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Antibacterial Activity of *Codium fragile* on Common Fish Pathogens

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Methanol, dichloromethane and hexane extracts of *Codium fragile* (Suringar) Hariot (Chlorophyta) were tested for antibacterial activity against common fish pathogens (*Aeromonas hydrophila*, *Yersinia ruckeri*, *Streptococcus agalactia* and *Enterococcus faecalis*) by microdilution method. The hexane extract of the alga has shown a strong antibacterial activity as MIC and MBC against *Aeromonas hydrophila* at 64 (>128) μg/mL concentrations and *Yersinia ruckeri* at 128 (128) μg/mL concentrations, respectively. While methanol extracts showed weak antibacterial activity against all bacterial pathogens, the dichloromethane extracts showed activity against the test microorganisms. The results demonstrate that the hexane extract of the alga has significant antibacterial activity and suggest that it may be useful in the treatment of bacterial fish diseases.

Key words: Macroalgae, *Codium fragile*, Antibacterial activity, Fish pathogens.

Seaweeds provide a rich source of structurally diverse secondary metabolites. There are numerous of compounds derived from sea weeds with a broad range of biological activities, such as antibiotics, antivirals, antitumorals and antiinflammatories[1], as well as neurotoxins[2]. In Western countries, sea weeds 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 sea weeds 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[3-5].

Codium fragile (Suringar) Hariot is a siphonous marine green alga belonging to the family Codiaceae (Cholorophyta). Generally this alga is called as Dead Man's Fingers[6]. This alga 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[7]. Also, it has antiviral[8], and anticoagulant properties[9-10]. Algal lectins, affect blood clothing and fibrinolysis, from *Codium* spp. are routinely used in biochemical studies[11-13].

Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes throughout the world. Most of these bacteria are naturally occurring opportunistic pathogens. Bacterial infections in fish may influence human health directly by induced disease. Also, the development of antibiotic resistance by pathogenic bacteria is a growing problem. The aim of this works was to evaluate the antibacterial activity of *Codium fragile* (Suringar) Hariot as wild growing in Turkey against common fish pathogens (*Aeromonas hydrophila*, *Yersinia ruckeri*, *Streptococcus agalactia* and *Enterococcus faecalis*).

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MATERIALS AND METHODS

Plant Material

Samples were collected at a depth of 1-2 m from the coast of Canakkale, Turkey in May, 2012 and were identified by Prof. Dr. Veysel Aysel from Dokuz Eylul University, Faculty of Science & Arts, Department of Biology, Izmir, Turkey. Algae samples were cleaned of epiphytes and necrotic parts were removed. Then the samples were rinsed with sterile water to remove any associated debris as described by Gonzales del Val *et al.*,[14].

Extraction of plant material

Freeze-dried *Codium fragile* samples were pulverized and samples (15 g for each solvent) were extracted using methanol, dicholoromethane and hexane (150 mL) for 24 h using a soxhlet apparatus[15]. The resulting extracts of *C. fragile* were concentrated to dryness under reduced pressure at 40-45 °C with a rotary evaporator (yield: 14.82% for methanol, 1.20% for dichloromethane, 0.8% for hexane). The dry extract, which was sticky and black, was stored in labeled sterile screw-capped bottles at 20°C pending use. Prior to testing, 1 g was dissolved in 0.2 L of dimethyl sulfoxide (DMSO) (5 mg/mL).

Microorganisms

in vitro Antimicrobial studies were carried out against *Aeromonas hydrophila* ATCC 7966, *Yersinia ruckeri* ATCC 29473, *Streptococcus agalactia* ATCC 13913 and *Enterococcus faecalis* ATCC 29212 which were obtained from bacterial stock collection of Biology Laboratory of Duzce University, Faculty of Science and Arts, Duzce, Turkey. For the purpose of antimicrobial evaluation, the microorganisms were cultured in Tryptone Soya broth (TSB) (Oxoid) at room temperature for 24 h and were adjusted to 10^7 cfu mL^{-1} with sterile saline. The optical density (OD) at 540 nm of each culture was measured by using ELISA microplate reader (Biorad, Japan).

Microdilution method

Determination of the Minimum Inhibitory Concentration (MIC) was carried out according to the method described by Zgoda and Porter[16] with some modification. 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 microorganisms, the bacteria strains were adjusted to 0.5 McFarland and diluted 1:100 in Mueller Hinton Broth. Plates were incubated 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) was determined by plotting samples from clear wells onto Mueller Hinton Agar. MBC was defined as the lowest concentration yielding negative subcultures. Ampicillin and Streptomycin were used as standard antibacterial agents. Their dilutions were prepared from 128 to 0.25 µg/mL concentrations in microtiter plates.

RESULTS AND DISCUSSION

The antibacterial activities of *Codium fragile* extracts against common fish pathogens examined in this study were assessed by the presence of MICs and MBCs (Table 1). The bacterial pathogens used in study are the ones commonly occur in aquaculture sector and cause serious infectious disease and mortality in fish[17] (Table 2).

None of the dichloromethane extracts showed activity against the test microorganisms. The highest antimicrobial activity as MIC and MBC were seen in the hexane extract against *Aeromonas hydrophila* at 64 (>128) µg/mL concentrations, respectively. The same extracts exhibited a strong effect against *Yersinia ruckeri* at 128 (128) µg/mL concentrations, respectively. Methanol extracts showed weak antibacterial activity against all bacterial pathogens, except *Enterococcus faecalis*. Consequently, all the extracts were presented weaker antibacterial activity than those of the standard antibacterial antibiotics Streptomycin and Ampicillin. The occurrence of antibiotic resistant strains of bacteria has been described in aquaculture systems[18-19]. Probably, the same mechanism involved in the antibiotic resistance should inhibit the deleterious action of the extracts on the bacterial cells.

Fatty acid composition of *Codium* species and bromophenols content of *C. fragile* were studied in previous studies[20-21]. In addition, the linear diterpene, phytol and its glycerol derivates, siphonein and siphomaxanthin contents of *Codium* species have been reported before[22-23]. However, antimicrobial activity studies of *Codium* are limited.

It was previously reported that, chloroform/ methanol (2:1) extracts of *C.isthmocladum* Vickers have antibacterial activity[24]. The extract of *C. dwarkense* Borg. exhibited antifungal activity[25]. Besides, methanol extract of *C. taylorii* have antibacterial activity against methicillin-resistant *S. aureus*[14]. In previous study, the solvent extracts and the essential oil were tested by microdilution to determine the MICs and MBCs. 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

Table 1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Codium fragile* extracts

Microorganisms	MIC (MBC) µg/mL				
	1	2	3	4	5
Aeromonas hydrophila	64 (>128)	250 (500)	-	4.0 (8.0)	8.0 (8.0)
Enterococcus faecalis	500 (>1000)	-	-	4.0 (4.0)	4.0 (4.0)
Streptococcus agalactia	250 (500)	500 (>1000)	-	2.0 (2.0)	2.0 (4.0)
Yersinia ruckeri	128 (128)	250 (500)	-	4.0 (4.0)	4.0 (8.0)

1: Hexane extract, 2: Methanol extract, 3: Dichloromethane extract, 4: Streptomycin, 5: Ampicillin, (-): no activity

Table 2. The most common bacterial pathogens in aquaculture[17]

Pathogen	Disease	Signs of disease	Host
Aeromonas hydrophila	Hemorrhagic septicemia, peritonitis, red sore diseases, fin rot, red-fin disease	Erosive or ulcerative dermal lesions, hemorrhage on fins and trunk, swelling of anus, erythema	Freshwater and ornamental fish, occasionally marine fish
Enterococcus faecalis	Streptococcosis, exophthalmia, hemorrhage	Bacteria in liver and kidney, ulcer on fins	Rainbow trout, catfish, brown bullhead
Streptococcus agalactia	Streptococcosis, exophthalmia, hemorrhage	Hemorrhagic areas on body, mouth, fins	Bluefish, cultured sea bream, wild mullet, striped bass, sea trout and ornamental fish
Yersinia ruckeri	Enteric red mouth disease, yersiniosis	Reddening of trout and mouth, hemorrhages on gills and fins	Salmonids, freshwater, ornamental and marine fish

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. Twenty four compounds were identified of *C. fragile* essential oil and n-tricosane (11.88%) was determined as major component[26]. In this study and above study, hexane was observed as the best solvent for extracting antimicrobial substances in *Codium fragile*. The mentioned substances especially n-tricosane obtained from the alga may be responsible for the antimicrobial activity.

There are some studies on the antimicrobial activity of various plants on fish pathogens. For instance, high antimicrobial activity

of the water extracts from the bulb of *Allium sativum* against *A. hydrophila* and *Pseudomonas fluorescens* and the leaves of *Calotropis gigantea* against *Edwardsiella tarda* was reported[27]. Also, *Hammada scoparia, Loranthus acacia* and *Peganum harmala* as desert plants exhibited antimicrobial effects against *A. hydrophila* and *Vibrio alginolyticus*[28]. *Psidium guajava* and *Momordica charantia* as traditional Thai herbs showed a strong antibacterial activity against *Vibrio harveyi* and *Vibrio parahaemolyticus*[29]. In another study, among the tested Turkish endemic plants, *Trifolium pannonicum* displayed the highest antibacterial activity against *A. hydrophila, Yersinia ruckeri, Streptococcus agalactia* and *Lactococcus garvieae*[30]. This study provides data about the antibacterial properties of *Codium fragile* using extracts at concentrations that would be able to study for therapeutically useful. Especially the hexane extracts may be applied for treating fish diseases to avoid the emergence of antibiotic resistance in aquatic and environmental bacteria.

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