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Comparison of biological activities of Chlorella and Spirulina algae before and after enzymatic hydrolysis

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Introduction

Microalgae have valuable medicinal metabolites such as carotenoids, polyphenols, fatty acids, phycobiliproteins and vitamins, which are part of the defense strategies of microalgae against stressors (Chu. 2012). Two *Spirulina* microalgae (*Artrospira platensis* (*Spirulina*))) and *Chlorella* (*Chlorella vulgaris*) are food-medicinal products that are usually consumed in the form of powder, tablets or capsules (Ferdous & Yusof. 2021). *Spirulina* has high protein, which is about 60-70% of its dry weight, and also contains all essential amino acids, minerals, vitamins, and photosynthetic pigments (Lee *et al*., 2017 and Yücetepe & Özçelik. 2016). *Chlorella* microalgae is rich in chlorophyll, protein, polysaccharides, vitamins, minerals, and essential amino acids, and it is very interesting because of its protein content, which is usually up to 60% (Enyidi. 2017).

Hydrolysis of microalgae proteins can increase several other interesting properties, as it leads to the release of bioactive peptides with several possible biological activities (Cunha & Pintado. 2022). Recently, bioactive peptides obtained from *Spirulina platensis* have been noticed due to their antihypertensive, antioxidant, antitumor, antiproliferative, and antimicrobial effects (Yücetepe & Özçelik. 2016). Several bioactive peptides have been produced from *Chlorella* that show interesting properties such as antioxidant, antihypertensive, anti-inflammatory, anticancer, and antimicrobial (Cunha & Pintado.2022).

In the present study, *Chlorella* and *Spirulina* algae were subjected to enzyme treatment, and the resulting hydrolysate was evaluated in terms of antioxidant activity, inhibition of cell proliferation, and antimicrobial activity, and then the antioxidant activity of algae extract was compared without any treatment.

Materials & Methods*:*

Spirulina and *Chlorella* microalgae were cultivated using Zarrouk culture medium (Rajasekaran *et al*., 2015) and Sorokin and Krauss culture medium (Sorokin & Krauss, 1958), respectively. *Spirulina* was cultured at a temperature range of 25±1°C and a pH of 9±1, and *Chlorella* was cultivated at a temperature of 20±1°C and a pH of 6±1. Exposure was done with 4 fluorescent lamps for 24 hours and continuous aeration. In order to check the microalgae growth of *Spirulina and Chlorella*, every 3 days samples were taken from the earless under sterile conditions, and cell counting was done with Neubaoer slides. After entering the descending course of cultivation, the biomass of *Spirulina* and *Chlorella* microalgae was collected and then dried at ambient temperature. A part of the dry powder was used to prepare the aqueous extract according to the method (Thiagarajan *et al*., 2019).

Dry biomass protein of *Spirulina* and *Chlorella* algae was extracted, and then algae protein (10% w/v) was mixed with alkaline protease and pepsin at a ratio of enzyme to the substrate of 1 to 10 at a temperature of 40°C and a pH of 10 at a temperature of 37°C. Grad was digested at pH 3 for alkaline protease and pepsin for 180 minutes, respectively. At the end of the reaction, the pH was adjusted to 7 to inactivate the enzyme. Then the resulting hydrolyzate was collected and dried (Sheih *et al*., 2009). Then the antioxidant activity was investigated by the DPPH method.

Colon cancer cell line Caco-2 and human breast cancer cell line MCF-7 were cultured in DMEM (Dulbecco's Modified Eagle's Medium) medium (Mirzaei *et al*., 2020). To investigate the effect of enzyme hydrolysate on cell proliferation, MTT colorimetric method was used based on the method (Khazraei-Moradian *et al*., 2014).

In order to investigate the antimicrobial activity of standard microbial strains of Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, diffusion methods in agar with the help of discs (disk diffusion) and agar wells were used based on the method (Zanganeh *et al*., 2020).

Average and standard deviation indices were used to show the distribution of data and also to summarize them. The results were analyzed using ANOVA statistical tests using Prism software and the significance level was evaluated at a P-value <0.05.

Results & discussion*:*

The antioxidant activity of the aqueous extracts of *Spirulina* and *Chlorella* microalgae shows that with increasing concentration, an increase in antioxidant properties has been observed, and in all concentrations, the antioxidant activity of *Spirulina* microalgae has been significantly different compared to *Chlorella* microalgae. The antioxidant activity of *Spirulina* microalgae protein before and after hydrolysis shows that with increasing concentration, an increase in antioxidant properties was observed and in all concentrations, the antioxidant property related to hydrolyzed protein and non-hydrolyzed protein had a significant difference. The antioxidant activity of *Chlorella* microalgae protein before and after hydrolysis shows that with increasing concentration, an increase in antioxidant properties has been observed, and the concentration of 200 μg/ml in each extract has the highest antioxidant property.

The survival percentage of MCF-7 and Caco-2 cancer cells after 24 and 48 hours of treatment with *Spirulina* and *Chlorella* microalgae protein before and after hydrolysis shows that

increasing the concentration of *Spirulina* and *Chlorella* microalgae protein has decreased the survival of cancer cells.

The results of the antimicrobial activity of *Spirulina* and *Chlorella* microalgae protein show that the antimicrobial activity of the hydrolyzed protein is higher than the non-hydrolyzed protein and the antimicrobial activity increased with increasing concentration.

According to the results, increasing the protein concentration of microalgae increases the antioxidant activity. The concentration of 200 µg/ml has the highest antioxidant activity and the antioxidant activity of the hydrolyzed protein is higher than the non-hydrolyzed protein of both microalgae, and enzymatic hydrolysis has increased the antioxidant property. Bermejo et al. proved the antioxidant activity of *Spirulina platensis* protein extract (Bermejo *et al*., 2008). Also, Yu et al. found a peptide with antioxidant properties by enzymatic hydrolysis of *Spirulina platensis* protein (Yu *et al*., 2016). These results are consistent with the results obtained from the present study.

According to the results obtained from the survival percentage of colon and breast cancer cells, it was found that hydrolyzed proteins have anti-cancer activity. Sediqi et al measured the inhibitory effect of peptides produced from *Chlorella* algae on *Escherichia coli* cells and breast cancer cell lines. Hydrolyzed peptides reduced the growth of *Escherichia coli* and the resulting peptides had a strong effect on the survival of breast cancer cells (Sedighi et al., 2016).

According to the obtained results, the enzymatic hydrolysis of the proteins of both microalgae has caused their antimicrobial properties. Sun et al obtained an antibacterial peptide from *Spirulina platensis* by enzymatic hydrolysis using alkaline protease and papain enzymes and stated that *Spirulina* peptides can be considered as potential promising antimicrobial agents. (Sun *et al*., 2016).

Conclusion*:*

Two microalgae, *Spirulina* and *Chlorella,* have diverse biological compounds, and enzymatic hydrolysis increases biological peptides with different properties. Enzymatic hydrolysis of protein increases antioxidant properties, and antibacterial and anticancer activity, and two microalgae, *Spirulina* and *Chlorella*, are good candidates in this field and can be used as food-drug supplements.

Keywords: *alkaline protease, antimicrobial, anti-cell proliferation, anticancer, pepsin*

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