

CHEMICAL COMPOSITION, SCOLICIDAL EFFECTS AND  
ANTIMICROBIAL ACTIVITIES OF GREEN MACROALGA  
*ULVA INTESTINALIS*

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**Abstract**

The aim of the study is to investigate the antimicrobial, biological, and scolicidal activities of *Ulva intestinalis* (*U. intestinalis*), which is one of the green algae. *U. intestinalis* was collected in appropriate amounts and some of the samples were distilled with water vapour to extract their essential oils, and some of them were extracted by Soxhlet extraction with hexane and dichloromethane. The values of lethal concentration doses (LD<sub>50</sub> and LD<sub>90</sub>) were calculated using the probit analysis. Antibacterial and scolicidal effects of the essential oil and solvent extracts of *U. intestinalis* against gram-positive, gram-negative and yeast microorganisms were studied. It was determined that essential oil and solvent extracts of *U. intestinalis* may have scolicidal effects. The highest scolicidal activity was observed in 15 000 µg/ml in all organic solvents. Antimicrobial activity was observed for *U. intestinalis*, with high potency with hexane extract against *Enterobacter aerogenes* and with dichloromethane extract against *Enterococcus faecalis* (inhibition zone  $\geq 12$  mm). It is suggested that controlled in vivo studies should be carried out in order to use this alga as a bactericidal and scolicidal medication.

**Key words:** *Ulva intestinalis*, antibacterial, scolicidal, probit

**Introduction.** Algae are high in vitamins A, B1, B2, B6, and C, as well as minerals such as niacin, iodine, potassium, iron, magnesium, and calcium, trace elements (Ga, Zn, Ni, Co, Fe, Mn, Ca, Cr, B, Na, Mg, Al, F, and K), and fatty

acids. Algae contribute significantly to the marine ecosystem by producing their own food via photosynthesis and serving as the first link in the food chain in the nutrition of sea creatures such as shrimp and jellyfish by supplying oxygen to water [1].

Essential oils are aromatic substances produced by plants or herbal sources that can easily volatilize at room temperature and are found abundantly in roots, stems, leaves, fruits, bark, and flowers. They are liquid at room temperature and can easily crystallize. Different studies have been conducted on essential oil analyses and the anticancer, antileukemic, antioxidant, antimicrobial, antifouling, antimalarial, and cytotoxic activities of algae [2-4].

Green macroalgae, mostly represented by Ulvophyceae, are among the important primary producers of marine and brackish coastal ecosystems [5]. It is well known that extracts of green algae such as *Ulva* spp. have many bioactive compounds [6]. It has been determined that the chemical composition of these macroalgae changes depending on the geographical distribution and seasons, and water temperature, salinity, light, nutrients, and mineral presence are the leading environmental factors affecting the composition [7].

In addition to dietary fibres, proteins, minerals, and vitamins, marine algae are a good source of polysaccharides, polyphenols, phytochemicals, and polyunsaturated fatty acids, some of which have potential therapeutic uses against inflammation, cancer, oxidative stress, allergies, diabetes, thrombosis, obesity, hypertension, lipidemia, and a wide variety of degenerative diseases [8]. There are studies on the use of algae, especially in anticancer research [9,10].

The aim of this study was to investigate the biological activities and scolicidal effects of chloroform, dichloromethane, hexane, and methanol, organic solvents of *U. intestinalis* using the essential oils of this alga collected from the vicinity of Sinop province, which is located on the Black Sea coastline.

**Materials and methods. Sample collection.** *U. intestinalis* samples were collected from the Karakum location in Sinop province in May 2018. The algae were preserved in plastic bottles and transferred via a cold chain to the laboratory.

**Preparation of different extracts.** Seaweed was rinsed with seawater to remove sand particles and cleansed of any remaining organisms. The seaweed material was dried in the shade at room temperature. They were then cut into smaller pieces and sliced using a mixer in the laboratory. After weighing out 30 grams of dried *U. intestinalis* parts, they were loaded into Soxhlet cartridges and placed in the reflux. For the soxhlet extraction, 250 mL of chloroform (Ch), dichloromethane (DCM), hexane (H), and methanol (MeOH) were added to each flask and left at room temperature for 24 h. After reaching a final solution, it evaporated. The leftover residue (3.2%, 2.5%, 2.5%, and 3.8%, respectively) was measured and kept at +4 °C until the results of the biological activity tests could be analyzed. We only ever utilized Sigma-Aldrich solvents, which were guaranteed

to be between 95% and 98% pure.

**Gas chromatography-mass spectrometry (GC-MS/FID).** The gas chromatography chromatography-flame ionization detector (GC-FID) analysis was carried out on a Shimadzu QP2010 plus gas chromatography coupled to a Shimadzu QP2010 ultra mass selective detector. Aromatic chemicals isolated from fibres were loaded into the GC-MS injector (split mode). With a 30 m long, 0.25 mm inner diameter, and 0.25 m phase thickness Restek Rxi-5MS capillary column, separation was achieved. The schedule for the oven started with an initial temperature of 60 °C for 2 min, which was raised to 240 °C for 3 min, and then to 250 °C for 4 min. Temperature of the injector was 280 °C, and the split ratio was 1:20. The carrier gas was helium at a constant flow rate of 1 mL/min, and the sample volume was 1 L. The detection method was electronic impact (EI), the ionization voltage was set at 70 eV, and the mass collection mode was scan (40–450 m/z).

**Analysis of components.** The retention indices (RI) of the components were determined with n-alkanes (C6-C30) using the Koyats method. Comparison of retention indices of the volatile components (relative to C6-C30 alkane standards) was made by comparing the mass spectra of the two libraries (FFNSC1.2 and W9N11).

**Antimicrobial analysis.** Antimicrobial activity was performed using the disc diffusion method as described by BERBER et al. [11]. For antimicrobial activity, a total of 20 bacterial species were used. Gram-positive bacteria included *Bacillus (B.) subtilis* ATCC 6633, *B. cereus* ATCC 10876, *B. megaterium* DSM32, *Staphylococcus (S.) aureus* ATCC 29213, *S. aureus* ATCC 25923, *Enterococcus (E.) faecalis* ATCC 29212, Metisillin Resistant *Staphylococcus aureus* (MRSA) ATCC 67101, MRSA 27R, *Staphylococcus (S.) epidermidis* ATCC 12228 and Gram-negative bacteria included *Cedecea (C.) neteri* ATCC 33855, *Escherichia (E.) coli* ATCC 25922, *E. coli* ATCC 36218, *Pseudomonas (P.) aeruginosa* ATCC 27853, *P. aeruginosa* ATCC 9027, *Klebsiella (K.) pneumoniae* ATCC 13883, *Acinetobacter (A.) baumannii* ATCC BAA-747, *Enterobacter (E.) aerogenes* ATCC 13048, *Citrobacter (C.) freundii* ATCC 43864, *Salmonella typhimurium* ATCC 14028, and *Proteus (P.) mirabilis* ATCC 43071. *Candida (C.) albicans* (ATCC 10231) was used for antifungal activity. In order to measure the antimicrobial effects of the Ch, MeOH, H, and DCM extracts of *U. intestinalis*, the microorganisms tested were inoculated in Mueller-Hinton broth and the yeasts in Sabouraud dextrose broth and were left for incubation at 37 °C for 18 h. Turbidity measurements were adjusted to McFarland No. 0.5 with a densitometer. A hundred µl of liquid broth adjusted to McFarland No. 0.5 under sterile conditions was placed on all Mueller-Hinton agar broths and distributed equally to the petri dishes with a sterile swab. Sterile blank discs (6 mm) were impregnated with 15 and 30 µL of extracts prepared in different solvents. The prepared discs were put into Petri dishes, and the microorganisms were added. They were

then left to grow at 37 °C for 24 h. The discs' zone diameters were measured, and the values were recorded in millimeters. As controls, conventional antibiotic discs of tobramycin (Bioanalyse, 10 g/disc), nystatin (Bioanalyse, 30 g/disc), and vancomycin (Bioanalyse, 30 g/disc) were utilized.

**Scolicidal analysis.** A 0.1% eosin solution was used to test the survivability of protoscolices isolated from a cow liver cyst hydatid. Depending on where the cyst hydatid is located, percutaneous procedures may be used to treat it. In these procedures, scolicial drugs may be utilized. 20% or 30% hypertonic saline, 95% ethanol, and 0.5% cetrimide are examples of scolicial agents. Percutaneous therapies, according to researchers, are both safe and effective. The solution was dissolved in sterile saline, its final concentration was adjusted to 15 000 µg/mL, and it was homogenized and sterilized by filtering the membrane. Sterile saline in the volume of 200 µL was added to 10 wells of the sterile microplate. Two hundred µL of *U. intestinalis* extracts were then added to the wells and diluted at 1/2 ratio. After serial dilution, a concentration of 3500 pieces per mL of 200 µL protoscolices was added to the wells. As a control, only 200 µL of protoscolices dilution were added to wells 9 and 10. The viability rates of protoscolices were determined by counting at certain times. Each count was performed twice, and the results were averaged. A NaCl solution of 30%, which is frequently used in routine practice, was used as the scolicial agent [12].

**Statistical analysis.** The data set's descriptive statistics were expressed as mean standard deviation (SD). The differences between means were compared by the one-way Kruskal–Wallis test followed by Dunn's post-hoc test. The values of lethal doses (LD50 and LD90) were determined using probit analysis for certain times. A *p*-value of  $\leq 0.05$  was considered statistically significant. All statistical analyses were performed using the SPSS v. 26 (IBM Inc., Chicago, IL, USA) and Minitab 19 (Minitab Inc., State College, PA, USA) statistical softwares.

**Results and discussion.** Essential oils are the primary active ingredients in many different products, and their biological properties have been known since ancient times. When it comes to biological qualities and medical applications, essential oils (EOs) are among the most significant natural compounds obtained from plants. A wide variety of sectors, including medical, pharmaceuticals, perfumery, cosmetics, agriculture, and the food industry, have made use of essential oils [13]. Volatile organic compounds (VOCs) are among the bioactive chemicals generated by macroalgae that have been linked to significant biological activity [14]. A variety of volatile metabolites, including hydrocarbons, ketones, aldehydes, alcohols, carboxylic acids, esters, halogenated chemicals, sulphur compounds, furans, pyrazines, pyridines, amines, and others, are found in seaweeds [15]. Hydrocarbons are the greatest class of volatile organic compounds included in essential oils [14]. Terpenes are basic hydrocarbons, however, terpenoids are a family of modified terpenes that include a variety of distinct functional groups. A broad variety of chemicals, including terpenes, polyphenolic compounds, and steroids,

have been documented to come from different types of marine green algae. The vast majority of these compounds are terpenoids [16]. The amount of oil obtained as a result of steam distillation of *U. intestinalis* with a cleverger apparatus was weighed as 112.9 mg. In the essential oil analysis of *U. intestinalis*, 11 compounds were identified and 80.02% of them were clarified. Hydrocarbon and alcohol class compounds are predominantly (51.69% and 28.10%) found in *U. intestinalis*. Pentanol was found by 28.10% and methyl cyclopentane by 18.85% in *U. intestinalis* (Table 1). Terpene and terpenoid class compounds (heptadecane and hexahydrofarnesyl acetone) were found by 6.34% and 0.082%, respectively. Our results are in agreement with a study by NAZARUDIN et al. [4] in Malaysia, where heptadecene, and octadecene were detected in 0.66% and 0.79% of *U. intestinalis*, respectively. In contrast, another Malaysian report [17] has identified neophytadiene (terpene, % 2.60) in the essential oil of *U. intestinalis*. The chemical composition of *Ulva intestinalis* varies depending on a number of variables, such as plant species, growth stage, season, geographical origin, and extraction technique.

In the present study, it was thought that hydrocarbon (51.69%) and alcohol (28.10%) class compounds in the structure of *U. intestinalis* may have a scolicidal effect. Accordingly, it was determined that the extract of algae in dichloromethane and hexane organic solvents decreased over time. It was also determined that a dose of 15 000 µg/ml in all organic solvents had a strong scolicidal effect. Similarly, researches were conducted with different substances (formol, hypertonic glucose solution, alcohol, hypertonic NaCl [3%, 10%, 20%], chlorhexidine, cetrimide, AgNO<sub>3</sub>, povidoniodine, alcohol, 3% H<sub>2</sub>O<sub>2</sub>, albendazole solution, iodine and compounds) as scolicidal agents. In addition, OZÇELİK et al. [18] reported that *Allium sativum* has a scolicidal effect. KARAMAN et al. [12] investigated the scolicidal effect of hexane, dichloromethane, chloroform and methanol extract of *Cystoseira barbata* alga. They showed that hexane extract of *C. barbata* alga showed a high scolicidal activity in vitro. In this study, the effect of *U. intestinalis* extracts prepared in hexane and dichloromethane organic solvents on protoscolex showed that it could be used as a scolicidal agent. No live parasites were observed in *U. intestinalis* in hexane organic solvent of *U. intestinalis* at 6th hour and in dichloromethane solvent at 4th hour (Table 2). According to the statistical analysis, it was determined that the application dose rates showed a significant change over time ( $p < 0.05$ ). In this study, LD50 and LD90 values were also examined by probit analysis (Table 3), and according to the results obtained, it was thought that *U. intestinalis* could also be used as a scolicidal agent.

Due to their considerable potential as antibacterial, anti-inflammatory, antiviral, and anti-tumour medications, a number of species of seaweed have generated a new area of research in biomedicine [9]. In the presented study, it was found that *U. intestinalis* extracts generally showed very low zone values, and the highest zone value of these algae extracts was 14 mm. The smallest zone was determined as 6.5 mm. As given in Table 4, it was determined that *U. intestinalis* extracts

T a b l e 1

Essential oil analysis values of *U. intestinalis*

No	Retention Time	Compound	<i>U. intestinalis</i> % Area <sup>b</sup>	Experi- mental RI <sup>a</sup>	Experi- mental RI <sup>b</sup>	Litera- ture RI
1	5.124	2-methyl pentane	17.938	–	565	563
2	5.229	3-methyl pentane	12.895	–	576	579
3	5.682	Methyl-cyclopentane	18.850	–	627	622
4	6.385	Pentanol	28.101	–	767	771
5	6.446	1-Octene	0.345	–	795	792
6	8.524	Hexanal	0.061	–	801	802
7	11.745	Heptanal	–	904	–	906
8	13.717	5-methyl-2-furfural	–	962	–	965
9	15.302	Furan-2-pentyl	0.103	998	991	999
10	15.752	Octanal	0.083	–	1002	999
11	16.153	(2 <i>E</i> ,4 <i>E</i> )-Heptadienal	–	1011	–	1012
12	18.114	2 <i>E</i> -Octenal	–	1046	–	1049
13	19.654	3,5-Octadiene-2-one	–	1072	–	1076
14	20.062	Nonanal	–	1101	–	1101
15	21.897	Pinocarveole	–	1134	–	1135
16	22.222	(2 <i>E</i> ,6 <i>Z</i> )- Nonadienal	–	1151	–	1150
17	22.477	2 <i>E</i> -Nonenal	–	1157	–	1157
18	23.997	$\alpha$ -Terpineol	-	1192	-	1191
19	25.324	$\beta$ -cyclocitral	–	1224	–	1220
20	29.089	(2 <i>E</i> ,4 <i>E</i> )-Decadienal	–	1314	–	1315
21	30.858	Decanoic acid	–	1359	–	1364
22	32.229	Tetradecane	–	1403	–	1400
23	32.511	Dodecanal	–	1404	–	1408
24	33.653	$\alpha$ - ionone	–	1429	–	1426
25	34.417	Nevil acetone	–	1439	–	1435
26	35.907	Trans- $\beta$ -ionone	–	1488	–	1487
27	36.000	Pentadecan	0.914	–	1499	1500
28	36.549	Tridecanal	–	1505	–	1509
29	38.515	Dodecanoic acid	–	1568	–	1565
30	40.242	Tetradecanal	–	1607	–	1611
31	42.517	Heptadecane	0.650	1682	1685	1680
32	43.798	Pentadecanal	–	1710	–	1710
33	45.540	Tetradecanoic acid	–	1762	–	1763
34	48.031	Hexahydrofarnesyl acetone	0.082	1848	1847	1847
35	49.689	(1 <i>E</i> ,4 <i>E</i> ,8 <i>E</i> )-Dodecatriene	–	1891	–	1890
36	51.311	9-Hexadecanoic acid	–	1943	–	1948
37	52.160	n-Hexadecanoic acid	–	1962	–	1966
38	56.731	Cis,cis- Linoleic acid	–	2128	–	2131
39	57.120	Oleic acid	-	2152	–	2152
		Total	80.02%			

Table 2

Time comparison of the viability rates in the hexane, dichloromethane and NaCl extract of *U. intestinalis* prepared at different concentrations

Hour	Control	Hexane extract										15000 mg/ml Mean	<i>p</i> *
		117 mg/ml Mean	234 mg/ml Mean	468 mg/ml Mean	937 mg/ml Mean	1875 mg/ml Mean	3750 mg/ml Mean	7500 mg/ml Mean					
1	100.00a	75.72Ab	61.43Ac	52.86Ad	45.71Ae	38.57Ae	34.29Ae	27.14Ag	17.14Ah				0.031
2	100.00a	70.00ABb	58.57ABc	50.00ABd	42.86ABe	35.72Af	30.00ABfg	24.29Ag	14.2B9h				0.031
3	100.00a	60.00BCb	55.72ABb	44.29BCc	38.57ABCcd	32.86ABde	25.71BCef	21.43ABf	10.00Cg				0.032
4	100.00a	55.72CDb	52.86BCb	41.43Cc	35.72BCc	27.14BCd	22.86Cd	15.72BCe	5.71Df				0.032
5	100.00a	50.00CDb	47.14CDb	38.57Cc	32.86CDc	24.29CDd	21.43CDd	10.00CDe	2.86Ef				0.032
6	100.00a	45.72DEb	41.43DEb	28.57Dc	27.14DEc	21.43CDEcd	17.14DEd	4.29DEe	0.00Fe				0.032
7	100.00a	37.14EFb	34.28EFb	25.71Dc	24.29EFc	18.57DEFcd	14.29EFd	2.86Ee	0.00Fe				0.033
8	100.00a	32.86FGb	28.57FGbc	24.29Dcd	21.43EFde	15.72EFGef	10.00Ff	0.00Eg	0.00Fg				0.032
9	100.00a	28.57FGb	25.71Gbc	21.43DEcd	18.57FGd	12.86FGe	4.29Gf	0.00Ef	0.00Ff				0.031
10	100.00a	25.71Gb	22.86Gb	15.72Ec	12.86Gcd	10.00Gd	0.00Ge	0.00Ee	0.00Fe				0.032
<i>p</i> *	-	0.027	0.027	0.028	0.028	0.030	0.026	0.027	0.025				

  

Hour	Control	Dichloromethane extract										15000 mg/ml Mean	<i>p</i> *
		117 mg/ml Mean	234 mg/ml Mean	468 mg/ml Mean	937 mg/ml Mean	1875 mg/ml Mean	3750 mg/ml Mean	7500 mg/ml Mean					
1	100.00a	61.43Ab	55.72Ab	47.14Ac	41.43Ac	35.72Ade	30.00Ae	22.86Af	12.86Ag				0.031
2	100.00a	60.00ABb	54.29ABb	44.29ABc	35.72ABd	32.86ABd	28.57ABde	21.43Ac	11.43Af				0.032
3	100.00a	55.72ABCb	52.86ABb	41.43ABc	32.86BCd	30.00ABCde	25.71ABce	18.57ABf	7.14Ag				0.032
4	100.00a	52.86BCDb	50.00ABb	38.57ABCc	30.00BCDd	27.14BCDd	24.29BCd	17.14ABe	0.00Bf				0.033
5	100.00a	50.00CDEb	47.14BCb	35.72BCDc	28.57BCDEd	25.71BCDEde	22.86CDe	14.29BCf	0.00Bg				0.031
6	100.00a	47.14DEb	41.43CDb	31.43CDEc	25.71CDEfcd	24.29CDEcd	18.57DEde	12.86BCe	0.00Bf				0.032

Table 2  
Continued

Dichloromethane extract																		
Hour	Control	117		234		468		937		1875		3750		7500		15000		p*
		mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	
7	100.00a	44.29EFb	35.72DEc	27.14DEFd	24.29DEFd	21.43DEFde	15.72EFef	14.29EFd	11.43FGf	10.00Gde	7.14Ge	0.028	0.028	0.00Bg	0.00Be	0.00Bf	0.028	0.032
8	100.00a	38.57FGb	32.86Eb	24.29EFc	21.43EFGc	18.57EFcd	15.72FGef	14.29EFd	11.43FGf	10.00Gde	7.14Ge	0.028	0.028	0.00Bg	0.00Be	0.00Bf	0.028	0.032
9	100.00a	35.72Gf	30.00EFc	22.86EFd	18.57FGde	15.72FGef	14.29EFd	11.43FGf	10.00Gde	7.14Ge	0.028	0.028	0.028	0.00Bf	0.00Bf	0.00Bf	0.028	0.031
10	100.00a	27.14Hb	24.29Fb	21.43Fbc	15.72Gcd	10.00Gde	0.031	0.029	0.029	0.031	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.033
p*																		
NaCl extract																		
Hour	Control	117		234		468		937		1875		3750		7500		15000		p*
		mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	mg/ml	Mean	
1	100.00a	68.57Ab	65.72Ab	64.29Ab	48.57Ac	42.86Acd	37.14Acd	32.86Ad	25.72Be	21.43Bfe	18.57Be	15.72Ae	15.72Ae	10.00Bf	10.00Bf	10.00Bf	10.00Bf	0.039
2	100.00a	61.43ABb	54.29ABbc	50.00ABbcd	44.29ABbcd	38.57ABd	31.43ABCd	25.72Be	20.00BCe	18.57Be	15.72Ae	15.72Ae	15.72Ae	10.00Bf	10.00Bf	10.00Bf	10.00Bf	0.034
3	100.00a	52.86BCb	50.00BCb	44.29BCbc	40.00ABCd	34.28BCbc	25.71CDc	22.86BCDcd	20.00BCe	18.57Be	15.72Ae	15.72Ae	15.72Ae	10.00Bf	10.00Bf	10.00Bf	10.00Bf	0.033
4	100.00a	50.00BCDd	47.14BCb	38.58BCDbc	34.28BCbc	32.86CDEbc	25.71CDc	22.86BCDcd	20.00BCe	18.57Be	15.72Ae	15.72Ae	15.72Ae	10.00Bf	10.00Bf	10.00Bf	10.00Bf	0.034
5	100.00a	44.29CDb	41.43CDb	32.86CDEbc	25.71CDc	22.86CDEbc	20.00BCe	18.57Be	15.72Ae	15.72Ae	15.72Ae	15.72Ae	15.72Ae	10.00Bf	10.00Bf	10.00Bf	10.00Bf	0.033
6	100.00a	37.14DEb	30.00DEbc	25.72DEFbcd	21.43CDcde	18.57CDEcd	15.72Ae	12.86DEFef	10.00DEde	7.14DEFef	0.028	0.028	0.028	0.00Ce	0.00Ce	0.00Ce	0.028	0.035
7	100.00a	28.57Eb	24.29EFbc	20.00EFbc	17.15Dcd	15.72Ae	12.86DEFef	10.00DEde	7.14DEFef	0.028	0.028	0.028	0.028	0.00Cf	0.00Cf	0.00Cf	0.028	0.034
8	100.00a	24.29Eb	14.29Fc	14.29FGc	12.86DEcd	7.14DEde	0.028	2.86EFef	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Cb	0.00Cb	0.00Cb	0.028	0.036
9	100.00a	0.00Fb	0.00Gb	0.00Gb	0.00Eb	0.00Eb	0.00Eb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Cb	0.00Cb	0.00Cb	0.028	0.030
10	100.00a	0.00Fb	0.00Gb	0.00Gb	0.00Eb	0.00Eb	0.00Eb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Fb	0.00Cb	0.00Cb	0.00Cb	0.028	0.030
p*																		

\*: Kruskal-Wallis test

Means that do not share a letter are significantly different ( $p < 0.05$ )



T a b l e 3

Lethal concentrations (LD50-90) of hexane, dichloromethane, and NaCl in 1 h

		Estimate	95% Confidence limits	
Hexane	LD <sub>50</sub>	3163.40	778.60	5823.70
	LD <sub>90</sub>	14931.00	10416.50	28511.40
Dichloromethane	LD <sub>50</sub>	24400.42	18735.78	38442.34
	LD <sub>90</sub>	11228.11	8516.42	15769.82
NaCl	LD <sub>50</sub>	22533.12	18802.90	29313.41
	LD <sub>90</sub>	11168.18	9279.13	13637.24

T a b l e 4

Antimicrobial effect of *U. intestinalis* on the studied microorganisms

Microorganisms	Dichloro- methane mg/disc		Hexane mg/disc		10 Tob	Standard mg/disc	
	20		20			30	30
	15 µl	30 µl	15 µl	30 µl		Nys	Va
<i>S. aureus</i> ATCC 25923	–	–	–	7	20	–	–
<i>S. aureus</i> ATCC 29213	–	–	–	7	20	–	–
<i>S. epidermidis</i> ATCC 12228	–	9	–	–			–
MRSA ATCC 67101	–	–	–	–	–		17
MRSA 27R	–	–	–	7			17
<i>Bacillus cereus</i> ATCC 10876	–	6.5	7	8	22		–
<i>Bacillus megaterium</i> DSM32	–	–	–	10	18	–	–
<i>Bacillus subtilis</i> ATCC 6633	–	–	–	–	18	–	–
<i>Enterococcus faecalis</i> ATCC 29212	–	12	–	11	16	–	–
<i>Pseudomonas aeruginosa</i> ATCC 27853	–	7	–	7	18	–	–
<i>Pseudomonas aeruginosa</i> ATCC 9027	–	8	–	8	20	–	–
<i>E. coli</i> ATCC 25922	–	6.5	6.5	6.5	20		–
<i>E. coli</i> ATCC 36218	–	–	–	–	20	–	–
<i>Klebsiella pneumoniae</i> ATCC 13883	–	8	–	9	19	–	–
<i>Acinetobacter Baumannii</i> ATCC BAA-747	–	7	–	7	22	–	–
<i>Enterobacter aerogenes</i> ATCC 13048	–	8	–	14	17	–	–
<i>Citrobacter freundii</i> ATCC 43864	–	–	–	7	20	–	–
<i>Cedecea neteri</i> ATCC 33855	–	–	–	7	18		–
<i>Salmonella typhi</i> ATCC 14028	–	–	–	–			–
<i>Proteus mirabilis</i> ATCC 43071	–	–	–	–	19	–	–
<i>Candida albicans</i> ATCC 10231	–	–	–	6.5	–	24	–

could show antimicrobial activity against *B. cereus*, *B. megaterium*, *S. aureus*, *S. epidermidis*, *MRSA 27R*, *E. faecalis*, *P. aeruginosa*, *E. coli* ATCC 25922, *K. pneumoniae*, *A. baumannii*, *E. aerogenes*, *C. neteri*, *C. freundii*, and *C. albicans*

strains. Berber et al. [11] prepared methanolic extracts by drying the seaweeds of *U. intestinalis* collected from Sinop coast in open air and mixing the samples in methanol in a water bath for 24 h. The antioxidant and antimicrobial activities of the extracts they obtained were examined. They found that *U. intestinalis* showed activity between 10–15 mm zone values. Similarly, in the presented study, the zone of *U. intestinalis* methanol extract was determined as 7 mm. Again, in their antimicrobial activity study on *U. intestinalis* collected from Pakistan, RIZWAN et al. [19] found that *U. intestinalis* methanol, chloroform, ethanol and n-hexane extracts gave effective values on *Shigella* spp., and no antimicrobial activity was recorded against *S. aureus* and *S. epidermidis*. Also, SIRBU et al. [20] prepared ethanol extract of *U. intestinalis* in algae samples collected from Romania in 24 h through continuous stirring and found a zone diameter value of 16.3 mm on *E. coli* ATCC 1053, and 7.8 mm on *S. aureus* ATCC 6538P as antimicrobial activity results.

**Conclusion.** The results of the current study showed that hexane and dichloromethane extracts of *U. intestinalis* may offer potential for use as antibacterial and scolicidal effect. It was determined that the dose of LD90 could be increased for a faster effect and decreased for a slower effect. GC-MS analysis of essential oil revealed the presence of hydrocarbons, and alcohol class compounds. This research draws attention to the need for more evaluation of the biological studies and chemical constituents of Turkish green algae *U. intestinalis*. This tested seaweed offers opportunities for producing new types of bioactive compounds. Accordingly, it was proposed that controlled experiments should be carried out in vivo in order to use *U. intestinalis* as a medicine in living cells, and more research will need to be done to confirm these results by testing the different extracts as a new scolicidal and antimicrobial agent in a clinical setting.

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