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Green synthesis, characterization and bioactivity of Ag-nanoparticles from algal polysaccharide of *Chnoospara minima*

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Abstract:

The synthesis of nanoparticles using biogenic material as a part of green chemistry is a recent attraction of nanotechnology. The current research aimed to test the cytotoxic efficacy of silver nanoparticles (Ag-NPs) synthesized by extract of polysaccharide from marine algae *Chnoospora minima* against Human Breast Cancer (MCF-7) Cells *in vitro*. The extracted polysaccharide was analyzed by Fourier-transform infrared spectroscopy (FTIR). Biosynthesized silver nanoparticles (Ag-NPs) were characterized using UV-spectrophotometry, dynamic light scattering (DLS), Zeta Potential, Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX). We demonstrated the dose-dependent cytotoxic effect of biosynthesized Ag-NPs in Human Breast Cancer cells (MCF-7) using Sulferhodamine B assay (SRB assay). An absorption peak at 420 nm in UV–vis spectrum proven the formation of Ag-NPs; DSL analysis confirmed the formed particles are within the nano scale with Z-Average of 84 d.nm and Zeta potential was -18.5 mV. SEM imaging showed biosynthesized Ag-NPs have a spherical shape with low aggregation and the EDX spectrometers confirmed the presence of elemental silver signal of the biosynthesized Ag-NPs. SRB assay demonstrated that the green synthesized Ag-NPs inhibit proliferation of breast cancer cell lines (MCF-7). The innovation of the present study is that the green synthesis of NPs, which is simple and cost effective, provides stable nano-materials and can be an alternative for the large-scale synthesis of silver nanoparticles.

Keywords: Ag-NPs; *Chnoospara minima*; FT-IR, DLS, SEM, EDX, Cytotoxicity

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Results and Discussion

- ❑ **Polysaccharide Extraction from Algae**
- ❑ **Silver nano-particle (Ag-NPs) Biosynthesis**
- ❑ **Characterization of synthesized Ag-NPs**
- ❑ **Bioactivity of Ag-NPs *VS* the Polysaccharide**

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Extraction of Polysaccharide

Chnoospora minima
Marian Brown Algae

Dried
↓
Powdered
↓
Depigmented (acetone)
↓
Hot water extraction (90–95 ^{0}C for 3–4 h)
↓
Filtered
↓
Concentrated
↓
Cooled
↓
Precipitated (95% ethanol)
↓
Centrifugation (1200rpm)
↓
Dried

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Biosynthesis of Silver nano-particle using extracted polysaccharide

Extracted algal polysaccharide
+
90 mL of sterile DW

↓

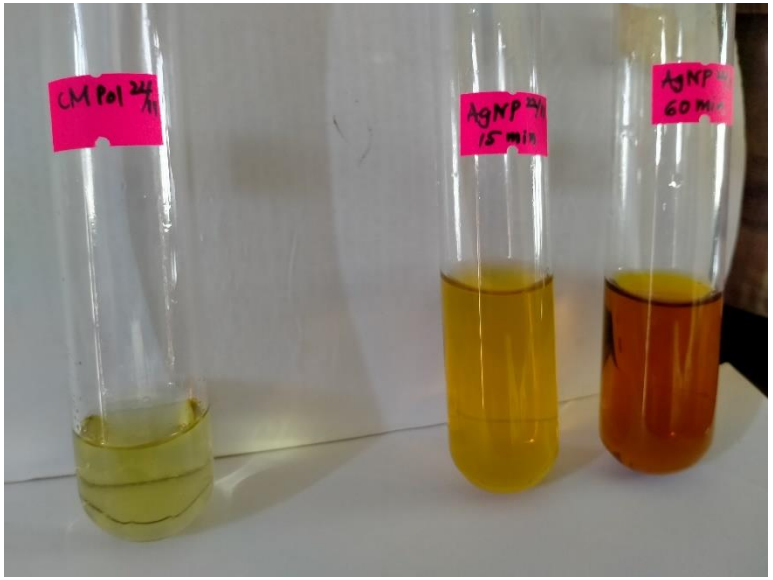
Drop wise add 1 mL of 0.1 mM $AgNO_3$

↓

stirrer at 70 ^{0}C for 20 min

↓

Ag-NPs



Sample and Ag-Np solution after 15 min & 60 min

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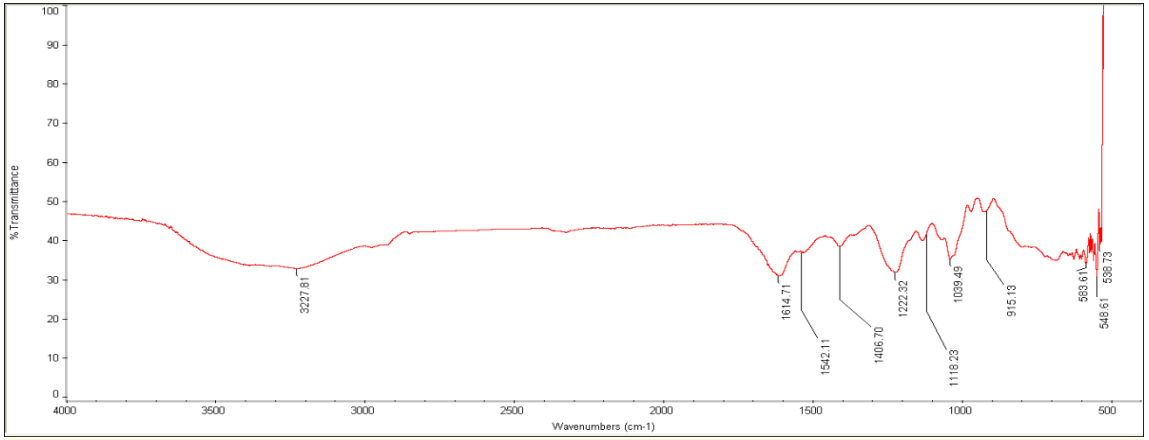
Characterization of synthesized Ag-NPs

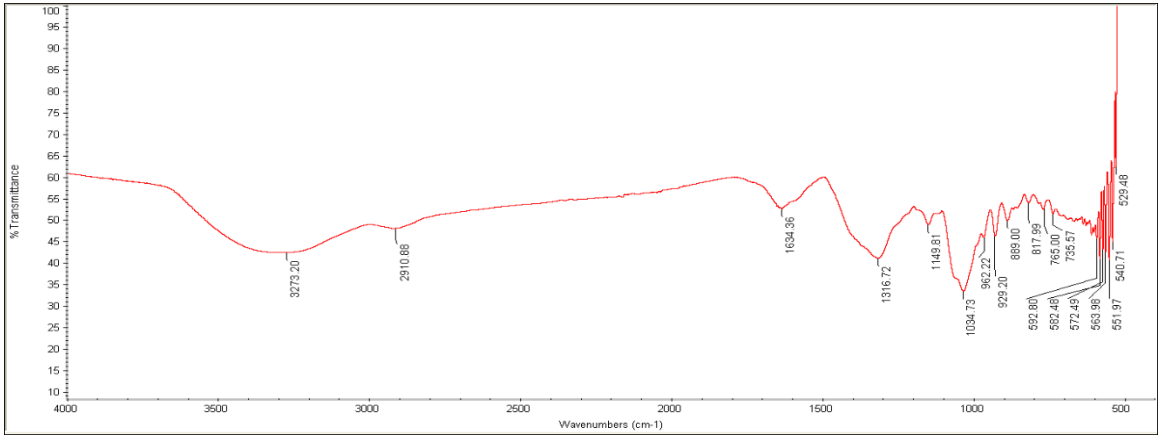
- Fourier Transform Infrared Spectroscopy (FTIR) Analysis



FT-IR spectra of the polysaccharides extracted from *C. minima*

500 cm^{-1} and 2000 cm^{-1} - fingerprint region for bond vibrational modes of polysaccharides.

Sharp peak at 1039 cm^{-1} – C-O-C stretching vibrations of the glycosidic bridges in polysaccharides.



FT-IR spectra of the biosynthesized Ag-NPs

1220 cm^{-1} and 1270 cm^{-1} – bending vibrations of C-O-S and stretching vibrations of S=O bonds in sulfate groups

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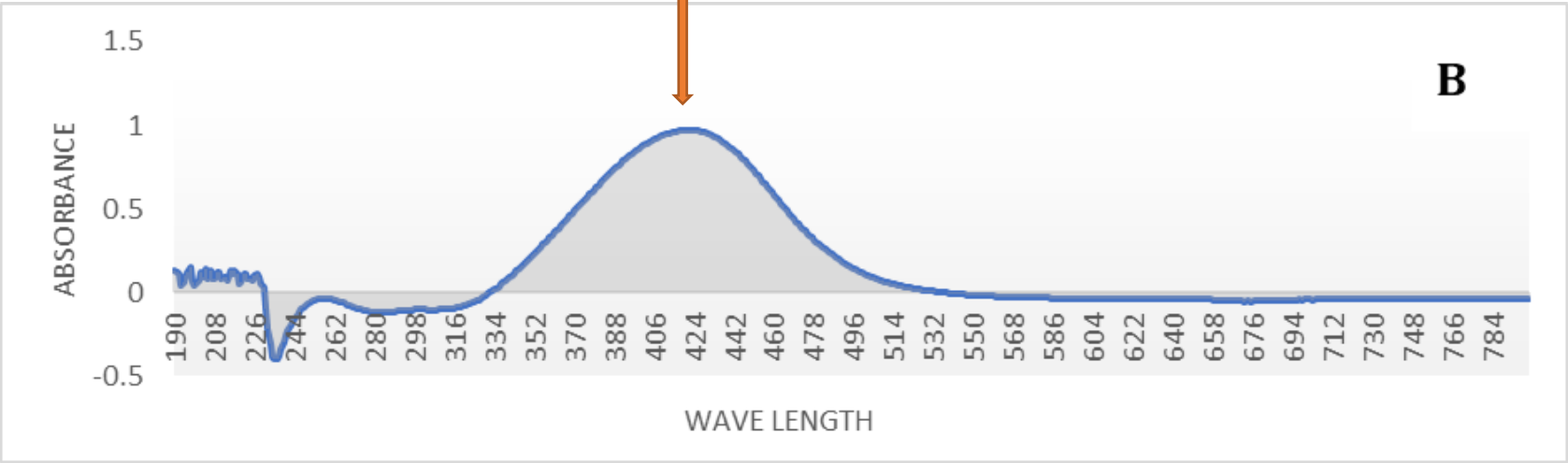
- UV-Visible Spectral Analysis

Broad peak at 420 nm - synthesis of Ag-NPs



UV-Visible Spectrum of Ag-NPs after 60 minutes

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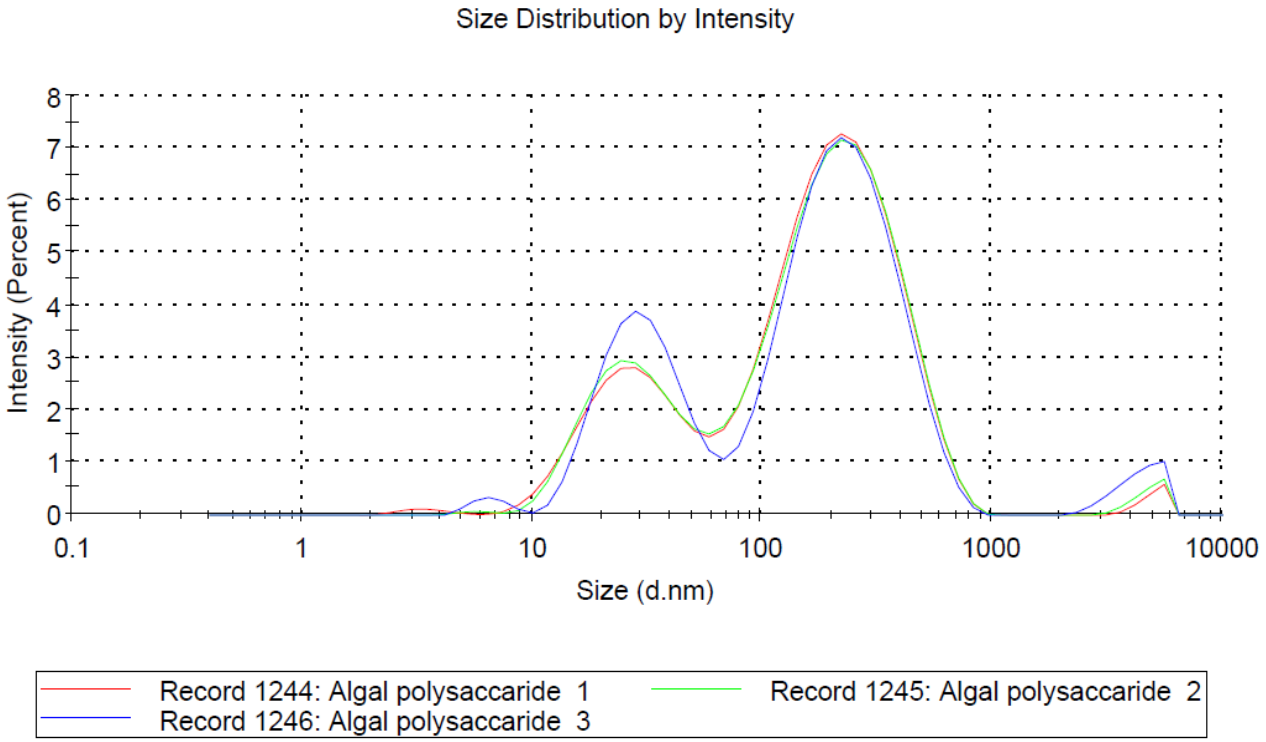
Dynamic Light Scattering (DLS) Analysis

DLS technique - **Identify the size distribution profile of Ag-NPs**

- Ag-NPs was in nanometer range
- Polydisperse mixture
- Z- Average of 84 d.nm



Size Distribution of Ag-NP soulution

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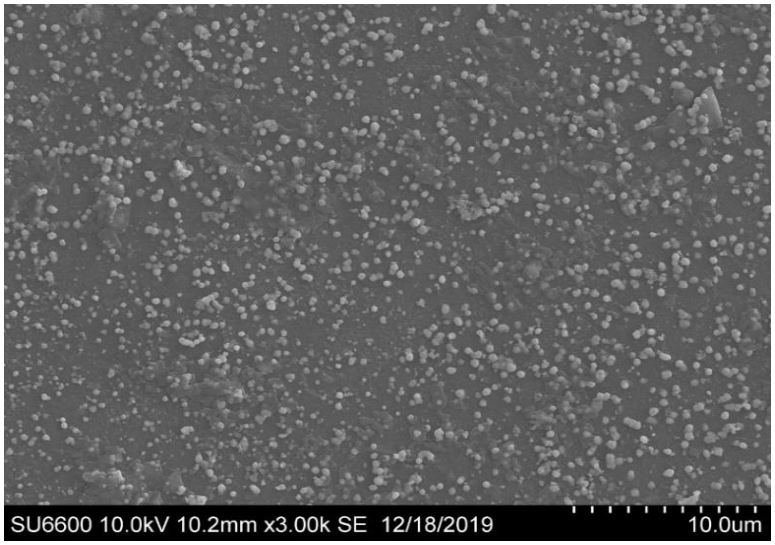
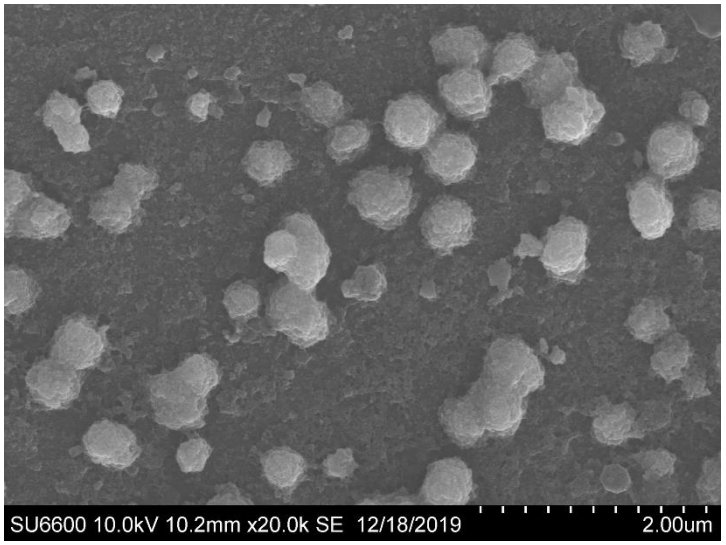
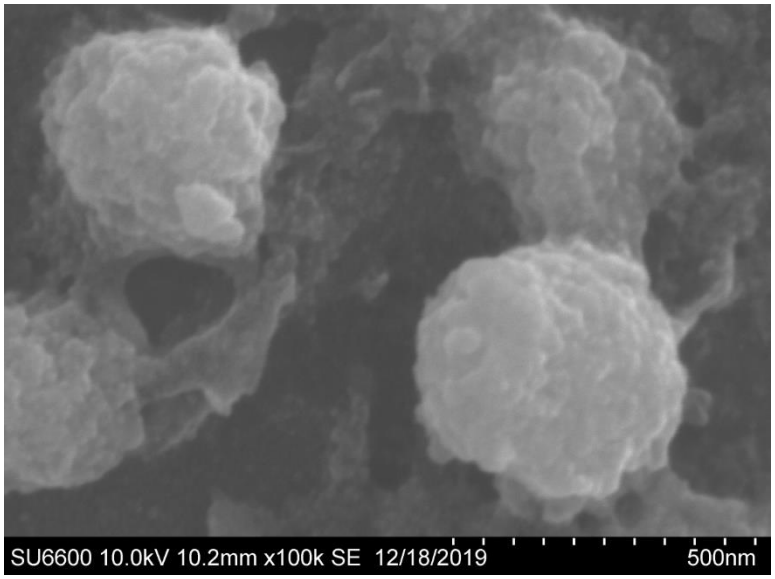
- Scanning Electron Microscopy (SEM) Imaging







SEM images of Biosynthesized Ag-NPs

- Ag-NPs - well dispersed
  - low aggregation
  - spherical in shape

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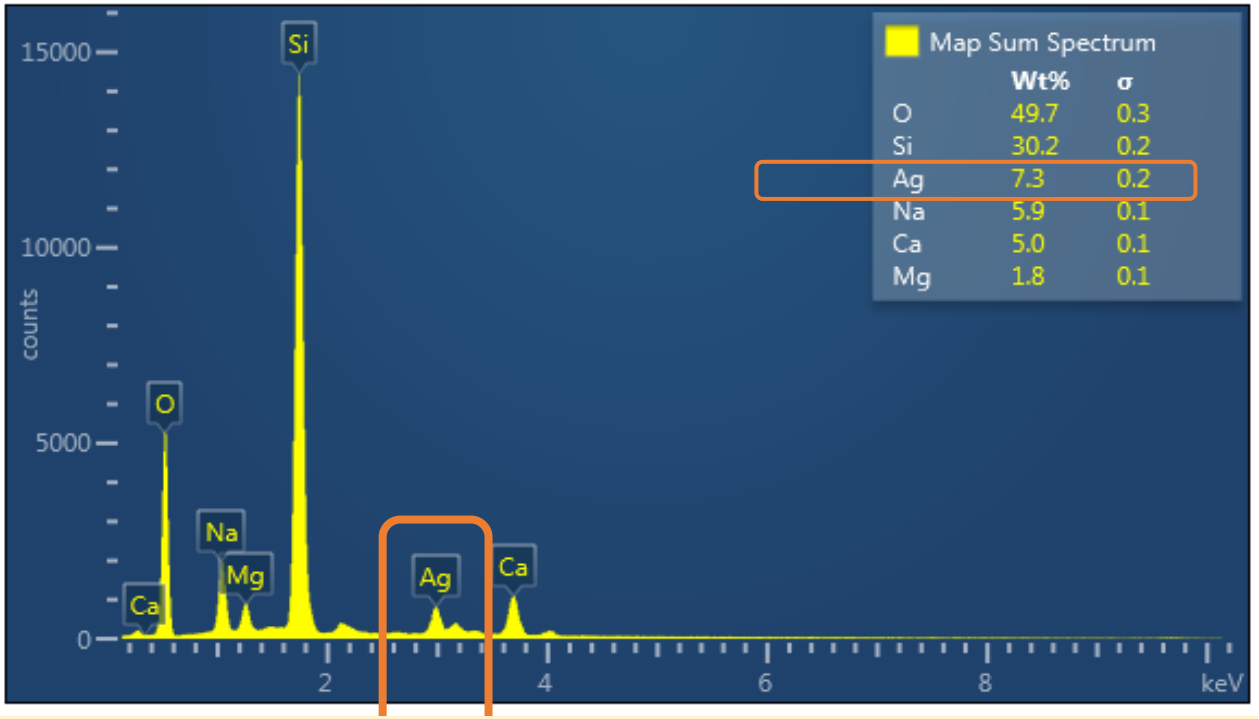
- Energy Dispersive X-Ray (EDX) Analysis

EDX analysis - **Qualitative and quantitative status of the elements involved in the formation of nanoparticles**



Energy Dispersive Spectroscopy (EDS) patterns of synthesized Ag-NPs

High peaks of Si and O – glass (SiO_2) slide used to mount the sample

Peak at 3 keV – metallic silver nanocrystals due to surface plasmon resonance

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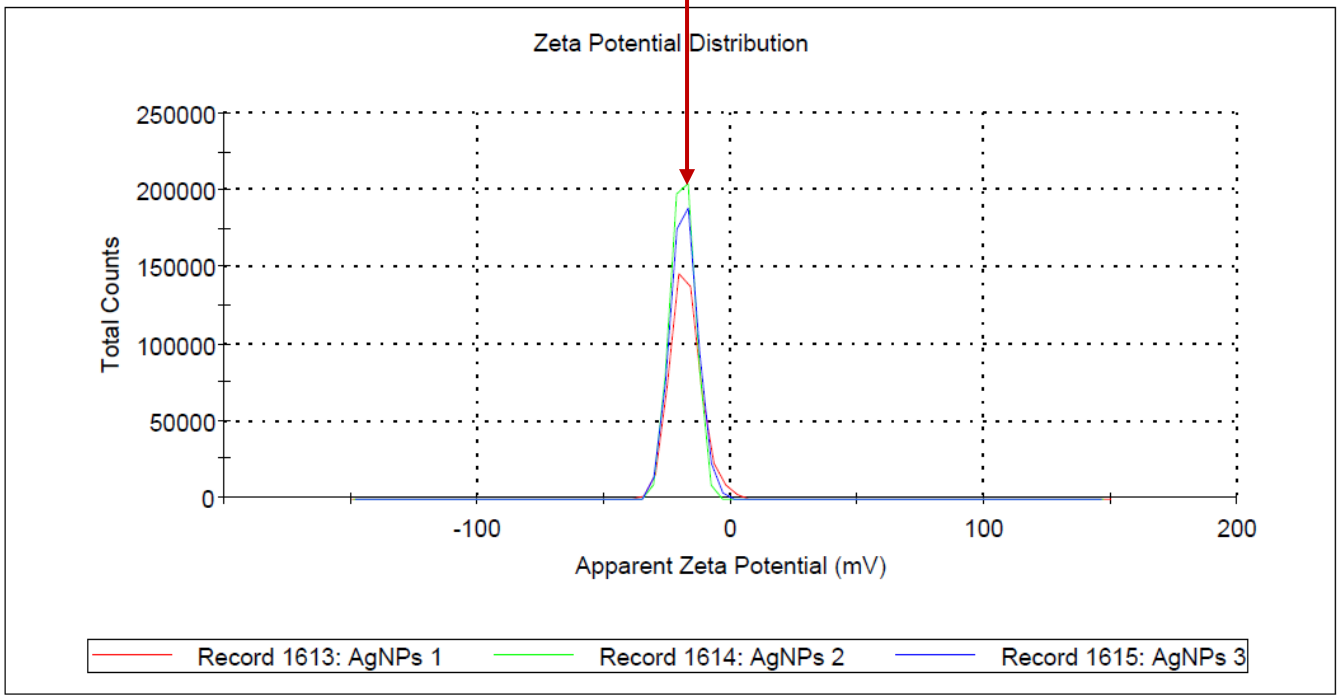
▪ Zeta Potential Analysis

Zeta Potential value -
suspension stability and particle surface morphology

Stable Ag-NPs at –18.5 mV



Zeta Potential Analysis of Ag-NP solution

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Bioactivity of Ag-NPs *VS* the Polysaccharide

The cytotoxicity of biologically synthesized Ag-NPs and crude polysaccharide extract were compared

Cell line	Polysaccharide based Ag nanoparticles (IC_{50})	Crude polysaccharide (IC_{50})
MCF-7	**3.921** $\mu g mL^{-1}$	**>200**$\mu g mL^{-1}$

IC_{50} values of the polysaccharide extract and biosynthesized Ag-NPs at 48 h post incubation

IC_{50} value < 10 $\mu g mL^{-1}$ –
cytotoxic,

IC_{50} value > 200 $\mu g mL^{-1}$ –
non-cytotoxic

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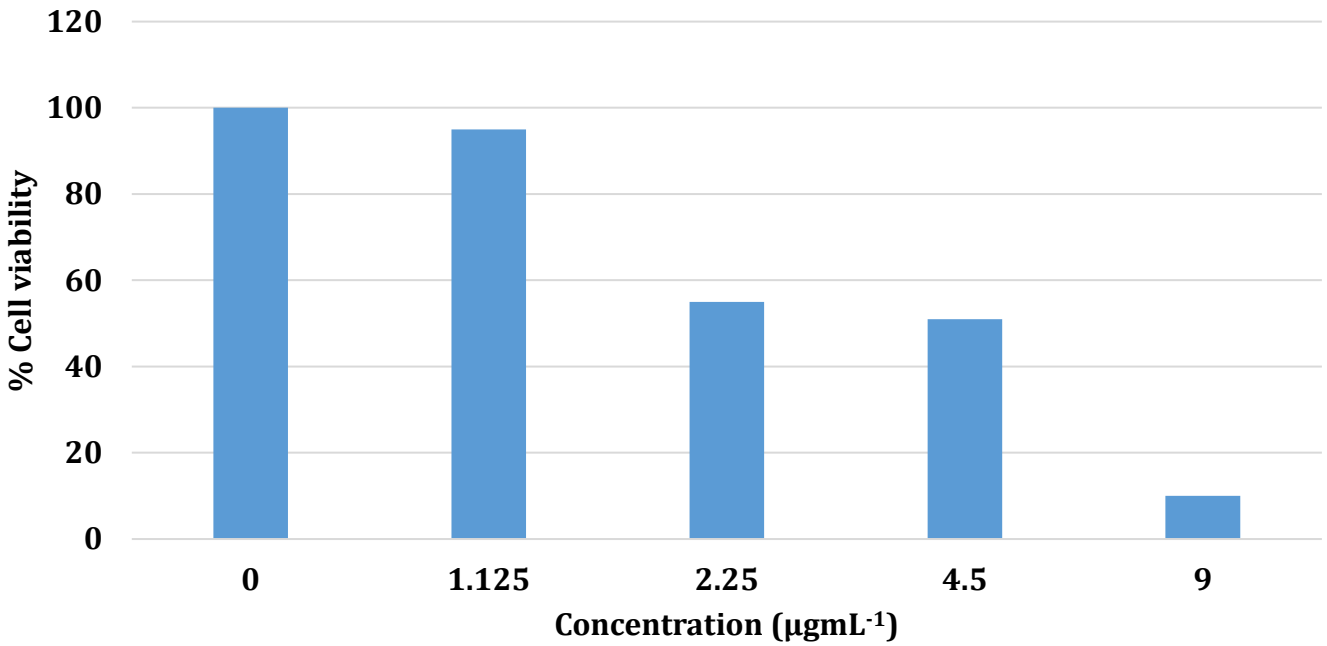
- *In-vitro* Anti-cancer activity Sulforhodamine B (SRB) assay - MCF-7

The sulforhodamine B (SRB) assay – **quantify cell density, based on the measurement of cellular protein content**

- Biosynthesized Ag-NPs shows **potent cytotoxic activity on MCF-7 cell line with increasing concentration**



SRB assay results - *in -vitro* cytotoxicity effect of biosynthesized Ag-NPs against the MCF-7 at different concentrations

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Conclusion

Silver nanoparticles are **effectively synthesized** from a silver nitrate solution through a simple green route using the **Polysaccharide extract of Sri Lankan Marian brown algae *Chnoospora minima.***

Further characterization of the prepared solution shows particles in **nano range** with **spherical shape**, are **well dispersed** and with an **average diameter of 84nm.**

Biosynthesized Ag-NPs show **IC_{50}** value of **3.921** $\mu g mL^{-1}$
compared to **>200**$\mu g mL^{-1}$ of the polysaccharide extract and **cytotoxic activity on MCF-7 cell line with increasing concentration**

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