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BIO - CAPPED SELENIUM NANOPARTICLES CONJUGATED WITH BOVINE SERUM ALBUMIN FOR TARGETED THERAPY

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ABSTRACT

To perform the green synthesis of selenium nanoparticles capped with plant extract nanoparticles are nano-sized particles with the dimension ranging from 1-100 nm. The production of selenium nanoparticles typically employs one of three techniques: physical, chemical, or biological techniques. We can characterize the synthesized selenium nanoparticles through UV-visible Spectrophotometer, FT-IR, and XRD. After the characterization of selenium nanoparticles, they are bio-conjugated with bovine serum Albumin and determined the concentration of bovine serum albumin for bio-conjugation against the cancer cell lines. Finally molecular dynamic of bovine serum albumin - plant extract through bioinformatics tools using molecular docking. Green chemistry procedures emphasize the use of biological systems, which include microorganisms, and plant extract. Biological systems are used as capping, reducing, and stabilizing agents in replacement of chemical biogenic synthesis of selenium nanoparticles is nontoxic and economical and uses environmentally benign non-hazardous material such as phytochemicals from plant extracts. The second biggest cause of death in the world is cancer. Still, cancer does not have a targeted drug delivery. Selenium nanoparticles may be used as a covering or directly in solution at dosages that inhibit bacterial and cancerous growth. With an atomic number of 34, selenium corresponds to group 16 of the periodic table. Selenium has achieved a different position in the area of nanotechnology because of its large potential in the delivery of drugs and proteins. Selenium has both crystalline and amorphous structures in nature. Although selenium is an essential trace element for human health, there is a very thin line separating it from harm. It has different physiological roles in the human body such as antioxidants and prevents the formation of cancer.

Key word: Selenium nanoparticles, Bovine serum albumin, Plant extracts.

1. INTRODUCTION

Nano oncology has emerged as the biggest boon to the field of science and technology in the last few decades and has shown rapid growth, which has dramatically transformed the material science, biomedical, environmental, agricultural, and industrial domains (Vanya Nayak et al.,2021). Because it needs a non-toxic solvent and a moderate temperature, green synthesis using plant extract has become common. It also prevents cellular damage stimulated by free radicals by incorporation into antioxidant enzymes (Krystyna Pyrzynska et al.,2021). The second biggest cause of death in the world is cancer. Still, cancer does not have a targeted drug delivery. Selenium nanoparticles may be used as a covering or directly in solution at dosages that inhibit bacterial and cancerous growth (Raisa L. Silveira et al.,2019). With an atomic number of 34, selenium corresponds to group 16 of the periodic table. Selenium has achieved a different position in the area of nanotechnology because of its large potential in the delivery of drugs and proteins (Vanya Nayak et al.,2021). Selenium has both crystalline and amorphous structures in nature. Although selenium is an essential trace element for human health, there is a very thin line separating it from harm (Neha Bisht, et al.,2022) It has different physiological roles in the human body such as antioxidants and prevents the formation of cancer (Neha Bisht et al.,2022). Here, we use the Withania Somnifera(ashwagandha), Vitis vinifera(grapes), and clitoria ternatea(butterfly pea plant) as a plant extract. The Fabaceae family includes the annual leguminous herbaceous plant known as Clitoria ternatea (butterfly pea). Around the globe, the genus Clitoria is widely dispersed in tropical and subtropical habitats. Ternatins, anthocyanins derived from C. ternatea, are responsible for the blooms' distinctively intense blue color. By using these plant extract we synthesize the selenium nanoparticles with the addition of sodium selenite (Krystyna Pyrzynska et al.,2021). After the selenium nanoparticle preparation, we bio-conjugated the SeNPs with the bovine serum albumin and used them for cancer therapy. Green chemistry procedure place emphasis on the use of biological systems which include microorganisms, and plant extract. Biological systems are used as capping, reducing, and stabilizing agents in replacement of chemical biogenic synthesis of selenium nanoparticles is non-toxic, and economical, and uses environmentally benign non-hazardous material such as phytochemicals from plant extracts (Krystyna Pyrzynska et al.,2021). Elemental selenium has more importance in the field of biological, physical, and chemistry (Neha Bisht et al.,2022). For various samples, TEM analysis showed the existence of NPs with essentially identical sizes and shapes, and all NPs were protected by a layer of unwrinkled BSA. The expanded BSA coating's isoelectric point on the NP surface was consistently close to 4.7, the same as unconjugated BSA. Controls included BSA-free and cationic surfactant-coated Au NPs. Under the same circumstances, they demonstrated high hemolytic activity and very poor cell viability. As a result, BSA-coated NPs

were thought to be the finest delivery systems for drugs and other potential biomedical uses. The sequencing of human DNA made possible by the human genome project has improved tools for detecting genomic, transcriptional, proteomic, and epigenetic alterations. The adoption of personalized medicine has been expedited by these technologies and innovative drug development. To individualize disease prevention, diagnosis, and therapy, personalized medicine makes use of ideas about the genetic and environmental causes of illness (Tarun Kumar Misra et al.,2009)**.** The promise of improving patient care lies in the optimization of treatment using targeted therapy, which uses molecules to target particular enzymes, growth factor receptors, and signal transducers to interfere with a variety of oncogenic cellular processes. This review summaries the most recent state-of-the-art uses of personalized medicine and focuses on targeted therapy for cancer therapeutics organized in accordance with the primary human carcinogenesis drivers (Tarun Kumar Misra et al.,2009). Nearly 7 million people die from cancer each year, making it one of the leading causes of mortality. The use of therapeutic antibodies or small molecules in new cancer targeted medicines has increased tumor specificity while reducing toxicity. Drug resistance, cancer stem cells, and high tumor interstitial fluid pressure are some of the ongoing difficulties in the fight against cancer. For instance, higher interstitial fluid pressure reduces the effectiveness of therapeutic drug absorption in many solid tumours. The use of ligand-targeted treatment, which can be utilised to make targeting more precise and deliver bigger dosages of anti-cancer medicine to tumour tissue, is one of the most promising approaches to overcoming such difficulties. Recent developments in the diagnosis and treatment of cancer are reviewed and discussed in this article.

2. MATERIALS AND METHOD

PLANT COLLECTION

The vitis vinifera seed, clitoria ternatea flower, withania somnifera root were collected from the outskirts of madurai and washed in water to remove dust. Then the seed, flower, root were shade dried for about 10-15 days.

SOLVENT EXTRACTION

The dried roots, seeds, flowers were coarsely powdered using an mortor and pestle and the dried powder of 4.8g of vitis vinifera, 0.6g of clitoria ternatea, 4.6g of withania somnifera were mixed with 50ml of distilled water. The solution were kept in 90˚c for 20 mins in water bath after the solution were filtered by using filter paper and then the extract was stored and used.

PREPARATION OF SODIUM SELENATE

0.315g of sodium selenate were mixed with 100ml of distilled water.

SYNTHESIS OF SELENIUM NANOPARTICLES

5 ml of plant extract mixed with 20 ml of 40mM selenate acid and mixed with 45 ml of distilled water. The mixed solution were kept in the water bath until the colour changes to reddish colour. After the colour changes the solution were kept in the centrifugation process for 20 minutes at 3500 rpm. Finally, the supernatant was removed and the pellet were diluted with distilled water.

BIO CONJUGATION OF BOVINE SERUM ALBUMIN WITH SeNPs

The synthesized nanoparticles are diluted with 8 ml distilled water and the PH was adjusted to 6.5. The bovine serum albumin solution was prepared(15mg BSA / 15 ml distilled water) and the PH was adjusted to 4.8 to 5.6. The 1ml of bovine serum albumin and 9 ml of plant derived selenium nanoparticles are mixed. Finally, the mixed solution were kept in the water bath for 45˚c.

3. RESULT AND DISCUSSION

PLANT COLLECTION

The vitis vinifera seed, clitoria ternatea flower, withania somnifera root were collected from the outskirts of madurai and washed in water to remove dust. Then the seed, flower, root were shade dried for about 10-15 days.

Fig.1 Withania somnifera **Fig.2** Withania somnifera powder

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Fig.3 vitis vinifera

Fig.4 vitis vinifera powder

Fig.5 Clitoriaternatea

Fig.6 Clitoria ternatea powder

4. SOLVENT EXTRACTION

The dried roots, seeds, flowers were coarsely powdered using an mortor and pestle and the dried powder of 4.8g of vitis vinifera, 0.6g of clitoria ternatea, 4.6g of withania somnifera were mixed with 50ml of distilled water. The solution were kept in 90˚c for 20 mins in water bath after the solution were filtered by using filter paper and then the extract was stored and used.

Fig7. Before filteration of plant extract

Fig.8 After filteration of plant extract

SYNTHESIS OF SELENIUM NANOPARTICLES

5 ml of plant extract mixed with 20 ml of 40mM selenate acid and mixed with 45 ml of distilled water. The mixed solution were kept in the water bath until the colour changes to reddish colour. After the colour changes the solution were kept in the centrifugation process for 20 minutes at 3500 rpm. Finally, the supernatant was removed and the pellet were diluted with distilled water.

Fig.9 Before synthesis of SeNPs

Fig.10 After synthesis of SeNPs

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5. CHARACTERAIZATION OF SELENIUM NANOPARTICLES

UV-VISIBLE SPECTROSCOPY

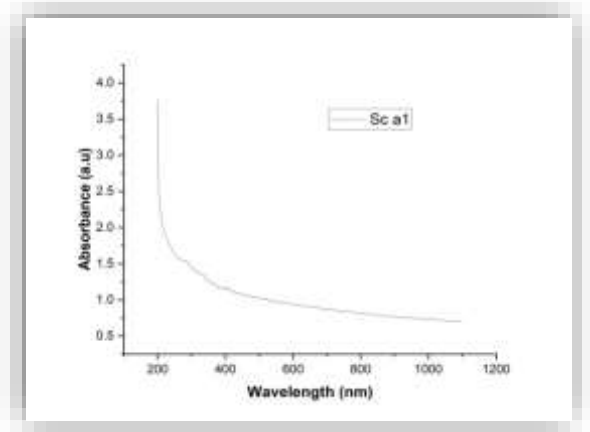

Fig.11 Characteraization of SeNPs in UV

Reduction of selenium ions into selenium nanoparticles during exposure to plant extracts and ascorbic acid was observed using UV-Vis spectra. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles. Chitosan stabilized selenium nanoparticles shown the SPR band at 450 and 310 nm indicates the formation of selenium nanoparticles. Some of the minor peaks were also observed due to the presence of biomolecules from chitosan Fig 11. Previous studies have shown that the spherical Se-NPs contribute to the absorption bands at around 250-400nm in the UV-Visible spectra reported max at 280 nm at 355 nm at 380 nm.

FT-IR

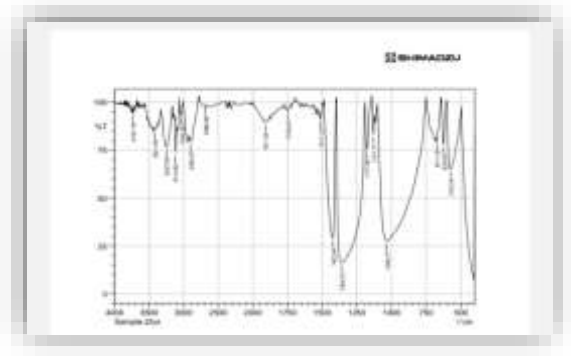

Fig.12 Characteraization of SeNPs in FT-IR

Table no. 1 Characteraization of SeNPs in FT-IR

LENGTH(cm^{-1})	FUNCTIONAL GROUP	VIBRATION
573.78	HALOGEN COMPOUND	-
623.93	ALKYNES	BENDING VIBRATION
671.18	ALKYNES	BENDING VIBRATION
1033.77	AMINES	STRETCHING VIBRATION
1131.17	AMINES	STRETCHING VIBRATION
1177.46	AMINES	STRETCHING VIBRATION
1359.72	ALKANES, ALCOHOLS, PHENOLS, ALDEHYDES & KETONES	BENDING VIBRATION
1422.4	ALKANES, ALDEHYDES & KETONES	BENDING VIBRATION
1511.12	CARBOXYLIC ACID & DERIVATIVE	BENDING VIBRATION

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1748.35	ALDEHYDES & KETONES	STRETCHING VIBRATION
1911.33	ALKENES	STRETCHING VIBRATION
2686.66	CARBOXYLIC ACID & DERIVATIVE	STRETCHING VIBRATION
2908.67	ALKANES	STRETCHING VIBRATION
3042.5	ALKENES	STRETCHING VIBRATION
3114.82	CARBOXYLIC ACID & DERIVATIVE	STRETCHING VIBRATION
3246.9	CARBOXYLIC ACID & DERIVATIVE ,ALCOHOLS AND PHENOLES	STRETCHING VIBRATION
3421.48	AMIDES	STRETCHING VIBRATION
3737.79	ALCOHOLS	-

The functional groups present in green synthesized chi-tosan-selenium nanoparticles were identified by FTIR spec-tra. FTIR analysed at different wavenumber range from 4000 to 500cm-1. The functional groups involved in the synthesis of selenium nanoparticles using chitosan were detected with the help of FT-IR analysis.

SEM

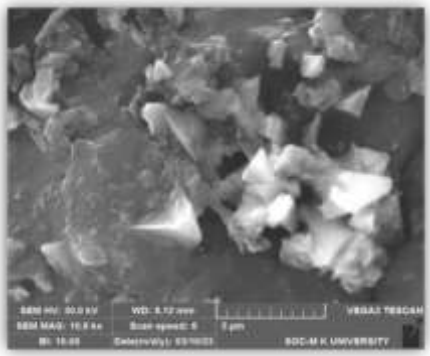

Fig.13 Characteraization of SeNPs in SEM

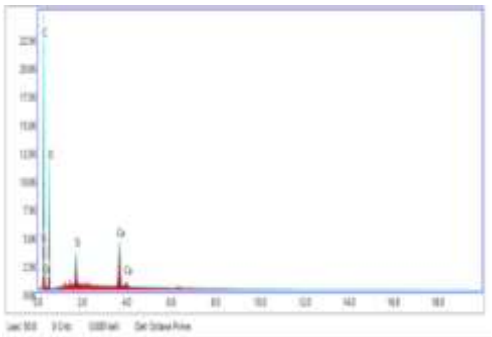

Fig .14 Selected area analysis

Table no.2 Selected area analysis

Element	Weight %	Atomic %	Net Int	Error %	K ratio	Z	A	F
CK	56.25	64.07	2400.61	5.44	0.3187	1.0184	0.5561	1.0000
OK	40.56	34.68	1169.28	9.96	0.0510	0.9804	0.1283	1.0000
SiK	1.06	0.52	444.90	5.06	0.0068	0.9018	0.7012	1.0057
CaK	2.12	0.72	864.83	2.42	0.0200	0.8585	1.0609	1.0338

BIO CONJUGATION OF BOVINE SERUM ALBUMIN WITH SeNPs

The synthesized nanoparticles are diluted with 8 ml distilled water and the PH was adjusted to 6.5. The bovine serum albumin solution was prepared(15mg BSA / 15 ml distilled water) and the PH was adjusted to 4.8 to 5.6. The 1ml of bovine serum albumin and 9 ml of plant derived selenium nanoparticles are mixed. Finally, the mixed solution were kept in the water bath for 45˚c.

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Fig.15 Plant derived selenium nanoparticle solution

Fig.16 Bovine serum albumin solution

Fig.17 BSA coated plant derived selenium nanoparticles solution

6. BIOCONJUGATION

Bio-conjugation of BSA with plant derived selenium nanoparticles are find by the protein estimation . Protein estimation done through Bradford method at 595 nm. The blue colour was observed based on the OD value of 1.02, We confirmed thepresence of BSA in nanomaterial after synthesis.

SELENIUM NANOPARTICLES AGAINST AGS CELL LINE STUDY

Table. No.3 SeNPs against AGS cell line (C1)

Samples(µg/ml)				**Average**	**Percentage**			**Average**	**stdvp**
VC	0.178	0.239	0.263	0.226667	78.52941	105.4412	116.0294	100	15.78538
10	0.2	0.259	0.266	-	88.23529	114.2647	117.3529	106.6176	13.05929
20	0.176	0.324	0.308	-	77.64706	142.9412	135.8824	118.8235	29.25842
40	0.221	0.322	0.296	-	97.5	142.0588	130.5882	123.3824	18.89119
80	0.256	0.315	0.185	-	112.9412	138.9706	81.61765	111.1765	23.44747
160	0.206	0.201	0.212	-	90.88235	88.67647	93.52941	91.02941	1.983932

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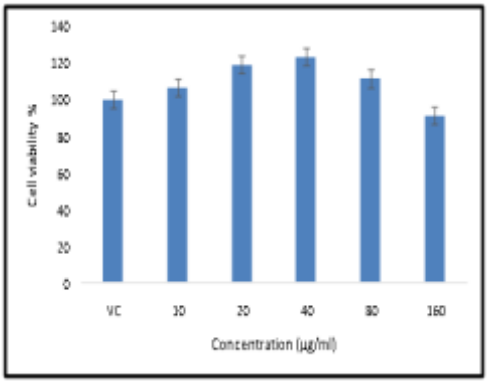

Fig .18 SeNPs against AGS cell line (C1)

Table no. 4 SeNPs AGAINST AGS CELL LINE(C2)

Samples(µg/ml)				Average	Percentage			Average	Stdvp
VC	0.263	0.22	0.195	0.226	116.3717	97.34513	86.28319	100	12.4262
10	0.23	0.276	0.287		101.7699	122.1239	126.9912	116.9617	10.92442
20	0.324	0.327	0.273		143.3628	144.6903	120.7965	136.2832	10.96417
40	0.268	0.276	0.282		118.5841	122.1239	124.7788	121.8289	2.537559
80	0.255	0.327	0.291		112.8319	144.6903	128.7611	128.7611	13.00614
160	0.212	0.29	0.285		93.80531	128.3186	126.1062	116.0767	15.77413

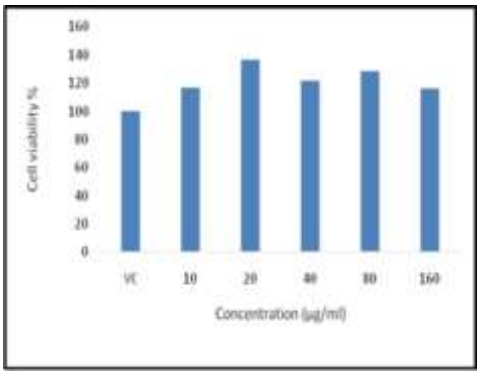

Fig 19 SeNPs AGAINST AGS CELL LINE (C2)

7. CONCLUSION

Sodium selenate acid combined with plant extract was used to create the selenium nanoparticles. The selenium nanoparticles' production was consistent with the red colour. The protein estimate determines the bio-conjugation of BSA with selenium nanoparticles generated from plants. Bradford method used to estimate protein. The OD value was used to determine the blue colour. After production, we verified the presence of BSA in the nanomaterial. This study's findings demonstrated the effectiveness of bio-conjugating bovine serum albumin with selenium nanoparticles against the AGS cell line. The AGS cell line is inhibited by it.

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