

MARINE ALGAE MEDIATED SYNTHESIS OF THE SILVER NANOPARTICLES AND ITS ANTIBACTERIAL EFFICIENCY

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ABSTRACT

Silver has been known since ancient history for its antimicrobial property. The silver nanoparticles were obtained by a green synthesis method in this study. Seaweeds were used as the bioreductant for the reduction of the silver salt to form the nanoparticles. The formation of the silver nanoparticles was confirmed with the dark brown colour development and it was characterised with UV-Visible spectroscopy. The Transmission electron micrographs indicate the size of the nanoparticles to be in the range of 25-40 nm and the nanoparticles were spherical in shape. The activity of the nanoparticles to inhibit the growth of bacteria was tested against a Gram positive *Bacillus cereus* and a Gram negative *Escherichia coli*. The silver nanoparticles exhibit good antimicrobial activity and hence their potential application in medicine as a wound dressing or for sterile clothing in hospital environment.

Keywords: Silver nanoparticle, Biosynthesis, Antibacterial activity

INTRODUCTION

In this era nanotechnology has gained wide acceptance and importance. The outcome of which is the production of nanomaterials like nanoparticles, carbon nanotubes, quantum dots and many more. A nanomaterial is any material that is synthesised under controlled conditions in the size ranges of 1-100nm [1]. Particles within this size range exhibit superior optical electrical and mechanical properties and therefore its extensive application. The nanoparticles are widely used in many fields and hence the production of nanoparticles is of importance. Although there are various chemical and physical methods employed there has been a biosynthesis route also that has been considered. There have been various reports on the biosynthesis of silver nanoparticles.

A review states the potential use of marine resources which have not been explored as much as plants for the synthesis of nanoparticles. A report states the biosynthesis of silver nanoparticles using the *Ocimum sanctum* leaf extract. They have reported a rapid synthesis of silver nanoparticles within a time span of 8 mins. The nanoparticles also exhibited antibacterial action against Gram positive and Gram negative microbes [2]. *Stevia rebaudiana* has been used for the biosynthesis of silver nanoparticles. They have reasoned the role of ketones to be the major responsible factor for the reduction [3]. Fruit bodies of the *T. terrestris* L. was used for the synthesis of silver nanoparticles. They have suggested the process to be rapid and the synthesised silver nanoparticles to exhibit good antibacterial efficiency [4]. The leaf extract of *Arbutus Unedo* was used for the synthesis of silver nanoparticles. These nanoparticles bio reduced was reported to be stable over long term [5]. Gold nanoparticle synthesis by using the aqueous extract of cypress leaves has been reported [6]. This process reported is very rapid with a high conversion rate of about 90% at room temperature. Silver nanoparticles have been synthesised using *Ulva fasciata* (Delile) ethyl acetate extract and they have been studied for its activity against a Gram negative bacterial pathogen *Xanthomonas campestris* pv. *Malvacearum* [1]. Sindhu et al have described an eco-friendly synthesis of nanoparticles using *Rhizophora apiculata*. They have proved the enhanced antimicrobial capability of the antibiotics when combined with the silver nanoparticles [7]. The synthesis of gold nanoparticle by the brown marine macroalga *Padina gymnospora* has been reported which uses seaweed for the process [8]. They have produced stable gold nanoparticles through this process and suggest the possibility of the application of these nanoparticles as a cancer therapeutic.

Silver nanoparticles are of great importance because of its high medicinal value that from ancient history it has been in use. So it is of high importance to manufacture these silver nanoparticles for use in medicine. In this report we have exploited the seaweed *Padina gymnospora* for the synthesis of silver nanoparticles.

MATERIALS AND METHODS

Seaweed processing

The seaweed *Padina gymnospora* was collected from the coast of Gulf of Mannar, Rameshwaram, Tamil Nadu. The seaweeds were repeatedly washed with water to remove the unwanted material. The seaweeds were then sorted out and shade dried. This shade dried seaweed was used for the synthesis of silver nanoparticles. The aqueous extract was prepared by dispersing 15grams in 100ml of Milli Q water.

Silver nitrate purchased from Merck, India was used as the salt solution for the synthesis. The pure aqueous extract of 10 ml thus obtained was interacted with 40ml 0.004M of silver nitrate. This mixture was kept in a shaker at 120rpm at 30°C. The interaction was allowed till the appearance of a dark brown colour which indicates the formation of silver nanoparticles.

UV-Vis spectroscopy

The reaction mixture was diluted appropriately for spectroscopic analysis in UV-Vis spectrophotometer (UV-Vis 2201, Systronics, India) at a resolution of 0.1 nm from 300–600 nm.

Transmission electron Microscopy

The nanoparticle colloidal solution was prepared and coated on the carbon coated copper grid. The grid was allowed to dry then observed for the presence of nanoparticles. The micrographs were taken using Tecnai G-10 (Philips) operated at 80 kV.

Antibacterial study

The bacterial cultures obtained from NCL, Pune, India *Bacillus cereus* 2458 and *Escherichia coli* 2809 (NCIM cultures) were used for the study. 100 µl of bacterial suspension containing 10⁸ cells was smeared uniformly on petri dishes with Muller-Hinton agar. The wells were punched and the extract, silver nitrate and silver nanoparticles were loaded in the respective wells. The plates were incubated at 37°C overnight. The zone of inhibition was observed for the nanoparticle and silver nitrate.

Results and Discussion

The aqueous extract of the seaweed *Padina gymnospora* when interacted with the silver nitrate salt solution forms a dark brown solution. This reaction results in the synthesis of nanoparticles marked by a colour change to dark brown from the pale yellow seen at the beginning of the reaction. The colour change was monitored visually and UV visible spectrophotometer was used to confirm the formation of silver nanoparticles. The peak at 410nm in the UV visible spectra indicates the presence of silver nanoparticles. (Fig.1)

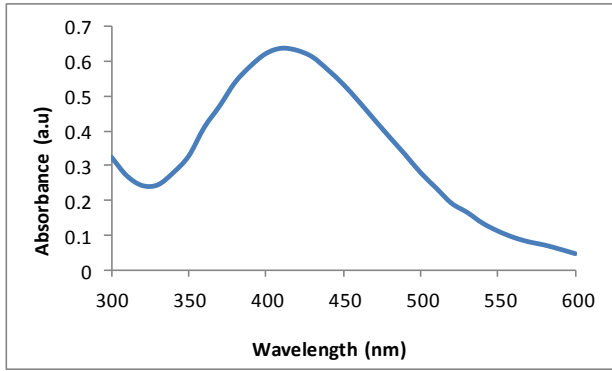


Fig. 1: It shows the UV-Vis spectrum of the biosynthesised silver nanoparticles

These nanoparticles were also found to be stable with no visible precipitation. The stability of the nanoparticles can be attributed to

the bio components present in the seaweed extract. The bio components cap the nanoparticles and thereby protecting the surface of the nanoparticle from interacting with other neighbouring particles and aggregate. This property is of much importance because of the unstable nature of the nanoparticles they may tend to aggregate/ agglomerate and cause undesired effects.

Electron micrographs were taken to study the surface morphology and size of the nanoparticles. The micrographs indicate spherical nanoparticles in the size range of 25-40nm. (Fig.2) The nanoparticles are seen to be distinctly separate which shows the stable nature of the nanoparticles synthesised.

The antibacterial activities of the silver nanoparticles were compared with the extract used and silver nitrate. (Table.1) Silver ions present in the silver nitrate solution exhibit activity and thus the zone of inhibition around the well with silver nitrate. The aqueous extract did not exhibit any antibacterial property. The biosynthesised nanoparticles also exhibit a significant inhibitory action against the tested microbes *Bacillus cereus* and *Escherichia coli*. (Fig.3)

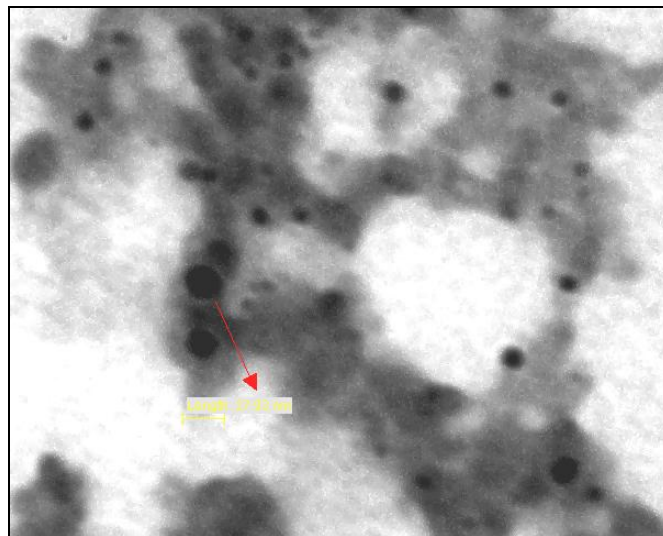


Fig. 2: It shows the transmission electron micrograph of the biosynthesised silver nanoparticles

Table 1: It shows the zone of inhibition of the microbes tested

Microorganism	Extract	Silver nitrate (mm)	Silver nanoparticle(mm)
<i>Bacillus cereus</i>	0	8.1 ± 0.1	13.06 ± 0.40
<i>Escherichia coli</i>	0	6.9 ± 0.25	9.5 ± 0.2

(values are expressed as Mean ±SD)

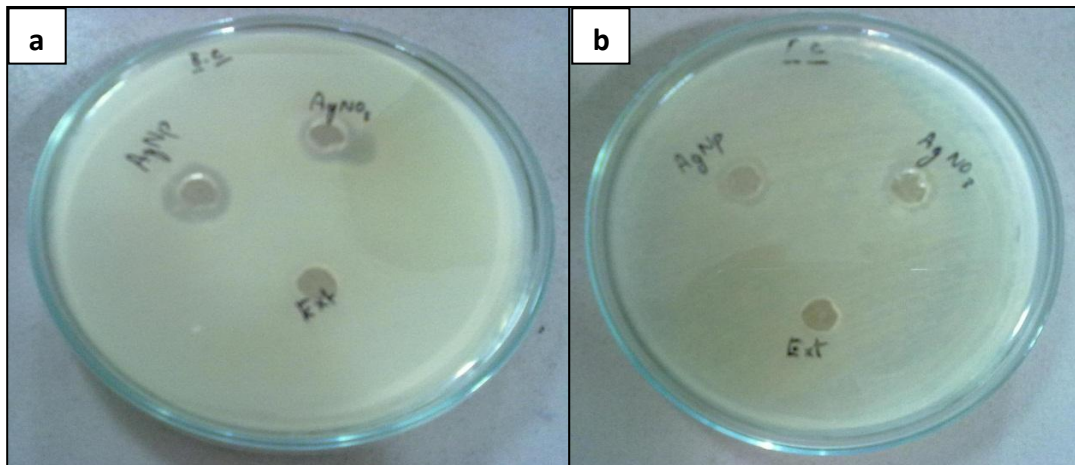


Fig. 3: It shows the antibacterial activity of the synthesised nanoparticle against a) *Bacillus cereus* b) *Escherichia coli*

CONCLUSION

The silver nanoparticles were synthesised through an environment friendly method. The aqueous extract of the seaweed *Padina gymnospora* acted as a reducing agent and capping agent. These nanoparticles were characterised using UV visible spectroscopy which exhibit specific surface plasmon resonance peak at 410 nm. The particle size was confirmed through transmission electron microscopy. The particles were within the range of 25-40 nm. The synthesised silver nanoparticles have proved to exhibit good antibacterial efficiency against the tested microbes, *Bacillus cereus* and *Escherichia coli*.

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