

Cytotoxic effect of fucoxanthin isolated from selected sargassum species of south east coast, india

Abstract

Background: Fucoxanthin is known for various biological activities and has been isolated for its bioactivity from *Sargassum fulvellum*, *Sargassum heterophyllum*, *Sargassum horneri*, *Sargassum siliquastrum*, *Sargassum wightii*, *Sargassum ilicifolium*, *Sargassum longifolium*. But there is no report on the isolation of fucoxanthin from *Sargassum polycystum* C. Agardh, *Sargassum swartzii* (Turner) C. Agardh and *Sargassum johnstonii* Setchell & N.L.

Aim: The present study was aimed to optimize the protocol for the isolation of fucoxanthin from *Sargassum polycystum* C. Agardh, *Sargassum swartzii* (Turner) C. Agardh and *Sargassum johnstonii* Setchell & N.L. Gardner and evaluate their cytotoxic potentials against brine shrimp (*Artemia salina*) naupli.

Methods: One gram of powdered seaweed was weighed and dissolved in 10 ml of 90% acetone. The fucoxanthin was observed by measuring the UV-Vis (UV-Vis = Ultra Violet- Visible light) spectra of the solution at 200–1100 nm (Lamda max) using Shimadzu spectrophotometer and the characteristic peaks were detected. Silica gel coated plates (Merck – 10×6 cm) and n-hexane: acetone was used as mobile phase for the separation with a ratio of 7:3. For cytotoxic analysis, fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* were taken in different concentrations viz., 50,100, 150, 200, 250µg/50ml. The LC50 (LC= Lethal concentration) was calculated for the isolated fucoxanthin of *S. johnstonii*, *S. swartzii* and *S. polycystum* using Software Package for Social Studies (SPSS).

Results: The quantitative occurrence of the fucoxanthin in the studied seaweeds as follows *S. swartzii* > *S. polycystum* > *S. johnstonii*. The UV-Vis analysis confirmed the existence of fucoxanthin in the thallus of *S. johnstonii*, *S. swartzii* and *S. polycystum*. Acetone extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* showed single fucoxanthin band with Rf value 0.4782, 0.4565 and 0.5 respectively. The fucoxanthin isolated from *S. johnstonii* demonstrated the lowest LC50 (LC= Lethal concentration) value with 338.2µg/50ml, next to that *S. swartzii* (423.47µg/50ml) and *S. polycystum* (434.57µg/50ml).

Conclusion: The results of the present study clearly validated the cytotoxic potentials of the *S. johnstonii*, *S. swartzii* and *S. polycystum* fucoxanthin.

Keywords: fucoxanthin, *sargassum*, cytotoxic, anti-oxidant, anti-tumor, anti-inflammatory

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Introduction

Fucoxanthin is known for anti-oxidant, anti-tumor, anti-inflammatory, and anti-obesity effects.¹⁻⁵ Fucoxanthin has been isolated for its bioactivity from *Sargassum fulvellum*.⁶ *Sargassum heterophyllum*.⁷ *Sargassum horneri*.⁸ *Sargassum siliquastrum*.³ *Sargassum wightii*, *Sargassum ilicifolium*, *Sargassum longifolium*.⁹ Cytotoxicity of aqueous and AgNPs of *S. johnstonii* and *S. polycystum* against Dalton's lymphoma ascites (DLA) cells and *Artemia salina*.^{10,11} and *Undaria pinnatifida* were reported.^{12,13} Anticancer activity of *Sargassum oligocystum*, *Sargassum swartzii*, *Sargassum pallidum*, *Sargassum turileand* and *Sargassum latifolium* were noted by the phycologist.¹⁴⁻¹⁸ But there is no report on the isolation of fucoxanthin from *Sargassum polycystum* C. Agardh, *Sargassum swartzii* (Turner) C. Agardh and *Sargassum johnstonii* Setchell & N.L. With this knowledge, the present study was aimed to optimize the protocol for the isolation fucoxanthin from *Sargassum polycystum* C. Agardh, *Sargassum swartzii* (Turner) C. Agardh and *Sargassum johnstonii* Setchell & N.L. Gardner and evaluate their cytotoxic potentials against brine shrimp (*Artemia salina*) naupli.

Materials and methods

The mature and healthy thallus of *Sargassum polycystum* C. Agardh, *Sargassum swartzii* (Turner) C. Agardh and *Sargassum johnstonii* Setchell & N.L. Gardner were collected from Manapad, Tirunelveli district, Tamil Nadu and Rasthacaud, Kanyakumari District Tamil Nadu. The collected seaweeds were shade dried at room temperature under dark conditions and the shade dried thallus was separately powdered. The powdered samples were stored in polythene bags and used for further analysis.

One gram of powdered seaweed was weighed and dissolved in 10 ml of 90% acetone (HiMedia, Mumbai, India). The samples were incubated for overnight at 4 °C in a dark place and then centrifuge at 8000 rpm for 15 min. Extraction was repeated three times till the sample become colourless. The procedure was carried out in triplicates. The sample was stored in amber colour bottles to avoid degradation by light. The fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* were centrifuged at 3000 rpm for 10 min and then filtered through Whatman No. 1 filter paper. The fucoxanthin was observed by measuring the UV-Vis spectra of the solution at 200–1100 nm

using Shimadzu spectrophotometer and the characteristic peaks were detected. Each and every analysis was repeated twice and confirmed the spectrum.

Thin layer chromatography was used to separate the pigments and find out the Rf value of the photosynthetic pigment fucoxanthin present in *S. johnstonii*, *S. swartzii* and *S. polycystum*. Pigments in the mixture are separated on the basis of their differences in solubilities and partition co-efficient in a binary solvent system. Silica gel coated plates (Merck – 10×6 cm) and n-hexane: acetone (HiMedia, Mumbai, India) was used as mobile phase for the separation with a ratio of 7:3.¹⁹

To know the cytotoxic potentials of selected seaweeds, the brine shrimp bioassay was performed.²⁰ 25mg of dried fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* was taken in 10 ml beaker and 500 µl DMSO (HiMedia, Mumbai, India) was added to it. Finally the volume (5ml) was adjusted by distilled water. The concentration of this solution was 5µg/µl. Fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* were taken in different concentrations viz., 50,100, 150, 200, 250µg /50ml. After 24 hours, the tubes were inspected using a magnifying glass and the number of survived nauplii in each tube was counted and the LC₅₀, 95% confidence limit, LCL and UCL were calculated.

Results

Fucoxanthin is one of the most profused carotenoids present in the edible brown algae. In the present study also the occurrence of fucoxanthin was validated in three studied *Sargassum* species. Among the three seaweeds studied, *S. swartzii* contained high content of fucoxanthin compared to other two studied *Sargassum* species. The quantitative occurrence of the fucoxanthin in the studied seaweeds were as follows *S. swartzii* (0.073 mg g⁻¹ DW) > *S. polycystum* (0.055 mg g⁻¹ DW) > *S. johnstonii* (0.014 mg g⁻¹ DW). The UV-Vis analysis confirmed the existence of fucoxanthin in the thallus of *S. johnstonii*, *S. swartzii* and *S. polycystum* (Figure 1 A–C). The fucoxanthin isolated from the thallus of *S. johnstonii* showed the optical peak at 664 and 404 nm with the absorption of 1.750 and 3.957 respectively (Figure 1A). *S. swartzii* illustrated the optical peak at 664 and 416 nm with the absorption of 2.153 and 4.000 respectively (Figure 1B). *S. polycystum* explained the optical peak at 664 and 412 nm with the absorption of 2.340 and 4.110 respectively (Figure 1 C). TLC separation of *S. johnstonii*, *S. swartzii* and *S. polycystum* acetone extracts confirmed the fucoxanthin occurrence (Figure 1D). Acetone extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* showed single fucoxanthin band with Rf value 0.4782, 0.4565 and 0.5 respectively. The fucoxanthin isolated from *S. johnstonii* demonstrated the lowest LC₅₀ value with 338.2017µg/ 50ml, next to that *S. swartzii* (423.47 µg/ 50ml) and *S. polycystum* (434.57µg/ 50 ml).

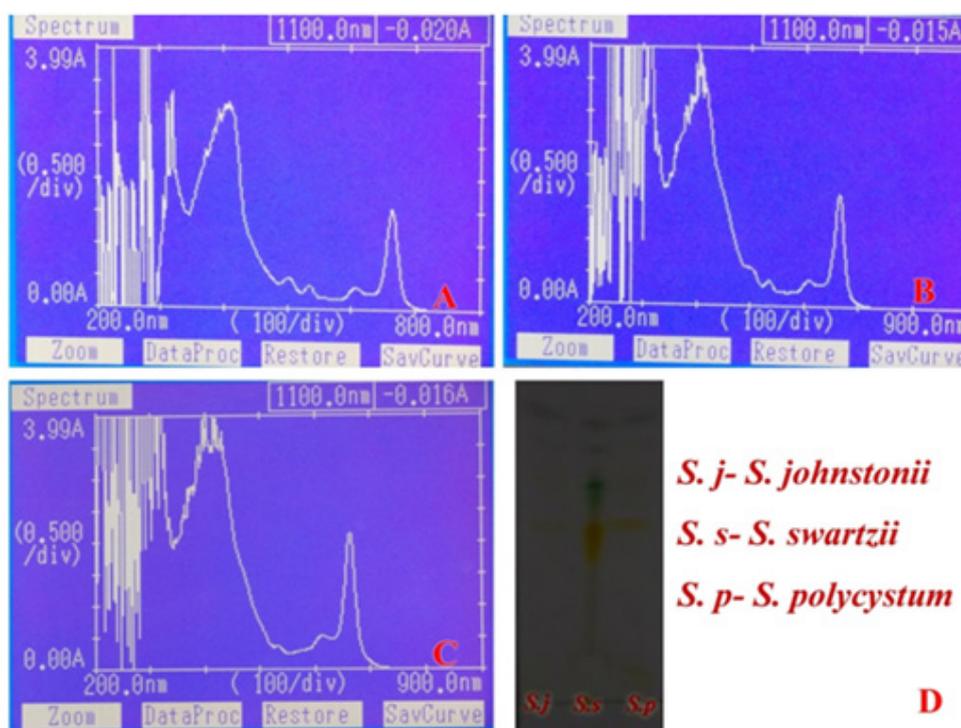


Figure 1 UV-Vis spectrum and TLC profile of Fucoxanthin.

A: UV-Vis spectrum of *S. johnstonii*; B: UV-Vis spectrum of *S. swartzii*; C: UV-Vis spectrum of *S. polycystum*; D: TLC profile of fucoxanthin extracts of studied *Sargassum* species; S. j- *S. johnstonii*; S. s - *S. swartzii*; S. p: *S. polycystum*.

Discussion

Globally phycologist and pharmacologist are paid attention to fucoxanthin due to their biopotentials especially as chemopreventive and chemotherapeutic agent.²¹ Similarly the same compound has been identified in the crude extract of brown seaweed *Himantalia elongate* at 331 nm, 446 and 468 using UV-visible spectroscopy.²²

Zailanie.²³ identified fucoxanthin in *Sargassum filipendula* at 450 nm. In addition to quantification, The UV-Vis analysis results exhibited novel markers for the identification of *S. johnstonii*, *S. swartzii* and *S. polycystum*. These UV-Vis spectroscopic peaks may be employed as pharmacognostical marker for the identification of the studied *Sargassum* species. The observed TLC profiles may be used as biochemical marker to distinguish *Sargassum* species from

its adulterants in the pharmaceutical industries. Fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum* showed different mortality rate of brine shrimp, which increased proportionally with the increasing concentration of the extracts. The inhibitory effect of the extract might be due to the toxic compounds present in the fucoxanthin extracts of *S. johnstonii*, *S. swartzii* and *S. polycystum*.

Chandra Kala et al.¹⁰ studied the cytotoxic properties of aqueous extracts and AgNPs of *Sargassum johnstonii*. They observed the cytotoxic potential of *S. johnstonii* silver nanoparticles showed least LC50 value at 656.89 µg/ml. Similarly the AgNPs of *Sargassum polycystum* also showed the cytotoxic effect with LC50 502.72 µl/ml against *Artemia salina* and with CTC50 at 188.64 µl/ml in AgNPs of *S. polycystum*.¹¹ In the present study also the fucoxanthin of *S. johnstonii* and *S. polycystum* showed least LC50 value compared to the aqueous extracts and AgNPs of *S. johnstonii* and *S. polycystum*. The results of the present study clearly validated the cytotoxic properties of fucoxanthin isolated from the *S. johnstonii* and *S. polycystum*. The results of the present study supplemented the previous observations. Khanavi et al.¹⁵ observed the anticancer activity in *Sargassum swartzii* against Caco-2 and T47D cell lines.

Conclusion

The results of the present study also supplemented the Khanavi et al.¹⁵ observations. Thus the results of the present study revealed the cytotoxic properties of *S. johnstonii*, *S. swartzii* and *S. polycystum*. These may be used as an alternative anticancer drug for the future. In addition the result of the spectroscopic and TLC analysis produced novel pharmacognostical and phytochemical markers for the medicinally important seaweeds *S. johnstonii*, *S. swartzii* and *S. polycystum*. These profiles may be used in the pharmaceutical industries to identify the medicinal source (*S. johnstonii*, *S. swartzii* and *S. polycystum*) from its adulterants.

Conflicts of interest

The authors declare that there is no conflict of the interest in the present study.

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