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**Original Article**

# Invitro Antioxidant activity of silver nano-particles from *Colpomenia sinuosa* and *Halymenia poryphyroides*

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**Abstract**

This study was conducted to investigate the in-vitro antioxidant activities of silver nano particles bio-synthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides*. The free radical scavenging activity of the extracts was measured by DPPH and ABTS and the antioxidant potential were compared with commercial antioxidants, such as Butylated Hydroxy Toulene (BHT) and L- ascorbic acid. The antioxidant activity of experimental algae showed a dose dependent increase when compared to commercial antioxidants. Spectro-photometric determination of the active compounds revealed that the antioxidant activity might be attributed to algal contents of carotenoids, free phenols and fatty acids.

Keywords: Antioxidant, *Colpomenia sinuosa*, *Halymenia poryphyroides*

## INTRODUCTION

Seaweeds and marine macro algae are rich sources of several compounds with biological effects including antioxidant activities (1). Seaweeds contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites including carotenoids ( α-and β carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporine-glycine), catechins (e.g., catechin, epigallocatechin,gallate,pholorotnnins,eckol and tocopherols( α-,χ-δ-tocopherols) (2)Oxidative stress is a crucial etiological factor to the pathophysiology of variety of degenerative or pathological conditions such as aging, cancer, coronary heart disease, Alzheimer's disease, atherosclerosis and inflammation (3,4).Multiple mechanisms of enzymatic and non-enzymatic antioxidant systems are present in human body to protect the cellular molecules against reactive oxygen species (ROS) induced damage(5).The over production of reactive species and inadequate antioxidant defense results in severe or continued oxidative stress. The harmful action of the free radicals can however be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism (6).The present study aimed to evaluate the in-vitro antioxidant activity of silver nano particle from marine algae *Colpomenia sinuosa* and *Halymenia poryphyroides* by measuring their scavenging activity against free radicals.

## MATERIALS AND METHODS

**Collection of algae:** The marine brown alga *Colpomenia sinuosa* and marine red alga *Halymenia poryphyroides* and were collected from leepuram coast and mandapam coast, Tamilnadu, South India respectively

**Preparation of algal extract:** The freshly collected samples were soaked and thoroughly cleaned in sea water to remove the sand and debris which was then shade dried. Dried seaweeds were powdered and soaked in Methanol (1:3 w/v) overnight and filtered to collect the methanol fraction. The residue was extracted two or more times and the filtrates were combined and concentrated to obtain the crude extract. All the fractions were concentrated by evaporating under vacuum in a rotary evaporator and the dried extract was used for its antioxidant potential.

**Invitro Antioxidant Assay:** The antioxidant activity of silver nano particle bio-synthesized from *Colpomenia sinuosa* and *Halymenia poryphyroides*

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were examined. The two free radical systems like DPPH and ABTS were used for assessing the free radical scavenging activity of the test extracts and the results were compared with the standard antioxidant Butylated hydroxytoulene and L-ascorbic acid.

**DPPH radical activity:** DPPH (2, 2 – diphenyl-1-picrylhydrazyl) scavenging activity was measured by spectrophotometric method (Sreejayan and Rao, 1996) (7). To a methanolic solution of DPPH (200 µM), 0.05 ml of the test compounds dissolved in methanol were added at different concentrations (100-900 µg/ml). An equal amount of methanol was added to the control. After 20 minutes, the decrease in the absorbance of the test mixture (due to quenching of DPPH free radicals) was read at 517nm and the percentage inhibition was calculated by using the formula (Prasanth Kumar et al., 2000). The experiment was repeated in triplicates.

**Inhibition (%) = (Control – Test) x 100**
**Control**

**ABTS radical cation decolonization assay:** In this improved version, $ABTS^{\cdot}$, the oxidant is generated by persulfate oxidation of 2, 2-azinobis (3-ethylbenzoline-6-sulfonic acid) – ($ABTS^{2-}$). ABTS radical cation was produced by reacting ABTS solution(7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in the dark at room temperature for 12-16 hrs before use (Sun et al., 2007)(8). For this study, different concentrations (100-900 µg/ml) of silver nano particle (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol to make 1.0 ml. The absorbance was read at 745 nm and the percentage inhibition was calculated using the formula

**Inhibition (%) = (Control – Test) x 100**
**Control**

All the determinations were carried out in triplicates. The $IC_{50}$, the antioxidant activity in terms of the amount (µg/ml) of the extracts necessary for inhibiting 50% of cell growth.

## RESULTS AND DISCUSSION

In the present study, the free radical scavenging activity of silver nano particle bio-synthesized from *C.sinuosa* and *H.poryphyroides* were carried out using hydro soluble radicals DPPH and ABTS.

**DPPH free radical scavenging activity:** DPPH is a stable free radical that accepts hydrogen to become a stable diamagnetic molecule which is used as a substrate to evaluate the anti-oxidant activity. (Elimastas et al., 2006)(9). the results showed a higher scavenging activity of DPPH at concentration of 900 µg/ml which were 77.73±0.02 for *Colpomenia sinuosa and* 75.63±0.02 for *Halymenia poryphyroides* (Table 1& 2). The $IC_{50}$values of silver nano particle bio-synthesized from Colpomenia *sinuosa* and *Halymenia poryphyroides* were 65±0.01 and 84±0.01 which was higher when compared to the standard BHT (33±0.01) and L-ascorbic acid 73±0.01 (Table 3) which was higher when compared to silver nano particle of *Colpomenia sinuosa.* The result indicates the hydrogen donating ability of the algal extracts Conforti et al (10).Brown algae and red algae contained higher amount of polyphenols and DPPH radical scavenging activity (Wang et al., (2009) and Yan et al., (1999)(11,12). However Chandini et al., (2008) (13) reported low levels of DPPH radical scavenging activity in brown seaweeds, in the range 17.79 to 23.16 % at a concentration of 1000µg/ml of the extract.

**ABTS free radical scavenging activity:** ABTS free radical scavenging activity showed a maximum % of 57.68±0.02 and 51.66±0.02 at a concentration of 900 µg/ml (Table 1& 2) for Colpomenia *sinuosa* and *Halymenia poryphyroides* respectively. The $IC_{50}$values of silver nano particle showed % of 82±0.01and 73±0.01 which were higher compared to the standards BHT (32.5±0.01) and L-ascorbic acid (45.1±0.01) (Table 3). The results of the present study indicate that the silver nano particle bio-synthesized from algae has higher and significant effect on scavenging of ABTS radicals. However the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and the slow reaction of many phenolics (Roginsky and Lissi, 2005) necessitate compatible evaluation of antioxidant activity using other assays as well.

## CONCLUSION

The results revealed that the silver nano particle bio-synthesized from Colpomenia *sinuosa* and *Halymenia poryphyroides* had active radical scavenging activity against DPPH and ABTS. Therefore these algae may be used as good sources of natural antioxidants. These studies suggest further studies for identifying active compounds in theses algae and also screen for their in vivo studies for their mechanism of action.

Table .1: Effect of silver nano-particle from *Colpomenia sinuosa* on DPPH and ABTS. (Free radical scavenging activity (inhibition %))

| S.No | Concentration (µg/ml) | DPPH radical | ABTS radical |
|---|---|---|---|
| 1 | 100 | 29.6±0.01 | 38.32±0.02 |
| 2 | 300 | 48.41±0.03 | 45.12±0.02 |
| 3 | 500 | 56.52±0.02 | 53.16±0.03 |
| 4 | 700 | 69.71±0.03 | 54.12±0.02 |
| 5 | 900 | 77.73±0.02 | 57.68±0.02 |
| **P – Value** | | 0.000 | 0.000 |
| **F – Value** | | 4.74344 | 1.10866 |

Values are expressed as mean±SD of triplicates

Table .2.Effect of silver nano-particle from *Halymenia poryphyroides* on DPPH and ABTS. (Free radical scavenging activity (inhibition %))

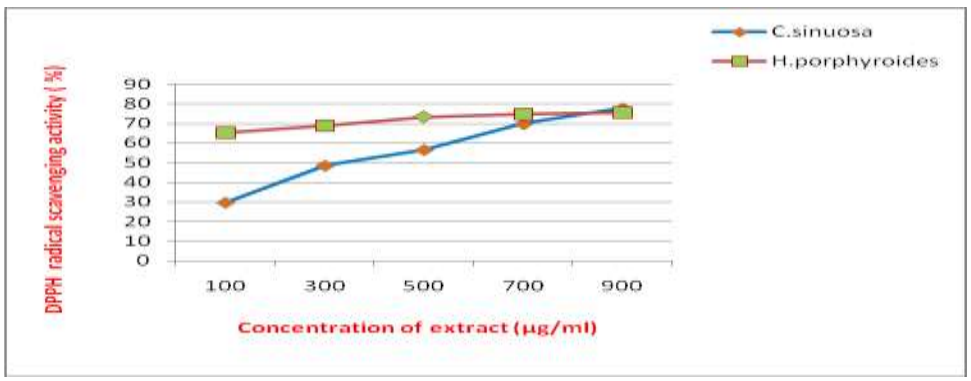
| S.No | Concentration (µg/ml) | DPPH radical | ABTS radical |
|---|---|---|---|
| 1 | 100 | 65.33±0.03 | 47.13±0.03 |
| 2 | 300 | 68.81±0.02 | 48.81±0.02 |
| 3 | 500 | 73.21±0.02 | 49.22±0.03 |
| 4 | 700 | 74.8±0.03 | 50.44±0.01 |
| 5 | 900 | 75.63±0.02 | 51.66±0.02 |
| **P – Value** | | 0.000 | 0.000 |
| **F – Value** | | 1.93555 | 7.5514 |

Values are expressed as mean±SD of triplicates

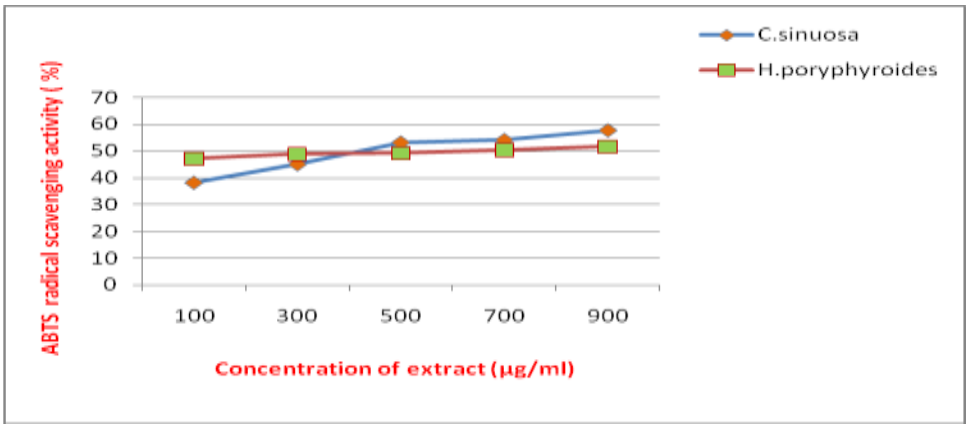
Table .3. $IC_{50}$ values of experimental algae *C.sinuosa* and *H. poryphyroides* with standard on free radical scavenging system. (Free radical scavenging assay $IC_{50}$ value (µg/ml))

| S.No | Experimental algae and Standard | DPPH radical | ABTS radical |
|---|---|---|---|
| 1 | *Colpomenia Sinuosa* | 65±0.01 | 82±0.01 |
| 2 | *Halymenia Poryphyroides* | 84±0.01 | 73±0.01 |
| 3 | BHT | 33±0.01 | 32.5±0.01 |
| 4 | L-ascorbic acid | 73±0.01 | 45.1±0.01 |

Values are expressed as mean±SD of triplicates




Graph. 1. DPPH radical scavenging activity of silver nano-particle from *Colpomenia sinuosa* and *Halymenia poryphyroides*




Graph. 2. ABTS radical scavenging activity of silver nano-particle from *Colpomenia sinuosa* and *Halymenia poryphyroides*

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