

Silver Nanoparticles Synthesized From a Seaweed Gracilaria Edulis Extract: Evaluation of Antibacterial Activity

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Abstract:

Metal nanoparticle synthesis is an interesting area in nanotechnology due to their remarkable optical, magnetic, electrical, catalytic and biomedical properties, but there needs to develop clean, non-toxic and environmental friendly methods for the synthesis and assembly of nanoparticles. Biological agents in the form of microbes have emerged up as efficient candidates for nanoparticle synthesis due to their extreme versatility to synthesize diverse nanoparticles. In the present study, an eco-favorable method for the biosynthesis of silver nanoparticles using *Gracilaria edulis* seaweed and evaluation of antibacterial activity against Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*) and Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*). The silver nanoparticles and *G. edulis* seaweed showed antibacterial activity and proved their efficacy in controlling the pathogenic bacterial strains. AgNPs exhibited strong antibacterial activity compared with seaweed extract. The synthesized silver nanoparticles using seaweed revealed interesting properties that could be potentially used for pharmaceutical applications.

Keywords: Silver nanoparticles, *Gracilaria edulis*, Gram positive and negative bacteria, Antibacterial activity.

INTRODUCTION

Nanotechnology has grown in popularity as a potential branch of applied research due to its numerous applications, which encompass many features of human existence and necessitate the use of nanoparticles. [1,2]. In recent years, the fields of biology and medicine have paid a lot of attention to biologically synthesised nanoparticles because of their distinct particle size and shape-dependent physical, chemical, and biological properties.This is due to the fact that these properties are all dependent on the particle.[3,4] Due to their strong antibacterial properties and high surface area to volume ratio, metal nanoparticles are the most promising agents. This interest in them among researchers is developing as a result of microbial resistance to metal ions, antibiotics, and the development of resistant strains. [5]. At the moment, there is a growing interest in the production of metal nanoparticles from marine sources. [cause and effect][6]. The biosynthesis of nanoparticles can be an environmentally benign and exciting process if marine macroalgae are used as the starting material. By utilising its biochemical qualities in drug delivery systems and cell encapsulation, such as its biocompatibility, biodegradability, immunogenicity, and non-toxicity, we may take advantage of its vast array of potential applications. [7].

Since more than a century ago, medical professionals have understood that silver possesses antimicrobial properties. The extraction of silver nanoparticles from seaweed is an environmentally friendly chemical process that has been shown to have effective antibacterial properties. [8]. One of the key renewable marine living resources that is used economically is seaweed or marine algae. In the past three decades, research has focused mostly on testing biologically active chemicals in diverse seaweeds against a range of human pathogenic viruses, bacteria, and fungi. [9, 10]. With the use of these prior details, the current study was conducted on the benefits of the bactericidal activity of the seaweed *Gracilaria edulis*, which is readily available year-round and can be harvested, and the manufacture of silver nanoparticles using its aqueous extract.

MATERIALS AND METHODS

Preparation of seaweed extract

The *Gracilaria edulis* that had been collected was given three washes in distilled and sterile water in order to remove any clinging dirt particles and salts. After being cleaned, the samples were allowed to air dry in the shade for seven days at room temperature. After being sliced into smaller pieces, the dried seaweed was then ground into a powder. After adding five grammes of plant powder to one hundred millilitres of deionized water and letting the mixture sit for twenty-four hours, a pure extract of seaweed was created. The extract was then filtered using Watman No. 1 filter paper, and the resulting supernatant was used and kept at 40 degrees Celsius for subsequent processing.

Biosynthesis of silver nano particles

For the purpose of this investigation, silver nanoparticles were created using the technique described by Abideen and Vijaya Sankar (2015). [11]. In a 250 ml conical flask, mix together 10 ml of pure *Gracilaria edulis* extract sample and 90 ml of 1 mM silver nitrate solution. This is the standard way to make silver nanoparticles. The mixture from the reaction was left to cool to room temperature. When the shift in colour was noticed, it provided conclusive evidence that nanoparticles had been formed.

***In vitro* antibacterial activity**

The disc diffusion method used by NCCLS [12] and Awoyinka *et al*.,[13] was used to test for antibacterial activity. Using samples and the disc diffusion method, an antibiogram was made. Into each Petri plate, 30 ml of Nutrient agar (NA) medium was poured. The bent glass rod is cleaned and then used to spread the microbe-filled liquid evenly on the Nutrient agar (NA) plates, where the bacteria have been growing for 24 hours. In short, inoculums containing *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were spread on Nutrient agar plates for bacteria strains. Using sterile forceps, the crude samples (30 l) on sterile filter papers with a diameter of 6 mm and placed on the surface of an inoculated agar plate. For 24 hours, the plates were kept at 37°C 2°C for bacterial strains. Every sample was examined in a total of three separate ways. In order to determine how effective the test chemicals were at inhibiting the growth of bacteria, the average diameter of the zone of inhibition that around the disc was measured in millimetres. On a scale measured in millimetres, the zones of inhibition caused by the chemicals on the microorganisms that were under study were calculated.

RESULTS AND DISCUSSION

Silver nitrate solution was mixed with an extract from the plant *Gracilaria edulis*. After 5 hours, a milky white to grey colour was seen, which is a sign that silver nanoparticles were forming (Figure 1). After 5 hours, the colour turned grey because free electrons in the reaction mixture were excited [14].

Figure 1: Changes in colour before (seaweed extract) and after (AgNPs), as well as a control, during the process of reducing Ag+ to Ag nanoparticles (AgNO3)

$AgNO_3$: 1 mM $AgNO_3$ (White colour)

AgNPs : 1 mM $AgNO_3$ in the presence of extract after 5 hours of incubation (Grey colour)

Gracilaria edulis extract was combined with a solution of silver nitrate. After five hours, a milky white to grey hue emerged, indicating the formation of silver nanoparticles (Figure 1). The colour changed to grey after 5 hours as a result of free electrons in the reaction mixture being stimulated [14]. The silver nanoparticles' surface free radical formations were what gave them their antibacterial properties [15]. It was made abundantly evident that in addition to this little particle connecting to the larger surface area, silver nanoparticles were clinging to the surface of the bacterium and acting against the cell wall protein, which controlled the power of the bacteria. [16]. Large contact areas of tiny, produced AgNPs and differing cell wall compositions in the two species of bacteria [17,18] may also contribute to this explanation. By using the agar disc diffusion method, individual silver nanoparticles that were biosynthesized from *Gracilaria edulis* seaweed extract were put to the test against test organisms to determine whether or not they have antibacterial activity. For this study, Gram positive bacteria (*Bacillus subtilis, Streptococcus pyogenes*, *Staphylococcus aureus*) and Gram negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*).

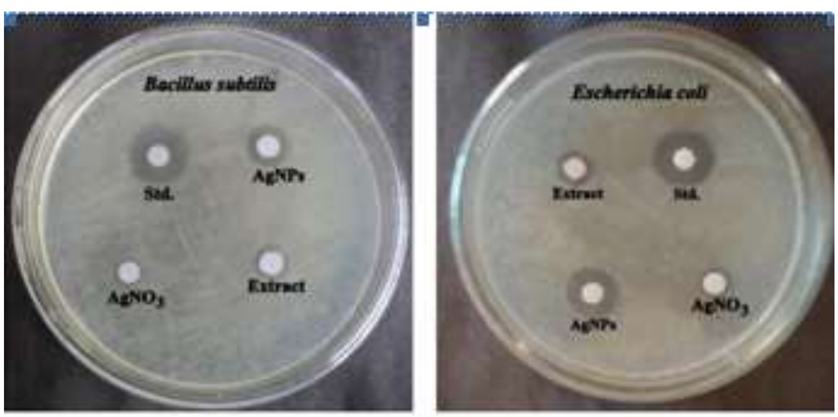
In order to accomplish this, the Zone of Inhibition (ZoI) was calculated. Calculating the Zone of Inhibition is an efficient and low-cost method for evaluating the degree to which a certain test organism is susceptible to an antimicrobial drug (Figure 2). After 24 hours of incubation, the inhibitory effect of AgNPs from *G. edulis* seaweed extract was significantly increased as compared to *G. edulis* seaweed extract and $AgNO_3$. The zone of inhibition (ZoI) was used as a measure for comparing the antibacterial activities of $AgNO_3$ 2.30mm, *G. edulis* extract 4.00mm and AgNPs 8.50mm against *E. coli.* The antibacterial activity of $AgNO_3$ was 0.90mm, *G. edulis* extract was 3mm and AgNPs was 8.00mm against *S. pyogenes.* The zone of inhibition (ZoI) of $AgNO_3$ was 0.70mm, *G. edulis* extract was 2.90mm and AgNPs was 7.70mm of zone against *S. aureus.*

The zone of inhibition (ZoI) observed on $AgNO_3$ was 0.50mm, *G. edulis* extract was 2.50mm and AgNPs was a 7.20mm zone against *Ps. aeruginosa.* The antibacterial activity of $AgNO_3$ was 0.40mm that of *G. edulis* extract was 2.10mm and AgNPs was 6.80mm against *K. pneumoniae.* Zone of inhibition (ZoI) was measured for $AgNO_3$ at 0.20mm, *G. edulis* extract was 1.70mm and AgNPs at 5.70mm zone against *B. subtilis.* The antibacterial activity was higher in AgNPs as compared with *Gracilaria edulis* seaweed extract and $AgNO_3$. The zone of inhibition of AgNPs is closest to that of standard Chloramphenicol. The antibacterial activity of AgNPs were observed in the following order: *Escherichia coli* > *Streptococcus pyogenes* > *Staphylococcus aureus* > *Pseudomonas aeruginosa* > *Klebsiella pneumoniae* > *Bacillus subtilis*. Table 1 represents the antibacterial activity of AgNPs, $AgNO_3$ and *Gracilaria edulis* seaweed extract.

Table 1: Antibacterial activity of AgNPs, $AgNO_3$ and *Gracilaria edulis* seaweed extract

Microbial Strains	**(30µl)**			
	$AgNO_3$	**Seaweed Extract**	**AgNPs**	**Std.**
Bacillus subtilis (mm)	0.20±0.01	1.70±0.11	5.70±0.0.39	9.40±0.65
Escherichia coli (mm)	2.30±0.16	4.00±0.28	8.50±0.59	10.60±0.74
Klebsiella pneumoniae (mm)	0.40±0.02	2.10±0.14	6.80±0.47	9.70±0.67
Pseudomonas aeruginosa (mm)	0.50±0.03	2.50±0.17	7.20±0.50	9.80±0.68
Staphylococcus aureus (mm)	0.70±0.04	2.90±0.20	7.70±0.53	10.10±0.70
Streptococcus pyogenes (mm)	0.90±0.06	3.00±0.21	8.00±0.56	10.40±0.72

Values expressed as Mean ± SD for triplicates, **Standard:** Chloramphenicol



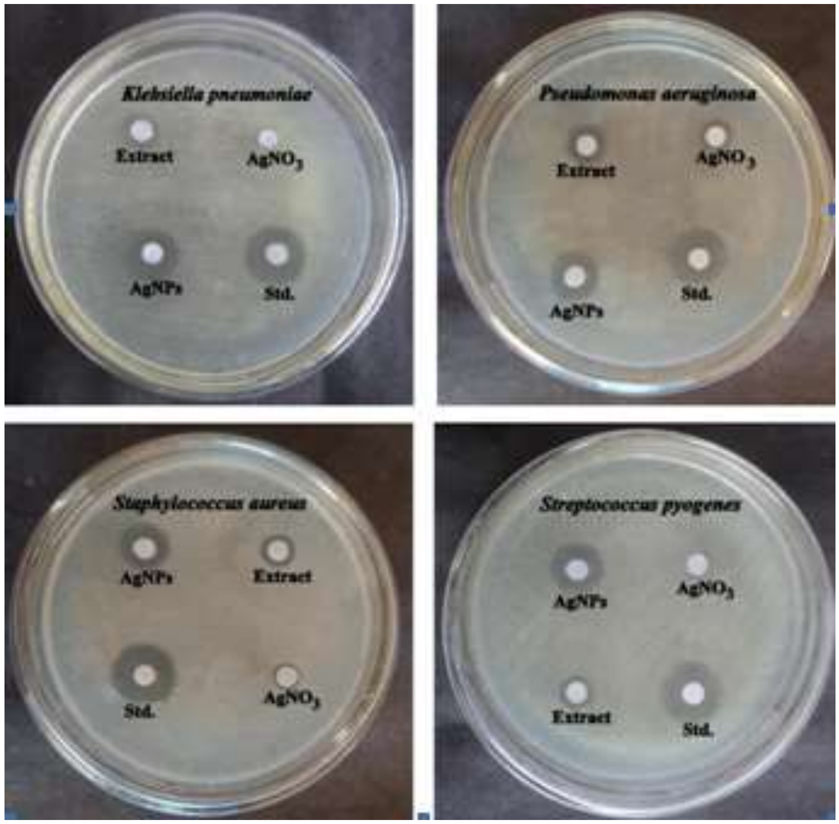


Figure 2: Shows the antibacterial activity of AgNPs, $AgNO_3$ and *Gracilaria edulis* seaweed extract

According to Cox *et al.*, [19], the existence of various bioactive components among algae species may have contributed to the variable antibacterial efficacy of algal NPs extracts. Major and minor minerals, cytokinins, auxin, amino acids, vitamins, and compounds that promote growth are present in seaweed extracts [20]. Marine macro-algal secondary metabolites have been linked to a wide spectrum of biological activities, including antibacterial, antiviral, and antifungal activity [21]. Several silver-based substances have been successfully used as antimicrobial agents [22]. There have been a significant number of studies carried out to evaluate and quantify the antibacterial potentials of silver and the products related to it. It has been discovered that these Ag-particles cause oxidative stress, protein dysfunction, membrane damage, and DNA damage, all of which are harmful to microbial cells. Despite the fact that antibacterial activity has been demonstrated by it. [23;24].

Compounds of silver have multiple applications in medicine, including the treatment of burn wounds and a wide variety of different illnesses. Due to the fact that silver nanoparticles have a greater specific area relative to their volume, which, when compared to bulk silver metal, will result in strong antimicrobial activity, these nanoparticles have been perfect candidates for investigation regarding their antibacterial quality. [25, 26, 16, 27]. Gram-positive bacteria, including

Staphylococcus aureus and *Bacillus subtilis*, as well as Gram-negative organisms such as *Klebsiella pneumonia* and *Salmonella typhus*, were both susceptible to the antibacterial effects of silver nanoparticles [28, 29]. Also, *Bacillus subtilis* and *Staphylococcus aureus* are more resistant to the effects of *Gracilaria verrucosa* extracts than *Pseudomonas aeruginosa* and *E. coli* [30].

CONCLUSION

In the current study, silver ions were bio-reduced using seaweed extract, and their antibacterial activity was also evaluated. The exposure of aqueous silver ions to the extracts and the formation of silver nanoparticles were verified by the colour change of plant extracts. According to the findings, silver nanoparticles effectively combat a variety of bacteria. The current research has demonstrated that silver nanoparticles possess potent antibacterial characteristics, and these properties hold a great deal of potential for the creation of medicines that can treat diseases that are brought on by bacteria.

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