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Novel antimicrobial activity of a dichloromethane extract obtained from red seaweed *Ceramium rubrum* (Hudson) (Rhodophyta: Florideophyceae) against *Yersinia ruckeri* and *Saprolegnia parasitica*, agents that cause diseases in salmonids



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ABSTRACT

Background: Enteric red mouth disease and Saprolegniasis, which are caused by the bacteria *Yersinia ruckeri* and the oomycete *Saprolegnia parasitica*, respectively, are important illnesses that affect salmonid farming. Sanitary problems in farms are addressed by the prevention of disease outbreaks or by the treatment of diseases with chemicals. Environmental and governmental restrictions, toxicity and high treatment costs limit the use of drugs. Marine organisms, such as algae, sponges and corals, have developed an antimicrobial defense strategy based on the production of bioactive metabolites. Among these organisms, seaweeds offer a particularly rich source of potential new drugs. Hence, many pharmacologically active substances have been isolated from seaweeds. In the *Ceramium* genus, *Ceramium rubrum* has been emphasized by several authors for its antimicrobial properties. Based on this background, the present study focused on the antimicrobial activity of a lipophilic extract of *C. rubrum* on *Y. ruckeri* and *S. parasitica*.

Results: The alga, collected from the Pacific coast of Chile, underwent an ethanol extraction, and the concentrated extract was partitioned between water and dichloromethane. From the dichloromethane extract, fatty acids, fatty acid esters, one hydrocarbon and phytol were identified by Gas Chromatography–Mass Spectrometry (GC/MS) analysis. The antimicrobial study showed that the whole extract was more active than the individual components, which suggests a strong synergistic effect among the components.

Conclusions: These results may constitute a basis for promising future applied research that could investigate the use of *C. rubrum* seaweed as a source of antimicrobial compounds against fish pathogens.

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1. Introduction

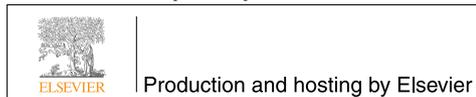
Enteric red mouth (ERM) disease and Saprolegniasis, which are caused by the Gram-negative bacteria *Yersinia ruckeri* [1] and the oomycete *Saprolegnia parasitica* [2], respectively, are important illnesses that affect salmonid species and some fish wildlife. ERM disease is controlled with antibiotics, such as quinolones, tiamulines, sulfonamides, oxytetracycline and erythromycin, and antiseptics,

such as iodophors [3]. Nevertheless, a decrease in drug efficacy and the development of bacterial resistance have limited their use [4]. A previous study showed that *Saprolegnia* infection could be easily controlled by the application of malachite green, a dye that can be applied alone or in combination with other fungicides [5]. However, since 2002, the use of malachite green has been banned worldwide due to both carcinogenic and toxicological effects, which has allowed *Saprolegnia* to become a major economic problem in freshwater fish farms [6]. Alternative treatments include bronopol (30–100 mg l⁻¹), sodium chloride (30 g l⁻¹), formalin (250 mg l⁻¹), potassium permanganate (10 mg l⁻¹) and hydrogen peroxide [7]. However, none of these chemicals are more effective than malachite green. In summary, sanitary problems, environmental and governmental restrictions, solubility, toxicity and high cost have limited the use of synthetic antimicrobials [8]; hence, research on new alternatives is necessary.

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Many bioactive substances have been isolated from algae. Extracts of seaweeds have been reported to exhibit antibacterial activity [9,10,11,12,13] and antifungal activity [10,14,15,16,17]. However, scarce literature is available on the antibacterial and antifungal effects of extracts from *Ceramium rubrum* (Hudson) (Rhodophyta: Florideophyceae). *C. rubrum* is a marine alga that is found in both hemispheres on tropical and polar coasts [18]. Hellio et al. [19] observed that the dichloromethane extract of *C. rubrum* that was collected from the French coast elicited antibacterial activity against several marine Gram-positive bacteria. Similar results were reported by Bansemir et al. [11] with samples collected from the German coast. Tuney et al. [20] observed that diethyl ether and ethanol extracts also showed antibacterial properties. Extracts obtained from the German samples using hexane and methanol inhibited the growth of a variety of marine and fish bacteria [21]. Salvador et al. [22] evaluated the antibacterial effect of fresh and lyophilized extracts and found that *C. rubrum* that was collected from the Mediterranean and Atlantic coasts of Spain was active against Gram-positive and Gram-negative bacteria. There are no reports regarding the antifungal activity of *C. rubrum*.

Seaweeds represent a great source of a variety of natural products [23], but their veterinary potential in fishes has barely been explored. Therefore, the aim of this study was to investigate the antimicrobial activity of the ethanol and dichloromethane extracts of *C. rubrum* against *Y. ruckeri* and *S. parasitica*, two of the main microbial diseases in Chilean farmed salmonids. The results obtained will form the basis for further studies on marine natural products.

2. Materials and methods

2.1. Plant collection

The alga *C. rubrum* was collected in the summer of 2010 at the mouth of the Maullín River, which is located to the northwest of the town Maullín (Chile, Los Lagos Region). The collected material was separated and washed with distilled water to remove traces of sand, seaweed and other marine organisms. Finally, the material was kept in plastic bags and frozen at -20°C .

2.2. Preparation of the extracts

The alga (20 kg) was extracted using 96% ethanol 3 times (each extraction period was 24 h). After filtration, the three extracted solutions were pooled, and the solvent was evaporated in a rotary evaporator, which produced the ethanol (E) extract. The dichloromethane (DCM) extract was obtained by the liquid–liquid separation of 250 g of the E extract that was suspended in 1000 ml of distilled water and subsequently extracted with three portions of dichloromethane (500 ml). The resulting portions were combined to yielding DCM.

2.3. Gas Chromatography–Mass Spectrometry (GC/MS) analysis

The GC/MS analysis of the DCM was performed through electron impact ionization (70 eV) using a Thermo Finnigan chromatograph (Milan, Italy) that was equipped with a BP-1 capillary column (30 m 0.22 mm by 0.25 μm ; SGE, Victoria, Australia) and used helium as the carrier gas. The GC oven was programmed to ramp from 40 to 300°C at $5^{\circ}\text{C min}^{-1}$ and held for 5 min. The injector and transfer line temperatures were fixed at 250°C . The column outlet was inserted directly into the electron ionization source block. The scan range was 40–500 Da. Kovats indices (KIs) of the DCM compounds were estimated by employing a series of homologous reference compounds. The retention indices were determined relative to an *n*-alkane series by means of linear interpolation. Compounds were identified through a comparison between the KI and the mass spectra with available commercial standards. For non-available standards, spectra were compared with the library mass spectral NIST.

3. Antimicrobial tests

3.1. Agar diffusion test

Bacterial inhibition was performed using the agar diffusion method [24]. A *Y. ruckeri* pure strain culture was grown on Tryptic Soy Agar (TSA) plates and incubated for 24 h at $26 \pm 1^{\circ}\text{C}$. Then, a Tryptic Soy Broth (TSB) suspension was prepared from 5 isolated colonies. The culture tube was incubated for 2 h at $26 \pm 1^{\circ}\text{C}$ [1], until reaching the turbidity equivalent to 0.5 McFarland standard (1.5×10^8 CFU ml^{-1}). Subsequently, TSA plates were inoculated with the bacterial suspension. Sterilized paper disks (6 mm, Whatman No. 1) were impregnated with 2 μl of a 500 mg ml^{-1} (1 mg disk^{-1}) solution of the E, the DCM and the DCM compounds. Finally, the disks were deposited on Petri dishes after solvent evaporation. Additionally, 0.02% of Tween 80 was gently mixed into the agar after autoclaving the medium at 45°C to enhance the solubility of the DCM and DCM compounds [25]. All antibiograms were carried out in triplicate. Oxytetracycline disks (30 μg) were used as positive control, and solvents and clean disks (without solvent) were used as control tests. The plates were incubated for 24 h at $26 \pm 1^{\circ}\text{C}$ [1], and the inhibition zones were measured taking into consideration the paper disk diameter.

3.2. Agar dilution test

The E, DCM and DCM compounds obtained from *C. rubrum* were tested against *S. parasitica* using the agar dilution method. Stock solutions (25,000 $\mu\text{g ml}^{-1}$) were prepared, and 200 μl of each solution and 19.8 ml of melted Sabouraud agar medium (SA) were pooled into 10 cm plates to achieve the desired concentration (250 $\mu\text{g ml}^{-1}$). Ten microliters of malachite green solution (500 $\mu\text{g ml}^{-1}$) and 19.99 ml of melted SA were pooled into positive control plates to achieve the desired concentration (0.25 $\mu\text{g ml}^{-1}$) [26]. Using the same method, we prepared control plates that contained SA alone or SA plus 200 μl of the solvent that was used in the stock solutions. Agar disks (6 mm diameter and 3 mm thick) taken from the margins of actively growing colonies of *S. parasitica* cultures were placed in the middle of the plates. The experiments were carried out in triplicate. All of the plates were incubated at $18 \pm 1^{\circ}\text{C}$ for 5 d in darkness. The fungal growth was measured in cm from the agar disk margin [27], and the inhibition of *S. parasitica* was obtained by comparing the fungal colony growth diameter with the controls (only SA) [28].

3.3. Broth dilution test

Minimal inhibitory concentration (MIC) values for *Y. ruckeri* and *S. parasitica* were determined by the broth dilution method. In the antibacterial assays, a two-fold serial dilution of DCM was prepared. Then, 800 μl of TSB, 100 μl of inocula compatible with 0.5 McFarland standard and 100 μl of each DCM dilution were added to an assay tube to achieve concentrations between 1 and 2048 $\mu\text{g ml}^{-1}$. Oxytetracycline (0.5–1 $\mu\text{g ml}^{-1}$) was used as the positive control. All of the mixtures were made in triplicate and incubated for 24 h at $26 \pm 1^{\circ}\text{C}$. Once the incubation time elapsed, the absorbance at 600 nm was monitored [29]. The bacterial inhibition was calculated according to the following equation: % growth inhibition = $100 - [\text{absorbance of the treatment tube} / (\text{absorbance of the growth control tube} - \text{the medium control tube})] \times 100$ [30]. To determine the minimum bactericidal concentration (MBC), a loopful of each assay tube that showed growth inhibition was transferred into a Petri dish that only contained TSA [31].

For the antifungal assays, *Saprolegnia* agar pieces excised from colonized SA plates were incubated in 500 ml of Sabouraud broth (SB) at $21 \pm 1^{\circ}\text{C}$ for 2 d. Subsequently, the grown mycelia mass was washed twice with sterile distilled water, transferred to an Erlenmeyer flask that contained 500 ml of sterile tap water and incubated at $21 \pm 1^{\circ}\text{C}$ for 24 h. The spore suspensions were filtered

through sterilized gauze into another flask [32], and the cysts were counted using a Neubauer chamber. The cyst suspension was adjusted to 5×10^5 spores ml^{-1} and transferred to the challenge tubes within 3 h of counting [33]. To determine the MIC value, a two-fold serial dilution of DCM was prepared, and 800 μl of SB, 100 μl of inocula and 100 μl of each dilution of DCM were added to an assay tube to achieve the desired concentrations between 250 and 2000 $\mu\text{g ml}^{-1}$. Malachite green (0.1–0.5 $\mu\text{g ml}^{-1}$) was used as the positive control [26]. All of the mixtures were made in triplicate and incubated for 5 d at $15 \pm 1^\circ\text{C}$ [34]. When the incubation time elapsed, the absorbance at 550 nm was monitored [35]. The fungicidal inhibition was calculated according to Leippe et al. [30], and the minimum fungicidal concentration (MFC) was calculated according to Sreenivasan et al. [31].

4. Results

4.1. GC/MS analysis

Twenty kilograms of *C. rubrum* yielded 250 g of the E extract. Subsequently, the E was partitioned to obtain 75 g of the DCM. GC/MS analysis of the DCM resulted in the identification of 14 compounds (one alkane, one ketone, fatty acids, fatty acid esters and one diterpene). Phytol (44.7%) and palmitic acid (25.8%) were the major components (Table 1). Seven of the compounds, representing 86.4% of the DCM, were tested in the antimicrobial assays.

4.2. Antimicrobial activity

The E, the DCM and the compounds identified in the DCM inhibited the growth of *Y. ruckeri* and *S. parasitica*, but all of these were less active than the positive controls ($p < 0.04$). The highest antibacterial inhibition was achieved with the DCM (14.7 mm), whereas the highest antifungal inhibition was achieved with the DCM and with stearic acid ($> 17.6\%$). None of the DCM compounds inhibited bacterial growth more than the DCM; however, the DCM and stearic acid had similar antifungal inhibition (Table 2). The MIC values of the DCM resulted in 507.7 $\mu\text{g ml}^{-1}$ and $> 2048 \mu\text{g ml}^{-1}$ for *Y. ruckeri* and *S. parasitica*, respectively.

Four DCM compound mixtures were tested: reconstituted sample, the main compounds, fatty acids and fatty acid esters. None of these mixtures were more active than the DCM. There is a direct relation between the antibacterial activity and the percentage of the compounds of the DCM tested, which suggests that the minor compounds could play an important role in the synergistic effect. In the antifungal assay, the highest activities were achieved when the mixture tested included stearic acid (Table 3).

Table 1
Compounds identified by GC/MS analysis in the DCM obtained from *C. rubrum*.

Compound	Rt ¹	Molecular ion	Mean % \pm SD ²	KI (exp) ³
Hexadecane	26.44	226	4.8 \pm 1.9	1694
Myristic acid	27.72	228	4.1 \pm 0.5	1756
Ethyl myristate	28.09	256	1.2 \pm 0.5	1763
Hexahydrofarnesyl acetone	29.13	268	0.6 \pm 0.1	1823
Hexadecenoic acid, Z-11	31.28	254	4.5 \pm 1.3	1932
Palmitic acid	31.96	256	25.8 \pm 4.9	1967
Ethyl palmitate	32.06	284	6.7 \pm 3.2	1972
Phytol	34.38	296	43.2 \pm 1.5	2097
Oleic acid	34.77	282	2.5 \pm 0.7	2120
Stearic acid	35.19	284	1.3 \pm 0.3	2144
Ethyl linoleate	37.07	308	0.6 \pm 0.4	2150
Ethyl linolenate	37.14	306	0.1 \pm 0.2	2165
DHA methyl ester	39.48	342	1.4 \pm 0.1	2260
EPA methyl ester	39.80	316	1.6 \pm 0.4	2274

Rt: Retention time; SD: Standard deviation; KI (exp): Kovats index (experimental).

Table 2

Antimicrobial activity of the investigated extracts and the DCM compounds against *Y. ruckeri* and *S. parasitica*.

Extract/compound	<i>Y. ruckeri</i> ¹ (mm of inhibition)		<i>S. parasitica</i> ² (% of inhibition)
	TSA	TSA + Tween 80	SA
E extract	8.7 \pm 0.6 ^{b*}	8.1 \pm 0.2 ^c	1.7 \pm 0.2 ^f
DCM extract	–	14.7 \pm 0.6 ^b	17.6 \pm 0.2 ^b
Ethyl myristate	nt	7.3 \pm 0.6 ^d	1.7 \pm 0.0 ^f
Ethyl palmitate	nt	8.0 \pm 0.0 ^c	10.8 \pm 0.1 ^c
Myristic acid	nt	8.0 \pm 0.0 ^c	2.9 \pm 0.2 ^e
Palmitic acid	nt	8.0 \pm 0.0 ^c	2.5 \pm 0.2 ^e
Oleic acid	nt	7.2 \pm 0.3 ^d	1.0 \pm 0.1 ^g
Stearic acid	nt	7.0 \pm 0.0 ^d	17.9 \pm 0.1 ^b
Phytol	nt	8.0 \pm 0.0 ^c	5.0 \pm 0.0 ^d
Oxytetracycline (30 μg)	28.3 \pm 0.6 ^a	27.9 \pm 0.5 ^a	nt
Malachite green (0.25 $\mu\text{g ml}^{-1}$)	nt	nt	44.2 \pm 0.1 ^a

¹ Concentration tested: 1 mg disk⁻¹; ² 250 $\mu\text{g ml}^{-1}$ per plate. (–) not active. (nt) not tested.

* The means that are followed by the same letter do not differ significantly at $p < 0.05$ (Conover–Inman test).

5. Discussion

A variety of organisms are capable of synthesizing substances that have antibacterial and antifungal activities. These properties have been observed in extracts obtained from a number of algae [9,10,11,12,13]. Nevertheless, previous studies evaluating this characteristic in *C. rubrum* are scarce. The results from the present study indicate that the antimicrobial ability of *C. rubrum* is primarily driven by the lipophilic extract. This is in agreement with the results reported by Hellio et al. [19], Bansemir et al. [11] and Dubber and Harder [21], who observed that lipophilic extracts (obtained using dichloromethane and hexane) inhibited the growth of a variety of marine bacteria. Whereas Bansemir et al. [11] did not find inhibitory effects against *Y. ruckeri*, Dubber and Harder [21] reported a growth inhibition between 50 and 90% on the same bacteria. The hexane extract of *C. rubrum* exhibited antibacterial activity at 10,600 $\mu\text{g ml}^{-1}$. In our research, the DCM of *C. rubrum* presented 100% bacterial inhibition on *Y. ruckeri* at approximately 500 $\mu\text{g ml}^{-1}$.

There are no reports regarding the antifungal activity of *C. rubrum*. The present study is the first study that has reported the antifungal activity of this alga against *S. parasitica*. The maximum activity for DCM and for stearic acid ($> 17.6\%$ of inhibition) occurred at 250 $\mu\text{g ml}^{-1}$. These results are consistent with other studies of antifungal inhibition in Rhodophyta seaweeds. Indeed, the ethyl acetate and chloroform extracts of *Solieria robusta* inhibited the growth of several fruit spoiling fungi [36]. The results reported by Stein et al. [17] indicated that hexane and chloroform extracts obtained from several *Laurencia* species possess antifungal properties.

The present results agree with those reported in other Rhodophyta algae studies. Bansemir et al. [37] observed that the dichloromethane extract of *Laurencia chondrioides* (2 mg disk⁻¹) was active on *Pseudomonas anguilliseptica* (Gram-negative) with an inhibition of 15.0 ± 7.3 mm, but it was not active on *Y. ruckeri*. In another study, the dichloromethane extracts (2 mg disk⁻¹) obtained from *Asparagopsis armata*, *Falkenbergia rufolanosa* and *Gracilaria cornea* were active on *Y. ruckeri* with inhibitions of 21.3 ± 1.7 , 13.3 ± 1.9 and 9.0 ± 2.6 mm, respectively, whereas *C. rubrum* extracts were not active [11]. Karabay-Yavasoglu et al. [38] found that an inhibition of 15 mm was obtained using a chloroform extract from *Jania rubens* (4 mg disk⁻¹) on *Enterobacter cloacae* (Gram-negative). It is difficult to assess the antimicrobial activity against *S. parasitica* because there are no reports about the fungal inhibition induced by algae extracts. Several studies have shown that medicinal plants, chitosan, humic substances and compounds isolated from bacteria have antifungal activity against *S. parasitica*. The active compound Oridamycin A, which was isolated from *Streptomyces* sp., exhibited an anti-*S. parasitica* activity with a MIC value of 0.3 $\mu\text{g ml}^{-1}$ [26].

Table 3
Antimicrobial activity of the DCM compound mixtures against *Y. ruckeri* and *S. parasitica*.

Compound	RS	MC	FA	FAE	IND
Hexadecane					
Myristic acid	•		•		•
Ethyl myristate	•			•	•
Hexahydrofarnesyl acetone					
Hexadecenoic acid, Z-11					
Palmitic acid	•	•	•		•
Ethyl palmitate	•			•	•
Phytol	•	•			•
Oleic acid	•		•		•
Ethyl linoleate					
Ethyl linolenate					
Stearic acid	•		•		•
Methyl DHA					
Methyl EPA					
% of DCM	86	71	34	8	–
¹ Antibacterial activity (mm)	9.0 ± 0.5 ^a *	8.5 ± 0.2 ^a	7.0 ± 0.1 ^b	7.0 ± 0.1 ^b	< 8.0 ± 0.0
² Antifungal activity (%)	15.3 ± 0.1 ^b	11.2 ± 0.2 ^c	18.2 ± 0.5 ^a	10.9 ± 0.2 ^c	< 17.9 ± 0.1

¹ Concentration tested: 1 mg disk⁻¹; ² 250 µg ml⁻¹ per plate. RS: reconstituted sample; FA: fatty acid; FAE: fatty acid esters; MC: main compounds; IND: single compounds (commercial standards).

* The means that are followed by the same letter do not differ significantly at p < 0.05 (Conover–Inman test).

Udomkusonsri et al. [39] reported that crude extracts from Thai plants produced a fungal inhibition growth between 11 and 100% at 250 µg ml⁻¹. Meinelt et al. [28] observed that humic substances from non-eutrophicated freshwater ecosystems produced a 40% inhibition of *S. parasitica* growth at 250 µg ml⁻¹.

Dubber and Harder [21] hypothesized that the antibacterial effect of *C. rubrum* extracts is due to the presence of significant quantities of unidentified fatty acids. Hence, this is the first report about active metabolites from the lipophilic extract of *C. rubrum*. In this study, 7 of the 14 identified metabolites were tested on the pathogens. These metabolites included fatty acids, fatty acid methyl esters and an acyclic diterpene alcohol. Stearic acid was the most active compound against *S. parasitica*, but none of the identified compounds were more active than the dichloromethane fraction against *Y. ruckeri*. According to Bansemir et al. [37], the antimicrobial activity of the extract could be a synergistic effect of its constituents because the pure compounds only showed a weak effect (the exception was the stearic acid activity against *S. parasitica*).

Many antibacterial metabolites have been isolated from aqueous or very polar extracts of red algae [40,41,42]. Despite numerous studies that show the biological activity of polar compounds, it is not uncommon to find antimicrobial activity based on lipophilic extracts obtained from algae. It seems that the use of lipophilic compounds in defense is an effective adaptation that ensures the presence of the active compound in the aquatic system as long as possible. In addition, water-insoluble compounds are also advantageous in that they diffuse slowly into the seawater, which ensures that the potency of the metabolite will remain intact for some period [43]. Several antimicrobial metabolites, including diterpenes, bromophenols, sesquiterpenoids and halogenated metabolites, share two aspects: their small molecular size and their lipophilic character. These physicochemical attributes are suggested to be of critical significance for metabolites to function in natural defense [44].

Other than the stearic acid activity against *S. parasitica*, none of the DCM compound mixtures were more active than the DCM. The findings that none of the DCM compounds were responsible for the antibacterial activity and that the reconstituted sample (86% of the DCM) was less active than the DCM indicate that a synergistic effect may be occurring. Although there are no reports of synergism among algae metabolites, this phenomenon is observed in plants. Sökmen et al. [45] observed synergism between the antimicrobial compounds obtained from the essential oil of *Achillea biebersteinii*. The essential oil was fractioned by column chromatography, and 9 extracts were obtained and tested. The

authors observed that the activity was mainly observed in the extracts that contained eucalyptol and camphor, followed by those containing borneol and piperitone. In addition, Carpinella et al. [46] observed an antifungal synergistic effect between scopoletin, vanillin, 4-hydroxyl-3-methoxycinnamaldehyde and pinoresinol that were isolated from *Melia azedarach* fruits. Although the major components of a natural sample generally represent its biological features, they are not necessarily responsible for the greatest activities [47]. Various minor components may contribute to the biological activities [48]. Chairgulprasert et al. [49] indicated that minor components of volatile oils may produce an antimicrobial effect when combined with other minor active components. Thus, it is possible that 2-decen-1-ol, a minor component of the essential oil of *Elettariopsis curtisii*, may enhance the essential oil's antimicrobial activity. In the DCM of *C. rubrum*, minority compounds (14% of DCM) or their mixtures could be responsible for activity.

The present results could indicate the occurrence of synergy between the DCM components because i) weak antibacterial activity was observed when the DCM compounds were evaluated separately, ii) the reconstituted sample (86%) had weak antibacterial activity, and iii) there was a trend toward increased antibacterial activity as more of the DCM compounds were included in the tested sample.

The possible mechanisms of the antimicrobial activity of fatty acids can explain the synergy between the DCM components. The antibacterial activity of fatty acids depends on their structure. Although there is disagreement in the literature, some general considerations are possible. The free carboxylic group is the most important structural requisite for antibacterial activity because it allows an optimal insertion into cells that have hydrogen-bond-acceptor groups in the membrane. The corresponding esters have little or no activity [50]. Other structural factors that modulate the antimicrobial activity of fatty acids are the length and degree of unsaturation of the carbon chain and the stereochemistry of the unsaturation.

Due to the amphipathic character of fatty acids, the microorganism's membrane is the target of fatty acids. After fatty acid-induced damage occurs, other molecules can penetrate the membrane and affect different metabolic processes [51].

C. rubrum is an interesting candidate to investigate for active metabolites against *Y. ruckeri* and *S. parasitica*. In summary, the growth of the *Y. ruckeri* and *S. parasitica* pathogens was inhibited to varying degrees by extracts from *C. rubrum*. Because of the natural origin of the extracts or pure compounds from *C. rubrum*, they have a lower hazard potential than drugs and are a potential alternative to prevent and control outbreaks of salmonid diseases.

Concluding remarks

The present study determined that *C. rubrum* contains metabolites that possess antibacterial and antifungal activities against fish pathogens. The lipophilic extract was shown to be active against *Y. ruckeri* and *S. parasitica*; thus, the results suggest the use of *C. rubrum* as a source of antimicrobial compounds.

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Author contribution

Proposed theoretical frame: AQ; Conceived and designed the experiments: YC, AQ; Contributed reagents/materials/analysis tools: LP, HL, AU; Wrote the paper: YC, AQ; Performed the experiments: YC; Analyzed the data: EH, AM.

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