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Research Article

ANTIFUNGAL ACTIVITY OF SEAWEED *ULVA LACTUCA* L. EXTRACTED CRUDE PROTEIN AGAINST PATHOGENIC FUNGI

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ABSTRACT

Objective: The objective of this study was to evaluate the antifungal activity of seaweed extracted protein against the pathogenic fungi.

Methods: Antifungal activity of seaweed *Ulva lactuca* L. extracted protein was determined against pathogenic fungi such as *Alternaria solani, Aspergillus clavatus, Aspergillus niger, Aspergillus flavus,* and *Fusarium oxysporum* by disk diffusion Method. Then, the potentially active protein was determined using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and circular dichroism (CD) spectroscopy.

Results: Seaweeds extracted protein checked for the antifungal activity against *A. clavatus, A. solani,* and *A. flavus* better activity compared to standard amphotericin-B and CD spectroscopy. Analysis of the extracts divulges the presence of entire protein compounds.

Conclusions: This study extracted seaweed protein sufficient of antifungal activity opposed to antifungal pathogens as compared with the standard. This is first report an activity of seaweed extracted protein against the plant and human pathogenic fungus bearing agricultural important.

Keywords: Bovine, Dichroism, Diffusion, Electrophoresis, Fungus, Protein.

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INTRODUCTION

Seaweeds are macroalgae which play an important role in ocean ecology and also being an important source of food, pharmaceutical as well as industrial products [1]. Bioactive compounds from seaweed are well known to have a great potential in pharmaceutical and biomedical applications. Several previously reported biomedical properties exhibited by seaweed include anti-inflammatory, antimicrobial, antioxidant, antihyperlipidemia, and anticoagulant [2-10]. Bioactivity of the compounds which has been isolated in recent years through the seaweed extract was used as novel drugs by the pharmaceuticals industries. Seaweeds are reported for their rich content of potential proteins The edible red seaweeds such as *Palmaria palmata* and Gracilaria cervicornis are having the highest protein content which possesses many potential biological activities [11,12] and seaweeds are good nutrition value such as carbohydrates, minerals, proteins, phenols, and good biofertilizer [13].

The ulvan species have been used as potential source for food preparation, bioenergy, feed and bioremediation etc. The nature content of proteins differs from one to another season and per species. The green seaweed *U. lactuca* L. possesses a protein level that reaches a maximum in August (32.7% of dry weight) and minimum in April (8.7% of dry weight) [14]. With this background of our knowledge and by using available reports the present studies was aimed to know the role of seaweed extracted protein against fungal pathogens.

MATERIALS AND METHODS

Materials

Ethanol, methanol, sulfuric acid, Whatman paper (No.1), ammonium hydroxide, diethyl ether, acetone, ammonium persulfate, acrylamide mix, tris, Coomassie Brilliant Blue (CBB)-R 250, acetic acid, sodium dodecyl sulfate (SDS), glycine, and amphotericin-B were used. All kinds of chemicals and kits were purchased from Sisco Research Laboratories Pvt., Ltd. (SRL), India.

Sample collection and preparation

The sample was collected from Ramanathapuram Mandapam region and authenticated Dr. M. Palanisamy, Scientist, Southern Regional Centre Botanical Survey of India, TNAU Campus, Lawley Road (PO), Coimbatore - 641 003, Tamil Nadu, India. The *U. lactuca* L. collected sample was washed with seawater for 5 times and further washed 2 times with distilled water to remove epiphytes, salts, sands, and contamination from other algae. The sample was air dried in shade condition at room temperature, and then, dried sample was homogenated and placed in plastic bags for further usage.

Extraction of protein from seaweed

The algal biomass (5 g) was homogenized in a solution containing distilled water (DW, 2 mL), 95% ethanol (10 mL), and concentrated sulfuric acid (0.72 mL) with uniform shaking for 20 min. Again, 13 mL DW and 95% ethanol (40 mL) were added, and the pH was adjusted to 1.7 using HCl. The suspension was then filtered using Whatman paper (No. 1), and then again, the pH was adjusted to 3.0 using NaOH. To this suspension, 140 mL of 95% ethanol and 190 mL diethyl ether were added and kept for 12 h at 4°C. After centrifugation at 3000× *g* for 10 min, the sediment was washed with acetone and diethyl ether before dissolving in 25% ethanol and the pH was adjusted to 8.5. The precipitate was collected by centrifugation after 18 h [15].

Fungi

Reference fungi name such as *Aspergillus clavatus* ATCC-9198, *Fusarium oxysporum* microbial type culture collection (MTCC)-1008, *Alternaria solani* ATCC-58177, *Aspergillus niger* MTCC-4285, and *Aspergillus flavus* MTCC 535 was obtained from MTCC, Chandigarh, India, and maintained in the laboratory. Other with ATCC was gifted by the Department of Microbiology, Bharathidasan University, *Tiruchirappalli*-24, Tamil Nadu, India.

Protein Determination

A modified Lowry *et al.* assay [16] described by Frolund *et al.* [17] was used to quantify the proteinaceous compounds in each seaweed

extract. The principle of this modification is that the omission of copper sulfate from the reagent enables determination of the auto-absorbance from humic compounds and chromogenic amino acids. Concentrations and volumes of Lowry and Folin–Ciocalteu's phenol reagents (Sigma F9252) were optimized for speed and sensitivity. Ultimately, a more concentrated reagent compared to that of Lowry *et al.* (1951) was prepared to maximize assay sensitivity. A standard curve was made using bovine serum albumin (BSA; 0, 0.0625, 0.125, 0.25, 0.5, and 1 g L^{-1}) and the absorbance was read at 650 nm.

SDS-polyacrylamide gel electrophoresis (PAGE)

SDS-PAGE generally used to fractionate protein based on their molecular weight. The separation medium is a discontinuous gel on the basis of polyacrylamide and a voltaic field is negatively charged nucleic acid, or proteins of voltaic fields appeal over roam of the gel toward the anode. Molecular mosses of the protein were isolated by SDS. Non-cleavage and cleavage linked in a collection of monomers although covalent disulfide bonds persist intact. To view the protein it was stained with CBB. The evaluation can be done qualitatively with a plotted molecular weight size marker of known molecular weight [18].

SDS PAGE was executed by a mini-Protean III cell electrophoresis unit (Biorad, Hercules, CA, USA) with stacking gel of 5% and separating gel 10%, acrylamide 30% in Tris–HCl 25 mM, pH 8.8, Glycine 0.18 M and SDS 10%. The separation was carried out at 100V for 2 Hrs. The Bovine serum albumin (67 kDa) polypeptides used as molecular mass marker. After migration, protein bands of algal protein extracts were detected by CBB staining. Protein bands were clearly viewed by CBB staining.

Circular dichroism (CD)

Far-UV CD scans were acquired using a Jasco J-815 spectrophotometer (Jasco, Inc., Easton, MD, USA) with a 10 µM protein concentration in 20 mM Na_2HPO_4/NaH_2PO_4, 100 mM NaCl, pH 8 buffer. Spectrum in the range of 260–190 nm was recorded with a bandwidth of 1 nm and an integration time of 1 s. Each spectrum was an average of 5 scans, with a scan rate of 50 nm/min. The mean residue ellipticity was calculated using the following formula (1):

$$[\theta]MRE = (\theta \times MRW)/(10 \times Xc \times Xd)$$

Where mean residue weight = molecular weight/ (N-1)

"c" is a concentration of protein (mg/ml), "d" is the path length in cm, and "N" is the number of amino acid.

Antifungal assay

Five fungal strains namely *A. clavatus*, *F. oxysporum*, *A. solani*, *A. niger*, *A. flavus* using for agar disc-diffusion method was followed to determine the antifungal activity. A suspension of inoculum was PDA medium (cooled to 40 - 45°C) swirl gently to mix well. After solidification, sterilized filter paper discs 6mm in diameter were impregnated with stock extracts and placed on the surface agar plate. Incubation period of (08-12) Hrs at 37°C. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth adjoining the seaweed protein extracts. For each test solution, two replicates were maintained (Sharma, 2011). The culture was incubated at 30°C for 1–5 days and examined for all culture plates at 24 h onward [19,20].

RESULTS AND DISCUSSION

The protein extraction and composition of different algal cell were studied (21) confirmation of the protein in seaweed were by first lane was obtained at 54-71 kDa, second lane at 33-43 kDa. The same band also confirmed at 2 and 3 rd lane sample. Sample (10, 15 and 20 µg) along with control were loaded and the targeted protein was identified. In comparison with control the thickness of the band was role in 20 µg sample Fig 1.

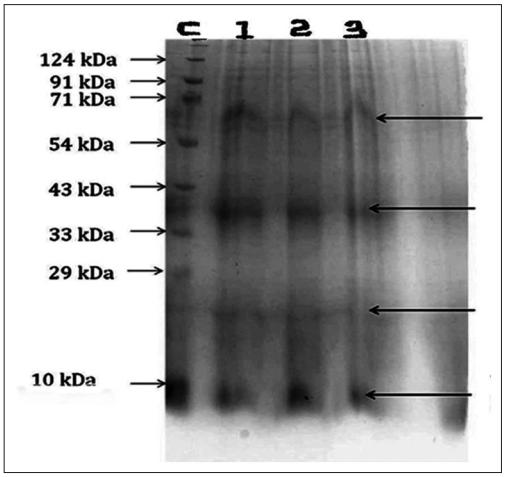

Fig. 1: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. C standard bovine serum albumin, 1–10 µg sample, 2–15 µg sample, 3–20 µg sample

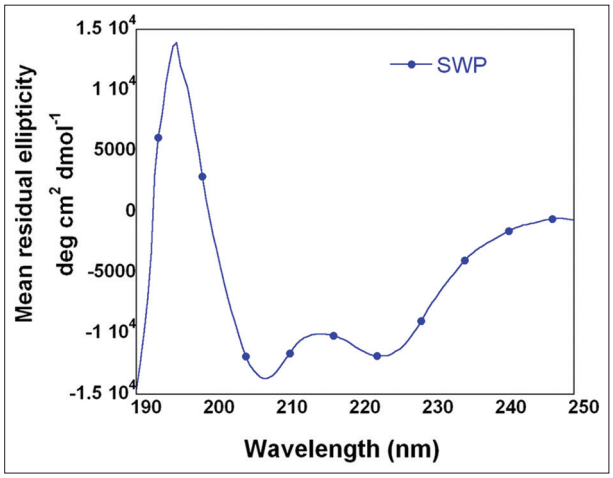

Fig. 2. *Ulva lactuca* L. crude protein secondary structure circular dichroism

A good resolution of proteins was observed for a gel with protein content starting with 15%. For the experiments, 15% resolving gels were chosen, as the most convenient due to the shortest length. It contains several protein bands proving protein bands corresponding to molecular masses of 68, 41, 27, and 6 kDa. A gel electrophoresis (Fig. 1) indicates the presence of proteins in several spots.

CD

Far UV CD studies were performed to understand the secondary structural characteristic of SWP protein. Spectra showed the absorption minima at 208 and 222 nm suggesting the characteristic of α-helical structure [22]. Further, the fractions of protein secondary structural elements were analyzed by K2D2, an online server for secondary structure estimation [23]. Secondary structure estimation showed 84.27% fraction of α-helix and 1.24% β strand as shown in Fig. 2.

Antifungal activity

Water-soluble protein has consequence against pathogenic fungal organisms in this selection faintly active against *A. solani,* then good and more active founds in *A. clavatus, A. niger,* and *A. flavus,* finally less activity of the fungi *F. oxysporum.* In comparing with standard, seaweed extracted protein high efficacy against fungal pathogen Fig. 3, and Table 1.

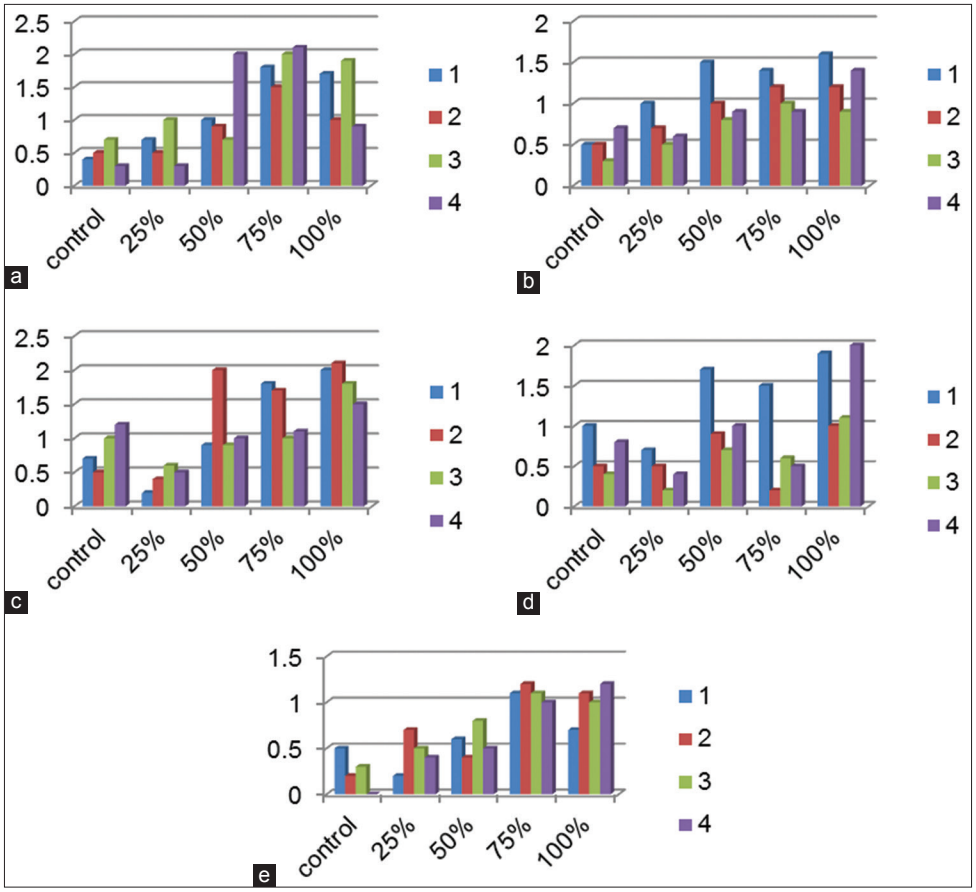

Fig. 3: Seaweed extracted protein in *Ulva lactuca* L. against fungus inhibition level. (a) *Aspergillus clavatus,* (b) *Aspergillus niger,* (c) *Alternaria solani*, (d) *Aspergillus flavus,* (e) *Fusarium oxysporum*

Table 1: Zone of inhibition extracted seaweed crude protein in *Ulva lactuca* L.

S. No.	Organism plant pathogens	Diameter of the zone of inhibition extract concentration (µg/ml) (mm value)				
		Control	25%	50%	75%	100%
1	*Aspergillus clavatus*	0.45±0.085	0.25±0.14	1.5±0.29	0.22±0.13	1.37±0.24+
2	*Fusarium oxysporum*	0.25±0.10	0.18±0.10	0.147±0.85	0.14±0.85	0.07±0.04-
3	*Alternaria solani*	0.55±0.155	0.14±0.08	0.15±0.09	0.15±0.09	0.07±0.04*
4	*Aspergillus niger*	0.5±0.81	0.18±0.10	1.5±0.155	0.19±0.11	1.27±0.14+
5	*Aspergillus flavus*	0.67±0.13	0.18±0.10	1.1±0.21	0.39±0.22	1.5±0.26+

C - Amphotericin+maximum inhibition *minimum inhibition - less inhibition

CONCLUSIONS

The finding of present study confirmed that, seaweed extract of *U. Lactuca L.* can be used/recommended as antifungal agent into preparing eco-friendly disinfectants. However further characterization showed be done. To the best of our knowledge this first report an *U. Lactuca L.* extract protein.

The water-soluble protein has consequence against pathogenic fungal organisms in this selection faintly active against *A. solani,* then good and more active found in *A. clavatus, A. solani,* and *A. flavus,* and finally, less activity of the fungi *F. oxysporum.* Moreover, antifungal activity effect expressed at this level. This statement provides strong conjectural evidence that antifungal potential plays a more important role in plants antifungal defense system. In study about anti-fungal activity further studies can be done by using other seaweed extract in to same pathogens to improve the metabolites quantities.

AUTHORS' CONTRIBUTIONS

R. Krishnamoorthi contributed in doing experiments and preparing the manuscript, and Dr. SR Sivakumar contributed in designing the experiment and put forwarded valuable suggestions.

CONFLICTS OF INTEREST STATEMENT

The author declared no conflicts of interest.

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