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# Antimicrobial activity of marine algal extracts

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## Abstract

Total, hydrophilic and lipophilic extracts of 21 marine algae species (2 species of Chlorophyta, 11 of Phaeophyceae and 8 of Rhodophyta) collected along the coast of Russian Far East were screened for antimicrobial activities (against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*). Among the macroalgal extracts analyzed, 95% were active against at least one of studied microorganisms, while 80% of extracts were active against two or more test strains. Broad-spectrum activity against three studied microorganisms was observed in the 35% of extracts from tested species of marine seaweeds. The results of our survey did not show clear taxonomic trends in the activities of the total extracts and their hydrophilic fractions against the studied microorganisms. Nevertheless, lipophilic red algal extracts had both the highest values and broadest spectrum of bioactivity among all survey algal extracts. More over, lipophilic extracts from red algal exhibited the highest activity against fungus *C. albicans* among tested algal extracts.

**Keywords:** Antimicrobial activity, extracts, marine algae.

## Introduction

Marine macro algae, or seaweeds as they are more commonly known, are one of nature's most biologically active resources, as they possess a wealth of bioactive compounds. Chemical compounds isolated from marine macro algae have demonstrated various biological activities, such as antimicrobial and anti-viral activity, antioxidant potential, anti-inflammatory properties, anti-coagulant and apoptotic effect [1-3]. As a result, seaweed – derived compounds have important applications in a range of products in food, pharmaceuticals, cosmetics, agriculture and in aquaculture against marine pathogens and saprophytes [4-7]. Russian Far East has a long coastline and abundant natural resources of marine algae. The biodiversity of Russian Far Eastern seas as well as variety of the environmental conditions provide important sources of chemical compounds having a broad range of biological activities. Unfortunately, despite huge potential of seaweed as source of biologically active molecules there are only few reports on the screening of marine algae for antibacterial and antifungal activities not only for algae from Russian Far-East but also around the world [1, 8-9]. The number of species so far screened is only a very small percentage of the total.

Recently the medicine has faced a number of problems concerned with appearance of antibiotic-resistant strains of the microorganisms or increase resistance of them to traditional drugs. This resistance has been a major factor increasing morbidity, mortality and health care cost to bacterial infection [10]. Overuse of antibiotic cannot solve this problem, so there is a constant search of the new classes of antibiotics with novel structure that are effective against human pathogens. According to numerous studies chemical compounds from marine seaweeds may be perspective in chemotherapy of infectious disease [11-12] chiefly in primary therapy. Moreover, in opposite to the synthetic drugs natural compounds have significantly lower side effects.

To date, there is a lot of information about antimicrobial activity of the total extractive substances of marine seaweeds extracted by solvents of different polarity such as acetone, methanol, ether, ethanol, chloroform or their mixture [12-15]. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kinds of solvent is the most effective and suitable for extraction of seaweeds. It was shown that both hydrophilic and lipophilic algal extracts have an antimicrobial activity [4,16-20]. In present study we tested three type of algal extracts (total extracts and their hydrophilic and lipophilic fraction) against gram-positive bacteria *Staphylococcus aureus,* gram-negative bacteria *Escherichia coli*, and yeast-like fungi *Candida albicans.*

## Materials and methods

### Sample collection

In the present study we tested antimicrobial activity of extracts from 21 species of marine seaweeds from Russian Far East. The full

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characteristic of the samples, place and time of their collection are given in the Table 1.

## Preparation of algal extracts

After collection algae were frozen and kept at -20 $^{o}$C until used. After samples defrosting, epibionts and other contaminants were removed; the algae were trice washed in distilled water. Then algae were dried during 2 – 3 h on absorbent paper at the room temperature. The dried algae were chopped and sequentially extracted at a 1:3 (w:v) ratio in ethanol (96%), then in 2:1 (v:v) solution of acetone – ethanol, and in 2:1 (v:v) solution of chloroform ethanol using homogenizer type «MPW-324» (Poland). The resulting organic extracts were filtered and combined. The combined extracts were concentrated in rotary evaporator. Dry weight was detected trice by weighing of its aliquot dried to constant weight.

Total ethanol extracts of each algae sample were separated into two parts. The first part was directly tested for their antimicrobial activity. Another part of the extracts was partitioned between chlorophorm and water to separate lipophilic (chlorophorm soluble) and hydrophilic (water-soluble) constituents. The lipophilic fraction was extracted twice from total extracts by chloroform. The chloroform extracts were separated from the water phase, combined and washed thrice in the distilled water. The content of hydrophilic and lipophilic substances in purified chloroform and water extract was detected trice by weighing of its aliquot dried to constant weight. Both lipophilic and hydrophilic fractions were tested in the antimicrobial activity.

In total 75 extracts were obtained to test antimicrobial activity of marine seaweeds.

## Antimicrobial assay

All extracts were screened for antimicrobial effects against the gram-positive bacteria *Staphylococcus aureus* ATCC 21027, gram-negative bacteria *Escherichia coli* ATCC 15034 and yeast *Candida albicans* KMM 453. These strains of microorganisms were provided by the laboratory of microbiology of Pacific Institute of Bioorganic Chemistry FEB RAS from the Marine Microorganisms Collection (Russia).

Antimicrobial activity was determined by the agar diffusion method as described in Gerasimenko [21]. Solid broth used for cultivation of fungi (pH 6.0) contained 3% glucose, 1% peptone, 1.7% agar. Solid broth for bacteria cultivation (pH 7.0) contained 0.1% glucose 0.5% peptone, 1.7% agar, 0.02% $K_2HPO_4$, 0.005% $MgSO_4$, 0.01% NaOH, 0.1% yeastnel. Both broths were prepared in distilled water. After autoclaving broths were cooled to 42 $^{o}$C and the microorganisms ($1x10^6$ cells/ml) were added. Then 25 ml of the seeded agar was poured into 90 mm sterile Petri dishes and allowed to solidify. The small holes (10 mm in diameter were punched out using a sterile cork borer in the solid broth inoculated with test microorganisms. Then 0.1 ml of either total algal extracts or their lipophilic or hydrophilic fractions dissolved in DMSO (100 mg/ml) were added into resulting holes. Negative solvent controls (DMSO) and positive controls containing nitrofungin and ampicillin (1 mg/ml) were used in each assay. The dishes with bacteria and fungi were incubated at 37 $^{o}$C and 28 $^{o}$C, respectively. After 24 h of incubation the zones of inhibition of the microorganisms' growth around the holes were measured. Each test was done in triplicate and the mean values were recorded.

## Results

In total, 75 extracts from 21 species of marine algae collected from Russian Far-East coastline (Table 1) were screened for antimicrobial activity against three human pathogen microorganisms. The results are summarized in Table 2. Overall, 95% of all extracts surveyed in this study were active against one or more assayed microorganisms; while 80% of extracts were active against two or more test strains. Broad-spectrum activity against three assay microorganisms was observed in the 35% of extracts from tested species of marine seaweeds.

**Table 1.** Characteristic of the collected samples.

| Species | Place of collection | Coordinates | Data of collection |
|---|---|---|---|
| CHLOROPHYTA | | | |
| **Ulotrichaceae, Ulotrichales** | | | |
| *Acrosiphonia sonderi* (Kützing) Kornmann | Sobol Bay, Sea of Japan | 43$^{o}$04' N, 131$^{o}$54' E | March, 2010 |
| **Ulvaceae, Ulvales** | | | |
| *Ulva lactuca* Linnaeus | Sobol Bay, Sea of Japan | 43$^{o}$04' N, 131$^{o}$54' E | March, 2010 |
| PHAEOPHYCEAE | | | |
| **Alariaceae, Laminariales** | | | |
| *Eualaria fistulosa* (Postels et Ruprecht) Wynne | Krasheninnikova Bay, Okhotsk Sea | 50$^{o}$18' N, 155$^{o}$18' E | August, 2008 |
| *Undaria pinnatifida* (Harvey) Suringar | Sobol Bay, Sea of Japan | 43$^{o}$04' N, 131$^{o}$57' E | March, 2010 |
| **Costariaceae, Laminariales** | | | |
| *Costaria costata* (C.Agardh) Saunders | Sobol Bay, Sea of Japan | 43$^{o}$04' N, 131$^{o}$57' E | March, 2010 |

| | | | |
|---|---|---|---|
| **Laminariaceae, Laminariales** | | | |
| *Saccharina japonica* (Areschoug) Lane, Mayes, Druehl et Saunders | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| *Saccharina cichorioides* (Miyabe) Lane, Mayes, Druehl et Saunders | Amursky Bay, Sea of Japan | 43º11' N, 131º54' E | March, 2010 |
| **Sargassaceae, Fucales** | | | |
| *Cystoseira crassipes* (Mertens et Turner) C.Agardh | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| *Sargassum pallidum* (Turner) C. Agardh | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| *Coccophora langsdorfii* (Turner) Greville | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| **Fucaceae, Fucales** | | | |
| *Fucus evanescens* C.Agardh | Cape Recorda, Okhotsk Sea | 50º18' N, 155º18' E | August, 2008 |
| *F. evanescens* C.Agardh | Misima Bay, Okhotsk Sea | 45º58' N, 150º11' E | August, 2008 |
| *F. evanescens* C.Agardh | Kraternay Bay, Okhotsk Sea | 43º31' N, 152º49' E | August, 2008 |
| *F. evanescens* C.Agardh | Krasheninnikova Bay, Okhotsk Sea | 50º18' N, 155º17' E | August, 2008 |
| **Desmarestiaceae, Desmarestiales** | | | |
| *Desmarestia viridis* (Müller) Lamouroux | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| **Scytosiphonaceae, Scytosiphonales** | | | |
| *Scytosiphon lomentaria* (Lyngbye) Link | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| RHODOPHYTA | | | |
| **Ceramiaceae, Ceramiales** | | | |
| *Ceramium kondoi* Yendo | Amursky Bay, Sea of Japan | 43º11' N, 131º54' E | March, 2010 |
| **Delesseriaceae, Ceramiales** | | | |
| *Delesseria serrulata* Harvey | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| Rhodomelaceae, Ceramiales | | | |
| *Neorhodomela larix* subsp. *aculeata* (Perestenko) Perestenko (1) | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| *N. larix* subsp. *aculeata* (Perestenko) Perestenko (2) | Lazurnaya Bay, Sea of Japan | 42º37' N, 131º07' E | September, 2010 |
| **Wrangeliaceae, Ceramiales** | | | |
| *Ptilota filicina* J.Agardh | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| **Gigartinaceae, Gigartinales** | | | |
| *Chondrus pinnulatus* (Harvey) Okamura | Amursky Bay, Sea of Japan | 43º11' N, 131º54' E | March, 2010 |
| *Mazzaella laminarioides* (Bory de Saint-Vincent) Fredericq | Amursky Bay, Sea of Japan | 43º11' N, 131º54' E | March, 2010 |
| **Tichocarpaceae, Gigartinales** | | | |
| *Tichocarpus crinitus* (S.G.Gmelin) Ruprecht | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |
| **Palmariaceae, Palmariales** | | | |
| *Palmaria stenogona* Perestenko | Sobol Bay, Sea of Japan | 43º04' N, 131º57' E | March, 2010 |

Among all studied microorganisms fungi were resistant to the most extracts. Only 59% of all surveyed extracts were active against yeast-like fungus *C. albicans,* whereas 89% and 64% of the extracts inhibited growth of gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*, respectively (Figure. 1).

Among tested extracts 54% of lipophilic extracts show of broad-spectrum activities inhibiting 3 studied microorganisms; more over 88% of liphophilic extracts were active against two and more strains. Both crude and hydrophilic extracts showed lower antimicrobial activities in compare to lipophilic ones. Only 79% of crude extracts and 71% of hydrophilic fractions were active against two and more strains, and less than 30% of both extracts inhibited growth of all tested microorganisms (Figure. 1). The similar results were obtained by Engel [4]. However, the results of present screening can not allow revealing the most effective solvent to obtain algal extracts for antimicrobial use. Although lipophilic extracts showed broad-spectrum activity, their inhibiting effect was not always higher in compare to either total extracts or hydrophilic ones. In the most cases zone of the microbial growth inhibition resulted by lipophilic extracts was smaller than those resulted by other extracts obtained from the same algal species (Table 2). In some species (such as *Eualaria fistulosa*) the inhibitory activity was only observed in the extract obtained with one or two kind of solvent but not in the extracts obtained in other solvent, which may suggest that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the percentage of inhibitory activity will go up when several solvents are used for extraction.

**Table 2.** Antimicrobial activity of the total extracts and their hydrophilic and lipophilic fractions, obtained from of marine algae.

| Species | Extracts | Antimicrobial acivity against: | | |
|---|---|---|---|---|
| | | *C. albicans* | *S. aureus* | *E. coli* |
| *Acrosiphonia sonderi* | T | 1.0±0 | 4.0±0 | 0.7±0.2 |
| | H | 1.2±0.2 | 1.0±0 | 0.7±0.2 |
| | L | 1.7±0.2 | 5.3±0.2 | 1.7±0.2 |
| *Ulva lactuca* | T | 0 | 7.3±0.3 | 0.7±0.3 |
| | H | 1.0±0 | 10.3±0.3 | 1.0±0 |
| | L | 0 | 5.5±0.3 | 1.0±0 |
| *Eualaria fistulosa* | T | 0 | 0 | 0 |
| | H | 0 | 1.8±0.2 | 0 |
| | L | 0 | 0.8±0.2 | 0 |
| *Undaria pinnatifida* | T | 1.0±0 | 1.0±0 | 1.0±0 |
| | H | 1.0±0 | 1.0±0 | 0 |
| | L | 1.0±0 | 2.0±0 | 1.0±0 |
| *Costaria costata* | T | 1.7±0.2 | 3.7±0.3 | 0.7±0.2 |
| | H | 1.2±0.2 | 0 | 1.2±0.4 |
| | L | 1.7±0.2 | 5.2±0.2 | 1.7±0.2 |
| *Laminaria cichoriodes* | T | 1.0±0 | 6.2±0.2 | 1.3±0.2 |
| | H | 0 | 3.0±0 | 1.0±0 |
| | L | 0.7±0.3 | 6.3±0.2 | 1.7±0.3 |
| *Laminaria japonica* | T | 0 | 2.8±0.3 | 1.8±0.2 |
| | H | 0 | 0 | 1.0±0 |
| | L | 1.0±0 | 4.5±0.3 | 1.7±0.3 |
| *Cystoseira crassipes* | T | 0 | 1.5±0 | 1.7±0.2 |
| | H | 1.0±0 | 1.5±0 | 1.0±0 |
| | L | 0 | 1.7±0.2 | 1.0±0 |
| *Fucus evanescens (1)* | T | 0 | 2.0±0 | 1.0±0 |
| | H | 3.5±0.2 | 6.5±0.5 | 0 |
| | L | 2.0±0 | 1.5±0.2 | 0 |
| *F. evanescens (2)* | T | 0 | 1.0±0 | 0.8±0.2 |
| | H | 2.3±0.2 | 2.5±0 | 0 |
| | L | 1.5±0.2 | 1.2±0 | 0 |
| *F. evanescens (3)* | T | 0 | 1.0±0 | 0.7±0.2 |
| | H | 2.2±0.3 | 5.0±0 | 0 |
| | L | 2.8±0.2 | 1.5±0.2 | 1.2±0 |
| *F. evanescens (4)* | T | 0 | 1.5±0 | 1.0±0 |
| | H | 2.0±0 | 3.0±0 | 0 |
| | L | 1.6±0.2 | 1.0±0 | 0 |
| *Coccophora langsdorfii* | T | 0 | 2.5±0 | 2.0±0 |
| | H | 1.5±0 | 3.5±0 | 2.0±0 |
| | L | 0 | 3.0±0 | 0 |
| *Sargassum pallidum* | T | 0 | 5.7±0.2 | 1.0±0 |
| | H | 0 | 6.8±0.3 | 2.0±0 |
| | L | 0 | 2.3±0.3 | 2.0±0 |
| *Desmarestia viridis* | T | 0 | 1.5±0 | 1.0±0 |
| | H | 0 | 1.0±0 | 0 |
| | L | 0 | 3.0±0 | 1.7±0.2 |
| *Scytosiphon lomentaria* | T | 2.0±0 | 1.7±0.2 | 1.0±0 |
| | H | 2.0±0 | 1.0±0 | 0 |
| | L | 2.0±0 | 4.8±0.2 | 1.5±0 |

| | | | | |
|---|---|---|---|---|
| *Ceramium kondoi* | T | 1.0±0 | 2.0±0 | 1.2±0.2 |
| | H | 1.5±0 | 0.7±0.3 | 1.0±0 |
| | L | 1.0±0 | 3.0±0 | 2.2±0.2 |
| *Delesseria serrulata* | T | 0 | 2.3±0.3 | +3.5±0.5 |
| | H | 0 | 0 | +4.0±0 |
| | L | 0 | 2.0±0 | +5.0±0 |
| *Chondrus pinnulatus* | T | 0 | 2.3±0.2 | 0 |
| | H | 0 | 0 | 0 |
| | L | 1.5±0.3 | 5.7±0.2 | 2.2±0.2 |
| *Mazzaella laminariodes* | T | 0 | 2.2±0.2 | 0 |
| | H | 0 | 0 | +5.0±0 |
| | L | 1.3±0.2 | 4.0±0 | 2.0±0 |
| *Neorhodomela larix* (1) | T | 2.0±0 | 9.7±0.2 | 2.0±0 |
| | H | 0 | 3.5±0.3 | 1.0±0 |
| | L | 2.0±0 | 10.8±0.2 | 2.5±0 |
| *N. larix* (2) | T | 0 | 1.0±0 | 0 |
| | H | 2.5±0.5 | 2.0±0 | 2.0±0 |
| | L | 1.3±0.3 | 3.3±0.2 | 2.8±0 |
| *Palmaria stenogona* | T | 1.8±0.2 | 0 | +5.0±0 |
| | H | 0.7±0.2 | 0 | +3.0±0 |
| | L | 2.8±0.3 | 2.0±0 | 1.7±0.3 |
| *Ptilota filicina* | T | 2.2±0.3 | 3.2±0.3 | +5.5±0.5 |
| | H | 1.3±0.2 | 2.5±0.5 | +7.0±0 |
| | L | 1.5±0 | 4.8±0.2 | +4.0±0 |
| Nitrofungin | | 7.0±0 | 14.0±0 | 3.0±0 |
| Ampicillin | | - | 20.0±0 | 6.5±0.2 |

Expressed in width of inhibition zones in mm; T – total extract, H – hydrophilic fraction, L – lipophilic fraction; «-» – no data.

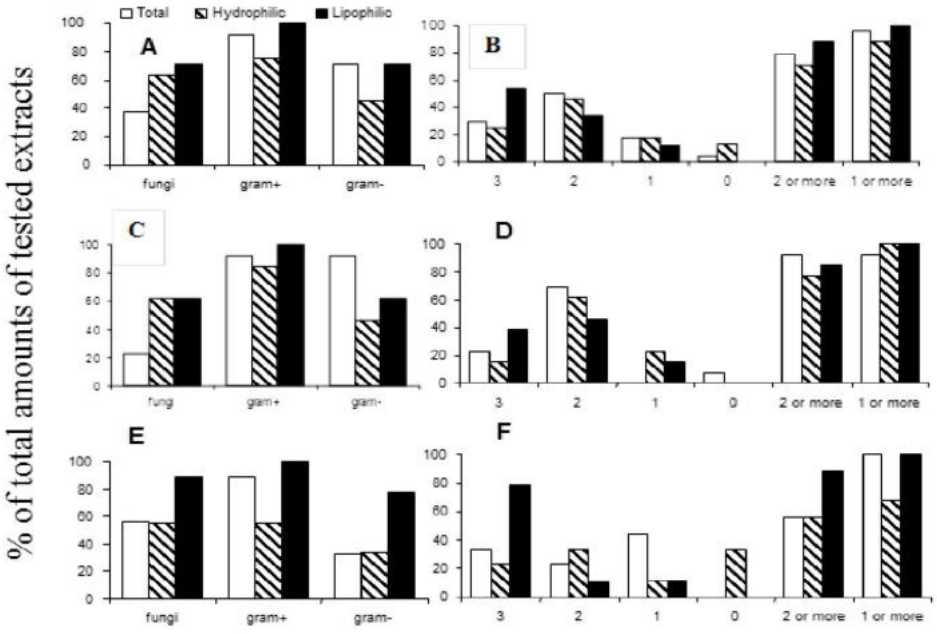


**Figure 1.** Antimicrobial activity of the algal extracts from all surveyed seaweeds (A and B, respectively), from the brow algae (C and D, respectively) and from the red (E and F, respectively) against assay microorganisms.

Gram-positive bacteria were most susceptible to extracts obtained by all used solvents. Growth of this strain was inhibited by over 92% and 100% of both crude extracts and lipophilic fractions, respectively, whereas only 75% of hydrophilic fractions were active against *S. aureus.* Gram-negative bacteria and fungi were less susceptible to extracts obtained by all used solvents. The highest activity against *E. coli* and *C. albicans* was shown by lipophilic fractions about 71% in both cases, and by total extract against gram-negative bacteria (71%). Hydrophilic fractions were less active against gram-negative bacteria and fungi (46 and 63%, respectively). Only 38% of total extract inhibited growth of *C. albicans* (Figure. 1).

## Antimicrobial activity of extracts from green algae (Chlorophyta)

Green algae were the less abundant in this study, comprising only two species (Table 1). All extracts obtained from green algae were active against both bacteria *S. aureus* and *E. coli*. Four of six tested extracts inhibited growth of yeast-like fungus *C. albicans*. Chlorophyta extracts showed the great activity against gram–positive bacteria. The zones of inhibition of the bacteria growth were in a range of 4–10 mm (Table 2). Hydrophilic extracts from *Ulva lactuca* showed the highest inhibitory effect against *S. aureus* (10.3 mm) among tested extracts. This activity was close to those showed by nitrofungin (14 mm). Similarly, Tüney et al. [14] reported that diethyl ether and ethanol extracts isolated from two *Ulva* species inhabited along the coast of Urla (Turkey) show a broad-spectrum antimicrobial activities inhibiting growth of three and more microorganisms. Similar results were obtained by Awad [22] studying antimicrobial action of sterols from *U. lactuca*. Mtolera and Semesi [23] suggested that the antimicrobial activity shown by *U. pertusa* of Tanzania might be due to acrylic acid commonly found in the genus.

## Antimicrobial activity of extracts from brown algae (Phaeophyceae)

Brown algae comprised 56% of all species surveyed in this study. This group included 11 species belonging to the seven families (Table 1). Overall 98% of survey extracts from brown algae were active against one or more assay microorganisms. Further, 86% of all survey extracts of brown algae were active against two or more studied microorganisms. Broad-spectrum activity against all studied microorganisms was observed for extracts from all species of the order Laminariales and some algae belonging to the orders Fucales and Scytosiphonales. Overall, 93% of all brown algae extracts were active against *S. aureus*, 69% yielded extracts were active against *E. coli*, and only 52% extracts inhibited growth of *C. albicans*.

The results of present screening can not allow revealing the most effective solvent for extraction of antimicrobial compounds from brown algae. Although higher percentage of lipophilic extracts showed broad–spectrum activity among tested extracts, there were not significant differences against individual pathogens. Total extracts were less effective against fungi and was most effective against gram–negative bacteria among all tested extracts, whereas hydrophilic and lipophilic fraction showed the similar activities against all studied pathogens (Figure. 1.). The zones of inhibition of microbe growth by tested extracts were in a range of 1–6.8 mm.

Growth of the gram–positive bacteria was suppressed in the highest degree among studied microorganisms (Table 2). The next extracts pointed out maximum activity against *S. aureus*: hydrophilic extract from *Fucus evanescens* (6.5 mm), lipophilic and hydrophilic extracts of *Costaria costata* (5.2 and 6.2 mm, respectively), lipophilic extracts from *Saccharina cichorioides*, *S. japonica* and *Scytosiphon lomentaria* (6.3, 4.5 and 4.8 mm, respectively), and total and hydrophilic extracts of *Sargassum pallidum* (5.7 and 6.8 mm, respectively) (Table 2).

Almost all extracts of brown seaweeds processed moderate activity against gram–negative microorganism *E. coli*. The widths of zones of inhibition of *E. coli* growth were generally less than 2 mm (Table 2). The strongest activity against fungi was obtained for lipophilic and hydrophilic extracts from fucoids as well as *C. costata* and *S. lomentaria*. Both extracts isolated from these seaweeds inhibited *C. albicans* growth in the high degree, resulting in more than 1.5 mm inhibition zones. For comparison, majority of extracts isolated from other tested brown algae produced only 1 mm inhibition zones. In should be pointed, that in the majority of cases the hydrophilic extracts have a slightly stronger activity against fungi than lipophilic ones (Table 2).

Among surveyed brown algae the strongest antimicrobial activity was obtained for species belonging to the families Laminariaceae, Costariaceae and Sargassaceae. The strong activity was also determined for *Fucus evanescens*. It should be noted, that antimicrobial properties were greatly variable among *F. evanescens* samples collected in the different places (Table 2).

## Antimicrobial activity of extracts from red algae (Rhodophyta)

Red algae comprised 33% of all species surveyed in this study. This group included 8 species belonging to the five families (Table 1). Majority of the survey extracts (79%) were active against *S. aureus*, while only 46% of all red algal extracts inhibited growth of *E. coli,* and 63% extracts of red algae were active against *C. albicans*.

Overall 88% of survey extracts from red algae were active against one or more assay microorganisms. Further, 58% of all survey extracts of red algae were active against two or more studied microorganisms. Broad-spectrum activity against all studied microorganisms was observed in 42% extracts isolated from red algae. The highest activity was found for extracts from *Ceramium kondoi* and *Neorhodomela larix* subsp. *aculeata.* The extracts only

from these algae strongly suppressed growth of the all tested microorganisms resulting wide inhibitory zones, while the extracts obtained from other tested Rhodophyta species were active against only one or two pathogens, showing moderate inhibitory strength (Table 2).

It should be noted, that extracts from several Rhodophyta species (such as *Delesseria serrulata*, *Palmaria stenogona*, *Ptilota filicina* and *Tichocarpus crinitus,* and hydrophilic extract from *Mazzaella laminarioides* stimulated growth of the *E. coli*.

Among tested extracts the lipophilic ones showed the highest activity, overall 75% of them inhibited growth of three studied microorganisms, while only 25% of both total and hydrophilic extracts had a broad-spectrum activity (Figure. 1). Moreover, lipophilic extracts exhibited highest activity against gram–positive bacteria and fungi among all surveyed extracts.

Comparison of antimicrobial properties of the *Neorhodomela larix* subsp. *aculeata* harvested in March and September appeared clear seasonal variation in activity against gram–positive bacteria *S. aureus*, with the extracts having strongest activity in spring and having moderate antibacterial activity during early fall (Table 2). No seasonal variation was observed in antifungal activity of the algal extracts tested against *C. albicans* and antibacterial activity against *E. coli*.

## Discussion

The results of present study clearly demonstrated that extractive compounds from marine algae inhabited Russian far-eastern seas can inhibit growth of the human pathogens such as gram–positive bacteria, gram–negative bacteria and fungi. About 95% of all algal extracts survey in this study was active against one or more assay microorganisms. Broad-spectrum activity against three studied microorganisms was observed in the 35% of extracts from tested species of marine seaweeds. These values were significantly higher that was found by large-scale screening program tested over 500 extracts from 159 species of marine plants against 12 human pathogens and reported that only 36% of all extracts showed antimicrobial activity [24]. These differences may be explained by two reasons. On one hand, studied algae from Russian Far-East may have a stronger antimicrobial activity in compare to seaweeds studied early due to high resistance of tested algae to bacteria associated with algae surface, which are diverse and abundant in the seas of the temperate zone. Moreover, due to pathogens and intense competition for space, especially in the greatly variable environmental conditions of the northern Pacific, seaweeds have developed an extensive chemical defense system, thus providing an untapped resource of bioactive natural products. Similar presumption was stated in study investigated antimicrobial activity of the extracts from Indo-Pacific seaweeds [25]. On the other hand, it is known that extraction procedures and method of storage of algae can significantly alter the composition of extract and may result differences in assay results [26].

Early it was repeatedly shown that antibacterial activities of seaweeds varied with the species division. However, data about distribution of antimicrobial activities among different taxa are strongly contradictive. Choi et al [27] found out that the highest antibacterial activity was exhibited by the brown algae. Karthikaidevi et al [28] reported that the species of Chlorophyta shows the strongest activities against the twelve studied microbes. Other researches reported that Rhodophyta exhibited both the highest value and the broadest spectrum of antimicrobial activity among tested seaweeds [29-30]. The results of our survey did not show clear taxonomic trends in the activities of the total extracts and their hydrophilic fractions against the studied microorganisms. However surprising, that 63% extracts of red algae tested in our study were active against fungus *C. albicans*. Obtained data suggests that biologically active compounds of red algae possess strong antimicrobial properties. Given suggestion is consistent with the results of other biomedical surveys. Our results agree with opinion of the date El Gamal [30], who noted that the large quantity metabolites with strong antimicrobial activity are typical for the red algae.

Comparing antimicrobial activity of extracts with different polarity it should be noted that lipophilic extract isolated by chlorophorm showed the broadest and highest activity against all tested microorganisms. This finding agrees with the results of the numerous studies, which suggested that biological activity of algae may be caused by high lipophilic nature of bioactive metabolites [12, 31-32]. The antimicrobial activity has been usually attributed to long-chain (mainly $C_{16}$ and $C_{18}$) unsaturated fatty acids, including palmitoleic, oleic, linoleic and linolenic acids; while the long-chain saturated fatty acids, including palmitic and stearic acids were less effective [31]. Moreover, Manilal et al [12] suggested that antimicrobial properties of methanolic extracts, having lipophilic nature, from red algae *Falkenbergia hillebrandii* is due to the presence of fatty acids mainly oleic and n-hexadecanoic acids. Antimicrobial properties of the fatty acids were also reported by Saravanakumar et al [32]. Early the strong antimicrobial properties was shown for monogalactosyldiacylglycerols (MGDG), sulfoquinovosyldiacylglycerols (SQDG), polyunsaturated fatty acids (PUFAs) and free fatty acids (FFA) extracted from *Laminaria cichorioides* (=*Saccharina cicchorioides*) [23]. The fact that lipophilic extracts obtained from red and brown algae were differ in their activity with the stronger inhibition action of red algae extracts in compare to Phaeophyceae extracts suggests that active components certainly different in the algae from different taxa. This difference was established for lipid composition of marine algae from different divisions. The red algae are characterized by high content of $C_{20}$ polyenic fatty acids, whereas $C_{18}$ and $C_{20}$ polyenic acids are prevailing in the brown seaweeds.

The total algal extracts obtained in the present study also exhibited the broad-spectrum antimicrobial activity, which was higher in compare to those obtained for hydrophilic extracts. Total extracts are the complex mixture of primary and secondary metabolites, including fatty acids and phenolic compounds, which can exhibit

antimicrobial activities. The antimicrobial properties of the fatty acids were considered above. As for phenolic substances, having hydrophilic nature, their antibacterial and antifungal properties were extensively discussed in the literature [32-34]. Thereby the higher activity of the total and hydrophilic extracts from brown seaweeds in compare to those from red and green algae is not surprising. The highest activity of hydrophilic extracts among all tested brown algae was detected for members of order Fucales. It is known that brown algae have a highest quantity of phenolic compounds among algae and fucoids are the most rich by these substances among Phaeophyceae. For example, phenol content in the different species of brown algae varies from < 1% to 14% of dry seaweed biomass [35]. Besides, phenolic and lipid compounds brown algae contain a high amount of alginic acids, which also show the strong antibacterial properties. They appeared to be effective against *E. coli* and *Staphylococcus* sp.

The results of the present study also showed that antimicrobial properties were greatly variable among samples of the same algal species collected in the different places and seasons. It is known that different kinds of biological activities of algae may be influenced by several endogenous and exogenous factors such as the variability of environmental conditions in the algal habitats, season of seaweeds collection, composition of the microflora on the thalli surface, herbivory activity, life history stage of plant or physiological state of the algae and other [11-12]. The data on the effect of environmental conditions on the biological properties of algae are very important for further screening algae as new sources of biological active compounds.

The high geographic variation of the antimicrobial activity of extracts from *Fucus evanescens*, collected from different locations in the Kurile Islands, was found in the present work. The region of Kurile Island belongs to the monsoon climate zone and characterized by broad-spectrum differences in abiotic (temperature, salinity, tidal motion, ice boundary, chemical composition and et al.) and biotic factors. Owing to this heterogeneity, ecotopes, where surveyed algae were collected, certainly were differed of environmental conditions, which affected chemical composition of the alga.

Comparison of two *Neorhodomela larix* samples collected in March and September showed differences in antimicrobial activity of their extracts with higher activity in March. Seasonal variations of bioactivity were reported by numerous investigations [11, 29]. Autumn was the season with the highest percentage of active taxa against microbes for Mediterranean and Indian species [29]; while South African species had highest antibacterial activity during late winter and early spring [11]. Some authors have associated peak activity with physiological phenomena such as growth or reproduction [29]. For example, *Falkenbergia rufolanosa* exhibited the peak of antimicrobial activity during autumn – winter, that may be related to the reproductive period of algae as authors suggested [29]. However, in the same study it was found that in other species *Osmundea truncate*, the peak of bioactivity (autumn-winter) occurred after the reproductive period. Also, seasonal change in activity may be caused by different quantities of a single compound in algal tissue, or the synthesis of different compounds due to different growth conditions. Unfortunately, sterile or fertile state of samples of *N. larix* collected in September was not established in the present study. However, it is known that in Peter the Great Bay (Sea of Japan) this species develops cystocarps from July till October. It is likely that lower antimicrobial activity of the *N. larix* extract, obtained in March, in compare to those isolated in September, was caused by plant reproduction.

## Conclusions

The results of this survey did not show clear taxonomic trends in the activities of the crude extracts as well as their hydrophilic and lipophilic fractions against the tested microorganisms, although Rhodophyta extracts showed highest inhibitory effect against fungi among all algae tested. Antimicrobial activities of extracts varied considerably among all studied algal species, and between assayed microorganisms, suggesting that microbial growth inhibition is mediated by a variety of antimicrobial metabolites. Overall, fewer extracts were active against fungi than gram-positive and gram-negative bacteria. In addition, larger more lipophilic than hydrophilic extracts inhibited the growth of all studied microbes and fungi indicating that liphophilic secondary metabolites may play a critical role in determination of antimicrobial properties of the algal extracts. *Neorhodomela larix* subsp. *aculeata*, *Fucus evanescens*, *Costaria costata*, *Saccharina cichorioides*, *S. japonica*, *Sargassum pallidum* and *Scytosiphon lomentaria* appear to be the most promising species for further investigation, as they have a broad spectrum of biological activity and so is likely to yield more than one active compound. These bioactive compounds will need further studies to identify the chemical structure of these active compounds and to examine their beneficial effect for inhibition of some pathogenic bacteria and fungi.

### Authors' contributions

EC executed experimental work, made statistical processing of experimental data and drafted the manuscript.

AS collected and identified the algal material, and helped to draft the manuscript.

MA conceived of the study and participated in its design and coordination, and helped to draft the manuscript.

DA involved in revising of manuscript for important intellectual content and given final approval for publication.



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