# Anticancer activity and green synthesized SeO<sub>2</sub> Nanoparticles from Cinnamomum verum bark extract

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Abstract- Selenium is an important component of human diet and numerous studies have declared its chemopreventive and therapeutic properties against cancer. However, very limited studies have been conducted about the properties of selenium nanostructured materials. Here, we have shown that the anticancer property of green synthesized selenium nanoparticles from Cinnamon. The optical, structural, morphological, elemental, and functional characterizations of the SeO2NPs were carried out using techniques such as UVvis spectrophotometry, electron microscopy, energy dispersive X-ray spectrometry, and Fourier transform infrared spectrophotometry, respectively. The results showed the presence of spherical shape of SeO2 nanoparticles with 149 nm of nanoparticle particle size. The MTT (3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay revealed that the biosynthesized SeO2NP induces cell death of Hep-G2 human cancer cells. The IC50 value was recorded at 55.16 µg/mL, respectively. Furthermore, the EtBr/Ao staining results confirmed that the selenium nanoparticles induced apoptosis of cancer cells. Therefore, the SeO2 NPs could be a promising candidate for the control of cancer cells.

*Keywords*- biosynthesis, selenium NPs, anticancer activity, Cinnamon

#### I. INTRODUCTION

Cancer is a disease in which abnormal cells divide uncontrollably and destroy body tissue. Cancer is a disease that occurs when cells grow and divide abnormally, causing a growth called a tumor. Cancer is caused by mutations to the DNA within cells, which can cause the cell to stop its normal function. There are more than 200 different types of cancer, which are divided into groups according to the type of cell they start from. The five main cancer groups are: Carcinomas, Lymphomas, Leukaemias, Brain tumors, and Sarcomas.

#### Hepatocellular Carcinoma (HCC)

Cancer that begins in the cells of the liver. The liver is the football-sized organ in the upper-right area of the stomach. Liver cancer, also known as hepatic cancer, is a malignant tumor that starts in the liver. It can be primary, meaning it starts in the liver, or secondary, meaning it spreads from another part of the body to the liver. The most common type of primary liver cancer is hepatocellular carcinoma (HCC), which affects the liver's main cell type, hepatocytes. Most common primary malignant tumor of the liver. Asia and Japan > United States. Etiology: Cirrhosis, Hepatitis B and C virus, Alcohol, AlfatoxinB1. Tendency for Hematogenous spread and invasion of portal and hepatic veins. Tumor marker: Alpha - Fetoprotein. HCC and CCC comprise the overwhelming majority of primary malignant hepatic neoplasms. Secondary or metastatic hepatic neoplasms are especially common owing to the blood filtration function of the liver. Both HCC and CCC are slow-growing tumors but majority of patients are unresectable at presentation.

Cinnamon has antioxidant, anti-inflammatory, antidiabetic, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering compound. Cinnamaldehyde exhibited potent antiproliferative effect on a liver cancer cell line, HepG2, in a dose- and time-dependent pattern where a concentration of 30  $\mu$ M of cinnamaldehyde inhibited approximately 71% of cell proliferation.

A nanoparticle is a particle of matter with a diameter of 1–100 nanometers (nm). Nanoparticles are spherical, polymeric particles composed of natural or artificial polymers. They range in size between 10 and 500 nm. As a consequence of their spherical shape and high surface area to volume ratio, these particles have a wide range of potential applications. Selenium nanoparticles (SeNPs) are a potential nutritional supplement due to their low toxicity and ability to gradually release selenium after ingestion. selenium can significantly suppress liver cancer carcinogenesis induced by aflatoxin B1, dimethyl azobenzene, or acetylamino.

Green synthesis is a sustainable and environmentally friendly method for producing nanoparticles. Green chemistry, similar to sustainable chemistry or circular chemistry, is an area of chemistry and chemical engineering focused on the design of products and processes that minimize or eliminate the use and generation of hazardous substances.

#### II. AIM

To find the anticancer property of SeO2 obtain using Cinnamomum verum against hepG2 cells

#### **III. OBJECTIVES**

- 1) Collection and Authentication of bark material
- 2) Extraction of Bark Material
- Green synthesis of SeO2 nanoparticles using process and plant different ratios
- 4) Bulk synthesis of SeO2 nanoparticles
- 5) Characterization of and SeO2 nanoparticles by
  - SEM (Scanning Electron Microscopy)
  - ✤ XRD (X-ray diffraction analysis)
  - ✤ FTIR (Fourier Transform Infrared spectroscopy)
  - DLS (Dynamic Light Scattering)
  - UV (Ultraviolet)
- Anticancer activity of nanoparticles by MTT assay in Hep G2 cell line.
- 7) EtBr/Ao staining of fluorescent imaging method.

#### **IV. METHODOLOGY**

#### 1) Collection and Identification of Plants

Cinnamon bark was collected from Mallasamudram, Namakkal district, TamilNadu, India in the month of August, 2023. The plant material was identified and authenticated by Department of Biomedical Dr. D.Vijayakumar Assistant Professor, Mahendra Institue of Technology Namakkal, TamilNadu, India.



Collection of Cinnamomum verum

2) Preparation of aqueous extract of Cinnamon:

1gm of Barks of Cinnamon were used for green synthesis of Cinnamon was homogenized into fine powder and boiled with 100 ml Double distilled water for 20 min at 100°C and the extract was obtained after filtration through Muslin cloth and No. 1 filter paper and was used for green synthesis experiments.



Figure.1 Plant extract (CM)

#### 3) Preparation of 1mM Sodium Selenite Solution:

For the preparation of ImM Sodium Selenite (Na2SeO3), 0.0421gms of Na2SeO3 was dissolved in 100 ml of double distilled water. The solution was mixed completely and stored in yellow colored bottle to prevent auto oxidation of sodium.



Figure.2 0.1 M of Sodium selenite

## Synthesis of Sodium Selenite Nanoparticles using Cinnamomum verum Barks Extract:

15ml of Cinnamomum verum plants barks extract was added to 35 ml of 1mM aqueous sodium selenite (Na2Seo3) solution and bolied at 100 c temperature at 10mins for 24hrs in Dark Conditions to produce sodium selenite nanoparticles.



Figure.3 Synthesis of SeO2 NPs using Sodium selenite + (CM) Plant extract in a differentratios



Figure.4 Alkaline treatment -NaOH (100µL)



Figure.5 Optimization of SeO2 NPs at various Ph



Figure.6 Bulk production using Sodium selenite + (CM) plant extract



Figure.7 Synthesized SeO2 NPs

#### 4) Characterization of synthesized Na2SeO3 nanoparticles:

The biosynthesized Sodium Selenite were characterized according to the method described by Gurunathan et al. UVvis spectra were measured using A The shape and size of sodium selenite nanoparticles were determined by TEM. For TEM, a drop of aqueous sodium selenite nanoparticles sample was fixed on a carbon coated grid, and let dry in room temperature; the micrographs were acquired using TEM. Fourier Transform infrared spectroscopy (FT- IR) (Thermo Scientific Smart ITR <sup>TM</sup>) was utilized to describe the changes and the number on the face of the synthesized nanoparticles.

#### 5) MTT ASSAY

#### Principle

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystal which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells [7]. Solubilization of cells by the addition of detergents (DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created [8]. The color can be quantified using a multi-well plate reader. Materials required: Fetal Bovine Serum (FBS) and antibiotic solution were from Gibco (USA), DMSO (Dimethyl sulfoxide) and MTT (3-4,5 dimethylthiazol-2yl-2,5-diphenyl tetrazolium bromide) (5 mg/ml) were from Sigma, (USA), DMEM medium, 1X PBS, (India) [9]. 96 well tissue culture plate and wash beaker were from Tarson (India) [10].

#### PROCEDURE

#### Cell culture

Hep-G2 liver cancer cell line was purchased from NCCS, Pune and were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100  $\mu$ g/ml penicillin and 100  $\mu$ g/ml streptomycin, and maintained under an atmosphere of 5% CO2 at 37oC.

#### MTT assay

The Test sample was tested for in vitro cytotoxicity, using Hep-G2 liver cells by MTT assay. Briefly, the cultured Hep-G2 liver cells were harvested by trypsinization and pooled in a 15 ml tube [11]. Then, the cells were plated at a density of  $1 \times 105$  cells/ml cells/well (200 µL) into the 96-well tissue

culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with the Test sample in a serum free DMEM medium [12]. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After incubation, MTT (10  $\mu$ L of 5 mg/ml) was added to each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope [13]. Finally, the medium together with MTT (220 µL) was aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min [14]. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value were calculated using Graph Pad Prism 6.0 software (USA).

Formula Cell viability % = Test OD/Control OD X 100

#### 6) ETBR/AO STAINING

#### **Principle:**

Fluorescent dyes with aromatic amino or guanidine groups, such as acridine orange (AO), interact with nucleotides to emit fluorescence. EtBr molecules intercalate inside the DNA double helix [15]. AO can form complexes with either double-stranded

DNA or single-stranded DNA and RNA. One molecule of AO can also interact with one phosphate group of single-stranded DNA or RNA to form an aggregated, or stacked, structure that emits red fluorescence with the maximum wavelength at 650 nm. This fluorescent dye is impermeable through the cell membranes of viable cells and can be used as fluorescent indicators of dead cells. Acridine orange is a vital dye and will stain both live and dead cells [16]. Necrotic cells stain orange but have a nuclear morphology resembling that of viable cells, with no condensed chromatin [17]. Ethidium bromide (EtBr) is only taken up by cells when cytoplasmic membrane integrity is lost and stains the nucleus red. EtBr also dominates over AO. Thus, live cells have a normal green nucleus; early apoptotic cells have a bright green nucleus with condensed or fragmented chromatin; late apoptotic cells display condensed and fragmented orange chromatin; cells that have died from direct necrosis have a structurally normal orange nucleus [18]. Ethidium re-emits this energy as yellow/orange light centered at 590 nm. The fluorescence of ethidium bromide in an aqueous solution is significantly lower than that of the intercalated dye.

#### Materials required

DMEM medium, Penicillin/Streptomycin antibiotic solution, Trypsin-EDTA was purchased from Gibco (USA), EtBr, and Acridine orange was purchased from Sigma Aldrich (USA), Fluorescent Imaging System, (ZOE, Bio-Rad, USA).

#### Procedure

#### Cell culture

Hep-G2 liver cell line was purchased from NCCS, Pune and was cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 u/ml penicillin, and 100  $\mu$ g/ml streptomycin, and maintained under an atmosphere of 5% CO2 at 37oC [19].

#### EtBr/AO staining

Briefly, 5 x 105 cells/ml of Hep-G2 liver cells were plated into a 96 well tissue culture plate and incubated for 24 hr in a DMEM growth medium. After incubation, the cells were treated with 44.85  $\mu$ g/ml of TiO2 sample in a serum-free DMEM medium [20]. The plate was incubated at 37oC at a 5% Co2 incubator for 24 hours [24]. After incubation, 10  $\mu$ l of 1 mg/ml acridine orange and ethidium bromide were added to the wells and mixed gently [21]. Finally, the plate was centrifuged at 800 rpm for 2 minutes and evaluated immediately within an hour, and examined at least 100 cells by a Fluorescent Imaging System, (ZOE, Bio-Rad, USA).

#### V. Results

## Characterization of synthesized Seo2 Nanoparticles

## 1) Scanning Electron Microscopy (SEM)

In the SEM images, a microscope is used to qualitatively identify the microstructural developments in the matrix of the stabilized soil specimens [21]. The SEM images of SeO2 are shown (different magnifications); thus, the spherical shape microstructure is easily observed since the pictures can be enlarged [22].



SEM (SeO2 NPs - Cinnamon) Figure.1



SEM (SeO2 NPs - Cinnamon) Figure.2



SEM (SeO2 NPs - Cinnamon) Figure.3



SEM (SeO2 NPs - Cinnamon) Figure.4

#### 2) X-ray diffraction (XRD)

X-ray diffraction is used most frequently to investigate the structure of bio composites with embedded nanostructure.

| Dataset Name                  | SEO-NPS-CM   |
|-------------------------------|--|
| File name                     | F:\16.10\VINCENT\SEO-NPS-CM.raw                    |
| Comment                       | Scan Mode: Continuous scan mode                    |
|                               | Scan Type: Locked Coupled                          |
|                               | Goniometer Stage: Phi                              |
|                               | Goniometer Control: Diffractometer Controller only |
|                               | Sample Changer: Unknown Sample Changer             |
|                               | Measurement Flag: Already measured                 |
|                               | Svnc. Axis: Unknown Svnc Axis                      |
|                               | Beam Optics: Unknown Beam Optics Flag              |
|                               | Monochromator: Unknown Monochromator               |
|                               | Analyzer: Unknown Analyzer                         |
| Measurement Date / Time       | 10/16/2023 1:04:35 PM                              |
| Raw Data Origin               | BRUKER-binary V/4 ( RAW)                           |
| Scan Avis                     | Gonio  |
| Start Position [°2Th ]        | 5 0000   |
| End Position [°2Th]           | 80 1600  |
| Sten Size [°2Th ]             | 0.0400   |
| Scan Sten Time [s]            | 13 4400  |
| Scan Type                     | Pre-set time                                       |
| Offset [°2Th ]                | 0 0000   |
| Divergence Slit Type          | Eived  |
| Divergence Slit Size [°]      | 9999 0000  |
| Specimen Length [mm]          | 10.00  |
| Receiving Slit Size [mm]      | 0.1000   |
| Measurement Temperature       | °C1 25.00  |
| Anode Material                | Cu   |
| K-Alpha1 [Å]                  | 1.54060  |
| K-Alpha2 [Å]                  | 1.54443  |
| K-Beta [Å]                    | 1.39225  |
| K-A2 / K-A1 Ratio             | 0.50000  |
| Generator Settings            | 30 mA, 40 kV                                       |
| Diffractometer Type           | Theta/Theta  |
| Diffractometer Number         | 0  |
| Goniometer Radius [mm]        | 240.00   |
| Dist. Focus-Diverg. Slit [mm] | 91.00  |
| Incident Beam Monochromat     | or No  |
| Spinning                      | No   |

XRD Results Chart





#### 3) Fourier Transform Infrared Spectroscopy (FTIR):

FTIR spectroscopy is a very powerful tool with many applications, however data interpretation is not straightforward [23]. By nature, the total spectrum generated is a series function of absorbed energy response (hence the Fourier Transform portion of the name).



#### 4) DLS (Dynamic Light Scattering):

Dynamic Light Scattering (DLS) analysis allows us to confidently measure the size distribution profiles of particles in the sub-micron range.

С

| Intensity Dis | stributi   | on           |              |           |               |                | S/            | N :      | 411615         |          |         |
|---------------|------------|--------------|--------------|-----------|---------------|----------------|---------------|----------|----------------|----------|---------|
| User          | : Con      | nmon         |              | Group     |               | :              |               |          | Repetition : 1 | /1       |         |
| Date          | : 13-9     | Sep-23       |              | File Nam  | ie            | : PSA-SeO N    | IPS           |          |                |          |         |
| Time          | . 11-      | 57.25        |              | Sample    | Information   |                |               |          |                |          |         |
| SOD Name      |            | Sizo Mothor  | 4            | Jumple    | anormadon     |                |               |          | Conurity · M   | la Cocur | b.      |
| SOP Wante     | 1.50       | Size metrio  | u            |           |               |                |               |          | security : r   | vo secur | ity     |
| Version 5.22  | 2/3.00     | Intensity    | / Distribut  | ion       |               |                |               | ACF      |                |          |         |
| F             |            |              |              |           | 200           | 100            |               |          |                |          |         |
|               | 1          |              |              | $\square$ | - 300         |                |               |          |                |          |         |
| 1             | 2          | -            | 1            | -         | "2            |                |               |          |                |          |         |
|               | Interior   |              |              | -         | 1 Income      | 8              |               | -        |                |          |         |
|               | for entite |              | 1            |           | muta          | 8              |               |          |                |          |         |
| 3             | 8          |              |              |           | я             |                |               |          |                |          |         |
|               | -          | 16.0         | 101.0        | NOM O     | 4             |                | 10 10         | 1000     | LONDO LONDO    | 1004804  |         |
|               |            | Utan         | ieser (nint) |           |               |                |               | Time (µ  | xec)           |          |         |
| Distributio   | n Resu     | lts (Contin) |              |           |               | Cumulant       | s Results     | (d)      | . 96.1         | 100      | 2       |
| -             |            |              |              |           |               | Dahad          | anality In de | (0)      | . 00.1         | (nr      | "       |
| Peak          |            | Diameter (r  | nm)          | Std. De   | v.            | Polyaisp       | ersity Inde   | x (P.I.) | : 0.337        |          |         |
| 1             |            | 163.2        |              | 149.9     | 8             | Diffusion      | n Const.      | (D)      | : 5.715e-008   | 3 (cn    | n*/sec) |
| 2             |            | 0.0          |              | 0.0       |               |                |               |          |                |          |         |
| 3             |            | 0.0          |              | 0.0       |               | Measuren       | nent Condi    | tion     |                |          |         |
| 4             |            | 0.0          |              | 0.0       |               | Tempera        | ature         |          | : 25.0         | (°C      | )       |
| 5             |            | 0.0          |              | 0.0       |               | Diluent I      | Name          |          | : WATER        |          |         |
| Average       |            | 163.2        |              | 149.9     |               | Refractiv      | ve Index      |          | : 1.3328       |          |         |
|               |            |              |              |           |               | Viscosity      | 1             |          | : 0.8878       | (CP      | )       |
| Residual      |            | 2.289e-00    | 03           | (O.K)     |               | Scatterin      | ng Intensit   | Y        | : 31552        | (cp      | s)      |
|               |            |              |              |           | ntoncity Dict | ribution Tab   | lo            | _        | : 10.12        | (%)      | )       |
| d (nm)        | f(%)       | f(cum.%)     | d (nm)       | f(%)      | f(cum.%)      | d (nm)         | f(%) f(c      | um.%)    | d (nm)         | f(%)f    | cum.%   |
| 1.0           | 0.0        | 0.0          | 6.6          | 0.0       | 0.0           | 44.1           | 2.1           | 16.9     | 292.5          | 2.0      | 85.5    |
| 1.1           | 0.0        | 0.0          | 7.2          | 0.0       | 0.0           | 47.5           | 2.3           | 19.2     | 315.5          | 1.9      | 87.4    |
| 1.2           | 0.0        | 0.0          | 7.7          | 0.0       | 0.0           | 51.3           | 2.4           | 21.6     | 340.3          | 1.7      | 89.2    |
| 1.3           | 0.0        | 0.0          | 8.3          | 0.0       | 0.0           | 55.3           | 2.5           | 24.1     | 367.1          | 1.6      | 90.8    |
| 1.4           | 0.0        | 0.0          | 9.0          | 0.0       | 0.0           | 59.6           | 2.7           | 26.8     | 396.0          | 1.5      | 92.2    |
| 1.5           | 0.0        | 0.0          | 9.7          | 0.0       | 0.0           | 64.3           | 2.8           | 29.5     | 427.1          | 1.3      | 93.6    |
| 1.6           | 0.0        | 0.0          | 10.5         | 0.0       | 0.0           | 69.4           | 2.9           | 32.4     | 460.7          | 1.2      | 94.8    |
| 1./           | 0.0        | 0.0          | 11.3         | 0.0       | 0.0           | 74.9           | 2.9           | 33.3     | 490.9          | 1.1      | 95.8    |
| 1.8           | 0.0        | 0.0          | 12.2         | 0.0       | 0.0           | 80.7           | 3.0           | 38.3     | 530.0          | 0.9      | 90.7    |
| 2.0           | 0.0        | 0.0          | 14.2         | 0.0       | 0.0           | 07.1           | 3.0           | 44 4     | 623.6          | 0.8      | 97.5    |
| 23            | 0.0        | 0.0          | 15.3         | 0.0       | 0.0           | 101 3          | 31            | 47.6     | 672 7          | 0.6      | 98.8    |
| 2.5           | 0.0        | 0.0          | 16.5         | 0.4       | 0.4           | 109.3          | 31            | 50.7     | 725.6          | 0.5      | 99.3    |
| 2.7           | 0.0        | 0.0          | 17.8         | 0.5       | 0.9           | 117.9          | 3.1           | 53.8     | 782.7          | 0.4      | 99.7    |
| 2.9           | 0.0        | 0.0          | 19.2         | 0.6       | 1.4           | 127.2          | 3.1           | 56.9     | 844.2          | 0.3      | 100.0   |
| 3.1           | 0.0        | 0.0          | 20.7         | 0.7       | 2.1           | 137.2          | 3.0           | 59.9     | 910.6          | 0.0      | 100.0   |
| 3.4           | 0.0        | 0.0          | 22.3         | 0.8       | 3.0           | 148.0          | 3.0           | 62.9     | 982.2          | 0.0      | 100.0   |
| 3.6           | 0.0        | 0.0          | 24.0         | 1.0       | 3.9           | 159.6          | 2.9           | 65.8     | 1059.5         | 0.0      | 100.0   |
| 3.9           | 0.0        | 0.0          | 25.9         | 1.1       | 5.0           | 172.2          | 2.8           | 68.7     | 1142.8         | 0.0      | 100.0   |
| 4.2           | 0.0        | 0.0          | 28.0         | 1.2       | 6.3           | 185.7          | 2.8           | 71.4     | 1232.7         | 0.0      | 100.0   |
| 4.5           | 0.0        | 0.0          | 30.2         | 1.4       | 7.6           | 200.3          | 2.7           | 74.1     | 1329.7         | 0.0      | 100.0   |
| 4.9           | 0.0        | 0.0          | 32.5         | 1.5       | 9.2           | 216.1          | 2.5           | /6.6     | 1434.3         | 0.0      | 100.0   |
| 5.3           | 0.0        | 0.0          | 37.9         | 1.8       | 10.9          | 253.1<br>251.4 | 2.4           | 81.3     | 1668.7         | 0.0      | 100.0   |
|               |            |              |              |           |               |                |               |          |                |          |         |
|               |            |              | 1            | 1         | ntensity Dist | ribution Tab   | le            |          |                |          |         |
| d (nm)        | f(%)       | f(cum.%)     | d (nm)       | f(%)      | f(cum.%)      | d (nm)         | f(%) f(c      | um.%)    | d (nm)         | f(%)f    | cum.%   |
| 6.2           | 0.0        | 0.0          | 40.9         | 2.0       | 14.7          | 271.2          | 2.2           | 83.5     | 1800.0         | 0.0      | 100.0   |
| D(10%)        | : 3        | 55.70 (nm)   | D(50%        | 1: 10     | (.50 (nm)     | D(90%):        | 353.80        | (nm)     |                |          |         |

| 3  | Operator      | TRI-BIOTECH |        |
|----|---------------|-------------|--------|
| 4  | Lamp          | 0           |        |
| 5  | Slit          | 0           |        |
| 6  | Scan From     | 1100.0nm    |        |
| 7  | Scan To       | 190.0nm     |        |
| 8  | Scan Step     | 1.0nm       |        |
| 9  | Sample Filter | 10          |        |
| 10 | memo          |             |        |
| 11 | WL(nm)        | Abs         | Т%     |
| 12 | 190           | -0.0109     | 102.53 |
| 13 | 191           | -0.011      | 102.56 |
| 14 | 192           | -0.0105     | 102.45 |
| 15 | 193           | -0.0102     | 102.37 |
| 16 | 194           | -0.0123     | 102.87 |
| 17 | 195           | -0.0112     | 102.61 |
| 18 | 196           | -0.0103     | 102.4  |
| 19 | 197           | -0.0121     | 102.82 |
| 20 | 198           | -0.0118     | 102.75 |
| 21 | 199           | -0.0128     | 102.99 |
| 22 | 200           | -0.0125     | 102.93 |
| 23 | 201           | -0.0139     | 103.24 |
| 24 | 202           | -0.0115     | 102.68 |
| 25 | 203           | -0.0115     | 102.69 |
| 26 | 204           | -0.011      | 102.56 |
| 27 | 205           | -0.011      | 102.57 |
| 28 | 206           | -0.0128     | 102.99 |
| 29 | 207           | -0.0143     | 103.35 |
| 30 | 208           | -0.0132     | 103.08 |
| 31 | 209           | -0.0107     | 102.5  |
| 32 | 210           | -0.0092     | 102.15 |
| 33 | 211           | -0.0097     | 102.25 |
| 34 | 212           | -0.0101     | 102.35 |

В

SeO NPs CM UV

September 20 16:54:58 2023

#### 5) Ultra Voilet (UV):

Titanium oxide nanoparticles confirmed by observing UV-Vis peaks at 350-365nm [25].

UV - VISIBLE SPECTROSCOPY ANALYSIS

UV - SeO<sub>2</sub> NPs

## 6) MTT ASSAY

## A. OD Value at 570 nm

A

Sample Name

Time

1

2

| S. No. | Tested sample<br>concentration<br>(µg/ml) | OD vai<br>(in triplic | lue at<br>ates) | 570 nm |
|--------|---|-----------------------|-----------------|--------|
| 1      | Control                                   | 2.891                 | 2.895           | 2.894  |
| 2      | 500 μg/ml                                 | 1.591                 | 1.619           | 1.629  |
| 3      | 400 µg/ml                                 | 1.631                 | 1.637           | 1.639  |
| 4      | 300 µg/ml                                 | 1.797                 | 1.741           | 1.774  |
| 5      | 200 µg/ml                                 | 1.812                 | 1.81            | 1.814  |
| 6      | 100 µg/ml                                 | 1.921                 | 1.928           | 1.93   |
| 7      | 80 μg/ml                                  | 1.945                 | 1.948           | 1.95   |
| 8      | 60 µg/ml                                  | 2.005                 | 2.024           | 2.028  |
| 9      | 40 µg/ml                                  | 2.058                 | 2.074           | 2.08   |
| 10     | 20 µg/ml                                  | 2.108                 | 2.117           | 2.129  |
| 11     | 10 μg/ml                                  | 2.339                 | 2.342           | 2.35   |



#### B. Cell Viability (%)

| S. No. | Tested sample<br>concentration (µg/ml) | Cell<br>(in triplic | viability<br>ates) | ( <u>%)</u> | Mean<br>Value (%) |
|--------|--|---------------------|--------------------|-------------|-------------------|
| 1      | Control                                | 100                 | 100                | 100         | 100               |
| 2      | 500 μg/ml                              | 55.0329             | 55.924             | 56.2889     | 55.74858          |
| 3      | 400 µg/ml                              | 56.4165             | 56.5458            | 56.6344     | 56.532216         |
| 4      | 300 μg/ml                              | 62.1584             | 60.1382            | 61.2992     | 61.198611         |
| 5      | 200 μg/ml                              | 62.6773             | 62.5216            | 62.6814     | 62.626758         |
| 6      | 100 μg/ml                              | 66.4476             | 66.5976            | 66.6897     | 66.578294         |
| 7      | 80 μg/ml                               | 67.2778             | 67.2884            | 67.3808     | 67.315658         |
| 8      | 60 μg/ml                               | 69.3532             | 69.9136            | 70.076      | 69.780943         |
| 9      | 40 µg/m1                               | 71.1864             | 71.6408            | 71.8728     | 71.56668          |
| 10     | 20 µg/ml                               | 72.9159             | 73.1261            | 73.566      | 73.202675         |
| 11     | 10 µg/ml                               | 80.9063             | 80.8981            | 81.2025     | 81.002283         |



Cell Viability Graph

#### C. IC50 Value of tested sample is 55.16 µg/ml

| log(inhibitor) vs. normalized response Variable slope |                   |
|---|-------------------|
| Best-fit values                                       |                   |
| LogIC50   | 1.742             |
| Hill Slope  | -1.062            |
| IC50  | 55.16             |
| Std. Error  |                   |
| LogIC50   | 0.03341           |
| Hill Slope  | 0.08924           |
| 95% CI (asymptotic)                                   |                   |
| LogIC50   | 1.673 to 1.810    |
| Hill Slope  | -1.245 to -0.8792 |
| IC50  | 47.12 to 64.57    |
| Goodness of Fit                                       |                   |
| Degrees of Freedom                                    | 28                |
| R squared   | 0.9286            |
| Sum of Squares  | 1853              |
| SX.x  | 8.135             |
| Number of points                                      |                   |
| # of X values   | 30                |
| # Y values analysed                                   | 30                |

#### D. Images of control cells and treated cells.







## 7) EtBr /AO STAINING:

| Samples  | Fluorescent images | Fluorescent images |
|--|--------------------|--------------------|
| Control  |                    | •                  |
| Treated with 55.16<br>µg/ml of SeO <sub>2</sub> sample |                    |                    |



Live and Apoptotic Cells Graph

| S.No | Dead cells | Necrotic cells | Pro-Apoptotic cells | Apoptotic cells | Live cells |
|------|------------|----------------|---------------------|-----------------|------------|
| 1.   | 2          | 10             | 20                  | 27              | 41         |
| 2.   | 1          | 13             | 22                  | 25              | 39         |

#### VI. RESULTS AND DISCUSSION

Selenium (Se) is an important trace element that plays a crucial role in human health and regulates many crucial cellular functions mediated through its incorporation into selenoproteins. The aim of the study was to green synthesis and characterization of SeO nanoparticles from Cinnamon and their anticancer property in Hep-G2 cells. The optical, structural, morphological, elemental, and functional characterizations of the SeONPs were carried out using techniques such as UV-vis spectrophotometry, electron microscopy, energy dispersive X-ray spectrometry, and Fourier transform infrared spectrophotometry, respectively. The results showed the presence of spherical shape nanoparticles with 149 nm of particle size and the presence carboxyl groups. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide) assay revealed that the biosynthesized SeONP induces cell death of Hep-G2 human cancer cells. The IC50 value was recorded at 55.16 µg/mL, respectively. Furthermore, the EtBr/Ao staining results confirmed that the selenium nanoparticles induced apoptosis of cancer cells. Therefore, the SeO Nps could be a promising candidate for the control of cancer cells. Another study on the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay revealed that the biosynthesized SeNrs induces cell death of Hep-G2 and MCF-7 human cancer cells. The lethal dose (LD50%) of SeNrs on Hep-G2 and MCF-7 cells was recorded at 75.96  $\mu$ g/mL and 61.86  $\mu$ g/mL, respectively.

#### **VII. CONCLUSION**

In conclusion, our results clearly show a selective cytotoxicity and apoptosis inductive effect SeO Nps from cinnamon on the human hepatoma (HepG2) cell line. The isolation of the active chemical constituents from the cinnamon and determination of their individual anticancer activity will be further performed.

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#### **Conflict of Interest**

The authors declare that they have "No conflict of interest".

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