

IN VITRO ANTIOXIDANT AND ANTICANCER ACTIVITY OF *ULVA LACTUCA* L. USING MOLT-3 CELL LINE

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ABSTRACT

Objective: The aim of the present investigation was to determine the *in vitro* antioxidant and anticancer activity of the ethanol extract of *Ulva lactuca* L.

Methods: The present study was to investigate the antioxidant and anticancer activity of *U. lactuca* L. The extract of *U. lactuca* L. was extracted by ethanol and subject to analysis. An *in vitro* antioxidant activity of the ethanol extract of *U. lactuca* L. was performed by 1, 1-diphenyl-2-picrylhydrazyl free radical scavenging assay. Simultaneously anticancer activity was also performed using blood cancer (MOLT-3) cell line, and the species showed a strong selective cell proliferation inhibition of the cancer cell line.

Results: The scavenging activity was measured and determined to be 78.5%. This might be due to high polyphenolic compounds and flavonoid contents of the extract, which showed maximum growth inhibition of 74.4%.

Conclusion: Thus, the study concludes that the constituents of seaweeds can act as potent in treating various diseases and can be used as an alternative for therapeutic treatment.

Keywords: Seaweed, *Ulva lactuca* L., Ethanol extract, Antioxidant activity, Anticancer activity, MOLT-3 (human peripheral blood T lymphoblast and acute lymphoblastic leukemia).

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INTRODUCTION

Many marine organisms live in complex habitats exposed to extreme conditions and in adapting to new environmental surroundings. To survive under extreme conditions, these organisms also produce a wide variety of secondary (biologically active) metabolites which cannot be found in other organisms and seaweeds are valuable sources of macronutrients [1,2]. *Ulva* or sea lettuce species are some of the most abundant representatives, being ubiquitous in coastal benthic communities around the world. The ubiquitous genus *Ulva* has been included in relatively numerous physiological marine macroalgal studies [3]. *Ulva lactuca* is a widespread macroalgae occurring at all levels of the intertidal zone, in calm and protected harbors as deep as 10 m and northern climates. *U. lactuca* grows along rocky or sandy coasts of oceans and estuaries. In parts of Britain and Asia, seaweed is consumed by humans and livestock as it is considered valuable to human nutrition. The elemental analyses of seaweeds have been carried out in several countries by various techniques [4]. Researchers determined the chemical composition of *Ulvaria oxisperma* (Kützing Bliding), *U. lactuca* (Linnaeus), and *Ulva fasciata* (Delile). It is known that *U. lactuca* is evaluated for its nutritional value as food for ruminants, and goats [5].

The potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, and carotenoid) (phenolic acid, flavonoid, and tannins). Seaweeds are noted that it contain not only labile antioxidants (i.e. ascorbate, glutathione) when it is fresh, but also has more stable molecules such as carotenoids, mycosporine-like amino acids and a variety of polyphenols (catechins and phlorotannins) [6,7]. The biochemical composition of marine seaweeds is generally known to be highly influenced by geographical location and local environmental condition [8]. Seaweed not only possesses nutrient potentials and but also have nutraceutical potentials such as antioxidant, antimutagenic, anticoagulant, anticancerous, and antibacterial activity [9]. Hence, seaweeds can be considered as

promising plants forming one of the important marine living resources of high nutritional value. 20% of the Asian diet is comprised seaweeds that are relished not for their nutritional viewpoint but of unique and enchanting flavor. Seaweeds are getting importance in various fields ranging from food to medical [10]. The seaweed extract of *U. lactuca* L. can be used and recommended as an antifungal agent into prepare eco-friendly disinfectants [11].

The present investigation was to determine the *in vitro* antioxidant and anticancer activity of the ethanol extract of *U. lactuca* L.

MATERIALS AND METHODS

Collection of seaweed

The seaweed *U. lactuca* L. (sea lettuce) was procured from the South coast areas, Rameswaram and Keelakarai, Latitude 9.280016N° and Longitude 79.129524E° Tamil Nadu, India. The collected edible seaweed was identified and authenticated by Botanical Survey of India, Coimbatore, Tamil Nadu.

Preparation of ethanolic extract

The collected seaweed was washed thoroughly and shadow dried for 7 days [12]. The dried samples were grounded and powdered finely. 20 g of air-dried seaweed powder was added to 100 ml of 70% ethanol and kept at 37°C for 6 h in an orbital shaker incubator. The final extract was obtained filtered by Whatman No.1 filter paper and concentrated to dry to yield crude extract residues. The final extract was diluted with solvents (50 mg/ml) and stored at 4°C was used for further analysis. The percentage of yield was calculated using the formula:

$$\text{Yield (\%)} = (W_1 \times 100) / W_2$$

Where W_1 was the weight of extract after evaporation and W_2 was the dry weight of the sample [13].

In vitro antioxidant activity**1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity**

The DPPH scavenging activity was estimated according to the method described [14]. To the extracts (20–100 µl), 1 ml of DPPH solution was added individually. The reaction mixture was incubated in the dark at for 30 min in room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. L-Ascorbic acid was taken as a standard antioxidant. The percentage of DPPH free radical scavenging activity was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \frac{A_{[\text{sample}]} - A_{[\text{control}]}}{A_{[\text{control}]}} \cdot 100$$

Anticancer activity**Cell culture**

The blood cancer (MOLT-3) cell line obtained from NCCS, Pune was maintained in humidified incubator at 37°C and in a 5% CO₂ atmosphere. RPMI-1640 contains 10% fetal bovine serum supplemented with antibiotics penicillin (100 units/ml) and Streptomycin (100 µg/ml).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

An *in vitro* cytotoxicity study was performed using *U. lactuca* L. ethanol extract [15]. The culture medium from the MOLT-3 cells was replaced with fresh medium. The samples in triplicates were added on the cells. After incubation at 37±1°C for 18 h, MTT (mg/ml) was added in all the wells and incubated for 4 h. After incubation, dimethyl sulfoxide was added in the wells and read at 570 nm using photometer. Cytotoxicity and cell viability were calculated using the formula:

$$\text{Cytotoxicity} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \cdot 100$$

$$\text{Cell viability} = \frac{\text{Treated}}{\text{Control}} \cdot 100$$

RESULTS AND DISCUSSION**Extraction of seaweeds**

The dried and powdered seaweeds (20 g) of *U. lactuca* L. were extracted with ethanol, which yielded a crude extract of 5 g. The percentage of yield in *U. lactuca* L. was found to be 25%.

IN VITRO ANTIOXIDANT ACTIVITY**DPPH free radical scavenging assay**

Several studies have reported the free radical scavenging capacity of macroalgae. Seaweeds are low in fats, but they also contain vitamins and antioxidant molecules, such as phlorotannins, ascorbic acid, tocopherols, carotenoids, phospholipids, and chlorophyll related compounds [16]. Antioxidant activity of *U. lactuca* L. was evaluated using ethanol solvent. The marine seaweed sample was checked for its antioxidant activity by DPPH method; the ethanolic extract of the sample was checked in different concentrations from 20 to 100 µg. L-ascorbic acid was used as a standard antioxidant. Scavenging activity was observed during an increase in concentration of extract.

In the present study, 100 µg of *U. lactuca* L. ethanol extract it shows the maximum antioxidant activity of 78.496% (Fig. 1). The higher activity in the ethanol extract of *Ulva* species may be due to their phenolic compounds within them. DPPH assay is one of the methods used for evaluating antioxidant activity [17]. The DPPH radical is a stable radical with a maximum absorbance at 517 nm due to the odd electron. The antioxidants can pair off this electron by hydrogen donation that causes a color change from purple to yellow, and the resulting decolorization is stoichiometric with respect to the number of electrons taken up [18].

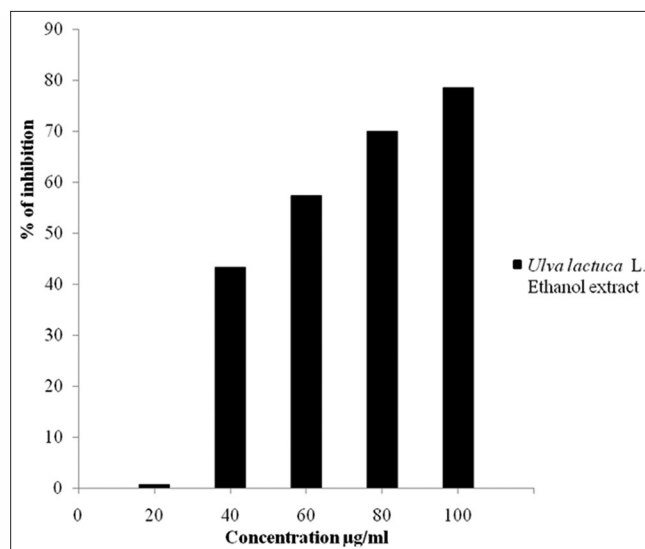


Fig. 1: 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity of *Ulva lactuca* L. ethanol extract

The green and red algae do not contain a large amount of total phenolic content in comparison with brown algae, although higher values have been found in green algae when compared with red species. The results of the green algae *Enteromorpha intestinalis* with an IC₅₀ value of 265.8 and *U. lactuca* with an IC₅₀ value of 236.5 were higher than those for the red ones *Palmaria palmata* with an IC₅₀ value of 76.9, *Gracilaria vermiculophylla* with an IC₅₀ value of 95.2, *Mastocarpus stellatus* with an IC₅₀ value of 102.5, and *Chondrus crispus* with an IC₅₀ value of 113.6. The extract from the green marine algae *Caulerpa cupressoides* contained higher content than the red marine algae *Cordillaris crenulata*, with the exception of extract from the green marine algae *U. fasciata* which was lower than that of the red marine algae *A. multifida* [19].

Anticancer activity

Blood cancer represents a large group of different malignancies. This group includes cancers of the bone marrow, blood, and lymphatic system, which includes lymph nodes, lymphatic vessels, tonsils, thymus, spleen, and digestive tract lymphoid tissue. Leukemia and myeloma, which start in the bone marrow, and lymphoma, which starts in the lymphatic system, are the most common types of blood cancer.

The abnormal cells in leukemia are usually found in the bone marrow and blood. The mechanism of leukemogenic and autonomous proliferation of leukemia cells are still unknown. The mechanism and control of proliferation of leukemia cell lines with the characteristics of cells at different stages of leukemic development have been established.

In this investigation, the effect of *U. lactuca* L. ethanol extract on MOLT-3 cell proliferation was studied. MOLT-3 cells were exposed to different doses as shown in Table 1 and Fig. 2 of the seaweed extracts for 24 h, and cell cytotoxicity and cell viability were checked by MTT assay. Cell viability was markedly decreased after treatment with different concentrations extract. The growth of cells was inhibited in a concentration and time-dependent manner.

The cytotoxicity and cell viability of MOLT-3 cell lines using green seaweed were investigated and the cytotoxic level of cells was performed by MTT assay. The tested algae extract *U. lactuca* L. ethanol extract, showed a strong selective cell proliferation inhibition of the cancer cell line. This might be due to high polyphenols and flavonoids contents of the 100 µg/ml extract, which showed maximum growth inhibition (74.4%). The experimental observation in detected that cell death was a concentration-dependent process; hence, the number of non-viable cells increased with increasing concentration of algal extracts, the *U. lactuca* L. seaweed extracts showed significant cytotoxic activity. It is assumed that the presence

Table 1: Cytotoxic activity of *Ulva lactuca* L. ethanol extract using MTT assay

Description	Concentration (μg)	Cytotoxicity (%)	Cell viability (%)	Cytotoxic reactivity
<i>Ulva lactuca</i> L.	5	10.8	89.2	Slight
	25	39.5	60.5	Mild
	50	63.7	36.3	Moderate
	75	71.9	28.1	Severe
	50	74.4	25.6	Severe

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

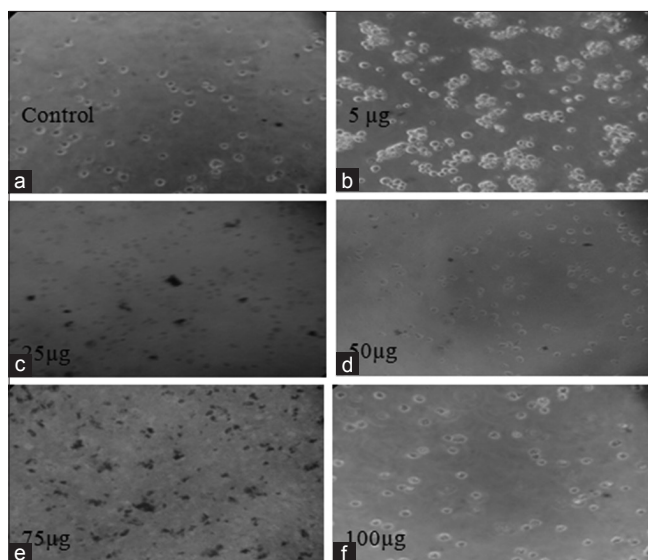


Fig. 2: (a-f) Cytotoxicity activity of *Ulva lactuca* L. ethanol extract on MOLT-3 cells

of polyphenolic compounds was the reason for observed free radical scavenging and anticancer activity. Mechanisms of antioxidant action can include suppression of reactive oxygen species (ROS) formation either by inhibition of enzymes or by chelating trace elements involved in the free radical generation, scavenging ROS, and upregulation or protection of antioxidant defenses [20]. The antioxidant activity of the methanolic extracts of *Halophila ovalis* and *Halophila beccarii* two seagrass species of Chilika lagoon was evaluated for DPPH and ABTS radical scavenging activities have strong antioxidant activity against ABTS and DPPH radicals. The seagrass species as a potential source of natural antioxidant for protection against several oxidative stress related diseases [21].

CONCLUSION

This study concludes that the ethanolic extract of *U. lactuca* L. seaweed possesses rich antioxidant property and may also contain polyphenolic compounds which may be responsible for the antioxidant property. The anticancer activity also shows that it has the capacity to kill the blood cancer cells in the human body. According to the results obtained from the study suggests that this seaweed can be the potential interest for food, development of novel drugs and functional foods, pharmaceutical and agricultural applications. Further research is needed to isolate and characterize the components responsible for their activities components and search for bioactive constituents with antimicrobial, antidiabetic and many other health-promoting activities.

The seaweed extracts *Ulva lactuca* L. showed slight to severe cytotoxic reactivity to MOLT-3 cells after 24 h contact. Control gave none cytotoxic reactivity as expected.

AUTHORS' CONTRIBUTIONS

Keerthana V (VK) conceived the project. Chidambararajan P (PCR) supervised and guided the research work and preparation of the

manuscript. Keerthana, Priyadharshini K (KP, and Sakthivel B (BS) collected and processed the samples and performed analysis, studied the scavenging activity, Performed and analyzed MTT assay. PCR and VK interpreted the data and prepared the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

All authors report no conflicts of interest regarding this manuscript.

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