

# Comparative Analysis of Antioxidant Potential of *Sargassum Sp* and *Lyengaria Sp*

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## ABSTRACT

**Aim:** To evaluate wide ranging bioactive ingredients using in-vitro assays for a better understanding of the protein networks which are targeted by natural agents.

**Methods:** *Sargassum sp* and *Lyengaria sp* were collected from southeast coast of Karachi. The powdered seaweed samples (500 g of *Sargassum sp* and *Lyengaria 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. ABTS radical scavenging assay and FRAP assays were used to analyze antioxidant potential.

**Results:** The methanolic extract of *Sargassum sp* showed higher potential of antioxidant activity as evidenced by ABTS radical scavenging assay. Methanolic extract of *Lyengaria sp* show the highest FRAP value 26.5 mM, indicating to highest antioxidant potential as evidenced by FRAP assay.

**Conclusion:** Identification of bioactive ingredients having considerable antioxidant potential will be helpful in better and deeper understanding of the underlying mechanisms inhibited or activated by natural agents to suppress carcinogenesis.

**Keywords:** Antioxidant, *sargassum Sp*, *Lyengaria Sp*

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## INTRODUCTION

Increasingly it is being realized that due to off target effects of chemotherapeutic drugs, drug discovery from marine natural products has re-gained tremendous appreciation in past few years. Rapidly accumulating experimental evidence regarding considerable and encouraging results obtained from in-vitro studies set stage for an interdisciplinary research to identify drugs with substantial efficacy and lesser off target effects<sup>1,2</sup>. It is appropriate to mention that translation of some of marine derived compounds from bench-top to the bedside including Ziconotide a peptide originally discovered in a tropical cone snail for the treatment of pain and Trabectedin (first marine anticancer drug) has opened new horizons for the researchers.

## MATERIALS AND METHODS

**Algal Material:** Seaweeds used in this study were *Sargassum sp* and *Lyengaria 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 *Lyengaria sp*) were extracted with methanol in conical flasks (1500 ml) (Volumetric flasks, (Pyrex) 1000 cm<sup>3</sup>) respectively at room temperature for three weeks. Filtration and evaporation give rise to a dark green viscous oily mass (17.34 g and 19.08 g respectively) of methanolic crude extract. The methanolic crude extract (5 mg) was mixed with (1 ml) of methanol for antioxidant activity.

### ABTS radical cation decolorization assay

ABTS assay can be used to determine the antioxidant efficacy of organic fluids, tissues, cells, synthetic and natural therapeutical compounds. Trolox was a water soluble form of vitamin E used for positive control for reducing the formation of radical cation in this assay. ABTS solution was prepared with mixing of 5 ml of 14 mM ABTS solution with 5 ml of 4.9 mM potassium per sulphate ( $K_2S_2O_8$ ) solution. After that the mixture was left to stand in dark at room temperature for 16 h for suspension. The absorbance of the reagent was then adjusted to  $0.700 \pm 0.02$  at 734 nm with deionized water and used for this assay.

### Ferric reducing antioxidant activity assay

The FRAP test was inexpensive, simple, wild and vigorous assay which uses antioxidants as reluctant in a redox associated colorimetric method to test the total antioxidant potential directly. FRAP reagent was prepared by mixing the reagents such as: 0.1 Molar acetate buffer with pH 3.6, 10 mili Molar of (TPTZ)

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and 20 mili Molar FeCl<sub>3</sub> with ratio of 10:1:1 (v/v/v). 5 mg of testing sample (5 mg/ml) was mingled with 300 ml of FRAP mixture, hatched at room temperature only for 15 min and the absorbance was noted with 593 nm. Assay involves FeSO<sub>4</sub>.7H<sub>2</sub>O as a standard reference and with different concentrations of which the standard curve was plotted. The FRAP values expressed in mM Fe<sup>2+</sup>/mg dry weight of the tested sample.

## RESULTS

Algal extracts of *Sargassum* sp and *Iyengaria* sp both scavenged the ABTS radical cation and shows antioxidant potential. ABTS assay results were expressed in TEAC values. TEAC values can be defined as the measure of active antioxidant activity of the biological sample. Higher TEAC value shows a higher antioxidant potential of sample. The methanolic extract of *Sargassum* sp showed higher potential of antioxidant activity having the TEAC value of 41.05 mM of Trolox equivalence. While the *Iyengaria* sp extracts indicates less antioxidant potential with TEAC Value of 37.98 mM. The overall trend of TEAC values of *Sargassum* sp and *Iyengaria* sp (Fig. 1).

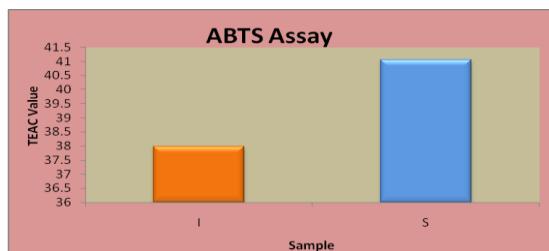


Fig. 1: ABTS assay of *Sargassum* and *Iyengaria* sp

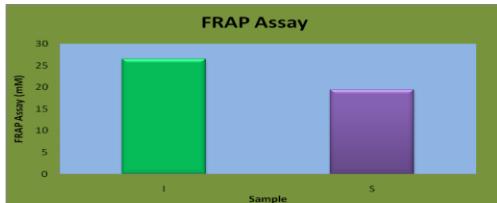


Fig. 2: FRAP assay of *Sargassum* and *Iyengaria* sp

**FRAP Assay:** FRAP assay results are usually expressed in FRAP units. A higher FRAP value indicated to a higher reducing aptitude of the sample which means a higher antioxidant potential. The FeSO<sub>4</sub> was used as a standard. The Methanolic extract of *Iyengaria* sp show the highest FRAP value 26.5 mM , indicating to highest antioxidant potential. *Sargassum* sp extract moderately less antioxidant potential with 19.4 mM value (Fig. 2). The TEAC and FRAP values for *Sargassum* and *Iyengaria* sp extracts were ranged from 36.0-41.058 mM of Trolox

equivalents and 36.0-37.98 mM of Trolox equivalents and 0.30-19.4 mM and 0.30-26.5 mM of FeSO<sub>4</sub> equivalents respectively.

## DISCUSSION

It is becoming progressively more understandable that antioxidant activity is essential to protect cells from oxidative stress induced dysregulated cellular activities. In line with this approach, there is an overwhelming list of bioactive ingredients reported to regulate cancer. Saponins isolated from the roots of *Platycodon grandiflorum* are potent antioxidants. Lung carcinoma A549 cells treated with Saponins displayed remarkably reduced activation Akt, ERK1/2, and GSK-3β upon TGFβ1 treatment. Moreover, downstream effectors Smad2/3 were also inhibited in saponins treated cancer cells<sup>5</sup>. It is noteworthy that different agents are being tested pre-clinically for efficacy. There is a recent report suggesting that α-Mangostin, isolated from pericarp of *Garcinia mangostana* L substantially suppressed pancreatic cancer cells derived orthotopic and ectopic xenograft tumors in athymic nude mice<sup>6</sup>. It has previously been convincingly revealed that *Sargassum* sp effectively induced apoptosis in breast cancer cell line (MCF7)<sup>7</sup>.

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