Original Article

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Chemical Contents and Bioactivities of Green Algae *Ulva rigida* C.Agardh Red Algae *Grateloupia turuturu* Yamada Extracts

Yeşil Alg *Ulva rigida* C.Agardh ve Kırmızı Alg *Grateloupia turuturu Yamada* Ekstrelerinin Kimyasal İçerikleri ve Biyoaktiviteleri

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ABSTRACT

Objective: Edible seaweeds, valued for their rich content of bioactive compounds, have gained recognition in complementary medicine beyond their traditional role in the food and pharmaceutical sectors. Notable examples include *Ulva rigida* C.Agardh and *Grateloupia turuturu* Yamada. These seaweeds offer a promising avenue for exploring their bioactive potential in medical and dietary contexts.

Methods: Hexane extracts were prepared to elucidate the fatty acid composition of seaweeds via Gas Chromatography-Mass Spectrometry analyses. Subsequently, investigations were conducted to assess the cytotoxicity on human breast cancer cell lines (MCF-7 and MDA-MB-231), as well as examine the antimicrobial and anti-cholinesterase activities of extracts obtained using hexane, dichloromethane:methanol (CH_2Cl_2:MeOH, 1:1), methanol (MeOH), and water solvents.

Results: The most abundant substances for the *U. rigida* and *G. turuturu* species were hexadecanoic acid, octadecenoic acid, and erucic acid compounds. The CH_2Cl_2:MeOH (1:1) extract of *G.*

ÖZ

Amaç: Zengin biyoaktif bileşik içeriği nedeniyle değer verilen yenilebilir deniz yosunları, gıda ve ilaç sektöründeki geleneksel rollerinin ötesinde tamamlayıcı tıpta da tanınmaktadır. Dikkate değer örnekler arasında *Ulva rigida* C.Agardh ve *Grateloupia turuturu* Yamada bulunmaktadır. Bu deniz yosunları, tıbbi ve diyetsel bağlamlarda biyoaktif potansiyellerini keşfetmek için umut verici bir yol sunmaktadır.

Yöntemler: Deniz yosunlarının yağ asidi kompozisyonunu Gaz Kromatografisi-Kütle Spektrometrisi analizleri yoluyla aydınlatmak için hekzan ekstreleri hazırlandı. Daha sonra, insan meme kanseri hücre dizileri (MCF-7 ve MDA-MB-231) üzerindeki sitotoksisiteyi değerlendirmek ve ayrıca hekzan, diklorometan:metanol (CH_2Cl_2:MeOH, 1:1), metanol (MeOH) ve su çözücüleri kullanılarak elde edilen ekstrelerin antimikrobiyal ve antikolinesteraz aktivitelerini incelemek için araştırmalar yapıldı.

Bulgular: *U. rigida* ve *G. turuturu* türleri için en bol bulunan maddeler hekzadekanoik asit, oktadekenoik asit ve erusik asit

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ABSTRACT

turuturu was found as the highest toxicity on MCF-7 and MDA-MB-231 breast cancer cells (IC_{50}: 28.7 µg/mL). *G. turuturu* extracts showed inhibition on *E. coli* and *C. albicans*. The hexane extracts of *U. rigida* and *G. turuturu* inhibited AChE enzyme in both algae species.

Conclusion: The results highlight seaweeds' bioactive potential for therapeutic and dietary products. Further research can explore medical and nutritional applications.

Keywords: *Ulva rigida*, *Grateloupia turuturu*, chemical composition, bioactivity studies

ÖZ

bileşikleridir. *G. Turuturu*'nun CH_2Cl_2:MeOH (1:1) ekstresinin, MCF-7 ve MDA-MB-231 meme kanseri hücreleri üzerinde en yüksek toksisiteye sahip olduğu bulundu (IC_{50}: 28,7 µg/mL). *G. turuturu* ekstreleri *E. coli* ve *C. albicans* üzerinde inhibisyon gösterdi. Her iki alg türünün hekzan ekstreleri AChE enzimini inhibe etti.

Sonuç: Deniz yosunlarının tedavi edici ve diyetsel ürünler için biyoaktif potansiyelini vurgulamaktadır. Daha fazla araştırma tıbbi ve beslenme uygulamalarını keşfedebilir.

Anahtar Sözcükler: *Ulva rigida*, *Grateloupia turuturu*, kimyasal içerik, biyoaktivite çalışmaları

Introduction

Edible seaweeds are recognized for their significant content of bioactive antioxidants, soluble dietary fibers, proteins, minerals, vitamins, phytochemicals, and polyunsaturated fatty acids. While traditionally utilized as gelling and concentrating agents in the food and pharmaceutical sectors, contemporary scientific inquiries have disclosed their auspicious prospects in the realm of complementary medicine (1,2). Two noteworthy examples are *Ulva rigida*, C.Agardh, a green macroalgae commonly referred to as "Sea lettuce", and *Grateloupia turuturu*, Yamada, a red macroalgae known colloquially as "Devil's Tongue Grass", "Jinuari" (Korean), and "Ratanho" (Portuguese), originating from Asia.

Marine macroalgae are known to produce a diverse array of volatile organic compounds, including hydrocarbons, terpenes, phenols, alcohols, aldehydes, ketones, esters, fatty acids, and halogen or sulfur-containing compounds. Macroalgae serve as valuable sources of antimicrobial compounds, omega-3 fatty acids, antioxidants, and other bioactive constituents, thus garnering increasing interest for integration into functional foods and nutraceutical products. Notably, polyphenols, flavonoids, and polysaccharides have been identified for their antioxidant and antimicrobial activities within brown, red, and green algae (3).

The use of macroalgae has an extensive history, with indigenous South Americans employing macroalgae for sustenance and medicinal purposes as far back as 12,000 years ago. The earliest recorded use of algae in medicinal contexts can be traced to Shen Nung's "Materia Medica" from 2700 BCE, although the systematic scientific investigation of algae-based products primarily emerged in the last century (4).

Despite approximately ten thousand identified algae species, only around 5% of them find use in human or animal diets. Nevertheless, over a hundred seaweed types are utilized worldwide, particularly in Asian nations where they serve as sea vegetables (5,6). Seaweed consumption is associated with various health benefits, including immunity enhancement, cholesterol reduction, blood sugar level regulation, antioxidant activity, memory enhancement, blood pressure control, fatigue alleviation, weight management, growth and development support, anemia prevention, liver protection, acne reduction, skin moisture improvement, intestinal flora regulation, digestion promotion, fecal excretion facilitation, and gastric mucosal injury prevention (7).

In previous studies conducted on *U. rigida* and *G. turuturu* species, antioxidant, antimicrobial, anti-inflammatory and anticancer activities of these algae were detected and secondary metabolites such as terpenic compounds, polysaccharides and fatty acids were revealed (8-17).

The primary objective of this study was to investigate the cholinesterase inhibitory activities, antimicrobial activities, and cytotoxic effects of *U. rigida* and *G. turuturu* species on MCF-7 and MDA-MB231 human breast cancer cells.

Methods

Algae Collection and Extraction

Ulva rigida and *Grateloupia turuturu* (Figure 1) were harvested from the coastal regions bordering the Mediterranean and the Aegean Sea. The algae specimens (E105 and E137) were deposited at the Faculty of Aquatic Sciences and Fisheries, Akdeniz University, Antalya, Türkiye, and their identification was verified by Dr. Emine Şükran Okudan.

Figure 1. *Ulva rigida* (left) and *Grateloupia turuturu* (right)

The dried and pulverized algae *Ulva rigida* (10 g) and *Grateloupia turuturu* (10 g) were extracted with Hexane, MeOH, water (200 mL × 5 days) respectively, and *Ulva rigida* (280 g) and *Grateloupia turuturu* (370 g) were percolated with dichloromethane:methanol (CH_2Cl_2:MeOH 1:1, 200 mL × 5 days) at room temperature. The water extract underwent lyophilization and drying, whereas the remaining extracts were concentrated using a rotary evaporator under low pressure and at 45 °C.

Gas Chromatography - Mass Spectrometry (GC-MS) Analysis

Thermo Scientific TSQ GC-MS/MS. TG-5MS 0.25 mm silica column was used. The GC oven temperature was kept at 80 °C and programmed to increase 300 °C. Using scan mode, a mass range from 50 to 950 m/z. The identification of components was conducted using commercial libraries such as Wiley and Nist, along with referencing literature data. Relative percentage amounts of the separated compounds were determined from Total Ion Chromatograms.

Cytotoxic Activity

Human breast cancer cell lines (MCF-7 and MDA-MB-231) and a healthy skin fibroblast cell line (CCD-1079Sk) were obtained from the American Tissue Culture Collection. The cells were cultured in T75 cell culture flasks in a controlled humidified incubator with a 5% carbon dioxide (CO_2) atmosphere at 37 °C, utilizing DMEM/F12 medium supplemented with 10% fetal bovine serum and 100 units per milliliter (U/mL) of penicillin/streptomycin. Upon reaching an appropriate confluency of 80%, the cells underwent PBS washing and were detached through trypsin/EDTA treatment. Prior to treatments, $5x10^3$ cells were seeded into 96-well plates for 24 and 48 hours. The plant extracts were introduced into the cell cultures at final concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.60 µg/mL. After 24 and 48 hours of incubation, MTT solution (5 mg/mL in PBS) was added to each well and further incubated at 37 °C with 5% CO_2 for 4 hours in the dark. Subsequently, the cell culture media was aspirated, and 100 µL of DMSO was added to each well to dissolve the formed formazan crystals. Absorbance values were measured at 540 nm using an Elisa microplate reader. The experiments were conducted in triplicate, and the results were presented as the mean ± standard deviation (SD).

Determination of Selectivity Index

Selectivity index (SI) is an equation calculated to evaluate the cytotoxic potential in cancer cells relative to toxicity in normal cells; high SI value indicates high potency and low cell toxicity. An SI value higher than 3 serves as a marker for the selection of extracts (18,19).

Anticholinesterase Activity Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity were measured using a spectrophotometric method described by Ellman (20). This procedure involved mixing 10 µL of the sample solution, 20 µL of AChE or BChE solution, and 130 µL of 0.1 M sodium phosphate buffer (pH 8.0). The mixture was incubated for 10 minutes at 25 ºC. Then, 20 µL of DTNB was added to the combination. Acetylthiocholine iodide or butyrylthiocholine iodide (20 µL) was added to start the reaction, which resulted in the enzymatic hydrolysis of the aforementioned iodides and the release of thiocholine. At a wavelength of 412 nm, the yellow-colored 5-thio-2-nitrobenzoic acid anion that resulted from the reaction of thiocholine with DTNB was detected using spectrophotometry. Three parallel studies were conducted for each sample and the following equation was used to compute the results:

% Inhibition = (A control - A sample) / A control x 100

Antimicrobial Activity Assay

Material

Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), Gram (-) bacteria *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028) yeast were used. Vancomycin and ciprofloxacin were used as antibiotics and amphotericin B was used as antifungals for the control (21).

Resazurin Microplate Test

The Resazurin microplate method was employed to assess the antibacterial and antifungal properties as well as the minimum inhibitory concentrations (MIC) of the compounds. Two replicates were conducted for the activity determination. The tested compounds were prepared as stock solutions at a concentration of 1000 µg/mL, which were then sterilized through a 0.22 µm diameter membrane filter. In each well of a 96-well microplate, 50 µL of Mueller Hinton Broth medium was initially added. The first well received diluted samples at 1000 µg/mL, while the control antibiotic and antifungal compound were added to the first well and further diluted in a series. One column of the plate was designated for DMSO as the negative control, and another column was assigned for 50 µL of standard bacteria and yeast as the positive control, also diluted in a series. A McFarland suspension with a turbidity value of 0.5 was prepared from microorganism colonies and diluted at a 1:100 ratio. Subsequently, 10 µL of the final suspension was added to the plate wells. The plates were covered with parafilm and subjected to incubation at 37 °C for 24 hours for bacteria and 48 hours for yeast. After incubation, 10 µL of a solution containing 33.75 mg of resazurin dissolved in 5 mL of distilled water and 10 µL of 20% Tween 80 were added to all wells. Visual evaluation of the results was performed after allowing the plates to incubate for an additional 2 to 4 hours. The MIC value was determined as the lowest concentration preventing a color change from purple to pink.

Statistical Analysis

IC_{50} values were obtained with the equation y=50 and statistical analyses were calculated with Graphpad Prism 10 (San Diego, USA). In the equation, log-transformations of the concentration values of the extracts were plotted on the X-axis and fluorescence absorbance values were plotted on the Y-axis to obtain a sigmodial curve. IC_{50} values had ± 95% confidence interval.

The results were represented as mean ± SD, $^{*}p<0.05$ (p-values were obtained paired t-test).

Results

The non-polar hexane extract of the edible seaweed *U. rigida* was subjected to GC-MS analysis to examine its fatty acid composition and other compounds. The analysis results revealed that among the fatty acids, hexadecanoic acid and octadecenoic acid were identified as major compounds (Table 1). Similarly, in the hexane extract of *G. turuturu*, fatty acids such as hexadecanoic acid was observed as major constituent (Table 2).

As shown in Table 3, *U.rigida* methanol extract (1) and *U. rigida* CH_2Cl_2:MeOH (1:1) extract (2) were the most toxic against MCF-7 and MDA-MB-231 cell lines. The most active extract from the *U. rigida* plant was extract 1, with IC_{50} values of 11.1 g/mL and 20.3 g/mL after 24 and 48 hours of exposure, respectively. In all cell lines, the most cytotoxic effect of the extracts obtained from *G. turuturu* plant was obtained by extract (4) on MCF-7 line. *U. rigida* water extract (5) and *G. turuturu* MeOH extract (3) had the least cytotoxic effect against a healthy cell line CCD-1079Sk. When the viability data of the extracts at 2 time slots in each cell line were compared with the pared t-test, significant differences were obtained in extracts 1, 2 and 3 (Figure 2). Since the plants from which the extracts were obtained could be consumed as food, high IC_{50} values were an expected result. Extracts 5 and 3 had the least cytotoxic effect on the healthy cell line CCD-1079Sk. Similarly, these extracts had the maximum IC_{50} values obtained after 48 hours of exposure in two cancer cell lines. In the graphs in (Figure 3), the percent viability data of the extracts according to time and concentration increased depending on the decrease in concentration. It was revealed that the percentage of time-dependent viability in healthy cell line and two cancer cell lines was increased. These findings indicated that the extracts showed their effects within twenty-four hours.

When the SI results were evaluated, it was determined that extracts 3, and 5 had the S>3 value, indicating that they had a specific effect on cancer cells; the S<3 value corresponds to the general toxicity of the other extracts. In 48 hours, the SI values of all extracts decreased relative to the 24 hour data. The SI values for extract 3, which had a significant effect on two cancer lines based on 24 hour treatment data, were 9.9 and 4.7, as shown in Table 4. The IC_{50} and SI values of extract 3 were consistent, and extract 3 demonstrated high selectivity against two cancer cell lines.

Table 1. Compounds detected in hexane extract of *Ulva rigida* by GC-MS

RT (min)	Name of the compound	Molecular formula	%
3.90	Benzaldehyde	C_7H_6O	0.65
4.08	Sabinene	$C_{10}H_{16}$	1.23
5.39	α-Terpinene	$C_{10}H_{16}$	0.16
6.06	Benzoic acid, methyl ester	$C_8H_8O_2$	0.43
6.33	Benzaldehyde dimethyl acetal	$C_9H_{12}O_2$	2.43
7.69	Terpinen-4-ol	$C_{10}H_{18}O$	0.54
9.61	Nonanal dimethyl acetal	$C_{11}H_{24}O_2$	0.4
11.85	Geranyl acetate	$C_{12}H_{20}O_2$	6.3
14.71	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	0.44
15.18	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	0.39
18.48	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	1.43
20.24	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	0.51
21.58	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	0.53
21.92	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	34.16
23.52	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	0.6
24.59	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	0.8
24.68	6-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2.19
24.76	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	2.46
25.06	Octadecenoic acid, methyl ester	$C_{19}H_{38}O_2$	24.21
27.94	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	0.75
30.29	Erucic acid	$C_{22}H_{42}O_2$	2.93
30.60	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	1.12
30.89	Mono(2-ethylhexyl) phthalate	$C_{16}H_{22}O_4$	3.98
		Total	88.64

GC-MS: Gas chromatography - Mass spectrometry

Table 2. Compounds detected in hexane extract of *Grateloupia turuturu* by GC-MS

RT (min)	Name of the compound	Molecular formula	%
4.09	Sabinene	$C_{10}H_{16}$	1.73
6.34	Benzaldehyde dimethyl acetal	$C_9H_{12}O_2$	4.73
11.86	Geranyl acetate	$C_{12}H_{20}O_2$	3.15
18.48	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	4.93
20.24	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	3.08
21.58	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	2.84
21.66	7-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	0.78
21.92	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	37.95
23.53	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	1.64
24.68	6-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.28
24.77	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	4.28
25.06	Octadecenoic acid, methyl ester	$C_{19}H_{38}O_2$	4.43
30.29	Erucic acid	$C_{22}H_{42}O_2$	1.53
30.88	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	5.86
		Total	81.21

GC-MS: Gas chromatography - Mass spectrometry

Table 3. IC_{50} values of the cytotoxicity of synthesized molecules at MCF-7, MDA-MB-231 and CCD-1079Sk cell line

IC_{50} value (µg/mL)

Extracts	Codes	CCD-1079Sk		MCF-7		MDA-MB-231	
		24h	48h	24h	48h	24h	48h
U. rigida MeOH	1	32.7±0.05	42.6±0.11	11.1±0.07	20.3±0.05	13.5±0.06	58.3±0.02
U. rigida CH_2Cl_2:MeOH (1:1)	2	27.1±0.06	36.5±0.09	12.8±0.12	14.1±0.1	10.1±0.15	31.2±3.81
G. turuturu MeOH	3	107.0±0.04	140.5±0.09	10.8±0.35	135.3±0.06	22.8±0.07	118.7±0.08
G. turuturu CH_2Cl_2:MeOH (1:1)	4	36.7±0.09	15.7±0.11	24.5±0.11	28.8±0.09	89.0±0.06	24.2±0.13
U. rigida water	5	86.8±0.06	25.8±0.09	384.7±0.05	166.6±0.02	10.7±0.77	221.5±0.1
G. turuturu water	6	15.1±0.19	9.1±0.18	79.2±0.05	69.7±0.06	54.1±0.13	17.3±0.15

According to the data obtained in this study, although antibacterial and antifungal activity was higher in methanol extracts for *Ulva rigida*, lower inhibition was observed for *S. aureus*. The MIC values for the inhibition of the methanol extract of *Ulva rigida* on *E. coli* and *C. albicans* were 125 µg/mL for both. There was also inhibition for hexane and CH_2Cl_2:MeOH (1:1) extracts of *Ulva rigida*. All of the *Grateloupia turuturu* extracts showed inhibition on *E. coli* at a concentration of 125 µg/mL, but lower on *S. aureus*. The highest antifungal activity was found in the methanol extract of *Ulva rigida* and *G. turuturu* at a concentration of 125 µg/mL (Table 5).

Hexane, CH_2Cl_2:MeOH (1:1), and MeOH extracts were investigated for the detection of AChE and BChE enzymes inhibitor activity. Due to their inability to penetrate the blood-brain barrier and their notably low AChE activities, water extracts were not included in this study of cholinesterase inhibitor activity (21). According to the results of cholinesterase enzyme inhibition experiments, 63-84% inhibition of AChE inhibition was observed in the range of 50-200 µg/mL on hexane extracts for both species. Inhibition values of control Galantamine under the same conditions were in the range of 75-84%. The results may be promising for future studies when compared with galantamine. BChE inhibition results were moderate to good only for the methanol extract of *Grateloupia turturu* (Table 6).

Discussion

The GC-MS is highly sensitive, allowing for the detection of trace amounts of compounds present in herbal extracts. This is important for identifying minor constituents that may contribute to the overall therapeutic effects or serve as markers for quality control. In prior chemical investigations, the chemical constituents of these algae were predominantly identified through GC-MS analysis of essential oils obtained via hydrodistillation. Notably, there is a dearth of literature on GC-MS analysis of hexane extracts. To address this gap, the chemical composition of hexane extracts, prepared using the maceration technique from both algae species, was determined through GC-MS analysis (12,17,18). Both hexadecanoic acid and octadecanoic acid act as energy sources for the body, as they are metabolized to produce energy needed for physical activities. Furthermore, these fatty acids are commonly incorporated into cosmetic product formulations, serving as moisturizing agents in skincare products

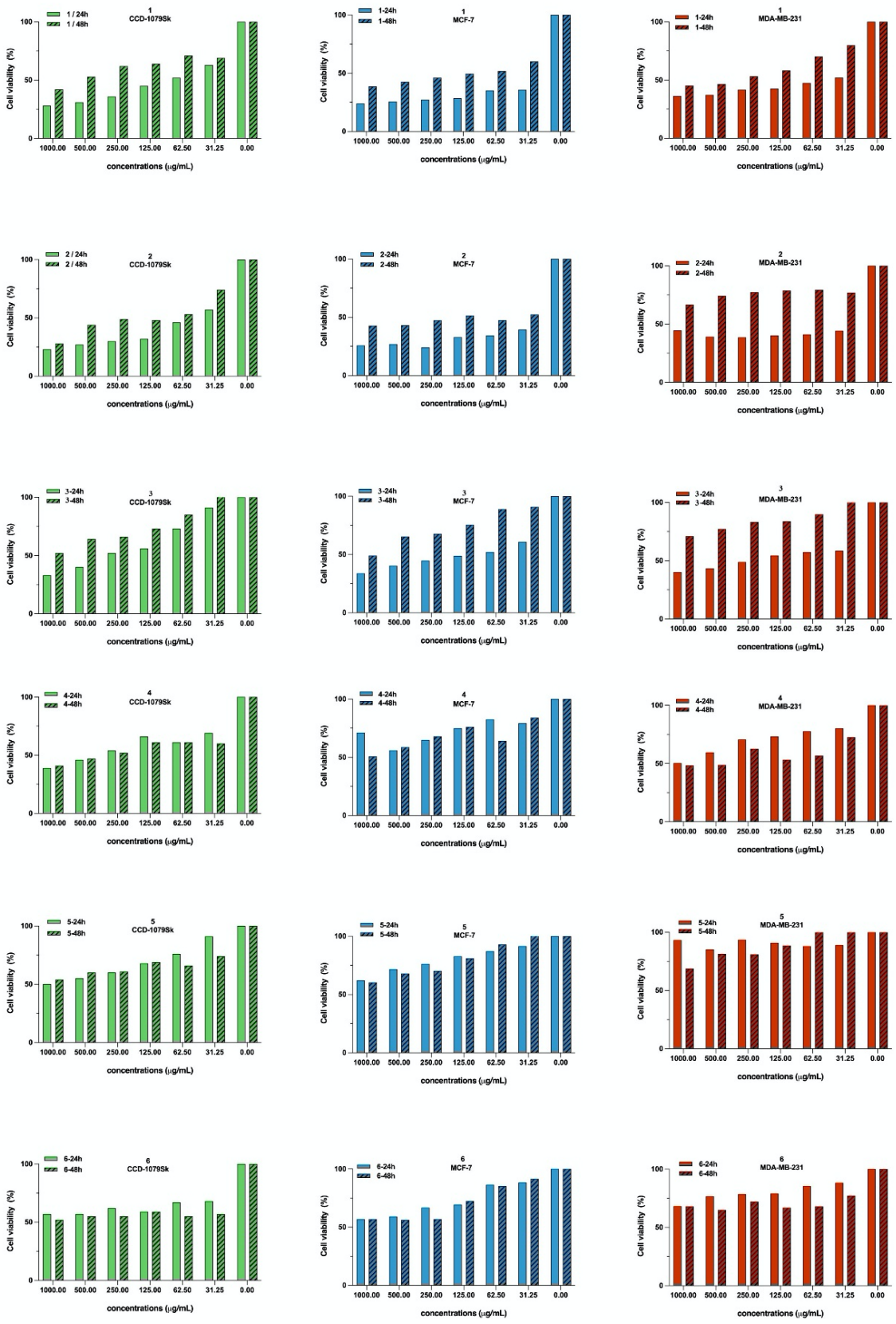

Figure 2. *In vitro* cytotoxicity of CCD-1079Sk, MCF-7 and MDA-MB-231 cells treated with extracts from *U. rigida* and *G. turuturu*. The cell viability was assessed by a MTT assay

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

and as foaming agents in soaps (8). The fact that *U. rigida* and *G. turturu* algae are rich in fatty acid composition and the major presence of hexadecanoic acid and octadecanoic acid in their composition gives importance to these algae.

The MCF-7 and MDA-MB-231 represent two distinct subtypes of breast cancer, each with unique characteristics. MCF-7 cells are estrogen receptor-positive (ER+) and typically respond to hormonal therapies, while MDA-MB-231 cells are triple-negative (ER-, PR-, HER2-) and tend to be more aggressive. Studying cytotoxic effects on both cell lines helps address the heterogeneity of breast cancer, allowing for a more comprehensive understanding of potential treatment options. Studying cytotoxic effects on breast cancer cell lines (MCF-7, MDA-MB-231) and a normal cell line (CCD-1079Sk) is important to observe any differential responses between cancerous and non-cancerous cells. This information is crucial for identifying compounds/extracts that selectively target cancer cells while sparing healthy ones.

U. rigida methanol extract (1) and *U. rigida* CH_2Cl_2:MeOH extract (2) were most toxic to cancer cells, with extract (1) showing significant cytotoxicity after 24 and 48 hours. Extracts (5) and (3) from *U. rigida* water and *G. turuturu MeOH* extracts, respectively, had minimal impact on healthy cells and the highest IC_{50} values in cancer cells after 48 hours. Statistical analysis revealed concentration-dependent effects, with significant differences in viability for extracts 1, 2, and 3 over time. Extract (3) demonstrated high selectivity against cancer cells (SI>3), maintaining consistency in efficacy at 48 hours. These findings suggest extract 3 as a promising candidate for further investigation in cancer treatment, highlighting the potential of natural compounds in therapeutic applications.

Table 4. Table of selectivity index for the two cell types

Extracts	Codes	Selectivity index			
		MCF-7		MDA-MB-231	
		24h	48h	24h	48h
U. rigida MeOH	1	2.9	2.1	2.4	0.7
U. rigida CH_2Cl_2:MeOH (1:1)	2	2.1	2.6	2.7	1.2
G. turuturu MeOH	3	9.9	1.0	4.7	1.2
G. turuturu CH_2Cl_2:MeOH (1:1)	4	1.5	0.5	0.4	0.6
U. rigida water	5	0.2	0.2	8.1	0.1
G. turuturu water	6	0.2	0.1	0.3	0.5

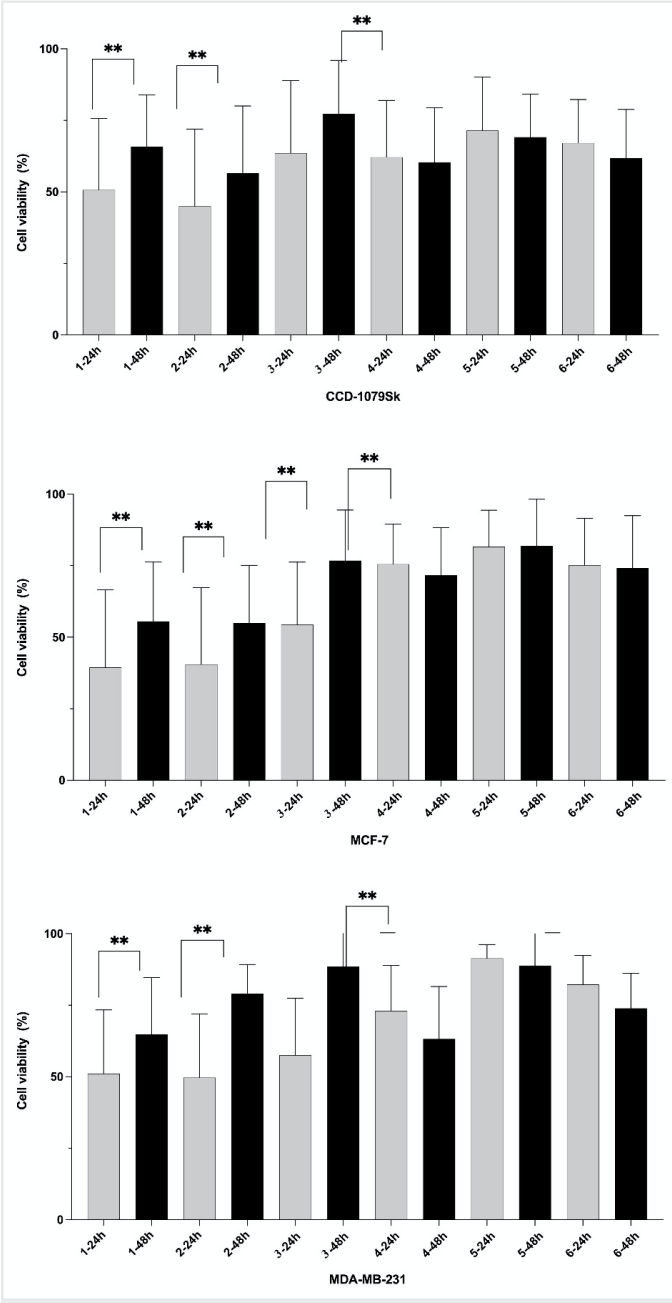

Figure 3. Cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Viability data obtained at two time points of extracts in each cell line. *p<0.05 (p-values were obtained paired t-test)

Table 5. Antibacterial and antifungal activities of *Ulva rigida* and *Grateloupia turuturu* extracts

	Ulva rigida MIC value (µg/µL)				***Grateloupia turuturu*** MIC value (µg/µL)			
	Hexane	CH_2Cl_2:MeOH (1:1)	MeOH	Water	Hexane	CH_2Cl_2:MeOH (1:1)	MeOH	Water
E. coli	125	250	125	NA	125	125	125	NA
S. aureus	250	250	250	NA	250	250	250	NA
C. albicans	250	125	125	NA	250	250	125	NA

Table 6. Acetylcholinesterase and butyrylcholinesterase inhibiton activities of *Grateloupia turuturu* and *Ulva rigida* extracts

Extracts	Inhibiton (%) Acetylcholinesterase			Inhibiton (%) Butyrylcholinesterase		
	200 µg/mL	100 µg/mL	50 µg/mL	200 µg/mL	100 µg/mL	50 µg/mL
G. turuturu Hexane	80	77	69	29	21	19
U. rigida Hexane	84	68	63	34	28	27
G. turuturu CH_2Cl_2:MeOH (1:1)	67	56	52	15	5	NA
U. rigida CH_2Cl_2:MeOH (1:1)	55	45	40	24	12	8
G. turuturu MeOH	NA	NA	NA	NA	NA	NA
U. rigida MeOH	NA	NA	NA	NA	NA	NA
Galantamine	84	80	75	79	78	68

The search for balanced foods and nutraceuticals is fueled by the growing evidence that dietary patterns can either increase or decrease the risk of developing certain cancers. Research has supported the protective effects of seaweeds against various neoplastic illnesses, including colorectal, breast, and pancreatic cancer (22). No previous research has explored the cytotoxic activities of either the *Grateloupia* species or the *Ulva* species on breast cancer. This study marks the first investigation into the cytotoxicity of both species on breast cancer cells.

In studies on the antimicrobial activity of algae, different results have been reported depending on various factors (23). According to a study investigating the effectiveness of extraction methods, it was found that methanol extraction provided higher antimicrobial activity than hexane and ethyl acetate. However, another study reported that chloroform was better than methanol and benzene (24,25). In general, it has been observed that Gram-positive bacterial strains are more sensitive to seaweed extracts than Gram-negative ones (26). This may be due to the presence of a more accessible web-like peptidoglycan layer in Gram-positive bacteria for the extracts to penetrate. Conversely, Gram-negative bacteria have a resistant barrier consisting of a thin lipopolysaccharide outer membrane that can restrict contact with the extract (27).

Methanol extracts of *Ulva rigida* exhibited higher antibacterial and antifungal activity, with notable inhibition against *E. coli* and *C. albicans* at a concentration of 125 µg/mL. However, *S. aureus* showed lower inhibition, indicating selectivity in the antibacterial effects. Interestingly, hexane and CH_2Cl_2:MeOH (1:1) extracts of *Ulva rigida* also demonstrated inhibitory effects, emphasizing the diverse bioactive compounds present in different extracts. *Grateloupia turuturu* extracts, on the other hand, exhibited inhibition on *E. coli* at 125 µg/mL, while demonstrating lower activity against *S. aureus*. Notably, the methanol extracts of both *Ulva rigida* and *Grateloupia turuturu* displayed the highest antifungal activity at a concentration of 125 µg/mL. This suggests the potential of these extracts in inhibiting fungal growth, particularly against *C. albicans.*

The observed variations in antibacterial and antifungal activities among different extracts and microbial strains highlight the complexity of the bioactive compounds present in these seaweeds. The consistent antifungal activity at a specific concentration indicates a potential therapeutic value against fungal infections. Further research is warranted to identify and isolate the specific bioactive compounds responsible for these activities and explore their potential applications in the development of antimicrobial agents. Additionally, understanding the selectivity of these extracts against different microbial strains is crucial for their targeted use in combating specific infections.

AChE and BChE play essential roles in regulating cholinergic neurotransmission by breaking down acetylcholine, ensuring proper neuronal signaling. Their balanced activity is critical for cognitive function, and alterations in AChE and BChE levels have implications in neurodegenerative diseases, emphasizing their importance in maintaining neurological health and cognitive integrity.

The investigation into AChE and BChE inhibitor activities of hexane, CH_2Cl_2-MeOH (1:1), and MeOH extracts revealed notable findings. Water extracts were excluded due to their limited ability to penetrate the blood-brain barrier and low AChE activities.

In the cholinesterase inhibition experiments, the hexane extracts of both species demonstrated substantial inhibition, ranging from 63% to 84% at concentrations between 50 and 200 µg/mL. The inhibition values for the control galantamine, under comparable conditions, fell within the range of 75% to 84%. These results suggest that the hexane extracts exhibit promising AChE inhibitory activity, comparable to the positive control galantamine. This raises the prospect of further investigations into the potential therapeutic use of these extracts in conditions associated with cholinesterase dysfunction. However, BChE inhibition results were only moderately to good for the methanol extract of *Grateloupia turuturu*. While not as pronounced as the AChE inhibition, these findings still indicate potential for the methanol extract in modulating cholinesterase activities.

The observed inhibitory effects, particularly with the hexane extracts, provide a foundation for future studies exploring the specific compounds responsible for cholinesterase inhibition and their potential neuroprotective effects. Understanding the molecular mechanisms underlying these inhibitory activities can contribute to the development of novel therapeutic agents for

conditions involving cholinergic dysfunction, such as Alzheimer's disease. Further investigations should focus on isolating and characterizing the bioactive compounds responsible for these inhibitory effects to advance the potential application of these extracts in neurodegenerative disorders.

Study Limitations

The isolation study for the master's thesis could not be conducted due to insufficient project support.

Conclusion

In this investigation, distinct from previous studies, the chemical composition of hexane extracts from *U. rigida* and *G. turuturu* was elucidated using GC-MS analysis, identifying hexadecanoic acid and octadecanoic acid as predominant metabolites. The study assessed the cytotoxic effects on previously unexplored MCF-7 and MDA-MB-231 breast cancer cell lines and examined their potential implications for Alzheimer's disease through the analysis of cholinesterase inhibitory activities. Additionally, antimicrobial activity was investigated. These results highlight the bioactive potential of the studied seaweed species, suggesting their prospective application in the development of therapeutic and dietary products. Further research could explore their applications in diverse medical and nutritional contexts, leveraging their diverse bioactive profiles. The varied compounds identified in these extracts present opportunities for future investigations and innovations in the realm of complementary medicine and beyond.

Ethics

Ethics Committee Approval: Ethics committee approval is not required.

Informed Consent: Informed consent is not required.

Authorship Contributions

Concept: G.Ö.A.T., G.T., H.Ş., Design: F.G., G.T., H.Ş., Data Collection or Processing: B.Z., G.Ö.A.T., R.S.Y., E.Ş.O., G.T., H.Ş., Analysis or Interpretation: B.Z., G.Ö.A.T., R.S.Y., F.G., H.Ö.D., G.T., H.Ş., Literature Search: B.Z., G.Ö.A.T., R.S.Y., F.G., G.T., H.Ş., Writing: B.Z., G.Ö.A.T., R.S.Y., F.G., G.T., H.Ş.

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References

1. Qin Y. 6 - Applications of Bioactive Seaweed Substances in Functional Food Products. Bioactive Seaweeds for Food Applications. 2018:111-34.
2. Ibañez E, Cifuentes A. Benefits of using algae as natural sources of functional ingredients. J Sci Food Agric. 2013;93:703-9.
3. Milinovic J, Mata P, Diniz M, Noronha JP. Umami taste in edible seaweeds: The current comprehension and perception. Int J Gastron Food Sci. 2021;23:100301.
4. McHugh DJ. A guide to the seaweed industry. Rome: Food and Agriculture Organization of the United Nations; 2003.
5. Gupta S, Abu-Ghannam N. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innovative Food Science & Emerging Technologies. 2011;12:600-9.
6. Roohinejad S, Koubaa M, Barba FJ, Saljoughian S, Amid M, Greiner R. Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. Food Res Int. 2017;99:1066-83.
7. LePape MA, Grua-Priol J, Demaimay M. Effect of Two Storage Conditions on the Odor of an Edible Seaweed, Palmaria palmata, and Optimization of an Extraction Procedure Preserving its Odor Characteristics. J Food Sci. 2002;67:3135-9.
8. Kalasariya HS, Patel NB, Yadav A, Perveen K, Yadav VK, Munshi FM, et al. Characterization of Fatty Acids, Polysaccharides, Amino Acids, and Minerals in Marine Macroalga *Chaetomorpha crassa* and Evaluation of Their Potentials in Skin Cosmetics. Molecules. 2021;26:7515.
9. Félix C, Félix R, Carmona AM, Januário AP, Dias PDM, Vicente TFL, et al. Cosmeceutical Potential of Grateloupia turuturu: Using Low-Cost Extraction Methodologies to Obtain Added-Value Extracts. Appl Sci. 2021;11:1650.
10. da Costa E, Melo T, Reis M, Domingues P, Calado R, Abreu MH, et al. Polar Lipids Composition, Antioxidant and Anti-Inflammatory Activities of the Atlantic Red Seaweed Grateloupia turuturu. Mar Drugs 2021;19:414.
11. Sánchez-García F, Mirzayeva A, Roldán A, Castro R, Palacios V, García-Barroso C, et al. Evolution of volatile compounds and sensory characteristics of edible green seaweed (Ulva rigida) during storage at different temperatures. J Sci Food Agric. 2019;99:5475-82.
12. Roussis V, Rapti T, Vagias C, Harvala C, Caberi H. Volatile Metabolites of the Green Alga Ulva rigida(C.Ag). J Essent Oil Res. 2000;12:201-4.
13. Olasehinde TA, Olaniran AO. Antiproliferative and apoptosis-inducing effects of aqueous extracts from Ecklonia maxima and Ulva rigida on HepG2 cells. J Food Biochem. 2022;46:e14498.
14. Celikler S, Tas S, Ziyanok-Ayvalik S, Vatan O, Yildiz G, Ozel M. Protective and antigenotoxic effect of Ulva rigida C. Agardh in experimental hypothyroid. Acta Biol Hung. 2014;65:13-26.
15. García-Márquez J, Moreira BR, Valverde-Guillén P, Latorre-Redoli S, Caneda-Santiago CT, Acién G, et al. In Vitro and In Vivo Effects of Ulvan Polysaccharides from Ulva rigida. Pharmaceuticals (Basel). 2023;16:660.
16. Trigui M, Gasmi L, Zouari I, Tounsi S. Seasonal variation in phenolic composition, antibacterial and antioxidant activities of Ulva rigida (Chlorophyta) and assessment of antiacetylcholinesterase potential. J Appl Phycol. 2013;25:319-28.
17. Yücel TB, Korkmaz B, Cebeci T, Karaman Ü, Arici YK, Fandakli S, et al. Analysis of Volatile Constituent by Hydrodistillation and Solid-Phase Microextraction Techniques and Antimicrobial and Scolicidal

Activities of Essential Oil and Soxhlet Extracts of Ulva rigida grown in Turkey. Chem Biodivers. 2023;20:e202300965.

18. Erol E, Orhan MD, Avsar T, Akdemir A, Okudan ES, Alim Toraman GO, Topcu G. Anti-SARS-CoV-2 and cytotoxic activity of two marine alkaloids from green alga Caulerpa cylindracea Sonder in the Dardanelles. RSC Advances. 2022;12(46):29983-29990.
19. Bézivin C, Tomasi S, Lohézic-Le Dévéhat F, Boustie J. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. Phytomedicine. 2003;10:499-503.
20. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88–95.
21. Chuah EL, Zakaria ZA, Suhaili Z, Bakar SA, Desa MM. Antimicrobial activities of plant extracts against methicillin-susceptible and methicillin-resistant Staphylococcus aureus. J Microbiol Res. 2014;4:6-13.
22. Tokudome S, Kuriki K, Moore MA. Seaweed and Cancer Prevention. Jpn J Cancer Res. 2001;92:1008-9.
23. Elnabris KJ, Elmanama AA, Chihadeh WN. Antibacterial activity of four marine seaweeds collected from the coast of Gaza Strip, Palestine. Mesopot J Mar Sci. 2022;28:81-92.
24. Sastry VMVS, Rao GRK. Antibacterial Substances from Marine Algae: Successive Extraction Using Benzene, Chloroform and Methanol. Botanica Marina. 1994;37:357-60.
25. Sukatar A, Karabay-Yavaşsoglu NU, Ozdemir G, Horzum Z. Antimicrobial activity of volatile component and various extracts ofEnteromorpha linza (Linnaeus) J. Agardh from the coast of Izmir, Turkey. Ann Microbiol. 2006;56:275-9.
26. Magallanes C, Córdova C, Orozco R. Actividad antibacteriana de extractos etanólicos de macroalgas marinas de la costa central del Perú. Rev Peru Biol. 2003;10:125-32.
27. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010;21:1199-218.