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GREENSYNTHESIS OF SILVER NANOPARTICLES FROM RED SEAWEED (*PORTIERIA HORNEMANNII*) AND ITS APPLICATIONS

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ABSTRACT
The present study aimed to green synthesis the nanoparticles using bioactive compounds from unique seaweeds and testing its application for anti-microbial, anti-diabetic, ovicidal, larvicidal and cytotoxic activity. Seaweeds are marine non- flowering plants have much of the secondary metabolites and had not been discovered much. Portieria hornemannii is a species of red algae in the family Rhizophyllidaceae, they are found on rocky shores. The green synthesis of silver nanoparticles (AgNPs) was synthesized from the seaweed *Portieriahornemannii*. The AgNPs were synthesized at 60°c at 5000rpm when a solution of silver nitrate was added to aqueous extract of *Portieria hornemannii*. The UV-Visible Spectroscopy, Fourier transform-infrared spectroscopy (FT-IR), Scanning electron microscope (SEM) were used to characterize the formation of AgNPs. The green synthesized silver nanoparticles were used to test the applications such as Anti-microbial, ovicidal, larvicidaland anti-diabetic and cytotoxicity using VERO cell lines.

KEYWORDS: *Portieria hornemannii*, UV-Vis, FT-IR, SEM, Cytotoxicity, VERO cell lines.

INTRODUCTION
Seaweeds are marine algae, multicellular organisms. They are present in the salt water and attached to rock or hard substrate in the coastal areas. They are complex organisms. They have specialized tissues and growth structures. They secrete pheromones and have different types of sex organs. Red algae have the most complicated sex known in plants. Seaweeds are used as food source and used in many industrial applications. Seaweeds contain proteins, vitamins, minerals and trace elements. They contain all the essential aminoacids for the human.

The seaweeds are used as food, cosmetics, fertilizer. They are also used extensively in the extraction of industrial gums and chemicals. It has the potential use of long and short chain chemicals with medicinal and industrial uses. They yield important products such as algin and agar-agar. Agar is used in cakes and also in moisturizing agent. Algin is used in textile and paper industry. Alginic acid and its salts are used as blood anti-coagulants.

Figure 1: *Portieria hornemannii.*

MATERIALS AND METHODS
COLLECTION OF SEAWEED
Portieria hornemannii was collected from the coast of Ramanathapuram district in Tamilnadu, India. The seaweeds were manually collected, the debris were removed and it was washed with tap water and again washed with distilled water and allowed to shade dried for 3 days at room temperature and using electric blender it was finely powdered. (Figure 1).

EXTRACTION OF SEAWEED

50 grams of the dried red seaweed were extracted in 500ml of Chloroform, Methanol, Hexane and Aqueous (1: 10 ratio) for 2 days in a conical flask. The solvents were filtered using a muslin cloth. The solvent were allowed to evaporate to obtain the concentrated residue. The residues were stored in the tight screw cap bottle and used for further studies.

SYNTHESIS OF SILVER NANOPARTICLES (Mohamed M *et al.*, 2014)

Silver nitrate GR used as such (purchased from Merck, India). 100 mL, 1 mM solution of silver nitrate was prepared in an Erlenmeyer flask. Then 20 mL of *Portieriahornemannii* extract was added separately to which 10 mL of silver nitrate solution under constant mixing at 60°C. This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of $Ag^{þ}$ to Ag^{0} was confirmed by the color change of solution from color-less to brown. Its formation was also confirmed by using UV- Visible spectroscopy.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Vis spectral analysis was done by using Shimadzu UV-visible spectrophotometer (UV-1800, Japan). UV-Visible absorption spectrophotometer with a resolution of 1 nm between 400 and 480 nm was used. One milliliter of the sample was pipetted into a test tube and subsequently analyzed at room temperature. Absorption of the ultra violet radiations results in the excitation of the electrons from the ground state to the higher energy state. Dynamic light scattering (Spectroscatter 201) was used to determine the average size of synthesized silver nanoparticles. FT-IR spectra of were recorded on **Shimadzu IRTracer-100.** FTIR Spectrophotometer sample preparation was done using **KBr Pellet Method**. The surface morphology was analyzed using Scanning electron microscopy (SEM), operated at an accelerated voltage of 120 kV. The preparation of the sample for SEM analysis is by adding 2ml of the nanoparticle and 8ml of distilled water. The prepared sample was kept in the sonicator for 30 minutes and then a drop was placed on the aluminium foil and allowed to dry and the sample was used for the SEM analysis.

APPLICATIONS OF SYNTHESIZED SEAWEED NANOPARTICLES

ANTIBACTERIAL ASSAY

Agar well Diffusion Method: (Jeyanthi *et al.*, 2013)

The stock cultures were maintained at 4°C on the nutrient agar slant slopes. Nutrient broth was prepared for 50ml. The loop-full of the stock cultures were transferred to 50ml of the nutrient broth and were incubated for 24 hrs at 37°C. The strains were inoculated in nutrient broth for 24 hours. 250ml of Muller Hinton agar was prepared and poured on petriplates.A sterile cotton swab was been dipped into the inoculums and spreaded over the Muller Hinton Agar.The wells were punctured on the inoculums spreaded plates using a sterile well borer.The algal extracts of (20µl, 40µl, 60µl, 80µl) were dispensed into each well using a micropipette. The petriplates were incubated at 37°C for 24hours and observed for the zone of inhibition. The diameter of the zone of inhibition was measured in mm.

ANTIDIABETIC ACTIVITY (Murugaesan S *et al.*, 2016)

Starch solution was prepared by adding 1g of starch in 100ml of sodium acetate buffer and added to the tubes. The algal extract was added in various concentrations (0.1, 0.2, 0.3, and 0.4ml).0.5ml of amylase was added to the test tubes and incubated for 10-15 minutes in room temperature.2ml of 3, 5-dintro salicylic acid was added to all the tubes. 0.5ml of amylase was added to control tubes and incubated in boiling water for 10-15 minutes and cooled.1ml of sodium potassium tartarate was added to all the tubes and the absorbance was read at 540nm.

% inhibition = OD of control- OD of test / OD of control * 100

OVICIDAL AND LARVICIDAL ACTIVITY (Kadarkarai M *et al.*, 2015)

*Aedesaegypti*eggs and larvae were placed in the distilled water and the silver nanoparticles algal extract and the seaweed extract were added in different concentrations (2, 4, 6, 8 µl). Larval food (yeast) was added to all the mosquito eggs and larvae.Control mosquito eggs and larvae were exposed for 24 hours. Percentage of mortality was calculated as follows:

Percentage of mortality = (number of dead individuals/ number of treated individuals)*100

IN-VITRO ASSAY FOR CYTOTOXICITY ACTIVITY (Leela K *et al.*, 2016)

MTT ASSAY

Cell lines such Vero cell lines were obtained from King's institute Guindy Chennai. The cells were maintained in Minimal Essential Media in a humidified atmosphere of CO_2 at 37°C. The cytotoxicity of the red seaweed was determined by the MTT assay. 1mg of sample is dissolved in 1ml of serum free MEM/DMSO. The stock is prepared fresh and filtered through 0.45µ filter before each assay. Working concentrations of sample ranging from 1000µg to 7.8µg are prepared. Cell (1×10^5/ well) (VERO CELLS) were plated in 24 well plates and incubated at 37°C with 5% CO_2 condition.After the cell reaches the confluence, media was removed from the wells carefully without disturbing the cells. The various concentrations of the samples (500µl) were added and incubated for 24 hours.After incubation, the sample was removed from the well and washed with phosphate buffered saline (pH-7.4) or MEM without serum.100µl / well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazoium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with ELISA reader using DMSO as the blank. Measurements were performed and the concentration

required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells/ A570 of control cells × 100

RESULTS AND DISCUSSION

EXTRACTION OF SEAWEED

The seaweed (*Portieriahornemannii*) was washed with marine water, the calcareous stones and epiphytes were removed my manual sorting and intensively washed with tap water and then again with distilled water. The seaweedis shade dried for 3-4 days. The bioactive compounds from seaweed were obtained by using the solvent extraction. The solvents such as Chloroform, methanol, hexane were taken in a conical flask and the seaweeds were weighed using an electronic weighing balance and added to the solvents in the ratio of 1:10 and left it for 2-3 days and the extract were filtered using a muslin cloth. The extracts were allowed to dry and the residues were collected and the dry weights of the samples were obtained. The residues were stored in a cork screw bottle and were used for the further analysis. (Figure 2).

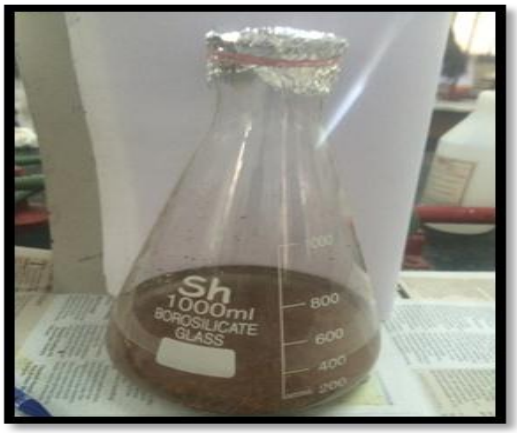

Figure 2: Red seaweed extracts

GREEN SYNTHESIS OF NANOPARTICLES

Figure 3: Silver nanoparticles *P.hornemannii*

Silver nanoparticles were formed by the reduction of Ag^+ by the action of the 1mM of $AgNO_3$. The solution was gradually heated and the colour change was observed which shows the presence of silver nanoparticles. The synthesis of silver nanoparticles was carried out in 30 minutes. The synthesized nanoparticles were stored in an air tight bottle at room temperature for further characterization studies. (Figure 3).

CHARACTERIZATION OF NANOPARTICLES

UV-VISIBLE SPECTROPHOTOMETER ANLYSIS

The newly synthesized nanoparticles were observed to show absorption peak at 400 to 480nm under UV-Visible absorption spectroscopy.

Table 1: UV-Visible Spectrophotometer Optical Density

SAMPLE	OD at 430nm
Portieriahornemannii	0.28

The green synthesized silver nanoparticles were subjected to UV-Visible spectroscopy and maximum absorption peak was obtained at 430nm. The OD value of red seaweed 0.28 OD indicates the synthesized silver nanoparticles. (Table 1).

The UV-Visible spectra recorded at the initiation of reaction with. It is generally recognize that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions. The appearance of the brown colour was due to the excitation of the Surface Plasmon Resonance (SPR), typical of silver nanoparticles having absorbance values which were reported earlier in the visible range of 430nm. (**Zeinab S *et al.*, 2014**).

SCANNING ELECTRON MICROSCOPE

In this present study, SEM was performed to study the morphology of the synthesized silver nanoparticles. The shapes of the silver nanoparticles were reported to be spherical. The synthesized nanoparticles were found in aggregations which were distributed evenly. The particles forms aggregate on the surface of the foil (Figure 4a & 4b). The size of the nanoparticles prepared are found to be between the range of 100-200µm.

SEM IMAGES OF RED SILVER NANOPARTICLES

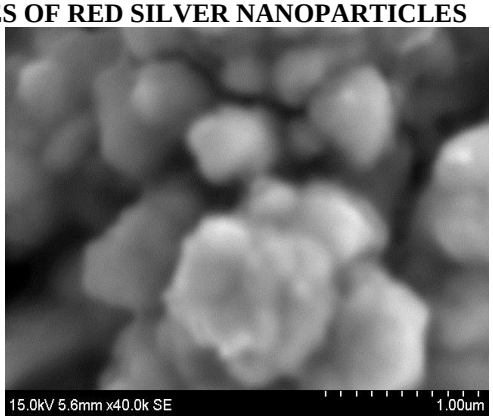

Figure 4(a)

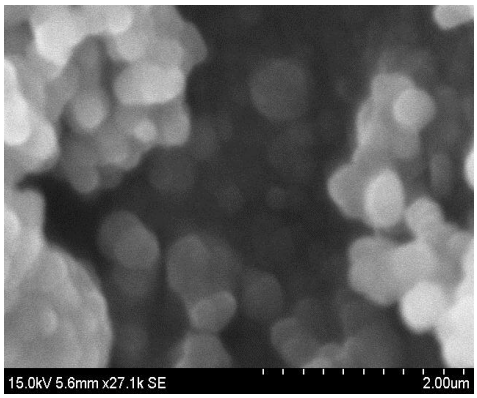

Figure 4(b)

FTIR SPECTRUM OF SYNTHESIZED NANOPARTICLES

FTIR Spectrum of Synthesized Nanoparticles from Red Seaweed

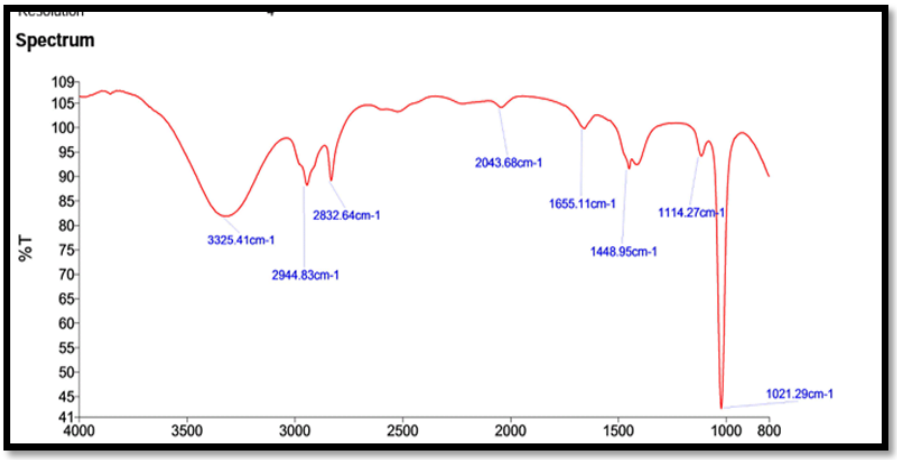

Figure 5: FTIR image of Red Nanoparticles

Table 2: FTIR for Red Silver Nanoparticle

S.NO	BAND SPECTRA	BONDS
1.	3325.41cm^{-1}	N-H_2
2.	2944.83cm^{-1}	C-H
3.	2832.64cm^{-1}	C-H
4.	2043.68cm^{-1}	C=C
5.	1655.11cm^{-1}	C=N
6.	1448.95cm^{-1}	C=C
7.	1114.27cm^{-1}	C-O
8.	1021.29cm^{-1}	C=O

The FTIR spectrum for the silver nanoparticles was analysed and absorption bands were observed at 3340cm^{-1} and 1638cm^{-1} which corresponds to O-H and C=O. The reduction of Ag^{+} to Ag^{0} was due to C=O stretch. The highest peak 1021.29cm^{-1} corresponds with C=O carbonyl group and the least peak is 2043.68cm^{-1}, 1655.11cm^{-1}, 1448.95cm^{-1} corresponds with C=C, C=N, C=C alkene. (Figure 5, Table 2).

ANTIMICROBIAL ACTIVITYOF RED SILVER NANOPARTICLES

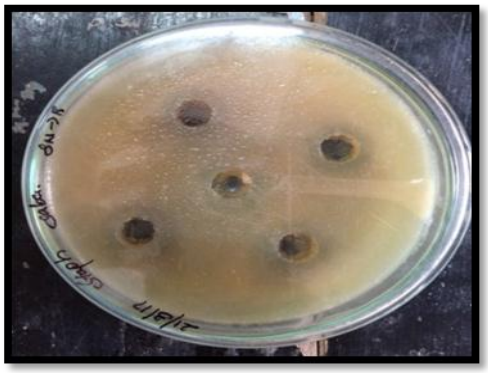

Figure 6(a) ***Staphylococcus aureus***

Figure 6(b) ***Klebsiellaspp***

Figure 6(c) ***Pseudomonas spp***

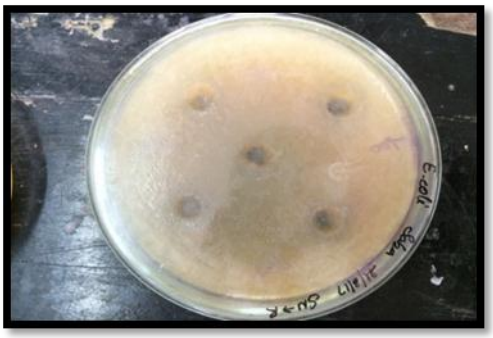

Figure 6(d) ***E. coli***

Figure 6(e) ***Proteus spp***

Table 3: Antibacterial activity of Red silver nanoparticles

ORGANISMS	SAMPLES			
	20µl	**40µl**	**60µl**	**80µl**
E. coli	10mm	13mm	14mm	19mm
Staphylococcusaureus	9mm	14mm	15mm	20mm
***Klebsiella*spp.**	8mm	12mm	14mm	16mm
***Proteus*spp.**	-	-	10mm	15mm
***Pseudomonas*spp.**	4mm	7mm	7mm	9mm
Control	1mm	1.5mm	1mm	2mm

The agar well diffusion method was used to enumerate the antimicrobial activity of the test microorganism but measuring the zone of inhibition. The activity of the test was done using different sample concentrations such as 20µl, 40µl, 60µl, 80µl of the red silver nanoparticles. In 80µl concentration 20mm of zone shows the highest activity against *E.coli*, *Staphylococcus aureus*. The maximum zone of inhibition was exhibited by the *Staphylococcusaureus*. The least activity of 15mm to 16mm was shown in *Klebsiella* spp. and *Proteus* spp.*S.aureus* shows the maximum zone of inhibition (Table 3).

ANTIDIABETIC ACTIVITY

Comparison between Aqueous Red Seaweed and Red Seaweed Nanoparticle

Figure 7(a)

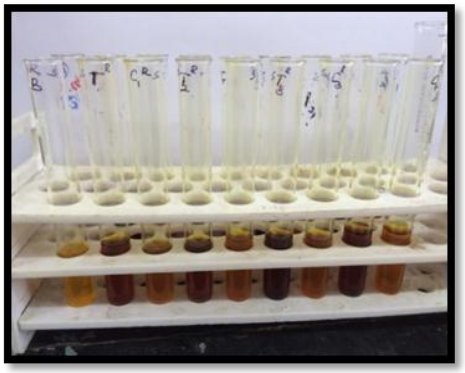

Figure 7(b)

Table 4: Comparison between Red and Red seaweed Nanoparticle

SAMPLE CONCENTRATION	% INHIBITION IN AQUEOUS RED SEAWEED	% INHIBITION IN RED SEAWEED NANOPARTICLE
0.1ml	8.3	9.0
0.2ml	14.2	13.3
0.3ml	18.75	17.6
0.4ml	26.31	53.1

ANTI-OVICIDAL ACTIVITY

Figure 8(a)

Figure 8(b)

COMPARISON OF RED AQUEOUS SEAWEED AND RED SEAWEEDNANOPARTICLE EXTRACT IN EGGS

Table 5: Comparison of Red Aqueous Seaweed and Red Seaweed Nanoparticle Extract in Eggs

NANOPARTICLE DILUTION	% OF MORTALITY OF MOSQUITO EGGS IN AQUEOUS RED SEAWEED	% OF MORTALITY OF MOSQUITO EGGS IN RED SEAWEED NANOPARTICLE
1:2	100	100
1:4	100	100
1:6	100	100
1:8	100	100

The extract of both the biosynthesized nanoparticles and the aqueous extracts were found to be effective in killing the mosquito eggs and the percentage of mortality was found to be 100%. The extracts can be further purified and used as the mosquito repellent.

LARVICIDALACTIVITY

Figure 9(a)

Figure 9(b)

COMPARISON OF AQUEOUS RED SEAWEED AND RED SEAWEED SILVER NANOPARTICLES IN LARVAE

Table 6: Comparison of Aqueous Red Seaweed and Red Seaweed Siler Nanoparticle in Larvae.

NANOPARTICLE DILUTION	% OF MORTALITY OF MOSQUITO LARVAE IN RED SILVER NANOPARTICLES	% OF MORTALITY OF MOSQUITO LARVAE IN AQUEOUS RED SEAWEED
1:2	28	40
1:4	41	50
1:6	56	56
1:8	66	76

The extract of the biosynthesized nanoparticles and the aqueous extract of the red seaweed were found to have the maximum mortality rate in killing the larvae than the red seaweed nanoparticle. The results were compared with the ulva lactuca extract which was found to be more effective than the nanoparticle synthesized using the extract. (**Kadarkari M *et al*., 2015**).

CYTOTOXICITY ASSAY (MTTASSAY)

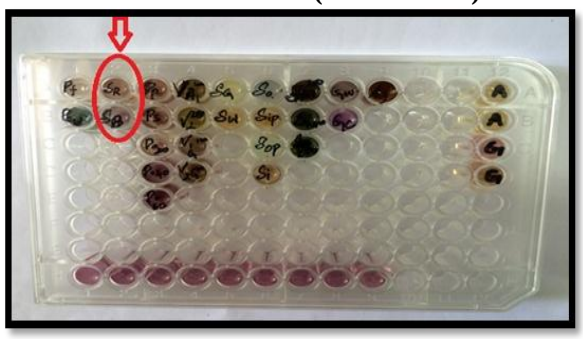

Figure 10: MTT Assay – Vero cell lines

Table 7: Cytotoxicity assay

SAMPLES	% CELL VIABILITY
Red silver nanoparticles	75.4%

The cytotoxicity assay was carried out using VERO cell lines. The percentage of cell viability was found to be 75.4%. This indicates the red seaweed silver nanoparticles were less cytotoxic against the VERO cell lines. The bioactive compound present in the red seaweed was found to contain the potential drug for treating the cancer cell lines.

DISCUSSION

The phytochemicals such as the alkaloids, phenols, tannins, flavonoids, saponins, phytosterols, proteins, glycosides, fats and oils were the secondary metabolites present in the seaweeds and used as a protective mechanism against the microorganisms, insects and acts as a defence mechanism in protecting the body. The red seaweed samples indicate the presence of bioactive compounds which has medicinal value and are been used for many applications.

The crude extract of alkaloids is considered harmful when they are purified and are combined with another compound and used for the synthesis of medicinal drugs. The alkaloid was compared with the value of the *G. cortica*and was found to be much high in the seaweeds (*P. hornemannii*). Phenols are also known as carbolic acid. They are mainly used in the development of drugs to treat diarrhoea associated with anti-HIV treatment. The phenolic content was compared with the phenol value of *Dictyota dichotoma* seaweed were found to be high in the red seaweed *P. hornemannii*. The total tannin content in *P.hornemannii*was compared with *Naravaliazeylanica*and was found to be much higher in the seaweeds *P. hornemannii.*

The silver nanoparticles synthesis was carried out and their formation was compared with the algal mediated synthesis of seaweeds **(Mohamed M *et al.*, 2014).** The biosynthesized nanoparticle extract was more effective when compared with the aqueous extract. With regard to anti-diabetic activity, the seaweeds *P.hornemannii* showed effective activity of anti-diabetic property.The *Ulva lactuca* leaf extract nanoparticles showed maximum mortality rate (**Kadarkari M *et al*., 2015**) which correlated with the finding of the present study where, the seaweed nanoparticles and extract exhibited maximum ovacidal activity. The aqueous extract of *P.hornemannii* showed better activity against the larvae when compared to the nanoparticles. The different concentrations of certain polysaccharides, include sulfated galactans, sulfated rhamnans or mannans, carrageenans and agars were also found to contribute to the different levels of lavicidal effects of the seaweeds which are bioreduced during the preparation of nanoparticles causing a reduced activity against the larvae.

CONCLUSION

The present aim is to study the synthesized silver nanoparticles usingthe red seaweed (*Portieriahornemannii).* The Phytochemical analysis was carried out by the qualitative and quantitative analysis and showed the presence of alkaloids, phenols and tannins. The synthesized nanoparticle was tested for its activity for anti-microbial, anti-diabetic, ovicidal, larvicidal and less cytotoxic. The red seaweed exhibited the maximum antimicrobial activity. This enhances a promising finding to utilize the bioactive compound in the field of medicine and pharmaceuticals which helps in enhancing the quality of the drugs.

About 50% of the anticancer drugs are been obtained from the natural sources such as medicinal plants, herbs

and spices. The active compounds such as alkaloids, phenols and tannins have shown to posses this activity. Anti-cytotoxicity testing of the silver nanoparticles obtained from the red seaweed extracts shows 75.4% anti-cytotoxic activity. Hence the red could be used in the treatment of the diseases as it is less cytotoxic.

This contributes to future pharmaceutical industry to obtain the active compound present in the red seaweed and prepare silver nanoparticles which aids in targeted drug delivery of the active compound which can be produced in large scale and used to treat diseases.

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