**ORIGINAL ARTICLE**

# Ferrous ion-chelating Assay for analysis of antioxidant potential of *Sargassum sp* and *Iyengaria sp*

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## ABSTRACT

**Aim:** To analyse antioxidant potential of Sargassum sp and Iyengaria sp by using ferrous ion chelating assay.
**Methods:** *Sargassum sp* and *Iyengaria sp* were collected from southeast coast of Karachi. The powdered seaweed samples (500g of Sargassum sp and Iyengaria sp) were extracted with methanol in conical flasks at room temperature for three weeks. The samples were filtered using Whatman filter paper to obtain clarified filtrates. Ferrous ion-chelating assay was used for evaluation of antioxidant potential.
**Results:** The methanolic extract of *Sargassum* sp showed higher potential as evidenced by Ferrous ion-chelating Assay.
**Conclusion:** This is a preliminary study highlighting potential of *Sargassum* sp and future studies must converge on in-vitro analysis of these species for a better understanding of the biological mechanisms regulated by *Sargassum* sp to control cellular activities.
**Keywords:** Ferrous ion chelating assay, methanolic crude extract, antioxidant potential

## INTRODUCTION

It is becoming sequentially more understandable that herbal extracts, bioactive ingredients isolated from various herbs possess potent anticancer activity. Algal extracts and bioactive ingredients derived from algae have also started to gain appreciation as anticancer agents as evidenced by in-vitro and in-vivo analysis. Rapidly mounting research work is deepening our understanding about role of algae in cancer suppression[1]. The study was designed to analyze antioxidant potential of *Sargassum sp* and *Iyengaria sp* using ferrous ion-chelating assay.

## MATERIALS AND METHODS

Seaweeds used in this study were Sargassum sp and Iyengaria sp. The seaweeds were collected during the winter season in the month of Feb. 2013, from, Sandspit, Hawkesbay, Buleji, Haji Goth and Paradise Point region on the southeast coast of Karachi Pakistan respectively. The seaweeds samples were dried, and powdered after washing thoroughly in fresh water to remove salt and other unwanted materials and stored in airtight containers at room temperature for further study. The powdered shad-dried seaweed samples (500 g of Sargassum sp and Iyengaria sp) were extracted with methanol in conical flasks (1500 ml) (Volumetric flasks, (Pyrex) 1000 cm3) respectively at room temperature for three weeks. The samples were filtered by Whatman filter paper (Whatmann filter paper no. 1, 2, 41, and 42.) to obtain clarified filtrates (1L and 800 ml respectively) which was evaporated (65°C -70°C) using rotary evaporator (Stuart RE300 Rotary evaporator, Germany equipped with Stuart RE3022C Vacuum pump, Germany and chiller) under vacuum for dryness to give rise a dark green viscous oily mass (17.34g and 19.08g respectively) of methanolic crude extract. The methanolic crude extract (5mg) was mixed with (1ml) of methanol for antioxidant activity.

Ferrous ion chelating assay can be used to test the antioxidants and it was stately by reduction in the absorbance at 562nm of the iron (II) and ferrozine complex. 1 ml of test sample (concentration 5 mg was mixed with 1 ml of methanol and 0.1 ml of 2 mM FeCl2 and the reaction was initiated by the addition of 0.2 ml of 5mM ferrozine. The mixture was hatched at room temperature for 10 min and the absorbance was determined at 562nm. Methanol without test sample was used as a control and methanol without ferrozine mixture was used as a sample blank. EDTA was used as a reference standard for the assay.

## RESULTS

Iron produces the free radicals through the Fenton and Haber-Weiss reaction. Oxyradical generation and the subsequent oxidative damage are inhibited by metal ion chelating activity of an antioxidant molecule. Ferrozine assay determined the chelation of iron Fe (II). % of bound iron was expressed as results of this assay. Ferrozine formed a colored

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complex with iron (II) which can be noted at 562 nm. EDTA was used as standard for this assay. The Methanolic fraction of *Sargassum sp* exhibited the maximal value of (76%) of bound iron. *Iyengaria sp* extracts have metal chelating value of % of bound iron (65%) which was lesser than the *Sargassum* methanolic extract as shown in figure.

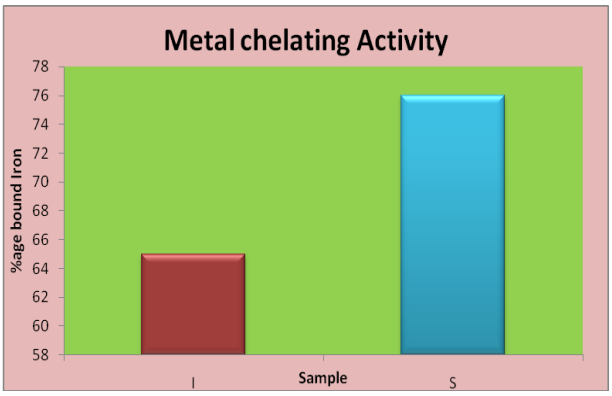


Fig. Metal chelating Activity of *Sargassum and Iyengaria sp*

## DISCUSSION

It has lately been shown that methanolic extract of *Sargassum muticum* is effective against MCF-7 and MDA-MB-231 breast cancer cell lines proliferation[2]. There is recent evidence suggesting that *Sargassum pallidum* aqueous extract is helpful in improving antioxidant activities in models of gastric cancer[3]. It is noteworthy that fucoxanthin has been tested for efficacy in mice inoculated with sarcoma 180 ascites tumor cells. Notably, Fucoxanthin at high-dose induced considerable regression of tumor load in xenografted mice[4]. Sargaquinoic acid isolated from *Sargassum siliquastrum* has also been shown to reduce inflammation via exerting inhibitory effects on NF-κB signaling pathway[5]. Meroterpenoids isolated from this particular specie have also shown substantial anticancer activity against different cancer cell lines including AGS, HT-29, and HT-1080[6]. This is a preliminary study highlighting potential of *Sargassum* sp and future studies must converge on in-vitro analysis of these species for a better understanding of the biological mechanisms regulated by *Sargassum* sp to control cellular activities.

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