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# Nano Cure Efficacy of Sargassum Wightii (SW) Mediated Silver Nanoparticles (Ag-Nps) For Antibacterial Efficacies Against Multidrug-Resistant (MDR) Bacteria

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***Abstract:*** *The spread of multidrug-resistant strains (MDR) pose serious threats to the environment and public health. Inappropriate use and indiscriminate release of antibiotics in the environment through un-metabolized form create a scenario for the emergence of virulent pathogens and MDR bugs in the surroundings. Eco-friendly synthesis of silver nanoparticles (Ag-NPs) prepared by Sargassum wightii (SW) were evaluated for antibacterial efficacies against Gram-positive and negative MDRs. SWAg-NPs phase purity, particle size/ morphology and composition were determined by UV-vis spectroscopy, X-ray diffraction (XRD), field-emission scanning electron microscope, energy dispersive spectroscopy (EDS) and chemical structure and functional groups was examined by using Fourier transformed infrared spectroscopy (FTIR).Antibacterial efficacies show maximum inhibition zones (16 mm) in Escherichia coli (B5 - NCIM 2931) and minimum inhibition zones of 10 mm in Bacillus subtilis (B1 - NCIM 2920) and Staphylococcus aureus (B4 - NCIM 2493) due to extracellular polymeric substance secretion in Gram-positive bacteria. The results demonstrate that SWAg-NPs act as effective which could cause drastic damages of MDRs in different clinical environments.*
***Keywords:*** *Green nanoparticles; Multidrug-resistant strains; Sargassum wightii; Antibacterial efficacies*.

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## I. Introduction

Multidrug-resistant strains (MDRs) are emerging pathogens that present a major challenge for public health in preventing its spread and impact on human health. Recently, there was an enormous increase of MDRs infections which were spreading around the globe, ranging from tropical and subtropical to temperate climates. These were causing substantial human and economic losses worldwide[1-3]. Currently, over 70% of bacterial nosocomial infections in the United States were resistant to one or more antibiotics that are traditionally used to eliminate them. In 2002, the United States (U.S) center for disease control and prevention (CDCP) estimated that, nearly 90,000 deaths per year occurred due to bacterial infection. More than half of them were caused by MDRs at least one commonly used antibiotic[4,5]. People who become infected with drug-resistant microorganisms usually spend more time in hospital and require a form of treatment that uses two or three different antibiotics in combination which are more effective but also more expensive[6]. The inability of current antibiotics to limit MDR infections coupled with the slow approval rate of new antibiotics necessitate the search for unconventional biocidals. There are several new strategies that have been employed to control microbial infections and are increasingly recognized as a useful outcome source of potential use in research and health related applications[7]. Hence, there is an urgent need to develop a sustainable path for an environmentally less harmful non-toxic antibiotic against MDRs.

Nanotechnology is a rapidly emerging field with applications in Science and Technology for the purpose of manufacturing new materials at nanoscale level[8]. Eco-friendly technologies for the synthesis of silver nanoparticles (Ag-NPs) are believed to be nontoxic, biosafe, and biocompatible and have been used as drug carriers, cosmetics, and fillings in medical materials[9-11]. The use of nanoparticles (NPs) as antibacterial agents have been the subject of several studies and Ag-NPs possesses natural antibacterial properties that are strengthened at nanoscale. Although physical and chemical methods are more popular in the synthesis of NPs, the use of toxic chemicals greatly limits their environmental applications[12]. The developments of reliable, non-toxic methods require extensive labour, and time in the synthesis of NPs. Furthermore, large quantities of secondary waste are generated, resulting from the addition of chemical agents for precipitation and reduction in these processes. An eco-friendly synthetic method employing plant extracts have drawing attention as a simple and viable alternative to chemical and physical methods. These advantages include lower cost, ease of synthesis,

white appearance NPs based antimicrobial formulations could be used as efficient bactericidal materials in modern medicine[13].

In the present study, an extract of brown seaweed *Sargassum wightii* (SW) was used for the eco-friendly synthesis of silver nanoparticles (Ag-NPs) obtained by using seaweed as both a reducing and stabilizing agent[14]. *S. wightii* (SW) is available throughout all seasons in large abundances at the Mandapam coastal region (latitude 78° 8' east and longitude 9° 17' north) in the Gulf of Mannar at the Bay of Bengal. This would allow a large scale production of SWAg-NPs. Biological approaches appear to be a cost-effective alternatives to conventional physical and chemical methods of synthesis. The obtained SWAg-NPs were characterized by UV-vis spectroscopic analysis followed by, Fourier Transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) equipped with energy dispersive spectroscopy (EDS), and Field-emission scanning electron microscopy (Fe-SEM). SWAg-NPs demonstrated significant antibacterial activity against Gram-positive and -negative strains such as methicillin-resistant *Bacillus subtilis*; *Staphylococcus aureus* and negative ampicillin-resistant *Escherichia coli*; *Vibrio cholera* was studied. Photocatalytic degradation of methyl orange was also investigated under different irradiation to find out the potential of the generated NPs to provide effective natural nano medicines active against microbial infections.

## II. Experimental Methods

### 2.1. Multi-drug resistant strains (MDRs) for testing

Gram-positive and -negative MDRs were procured such as *Bacillus subtilis* (B1 - NCIM 2920); *Micrococcus luteus* (B2 - NCIM 2871); *Staphylococcus aureus* (B3 - NCIM 5021), and *Staphylococcus epidermis* (B4 - NCIM 2493), and negative *Escherichia coli* (B5 - NCIM 2931); *Klebsiella pneumonia* (B6 - NCIM 2883); *Proteus mirabilis* (B7 - NCIM 2241); *Pseudomonas aeruginosa* (B8 - NCIM 5029); *Salmonella typhimurium* (B9 - NCIM 2501); *Vibrio cholera* (B10 - MTCC 2501). These cultures were obtained from the Council of Scientific and Industrial Research - National Chemical Industrial Microorganisms (CSIR-NCIM), Pune, India, and the Council of Scientific and Industrial Research - Microbial Type of Culture Collection and Gene Bank (CSIR-MTCC), Chandigarh, India.

### 2.2. Collection and extraction of seaweed

Fresh specimens of brown seaweed *S. wightii* (SW)were collected from the Mandapam coastal region (latitude 78° 8' east and longitude 9° 17' north) in the Gulf of Mannar at the Bay of Bengal by using sterile polyethylene bags. Then the collected samples were cleaned thoroughly with seawater followed by tap water and distilled water to remove adhering debris, associated epifauna/ epiphytes[15]. After cleaning, seaweed was dried in the shade at room temperature ($28 \pm 2\ ^0C$) for a week. The shade dried SW was macerated as to make a coarse powder using mortar and pestle. From that sample 20 g of SW powder were mixed with 200 ml of Milli-Q water (Millipore, USA) and kept in a boiling water bath at 120 $^0C$ for 15 min. After cooling, the crude seaweed extract [*S. wightii* (SW); SE] was filtered through a Whatman No.1 filter and was stored in a refrigerator at 4 $^0C$ until further study[16].

### 2.3. Synthesis and characterization of silvernanoparticles (Ag-NPs)

An aqueous solution of 1 mM silver nitrate ($AgNO_3$) (analytical grade - Merck, India) was used for the synthesis of silver nanoparticles (Ag-NPs). The reaction mixture was prepared by adding 5 ml of SE and 95 ml of 1 mM $AgNO_3$ solution in a 250 ml Erlenmeyer flask and kept in a boiling water bath at 70 $^0C$ until the color changed to dark brown[17]. The formation of dark brown color indicates an eco-friendly synthesis of *S. wightii* (SW) mediated silver nanoparticles (SWAg-NPs).

These were confirmed by UV-vis spectroscopy (Shimadzu 1700, America Varian Cary 5000 spectrophotometer), the size and morphology were elucidated by scanning electron microscopy (SEM; HITACHI, S-3000H). Further confirmation was obtained by X-ray diffraction (Nicolet Model: 6700) and a field-emission scanning electron microscopy (ZEISS, Fe-SEM), equipped with energy dispersive spectroscopy (EDS), measured at 20kV accelerating voltage. Composition and functional groups were studied by fourier transform infrared spectroscopy (FTIR). Analysis was taken place using the KBr pellet technique at a range of 4000 $cm^{-1}$ to 400 $cm^{-1}$ (Made spectrum RX 1, Male Perkin Elmer) at the CSIR-CECRI laboratory in Karaikudi, Tamil Nadu, India. The UV-visible (UV-vis) diffuse reflectance spectra were obtained by an America Varian Cary 5000 spectrophotometer.

### 2.4. *In-vitro* antimicrobial efficacy against MDRs

All the media, standard disks, sterile swabs and HiAntibiotic ZoneScale - C were purchased from Hi-Media (Mumbai, India). Isolates of MDRs were grown in nutrient agar medium, then followed by frequent sub-culturing into fresh nutrient broth medium incubated at $37 \pm 1\ ^0C$ for 24 - 48 hrs for an antimicrobial efficacy test. *In-vitro* antimicrobial sensitivity assays were carried out using a well diffusion assay to test samples

SWAg-NPs against certain MDRs plated on a Muller Hinton Agar (MHA) medium. Sterile cotton swabs were used to inoculate standardized bacterial suspensions (test culture suspensions prepared in sterile 0.85% saline matching an optical density of 0.5 McFarland standards corresponding to $10^8$ CFU/mL) on the surface of agar plates for homogeneous growth[18-21].

Lyophilized SWAg-NPs were dissolved in Milli-Q water (Millipore, USA) and sonicated in order to prevent the agglomeration of particles. Four wells each of 6 mm diameter were made on each plate with different concentrations of eco-friendly synthesized SWAg-NPs solutions (20, 40, 60 and 80 µg $ml^{-1}$) were loaded into each well. These plates were incubated at 37 ± 1 $^0$C for 24 - 48 hrs after incubation. Zones of inhibition were measured by a ruler/HiAntibiotic ZoneScale-C. Assays were performed in triplicate and average values were recorded[22].

## III. Results and discussion

### 3.1. Green Synthesis and characterization of silver nanoparticles (Ag-NPs)

The ultimate challenge/aim to achieve appropriate disinfection to deform harmful disinfection byproducts by conventional chemical disinfectants, including growing demand for decentralized or point-of-use MDRs bactericidal as well as photocatalytic degradation of MO dye treatment and recycling systems calls for new technologies for/towards efficient disinfection and microbial control. Several natural and engineered NPs have demonstrated strong antimicrobial properties through diverse mechanisms including the photocatalytic production of reactive oxygen species that damage cell components and viruses, compromising the bacterial cell envelope, interrupting energy transduction, and inhibiting enzyme activity and DNA synthesis[21].

Muthukumar and co-workers[17]reported that seaweeds are extremely different from terrestrial plants with the ability to reduce silver to silver ions ($Ag^+$). However, when 1 mM $AgNO_3$ solution was added to aqueous *S. wightii* (SW) seaweed extract (SE), no reaction occurred. Instead, after 48 hrs of incubation at room temperature, the color of the solution intensified to dark brown indicating the formation of Ag nanoparticles as shown in Fig. 1(a). This characteristic color change may be due to the excitation of surface plasmon resonance (SPR) and reduction of biosynthesized SWAg-NPs. The $AgNO_3$ solution control remained as such without any change in color[22]. This suggests that the color intensity of the biosynthesized SWAg-NPs studied by UV–visible spectroscopy is a convenient tool for measuring the reduction of metal ions based on optical properties called SPR[23]. The reaction mixture has an absorption maximum of 430 nm through an increase of color extinction with time and the colorless product indicates a cessation of the reduction reaction shown in Fig. 1(a).

Intense FTIR analysis bands were observed at 3429 $cm^{-1}$, 1637 $cm^{-1}$ and 685 $cm^{-1}$, which indicate the presence of molecular functional groups that are responsible for the reduction of biomolecules for capping and stabilization of SWAg-NPs in Fig. 1(b). FTIR peaks unveil the presence of phenolic compounds (3429 $cm^{-1}$) with a hydroxyl group (OH) bonded directly to an aromatic hydrocarbon group (685 $cm^{-1}$). The band peak at 685 $cm^{-1}$ could be assigned to the stretching vibration of aromatic rings that may be attached to a free OH-group reported as silver ions ($Ag^+$), which possibly bind to phenolic compounds with one or more aromatic rings resulting in the formation of Ag-NPs[24].XRD data showed four strong peaks at $32.5^0$, $46.4^0$, $57.4^0$ and $76.3^0$. These values were comparative with the original XRD pattern of $AgNO_3$ crystals and pure silver (Ag) [that was published by the Joint Committee on Powder Diffraction Standards (JCPDS - file no. 84-0713 and 04-0783)]. Intense peaks at $38.1^0$, $44.3^0$, $64.4^0$, and $77.3^0$ were indexed with the 111, 200, 220 and 311 planes of Ag, respectively (Fig. 1c). The XRD spectra indicated that particles were of acceptable crystallinity with the cubic structure form of Ag-NPs and aggregations formed due to the action of stabilizing agents in the algal extract. The diffraction angles of Ag-NPs were quite close to Ag crystals.This was also confirmed by Fe-SEM analysis that demonstrated the spherical, crystalline and poly-dispersed SWAg-NPs of 22 nm sizes with minimal agglomeration due to the presence of organics of *S. wightii* (SW) as stabilizing agents (Fig. 1d). An earlier study by Morones and co-workers[25]showed bactericidal effects of Ag-NPs of 1–100 nm size, proving broad spectrum bactericidal activity.

### 3.2. Antibacterial efficacies of green synthesized SWAg-NPs

Antibacterial activities of biosynthesized SWAg-NPs samples were found to reveal considerable activity against most of the MDRs. That concentration and species-specific characteristics play an important role in antimicrobial screening clearly depicts the potent nature of the synthesized SWAg-NPs. A considerable difference in the diameter of inhibition zone was observed with different concentrations of SWAg-NPs as displayed in Table 1 and Fig. 2. Antibiotics are among the most successful drugs used in human therapy. However, since they can challenge microbial populations, they must be considered as crucial pollutants as well. Besides being used for human therapy, antibiotics are extensively used for both animal farming and other agricultural purposes. Residues from the human environment and farms may contain antibiotics and antibiotic resistance genes that can pollute natural environments[26].

In addition, higher SWAg-NPs concentrations than 80 μg $ml^{-1}$ showed a greater sensitivity than lower concentrations (20 μg $ml^{-1}$) of all tested microorganisms. That concentration and species-specific characteristics play an important role in the antimicrobial screening clearly depicts the potent nature of the synthesized SWAg-NPs. A maximum growth inhibition zone of 18 mm was observed in Ampicillin-resistant *Escherichia coli* (NCIM 2931) and a minimum of 12 mm in Methicillin-resistant *Bacillus subtilis* (B1 - NCIM 2920) due to extracellular polymeric substance (EPS) secretion in Gram-positive bacteria. Amro and co-workers[27]reporting on the mechanisms of Ag-NPs toxicity suggested that the attachment of particles to the surface of cell membranes caused a disturbance of permeability and respiration. SWAg-NPs are known to bind with thiol groups of DNA and RNA affecting the protein biosynthesis of bacteria. Studies have demonstrated that silver ions ($Ag^{+}$) interact with sulfhydryl (SH) groups of proteins as well as the bases of DNA, leading either to respiratory inhibition or to the unwinding of DNA. The bactericidal activity was dependent on the shape and size of the nanostructures and their concentrations. Besides, SWAg-NPs react with the thiol groups of proteins and interfere with DNA replication leading to bacterial inactivation.

## IV. Conclusions

We were able to biosynthesize Ag-NPs from *S. wightii* (SW) at room temperature within 48 hrs of incubation. The formation of SW-AgNPs was confirmed by UV-spectra, FT- Fe-SEM and EDS studies. The suggested procedure was effective against Gram-negative MDR isolates with a maximum inhibition (16 mm) of *E. coli* and a minimal (14 mm) of *P. aeruginosa*, whereas, Gram-positive a maximum inhibition (12 mm) of *S. epidermis*anda minimal (10 mm) of *B. subtilis* and *S. aureus* by rupturing the membrane of the Gram-positive and negative bacteria cell walls as well as extracellular polymeric substance (EPS) secretion from binding to intracellular material. Moreover, biosynthesized SWAg-NPs exhibited an excellent stability and long-term usability. The concentration of the photocatalyst has a significant impact on the efficiency of bacterial inactivation. The results demonstrated that could cause drastic damage to MDRs and provides water purification at the same time.

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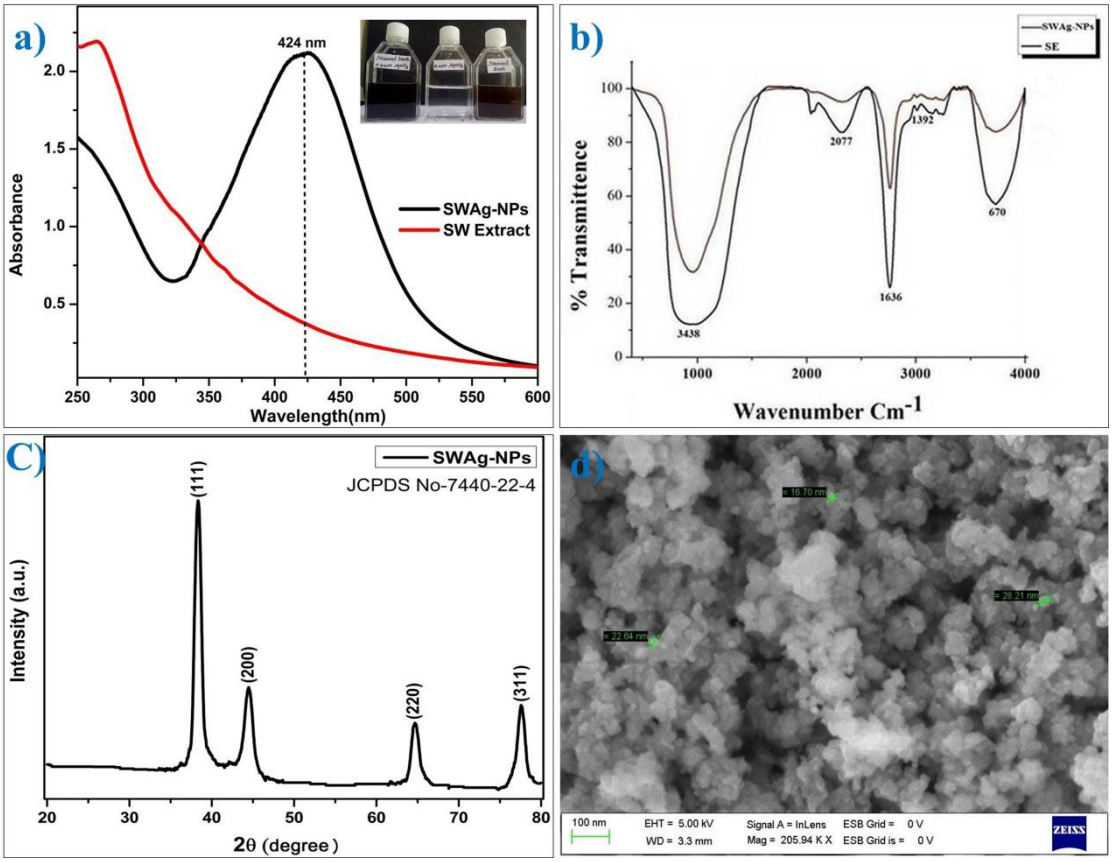
**Captions of Tables and Figures:**

**Figures**

Figure 1: (a) Visible color change and UV–Vis spectroscopy absorbance; (b) FTIR spectra of seaweed extract (alone) and green synthesized SWAg-NPs from *Sargassum wightii* (SW) seaweed extract; (c) XRD pattern mixed phase of face-centred cubic (fcc) structures; (d) Fe-SEM and SADE images of randomly selected SWAg-NPs observation.



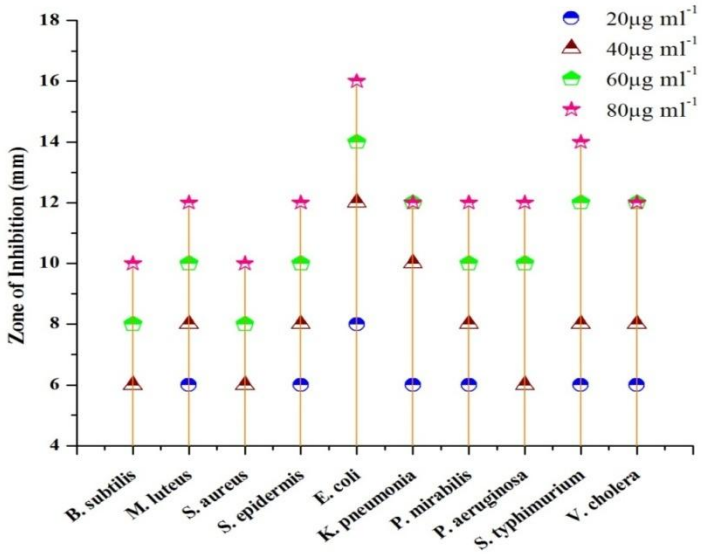




**Figure 2**: *In-vitro* antibacterial efficacy of green synthesized SWAg-NPs against multi-drug resistant strains (MDRs).

**Table:1**

**Table 1: *In-vitro* antibacterial efficacy of green synthesized SWAg-NPs against multi-drug resistant strains (MDRs).**

| S.No | Multi-drug resistant strains (MDRs)for testing organisms | Evaluation of bactericidal Zone of inhibition (mm) efficacy on SWAg-NPs nanoparticles | | | |
|---|---|---|---|---|---|
| | | 20 μg $ml^{-1}$ | 40 μg $ml^{-1}$ | 60 μg $ml^{-1}$ | 80 μg $ml^{-1}$ |
| **Gram- Positive** | | | | | |
| 1 | *Bacillus subtilis* | -- | 06 | 08 | 10 |
| 2 | *Micrococcus luteus* | 06 | 08 | 10 | 12 |
| 3 | *Staphylococcus aureus* | -- | 06 | 08 | 10 |
| 4 | *Staphylococcus epidermis* | 06 | 08 | 10 | 12 |
| **Gram- Negative** | | | | | |
| 5 | *Escherichia coli* | 08 | 12 | 14 | 16 |
| 6 | *Klebsiella pneumonia* | 06 | 10 | 12 | 12 |
| 7 | *Proteus mirabilis* | 06 | 08 | 10 | 12 |
| 8 | *Pseudomonas aeruginosa* | -- | 06 | 10 | 12 |
| 9 | *Salmonella typhimurium* | 06 | 08 | 12 | 14 |
| 10 | *Vibrio cholera* | 06 | 08 | 12 | 12 |

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