Determination of Biological Properties of *Chlorella vulgaris* C1 Extracts

Hikmet KATIRCIOĞLU[1], Minoo POURHASSAN SHAMCHI[2], Selçuk ÇEKER[3]*, Özel ÇAPİK[4] and Güleray AĞAR[5]

[1] *Gazi University, Faculty of Education, Department of Biology Education, Ankara, Turkey,* hturk@gazi.edu.tr
[2] *Hacettepe University, Faculty of Science, Department of Biology, Ankara, Turkey,* minoopourhassan@yahoo.com
[3] *Department of Medical Biology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey,* selcuk.ceker@ibu.edu.tr
[4] *Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Turkey,* ozlcpk.36@gmail.com
[5] *Ataturk University, Faculty of Science, Department of Biology, Erzurum, Turkey,* gagar@ataunii.edu.tr

Abstract

In this study, anti-genotoxic and anti-oxidant activities of algal type *Chlorella vulgaris* C1 were investigated. Dried samples in the Soxhlet device; It was extracted with acetone, methanol, distilled methanol and dimethyl sulfoxide. The anti-oxidant and anti-mutagenic properties of the extracts were determined by micronucleus and Trolox equivalent anti-oxidant capacity methods. It was observed that the extracts investigated showed anti-genotoxic properties by preventing the mutagenic activity of AFB_1 in the tested concentrations. In addition, methanol and acetone extracts were found to show relatively higher anti-oxidant and anti-mutagenic activity compared to other extracts. The results show that these natural algae have the ability to reduce the mutagenic activity of AFB_1, suggesting that anti-oxidant activities may play a role in anti-genotoxic activity mechanisms. Due to these properties, the use of *Chlorella vulgaris* C1 in the pharmaceutical and food industry can be expanded.

Keywords: Aflatoxin B_1, *Chlorella vulgaris* C1, Micronuclei, Oxidative.

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Corresponding author: Selçuk ÇEKER, *PhD*
Department of Medical Biology, Faculty of Medicine, Bolu Abant Izzet Baysal University, Bolu, Turkey
E-mail: selcuk.ceker@ibu.edu.tr
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1. Introduction

Algae are simple chlorophyll-containing organisms composed of one cell or cells grouped together in colonies (Wynne and Bold 1985). They are consisting of proteins, lipids, essential amino acids, anti-oxidant pigments, fatty acids, vitamins, carotenoids and other bioactive compounds that express unique features for the development of pharmaceuticals, nutraceuticals, cosmetics, biofuel industry and CO_2 fixation ability (Jin et al. 2006; Oncel 2013; Pulz and Gross 2004). *Chlorella vulgaris* (CV) is a microscopic single-celled green alga. It is known that microalgal phyla provides pharmacological and chemical diversity and innovation. In addition, microalgae are considered to be important producers of some bioactive compounds that are highly found in marine resources (Shimizu 1996).

Previous studies have been reported that CV has rich source of anti-oxidants, such as carotene and tocopherol, lutein and ascorbic acid, also supplying large quantities of vitamins, minerals, dietary fiber and essential fatty acids (Rodriguez-Garcia and Guil-Guerrero 2008). In additional, some researchers showed that treatment with CV has protective activities against stress, tumors, infections and high-fat-diet-induced insulin resistance (Dantas and Queiroz 1999; Ramos et al. 2010). Therefore, Aremu et al. (2016) reported that phytochemical content and biological activities of the *Chlorella* strains were affected by strain, harvest time and N levels.

Negishi et al. (1991) demonstrated that a chlorophyll sample prepared from CV has anti-

genotoxic activity using *Salmonella* and wing spot *Drosophila* test systems. There have been many studies reporting related to especially anti-oxidant activity utilization of CV. This organism showed a shielder effect against heavy metals and other harmful components (such as lead, cadmium, and naphthalene) by decreasing remarkably the oxidative stress reduced by these detrimental compounds, and increasing the anti-oxidant activity in the organisms of tested animals (Shim et al. 2008; Yun et al. 2011).

Recently, there has been an increase in naturally occurring anti-oxidants research for their use in food or medicinal compounds to replace synthetic anti-oxidants, which are being restricted due to their carcinogenicity (Velioglu et al. 1998).

Research and determination of anti-mutagenic and anti-oxidant activities of these compounds has become an important strategy in the treatments of many human diseases related to mutations (Celikler et al. 2009; Okai et al. 1996).

In addition, owing to the changefulness in chemical and aromatic compounds, seaweeds are conventionally used in the cosmetic and pharmaceutical industries as culinary herbs. They have also been used as a traditional food and medicine for healing helminths infections, eczema and gout, most especially by people in coastal areas of several countries (Hoppe 1979). Especially, some metabolites from these seaweeds act as protective compounds against endogenic and exogenic agents threatening the genome. There are several studies conducted to designate the biological activity of these metabolites including immunostimulant, anti-microbial, anti-oxidative, anti-ulcerogenic, anti-inflammatory, analgesic/antipyretic, anti-tumor and anti-mutagenicity activity assays (Khan et al. 1988; Leitte-Silva et al. 2007).

Therefore, in this study, we investigated genotoxic and anti-genotoxic properties of the extracts of *C. vulgaris* C1 by using micronuclei (MN) assay. In addition, the total anti-oxidant activity was measured in order to clarify the mechanism underlying the anti-genotoxic effects of CV. It is also the first study to determine the mutagenic and anti-mutagenic activity depending on the biological activity of *C. vulgaris* C1 isolated and identified from Mogan Lake (Turkey-Ankara). On the other hand, the mutagenic and anti-mutagenic effects of different extracts obtained from the same strain were also evaluated in terms of the antagonistic and synergistic effects of the extracts.

2. Materials and Methods

2.1. Culturing and growth conditions

The seaweeds were collected from Mogan Lake in Ankara, Turkey. Micromanipulation technique was used to isolate CV from mix culture and the axenic cultures were obtained. Collection and isolation of microalgae were made in compliance with Rippka (1988) and Attalah et al. (2019).

2.2. Preparation of the extracts

The extracts were prepared according to the methods of Khan et al. (1988) and Vlaschos et al. (1996). The dried extracts were resuspended in 3 ml of each solvent and preserved at 4 °C for further use in different bioassays (Deshmukh and Puranik 2010; Prakash et al. 2011).

2.3. Quantitative analysis of chemical constituents

TOF-LC / MS was used for quantitative analysis and was performed at Çankırı Karatekin University (Erenler et al. 2015).

2.4. Micronuclei tests

For the micronucleus test, Nartop et al. (2020) determined procedure was applied. Experiments on different extracts of Chlorella vulgaris C1 (10, 20 and 40 µg / ml) were carried out with 6 groups as follows:

Culture 1: Control
Culture 2: AFB1 (5 µM)
Culture 3: CVE (20 µg/ml)
Culture 4: AFB1 (5 µM) + CVE (10 µg/ml)
Culture 5: AFB1 (5 µM) + CVE (20 µg/ml)
Culture 6: AFB1 (5 µM) + CVE (40 µg/ml)

2.5. Total antioxidant status

Total anti-oxidant activity was measured by Trolox equivalent anti-oxidant capacity (TAC) assay. The assay is calibrated with a stable anti-oxidant standard solution called Trolox Equivalent, that is a vitamin E analogue (Yıldırım et al. 2013). The automated TAS experiment was carried out by commercially available kits (Total Antioxidant Status, Rel Assay Diagnostics, Turkey; Turkez et al. (2010). Regents from the kits were added on to the samples from the extracts.

2.6. Statistical analysis

Data were analyzed and treatments compared using the one-way ANOVA with 95% confidence limits ($p<0.05$), according to Duncan's multiple range tests (SPSS 15.0 Version).

3. Results

The seaweed extracts showed anti-oxidant activity to various degrees (Tab. 1). As shown in Tab. 1, acetone and methanol extracts of CV exhibited high anti-oxidant activity which was significantly different compared with other CV extracts.

MN frequencies of the experimental groups are given in Tab. 2. MN frequency in AFB_1 treated group (5 µM) was higher than that in the control group ($p <0.05$). There was a significant decrease in the MN frequency in CV extracts - treated group when compared with the groups receiving AFB_1. In additional, the composition of CV extracts and the relative amounts of the components are analysis as shown in the Tab. 3. According to these results, salicylic acid (0.893 mg / kg) was found higher in methanol extract while vanillic acid (2.107 mg / kg) higher in acetone extraction. It is also seen that vanillic acid (0.714 mg / kg) compounds in dimethyl sulfoxide (DMSO) extract are higher than other compounds. This result shows that alone the activity of anti-oxidant of methanol extract was the same DMSO + acetone mixture (4.14 mmol / L) (4.37 mmol/L).

Table 1. Total antioxidant status of Chlorella vulgaris C1 extracts

Algal species	Extract	Abbreviation	Antioxidant (mmol/L)
C. vulgaris C1	DMSO+ Acetone	CVDA	4.37
	DMSO	CVD	0.23
	DMSO+ Methanol	CVDM	0.92
	Distillate Methanol	CVdM	0.23
	Methanol	CVM	4.14

Table 2. The effects of AFB1 and extracts of Chlorella vulgaris C1 (CVE) on MN

Test Items	Concentrations	MN numbers ± S.E (CVDA)	MN numbers ± S.E (CVD)	MN numbers ± S.E (CVDM)	MN numbers ± S.E (CVdM)	MN numbers ± S. E (CVM)
Control		2.45 ± 0.01^{a}	2.45 ± 0.01^{a}	2.45 ± 0.01^{a}	2.45 ± 0.01^{a}	4.45 ± 0.01^{a}
AFB_1	5 µM	4.72 ± 0.48^{d}	4.72 ± 0.48^{d}	4.72 ± 0.48^{d}	4.72 ± 0.48^{d}	6.72 ± 0.48^{d}
CVE	20 µM	2.56 ± 0.13^{a}	2.52 ± 0.13^{a}	2.63 ± 0.13^{a}	2.64 ± 0.13^{a}	4.82 ± 0.22^{a}
AFB_1 + CVE	5 µM + 10 µM	3.21 ± 0.36^{bc}	4.35 ± 0.36^{d}	3.89 ± 0.36^{c}	4.28 ± 0.36^{cd}	5.58 ± 0.31^{c}
AFB_1 + CVE	5 µM + 20 µM	2.96 ± 0.27^{ab}	4.14 ± 0.27^{cd}	3.70 ± 0.27^{c}	4.03 ± 0.27^{c}	5.27 ± 0.08^{b}
AFB_1 + CVE	5 µM + 40 µM	2.72 ± 0.19^{a}	3.98 ± 0.19^{bc}	3.61 ± 0.19^{cb}	3.92 ± 0.19^{cb}	4.90 ± 0.43^{a}

AFB_1 was used as positive controls for human blood cells. Values of MN (a, b, c, d) are significantly different compared to negative control ($P < 0.05$).

Table 3. Chemical composition of C. vulgaris C1 with different extracts. (mg/kg)

Compound	DMSO	Methanol	Acetone
Gallic acid	0.048	-	-
Gentisic acid	0.1488	-	-
Caftaric acid	-	0.0341	-
Chlorogenic acid	-	-	-
Catechin	1.84	-	-
P-hydroxybenzoic acid	1.9247	0.6028	0.8824
Protocatechuic acid	-	-	-
Caffeic acid	0.1734	0.5572	0.5209
Rutin	0.7168	-	-
p-coumaric acid	0.067	0.02486	0.6318
Chicoric acid	-	-	-
Ferulic acid	0.0471	0.3646	-
Hesperidin	-	-	-
Apigenin-7-glucoside	-	-	-
Rosmarinic acid	0.0023	-	-
Protocatechuic acid ethyl ester	0.5312	0.562	0.962
Salicylic acid	0.498	0.893	0.163
Quercetin	-	-	-
Cinnamic acid	-	-	-
Naringenin	-	-	-
Kaempferol	-	0.037	-
Vanillic acid	0.714	0.0374	2.107
Caffeic acid	0.1734	0.5572	0.5209
Rutin	0.7168	-	-
p-coumaric acid	0.067	0.02486	0.6318

4. Discussion

CV is known as a functional food source. Therefore, in this study, the antagonistic effects of extracts of CV were studied against AFB_1 mutation agents in the peripheral blood lymphocytes using MN test systems. This agent is known to stimulate the release of free radicals, including reactive oxygen species that cause chromosomal aberrations (Ceker et al. 2018; Orhan et al. 2016). CVE showed great anti-mutagenic potential against AFB_1. This anti-mutagenic activity may be explained with inhibitor activities of the CVE on the formation of free radicals. In order to elucidate the anti-mutagenic activities of CVE, the amount of anti-oxidants was determined. Our results showed anti-oxidant activity to various degrees all extracts.

As shown in Tab. 2 methanol and acetone extract exhibited relatively high anti-oxidant activity. This is the first scientific report on the anti-genotoxic and protective potential of CVE.

Recent studies have reported that several algal extracts have anti-cancer, immunoregulator, immunostimulant, anti-oxidant, anti-microbial activities and strong anti-genotoxic activity in human lymphocytes in vitro (Celikler et al., 2008; Faulkner 2000; Koyanagı et al. 2003; Leitte-Silva et al. 2007; Okai et al. 1996; Varella et al. 2004; Yamamoto et al. 1986).

Several authors have reported that algal extracts have anti-oxidant and anti-mutagenic/anti-carcinogenic activities due to compounds, such as β-carotene, lutein and chlorophyll-related derivatives isolated from this species (Benedetti et al. 2004; Kotake-Nara et al. 2001).

The phenolic compounds of CV such as vanillic acid, caffeic acid, ferulic acid, protocatechuic acid, p-coumaric acid, 4-hydroxybenzoic acid, salicylic acid, caftaric acid, camphenol have been determined in our previous and unpublished studies. Among the contents of CV vanillic, fumaric, caffeic, protocatechuic and caftaric acid have been reported that have to anti-mutagenic activities in previous studies and these effects could be related to its anti-oxidant potential. Other phenolic compounds have determined anti-oxidant activities but there have not been sufficiently studies related to anti-mutagenic potential (Chen et al. 2016; Safaeian et al. 2016; Zhang et al. 2011).

In addition, the results obtained in this study suggest that to understand the anti-oxidant activities of extracts obtained from CV may not suffice compounds derived from a single solvent. It has been demonstrated that synergistic and antagonistic effects

of the compounds obtained from these solvents may also be present. Anti-mutagenic potentials and changes were also investigated with these interactions.

The algal extracts examined in our study showed a great anti-oxidant property. According to the results obtained from MN test system, CV has a significant anti-genotoxic effect. Taken together, the results of our study indicate that anti-genotoxic effect of CV could be related to its anti-oxidant potential. Although the performed test systems showed important data including anti-genotoxic and anti-oxidant potential of the CV, further studies are needed.

The clarification of each of the contents of CV can extend the usage of it in treating some diseases. These compounds are valuable towards an extension of the use of drugs as new phytotherapeutic or preservative ingredients, besides their consolidated ethno medical use. In addition, CV and compounds can be use in chemotherapeutic drugs with the purpose of more effective curation with least toxicity.

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