

# An *in Vitro* Study on The Antimicrobial Activity and Antioxidant Activities of The Extract of A Seaweed, *Enteromorpha Intestinalis* Against Certain Pathogens

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#### **Research Article**

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# Abstract

Seaweeds are potential bio resources of marine ecosystem and they are the producers of marine ecological energy chain and also possess many bioactive compounds with them. The seaweed *Enteromorpha intestinalis* is the plant material chosen for the study and was collected from the Pulicat estuary. The collected seaweeds were processed to synthesise nanoparticles out of them and the synthesised silver nanoparticle's *in vitro* antimicrobial and antioxidant activity was evaluated. The antibacterial activity was determined by the action against *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Vibrio parahaemolyticus* and *Micrococcus luteus*. Similarly the *in vitro* antifungal effect was also explored against *Candida albicans, Trichoderma viridae, Aspergillus niger, Rizhopus* sp and *Penicillium notatum*. Among them, *Staphylococcus aureus* and *Aspergillus niger* were found to be highly susceptible to the synthesised nanoparticles. When the antioxidant efficacy of synthesized nanoparticle was tested using different methods such as DPPH and ABTS it was observed that they possessed appreciable antioxidant property whose efficiency was correlated with their respective positive standards. These investigations illustrated the potential effects of the seaweed E. *intestinalis* for using them as an active antimicrobial and antioxidant agent.

# Introduction

In India, seaweed resources are exclusively utilized for the production of commercially and industrially important phycocolloids (Reddy et al., 2014). Seaweeds are a diverse and large group of macro algae which are broadly classified into various types based on the presence of photosynthetic pigments as rhodophyta (red algae), chlorophyta (green algae), and phaeophyta (brown algae) (Hemasudha et al., 2019). Seaweed extracts contains major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances (Mooney and Staden, 1986). Agar, carrageenan and alginate are popular examples of seaweeds these have been used as food for human beings, fertilizers for plants and source of various chemicals (Shelar *et al.*, 2012).

Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and ageing processes (Kohen and Nyska, 2002). Some researchers have also worked and reported the same (Mhadhebi et al., 2014; Venkatesan *et al.*, 2016; Keshari et al., 2018; Hemasudha et al., 2019). Nanoparticles extremely attractive for a large range of applications, including catalysis, gas and energy storage, photovoltaic, electrical and optical devices and biological and medical technologies (Goesmann *et al.*, 2010; Lin *et al.*, 2015, Kunjachan et al., 2015, Chinen et al., 2015; Pelaz *et al.*, 2017; Wuttke et al., 2017; Peller *et al.*, 2017; Li et al., 2017; Freund et al., 2018; Rosenblum et al., 2018). Several characterization methods have been devised to investigate size, distribution, shape, surface charge and porosity of nanoparticles in different environments (Modena et al., 2019). Silver nanoparticle (AgNPs) are known for their antimicrobial properties, being effective against pathogens, which explain their potential for several biotechnological application, in addition to their electrical, thermal, magnetic and catalytic characteristics (Vigeshwaran *et al.*, 2008; Thakkar et al., 2010; Konwarh et al., 2011; MubarakAli et al., 2011; Mohanty et al., 2012; Chen *et al.*, 2017). The AgNPs

possess unique attributes which are breakthrough myriad applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities (Jannathul and Lalitha, 2015). The biosynthesis of nanoparticles has been proposed as a cost-effective and environmental friendly alternative to chemical and physical methods (Anju et al., 2015). Biological synthesis of AgNPs has an outstanding numerous benefits without the use of toxic chemicals and possess advancement over both physical and chemical methods (Bhainsa and Souza, 2006; Song and Kim, 2009; Parashar et al., 2009; Saifuddin et al., 2009).

Many marine algae were screened for their antimicrobial activity by Reichelt and Borowitzka (1984) & Latha and Latha (2011). Silver nanoparticles attach with the microbial cell wall membrane by electrostatic attraction (Dibrov et al., 2002; Lara et al., 2010) and subsequently penetrate it, thereby changing the permeability of the cell membrane and causes cell death (Sondi and Salopek-Sondi, 2004). Extracted substances from seaweeds have antibacterial actions and other properties include antifungal activities and growth inhibition of plants (Burkholder and Sharma 1969; Su et al., 1973; Abdussalam, 1990; Scheuer, 1990; Rizvi and Shameel, 2003). Seaweeds are excellent source of bioactive compounds which demonstrated a broad range of biological activities such as: anti-inflammatory, antibiotics, antiviral, cytotoxic and antimitotic activities (Nagvi et al., 1980; Bhosale et al., 2002), anticoagulants, anti-ulcer (Fayaz et al., 2005). Enteromorpha sp have also been shown to contain certain bioactive substances (Dhawan 1992; Chauhan and Siddhanta 1992). Many investigations revealed that macroalgae have a broad range and potential use in pharmacology researches as antibacterial and antifungal(Karabay-Yavasoglu et al., 2007; Zbakh et al., 2012; Jeyaseelan et al., 2012; Alghazeer et al., 2013; Oumaskour et al., 2013; Abo-State et al., 2015). In this present study the seaweed E. Intestinalis was collected from the Pulicat estuary and the nanoparticles of the same were tested for various bioactivities to illustrate its potential utilizations.

# **Materials And Methods**

# Enteromorpha intestinalis

The marine seaweed *Enteromorpha intestinalis* was collected from the Pulicat estuary at the Thiruvallur district of Tamil Nadu and were processed for extraction using distilled water as solvent to obtain the aqueous extract by following standard methods (Rajeshkumar et al., 2016).

**Synthesis of silver nanoparticles** Silver nanoparticles were synthesized by adding standard amounts of silver nitrate solution to the prepared aqueous extract (Rajeshkumar et al., 2016). The reaction was characterized by the colour change and monitored by UV spectroscopy.

# Characterization

The morphology of the synthesised silver nanoparticle was identified and confirmed using different characterisation techniques such as Fourier Transform Infrared spectroscopy (FTIR), UV–VIS Spectroscopy and Scanning Electron Microscopy (SEM).

# **Antioxidant Activity**

### DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay

DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was performed to evaluate the radical scavenging activity of the synthesised silver nanoparticles (Molyneux, 2004). BHT was taken as the standard for the evaluation. About 100  $\mu$ l of respective samples were added to all tubes marked as tests except one which received BHT. 200  $\mu$ l of DPPH reagent was added to all the test tubes including blank and all the test tubes were incubated at room temperature in dark condition for 30 min. The absorbance were read at 517 nm and the anti-oxidant activity was determined using the given formula



#### ABTS assay

The radical scavenging activity was determined as described by Re et al. (1999).The decolourisation assay involves the generation of the ABTS<sup>+</sup> chromophore by the oxidation of ABTS with ammonium persulphate. The scavenging activity of the plant extracts on ABTS radical action were measured at 734 nm. Seaweed samples were diluted to produce 20, 40, 60, 80, 100 $\mu$ g/ml. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10  $\mu$ l of different concentration of the sample and 10 $\mu$ l of methanol as control. BHT was used as standard. The absorbance was read at 734 nm and the percentage inhibition was calculated. The percentage inhibition was calculated according to the equation.

### ABTS scavenging activity (%) = $(A_0 - A_1) / A_0 \times 100$

Where,

 $A_0$  is the absorbance of the control,

 $A_1$  is the absorbance of the sample

# **Antibacterial Activity**

The disc diffusion method on Muller Hinton agar (MHA) medium was followed to determine the antibacterial activity. The pathogens are *Salmonella typhi, Seudomonas aeruginosa, Staphylococcus aureus, Vibrio parahaemolyticus* and *Micrococcus luteus* were used as test bacteria for evaluating the antibacterial efficacy at different concentrations (1000µg, 750µg, 500µg and 250 µg), with ampicillin (20µg/ml)as the positive standard. Muller Hinton Agar (MHA) medium was used for the bacterial culture

and the plates were incubated at 37°C for 24 h (Bauer et al., 1966). The antimicrobial activity was determined by measuring the diameter of zone of inhibition.

# Antifungal Activity Assay

The antifungal activity of the synthesised silver nanoparticles was determined by disc diffusion method on Sabouraud Dextrose agar (SDA) medium. The fungal pathogens like *Candida albicans, Trichoderma viridae, Aspergillus niger, Rizhopus* sp and *Penicillium notatum* were tested using amphotericin-B is taken as positive control. 20 µl of respective concentrations of test samples and positive control were added in SDA plates. The plates were incubated at 28°C for 24 h. The antifungal activity was determined by measuring the diameter of zone of inhibition (Bauer et al., 1966).

## **Results**

The seaweed *Enteromoprha intestinalis* (Fig. 1.) was processed and the silver nanoparticles were synthesized, which were identified and confirmed by following characterisation methods. The FTIR peak values indicated the presence of characteristic bioactive compounds (Fig. 2.). UV visible absorption spectroscopy through UV light was absorbed by the molecule and the range of peak value was 380 to 480 nm. The synthesized silver nanoparticle was monitored at the peak value of 420 nm (Fig. 3). The size of the nanoparticles synthesized from the collected seaweed varied from 57.87 and 82.09 nm in diameter (Fig. 3).

The silver nanoparticles synthesized from the seaweed *Enteromorpha intestinalis* was evaluated for its radical scavenging activity using DPPH assay and the maximum antioxidant activity was found was 40.86% at  $100 \mu$ g/ml.

The results of the ABTS assay showed maximum activity of 23.04% at 100  $\mu$ g/ml and minimum of 2.38% at 20  $\mu$ g/ml concentrations. The overall efficacy of synthesized nanoparticles was found to approximately similar to the standard (Fig. 4).

The *in vitro* antibacterial activity of E. *intestinalis* seaweeds using silver nanoparticle showed maximum activity (Zone of inhibition - 8.66 ± 1.15 mm) against *Staphylococcus aureus* at 1000 mg/ml (Fig. 7.) (Table 1).

On evaluation, *in vitro* antifungal activity of E. *intestinalis* silver nanoparticles showed highest inhibitory action on *Aspergillus niger* at (10 ± 1 mm of inhibitory zone) 1000 mg/ml (Fig. 8)(Table. 1).

#### Table 1. Antimicrobial activity

Microbial species	Zone of Inhit	Zone of Inhibition			
	(mm)				(1mg/ml)
	Sample (1mg/ml)				
	1000	750	500	250	
Bacterial pathogen					
Pseudomonas aeruginosa	8.33 ± 0.57	8 ± 0.00	7.33 ± 0.57	7 ± 0.00	11 ± 1
Staphylococcus aureus	8.66 ± 1.15	8 ± 0.00	7.33 ± 0.57	7 ± 0.00	14.33 ±0.57
Salmonella typhi	8.33 ±0.57	7.66 ± 0.57	7.33± 0.57	7 ± 0.00	17.66 ±1.52
Vibrio parahaemolyticus	8.33 ±0.57	7.66 ± 0.57	7 ± 0.00	7 ± 0.00	13.33 ±7.50
Micrococcus luteus	8 ± 0.00	7.33± 0.57	7 ± 0.00	7 ± 0.00	16.66 ±0.57
Fungal pathogen					
Candida albicans	9.33 ± 0.57	8.66 ± 1.15	766 ± 1.15	7 ± 0.00	17.33 ±0.57
Trichoderma viridae	8.66 ± 1.15	8 ± 0.00	7.33 ± 0.57	7 ± 0.00	14.33 ±0.57
<i>Rhizopus</i> sp	7.6 ±0.57	7 ± 0.00	7 ± 0.00	7 ± 0.00	17 ± 1
Aspergillus niger	10 ± 1	9.33 ± 1.15	8 ± 1	7.33±0.57	8.33 ± 0.57

## Discussion

Seaweed extracts contains major and minor nutrients, amino acids, vitamins, cytokinins, auxin and abscisic acid like growth promoting substances (Mooney and Staden, 1986). The secondary metabolites derived from marine macro-algae have been associated with a broad range of biological activities such as antibacterial, antiviral, antifungal, antifouling and anti-inflammatory effects as well as cytotoxic and antimitotic activities (Mayer *et al.*, 2009). Several silver-based compounds have been utilized effectively as antimicrobial specialists (Nomiya et al., 2004). The compounds of silver are also used in the medical field to treat burnt wounds and various other types of infections. Nanoparticles of silver have aptly been investigated for their antibacterial property because the silver nanoparticles have high specific area than their volume, which will lead to excellent antimicrobial activity as compared with bulk silver metal (Morones et al., 2005; Baker et al., 2005; Panacek et al., 2006; Rai et al., 2009). Silver nanoparticles showed potential antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative organisms such as *Klebsiella pneumonia* (Mahitha et al., 2011) and *Salmonella typhus* (Tripathi et al., 2010). The extracts of *Gracilaria verrucosa* were also reported to be effective against the Gram negative bacteria (*Pseudomonas aeruginosa* and E. *coli*) than Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) (Adaikalaraj et al., 2010).

In the present study was *in vitro* antibacterial activity of *Enteromorpha intestinalis* seaweeds using silver nanoparticle showed good maximum activity against Staphylococcus aureus species of pathogen at 1000 mg/ml due to the presence of phytochemicals like flavonoids, tannins, phenols and proteins which may act as an antimicrobial agent (Priyadarshni and Mahalingam, 2017) by attaching to the microbial cell wall membrane by electrostatic attraction (Dibrov et al., 2002; Lara et al., 2010) Antifungal activity of Enteromorpha intestinalis was Maximum against Aspergillus niger because of the bioactive compounds of the nanoparticle. Cyril et al. (2017) reported the antifungal activity against the pathogen seaweed has antifungal property. The antibacterial (Latha and Latha, 2011; YokeshBabu et al., 2013; Venkatesan et al., 2016; Pérez et al., 2016; Jayaraman et al., 2018; Hemasudha et al., 2019) and antifungal activities of several of the marine algae were reported by various researchers have illustrated the same mechanism (Caccamese et al., 1980; Perry et al., 1991; Val et al., 2001; Oumaskour et al., 2012; Bouhraoua et al., 2018). Seaweeds possess various and abundant secondary metabolites which has good radical scavenging activity and therefore could be utilised as antioxidant source (Diplock, 1997). The present study explored the antioxidant efficacy of synthesized nanoparticle from Enteromorpha intestinalis using DPPH and ABTS methods. Silver nanoparticle of Enteromorpha intestinalis showed antibacterial, antifungal and antioxidant activity, thereby could be effectively used as a therapeutic agent.

# Declarations

### AUTHOR'S CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author JG and LJ carried out the assays and experiments of the study. Author JJ designed the experimental setup for this study. Author MGR supervised the whole research work and corrected the manuscript draft. All authors read and approved the final manuscript.

### DECLARATION OF COMPETING INTERESTS

Authors have declared that no competing interests exist.

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## Enteromorpha intestinalis



FTIR spectroscopy.



#### Figure 3

UV spectroscopy



SEM analysis of silver nanoparticles.



DPPH radical scavenging activity



#### Figure 6

ABTS radical scavenging activity



Pseudomonas aeruginosa





Staphylococcus aureus

Salmonella typhi



Micrococcus luteus



Vibrio parahaemolyticus

Antibacterial activity by disc diffusion method



Rhizopus



Candida albican



Trichoderma viridae



Aspergillus niger

Antifungal activity by disc diffusion method