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Green Synthesis And Characterization Of Sargassum Polycystum-Mediated Copper Nanoparticles For Anti Oxidant And Anti Cancer Applications

Sarika P.V* [1]

[1]M Pharm student, Jamia Salafiya Pharmacy college Pulikkal, Malappuram District, Kerala, India. 673637.
Email : sarikapv63@gmail.com

ABSTRACT :

This study reports the phytochemical analysis, synthesis, and characterization of *Sargassum polycystum*-conjugated copper nanoparticles (*Sargassum polycystum*-CuNPs). The nanoparticles exhibited significant antioxidant and cytotoxic activities against lung cancer cell lines, with an IC50 value of 82.90 µg/ml. Phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, steroids, terpenoids, and carbohydrates. Characterization techniques, including UV-Vis spectroscopy, XRD, EDAX, FT-IR, FE-SEM, and HRTEM, confirmed the formation of *Sargassum polycystum*-CuNPs. These findings suggest the potential of *Sargassum polycystum*-CuNPs as a novel anticancer agent, warranting further investigation.

Keywords: *Sargassum polycystum*, Copper nanoparticles, Phytochemical analysis, Antioxidant activity, Cytotoxicity, Lung cancer, Green synthesis

INTRODUCTION :

Nanotechnology has a vast applicability in areas such as environmental sciences, bio-nanotechnology, applied microbiology, medicine and drug- gene delivery systems, quantum dots, surface – enhanced Raman scattering (SERS), chemistry, space and chemical industry, energy science, mechanics, electronics, optics and opto-electronic devices. Among them, bio-nanotechnology (green synthesis) is an eco-friendly and cost-effective method for the formation of nanoparticles by using simple prokaryotic bacterial cells or complex eukaryotic plants, because they do not involve the usage or production of toxic chemicals and can easily cope with harsh environmental conditions, scalability, and diverse nanoparticle specifications, thereby reducing environmental impact, production costs, and enhancing biocompatibility and nanoparticle stability.Copper nanoparticles extracted from medicinal plants have numerous benefits, including catalysis, photocatalysis, antibacterial activity, DNA binding and sensing, anticancer properties, and free radical scavenging.Copper nanoparticles synthesized using medicinal plant extracts are biodegradable, cost-effective, and stable. Plant extracts contain biomolecules such carbohydrates, phenols, tannins, flavonoids, and others that play a key role in reducing, aggregating, and capping nanocomposites. Herbal supplements for health care are becoming increasingly popular.

Sargassum polycystum, a brown seaweed, includes several minerals and bioactive substances with antioxidant and therapeutic qualities.Sargassum polycystum is a brown algae that is extensively dispersed in tropical places. In Asian countries, it has great economic importance because it is used as food, traditional medicine, animal fodder, and chemical compounds.Paniya tribes treat stomach issues.Marine algae are an abundant source of structurally varied bioactive chemicals with a wide range of biological functions. There are two major types of algae: macroalgae and micro algae. Floating tropical brown macro algae, generally known as Sargassum, are tropical and subtropical seaweeds found in shallow marine meadows. These are nutrient-dense foods high in bioactive substances such vitamins, carotenoids, dietary fiber, proteins, and minerals. Sargassum seaweed has a variety of biological actions, including analgesic, antiinflammatory, antioxidant, antibacterial, anticancer, fibrinolytic, anticoagulant, antiviral, and immunological modulatory properties.

Material and Methods

Materials

Sargassum polycystum, Copper sulfate, and HMDS were purchased from Sigma Aldrich (Mumbai, India). DPPH (2,2-diphenyl-1-picrylhydrazyl),a549 human cell line,DMEM, DMSO

Plant collection

The fresh and healthy specimen (whole plant) of *Sargassum polycystum* were from Rameswaram sea, Ramanathapuram Dist., Tamil Nadu, India, during the month of December 2023. The plant *Sargassum polycystum* was identified and authenticated by,Dr. M Shamina assistant professor, Department of Marine science, University of Calicut.

Preparation of plant extract

Distilled water is used to clean plant and remove any particulates. To make a fine powder from whole plant, use a mortar and pestle. The extract (methanol) was prepared by soxhlet extraction method using *Sargassum polycystum* powder (100 g) It was then allowed to dry at normal temperatures. The extract was purified and stored at - 4 °C for further analysis.

Phytochemical screening

The extract underwent a preliminary phytochemical examination to identify active components using basic methods.

Green synthesis of copper nanoparticle (CuNPs)

Sargassum polycystum metallic nanoparticles were synthesized. Erlenmeyer flask 5mM 50 ml of CuSo4.5H_2O solution was prepared. Fifty milliliters of 10 mg/ml *Sargassum polycystum* solution were prepared and slowly added to the copper sulfate solution with magnetic stirring for 30 min at 10,000 rpm. The color of the solution changed from blue to light green, which confirmed that *Sargassum polycystum* nanoparticles were formed and identified visually. The nanoparticle solution was centrifuged (30 min and 12000 rpm), and the *Sargassum polycystum* copper nanoparticles (*Sargassum polycystum* -CuNPs) were collected. The *Sargassum polycystum* -CuNPs were washed with sterile water for further characterization and antioxidant activity.

Characterization of Sargassum polycystum-CuNPs

CuNPs were initially explored using UV spectroscopy in the 200-800 nm range.
FTIR was utilized to determine structural characteristics and select phytochemical components. The results predicted a range of 4000-400 cm−1. The nanoparticle powder was subjected to CuKα1X-Ray diffractometer radiation (λ = 1.5406 A°) at 40 kV, 30 mA, and 2θ rang. CuNPs were placed on the sample holder and sputter-coated with gold. The nanoparticles' average particle size and shape were then investigated utilizing the TESCAN MIRA3 LMH Schottky FE-SEM (Japan).

Determination of antioxidant activity (CuNPs)

The ascorbic acid and CuNPs sample stocksolutions prepared have a strength of 1.0 mg/ml.*Sargassum polycystum, Sargassum polycystum* - CuNPs and ascorbic acid concentrations of 10, 20,40, 60, 80, 100 µg/mL, in methanol solution. CuNPs (0.5 ml), ascorbic acid transformed into 0.5ml of0.5 mM DPPH in methanol solvent. After 30 min of storage at in the dark place at room temperature,the optical density was measured at 517 nm using Stat Fax 4200 Elisa reader (USA). Each experiment was performed in duplicate. Inhibition (I%) calculated as follows

I% antioxidant activity =

$$\frac{\text{Abs control} - \text{Abs of antioxidant}}{\text{Abs control}} \times 100$$

CYTOTOXICITY SCREENING OF Sargassum polycystum Cu NPs.

Cytotoxic Effects of *Sargassum polycystum*-Derived Copper Nanoparticles on Lung Cancer Cells (A549) Using Neutral Red Assay
The neutral red uptake assay is a widely used cytotoxicity test evaluating cell viability based on pH gradients and ATP production. A549 human lung carcinoma cells (National Centre for Cell Sciences, Pune, India) were cultured in Dulbecco's Modified Eagles Medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotic cocktail. Cells (10,000/well) were seeded in 96-well plates and incubated for 24 h.Test samples were prepared in DMSO (10 mg/mL), diluted in DMEM, and added to cells for 24 h. Neutral red working solution (40 µg/mL) was incubated overnight, centrifuged, and added to cells for 2 h. After washing with PBS, neutral red destain solution was added, and OD was measured at 540 nm. Cell viability was calculated as (Average absorbance of treated / Average absorbance of control) × 100. Experiments were performed in triplicates using a microtiter plate reader spectrophotometer

RESULT AND DISCUSSION :

Phytochemical analysis

All the findings of the phytochemicalinvestigation are shown in Table 1. Methanol extractgave promising outcomes for steroids, terpenoids confirmed by Salkowski and Liebermann- Burchard's test in the present study. The presence of terpenoids, phenols and flavonoids were also confirmed in *Sargassum polycystum* extract.

Evaluation of CuNPs
UV - Vis spectroscopic analysis

U.V. spectroscopy within the 30-100 nm range initially verified the development of CuNPs.A characteristic peak of 650 nm showed the absorption spectrum of green synthesized CuNPs.

FTIR spectroscopic analysis

A Fourier transform infrared spectroscopy was utilised as an affirmative method for the construction of nanoparticles. This study offers an imprint of current molecules vibrational and rotational modes, thus helping to classify the functional and potential plant molecules involved in CuNPs nanoparticles reduction**.**

XRD Analysis

The structure of the nanoparticles is indicated by the XRD analysis of the synthesised CuNPs.At 2θ values of 28.70°, 26.07°, and 28.96° degrees, diffraction peaks were observed **.**

FE-SEM analysis

The SEM study was carried out using the Schottky FE-SEM (Japan) TESCAN MIRA3 LMHmodel. FE-SEM analysis was used to classify the structure and scale of CuNPs. Microscopy ofCuNPs has shown that they have a nano-rangeparticle size (500nm) spherical and homogeneous in distribution under the FE-SEM Microscope .

EDAX analysis

The elemental analysis of the CuNPs from the EDAX spectrum of the FE-SEM image is shownin Fig-5. The percentage of molecular mass and atomic value of Cu respectively 69.7%. The EDAXspectrum is consistent with the presence of copperin the nanoparticle.

Antioxidant activity

The antioxidant properties of CuNPs using DPPH were evaluated and compared to ascorbic acid. The ratio of free radical inhibition of CuNPs was observed at different concentrations. The free radical inhibition visually detected colour transformation from purple to yellow suggests that DPPH reduced exhibiting better scavenging action**.**

Phytochemical analysis

Table no.1 phytochemical screening of Sargassum polycystum

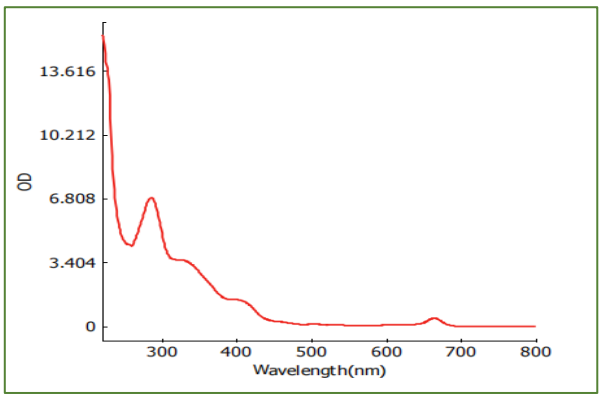
Sl.no.	Phytoconstituents	Chemical test	Inference
1.	Alkaloid	Dragendroff's test	Positive
2.	Phenols	Ferric chloride test	Positive
3.	Saponins	Foam test	Negative
4.	Flavonoids	Lead acetate test	Positive
5.	Steroids	Salkowski test	Positive
6.	Terpenoids	Libermann Burchard	Positive
7.	Carbohydrate	Molisch's test	Positive

Characterization of Sargassum polycystum Copper Nanoparticles

UV spectroscopy

UV Visible spectroscopic analysis of Sargassum polycystum - CuNPs

The surface plasmon resonance (SPR) of *Sargassum polycystum*-CuNPs was investigated utilizing a UV-visible spectrophotometer across the absorbance range of 100–800 nm. Analysis revealed a distinct absorption peak at 650 nm, as depicted in Figure confirming the formation of nanoparticles. This characteristic SPR peak indicates the collective oscillation of free electrons on the surface of the nanoparticles upon interaction with incident light. The precise measurement of the SPR peak at 650 nm provides valuable quantitative information about the size, shape, and composition of the *Sargassum polycystum*-CuNPs, enabling further understanding of their optical properties and potential applications in fields such as sensing, imaging, and catalysis.



OD800	: 0.016
OD650	: 0.197
OD542	: 0.090
OD540	: 0.098
OD537	: 0.095
OD450	: 0.282
OD276	: 6.086
OD220	: 15.547

Fig.1: UV Spectrum of copper nanoparticle

X-RD analysis of Sargassum polycystum - CuNPs

X-ray diffraction (XRD) analysis of Sargassum polycystum-conjugated copper nanoparticles (*Sargassum polycystum*-CuNPs) revealed a distinct peak at 2θ = 26.07°, confirming their crystalline nature. The peak's sharpness and intensity indicate a well-defined crystalline structure, suggesting an ordered arrangement of atoms within the nanoparticles. This crystallinity is crucial for their functionality and stability in various applications. The XRD results provide valuable insights into the structural properties of Sargassum polycystum-CuNPs, enabling confident investigation into their potential roles in biomedicine, catalysis, and nanotechnology, ensuring structural integrity and optimal performance.

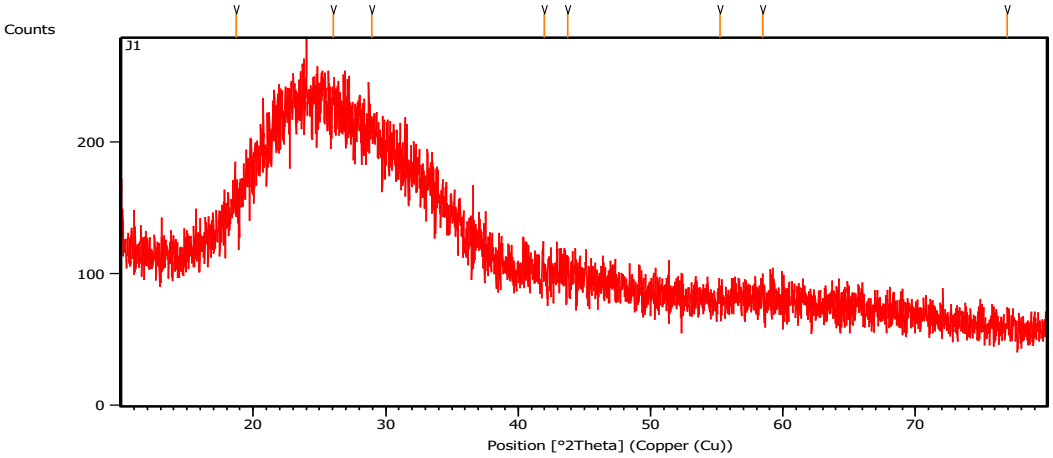


Fig.2: XRD Spectrum of copper nanoparticles

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
18.7020	0.94	0.3346	4.74474	7.18
26.0735	9.98	0.1673	3.41763	76.55
28.9656	8.62	0.8029	3.08265	66.11
41.9475	3.65	0.2007	2.15382	27.98
43.7445	7.84	0.1338	2.06942	60.18
55.2234	9.41	0.2007	1.66338	72.17
58.4832	13.04	0.1673	1.57819	100.00
76.9365	12.12	0.2007	1.23929	92.98

Table no.2. peak position and peak height of Copper nanoparticles

EDAX analysis of the Sargassum polycystum - CuNPs

EDAX spectroscopy (Graconfirmed the composition of *Sargassum polycystum*-conjugated copper nanoparticles (Sargassum polycystum-CuNPs), revealing elemental constituents including copper (Cu), calcium (Ca), chlorine (Cl), and potassium (K). The quantitative EDAX analysis provided valuable insights into the nanoparticles' stoichiometry and composition, verifying the successful incorporation of copper into the Sargassum polycystum-based nanomaterial and affirming the synthesis of *Sargassum polycystum*-CuNPs with a multielemental structure.

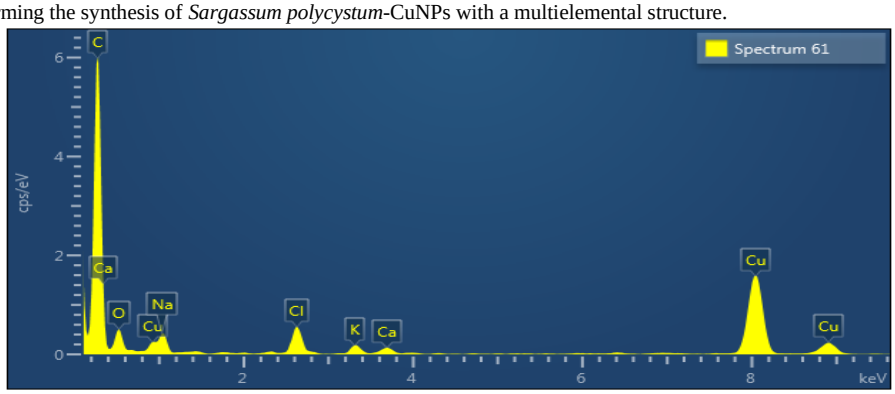
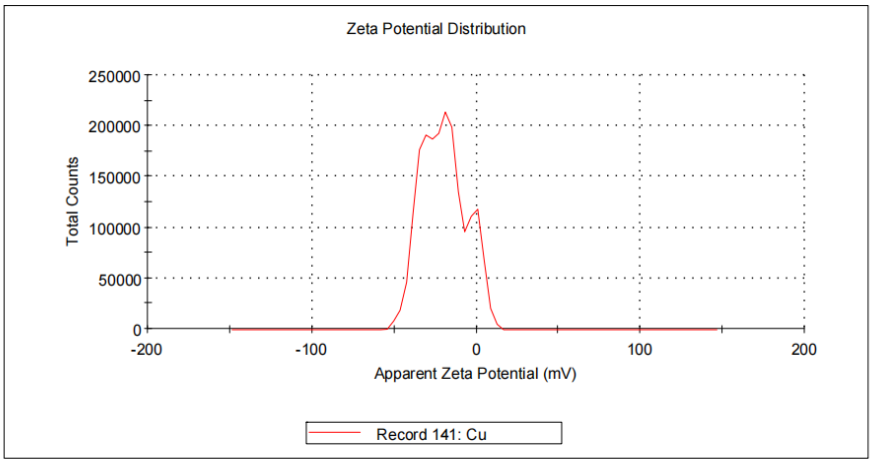


Fig.3 .Revealing elemental constituents in copper nanoparticles

Sargassum polycystum-CuNPs Zeta Size and potential analysis

Sargassum polycystum-CuNPs average zeta size 104.6 d. nm and -19.9 mV zeta potential indicate that the synthesised *Sargassum polycystum* -CuNPs were in nano size. The surface charge potential was measured using zeta potential, a major parameter determining the stability of *Sargassum polycystum*-CuNPs in a methanol solutions.

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -19.9	Peak 1:	-18.1	46.8	6.15
Zeta Deviation (mV): 13.2	Peak 2:	-33.2	34.0	5.51
Conductivity (mS/cm): 0.0382	Peak 3:	-0.835	19.2	4.78
Result quality Good				



		Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm): 104.6	Peak 1:	147.4	100.0	90.87
PdI: 0.269	Peak 2:	0.000	0.0	0.000
Intercept: 0.921	Peak 3:	0.000	0.0	0.000
Result quality Good				

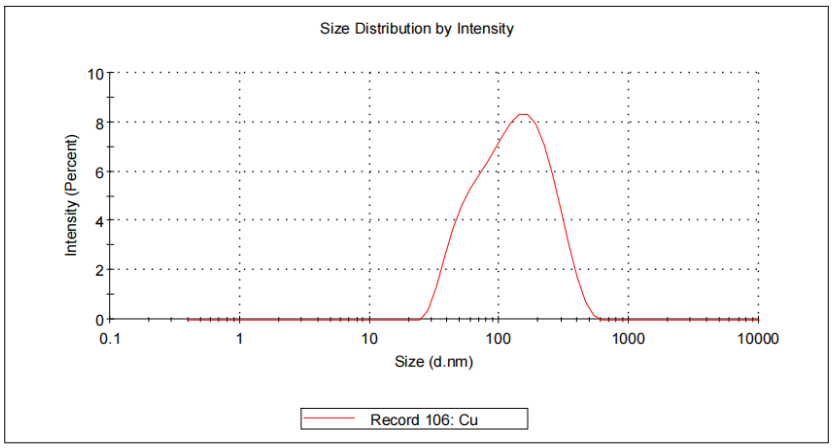


Fig.4 Zeta potential analysis of Sargassum polycystum- CuNPs

FT-IR analysis of Sargassum polycystum- CuNPs

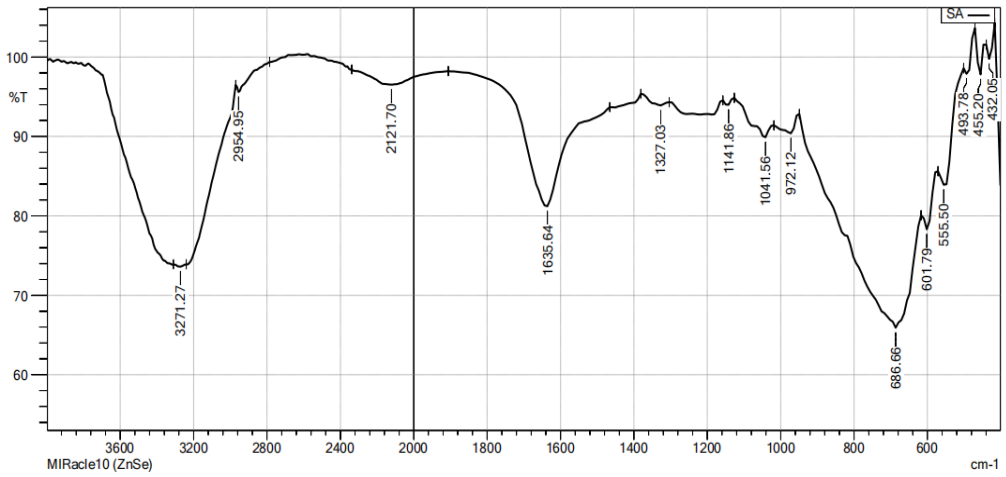


Fig.5. FT-IR potential analysis of Sargassum polycystum- CuNPs

The FT-IR spectrum of *Sargassum polycystum*-CuNPs revealed a complex profile, indicating the involvement of various functional groups in nanoparticle formation, with prominent peaks at 3271.27 cm^-1 (O-H stretching), 2954.95 cm^-1 (C-H stretching), 2121.70 cm^-1 (N-H stretching), 1741.86 cm^-1 (C=O stretching), 1635.64 cm^-1 (C=O stretching), 1327.03 cm^-1 (C-N stretching), 1041.56 cm^-1 (C-O stretching), 972.12 cm^-1 (C-H bending), 686.66 cm^-1 (C-C stretching), 601.79 cm^-1 (C-S stretching), 555.50 cm^-1 (Cu-O stretching), 493.78 cm^-1 (Cu-N stretching), 455.20 cm^-1 (C-C stretching), and 432.05 cm^-1 (Cu-O stretching). These peaks indicated hydroxyl, aliphatic, aromatic, carbonyl, and amine groups, suggesting reduction of Cu(II) to Cu(0) by hydroxyl and amine groups, coordination of Cu(0) with carbonyl and amine groups, and stabilization of CuNPs by Sargassum polycystum's bioactive compounds, confirming successful nanoparticle synthesis and providing insights into molecular interactions and chemical changes for optimizing synthesis protocols.

Sargassum polycystum - CuNPs observation by electron microscope (FE-SEM and HRTEM).

Electron microscopy (FE-SEM and HRTEM) was used to confirm that the *Sargassum polycystum* -CuNPs were nano-sized, and the particles were oval and spherical in shape. Figure 7 shows the *Sargassum polycystum* -CuNP size from 36.71244 nm to 100 nm.

Sargassum polycystum - CuNPs - Scanning Electron Microscopy (SEM) analysis

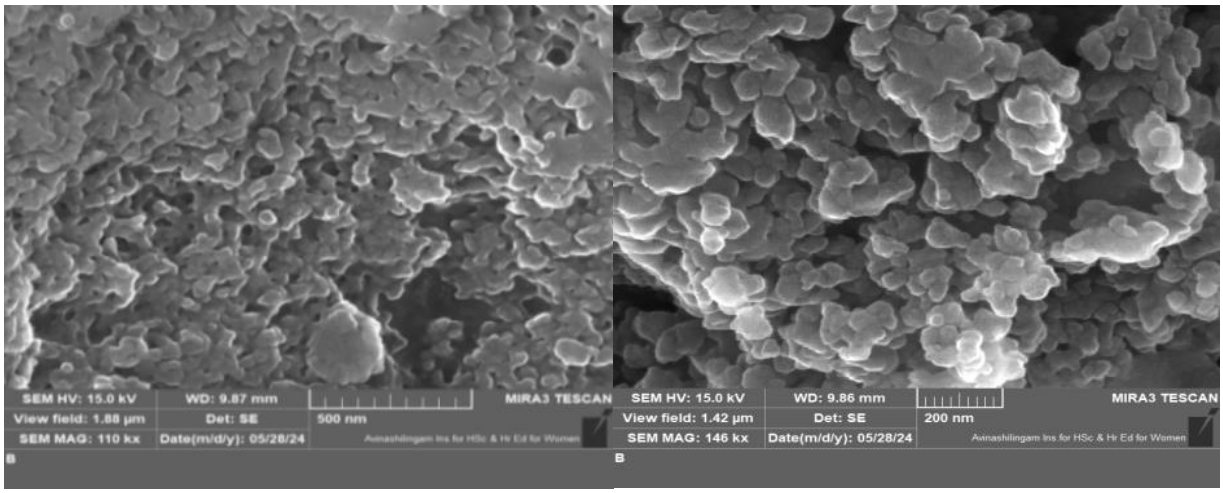


Fig: 6. Sargasuum polycystum-CuNPs FE-SEM obsevation

Sargassum polycystum - CuNPs - Transmission Electron Microscopy (TEM) analysis

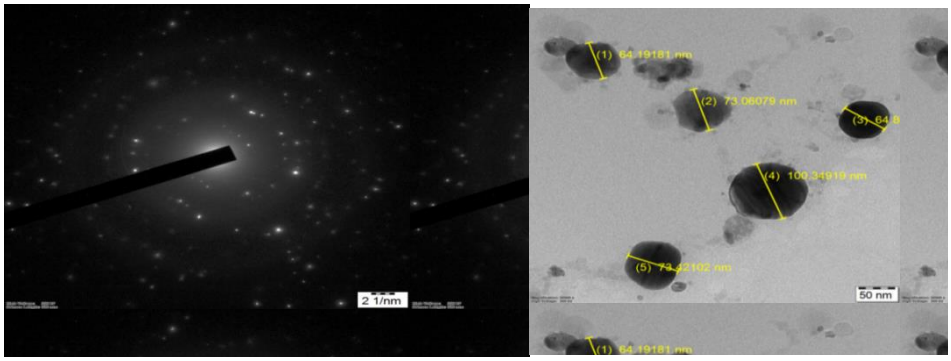


Fig: 7. Sargasuum polycystum-CuNPs HR-TEM obsevation

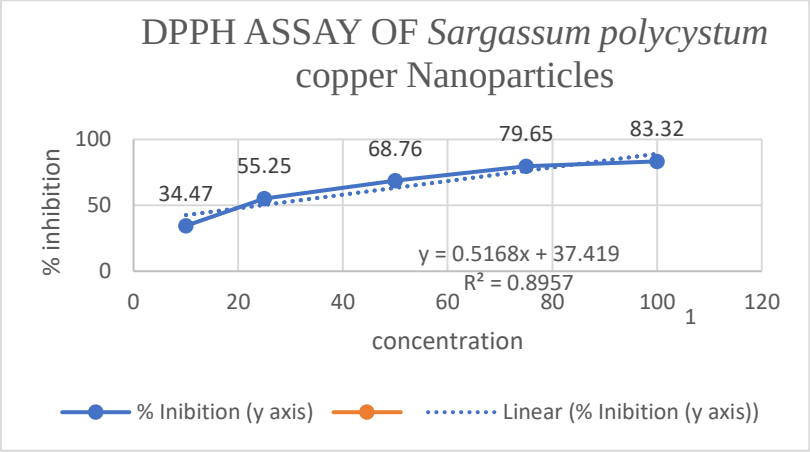
Antioxidant activity

Table 3: Free radical scavenging activity of standard- Ascorbic acid.

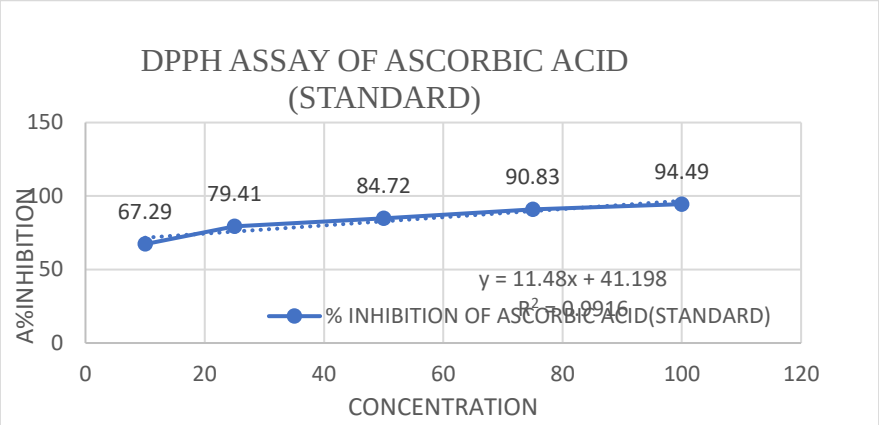
	Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean Absorbance	% Inhibition
Ascorbic Acid	**10**	**0.554**	**0.541**	**0.543**	**0.55**	**67.29**
	25	**0.345**	**0.349**	**0.337**	**0.34**	**79.41**
	50	**0.267**	**0.254**	**0.244**	**0.26**	**84.72**
	75	**0.154**	**0.148**	**0.157**	**0.15**	**90.83**
	100	**0.089**	**0.099**	**0.088**	**0.09**	**94.49**

Table 4: Free radical scavenging activity of sample Nanoparticle.

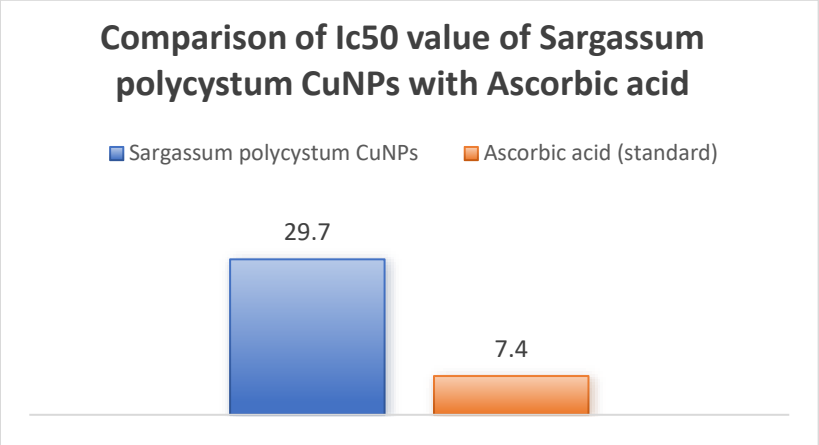
	Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Mean Absorbance	% Inhibition
Sample NP	**10**	**1.098**	**1.096**	**0.99**	**1.06**	**34.47**
	25	**0.752**	**0.775**	**0.765**	**0.76**	**55.25**
	50	**0.568**	**0.569**	**0.587**	**0.57**	**65.77**
	75	**0.325**	**0.396**	**0.332**	**0.35**	**79.65**
	100	**0.189**	**0.194**	**0.201**	**0.19**	**83.32**



Graph.1 DPPH assay of Sargassum polycystum copper nanoparticles



Graph.2 DPPH assay of Ascorbic acid



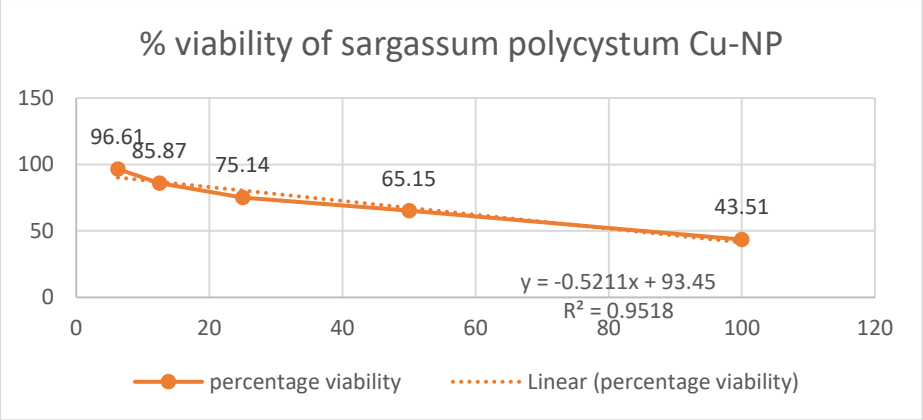
Graph.3 comparison of IC50 value

Cytotoxic activity of *Sargassum polucystum* copper nanoparticles on Lung cancer cell lines using Neutral red assay method.

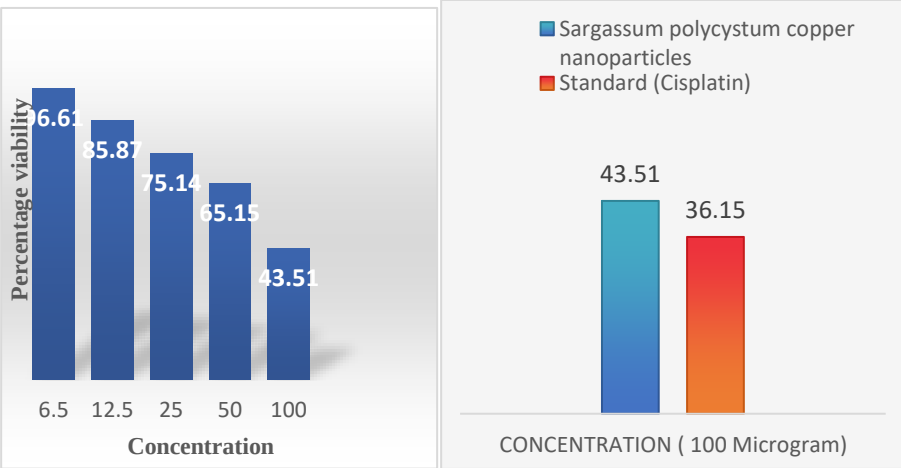
Table no.5: assay results for varying concentration of test sample

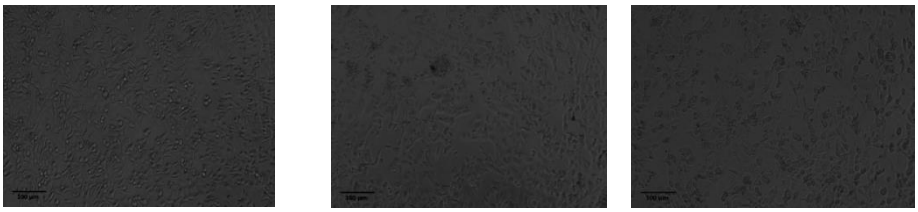
Samples	Triplicate 1	Triplicate 2	Triplicate 3	Average
Control	0.172	0.18	0.179	0.177
6.5	0.171	0.167	0.175	0.171
12.5	0.153	0.154	0.149	0.152
25	0.136	0.131	0.133	0.133
50	0.114	0.114	0.118	0.115
100	0.079	0.075	0.077	0.077
Cisplatin Standard (100)	0.064	0.066	0.062	0.064

concentration	percentage viability
6.5	96.61
12.5	85.87
25	75.14
50	65.15
100	43.51
IC 50	82.90



Graph .4.percentage viability of copper nanoparticles





CONTROL **TEST 100 µg/ml** **STANDARD 100 µg/ml**

Fig.8 Lung cancer cell line after treatment

CONCLUSION :

In conclusion, the present study demonstrates the successful synthesis and characterization of Sargassum polycystum-conjugated copper nanoparticles (Sargassum polycystum-CuNPs), highlighting their potential antioxidant and cytotoxic properties against lung cancer cell lines. The phytochemical analysis revealed a rich composition of bioactive compounds, including alkaloids, phenols, flavonoids, steroids, terpenoids, and carbohydrates. The *Sargassum polycystum*-CuNPs exhibited significant free radical scavenging activity and cytotoxicity, with an IC50 value of 82.90 µg/mL. These findings underscore the potential of Sargassum polycystum-CuNPs as a novel anticancer agent.

Future investigations will focus on elucidating the specific mechanisms of action, evaluating in vivo efficacy and safety, and developing Sargassum polycystum-CuNP-based formulations for cancer therapy, as well as exploring their potential applications in other biomedical fields, such as antimicrobial and anti-inflammatory therapies, to fully harness the therapeutic potential of these nanoparticles.

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CONFLICT OF INTEREST

Nil

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