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Antioxidant and Antimicrobial Activities of *Codium fragile* (Suringar) Hariot (Chlorophyta) Essential Oil and Extracts

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Macroalgae are rich sources of natural bioactive products. Their secondary metabolites are attracted attends in many fields of life *i.e.*, food, animal feeding, textile, cosmetics, medicine *etc.* In this study, methanol, dichloromethane and hexane extracts of *Codium fragile* (Chlorophyta) were tested for antimicrobial and antioxidant activities. Antioxidant effects were evaluated by hydroxyl radical scavenging assay (deoxyribose degradation assay) and β-carotene bleaching assay. The extracts indicated relatively little antioxidant activities, as compared to commercial antioxidants. The composition of the essential oil of *C. fragile* was also analyzed by GC and GC-MS. Twenty four compounds were identified of *C. fragile* essential oil and *n*-tricosane (11.88 %) was determined as major component. Essential oil of *C. fragile* showed weak antibacterial activity against all gram positive bacteria tested, except methicillin-oxacillin resistant *Staphylococcus aureus* ATCC 43300. Similarly, all the extracts of *C. fragile* showed weak antimicrobial activity on tested organisms.

Key Words: Macroalgae, *Codium fragile*, Essential oil, Extracts, Composition, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

Seaweeds have been used since ancient times as food, fodder and fertilizer especially in Asian countries because of their high content of essential and free amino acids, also utilized as sources of medicine (*e.g.* for the treatment of diarrhea, cough and fever) and vitamins[1]. In Western countries, seaweeds are mainly use as sources of alginate, carrageenan and agar in addition to ingredients in the content of many beauty products. The greatest use of seaweeds in the worldwide is for food, most probably by reason of rich in non-digestible fibers, mineral salts, vitamins and protein, but low in fat content[2-4]. Furthermore, marine algae are potential suppliers of fatty acids such as polyunsaturated fatty acids (PUFA), sterols and hydrocarbons which are found in marine sediments[5,6].

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As known, recent researches were focused on important bioactive chemicals identified in macroalgae and described the range of biological and pharmacological activities[7]. Antibiotic activity is fairly common in macroalgae that synthesize bioactive secondary metabolites. Several extractable compounds, such as cyclic polysulfides and halogenated compounds had shown the toxic effects on micro-organisms, therefore they are responsible for the antibiotic activity of some marine algae[8,9]. Besides, more recently several screening studies on marine algae draw attention for antioxidant activity found in several seaweeds[10-12]. Phenolic compounds in the contents of the algal extracts are particularly effective antioxidants to preservation of PUFA[11]. Antioxidant and antimutagenic effects of dietary macroalgae have been observed in rodent model studies[13,14].

Codium fragile (Suringar) Hariot is a siphonous marine green alga belonging to the family Codiaceae (Chlorophyta). *C. fragile* is a dark green alga, ranging from 10 to 40 cm high and consists of repeatedly branching cylindrical segments. It grows in fuzzy patches of tubular fingers that hang from the rocks, at 1-2 m depth. It seems to prefer east-facing surfaces. Generally this alga is called as *Codium* or Dead Man's Fingers[15]. *C. fragile* is consumed by humans and used as invertebrate food by mariculture industry. In China, it is used as anticancer, antipyretic and helminthic agents in Chinese traditional medicine[16]. It also has antiviral[17] and anti-coagulant properties[18,19]. Algal lectins, affect blood clotting and fibrinolysis, from *Codium* sps are routinely used in biochemical studies[7,20,21]. In addition, high levels of PUFA (47.6 %) are found in *Codium* sps.[22].

The objectives of present study were to analyze the chemical composition by GC and GC/MS systems and the antimicrobial activity of a hydrodistilled essential oil of *C. fragile* collected from the Izmir Bay (Turkey) and to investigate the anti-oxidant and antimicrobial activities of various extracts from *C. fragile*.

EXPERIMENTAL

Field collections of seaweeds were made from several reefs (depths of 1-2 m) along the Izmir coast during September 2004 and identified by Prof. Dr. Atakan Sukatar. Voucher specimen (EGE 40761) was deposited in the herbarium of Ege University, Department of Biology, Izmir, Turkey. After the seaweed samples were collected, salt, epiphytes and sand were removed using tap water. They were rinsed carefully in freshwater, frozen immediately and freeze-dried, then stored at - 20 °C until experiments.

Preparation of extracts: Preparations of methanol, dichloromethane and hexane extracts. Freeze-dried *C. fragile* samples were pulverized and samples (15 g for each solvent) were extracted using methanol, dichloromethane and hexane (150 mL) for 24 h using a Soxhlet extraction apparatus[23,24]. The resulting extracts of *C. fragile* were concentrated to dryness under reduced pressure at 40-45 ºC with a rotary evaporator (yield: 15.67 % for methanol, 1.05 % for dichloromethane, 0.8 % for hexane) and stored at -20 °C until use. All solvents used were of analytical reagent grade and obtained from Sigma Aldrich Chemical Co. (St. Louis, USA).

Isolation of the essential oil: Freeze-dried samples of *C. fragile* (50 g) were subjected to steam distillation for 4 h using a Clevenger-type apparatus according to the European Pharmacopoeia (1975)[25]. The obtained distillate was diluted with hexane. and the volume was reduced 100-fold prior to analysis.

GC-MS analysis: The steam-distilled components were analyzed by GC and GC/MS. A HP 6890 gas chromatograph equipped with a FID and a 5 m × 0.2 mm HP-1 capillary column (0.33 μm coating) was employed for the GC analysis. GC/MS analysis was performed on a HP 5973 mass selective detector coupled with a HP 6890 gas chromatograph, equipped with a HP-1 capillary column. The column temperature was programmed from an initial temperature of 70 °C to a final temperature of 280 °C at 10 °C/min. The injector temperature was 150 °C (1 μL injection size), whereas the detector temperature was 250 °C. The carrier gas was helium (2 mL/min). Identification of the individual components was performed by comparison of mass spectra with literature data and by a comparison of their retention indices (RI) relative to a C8-C32 *n*-alkenes mixture[26]. A computerized search was carried out using the Wiley 275 L. GC/MS library and ARGEFAR GC/MS library created with authentic samples.

Antioxidant activity

Ferric chloride, thiobarbituric acid and hydrogen peroxide were purchased from Merck Co. (Darmsradt, Germany), 2-deoxyribose, trichloroacetic acid, butylated hydroxytoluene, linoleic acid, β-carotene were purchased from Fluka Chemie AG (Buchs, Switzerland), ethylenediamine tetra-acetic acid and α-tocopherol (vitamin E) were purchased from Sigma Aldrich Chemical Co. (St Louis, USA). All the organic solvents and the other chemicals used were analytical grade of 99 % or greater purity.

Hydroxyl radical scavenging assay (deoxyribose degradation assay): Hydroxyl radical scavenging assay was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fenton reaction (Fe^{3+}/ascorbate/EDTA/H_2O_2) system[27-30]. The attack of the hydroxyl radical on deoxyribose leads to thiobarbituric acid reactive substances formation. Various concentrations of the extracts were added to the reaction mixture containing freshly prepared solution of 28 mM deoxyribose (dissolved in 20 mM phosphate buffer pH 7.4), 10 mM $FeCl_3$, 1 mM EDTA, 1 mM H_2O_2 and 1 mM ascorbic acid, making up a final volume of 1.0 mL. The reaction mixture was incubated at 37 ºC for 1 h. 1 mL of thiobarbituric acid (0.5 %, w/v in 0.05 M NaOH) and 1.0 mL trichloroacetic acid (2.8 %, w/v) were added to test tubes and incubated at 90 ºC for 10 min. After cooling, the absorbance of pink chromogen formed in the reaction was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate. Inhibition (I) of deoxyribose degradation in per cent was calculated in following way:

$$I = \left(\frac{A_0 - A_1}{A_0} \right) \times 100$$

where A_0 = absorbance of the control reaction (containing all reagents except the test compound) and A_1 = absorbance of the test compound.

β-Carotene bleaching assay: The β-carotene bleaching assay is mainly based on preserving the yellow colour of β-carotene-linoleate system in case of antioxidants present in the test material[12,31,32]. 1 mL of β-carotene solution (0.2 mg/mL chloroform) was pipetted into a round-bottom flask (50 mL) containing 0.02 mL of linoleic acid and 0.2 mL of 100 % Tween 20. Chloroform was evaporated at 40 °C for 15 min and was added some water to the mixture to make an emulsion. Sample solutions in methanol (0.2 mL) were added in 5 mL of emulsion and 0.2 mL methanol was used as a control instead of samples. The tubes were kept at 50 °C in a water bath for 2 h. Absorbance was taken at zero time (t = 0) and after every 15 min against a blank. The absorbance of the samples was measured at 470 nm using a spectrophotometer (Varian Carry 300 Bio UV/VIS spectrophotometer). Absorbance measurements were continued until the colour of β-carotene disappeared in the control reaction (t = 120 min).

The antioxidant activity (AA) was determined as per cent inhibition relative to control samples,

$$AA = \left[1 - \left(\frac{A_0 - A_t}{A_0^o - A_t^o}\right)\right] \times 100$$

A_0 and $A_0°$ are the absorbance values measured at the initial incubation time for sample and control. A_t and $A_t°$ are absorbance values measured in the samples or standards and control at t = 120 min.

Synthetic antioxidant reagent butylated hyroxytoluene (BHT) and Vitamin E were used as positive controls in both assays.

Statistical analysis: Results are shown as mean ± SEM. Statistical analysis was performed by ANOVA with LSD test and student's t test. P value of 0.05 or less was taken to indicate statistical significance.

Antimicrobial activity

Test microorganisms: *In vitro* antimicrobial studies were carried out against ten bacteria strains (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 7064, *Streptococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 6538 p, *Enterobacter aerogenes* ATCC 13048, *Enterobacter cloacae* ATCC 13047, *Escherichia coli* ATCC 29998, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 6897, *Salmonella typhimurium* CCM 583), two specific pathogenic strains (methicillin-oxacillin resistant *Staphylococcus aureus* ATCC 43300, hemorrhagic *Escherichia coli* (O157:H7) RSSK 232) and one yeast strain (*Candida albicans* ATCC 10239) which were obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science (Turkey).

Disc diffusion method: The paper disc diffusion method was employed[32,33]. Sterile 6 mm diameter filter paper discs (Schleicher and Schül, Nr 2668, Dassel,

Germany) were impregnated with 20 µL of the *C. fragile* solvent extracts (0.25 and 0.50 mg $disc^{-1}$) and essential oil (0.4 and 0.6 µL $disc^{-1}$).

The bacteria strains were inoculated on nutrient broth (Oxoid) and incubated for 24 h at 37 ± 0.1 °C, while the yeast strain was inoculated on Malt Extract Broth (Oxoid) and incubated for 48 h at 28 ± 0.1 °C. Adequate amounts of Muller Hinton Agar (Oxoid) and Malt Extract Agar (Oxoid) were dispensed into sterile plates and allowed to solidify under aseptic conditions. The counts of bacteria strains and yeast strain were adjusted to yield *ca.* 1.0×10^7–1.0×10^8 mL^{-1} and 1.0×10^5–1.0×10^6 mL^{-1}, respectively, using the Standard McFarland counting method. The test organisms (0.1 mL) were inoculated with a sterile swab on the surface of appropriate solid medium in plates.

The agar plates inoculated with the test organisms were incubated for 1 h before placing the extract impregnated paper discs on the plates. Following this, the sterile discs impregnated with the different extracts were placed on the agar plates. The bacterial plates were incubated at 37 ± 0.1 °C for 24 h while the yeast plates were incubated at 28 ± 0.1 °C for 48 h. After incubation, all plates were observed for zones of growth inhibition and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated 3 times. Tobramycin discs (Bioanalyse, 10 µg/disc) and nystatin discs (Oxoid, 30 µg/disc) were used as positive controls.

Microdilution method: Determination of the minimum inhibitory concentration (MIC) was carried out according to the method described by Zgoda and Porter[34] with some modifications.

Dilution series of the extracts were prepared from 10 to 0.5 mg/mL in test tubes then transferred to the broth in 96-well microtiter plates. Final concentrations were 1000 to 50 µg/mL in the medium. Before inoculation of the test organisms, the bacteria strains and yeast strain were adjusted to 0.5 McFarland and diluted 1:100 in Mueller Hinton broth and Malt Extract broth, respectively. Plates were incubated at 35 °C for 18-24 h and at 30 °C for 48 h for the yeast. All the tests were performed in broth and repeated twice. Whereas the MIC values of the extracts were defined as the lowest concentration that showed no growth, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by plotting samples from clear wells onto Mueller Hinton agar and Malt Extract agar, respectively. Minimum bactericidal concentration and minimum fungicidal concentration were defined as the lowest concentration yielding negative subcultures.

Ampicillin (Sigma Aldrich Chemical Co. St Louis, USA) and streptomycin (Sigma Aldrich Chemical Co. St Louis, USA) were used as standard antibacterial agents, whereas nystatin (Sigma Aldrich Chemical Co. St Louis, USA) was used as a standard antifungal agent. Their dilutions were prepared from 128 to 0.25 µg/mL concentrations in microtiter plates.

RESULTS AND DISCUSSION

In present study, the extracts of *C. fragile* for their antioxidant and antimicrobial activity and its essential oil was also investigated for providing clarification to the chemical constituents. Macroalgae are ecologically and biologically important natural sources. They are important basis for the therapeutically useful substances. Due to their biological and chemical variations, the marine environment may be a source of novel types of antimicrobial agents and biologically active compounds[35,36].

The composition of *C. fragile* volatile components was analyzed by employing GC and GC-MS, leading to compare the relative retention times, the mass spectra of oil components with those of authentic samples and from data library. 24 Compounds were identified in the essential oils of *C. fragile* accounting for > 56 % of the total composition of the essential oil. The components are listed in Table-1. Many of the compounds in the volatile oil of *C. fragile* were identified as hydrocarbon compounds. The major components were *n*-tricosane (11.88 %), 1,3-dioxolane, 2,2,4-trimethyl (8.53 %) and *n*-hexadecane (4.07 %). The other abundant components were identified as *n*-eicosane (3.83 %), *n*-tetracosane (3.48 %), 3-ethyl-3-methylheptane (2.36 %),

TABLE-1
ESSENTIAL OIL COMPONENTS OF *C. fragile* (GC/MS ANALYSIS)

Rt (min)	Compound	Area (%)
6.18	1,3-Dioxolane, 2,2,4-trimethyl	8.53
6.41	2,3,4-Trimethyl hexane	0.30
8.37	*n*-Heptane	0.67
8.57	*n*-Octane	0.89
8.64	Prolamine	1.19
9.46	2,4,6-Trimethyl octane	2.34
9.57	2-Decyloxyethanol	0.77
9.88	1-Tetradecanol	1.32
9.96	1-Hexadecanol	1.33
10.19	*n*-Undecane	0.53
10.29	*n*-Tridecane	2.06
10.40	2,4-Dimethyl heptane	1.02
13.91	Farnesane	1.72
14.43	Dihexyl sulfide	1.34
14.61	*n*-Pentadecane	1.85
14.80	*n*-Hexadecane	4.07
18.10	3-Ethyl-3-methyl heptane	2.36
18.62	*n*-Nonadecane	0.79
18.89	*n*-Eicosane	3.83
19.08	*n*-Heneicosane	1.77
19.31	2,4-Bis(1,1-dimethyl ethyl) phenol	0.88
19.64	*n*-Docosane	1.14
21.60	*n*-Tricosane	11.88
21.95	*n*-Tetracosane	3.48
	TOTAL	56.06

octane, 2,4,6-trimethyl (2.34 %) and *n*-tridecane (2.06 %). Here we report for the first time on the essential oil composition of *C. fragile*. However, fatty acid compositions of *Codium* species and bromophenols contents of *C. fragile* were studied by several investigators previously[37-39]. In addition, the linear diterpene, phytol and its glycerol derivatives, siphonein and siphonaxanthin contents of *Codium* species have been reported before[40,41]. However, studies on the secondary metabolites of *C. fragile* are limited.

In respect of the deoxyribose degradation assay (Fig. 1), all the extracts showed dose dependent inhibition, indicating lesser antioxidant capacity than the positive controls ($p < 0.05$). Moreover, free radical scavenging activity of the hexane extract greater than the other extracts. As shown in Fig. 1, 55, 61, 69 and 72 % inhibitions of the hexane extract were achieved at 0.5, 1, 2 and 4 mg/mL concentrations, respectively.

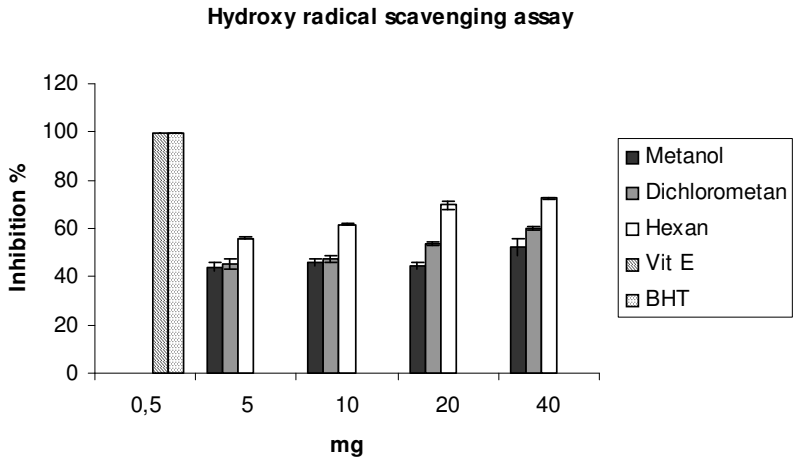

Fig . 1. Inhibitory effect of *C. fragile* extracts on deoxyribose degradation, owing to their hydroxyl radical scavenging capability. BHT and vitamin E used as control at the lowest concentration. * $p < 0.05$ compared with four doses of three *C. fragile* extracts

On the other hand, there was a little decrease in the absorbance values of β-carotene of the algae extracts due to the oxidation of β-carotene and linoleic acid. Because the initial absorbance values were maintained during the 2 h, it was accepted that the extracts includes antioxidant compounds. In the case of inhibition of β-carotene bleaching, all extracts were able to effectively inhibit the linoleic acid oxidation. As shown in Fig. 2, as similar to hydroxyl radical scavenging assay, all the extracts showed dose dependent inhibition in β-carotene bleaching assay. Likewise, the hexane extract has more antioxidant activity than the other extracts and 57, 60, 65 and 75 % inhibitions were achieved at 5, 10, 20 and 40 mg/mL concentrations, respectively.

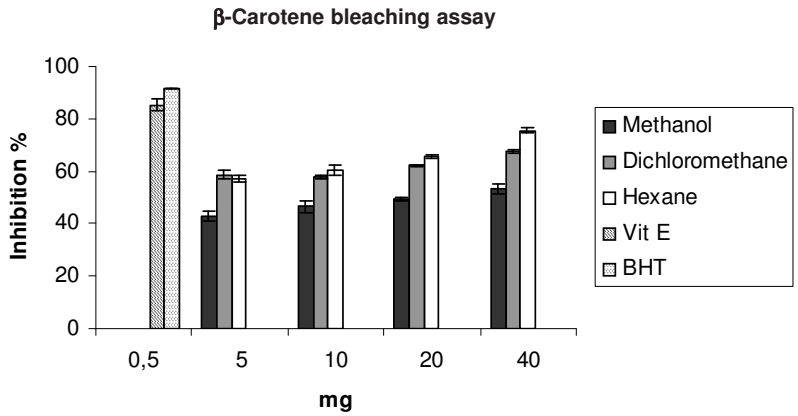

Fig. 2. Protective activity of the extracts against lipid oxidation in β-carotene bleaching assay. BHT and vitamin E used as control at the lowest concentration. * $p < 0.05$ compared with four doses of three *C. fragile* extracts

Basically antioxidants delay the oxidation and reduce the oxidative damage which is significant causative factor in the development of many chronic diseases. Oxidative stress is an important factor in the pathogenesis of various diseases such as atherosclerosis, cancer *etc.* and aging. Naturally generated reactive oxygen species (ROS) can attack cell components then create several types of biological damage and oxidative stress. Antioxidants protect against these reactions which occur in vital systems and increase shelf life when added to lipid and lipid containing foods[42]. Although synthetic antioxidants such as butylatedhydroxyanisole, butylatedhydroxy-toluene and propyl gallate have been used for many years, they have started to restrict in recent years because of their carcinogenicity. Thus, there is a gradual increase in the investigations to identify new natural antioxidants. Seaweeds are considered to be important sources of antioxidants[43]. The results from antioxidant activity screening in the extracts suggested that plant extracts have radical scavenging and lipid peroxidation inhibitor activity. Hydroxyl radical scavenging capability mainly derived some phenolic compounds[44,45]. Flavanoids and other phenolic compounds have been reported as scavengers and inhibitors of lipid peroxidation[46,47].

In present study, two different methods were used to investigate the antioxidant potential of *C. fragile* extracts. As seen, all *C. fragile* extracts showed a protective activity from oxidative damage and decreased the percentage of the rate deoxyribose degradation and lipid oxidation. Because the β-carotene bleaching assay is carried out in an emulsion, a situation usually in foods, was selected for total antioxidant activity determination in this study. β-Carotene bleaching assay is especially appropriate for inquiring essential oil and lipophilic antioxidants. The test materials, mainly contain polar compounds, could be evaluated as weak antioxidants if they are tested only by the β-carotene bleaching assay. On the other hand, hydroxyl radical scavenging assay allow testing both lipophilic and hydrophilic substances[31]. In present study,

there is no statistically significant difference ($p > 0.05$) between two different assays tested for antioxidant activity in all concentrations of methanol and hexane extracts. However, it was determined that there was a statistically significant difference ($p < 0.05$) between these assays in all concentrations of dichloromethane extract.

The antimicrobial activities of *C. fragile* essential oil and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones, MIC, MBC and MFC. In this study, as reported that in the Tables 2 and 3, the 0.4 µL/disc essential oil of *C. fragile* did not remarkably inhibit the growth of tested microorganisms except *Bacillus subtilis* ATCC 6633 (inhibition zone is 6.5 mm). Similarly, 0.6 µL/disc essential oil of *C. fragile* showed weak antibacterial activity on only *B. subtilis* ATCC 6633 and *B. cereus* ATCC 7064 (inhibition zones are 7 and 6.5 mm). These values are far below than the positive control tobramycin.

TABLE-2
ANTIMICROBIAL ACTIVITY OF *C. fragile* VOLATILE COMPONENTS

Miroorganisms	Gram	Diameter of zone of inhibition (mm)[a]			
		C. fragile (µL/disc)		Standards	
		0.6	0.4	Tob	Nys
B. subtilis (ATCC 6633)	+	7.0	6.5	24	NT
B. cereus (ATCC 7064)	+	6.5	–	18	NT
S. epidermidis (ATCC 12228)	+	–	–	7	NT
S. aureus (ATCC 6538-p)	+	–	–	16	NT
S. aureus (methicillin-oxacillin resistant) (ATCC 43300)	+	–	–	7	NT
E. aerogenes (ATCC 13048)	–	–	–	19	NT
E. cloaceae (ATCC 13047)	–	–	–	13	NT
E.coli (ATCC 29908)	–	–	–	10	NT
E. coli (Hemorrhagic, O157:H7) (RSSK 232)	–	–	–	25	NT
P. aeruginosa (ATCC 27853)	–	–	–	12	NT
P. vulgaris (ATCC 6897)	–	–	–	13	NT
S. typhimurium (CCM 5445)	–	–	–	10	NT
C. albicans (ATCC 10239)		–	–	NT	18

[a]Zone of inhibition, including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; Tob = tobramycin (10 µg/disc); Nys = nystatin (30 µg/disc); NT = not tested; G = gram reaction; – = no activity.

Methanol, dichloromethane and hexane extracts of *C. fragile* showed weak antimicrobial activity on *B. subtilis* (ATCC 6633), methicillin-oxacillin resistant *S. aureus* (ATCC 43300), *E. aerogenes* (ATCC 13048) and *E. coli* (ATCC 29908). All the extracts showed similar antimicrobial activity. When compared with the standard antibiotic tobramycin, all the extracts of *C. fragile* exhibited low antimicrobial activity. Antimicrobial activity studies of *Codium* are limited. It was previously reported that, chloroform/methanol (2:1) extracts of *C. isthmocladum* Vickers

TABLE-3
ANTIMICROBIAL ACTIVITY OF *C. fragile* EXTRACTS

Microorganisms	Diameter of zone of inhibition (mm)[a]					
	Methanol (mg/disc)		Dichloromethane (mg/disc)		Hexane (mg/disc)	
	0.25	0.50	0.25	0.50	0.25	0.50
B. subtilis (ATCC 6633)	6.5	7	6.5	7	6.5	7
B. cereus (ATCC 7064)	–	–	–	–	–	–
S. epidermidis (ATCC 12228)	–	–	–	–	–	–
S. aureus (ATCC 6538-p)	–	–	–	–	–	–
S. aureus (methicillin-oxacillin resistant) (ATCC 43300)	7.5	8	–	6.5	6.5	7
E. aerogenes (ATCC 13048)	7	8	7	8.5	7	8.5
E. cloaceae (ATCC 13047)	–	–	–	–	–	–
E.coli (ATCC 29908)	7.5	8.5	7	8	–	6.5
E. coli (Hemorrhagic, O157:H7) (RSSK 232)	–	–	–	–	–	–
P. aeruginosa (ATCC 27853)	–	–	–	–	–	–
P. vulgaris (ATCC 6897)	–	–	–	–	–	–
S. typhimurium (CCM 5445)	–	–	–	–	–	–
C. albicans (ATCC 10239)	–	–	–	–	–	–

[a]Zone of inhibition, including the diameter of the filter paper disc (6 mm); mean value of three independent experiments; – = no activity.

have antibacterial activity[48]. It was also exhibited that the extract of *C. dwarkense* Børg. exhibited antifungal activity by Aliya and Shameel[49]. Besides, methanol extract of *C. taylorii* have antibacterial activity against methicillin-resistant *S. aureus*[50]. Equally, in this study all the extracts of *C. fragile* were presented weak antibacterial activity to methicillin-resistant *S. aureus*.

The solvent extracts and the essential oil were further tested by microdilution to determine the MICs and MBCs (Table-4). Essential oil of *C. fragile* showed weak antibacterial activity against all Gram-positive bacteria tested, except methicillin-oxacillin resistant *S. aureus* ATCC 43300. The lowest MICs and MBCs of essential oil were < 50 µg/mL against *B. subtilis* (ATCC 6633), *B. cereus* (ATCC 7064), *S. epidermidis* (ATCC 12228). However, MIC value against *S. aureus* was < 50 µg/mL (ATCC 6538-p), MBC was 500 µg/mL. The highest antimicrobial activity as MIC and MBC were seen in the hexane extract against *P. aeruginosa* (ATCC 27853) at < 50 µg/mL concentrations. None of the dichloromethane extracts showed activity against test microorganisms. As herein defined, the solvent extracts and the essential oil of *C. fragile* have potential antimicrobial activity. Consequently, it could be expected to gain more effective active pure compounds from the extracts by the advanced investigations, even if they were applied very little amounts.

As previously shown in many studies antimicrobial component-producing marine macro algae inhibited the growth of some bacteria[50-53]. Also, as reported that the efficacy of macro algae extracts against microorganisms is mostly influenced by factors such as location and seasonality[54] and another study that macro algae showed a high percentage of species with antimicrobial activity, 73 % in the case of Chlorophyta (green algae), 69 % in Rhodophyta (red algae) and 53 % in Phaeophyta (brown algae)[53].

TABLE-4
MINIMUM INHIBITORY CONCENTRATION (MIC), MINIMUM BACTERICIDAL CONCENTRATION (MBC), MINIMUM FUNGICIDAL CONCENTRATION (MFC) OF *C. fragile* EXTRACTS AND ESSENTIAL OIL

Microorganisms	MIC (MBC or MFC) µg/mL						
	1	2	3	4	5	6	7
B. subtilis	250(500)	250 (500)	–	<50(<50)	0.5 (0.5)	0.5 (2.0)	NT
B. cereus	1000(1000)	500(>1000)	–	<50(<50)	4.0 (4.0)	8.0 (8.0)	NT
S. epidermidis	–	500(>1000)	–	<50(<50)	0.5 (0.5)	0.5 (2.0)	NT
S. aureus	–	500(>1000)	–	<50(500)	2.0 (4.0)	<0.25(<0.25)	NT
S. aureus (methicillin-oxacillin resistant)	–	–	–	–	4.0 (4.0)	32(>128)	NT
E. aerogenes	1000(1000)	–	–	–	4.0 (8.0)	–	NT
E. cloaceae	–	–	–	–	–	2.0 (2.0)	NT
E.coli	500(>1000)	–	–	–	4.0 (4.0)	64 (128)	NT
E. coli (Hemorrhagic, O157:H7)	500 (500)	–	–	–	4.0 (4.0)	4.0 (4.0)	NT
P. aeruginosa	<50 (<50)	250(>1000)	–	–	1.0 (1.0)	16 (32)	NT
P. vulgaris	250 (500)	250(>1000)	–	–	8.0 (8.0)	0.5 (0.5)	NT
S. typhimurium	–	–	–	–	16 (32)	1.0 (4.0)	NT
C. albicans	–	–	–	–	NT	NT	8.0(16)

1 = Hexane extract, 2 = Methanol extract, 3 = Dichloromethane extract, 4 = Essential oil, 5 = Streptomycin, 6 = Ampicillin, 7 = Nystatin; – = no activity; NT = not tested.

In conclusion, the present results did not declare conspicuous antioxidant and antimicrobial activities. After all, it should be considered that compositions and amounts of volatile compounds and other secondary metabolites are influenced from various climate regions and different seasons. Still, it may be accepted that *C. fragile* is a potential for enhancing new pharmaceutical applications.

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