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Green Synthesis of Silver Nanoparticles using *Dictyota bartayresiana* J.V. Lamouroux and their Cytotoxic Potentials

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The present study was intended to standardize the protocol for the synthesis of silver nanoparticles (AgNPs) using aqueous extract of *Dictyota bartayresiana* J.V. Lamouroux and evaluate cytotoxic potentials using brine shrimp bio-assay activity and trypan blue dye exclusion method. The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the solution at 200-900 nm and the characteristic peaks were detected. FTIR analysis was used to detect the characteristic peaks and their functional groups. The powder method of diffraction was employed for structural characterisation of AgNPs . The cytotoxic and anticancer potentials were evaluated by, the brine shrimp bio-assay and trypan blue dye exclusion method against DLA cell lines, repectively . When the D. bartayresiana aqueous extract was mixed with 1 mM AgNO3 solution, the colour of the solution changed from pale yellow to yellowish brown colour. The AgNPs synthesized from aqueous extract of D. bartayresiana showed an absorption of 0.639 at 410 nm. The broadening of peaks indicated that the particles are polydispersed. The capping was confirmed by the existence of bands at 1019.19, 1642.09, 1643.05 and 3401.82 cm–1. The D. bartayresiana AgNPs illustrated nine peaks at 2θ values were 26.661°, 28.388°, 29.947°, 32.244°, 40.553°, 46.223°, 50.19°, 54.78°, 57.448° and 76.67° corresponding to 208, 509, 149, 1681, 257, 833, 178, 275, 272 and 256 planes of silver, respectively. The AgNPs of D. bartayresiana revealed 50% mortality (LC50) of brine shrimp nauplii at 196.5 µl/l. Concentration needed for 50% inhibition of growth of DLA cells was found to be 296.14 µl/l of D. bartayresiana AgNPs. The results of the present study demonstrated a simple, rapid and economically cheap route to synthesize AgNPs using aqueous extract of *Dictyota bartayresiana* thallus. Cytotoxic studies against Artemia salina confirmed that AgNPs are capable of rendering high cytotoxic activity and hence has a great potential in the preparation of anti-cancer drugs. The synthesized AgNPs may improve the therapeutic and medicinal values of *Dictyota bartayresiana*.

Keywords: Silver nanoparticles, seaweeds, Dictyota, cytotoxic

At present, nanotechnology research is flourishing due to its potential applications in various sciences such as physics, chemistry, biology and medicine. Generally, metal nanoparticles are synthesized and stabilized by various methods viz., chemical reduction (1), electrochemical techniques

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(2), photochemical reactions in reverse micelles (3), and nowadays via green chemistry (4). Synthesis of nanoparticles from the biological organisms is a good, eco-friendly and economically alternative method. Biologically synthesized silver nanoparticles (AgNPs) have wide range of applications in medicine because of their notable physical and chemical properties (5, 6). There is a very limited data on the extra cellular biosynthesis of AgNPs using plants and their isolated pure compounds (7- 9) and extracellular synthesis of AgNPs by using seaweeds was rarely reported (10, 11). There are some reports on antioxidant properties of methanolic extract of *Dictyota bartayresiana* from South East coast of India (12), the phytochemical composition as well as the antibacterial, cytotoxic and larvicidal potential of *Dictyota bartayresiana* (13, 14). The antifungal activity of AgNPs as well as gold nanoparticules of aqueous extract of brown seaweed *Dictyota bartayresiana* was also studied (15, 16). But there is no report on the cytotoxicity of AgNPs of marine brown seaweed *Dictyota bartayresiana*. With this knowledge the present study was intended to standardize the protocol for the synthesis of AgNPs using aqueous extract of *Dictyota bartayresiana* J.V. Lamouroux (Class: Phaeophyceae, Order: Dictyotales, Family: Dictyotaceae) from Gulf of Mannar Southeast coast of India and evaluate cytotoxic potetnials using brine shrimp bio-assay activity and trypan blue dye exclusion method.

Materials & Methods

Collection of plant material

The mature and healthy thallus of *Dictyota bartayresiana* J.V. Lamouroux was collected from Manapad, Tirunelveli district, Tamil Nadu, India during November, 2014. The collected plant material was washed with tap water followed by distilled water to remove the unwanted debris.

Synthesis and characterization of silver nano particles of *D. bartayresiana*

The aqueous extract was prepared directly by boiling 10 g of *D. bartayresiana* thallus with distilled water for 3 h and filtering using Whatman No.1 filter paper. The aqueous extract of *D. bartayresiana* was used for the synthesis of AgNPs. To this end, $AgNO_3$ was dissolved in 100 ml distilled water (10^{-3} M). The aqueous extract was added to $AgNO_3$ solution in 1:10 ratio for reduction of Ag^+ ions. After reduction, incubated solution was centrifuged at 10,000 rpm for 15 min.

UV-Vis analysis

The aqueous extract of *D. bartayresiana* was centrifuged at 3000 rpm for 10 min and then filtered through Whatman No. 1 filter paper using high pressure vacuum pump. The sample was diluted to 1:10 with the same solvent. The crude extract containing the bioactive compound was analyzed spectroscopically for further confirmation. The extract was scanned in the wavelength ranged from 200-1100 nm using Shimadzu spectrophotometer and the characteristic peaks were detected. Each and every analysis was repeated twice. The supernatant containing AgNPs of *D. bartayresiana* was analyzed spectroscopically for further confirmation. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the solution at 200-900 nm using Shimadzu spectrophotometer and the characteristic peaks were detected.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis was performed using Perkin Elmer spectrophotometer system, in order to detect the characteristic peaks and their functional groups. The AgNPs of *D. bartayresiana* were passed into the FTIR and the peak values were recorded. Each and every analysis was repeated twice.

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X-ray diffraction analysis

To know the structural characteristics of AgNPs, the powder method of diffraction was employed. The peaks of the X-ray diffraction pattern were compared with the standard available data using Willars Hand book for the confirmation of the structure.

Cytotoxic activity

25 mg of dried aqueous extract was taken in 10 ml beaker and 500 µl DMSO was added. Finally the volume (5 ml) was adjusted by distilled water. The concentration of this solution was 0.5 µg/µl. Artificial sea water (38 g NaCl/1000 ml tap water) was taken into small tank and shrimp eggs were added to one side of the divided tank and the side was covered. The shrimps were allowed to hatch and mature as nauplii for 48 h. During this period, constant oxygen supply, temperature (around 37˚C) and light supply was maintained. The hatched shrimps were taken for bioassay.

30 clean test tubes were taken and separated by 10 ml in each test tube. 25 tubes were used for the samples in five different concentrations (five test tubes for each concentration) and 5 tubes for control. With the help of a Pasteur pipette, 10 living shrimps were dropped into each test tube (17). The aqueous extract and AgNPs of *D. bartayresiana* were taken in different concentrations (1, 2, 3, 4, 5 µl/ml into the sample tubes). 50 µl of DMSO was added to the control tubes containing 5 ml of mother solution and 10 shrimp nauplii. After 24 h, the tubes were inspected using a magnifying glass and the number of survived nauplii in each tube was counted and the LC_{50}, 95% confidence limit, LCL and UCL were calculated.

Anticancer activity of aqueous and AgNPs of *D. bartayresiana* against Dalton's lymphoma ascites cells

Dalton's lymphoma ascites (DLA) cells were used for short term *in vitro* cytotoxicity experiments. For the cytotoxicity analysis, aqueous extract and AgNPs of *D. bartayresiana* were tested at different concentrations viz., 10, 20, 50, 100, 200 µl/ml in the sample tubes. Cells were aspirated from the peritoneal cavity of tumor bearing mice and washed three times using phosphate buffered saline (PBS). The viability of cells were checked using trypan blue (cell viability should be above 98%). The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using PBS. Control tubes contained only cell suspension. These assay mixtures were incubated for 3 h at 37 °C and then 1 ml of trypan blue was added and the number of dead cells was counted using a haemocytometer (18). The percentage of cytotoxicity was calculated using the formula as follows:% of cytotoxicity = Number of dead cells / Total number of cells (Dead and live cells) * 100

Results

Synthesis of silver nanoparticles

When the *D. bartayresiana* aqueous extract was mixed with 1 mM $AgNO_3$ solution, the colour of the solution changed from pale yellow to yellowish brown colour indicating the presence of AgNPs (Fig. 1 A).

UV-Vis analysis

The results of UV-Vis spectrum of aqueous extract and AgNPs synthesized from *D. bartayresiana* aqueous extract were depicted in Fig. 1 B. The AgNPs synthesized from aqueous extract of *D. bartayresiana* illustrated the optical peak at 410 nm with the absorption of 0.639. The broadening of peaks indicated that the particles are polydispersed. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the

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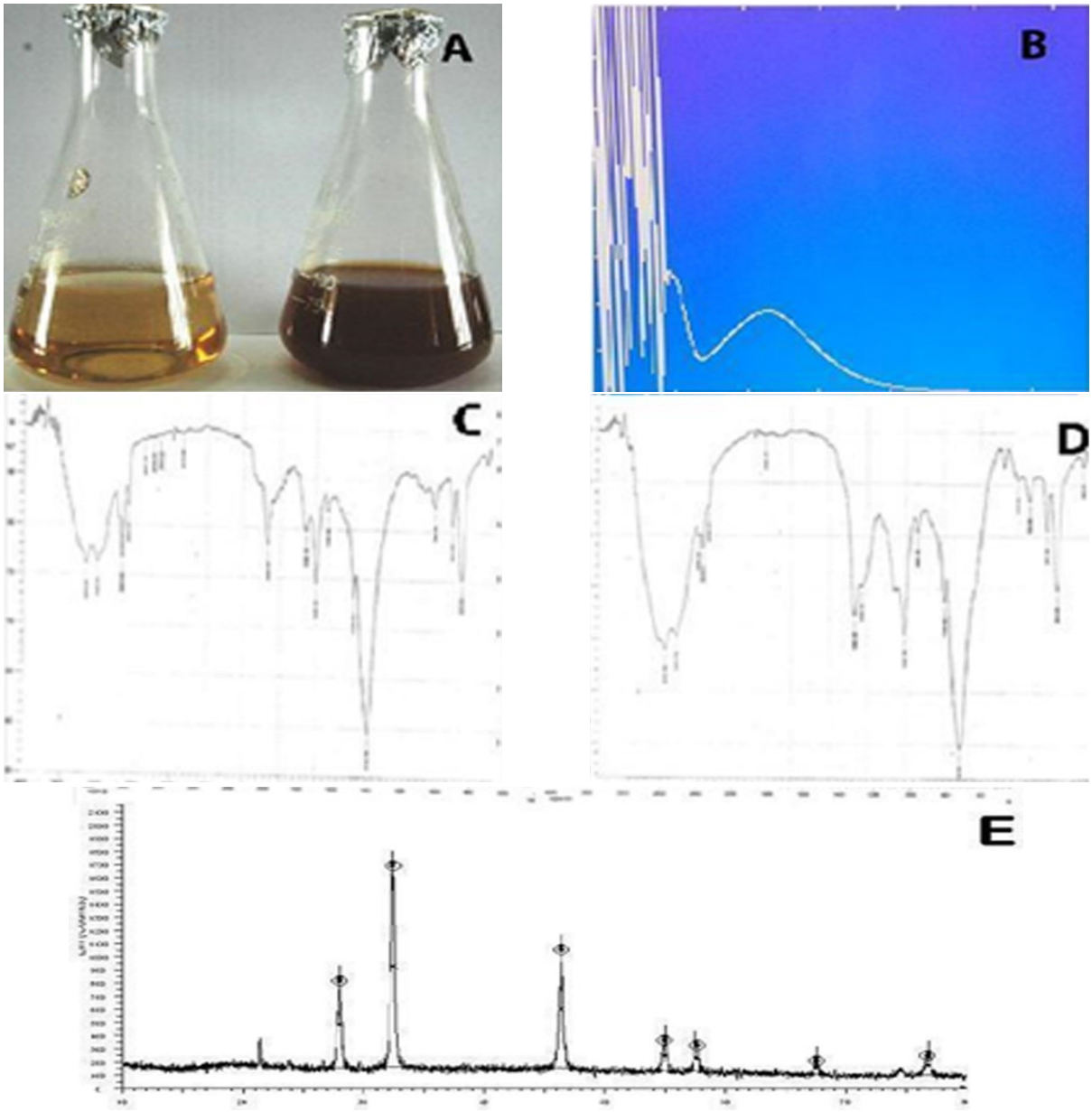


Figure 1. Synthesis and characterization of silver nanoparticles - *Dictyota bartayresiana* J.V. Lamouroux; A- Synthesis of AgNPs from aqueous extract; B- UV-Vis spectrum of AgNPs; C- FTIR spectrum of aqueous extract; D- FTIR spectrum of AgNPs extract; E- XRD pattern of AgNPs extract.

dielectric constant of the metal itself and the surrounding medium.

FTIR

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The aqueous and $AgNO_3$ extracts of *D. bartayresiana* were passed into the FTIR and the functional groups of the components were separated based on their peak ratio. The results of FTIR analysis of *D. bartayresiana* AgNPs confirmed the presence of alkyl halides, aromatic amines, alkynes, 1° amines and 1°, 2° amines, amides with the peak values from 603.61 to 3375.78 (Fig. 1 C-D). The existence of 1° amines was confimred by the occurrence of bands at 1642.09 and 1643.05 cm^{-1} corresponding to N–H bond. The peak at 1019.19 cm^{-1} corresponds to C–N stretching of aromatic amines group. The peak at 603.61 and 626.560 cm^{-1} correspond to C–Br stretch of alkyl halides. The

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peak at 2109.74 cm^{-1} corresponds to –C≡C– stretch of alkynes. The capping was confirmed by the existence of bands at 1019.19, 1642.09, 1643.05 and 3401.82 cm^{-1}.

X-ray diffraction (XRD)

XRD pattern of derived AgNPs (Fig. 1 E) showed nine intense peaks in the whole spectrum of 2θ values ranged from 20° to 70°. The *D. bartayresiana* AgNPs illustrated nine peaks at 2θ values were 26.661°, 28.388°, 29.947°, 32.244°, 40.553°, 46.223°, 50.19°, 54.78°, 57.448° and 76.67° corresponding to 208, 509, 149, 1681, 257, 833, 178, 275, 272 and 256 planes of silver respectively. Among these, only one peak with 32.244° 2θ value showed 100% intensity count. A comparison of the XRD pattern with standard confirmed that silver particles are formed in the XRD analysis.

Brine shrimp lethality bioassay

The aqueous extract and AgNPs of *D. bartayresiana* showed different mortality rate of brine shrimp which increased proportionally with the increasing concentration of the extract. The inhibitory effect of the extract might be due to the toxic compounds present in the aqueous extract and AgNPs of *D. bartayresiana*. The AgNPs of *D. bartayresiana* were found to be most effective, with 50% mortality (LC_{50}) of brine shrimp nauplii being 196.5µl/l. Next to that, aqueous extract showed moderate cytotoxicity with LC_{50}= 302.6 µg/l.

Discussion

Similar to Senthil Kumar and Sudha (15), we also observed the formation of AgNPs by reduction of aqueous Ag+ during exposure to the *D. bartayresiana* thallus aqueous extract with reddish-brown colour appearence. The change of colour confirmed the formation of AgNPs in solution. The results of the present study directly coincided and was in agreement with other reports (15, 19) . The colour's change was due to the excitation of outer plasmon vibrations in the AgNPs (20). Furthermore, the formation of AgNPs of *Dictyota bartayresiana* extract was confirmed by UV-Vis and FTIR spectroscopy. The AgNPs synthesized from aqueous extract of *D. bartayresiana* illustrated an optical peak at 410 nm with the absorption of 0.639. The UV-Vis analysis results suggest that the AgNPs were poly dispersed. The results of FTIR analysis confirmed the occurrence of absorbance bands at 1019.19, 1642.09 and 1643.05 cm^{-1} which were assigned to the stretching vibrations of 1° amines and aromatic amines group. The capping was confirmed by the existence of bands at 1019.19, 1642.09, 1643.05 and 3401.82 cm^{-1}. The bands at 1642.09 and 1643.05 cm^{-1} were known to be associated with the bending vibrations. The results revealed that the capping ligand of the AgNPs may be an aromatic compound or alkanes or amines or alkynes. The XRD analysis explained the crystalline nature of silver (21). In the present study, the aqueous extract of *D. bartayresiana* mediated silver particles showed nine peaks with corresponding lattice planes confirming the crystalline nature of AgNPs. Many studies have shown the cytotoxic effects of AgNPs (19, 22- 26), but there is no report on the cytotoxic effects of AgNPs synthesized using the aqueous extract of *D. bartayresiana*. The green synthesis of nanoparticles using *Dictyota bartayresiana* aqueous extract was found highly toxic against *Artemia salina* and DLA cell lines. Concentration needed for 50% inhibition of growth of DLA cells was found to be 296.14 µl/l of *D. bartayresiana* AgNPs. The concentration AgNPs of *D. bartayresiana* at which 50% mortality (LC_{50}) of brine shrimp nauplii occurred was 196.5 µl/l. Similarly, our previous study proved that the AgNPs synthesized from seaweeds showed cytotoxic effect against brine shrimp (19, 22- 26). The results of the present study demonstrated a simple, rapid and

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cheap route to synthesize AgNPs using aqueous extracts of *Dictyota bartayresiana* thallus. In the present study the dead cells are identified by penetration of trypan blue. Similarly, Kuttan et al. also employed the trypan blue staining method to identify the viable cells (27). The results of cytotoxicity studies illustrated different LC_{50} and CTC_{50} values. It clealry indicated that AgNPs have cytotoxic potenitals against *Artemia salina* and DLA cells. Cytotoxic studies against *Artemia salina* confirmed that AgNPs are capable of rendering high cytotoxic activity and hence has a great potential in the preparation of anti-cancer drugs. The synthesized AgNPs improve the therapeutic and medicinal values of *Dictyota bartayresiana*. In conclusion, the present results showed that the spectroscopic profiles will act as pharmacognostic marker to distinguish the *Dictyota bartayresiana* from its adulterants using relatively simple, cost effective spectroscopic profile in the pharmaceutical industries. The biological analysis of *Dictyota bartayresiana* showed that its anticancer property can be used in pharmaceutical industries.

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Conflict of interest

The authors declared no conflict of interests.

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