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Determination of antioxidative potential of the compounds isolated from *Sargassam wightii*

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A B S T R A C T

Two compounds were isolated and purified from the seaweed *Sargassam wightii*. These compounds were studied for their potential antioxidative activities using DPPH assay, ABTS assay, FRAP assay and reducing potential determination. When the compounds were assessed, the compounds showed increase in the antioxidative activity with an increase in the concentration of the compounds on all the four assays. This study shows that apart from antimicrobial activity, these compounds elicit antioxidative activity too.

Introduction

Diabetic foot ulcer (DFU) is one of the complications induced by Diabetes. This occurs due to the infection at the extremities and may result in amputation of the extremity (Dowd *et al.*, 2008). Although infection plays a major role in development of DFU there are involvement of other factors that contribute the dispersal of ulcer. One of the most important factors that favor the DFU is oxidative stress. Various studies have shown that oxidative stress aids spreading of DFU, which eventually result the morbidity in the patients. Therefore there is a need for agents which possess antioxidative properties apart from eliminating the infection (Vairamon *et al.*, 2009).

In patients with DFU various factors have been found to be affected. There was a reduction in the levels of antioxidative enzymes like superoxide dismutase, Glutathione peroxidase and antioxidants like Vitamin E, reduced glutathione (Bolojako *et al.*, 2009). Generally, the oxidative stress is elevated in patients with diabetes owing hyperglycemia. This hyperglycemia results in reduced endogenous antioxidants and elevated reactive oxygen species (Singh *et al.*, 2008). This results in reduced capacity for neovascularization in the wound affected areas. Therefore there is a need for an antioxidative substance which would supplement the treatment of DFU. Previous

studies have shown that supplementing diabetic patients with antioxidants during treatment for DFU, resulted in healing of wound faster that the non-supplemented control (Park and Lim, 2011). Genistein supplementation has been shown to enhance the antioxidant status in mice and resulted in an accelerated closure of wound in them (Eo *et al.*, 2015).

Sargassam wightii is a kind of seaweed, a kind of brown colouredmacroalga present in the coastal areas. Previously various studies have been performed in this species and the compounds isolated from *Sargassam* have been reported to possess antibacterial, antioxidant and other health benefits. A review by Liu *et al.*, (2010) shows the uses of *Sargassam wightii* in traditional Chinese medicine. Here *Sargassam wightii* has been reported to be used for various complications like arteriosclereosis, goitre, skin disease, high blood pressure *etc*. Another review by Smit, 2004 summarizes the antiviral, antibiotic, anticoagulative and anti-inflammatory properties of compounds isolated from *Sargassam wightii*.

We in this study report the antioxidative properties of two purified compounds from *Sargassam wightii*. These compounds were reported for their antimicrobial activity previously. Here the antioxidative properties of these compounds were determined by using Free radical scavenging by using DiPhenylPicrylHydrazyl (DPPH) assay, Ferrous reducing antioxidative potential (FRAP) assay, determination of reducing potential by potassium ferricyanide and ABTS reduction assay.

Materials and Methods

Extraction and Purification of Compounds from *Sargassam wightii*

The extraction of various compounds from *Sargassam wightii* has been previously reported. The extract has been purified by column chromatography and resulting compounds were separated and purified. The purified compounds were then tested for the antioxidative activity.

DPPH Scavenging Assay

The ability of compounds to scavenge the free electrons from DPPH is determined by the method as reported (Floegel *et al.*, 2011). To 3.9 ml of DPPH solution, 0.1 ml of the extract was added and incubated under darkness for 30 min. After incubation, the absorbance of the solution was measured in a spectrophotometer at 517 nm and the readings were noted. Adding DMSO alone served as control and ascorbic acid was used as positive control. The antioxidant capacity of the compounds extracted from *Sargassam wightii* is directly proportional to the reduction in the absorbance of the reaction mixture. The antioxidative activity was measured by the formula

$$\text{Antioxidative activity} = \frac{Ac - At}{At} * 100$$

Where Ac – Absorbance of Control; At – Absorbance of reaction mixture at time t=30 min.

ABTS Assay

Using ABTS 2,2′-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) assay, the cation scavenging ability of the compounds were determined (Thaipong *et al.*, 2006). Briefly, ABTS (7 mM) was mixed with potassium persulfate (2.45 mM) and incubated at room temperature overnight. After incubation, the resulting dark coloured solution containing ABTS radical was diluted in 50% methanol. The absorbance was measured at 745 nm. The reaction mixture essentially contained 300

µL of the extracted compound and 3.0 mL ABTS. The absorbance was noted at 0^{th} minute and after 5 min. Ascorbic acid was used as positive control. The amount of decrease in the absorbance is directly proportional to the antioxidative ability of the compound. The antioxidative effect was determined using the formula

Antioxidative activity = $\frac{Aca-Ata}{Ata}$*100

Where Aca – Absorbance of Control; Ata – Absorbance of reaction mixture at time t=30 min.

Determination of the Reducing Potential

The reducing power of the compounds isolated from *Sargassam wightii* was determined by the method that has been previously reported (Arora and Chandra, 2011). Essentially a reaction mixture was prepared by mixing 0.5 mL of the compounds at various concentration with 0.1 ml of potassium ferricyanide and incubated for 30 mins at 50 ^{o}C. After incubation 0.1 mL of 1 % trichloroacetic acid was added followed by the addition of 0.1 mL of 0.1 % $FeCl_3$ and incubated for 20 mins. The absorbance of the resulting solution was read at 700 nm. The antioxidative ability of the compounds was proportional to the absorbance of the final reaction mixture. Ascorbic acid was used as control.

Ferric Reducing Antioxidant Potential (FRAP)

For FRAP assay, FRAP reagent was prepared which consists of 300 mM acetate buffer (pH 3.6, 20 mM FeCl3 and 1 mL TPTZ in the ratio of 10:1:1. For determination of the antioxidant potential 0.5 mL of the compounds were mixed with 1 mL of distilled water, 0.5 mL of extract and incubated for 10 mins. After incubation the absorbance of the resulting solution was read at 593 nm. Ascorbic acid was used as control (Pal *et al.,* 2013). The antioxidant ability of the compounds was directly proportional to the absorbance of the final solution.

Results and Discussion

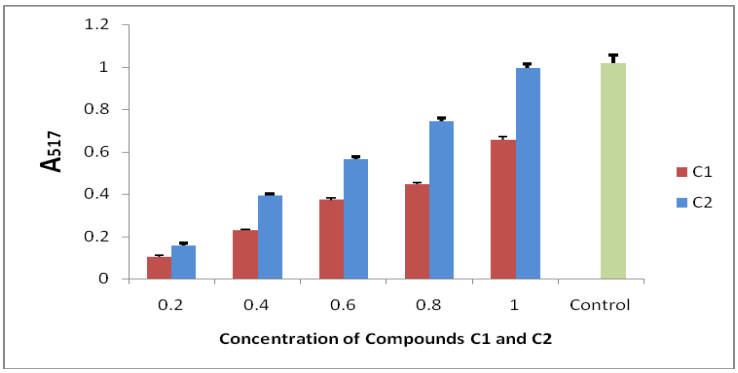
Extraction and Purification of Compounds from *Sargassam wightii*

As reported previously, various compounds have been isolated from *Sargassam wightii* (Ashtalakshmi and Prabhakaran, 2015). These compounds were tested for antioxidative properties further. Two compounds which showed good antimicrobial activity was further studied for antioxidant activity. The compounds were termed as C1 (FallaQuinone derivative) and C2 (Trimethyl Teterahydrobenzofuranone).

Purified Compounds Scavenged DPPH

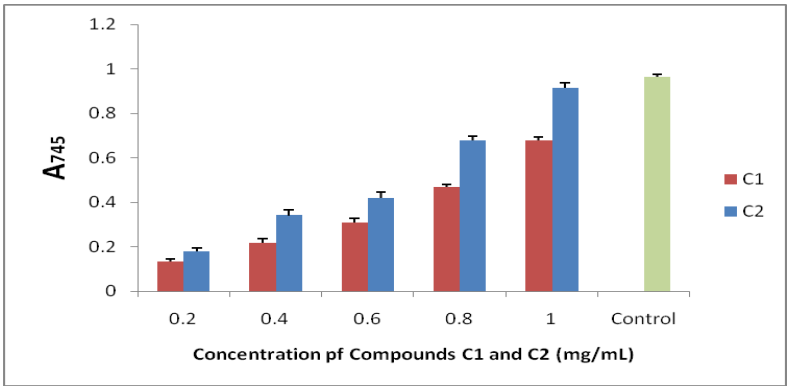
When compounds C1 and C2 were treated with DPPH, the antioxidative effect increased with the increase in the concentration of the compounds. This is marked by the reduced absorbance of the reaction mixture. Fig. 1 shows the effects compounds C1 and C2 on DPPH radical scavenging. Scavenging DPPH radical is one of the widely used methods to determine the antioxidative ability of a moiety under study. DPPH is a stable free radical. DPPH is orange in colour when it is in radical form. Yet, when the radical is neutralized, DPPH becomes colourless. The ability of compound to reduce the colour is directly proportional to its ability to neutralize DPPH radical (Floegel *et al.,* 2011). Previously DPPH has been used for determining the antioxidative potential of various phytochemicals, whole microorganism and enzymes.

Fig.1 Determination of Antioxidative Activity by DPPH Assay



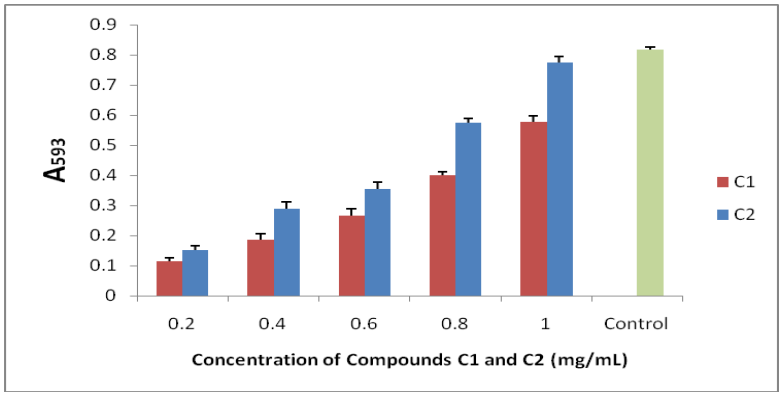
Here increase in the concentration of compounds C1 and C2 proportionally scavenged more DPPH ions where control is ascorbic acid

Fig.2 Determination of Antioxidative Activity by ABTS Assay



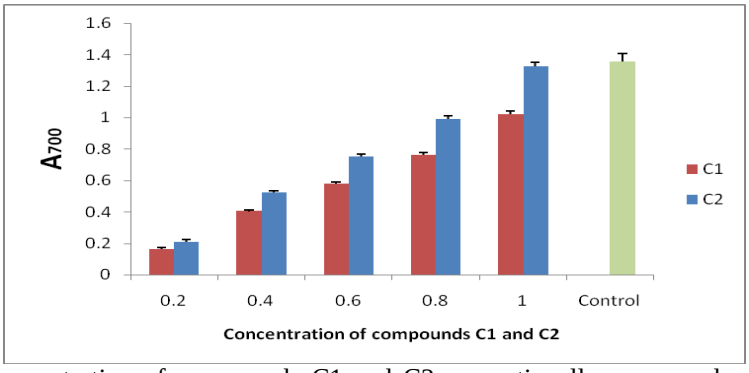
Here increase in the concentration of compounds C1 and C2 proportionally scavenged more ABTS ions where 1 mg/mL ascorbic acid served as control

Fig.3 Determination of Antioxidative Activity by FRAP Assay



Here increase in the concentration of compounds C1 and C2 proportionally scavenged more DPPH ions where control is ascorbic acid

Fig.4. Determination of Antioxidative Activity by Determination of Reducing Potential



Here increase in the concentration of compounds C1 and C2 proportionally scavenged more DPPH ions where control is ascorbic acid

Purified Compounds Scavenged ABTS Radical

ABTS is also a stable radical similar to DPPH. The antioxidative effect increased with the increase in the concentration of the compounds (Fig. 2). ABTS similar to DPPH, forms a stable radical which is blueish green in colour. The radical is formed when ABTS is mixed with potassium persulphate. When the moieties under study neutralize the radical, the intensity of the colour is reduced. The reduction in the colour is directly proportional to the reduction in the oxidative state of ABTS which is directly proportional to the antioxidative activity of the compound(Thaipong *et al.*, 2006). ABTS is also a widely used method to determine the antioxidative activities of various compounds including phytochemicals, proteins *etc*.

Antioxidative Activity by FRAP Assay

FRAP assay is based on the ability of the compound to produce colour when the colourless Fe^{3+}-TPTZ complex become blue in colour when TPTZ forms complex with Fe^{2+}(Pal *et al.*, 2013). Here when the concentration of the compounds C1 and C2 extracted from *Sargassamwightii*. was increased, an increase in the intensity was noted which indicates that Fe^{3+} gets converted to Fe^{2+} by the activity of the compounds extracted (Fig. 3). This shows that the compounds have the ability to reduce the oxidative state of iron thereby making it less reactive. This is also a widely used method for the determination of the antioxidative ability of various compounds.

Antioxidative Determination by Measuring Reducing Potential

This method is based on the conversion of yellowish potassium ferricyanide to blue coloured potassium ferrocyanide by the compounds under study. If the compound under study had the ability to convert the oxidative state of iron, it is marked by the blue colour formation(Arora and Chandra, 2011). Here both the compounds C1 and C2 formed blue colour when treated with potassium ferricyanide indicating the antioxidative ability of the compounds (Fig. 4).

In conclusion, the compounds C1 and C2 from *Sargassam wightii* were found to have excellent antioxidative ability apart from their potential antimicrobial activity. Therefore, these compounds will be studied for their ability in the treatment of diabetic foot ulcer.

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