, the flask was thoroughly washed with distilled water and rinsed with ethanol to ensure the removal of any impurities. Since cerium oxide is insoluble in ethanol, approximately 10 mL of concentrated hydrochloric acid (HCl) was added to facilitate dissolution until the pH shifted from neutral (blue litmus) to acidic (red litmus). The mixture was then gently swirled to obtain a clear homogeneous solution, confirming the complete dissolution of CeO₂ with no visible solid residue remaining. (Fig. 1)

2.2.C) Biosynthesis of the alga *Laminaria japonica* Seaweed extract with Ethanolic solution of CeO₂ NPs:

For the biosynthesis of cerium oxide (CeO₂) nanoparticles, the isolated *Laminaria japonica* seaweed extract was utilized as a natural reducing and stabilizing agent. A 100 mL ethanolic solution of CeO₂ nanoparticles (20 mM) was first prepared, and 20 mL of the seaweed extract was transferred into a clean conical flask and placed on a magnetic stirrer. During continuous stirring, 100 mL of the 20 mM CeO₂ ethanolic solution was added dropwise to the extract. The progression of the biosynthetic reaction was visually indicated by a noticeable colour change from a Dark Brown coloured solution to a Light greenish-grey colour, confirming the formation of CeO₂ nanoparticles (Fig. 1). The synthesized CeO₂ nanoparticle suspension was collected in 5 mL vials for characterization using UV–Visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy[17]. The remaining solution was gently evaporated to obtain solid residue pellets, which were further analysed by X-ray Diffraction (XRD) to determine the crystalline structure and particle size, and by Scanning Electron Microscopy (SEM) to study surface morphology.

3. CHARACTERIZATION

UV–Visible Spectroscopic Analysis:

The successful synthesis of *Laminaria japonica*–mediated CeO₂ nanoparticles was verified through UV–Visible spectrophotometric analysis using a LABINDIA ANALYTICAL UV-3092 spectrometer equipped with a 1 cm quartz cuvette. The absorbance spectrum was recorded over a wavelength range of 400–4000 nm to confirm nanoparticle formation.

Fourier transform InfraRed (FT-IR) analysis

Fourier Transform Infrared (FT-IR) spectroscopy was performed to identify the functional groups present in the synthesized nanoparticles. The analysis was carried out using the KBr pellet method in a 1:100 sample-to-KBr ratio. The spectra were recorded in the wavenumber range of 500–4500 cm⁻¹ using a Shimadzu IR Affinity-ICE spectrometer (Model IV) at standard resolution.

X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) analysis was carried out using a Bruker D8 Advance diffractometer to determine the crystalline structure and phase purity of the synthesized cerium oxide nanoparticles. For the measurement, the nanoparticle powder was finely ground and uniformly spread on a glass slide. The diffraction patterns were recorded using Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) over a 2θ range of 10° – 80° , with a step size of $0.02^\circ/\text{s}$. The obtained data were analysed using X'Pert Highscore software (version 3.0). The average crystallite size was calculated from the full width at half maximum (FWHM) of the prominent peaks using the Debye–Scherrer equation.

Scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX) analysis

Scanning Electron Microscopy (SEM) was employed to study the morphology and particle size of the synthesized cerium oxide nanoparticles. The samples were mounted on aluminum stubs using double-sided carbon tape and subsequently coated with a thin layer of gold to enhance surface conductivity. SEM images were captured at an accelerating voltage of 20 kV. The elemental composition of the nanoparticles was analyzed using Energy Dispersive X-ray (EDX) spectroscopy, performed with the same instrument used for SEM imaging. The EDX spectra were obtained at an energy range around 1.492 keV to confirm the presence of constituent elements.

4. Result and discussion

UV–Visible spectroscopy of *Laminaria japonica* - CeO₂ nanoparticles

The UV–Visible absorption spectrum of *Laminaria japonica*–mediated ethanolic solution of cerium oxide (CeO₂) nanoparticles displayed a distinct absorption maximum at 300 and 410 nm(Major peak), confirming the formation of CeO₂ nanoparticles. This absorption band corresponds to the charge-transfer transition between O (2p) and Ce (4f) orbitals, a characteristic feature of cerium oxide in its Nano crystalline state. The presence of a prominent peak in the visible region indicates surface modification and stabilization of nanoparticles by the bioactive[10] compounds in the *L. japonica* extract, which act as natural reducing and capping agents facilitating the reduction of cerium ions to nanoparticles while simultaneously preventing their aggregation through surface capping. These results confirm the successful biosynthesis of stable, crystalline CeO₂ nanoparticles through an environmentally benign green synthesis route using *L. japonica* seaweed extract[15][17][18].

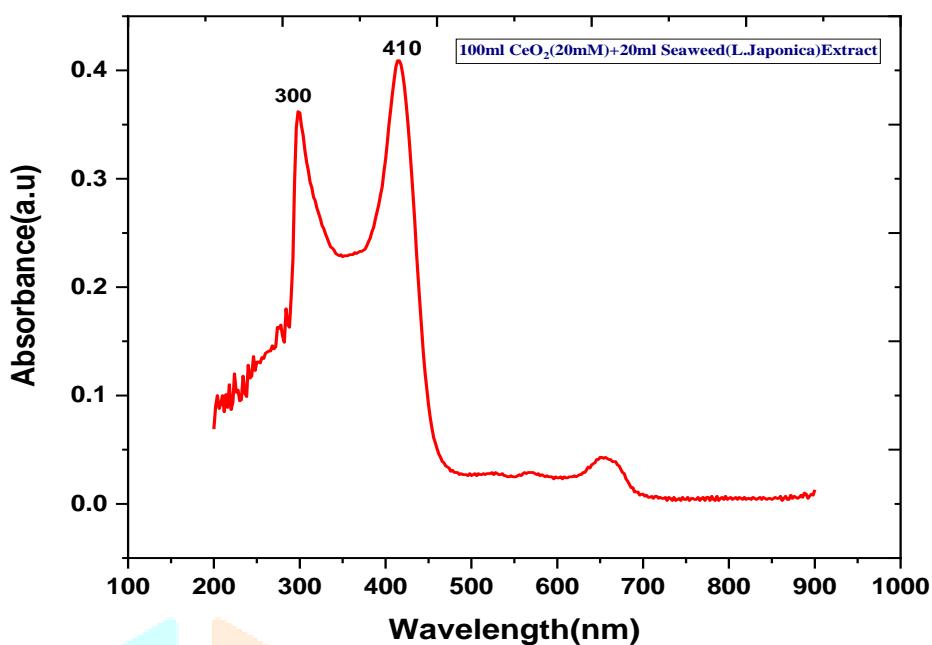


Figure 2: UV-Visible spectrum of Seaweed extract of *Laminaria japonica* biosynthesized with CeO_2 Nps

FT-IR evaluation of *Laminaria japonica* - CeO_2 nanoparticles

The Fourier Transform Infrared (FTIR) spectrum of the biosynthesized cerium oxide nanoparticles using *Laminaria japonica* extract reveals the presence of several characteristic functional groups that play a vital role in nanoparticle formation and stabilization. The broad absorption band at **3338.85 cm^{-1}** corresponds to the O–H stretching vibration of hydroxyl groups, indicating the presence of phenolic compounds, alcohols, and polysaccharides in the seaweed extract[18]. The peak at **2974.23 cm^{-1}** is attributed to C–H stretching vibrations of aliphatic groups derived from proteins and lipids. A distinct peak observed at **1722.43 cm^{-1}** is assigned to the stretching vibration of carbonyl (C=O) groups, suggesting the involvement of aldehydes, ketones, or carboxylic acids in reducing Ce^{4+} ions to CeO_2 nanoparticles. The absorption band at **1201.65 cm^{-1}** corresponds to C–O–C or C–O stretching vibrations of polysaccharides and other carbohydrate derivatives that act as stabilizing agents[17]. The peak at **1010.70 cm^{-1}** indicates C–N stretching vibrations, confirming the presence of amine groups from proteins or peptides, which may help cap the nanoparticles. The characteristic band around **694.37 cm^{-1}** corresponds to Ce–O stretching vibrations, confirming the successful formation of cerium oxide nanoparticles[10]. Overall, the FTIR analysis confirms that the bioactive compounds in *Laminaria japonica* extract act as natural reducing and stabilizing agents, facilitating an eco-friendly synthesis of CeO_2 nanoparticles and acted as natural stabilizers, preventing nanoparticle agglomeration[16].

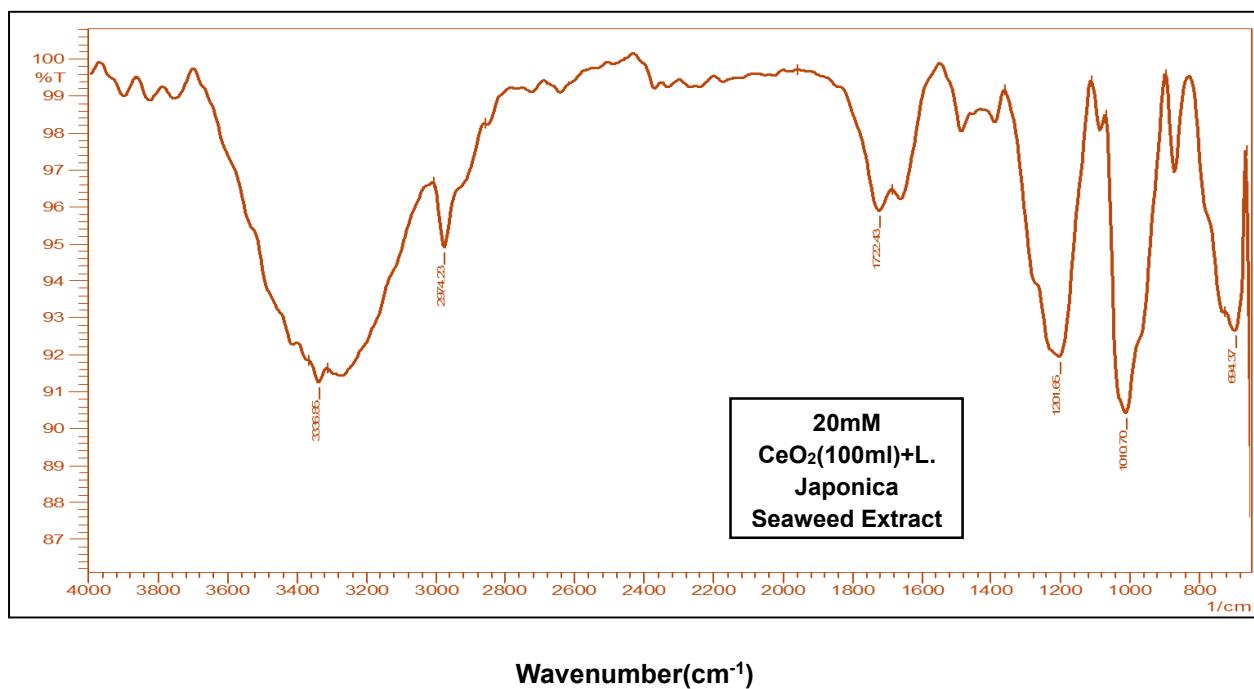


Figure 3: FTIR spectrum of Seaweed extract of *Laminaria japonica* biosynthesized with CeO_2 NPs

X-ray Diffraction (XRD) Analysis

The crystalline structure of the cerium oxide nanoparticles synthesized using *Laminaria japonica* seaweed extract was confirmed by X-ray diffraction (XRD). The recorded diffractogram exhibited several characteristic diffraction peaks at $2\theta = 28.55^\circ$, 33.06° , 47.51° , and 56.37° , which correspond to the (111), (200), (220), and (311) planes, respectively. These reflections are well indexed to the cubic fluorite phase of CeO_2 (JCPDS No. 34-0394)[15]. Additional low-intensity peaks appearing at higher angles (65.18° , 69.46° , and 76.71°) also matched the (400), (331), and (420) planes, further confirming the crystalline nature of the nanoparticles. From Bragg's law ($n\lambda = 2d \sin\theta$) the interplanar spacings (d) calculated for the observed peaks are consistent with a cubic lattice. The calculated interplanar spacing (d) values for the dominant peaks were 3.125 \AA (111), 2.709 \AA (200), 1.913 \AA (220), and 1.632 \AA (311)(Table.1). Using the relation $a = d\sqrt{h^2+k^2+l^2}$ for the cubic system, the lattice parameter obtained from each indexed reflection is close to 5.41 \AA matching the standard JCPDS No. 34-0394 value for cubic CeO_2 , consistent with reported values for fluorite CeO_2 and confirming the oxide adopts the expected cubic fluorite structure. The narrow and symmetric nature of the peaks suggests high crystallinity and the absence of secondary phases or cerium hydroxide impurities, indicating that the biosynthetic process yielded phase-pure CeO_2 nanoparticles. The average crystallite size (D) can be estimated from the peak broadening using the Scherrer equation, $D = \frac{K\lambda}{\beta \cos\theta}$ where $K=0.9$ is the shape factor, $\lambda=1.5406\text{\AA}$ is the wavelength of $\text{Cu K}\alpha$ radiation, β is the full width at half maximum (FWHM) in radians, and θ is the Bragg angle[15]. Using the observed FWHM values ($\sim 0.47^\circ$ – 0.63° 2θ) from the dominant peaks, the average crystallite size was found to be in the range of 20–40 nm, indicating nanoscale dimensions of the synthesized particles. The XRD pattern clearly confirms that the *Laminaria japonica* seaweed extract effectively reduced and stabilized Ce^{4+} ions to form highly crystalline cerium

oxide nanoparticles with a pure cubic fluorite structure. The absence of any additional peaks related to impurities further demonstrates the efficiency of the green synthesis route in producing high-purity CeO₂ NPs[17].

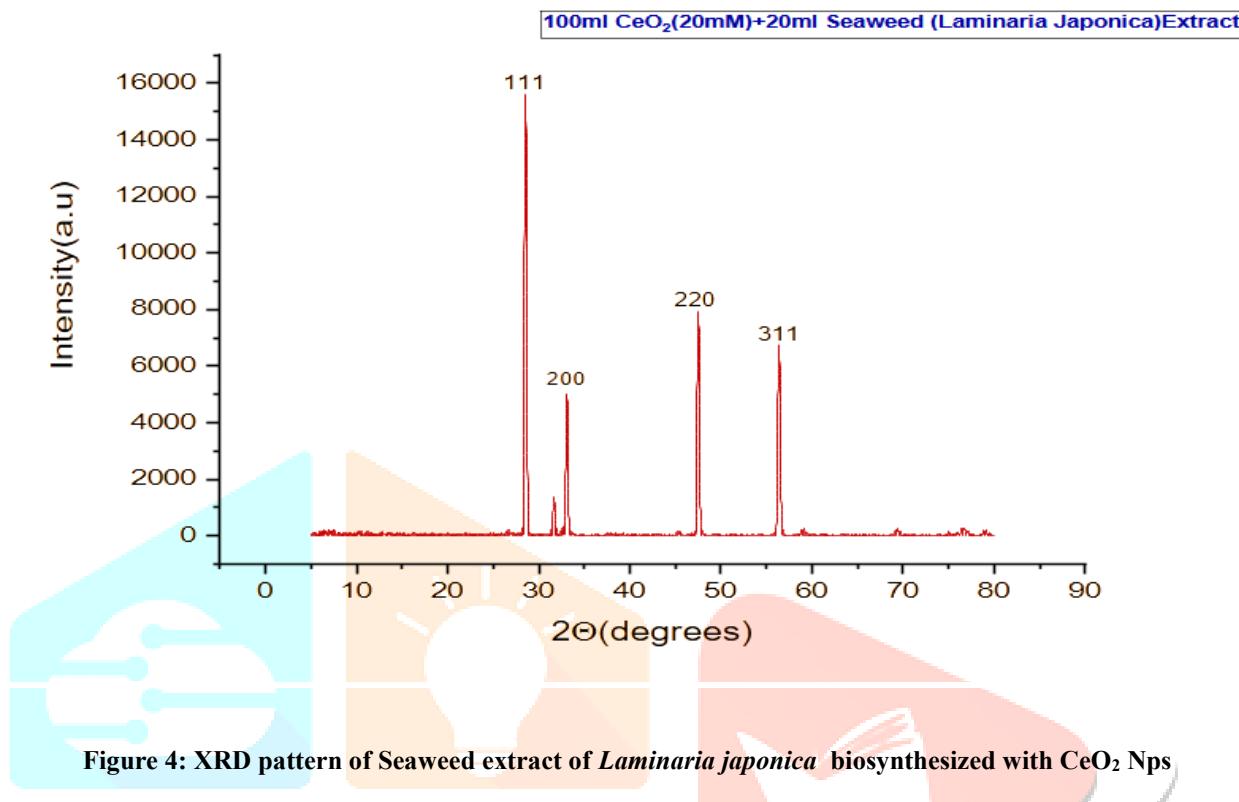


Figure 4: XRD pattern of Seaweed extract of *Laminaria japonica* biosynthesized with CeO₂ NPs

Table.1

2θ (°)	d-spacing (Å)	FWHM (°)	Assigned Plane (hkl)	Phase Identification	Crystallite Size (nm)
28.55	3.125	0.25	(111)	CeO ₂ (Cubic, JCPDS 34-0394)	32.8
33.06	2.709	0.25	(200)	CeO ₂ (Cubic)	33.2
47.51	1.913	0.25	(220)	CeO ₂ (Cubic)	34.7
56.37	1.632	0.25	(311)	CeO ₂ (Cubic)	36.0

Average D = 34.2 nm

Structural analysis of SW-CeO₂ NPs (Scanning Electron Microscope (SEM)):

Fig.5 Shows the Scanning Electron Microscope (SEM) of the prepared CeO₂ nanoparticles. The nanoparticles exhibit an irregular shape with slight agglomeration, indicating that the biomolecules from the seaweed extract effectively contribute to their stabilization and capping during biosynthesis[17]. The aggregation may be attributed to the interaction between hydroxyl and carbonyl groups of polysaccharides, proteins, and phenolic compounds that act as reducing and stabilizing agents during the biosynthesis process. At 5.00 KX(5,000 times magnification) magnification, the nanoparticles display a **spherical to semi-spherical shape** with slight agglomeration attributed to natural capping agents present in the seaweed extract[10]. The average particle size is estimated to be in the range of **20–50 nm**. The relatively smooth texture and Nano scale dimensions confirm the successful formation of CeO₂ nanoparticles[1]

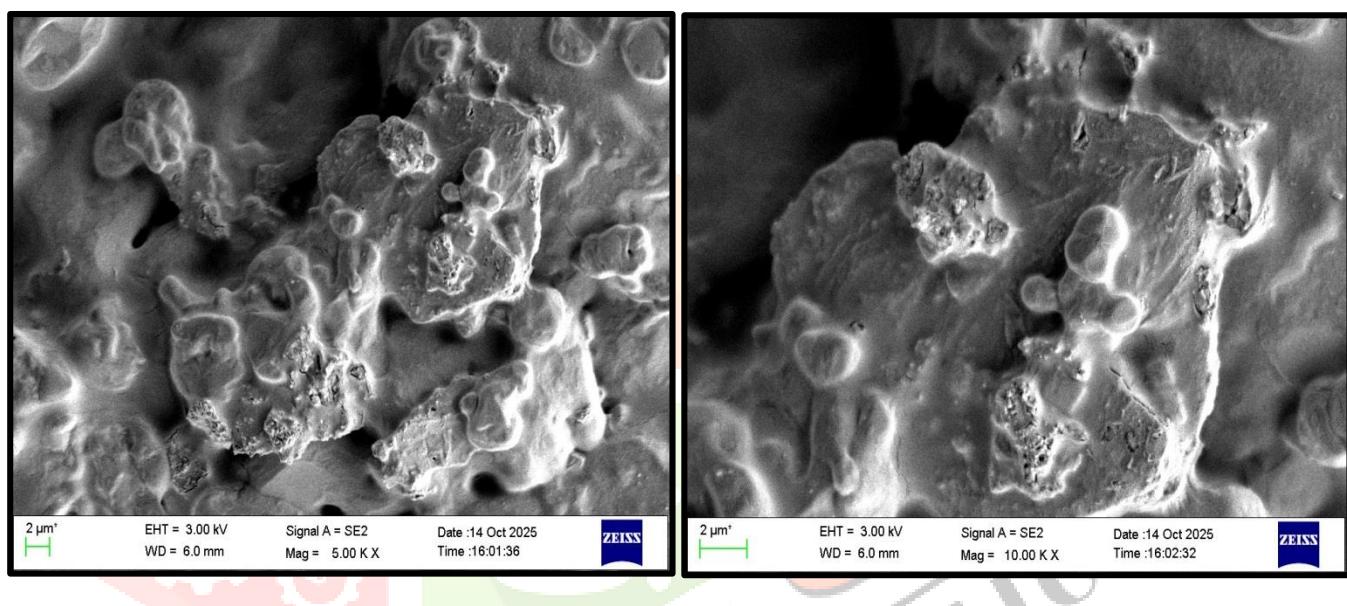


Fig. 5: SEM images of the synthesized CeO₂ NPs by L.Japonica

Energy Dispersive X-ray (EDX) Analysis

The EDX spectrum (Fig.6.a)) confirm the successful biosynthesis of cerium oxide (CeO₂) nanoparticles using *Laminaria japonica* extract. The prominent peaks corresponding to cerium (Ce) in 26.75 W% and oxygen (O) in 11.81 W % validate the formation of cerium oxide as the major phase in the synthesized material. The presence of these two elements indicates that the Ce³⁺ ions from the precursor were effectively reduced and stabilized by the phytochemicals present in the seaweed extract, leading to the formation of CeO₂ nanoparticles[18].

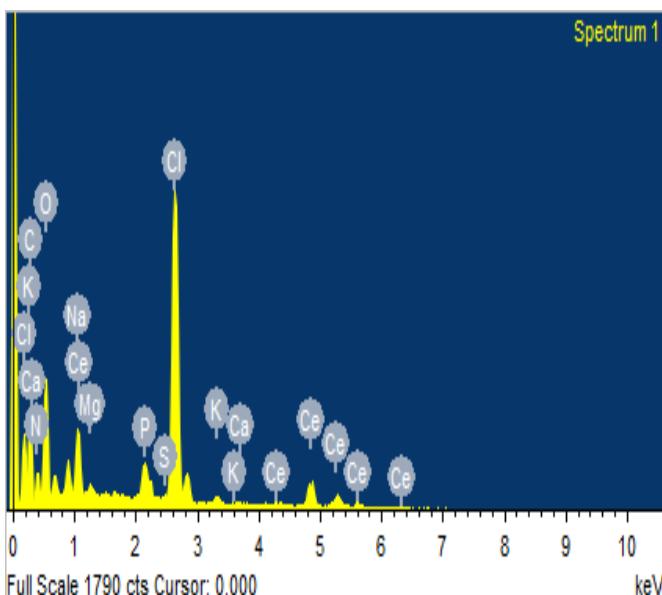


Fig.6.a

Table.1

Element	Weight %	Atomic %
C K	21.89	43.30
N K	6.97	11.82
O	11.81	17.54
Na K	2.76	2.85
Mg K	0.38	0.37
P K	-0.60	-0.46
S K	-0.38	-0.28
Cl K	29.33	19.66
K K	0.87	0.53
Ca K	0.23	0.14
Ce	26.75	4.54
Totals	100.00	

Elemental Composition

The quantitative bar chart (Fig.6.b) shows the weight percentage of various elements detected on the nanoparticle surface. The major constituents are Ce (~27%) and O (~10–12%), confirming CeO₂ as the main compound. The peaks of C, N, Na, Mg, P, S, Cl, K, and Ca are also visible in smaller quantities, which are typically attributed to the biomolecules and residual organic compounds from the *Laminaria japonica* extract. These elements originate from seaweed metabolites such as polysaccharides, proteins, phenolic, and alginates, which serve as natural capping and stabilizing agents during nanoparticle formation[10].

4. Biological Applications

4.1. Antibacterial activity

4.1.a) Testing of Antibacterial activity

The paper disc method is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous samples. An agar plate is uniformly inoculated with the test organism and a paper disc was impregnated with a fixed concentration is placed on the agar surface. Growth of the organism and diffusion commence simultaneously resulting in a circular zone of inhibition in which the amount of it exceeds inhibitory concentrations. The diameter of

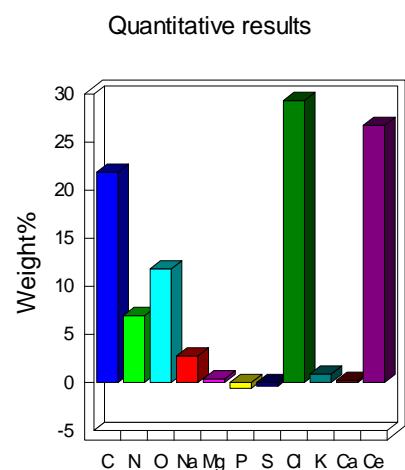


Fig.6.b

the inhibition zone is a function of the amount of drug in the disc and susceptibility of the microorganism.

This test must be rigorously standardized since zone size is also dependent on inoculum size, medium composition, and temperature of incubation, excess moisture and thickness of the agar. The Antibacterial activity of the prepared samples was determined by using paper disc method. The inoculated samples were then examined for inhibition zones (in mm) by zone reader, which indicates Antibacterial activity.

4.1.b)Procedure:

Preparation of nutrient agar:

Agar medium is prepared by mixing nutrient medium with commercially available agar powder if nutrient medium is not available then it can be individually prepared by water, peptone, beef extract and agar. 1gm of agar was weighted and was mixed with nutrient medium in 100ml of water and poured into petri plates which are kept for autoclave at 121°C at 15lbs.

Using a standard Make a suspension at an appropriate turbidity of the bacterial culture to be tested. 1 to 2 drops of gram positive and gram negative bacteria was placed on sterile nutrient agar plate each for spreading. Spreading was done with the help of sterile spreader. Plates were incubated within 15 minutes after applying the disks. The plates should be incubated soon after placing the disks since the test is standardized under conditions where diffusion of the test samples and bacterial growth commences table of antibacterial susceptibilities, determined if the strain is resistant, intermediate, or susceptible to the samples tested[29].

Antibacterial activity of samples

Following inoculation, the bacteria were incubated for a standardized period of 24 hours to allow for the development of observable zones of inhibition around sample discs [25].

The results of the Antibacterial activity test were systematically documented and are summarized in the table. The zones of inhibition, indicative of the effectiveness of each sample against the respective isolates, were measured and recorded.



Fig.7 : Sample : Biosynthesised 20mM ethanolic Cerium Oxide nanoparticles by *Laminaria Japonica* Seaweed Extract

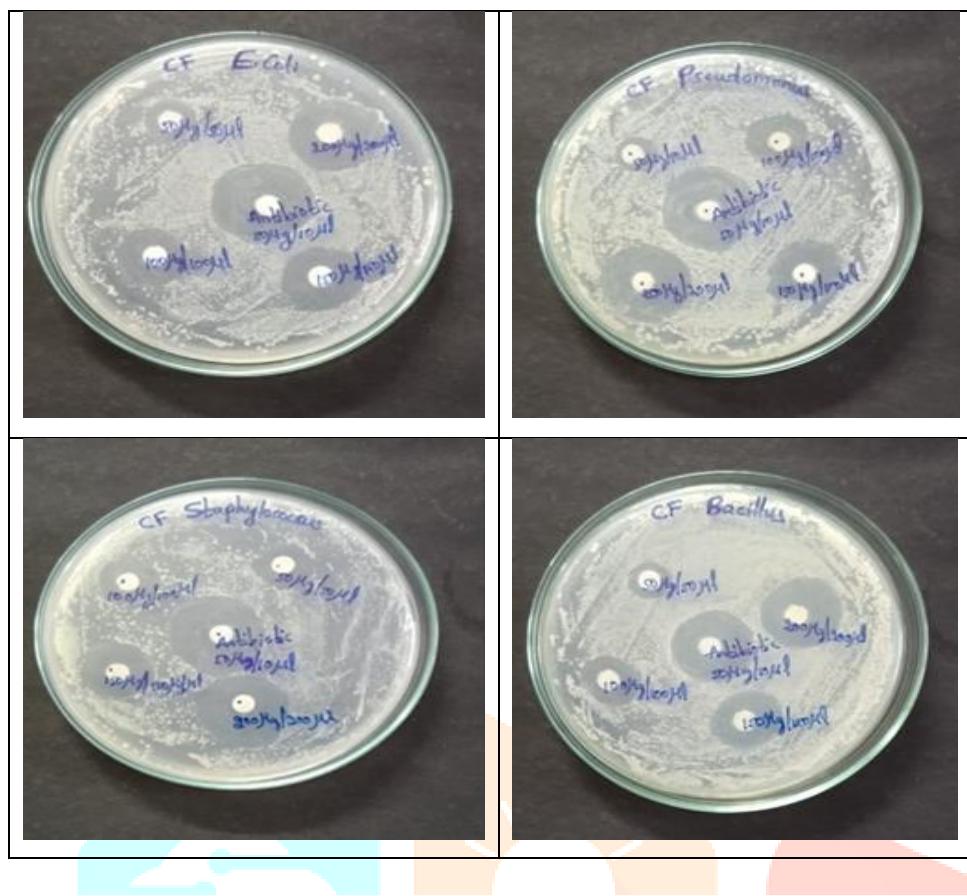


Fig.8 : Antibacterial activity of *Laminaria Japonica*-CeO₂ NPs against *E.Coli*, *Pseudomonas*, *Staphylococcus*, *Bacillus* species bacteria at different concentrations.

S.No	Bacterial strain	Concentration level (µl)Zone of inhibition (mm)					Antibiotic AMP 50µg/10µl
		50µg/50µl	100µg/100µl	150µg/150µl	200µg/200µl		
1	E.Coli(gram negative)	0.3	0.7	0.8	1		1.4
2	Psedomonas(gram negative)	0.2	0.7	0.8	1.1		1.3
3	Staphylococcus(gram positive)	0.3	0.4	0.7	1		1.2
4	Bacillus species (gram positive)	0.2	0.4	0.8	0.9		1.1

Table:1 Showing Antibacterial activity of *Laminaria Japonica*-CeO₂ NPs against selected bacteria at different concentrations.

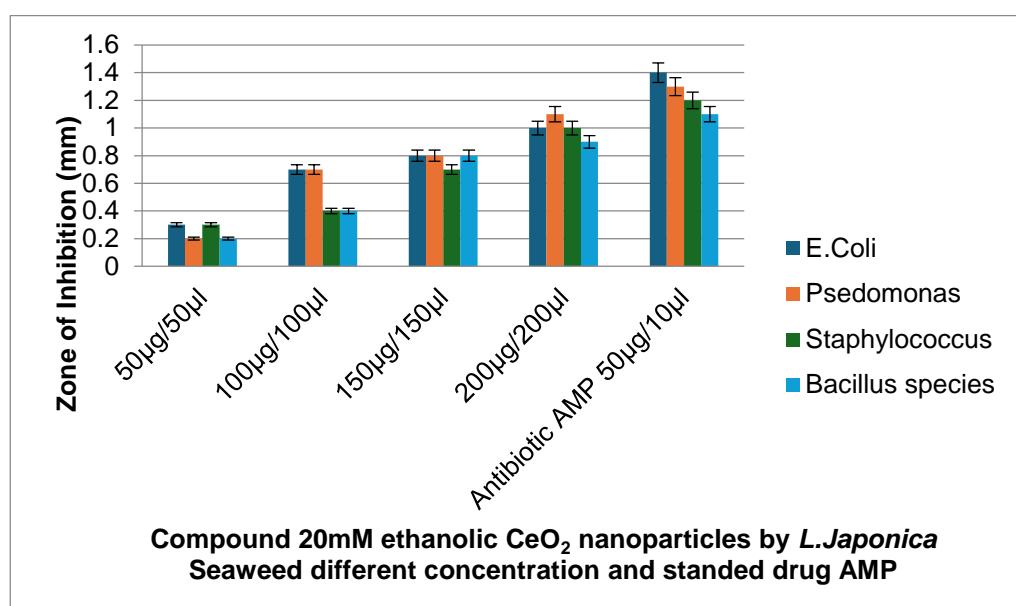


Figure:9. Showing Antibacterial activity of Biosynthesised 20mM ethanolic Cerium Oxide nanoparticles by *Laminaria Japonica* Seaweed Extract against bacterial strains with different concentration.

The synthesized nanomaterial exhibits broad-spectrum antibacterial activity against both Gram-positive (*Staphylococcus*, *Bacillus*) and Gram-negative (*E. coli*, *Pseudomonas*) bacteria. Activity increases with concentration, confirming that nanoparticle dosage influences antibacterial efficiency[29]. *E. coli* and *Pseudomonas* were the most sensitive, while *Bacillus* showed slightly lower response. The material can be considered a potential Antibacterial nanocomposite, especially useful in biomedical coatings or wound dressings.

4.2. Antioxidant Potential (DPPH ASSAY)

The antioxidant potential of synthesis nanoparticles Biosynthesised 20mM Cerium Oxide nanoparticles by *Laminaria Japonica* Seaweed Extract was evaluated using the DPPH radical scavenging assay. The absorbance values obtained at different concentrations are summarized below. For the synthesis nanoparticles, the recorded absorbance values were 0.43, 0.46, 0.59, 0.44, and 0.67 across the tested concentrations. A progressive change in absorbance was observed, indicating moderate free radical scavenging activity compared with the standard and other solvent extracts. The lowest absorbance value was 0.43, while the highest was 0.67, suggesting dose-dependent variation in antioxidant activity[15].

These findings demonstrate that the synthesis nanoparticles DPPH scavenging ability, though less pronounced compared to of Biosynthesised 20mM Ethanolic Cerium Oxide NPs by *Laminaria Japonica* Seaweed Extract.

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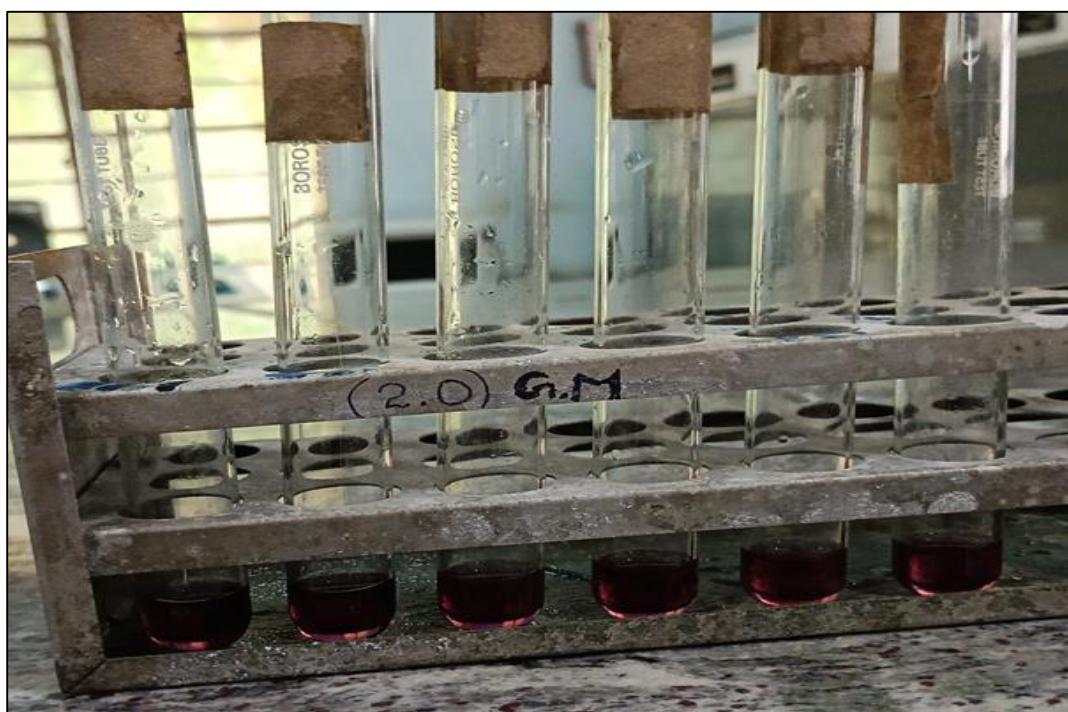


Figure:10. Biosynthesised 20mM ethanolic Cerium Oxide NPs by *Laminaria Japonica* Seaweed Extract different concentration with DPPH assay

S.No.	Sample	Concentration ($\mu\text{g}/\mu\text{L}$)	% Inhibition of DPPH (or) % Radical Scavenging Activity (%RSA)
1	L.J-CeO ₂ NPs	22	34.2
		50	39.8
		75	44.5
		100	47.2
		200	53.7
2	Ascorbic acid (Standard antioxidant)	22	28.06
		50	36.67
		75	72.21
		100	78.74
		200	82.90

Table:2. Biosynthesised 20mM Cerium Oxide nanoparticles by *Laminaria Japonica* Seaweed Extract different concentration and standard ascorbic acid

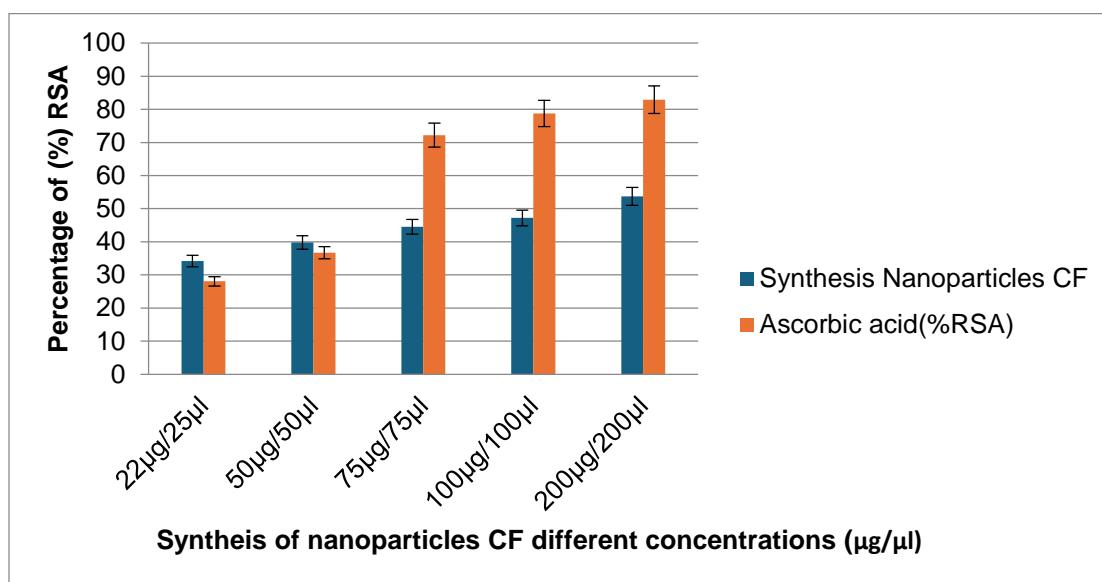


Figure:11. Biosynthesised 20mM ethanolic Cerium Oxide nanoparticles by *Laminaria Japonica* Seaweed Extract different concentration and standard ascorbic acid

Both L.J–CeO₂ NPs and ascorbic acid show an increase in %RSA with increasing concentration, confirming that higher nanoparticle or antioxidant levels lead to stronger free-radical scavenging. At lower concentrations (22–50 µg/µL), the L.J–CeO₂ NPs show better %RSA than ascorbic acid. This suggests that CeO₂ NPs possess rapid electron-donating ability at low doses due to the Ce³⁺/Ce⁴⁺ redox pair. At higher concentrations (75–200 µg/µL), ascorbic acid shows much stronger antioxidant activity than CeO₂ NPs[15]. This is expected since ascorbic acid is a pure antioxidant, while CeO₂ NPs act mainly through catalytic redox cycling, not direct radical neutralization. L.J–CeO₂ NPs exhibit significant, concentration-dependent antioxidant activity, demonstrating their potential as eco-friendly antioxidant nanomaterials. While not as potent as ascorbic acid, they provide sustained radical scavenging ability with additional biocompatibility and stability advantages for biomedical and catalytic applications.

Conclusion:

In this study, cerium oxide (CeO₂) nanoparticles were successfully synthesized through an environmentally benign green approach using *Laminaria japonica* seaweed extract as both a natural reducing and stabilizing agent. The **UV–Visible** spectral analysis exhibited a distinct absorption peak at **410 nm**, confirming the generation of CeO₂ nanoparticles corresponding to the O (2p) → Ce (4f) electronic transition[17]. **FTIR** spectroscopy verified the presence of key functional groups such as hydroxyl (absorption band at **3338.85 cm⁻¹**), carbonyl (peak observed at **1722.43 cm⁻¹**), and amine (peak at **1010.70 cm⁻¹**), originating from the phenolic compounds, proteins, and polysaccharides in *L. japonica*, which facilitated the reduction of Ce⁴⁺ ions and subsequent stabilization of the nanoparticles. The X-ray diffraction (XRD) pattern displayed sharp and well-defined peaks indexed to the (111), (200), (220), and (311) planes, characteristic of the cubic fluorite structure of CeO₂ (JCPDS No. 34-0394). The calculated crystallite size ranged between **20–40 nm**, as determined using the Scherrer equation, indicating the formation of highly crystalline nanoparticles[18]. Scanning Electron Microscopy (SEM) further confirmed the presence of nearly spherical particles with slight aggregation, a result of the capping effect exerted by the biomolecules in the extract and Average particle size **20-50nm**. Overall, these findings establish that *Laminaria japonica*-assisted synthesis offers a cost-effective, eco-friendly, and efficient method for producing stable and crystalline CeO₂ nanoparticles, suitable for diverse applications in catalysis, biomedicine, and environmental purification. The synthesized nanoparticles exhibited effective antibacterial activity against both Gram-positive and Gram-negative bacteria, with

E.coli and *Pseudomonas* showing the highest sensitivity. Additionally, the antioxidant (DPPH) assay revealed concentration-dependent radical scavenging activity, attributed to the $\text{Ce}^{3+}/\text{Ce}^{4+}$ redox behaviour[15]. Overall, the *L. japonica*–mediated CeO_2 NPs demonstrate excellent biological potential, making them promising candidates for biomedical, antioxidant, and Antibacterial applications[29].

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