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Determination of Biological Activity of Some Macro/Micro Algae

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Abstract: Algae are primary producers in the aquatic environment and from an important part of the food chain. Algae have secondary metabolites with high biological activity, which are especially important in the creation of novel pharmaceutical agents. Main purpose of the research was to ascertain antioxidant, anti-alzheimer, anti-diabetic and tyrosinase inhibitory activities of ethanol, hexane and water extracts of some macro/micro algae. Maceration was used as the sample prepatation method. Antioxidant activity of algea for different radical and compound was assessed. Anti-Alzheimer activity of the obtained calluses were determined using Ellman method which is a spectrophotometric method. Anti-diabetic activities were determined using the α-glucosidase inhibition method. It was discovered that the water extract contained more antioxidant phytochemicals, including phenolic (587.54±2.12 mg PEs/g DW) and flavonoids (618.39±2.47 mg QEs/g DW). This study confirmed that the water extract contained high levels of biological acitivty. This extract may be utilized as a possible source of beneficial nutrients or antioxidants.

Keywords: Macro/micro algae, antioxidant activity, anti-alzheimer activity, anti-diabetic activity

Öz: Algler su ortamındaki birincil üreticilerdir ve besin zincirinin önemli bir parçasıdır. Algler, özellikle yeni farmasötik ajanların geliştirilmesinde önemli olan, yüksek biyolojik aktiviteye sahip ikincil metabolitlere sahiptir. Bu çalışmada bazı makro/mikro alglerin etanol, hekzan ve su ekstraktlarının antioksidan, anti-alzheimer, anti-diyabetik ve tirozinaz inhibitör aktivitelerinin belirlenmesi amaçlandı. Numune hazırlama yöntemi olarak maserasyon kullanıldı. Farklı radikal ve bileşikler için alglerin antioksidan aktivitesi değerlendirildi. Elde edilen ekstraktların anti-alzheimer aktivitesi spektrofotometrik bir yöntem olan Ellman yöntemi kullanılarak belirlendi. Anti-diyabetik aktiviteler α-glukozidaz inhibisyon yöntemi kullanılarak belirlendi. Su ekstrelerinin fenolik (587.54±2.12 mg PEs/g DW) ve flavonoidler (618.39±2.47 mg QEs/g DW) gibi antioksidan fitokimyasallar açısından daha zengin olduğu bulundu. Bu çalışma, su ekstraktının yüksek düzeyde biyolojik aktivite içerdiğini doğruladı. Bu ekstrakt potansiyel bir antioksidan veya fonksiyonel besin kaynağı olarak kullanılabilir.

Anahtar Kelimeler: Makro/mikro algler, antioksidan aktivite, anti-alzheimer aktivite, anti,diyabetik aktivite

1. Introduction

Algae, one the most important living resources of the seas, ise used in food, agriculture, cosmetics, medicine, pharmacy and industry. Algae have secondary metabolites with high biological activity, which are especially important in the development of new pharmaceutical agents [1]. It is usually found in oceans, rivers, freshwater lakes, streams, arctic lakes, puddles, etc. Algae, which can live in a wide range of aquatic and semi-aquatic environments, are autotrophic and photosynthetic organism. In the food chain, algae play a major role as producers. Algae are becoming more popular since they are used as food in island countries and the Far East. Studies show that seaweed has high nutritional value [2]. Humans have been eating seaweed for thousands of years, and the global market currently stands at over $6 billion annually, with an estimated 12 million tons of seaweed produced in 2018. Coastal habitats are home to a wide range of macroalgea, which are rich sources of bioactive metabolites with diverse biological activities that can affect the survival, dispersion, and abundance of marine species. Based on their color, Seaweeds are categorized into three main categories: Rhodophyta (red algae), Ochrophyta (brown algae), and Chlorophyta (green algae). It is predicted that the maritime environment has 1800 green macroalgae, approximately 1800 brown macroalgae, and 6200 red macroalgae [3].

Antioxidants are frequently introduced into food as food additives to guard against free radicals' oxidative degradation of food. Several naturally occurring compounds found in plants have gained significant interest as safe antioxidants due to their long-standing use by both humans and animals. Thus, it is desirable to create and use more potent antioxidants derived from natural sources [4]. Alzheimer's disease is defined as the knotting of neurofibrils and the formation of amyloids connected to cholinergic neuron loss in regions of the brain related to learning and memory. Acetylcholine level decreases in Alzheimer's patients. One of the ways to treat this is by inhibition of the enzyme responsible fort he hydrolysis of acetylcholine (acetylcholinesterase) [5].

Diabetes mellitus is a comlex illness that can cause serious complications. As a result, the treatment incorporates a variety of therapeutic modalities. Following a meal, postprandial hyperglycemia in diabetes people happens as a result of the gastrointestinal system absorbing glucose. Reducing blood glucose levels in postprandial hyperglycemia, a common condition among diabetics, can be achieved by inhibiting intestinal glucose absorption and promoting tissue glucose absorption [6]. The purpose of this investigation was to ascertain the antioxidant, anti-alzheimer, anti-diabetic and tyrosinase inhibitory activities of ethanol, hexane and water extracts of some macro/micro algae (*Dunaliella salina*, *Microcystis aeroginosa*, *Myriophyllum spicatum*, *Chrolella vulgaris*, *Aphanizomenon flasaque*, *Spiruline palatensis* and *Cladophora glomerata*)..

2. Material and Method

Chemicals and spectral measurements

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), 5,5'-dithiobis (2-nitrobenzoic) acid (DTNB), acetylthiocholine iodide (AcI) and butyrylthiocholine chloride (BuCl), phosphate buffer (pH 6.8-8.0), galantamine, p-nitrophenyl-α-D-glucopyranoside (p-NPG), genistein, linoleic acid, α-tocopherol, potassium persulfate, nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), 1,1-diphenyl-2-picrylhyrazyl (DPPH), pyrocatechol, quercetin and 3-(-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). α-glucosidase, dimethyl sulfoxide (DMSO), methanol, ethanol were purchased from Merck (Darmstadt, Germany). The BioTek Power Wave XS (USA) 96-well microplate was used for the bioactivity measurements. Utilizing Gen5 Data Analysis software, the measurements and computations of the activity data were assessed. Ammonium thiocyanate, ferrous chloride, polyoxyethylenesorbitan monolaurate (Tween-20), tricholoroacetic acid (TCA), ethanol (EtOH) and hexane were acquired from Merck. The remaining substances were acquired through Sigma-Aldrich or Merck and were all of analytical quality. Water was puridifed by Human (Japan) ultrawater purification system.

Materials and extraction procedures

Dunaliella salina, *Chrolella vulgaris* and *Spirulina platensis* were obtained from Çukurova University, Faculty Aquaculture. *Cladophora glomerata*, *Microcystis aeroginosa* from Sanlıurfa Birecik Karkamış Dam; *Aphonizomenon flasaque* and *Myriophyllum spicatum* from Sanlıurfa Siverek Çamçayı Dam. collected by Göksal Sezen. The collected algae were cleaned of stones, sand and invertebrates (mussels and snails), dried in the shade, pulverized with a 46000 rpm blender (Tefal Ultra High Speed Blender) and stored in the deep freezer at -20 ºC. Algae were placed in 20 liter plastic bottles in the Hydrobiology-Algology laboratory of the Biology Department of the Faculty of Science and Letters, Harran University. Preparation of the extracts was done according to the method we developed in our previous study [4]. To prepare the water extract, 25 g algae sample and 500 mL of boiling water were mixed in a magnetic stirrer for quarter of an hour. The extract was then filtered and evaporated to dryness in a rotary evaporator under lower pressure and controlled temperature (40-50 ºC). To prepare ethanol and hexane extracts, 25 g algae sample was mixed with 500 mL of solvent. Then, it was incubated for 3 hours in a shaking water bath at 100-150 rpm at room temperature (25 ºC). The residue was re-extracts until extraction solvents are colerless Using filter paper, the extracted materials were purified (Whatman No.1 paper) and the solvents of the filrates (ethanol and hexane) were evaporated in a rotary evaporator (Buchi R-200, Switzerland) at 40-80 ºC. Following the creation of the water extracts, the filtrate was lyophilized after being filtered via filter paper (at 5 µm Hg pressure at – 50 ºC [Labconco, Freezone 1 L]). Before being used, each extract was dissolved in solvent or water and stored at -20 ºC.

Total phenolic and flavonoid contents

The analysis of the specimens revealed that the overall phenolic [7] and flavonoid [8] contents were comparable to quercetin and pyrocatechol, respectively. For fermented non-alcoholic drinks, the overall levels of phenolic and flavonoid compounds were determined using the following formulae.

Absorbance = 0.001x - 0.0012 pyrocatechol (µg) (r^2 = 0.9978)

Absorbance = 0.0007x + 0.0012 quercetin (µg) (r^2 = 0.9983)

Antioxidant activity

The β-carotene/linoleic acid bleaching assay, ABTS cation radical scavenging, DPPH free radical scavenging assays, superoxide anion radical scavenging, hydrogen peroxide scavenging activity, and CUPRAC (Cupric reducing antioxidant capacity) assay were a total of six methods were employed to evaluate the specimen's antioxidant activity (4). To

determine the samples' IC50 (50% inhibition), doses of 100, 50, 25, and 10 µg/mL were generated. As standards, BHA, BHT, and α-tocopherol were employed in these six antioxidant test techniques.

Anticholinesterase activity

The spectrophotometric technique created by Ellman et al [9] was slightly modified to evaluate the inhibitory activities of acetyl- and butyryl-cholinesterase. The reaction's substrates were acetylthiocholine iodide and butyrylthiocholine iodide, and DTNB was employed to gauge the anticholinesterase activity. Methanol was used to dissolve each callus culture in order to create stock solutions at a concentration of 4000 g/mL. The following ingredients were combined and incubated for 15 minutes at 25 ºC: 150 microliters of 100 mM sodium phosphate buffer (pH 8.0), 10 g/mL of sample solution, 20 l of AChE (or BChE) enzyme solution, and 10 g/mL of DTNB. Acetylthiocholine iodide (or butyrylthiocholine iodide) 10 g/mL was then added to start the reaction. The callus culture's final concentration in solution was 10, 25, 50, and 100 g/mL. The production of yellow 5-thio-2-nitrobenzoate anion with a wavelength of 412 nm, which occurs when DTNB reacts with thiocholine produced by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine iodide, served as a marker for the hydrolysis of these substrates. The samples and controls were dissolved in methanol, which served as a solvent. Galantamine was used as standard.

α-glucosidase inhibitory activity

The callus culture was evaluated for the inhibition of Saccharomyces cerevisiae's -glucosidase using a technique somewhat modified by Tsujii et al [10]. In a nutshell, a 40 L solution of -glucosidase (3.0 U/mL, dissolved in phosphate buffer, pH 6.8) was pre-incubated at 37 ºC for 30 min with 10 L of each callus culture in DMSO. P-nitrophenyl-D-glucopyranoside (p-NPG; final concentration 0.5 mM) was added to the mixture to start the enzymatic reaction, which continued for another 30 minutes. Observing the p-nitrophenol produced from p-NPG at 405 nm allowed researchers to ascertain the -glucosidase activity. The positive control utilized was genistein.

Statistical analysis

A power analysis was done to figure out how many extracts of algae there were. The findings were shown as means ± standard deviation as n=3 for each test specimen.

3. Result

Extraction yield, total phenolic and flavonoid contents

The percentage yields of algae extracts displayed in Table 1. The highest extraction efficiency was seen in water extracts and the percent extraction yields of the water extracts varied between 66.44% and 60.10%. The percent extraction yields of the ethanol extracts varied between 42.66% and 38.93%. The percent extraction yields of the hexane extracts varied between 22.90% and 18.66%. Therefore, all of the extractable components were greater in the water extract. Food secondary metabolites known as phenols or polyphenols are significant because of their antioxidant activity, which involves chelating redox-active metal ions, initiating lipid free radical chains, and blocking the process of transformation of hydroperoxide into reactive oxygen radicals. The total phenolic content of the ethanol extracts differentiating between 407.44±1.38 and 420.47±1.66 µg PEs/mg extract. The extract from *Myriophyllum spicatum* has the greatest total phenolic content among the ethanol extracts (163.61±0.94 µg PEs/mg extract). Total flavonoid content od hexane extracts ranged from 368.60±0.72 to 382.66±0.82 µg QEs/mg extract. The highest total flavonoid content of hexane extracts was detected in *Myriophyllum spicatum* extract (382.66±0.82 µg QEs/mg extract). These quantities were similar to the outcomes for the other algae product extracts reported in the available research. Keramane et al [11] in their study found the extraction efficiency of *Padina pavonica* (Brown algae) to be 4.25% and the total phenolic content to and 49.82±1.40 mg GAE/g extract [11]. Since The water extract had the most extraction efficiency, as shown, this extract was used in antioxidant, anti-Alzheimer and anti-diabetic acitivity determinations.

Table 1. Extraction yields and contents of total phenols, total flavonoids in algae extracts

Algae samples	Extraction solvent	Extraction yield (%)	Total phenolic content (µg PEs/mg extract)[a]	Total flavonoid content (µg QEs/mg extract)[b]
Dunaliella salina	Hexane	22.53	345.84±0.94	380.98±0.61
	Ethanol	41.16	418.77±1.25	472.59±1.51
	Water	66.44	587.54±2.12	618.39±2.47
Chrolella vulgaris	Hexane	20.08	340.24±0.78	371.24±0.83
	Ethanol	38.93	407.44±1.38	463.80±1.56
	Water	62.75	579.81±2.17	614.75±2.33
Spirulina platensis	Hexane	18.66	339.79±0.77	365.32±1.99
	Ethanol	42.40	415.63±1.82	473.11±2.80
	Water	60.10	570.39±2.43	600.67±2.65

	Hexane	21.85	341.35±0.71	375.63±0.58
Cladophora glomerata	Ethanol	40.38	410.94±1.58	466.40±1.64
	Water	64.10	581.77±2.40	616.82±2.80
	Hexane	19.37	343.26±0.79	368.60±0.72
Microcystis areroginosa	Ethanol	41.93	416.02±1.74	296.33±1.70
	Water	65.75	584.14±2.87	617.52±2.31
	Hexane	20.66	342.60±0.63	372.69±0.74
Aphonizomenon flasaque	Ethanol	40.90	411.88±1.45	468.23±1.59
	Water	63.18	583.61±2.33	612.60±2.70
	Hexane	22.90	347.58±0.84	382.66±0.82
Myriophyllum spicatum	Ethanol	42.66	420.47±1.66	475.18±1.60
	Water	61.34	572.50±2.22	607.42±2.88

[a]*Phenolic content equivalent to pyrocatechol ($y=0.001x-0.0012$ $R^2=0.9978$)*
[b]*Flavonoid content equivalent to quercetin ($y=0.0007x+0.0012$ $R^2=0.9983$)*

Antioxidant activity

The antioxidant activity of algae was tested by the β-carotene/linoleic acid bleaching assay, ABTS cation radical scavenging, DPPH free radical scavenging assays, superoxide anion radical scavenging, hydrogen peroxide scavenging activity, CUPRAC (Cupric reducing antioxidant capacity) assay for reducing antioxidant activty. The antioxidant activity test results are given in Table 2. In DPPH• radical scavenging activity, all algae water extracts exhibited higher (stronger) antioxidant activity than standards (except *Cladophora glomerata*). Among all algae, *Cladophora glomerata* exhibited the lowest antioxidant activity.

Table 2. IC_{50} values(μg/mL) of DPPH• free radical scavenging activity, ABTS•+ cation radical scavenging activity, hydrogen peroxide scavenging activity and superoxide anion scavenging activity of algae water extracts (100 μg/mL)

Extracts and standards	**Scavenging ability on DPPH• free radicals**	**Scavenging ability on $ABTS^{\bullet+}$ cation radicals**	**Scavenging ability on hydrogen peroxide**	**Scavenging ability on superoxide anion**	**CUPRAC assay**
Dunaliella salina	57.14±1.32	35.22±0.13	41.26±1.46	63.85±2.59	21.28±1.03
Chrolella vulgaris	43.27±1.33	36.40±0.55	40.81±1.33	65.48±2.29	20.93±1.14
Spirulina platensis	50.28±2.11	38.23±0.62	42.38±1.77	67.50±2.30	19.85±0.97
Cladophora glomerata	36.15±0.49	33.17±1.05	38.40±1.18	60.47±2.83	12.53±0.81
Microcystis aeroginosa	54.35±1.48	34.38±1.30	44.61±0.71	64.93±2.08	18.87±0.91
Aphanizomenon flasaque	48.63±1.22	35.96±0.78	41.90±0.96	62.66±2.61	20.34±1.20
Myriophyllum spicatum	44.75±0.95	36.05±1.11	43.26±0.58	63.01±2.18	19.08±0.63
BHA	40.80±1.73	28.67±0.49	37.62±1.30	57.14±2.23	10.85±0.60
BHT	40.28±1.45	30.23±0.52	36.40±0.83	58.63±2.40	11.02±0.20
α-tocopherol	39.53±1.19	32.81±0.85	35.30±0.44	55.50±2.33	10.39±0.12

Values are given as the mean and standard deviation of three parallel measurements.

Anti-alzheimer activity

Acetylthiocholine iodide is utilized as the substrate and acetylcholinesterase enzyme is employed as the inhibitor of acetylcholinesterase activity. The reaction is based on the formation of the yellow 5-thio-2-nitrobenzoate anion with DTNB of the thiocholine formed as a result of the acetylcholine iodide decomposition by the acetylcholinesterase enzyme and its spectrophotometric measurement at 412 nm. The standard is galantamine, an isolated alkaloid substance from the Galanthus plant. Ethanol was used as a control. Anticholinesterase activity was determined as % inhibiton relative to control using the following equation.

% inhibition = (Acontrol – Asample / Acontrol) × 100

Acontrol = Ethanol absorption

Asample = Algae water extracts

Algae extracts showed anti-Alzheimer activity in direct proportion to the increase in concentration. According to table 3, although galantamine was used as a standard, it did not show 100% inhibition. Therefore, assuming 100% inhibition of

galantamine, algae water extracts showed moderate anti-alzheimer's activity relative to galantamine. Among all algae, *Cladophora glomerata* exhibited the lowest anti-Alzheimer activity in Table 3.

Table 3. Anticholinesterase inhibition of algae extracts and standard

Algae Samples (water extracts)	**% inhibition of acetylcholinesteras**
Dunaliella salina	57.14±1.20
Chrolella vulgaris	53.27±1.30
Spirulina platensis	52.18±1.11
Cladophora glomerata	41.45±1.49
Microcystis aeroginosa	53.35±1.28
Aphanizomenon flasaque	54.80±1.53
Myriophyllum spicatum	52.28±1.75
Galantamine	80.53±1.49

Anti-diabetic activity

A class of medications used to treat diabetes prevents the conversion of carbohydrates into glucose by blocking the enzymes -amylase and -glucosidase, which are included in the analysis of carbohydrates in metabolism. Extracted callus tissue cultures were made according to the method described for inhibition of α-glucosidase from Saccharomyces cerevisiae. Genistein was used as a standard. The control group was analyzed by changing samples with phosphate buffer. α-glucosidase inhibition activity was calculated as % inhibiton relative to control using formulation below.

% inhibition = (Acontrol – Asample / Acontrol) × 100

Acontrol = Phosphate buffer

Asample = Callus culture extracts

Algae extracts showed anti-diabetic activity in direct proportion to the increase in concentration. According to table 4, although genistein was used as a standard, it did not show 100% inhibition. Therefore, assuming 100% inhibition of genistein, algae water extracts showed moderate anti-alzheimer's activity relative to genistein. *Dunaliella salina* exhibited the highest anti-diabetic activity among algae samples in Table 4.

Table 4. α-glucosidase inhibition of algae extracts and standard

Algae Samples (water extracts)	**% inhibition of α-glucosidase**
Dunaliella salina	66.64±2.70
Chrolella vulgaris	63.07±2.33
Spirulina platensis	62.28±2.51
Cladophora glomerata	51.25±2.20
Microcystis aeroginosa	63.75±2.48
Aphanizomenon flasaque	61.20±2.23
Myriophyllum spicatum	60.14±2.35
Genistein	88.63±2.09

4. Discussion and conclusion

The results of this research reveal the early details on the anticholinesterase, antidiabetic, and antioxidant properties of algal extracts. The aqueous extract of the algae sample (*Spirulina platensis*) showed the highest effectiveness among the three evaluated techniques for inhibiting scavenging ability on superoxide anion. The aqueous extracts had much more DPPH-scavenging activity than the benchmarks BHA, BHT, and α-tocopherol. As is well known, phenolic content and free radical-scavenging activity—particularly with regard to the DPPH radical—have a strong linear relationship. The water extracts also showed strong scavenging ability on ABTS cation radicals. The water extract showed results close to the standards (galantamine and genistein) in anti-Alzheimer and anti-diabetic activity. Zaid et al [12] found the total phenolic substance content of *Spirulina platensis* extract to be 40 mg/g [12]. Trigui et al [13] found the acetyl choline esterase activity of ethyl acetate extract of *Ulva rigida* to be 7.6 mg/mL [13]. Therefore, the algae may shield humans from the damaging effects of free radicals and lipid peroxidation, and its extracts are likely be useful in the creation of

safe food additives and goods. This algae's ability to scavenge free radicals could represent one of the ways that it serves both a culinary and traditional medicinal purpose. It is necessary to conduct more research on specific molecules (secondary metabolites), their biological actions in vivo, and various biological pathways. The usage of algal water extracts as a natural source of antioxidants was determined.

Conflict of Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics Committee Approval

Ethics committee approval is not required.

Author Contribution

Conceptization: BC, GS; methodology and laboratory analyzes: BC, GS; writing draft: BC, GS; proof reading and editing: Other: All authors have read and agreed to the published version of manuscript.

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