ANTIBACTERIAL AND ANTICANCER ACTIVITY OF THE SELENIUM NANOPARTICLES OF RED SEAWEED *JANIA RUBENS* (L) J.V.Lamouroux

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Abstract

Red seaweeds a significant class of macroalgae is a rich source of structurally varied bioactive components, such as protein, sulfated polysaccharides, pigments, polyunsaturated fatty acids, vitamins, minerals, and phenolic compounds with industrial, medical, and and nutritional significance. Red coral seaweed *Jania rubens* has a strong pharmacological component. The natural macromolecules that are derived from these algae have not been well recognized, despite its potential for medicinal use.Our present investigation was aimed to study the antibacterial, antioxidant and anticancer activity of Selenium Nanoparticles (SeNPs) synthesized from the aqueous extracts of *J. rubens*. The SeNPs synthesized by using Sodium seleniteand SeNPs formation was confirmed by the brown color change followed by the UV Vis spectroscopy absorbance maximum at 225nm and 230nm. Furthermore, the antibacterial activity of SeNPs was studied against human pathogenic bacteria such as *Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis* and the results indicated that a synthesized SeNPs showed potential antibacterial activity against gram negative bacteria *P. aeruginosa* and gram positive bacteria *B.subtilis* with the zone of inhibition of 23mm. On the other hand the antioxidant activity against the DPPH radical and anticancer activity against the human adenocarcinomic cells (A549 cell line) Collectively the present study results explored the strong biological applications of the *Jania rubens* based SeNPs and its importance in the pharmaceutical filed for the development of new drug.

Keywords: *Jania rubens,* Selenium Nanoparticles; Antibacterial, Antioxidant, Anticancer

Introduction

Marine life makes up 70% of the earth's surface, its enormous diversity and biodiversity are only partially understood. Nevertheless, marine life is a rich source of novel metabolites with a variety

of uses, such as pharmaceuticals, cosmeceuticals, nutraceuticals, agrochemicals, and other chemicals with industrial relevance (Rengasamy et al., 2020). Seaweed extracts and their natural products are becoming more and more popular in pharmacology and cosmetics, among other areas of healthcare. Macroscopic sea algae have been closely linked to human life from ancient times and have been utilized extensively in a variety of ways as a source of food, feed, fertilizer, and medicine. They are mostly utilized for commercially significant phycocolloids (Compere, 1970). Over 60 trace elements are present in marine algae at concentrations far higher than those found in terrestrial plants. They also contain vitamins, protein, iodine, bromine, and compounds that are both stimulatory and antibacterial (El-Din and El-Ahwany, 2016). Seaweeds include a wide variety of pharmaceutically useful chemicals from many chemical classes, including pigments, polyphenols, polysaccharides, and polyunsaturated fatty acids (Pradhan et al., 2022; Lomartire and Goncalves, 2022).

The red seaweed species *Jania rubens* is one of the most prevalent in the Eastern Mediterranean intertidal zone. *J. rubens* is a tiny, branching seaweed with thin, segmented, dichotomous, cylindrical thalli that, when bleached during low tide, form dense mats covering the abrasion platforms. These mats can range in color from dark red to pure white. It can grow as an epiphyte on other seaweeds or connected to the substrate (Krupnik et al., 2023). The algae are well known for the abundant in various phytochemicals, such as diterpenes and polyphenols includes flavonoids and tannins being the two main types of polyphenols (Ahmed et al., 2011). The phytochemicals that can be extracted from *J. rubens* can be added to food items, makeup, and supplements (Fathi and Varshosaz, 2013).

Selenium is a trace and necessary micronutrient for the well-being of people, animals, and microbes. Many researchers have recently become interested in selenium nanoparticles (SeNPs) because of their low toxicity, biocompatibility, and bioavailability (Bisht, 2022). Selenium is a necessary trace element that has numerous pharmacological effects, physiological processes, antioxidative and prooxidative effects, and nutritional benefits. The field of nanotechnology is a new one that is radically altering the way materials are created at the nanoscale (Zhang et al., 2004). Comparing nano selenium to its bulk form reveals that it has superior reactivity, low toxicity, low dosage, and great biological activities. This crucial vitamin is harmful at high concentrations but necessary at low concentrations for the majority of living things. Since SeNPs have a very high surface to volume ratio, gradual release of Se ions, and are

known to bind proteins, nanoparticle formulations have been designed to give more efficient selenium dosing regimens. Additionally, the surface of the nanoparticles may catalyze processes in efficiently (Zhang et al., 2008; Patil et al., 2019). Based on this background information the present study was aimed to study the antibacterial, antioxidant and anticancer activities of selenium nanoparticles synthesized by red seaweed *Jania rubens*.

Materials and Methods

Sample Collection and extraction

The red algal seaweed, Jania rubens, was gathered from the Gulf of Mannar's Mandapam coastal region on India's southeast coast. The algae were prepared for additional aqueous extraction by being cleaned with distilled water, dried, and ground into a coarse powder.

Preparation of Selenium nanoparticles (SeNPs)

Sodium selenite (0.1 g) was added to the 10 ml of distilled water and the mixure was heated on a hot plate with a magnetic stirrer for 30 minutes. Then, added 5ml of algal extract, and agitate it magnetically for 1 hour. After overnight incubation the colour changes wasnoticed and the absorbance is calculated using UV Vis spectroscopy (200-700nm) (Francis et al., 2020).

Antibacterial activity

Antibacterial activity *J. rubens* algal extract synthesized SeNPs was performed against both gram positive (*Staphylococcus aureus and Bacillus subtilis*) and gram negative bacteria (*Pseudomonas aeruginosa*) by well diffusion method. Different bacteria cultures were swabbed onto the Muller Hinton agar media plate and the 6mm of wells were prepared with the help of cork borer. Different concentrations of extracts (25, 50, 75 and 100µg /mL) were put into the well and Gentamicin was used as a positive control (20µl). The plates were incubated at 37°C for 24 hours and the zone of inhibition. The zone formation surrounding the wells was measured in order to determine the zone of inhibition (Francis et al., 2020).

Anticancer activity

Human epithelial cell line (A549) was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Using Dulbecco's modified eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin, cells were kept in the logarithmic phase of growth. They were kept in an incubator with 95% air humidity at 37°C and 5% CO_2.

The sample's anticancer properties were evaluated using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) on A549 cell lines (Mossman (1983). The cells were seeded in 96-well microplates (1 x 106 cells/well) and allowed to develop to 70–80% confluence after 48 hours of incubation at 37°C in an incubator with 5% CO2. After that, the medium was changed, and the cells were cultured for 24 hours with varying sample concentrations. Following a 24-hour period, the cells were rinsed with phosphate-buffer saline (PBS, pH -7.4), and each well received 20 µL of MTT solution (5 mg/mL in PBS). The plates were then left in the dark for two hours at 37ºC. After dissolving the formazan crystals in 100 µL DMSO, the absorbance was measured at 570 nm using spectrometry. Percentage of cell viability was calculated using the formula,

$$\text{Cell Viability} = \frac{Absorbance of sample}{Absorbance of control} X100$$

Results

Synthesis of Selenium nanoparticles (SeNPs)

Selenium nanoparticles (SeNPs) were synthesized by using the *J. rubens* algal aqueous extracts. The first stage of characterization of nanoparticles was confirmed by colour change. Our present study showed that the colous change of pale yellow to brown which indicates the presence of SeNPs and the absorbance of SeNPs is maximum at 225nm and 230nm in the UV Vis spectroscopy (Fig. 1A and B).

Antibacterial activity

The zone of inhibition of *J. rubens* SeNPs against the gram positive and gram negative bacteria was assessed and the results showed that the maximum zone of inhibition (23mm) was noticed at the 100µg /mL concentration against the *P. aeruginosa* and *B. subtilis* followed by 75µg /mL concentration against the *P. aeruginosa* (21mm) and *B. subtilis* (18mm). The control Gentamicin zone of inhibition showed 21mm against *P. aeruginosa* and 22mm against *B. subtilis*. Additionally, there is no zone of inhibition was observed against *S. aureus* at any concentrations of *J. rubens* SeNPs (Table 1 and Fig. 2).

Anticancer activity

Anticancer activity of different concentration of (6.25, 12.5, 25 and 50µg/mL) *J. rubens* SeNPs against human adenocarcinomic (A549) cell were analyzed. The results showed in the Figure 4 and 5 demonstrated that the A549 cell viability was significantly debited in the SeNPs treated cells than the control after 24 hours by dose dependently. After 24 hours of the inhibition time the 50µg/mL concentration of SeNPs treatment reduced the A549 cell viability to 15.63% followed by 25, 12.5 and 6.25µg/mL of SeNPs treatment showed the cell viability of 29.25%, 54.36% and 88.68% respectively.

Discussion

The abundance of biologically active natural products found in marine resources is unparalleled, and many of these chemicals have structural characteristics not seen in terrestrial creatures. Numerous studies have documented the presence of chemicals obtained from macro algae that have a wide range of biological activities, including antibacterial, antiviral, anti-tumor, anti-inflammatory, and neurotoxic properties (Saritha et al., 2013; Osman et al., 2013). Many scientific communities have been interested in nanotechnology for many years. Numerous industries, including agriculture, medicine, biocontrol agents, therapeutics, anticancer medications, and targeted drug delivery, have found favor with nanomaterials due to their intriguing characteristics, including size, surface area to charge ratio, reactive surfaces, solubility, and consequently enhanced bioavailability (Khurana et al., 2019). Our present study results revealed that the Selenium nanoparticles (SeNPs) were synthesized by using the *J. rubens* algal aqueous extracts and the absorbance maximum was noticed at 225nm and 230nm in the UV Vis spectroscopy. Similar to our study results Selenium nanoparticles synthesized by using the marine seaweed *Sargassum fusiforme* polysaccharides showed the absorbance spectrum at 228nm and 232nm.

Nanoparticles have been considered as a treatment for infections because they kill bacteria through different pathways than traditional antibiotics and have very little damage to human cells. Therefore, in order to control bacterial infections, nanomaterials can be viewed as a possible substitute for antibiotics. Selenium, a dietary element having a significant role in biological systems is one of the intriguing substances to incorporate with antibacterial agents (Alpaslan et al., 2017; Beyth et al., 2015). Selenium has bactericidal properties because it can catalyze the oxidation of intracellular thiols, which kills tiny organisms.SeNPs were thought to

exhibit antibacterial action as a result of oxidative stress.Our present study results on the antibacterial activity of SeNPs against the pathogenic bacteria demonstrated that, the SeNPs having the potential to kill the tested pathogenic bacteria species such as *P. aeruginosa* and *B. subtilis* which was confirmed in the zone of inhibition assay. Numerous studies contrasting the antibacterial efficacy of chemically and environmentally produced SeNPs have shown that the former is noticeably more potent (Mariadoss et al., 2022). Accordance with our study results Khudier et al. (2023) reported the synthesized selenium nanoparticles by using the *Vaccinium arctostaphylos* (L.) fruit extract was noticed the high percentage of bacterial death against the *S. aureus, C. diphtheria* and *E. coli* was 44.6%, 29.49%, and 24.4% respectively. Numerous researches postulated that positively charged SeNPs have a greater chance of inhibiting bacterial development because of their enhanced adherence to bacterial surfaces (Alghuthaymi, 2022).

One of the essential minor components, selenium, has been shown to enhance or restore the activity of glutathione peroxidase and selenium-catalyst in preventing free radical damage to cells and tissues *in vivo*. In addition to that selenium is a crucial biochemical component of glutathione peroxidase, an enzyme that functions as an antioxidant by protecting vital SH-groups and breaking down peroxides (Ramos and Webster, 2012; Sieber et al., 2005). On diverse human body cells, the surface of SeNPs adorned with different chemical compounds and plant extracts functions as a pro-oxidant and antioxidant (Menon and Shanmugam, 2020). Antioxidant result of our present investigation revealed that the synthesized SeNPs has greatly inhibited the formation of DPPH free radicals at different concentrations. Our study results were supported by the green synthesis of SeNPs by *Emblica officinalis* fruit extract showed a potential free radical scavenging activity against DPPH and ABTS radicals (Gunti et al., 2019). Another study on Kong et al. (2014) reported that SeNPs were prepared by using gum arabic as the stabilizer in a facile synthetic approach showed significant antioxidant activity against the hydroxyl and DPPH radical scavenging.

Selenium nanoparticles are currently being extensively studied for their anti-cancer properties against osteosarcoma, lung, kidney, and breast malignancies based on in vitro and in vivo studies (Ramamurthy et al., 2013). Our present study results explored thata notable decrease in the cell viability was noticed against the Human lung carcinoma (A549) cells by different concentration *J. rubens* SeNPs treatment.Numerous investigations have demonstrated that selenium nanoparticles can prevent harm to healthy cells while specifically inducing apoptosis in

a variety of cancer cell types.The most often used mechanisms for SeNPs' anticancer effects include internalization of the nanoparticles, control of reactive oxygen species generation, autophagy induction, and activation of the intrinsic apoptotic machinery. In accordance with our study results a phytofabricated selenium nanoparticles prepared with the *Gracilaria lemaneiformis* Polysaccharide showed potential anticancer activity against the U87 glioma cell (Jiang et al., 2014). Another study on Tang et al. (2019) reported that, arabinogalactans incorporated selenium nanoparticles showed significant anticancer activities against the different types of cancer cells such as A549, HepG2 and MCF7 cells with a dose-dependent effect.

Conclusion

Our present study results concluded that, the selenium nanoparticles synthesized from the red seaweed *Jania rubens* aqueous extracts showed the proficient antibacterial activity against the human pathogenic bacteria species and promising effect against the Human adenocarcinoma A549 cells.Overall the present study results demonstrated that, they are seen as interesting natural candidates for anticancer and antibacterial treatments.It is advised that in the future, more applied research be done on the bioactive chemicals causing the actions in order to further explore the species under study in vivo and for therapeutic and medical purposes.

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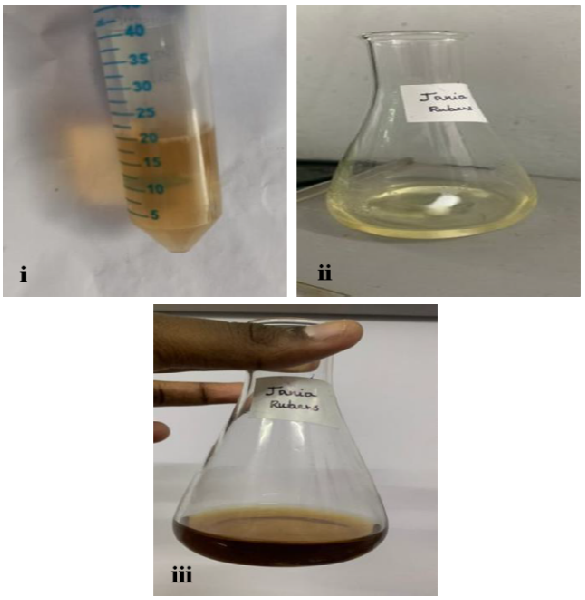
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Figure legends

(A)



(B)

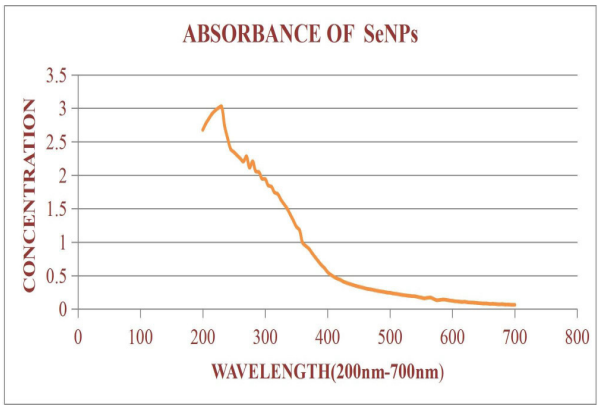


Figure 1: (A) Synthesis of Selenium nanopartilces from the aqueous extracts of red seaweed *Jania rubens*; (i) Aqueous extract of *Jania rubens*; (ii) Sodium selinite solution and (iii) synthesized Selenium nanopartilces color changed to brown color. (B) UV Vis spectrum of synthesized Selenium nanopartilces.

S.No	Name of the Bacteria	Zone of inhibition (mm)				
		Control (Gentamicin) (20µl)	Concentration of Selenium nanopartilces (µg /mL)			
			25	50	75	100
1	*Pseudomonas aeruginosa*	21	Nil	Nil	21	23
2	*Staphylococcus aureus*	Nil	Nil	Nil	Nil	Nil
3	*Bacillus subtilis*	22	Nil	Nil	18	23

Table 1: Zone of inhibition of *J. rubens* Selenium nanopartilces against the Human pathogenic bacteria.

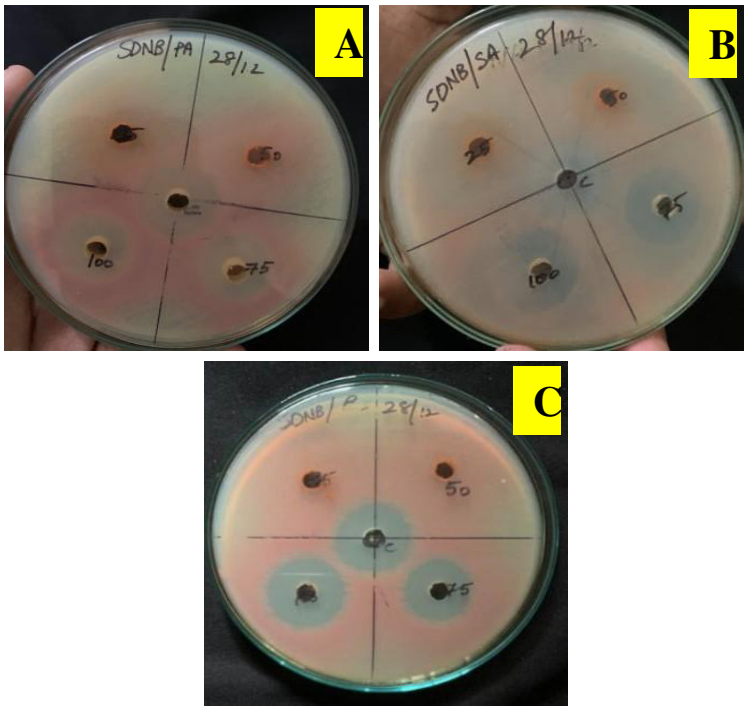


Figure2: Impact of different concentration of *J. rubens* Selenium nanopartilces against the bacteria

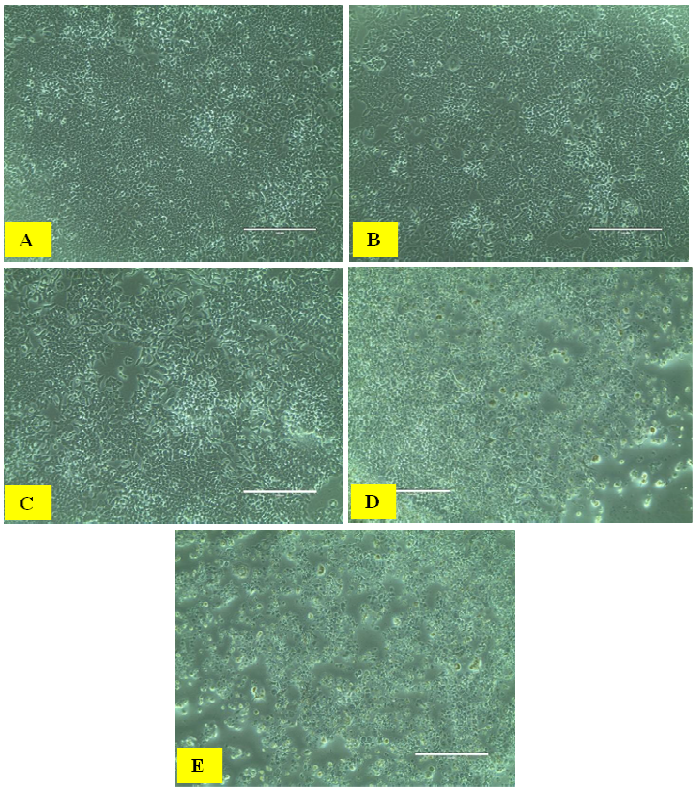


Figure 3: Anticancer activity of different concentration of *J. rubens* Selenium nanopartilces against the cell viability of human adenocarcinoma cells (A549). (A) Control (B) 6.25 µg/mL treated cells (C) 12.5 µg/mL treated cells (D) 25 µg/mL treated cells (E) 50 µg/mL treated cells.