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 Ulva lactuca L. The extract of Ulva lactuca L. was extracted by ethanol and subject to analysis. An in vitro antioxidant activity of the ethanol extract of Ulva 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). 

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. Ulva 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 oxysperma (Kützing Bliding), Ulva lactuca (Linnaeus), and Olva fasciata (Delle). It is known that Ulva 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 but also have nutraceutical potentials such as antioxidant, antimutagenic, antiagulant, 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 Ulva 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 Ulva lactuca L.

MATERIALS AND METHODS

Collection of seaweed
The seaweed Ulva lactuca L. (sea lettuce) was procured from the South coast areas, Rameswaram and Keelakarai, Latitude 9.280016°N and Longitude 79.129524°E 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 for further analysis. The percentage of yield was calculated using the formula:

\[ \text{Yield (\%) = } \left( \frac{W_1 \times 100}{W_2} \right) \]

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:

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

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% CO\(_2\) atmosphere. RPMI-1640 contains 10% fetal bovine serum supplemented with antibiotics penicillin (100 units/ml) and Streptomycin (100 ug/ml).

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

An in vitro cytotoxicity study was performed using Ulva 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°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:

\[
\text{Cytotoxicity} = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100
\]

\[
\text{Cell viability} = \frac{\text{Treated}}{\text{Control}} \times 100
\]

RESULTS AND DISCUSSION

Extraction of seaweeds

The dried and powdered seaweeds (20 g) of Ulva lactuca L were extracted with ethanol, which yielded a crude extract of 5 g. The percentage of yield in Ulva 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 macroalgal. 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 Ulva lactuca L was evaluated using ethanol solvent. The marine seaweed sample was checked for compounds [16]. Antioxidant activity of tocopherols, carotenoids, phospholipids, and chlorophyll related compounds in the ethanol extract of Ulva lactuca L was found to be 25%. The higher activity in the ethanol extract of Ulva species may be due to their phenolic compounds within DPPH. 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].
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 and strong antioxidant activity against ABTS and DPH radical. 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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