Electronic Journal of Biotechnology 57 (2022) 12–23

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

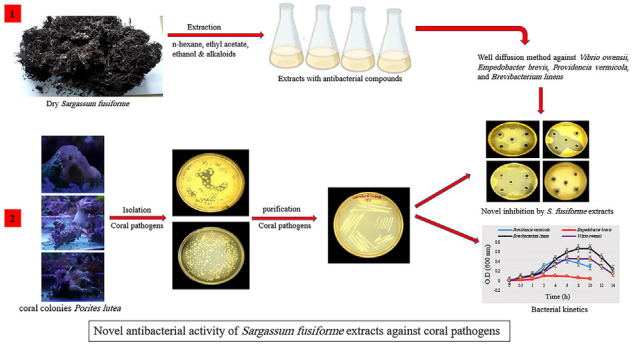
Research Article

Novel antibacterial activity of *Sargassum fusiforme* extract against coral white band disease ☆

Nedaa Ahmed [a,b,c], Hala F. Mohamed [b,c], Changan Xu [b,*], Xiaohui Sun [d], Lingfeng Huang [a,*]

[a] Xiamen University, College of the Environment & Ecology, Xiang'an District, Xiamen 361102, People's Republic of China
[b] Third Institute of Oceanography, Ministry of Natural Resources, Xiamen 361005, People's Republic of China
[c] Al-Azhar University (Girls Branch), Faculty of Science, Botany & Microbiology Department, Cairo, Egypt
[d] Huaqiao University, College of Chemical Engineering, Xiamen 361021, People's Republic of China

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 10 October 2021
Accepted 15 March 2022
Available online 19 March 2022

Keywords:
Alkaloids
Antibacterial
Coral pathogens
Coral white band disease
Crude extracts
Flavonoids
Herbal plants
Natural resources
Porites lutea
Sargassum fusiforme
Seaweeds

ABSTRACT

Background: Coral diseases are one of the serious threats embroiling in the imbalance of the coral holobiont integrity through disruption of the complex symbiotic relationship between endobiotic alga, coral animal, and a group of microorganisms. Such diseases are usually associated with many bacterial pathogens inflicting gross lesions in corals which show resistance against antibiotics. Therefore, this has led scientists to draw more attention towards the curative compounds from natural resources like herbal plants and seaweeds as substitutes for chemical antimicrobial agents. This study aimed to evaluate the antibacterial activity of the crude extracts (n-hexane, ethyl acetate, and ethanol), alkaloids, and flavonoids from *Sargassum fusiforme* through Well Diffusion Assay against different isolated bacterial coral pathogens such as (*Vibrio owensii, Empedobacter brevis, Providencia vermicola,* and *Brevibacterium linens*) which cause white band disease to coral reef *Porites lutea.* This study was also validated by bacterial growth kinetics using optical density, dry weight, and plate count method for the isolated coral bacterial pathogens.
Results: The results indicated that the crude extract with n-hexane and alkaloid extract showed prominent inhibiting activity against the tested bacterial pathogens compared to other extracts.
Conclusions: Here we report *S. fusiforme* extracts as a novel antibacterial agent against four *Porites lutea* bacterial pathogens and further investigation is recommended against other coral pathogens. Overall, *S. fusiforme* extracts might be able to improve the health status of commercially important coral species.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
* Corresponding authors.
E-mail addresses: xuchangan@tio.org.cn (C. Xu), xuchangan@tio.org.cn (L. Huang).

https://doi.org/10.1016/j.ejbt.2022.03.002

How to cite: Ahmed N, Mohamed HF, Xu C, et al. Novel antibacterial activity of *Sargassum fusiforme* extract against coral white band disease. Electron J Biotechnol 2022;57. https://doi.org/10.1016/j.ejbt.2022.03.002.

1. Introduction

Coral reefs are one of the most important economic marine ecosystems [1] old back to 500 million years ago [2]. They constitute significant biological substrates that supply shelter and food for reef-associated microorganisms [3]. Anthropogenic disorders in physical and chemical atmospheric dynamics, sedimentation [4], overfishing, and decrease calcification due to ocean acidification, climate changes [5], and most importantly, infectious microbial diseases [6] are much known for triggering their decline and death. In this study, we investigate the ability of *S. fusiforme* to inhibit different pathogenic bacteria that cause infectious diseases to corals which are considered one of the elementary reasons for the current global degradation of coral reef ecosystems [7]. The diseases caused by microbes can be isolated and distinguished in many cases as *Vibrio* species which are widely distributed in the marine environment and frequently associated with the bivalves, shrimp, fish, [8] corals, and shellfish species [9,10]. As mentioned in the previous studies, *Vibrio coralliilyticus* was detected for lysis and bleaching of *Pocillopora damicornis* [11], *Serratia marcescens* for white pox disease [12], *Aurantimonas coralicida* for white plague Type II [13], *Vibrio shiloi* for bleaching *Oculina patagonia* [14], *Vibrio harveyi* [15] and *Vibrio alginolyticus* [16] for White Syndrome in tropical stony corals in the Caribbean and Indo-Pacific. These bacterial diseases detected are known to be pathogenic to corals [17].

Diseases caused by such pathogenic bacteria are the major problem in aquacultures. However, various vaccines, chemotherapeutics, probiotics, and immune stimulants have been used to treat bacterial infections, but mutants and drug-resistant microorganisms have become a great problem [18]. Therefore, worldwide detection and recognition of the efficiency of natural phytopharmaceutical compounds from natural sources like seaweeds seem to be the model of therapeutic medication [19].

Seaweeds have been long known for their high potential production of natural bioactive secondary metabolites such as alkaloids, flavonoids, tannins [20], phenolics, proteins, fatty acids, terpenes, etc., [21,22] which demonstrated considerable antibacterial, anthelmintic, antiviral, antifungal [23], anti-inflammatory, antioxidant and antibiotics activities [24]. Among marine seaweeds, brown algae have been shown to have a higher content of marine phenolic compounds [25] as well as flavonoids and alkaloids in which marine algal alkaloids are relatively rare, when compared to terrestrial plant alkaloids and mainly belong to the indole and phenylethylamine groups [26]. The same authors also added that the biological activities of marine seaweed alkaloids were not wholly detected. On the other hand, flavonoids are the most significant natural phenol due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties [27].

One of the most important and vital seaweed is *S. fusiforme* or Hizikia fusiformis which is the most common brown macro-alga with high economic value and market demand [28]. It has been grown in China, Japan, and Korea and consumed for thousands of years as food [29] and is common in prolonging expected life [30]. The biological activities of crude extracts from *S. fusiforme* in many studies were examined, and the results reported that *S. fusiforme* possessed significant-high antimicrobial [31], anti-allergic [32], antioxidant [33], anti-diabetic [34] anti-inflammatory [32,34] and HIV-1 inhibitor activities [35]. To our knowledge, there is no report on the evaluation of crude extracts, alkaloids, and flavonoids from *S. fusiforme* as antibacterial agents against coral pathogens. Therefore, this study aimed to extract the crude (n-hexane, ethyl acetate, and ethanol), total alkaloids, and total flavonoids from *S. fusiforme* and determine their antibacterial activity against *Porites lutea* bacterial pathogens. The results obtained in this study will help understand the significant bioactivity of the extracted crudes, alkaloids, and flavonoids as natural antimicrobial agents against coral aquarium pathogens.

2. Materials and methods

This experiment was conducted in the marine laboratory at Third Institute of Oceanography, Ministry of Natural Resources (China) to investigate the effect of *S. fusiforme* crude extracts (n-hexane, ethyl acetate, and ethanol), alkaloid and flavonoid extract as antimicrobial agents against some coral bacterial pathogens isolated from diseased coral *Porites lutea*.

2.1. Sample collection

2.1.1. Materials

Five kg of well-identified *S. fusiforme* sample was collected in the summer of 2020 from Wenzhou Dongtou Xinrui mariculture professional cooperation, placed in ice bags, and transported to the laboratory.

Four different diseased coral colonies from *Porites lutea* were collected from an aquarium in the laboratory of coral conservation, Third Institute of Oceanography, Ministry of Natural Resources (Fig. 1). They were originally collected from Luhuitou, Sanya bay about three meters deep. The coral colonies were scratched using a suitable tool, the samples were collected, and placed in prelabeled sterilized plastic bags, and transported to the laboratory where the experiment was carried out.

2.1.2. Strains, media, and chemicals

Bacterial strains were obtained from diseased coral *Porites lutea* from the coral aquarium in the coral conservation lab, Third Institute of Oceanography, Ministry of Natural Resources. Solvents and reagents in this study (ethanol, n-hexane, ethyl acetate, acetic acid, methanol, and ammonium hydroxide) were of analytical grade purity. The Luria-Bertani medium was used for isolation, antimicrobial activity, and bacterial growth curve.

2.2. Isolation of coral bacterial pathogens

Samples collected from diseased *Porites lutea* colonies were subjected to serial dilution method to separate different colonies for molecular identification of involved coral pathogenic bacteria from coral samples *Porites lutea* A, B, C, and D. Coral diseased pathogens were scratched and cultured on plates containing solid LB media for 24 h at 37°C. After the growth of different bacterial strains, a swab from each bacterial group was cultured in LB liquid media which in turn was used for serial dilution as mentioned by Throndsen [36]. One mL of each bacterial sample was diluted by the addition of 9 mL of sterilized water (diluent). A serial dilution was carried out by the dilution of a sample, in 10-fold dilutions. These

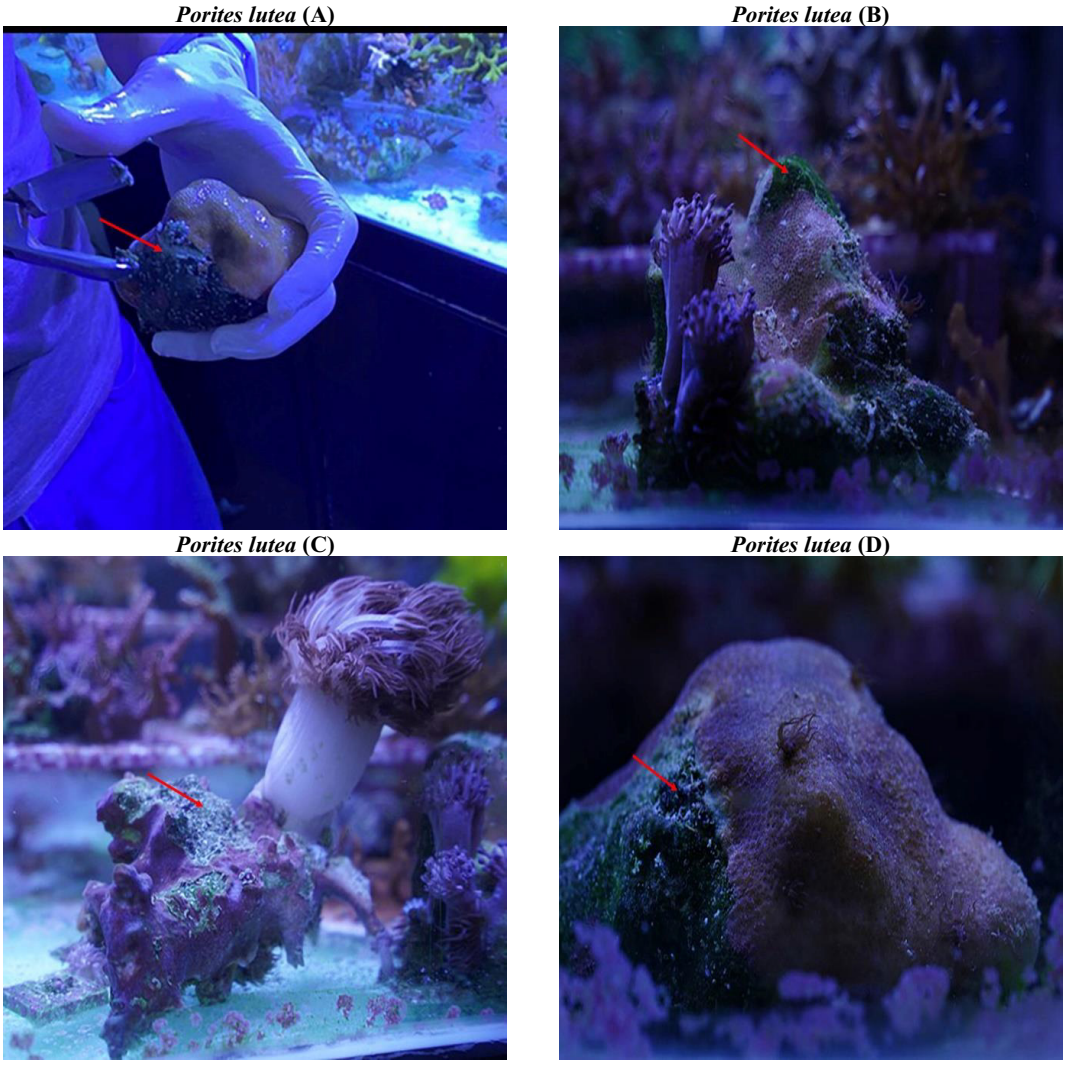


Fig. 1. White band diseased coral colonies from *Porites lutea* A, B, C, and D samples. Coral pathogens (*Vibrio owensii, Alcaligenes faecalis*, and *Bordetella trematum*), (*Brevibacterium linens*, and *bacillus cereus*), (*Alcaligenes faecalis, Providencia vermicola*, and *Ochrobactrum pseudogrignonese*), (*Empedobacter brevis*) were isolated from samples A, B, C, and D, respectively.

steps were repeated for as many dilutions as needed. Both samples *Porites lutea* (A & B) were subjected to six repeated dilution steps (10^{-6} dilution), while with *Porites lutea* (C & D) samples, the growth of the colonies was so heavy, therefore it was needed to subject both samples to more than six dilution steps (10^{-7} with C sample and 10^{-8} dilution with D sample). After the preparation of different dilutions, 50 µl from each of the tubes labeled coral bacterial pathogens 1, 2, 3, and 4 was spread on a solid LB media and incubated for 24 h at 37°C. Morphologically different bacterial colonies were selected on fresh media for further identification using 16S rDNA sequencing.

2.3. Identification of coral bacterial pathogens using PCR and 16S rDNA sequencing

Genomic DNA extraction was performed according to the instructions of "Ezup Column Bacterial Genomic DNA Extraction Kit (Shanghai Shenggong)"; using PCR amplification bacterial 16s rDNA. 25 µl reaction system was used in which 0.5 µl template, 2.5 µl (10 × Buffer), 1 µl dNTP, 0.2 µl Taq enzyme, 0.5 µl 27F, 0.5 µl 1492 R, and 19.8 µl DD H_2O reacted under the following conditions: 94°C for 4 min, 94°C for 45 s, 55°C for 45 s, 72°C for 1 min, 72°C for 10 min, and finally 4°C. Gel electrophoresis detection uses 1% agarose carbohydrate electrophoresis, run at 150 V, 100 mA for 20 min, and observed the electrophoresis results of PCR products. After sequencing analysis and electrophoresis, detection showed a single bright band of about 1500 bp, using the "SanPrep Column DNAJ Gel Recovery Kit (Shanghai Shenggong)" to pair the strips. After the belts were recovered and purified, they were sequenced, and the sequencing results were uploaded to NCBI for BLAST analysis. Oligonucleotide primers used to amplify the 16S rRNA of *Porites lutea* bacterial pathogens are shown in (Table 1).

2.4. S. fusiforme extractions

2.4.1. Crude extractions

Samples of *S. fusiforme* were dried in shade for 6 weeks to remove all water content, pulverized by a grinder into powder,

Table 1
Oligonucleotide primers used to amplify the 16S rRNA from coral bacterial pathogens.

Primers	Sequences
27 F	AGTTTGATCMTGGCTCAG
1492 R	GGTTACCTTGTTACGACTT

and sieved by 0.45 mesh sieve. Crude extracts with 70% ethanol, n-hexane, and ethyl acetate were carried out according to Bolaños et al. [37] 1000 mL from each solvent was added separately to 200 g of *S. fusiforme* powdered material (1:10 g/mL) with continuous shaking at 40°C for 24 h. The sample was filtered, and a fresh solvent was added again into the sample and was shaken for another 24 h. The collected crude extracts were reduced and concentrated using a rotary evaporator at 45°C, lyophilized, weighed, and introduced as gram dry plant material then 50 mg/mL re-dissolved again in the appropriate solvent to be used as antimicrobial agent against different bacterial coral pathogens.

2.4.1.1. Extraction of total alkaloids. Total alkaloids were determined according to Harborne [38]. 200 g of the dry *S. fusiforme* sample was weighed and 1000 mL of 10% acetic acid in ethanol was added, sheeted, and allowed to stand for 4 h. The extract was filtered and concentrated on a water bath until it reached one-quarter of the original amount. Concentrated ammonium hydroxide was added drop by drop to the extract until the precipitation occurred. The whole extract was filtered, and the residue was collected. The collected residue is the alkaloid, which was dried, weighed, and represented as gram dry plant material; then 50 mg/mL re-dissolved again in ethanol to be used as an antimicrobial agent against different bacterial coral pathogens.

2.4.1.2. Extraction of total flavonoids. Total flavonoids were determined according to Bohm and Koupai-Abyazan [39]. 200 g of the dry *S. fusiforme* sample was weighed and extracted repeatedly with 1000 mL of 80% methanol at room temperature. The whole solution was filtered using a filter paper, number 42 (125 mm); the filtrate was later transferred to the oven and evaporated to dryness, until a constant weight was obtained. The amount of flavonoids was calculated as gram dry plant material then 50 mg/mL re-dissolved again in 80% methanol to be used as antimicrobial agent against different bacterial coral pathogens.

The amount of different crudes, total alkaloids, and total flavonoids was weighed and calculated according to the following equation:

$$Y(\%) = \frac{\text{Weight of the obtained dry extract}}{\text{Weight of dry } S.fusiforme} \times 100$$

2.5. Assay of anti-microbial activity of different extracts from S. fusiforme against coral bacterial pathogens

The anti-microbial effect of *S. fusiforme* crude extract with (n-hexane, ethyl acetate, and ethanol), total alkaloids, and total flavonoids was found using the Well-Diffusion method. Sterilized Luria-Bertani medium was mixed quickly with coral bacterial pathogens (*Vibrio owensii, Empedobacter brevis, Providencia vermicola*, and *Brevibacterium linens*) and poured into the plates under aseptic conditions, which was then was let to solidify. Solidified cultures media were perforated using Φ 8 mm perforator to make holes. 10 μL, 20 μL, 30 μL, 40 μL, 50 μL of different extractions of *S. fusiforme* were added to the holes in the agar media while tetracycline (500 μg/mL) was used as a positive control and concerned solvents were used as a negative control. The cultured media were then incubated in an incubator for 24 h at 37°C. The anti-microbial activity was determined by measuring the diameter of the inhibition zone and the mean values were calculated. The assays were performed in duplicate with three repetitions under strict aseptic conditions. The microbial index was also calculated using the following equation:

$$\text{Microbial Index}(mm) = \frac{\text{Inhibition zone-Diameter of the well}}{\text{Diameter of the well}}$$

2.6. Determination of minimum inhibitory concentration (MIC)

MIC is known as the lowest concentration of the antimicrobial agent that inhibits the visible growth of microbes after incubation overnight. From each extract (Alkaloids, n-hexane, ethyl acetate, and ethanol) 50 mg/mL was weighted. 100 μl from each extract was pipetted into well column 1 separately, then 100 μl from LB media was pipetted into all the wells from columns 1 to 12. The extract and LB media were mixed well by sucking 5–8 times up and down. Then 100 μl from column 1 was transferred to column 2, mixed well and another 100 μl was transferred to column 3. The previous steps were repeated until column 10 to make (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 and 0.048 mg/mL) from each extract. Finally, 100 μl from column 10 was discarded. 5 μl of the diluted bacterial strain was pipetted into wells except column 12 which was the blank control. These steps were repeated separately with each extract and with different bacterial strains. The results were manually taken using a black card after incubation at 37°C for 12–18 h.

2.7. Microbial growth curve

The purified colonies of different bacterial coral pathogens such as (*Vibrio owensii, Empedobacter brevis, Providencia vermicola,* and *Brevibacterium linens*) were cultured in LB media and incubated with shaking incubator at 37°C, 180 rpm, and overnight. The optical densities of bacterial cultures were measured at regular intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 14 h) by the spectrophotometer (SpectraMax M5) at OD 600 nm.

The dry weight of bacterial strains was obtained by the filtration method in which 1 ml of the bacterial broth was placed on a pre-weighted dry filter paper. After the dryness of the filter paper at 40°C, the growth of bacterial strains was calculated according to the following formula:

$$\text{Dry weight} = (X_2 - X_1).$$

where X_2 represents the final weight of filter paper (g L^{-1}) while X_1 represents the initial weight of the dry filter paper.

The viable bacterial cells were performed using the plate count method, in which 100 μL of the bacterial broth was inoculated using the spread plate method; the plates were incubated at 37°C for 24 h and the number of bacterial colonies was counted.

3. Results

3.1. 16S rDNA sequencing identification of tested bacterial strains isolated from Porites lutea

All isolated bacterial pathogens cultured on LB media were then analyzed by extraction of their DNA followed by sequencing of their 16S rDNA. Molecular analysis showed that bacterial strains (*Vibrio owensii, Bordetella trematum, Empedobacter brevis, Providencia vermicola, Brevibacterium linens, Alcaligenes faecalis, Ochrobactrum pseudogrignonese,* and *bacillus cereus*) were obtained from white band diseased *Porites lutea* samples A, B, C, and D from the coral aquarium. Only four coral bacterial pathogen strains (*Vibrio owensii, Empedobacter brevis, Providencia vermicola,* and *Brevibacterium linens*) obtained from coral aquarium diseased *Porites lutea* samples were selected for anti-microbial activity. (Fig. 2) shows 16S rRNA alignment reference trees for selected bacterial pathogens *Vibrio owenssi, Empedobacter brevis, Providencia vermicola,* and *Brevibacterium linens.*

3.2. Yield of total alkaloids, total flavonoids, and total secondary metabolites from different crude extracts obtained in vitro from seaweed S. fusiforme

The yield from *S. fusiforme* using different solvents showed that the highest yield was recorded with ethanol (49.9 g – 23.9%) followed by n-hexane (3.30 g – 1.7%) and ethyl acetate (0.91 g – 0.45%). The present study was also performed to extract alkaloids and flavonoids from the same species in which the yield was (0.679 g – 0.33%) and (39.1 g – 19.05%) respectively. The yield of total secondary metabolites obtained from seaweed *S. fusiforme* is shown in (Fig. 3).

3.3. Anti-microbial activity of crude extracts (n-hexane, ethyl acetate, and ethanol), total alkaloids, and total flavonoid extracts from S. fusiforme against Porites lutea bacterial pathogens

In the present study, we evaluated the antibacterial activity of the crude extracts (n-hexane, ethyl acetate, and ethanol), alkaloids, and flavonoid extracts from *S. fusiforme* against different White band diseased *Porites lutea* pathogenic bacteria namely: *Vibrio owensii, Empedobacter brevis, Providencia vermicola,* and *Brevibacterium linens* isolated from coral reef aquarium. Each bacterial species has shown different resistance against different solvent extracts, alkaloids, and flavonoids.

As for the solvent extracts, n-hexane exhibited a higher antibacterial activity against tested coral pathogenic bacteria compared to ethanol and ethyl acetate extracts in which n-hexane extract inhibited all the tested bacterial pathogens at different concentrations. The highest inhibition was (18 mm) against *Vibrio owensii* then *Brevibacterium linens* (17 mm), *Providencia vermicola* (16 mm), *Empedobacter brevis* (16 mm) at 50 µl extract. On the other hand, the crude extract with ethanol followed n-hexane extract which was able to inhibit only *Empedobacter brevis* at 20 µl, 30 µl, 40 µl, 50 µl with inhibition zones 14, 15, 17, 19 mm, respectively. As regards ethyl acetate extract, the results showed that it was able to inhibit *Vibrio owensii* at 40 µl and 50 µl by 12 mm. In the present study, it has been observed that the tested solvent extracts (n-hexane, ethyl acetate, and ethanol) had antibacterial properties against coral bacterial pathogens. Regarding total alkaloids and total flavonoids, the latter was not able to inhibit any of the tested coral bacterial pathogens. As regards alkaloid extract from *S. fusiforme,* it showed prominent inhibition against tested *Porites lutea* bacterial pathogens. The highest inhibition (38 mm) against *Bre-*

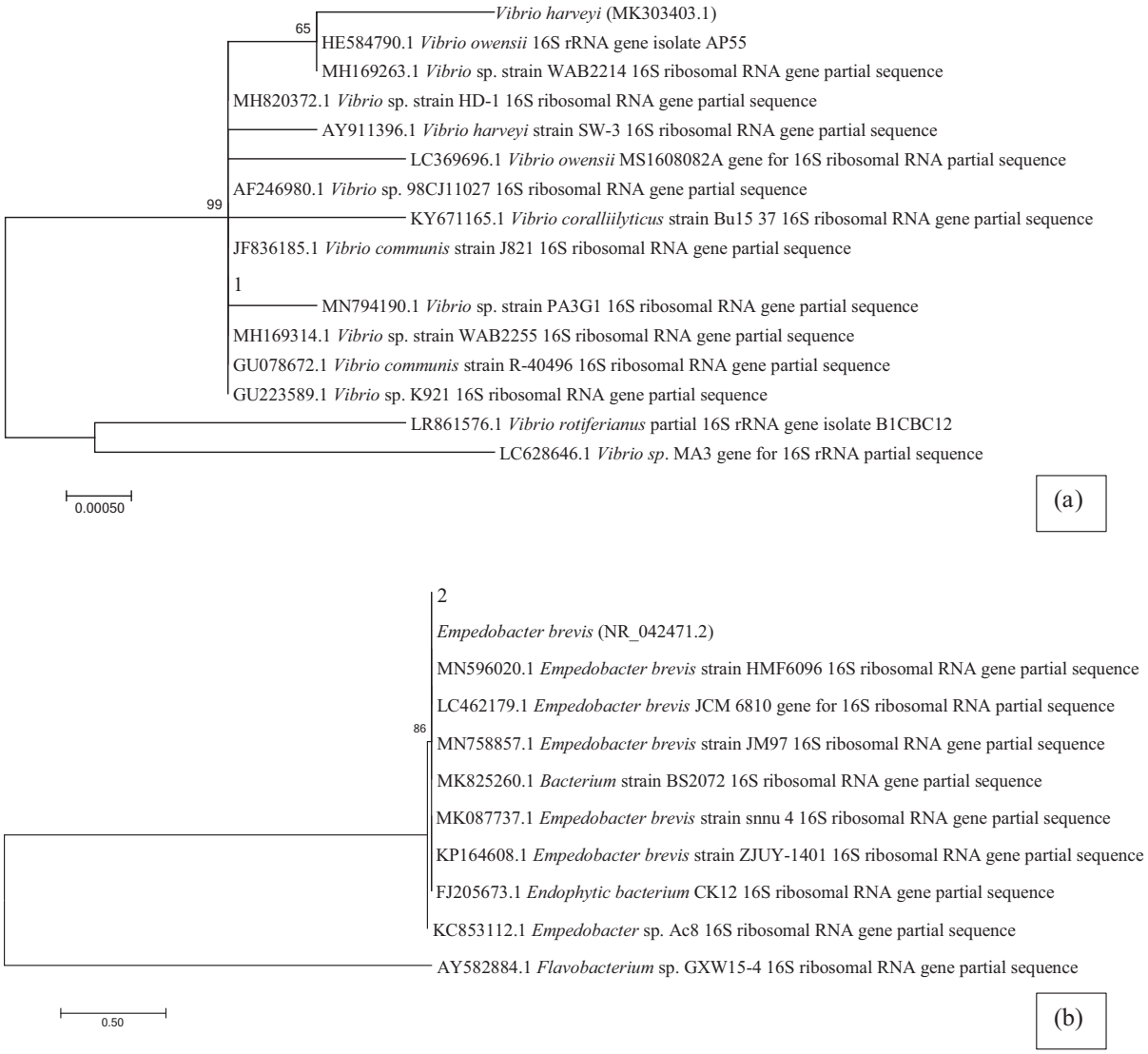


Fig. 2. 16S rRNA alignment reference trees for (a) *Vibrio owenssi,* (b) *Empedobacter brevis,* (c) *Providencia vermicola,* and (d) *Brevibacterium linens.*

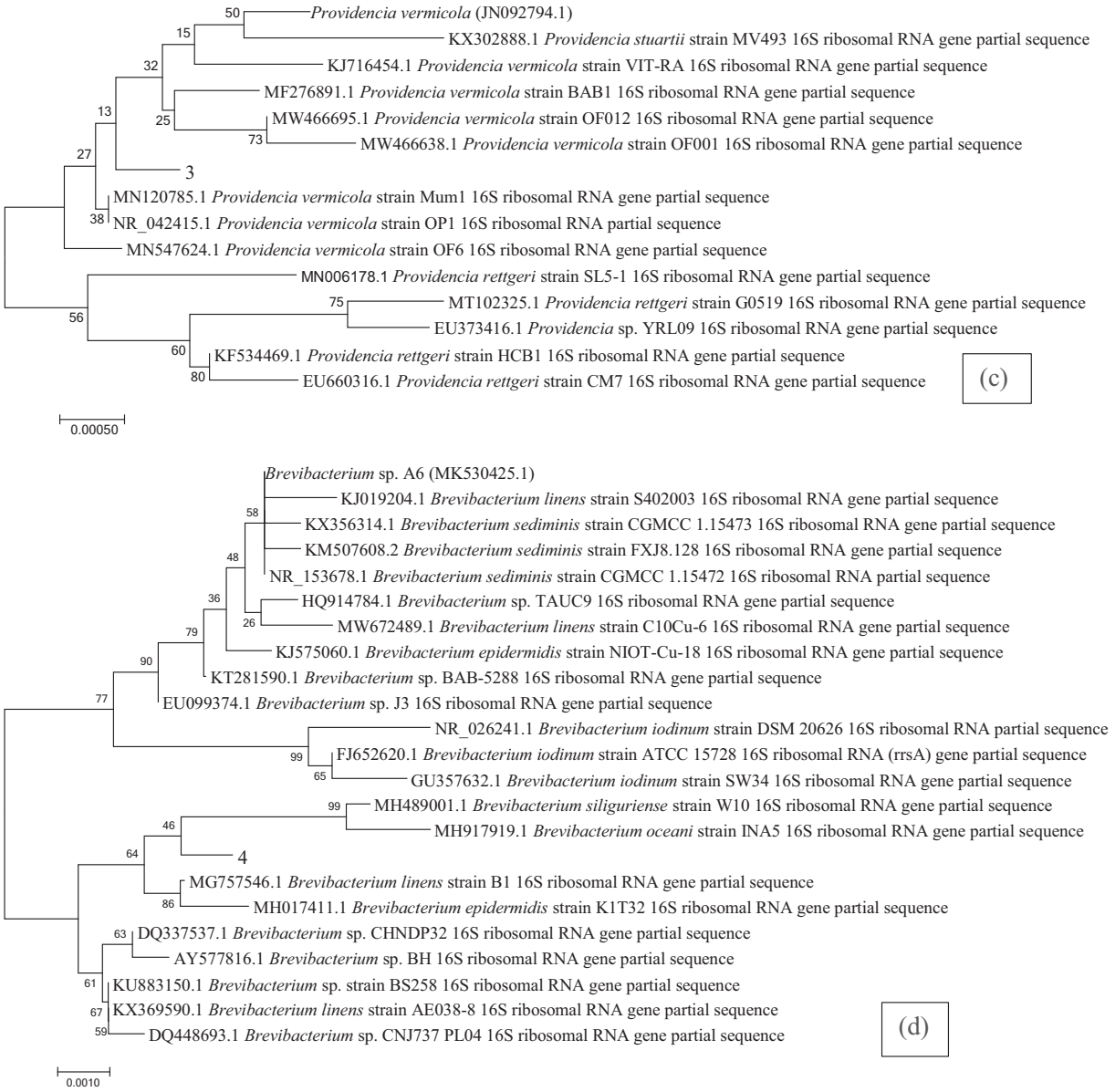


Fig. 2 (*continued*)

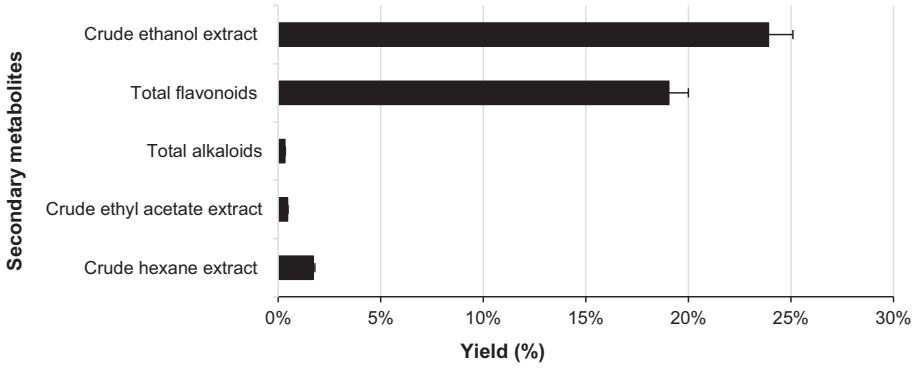


Fig. 3. The yield of total secondary metabolites obtained *in vitro* from seaweed *S. fusiforme*. The yield of metabolites extracted from *S. fusiforme* /200-gram dry biomass was (49.9 g – 23.9%), (3.30 g – 1.7%), (0.91 g – 0.45%), (0.679 g – 0.33%) and (39.1 g – 19.05%) from ethanol, n-hexane, ethyl acetate, alkaloids and flavonoids, respectively.

vibacterium linens then *Vibrio owensii* (30 mm) *Empedobacter brevis* (28 mm) and *Providencia vermicola* (19 mm) at 50 µl extract. All the tested strains were inhibited by alkaloid extract at all concentrations except *Vibrio owensii,* alkaloid extract unable to inhibit it at 10 µl. The results illustrated in (Fig. 4 and Fig. 5) are dealing with the calculation of microbial indices from LB cultures of *Porites lutea*

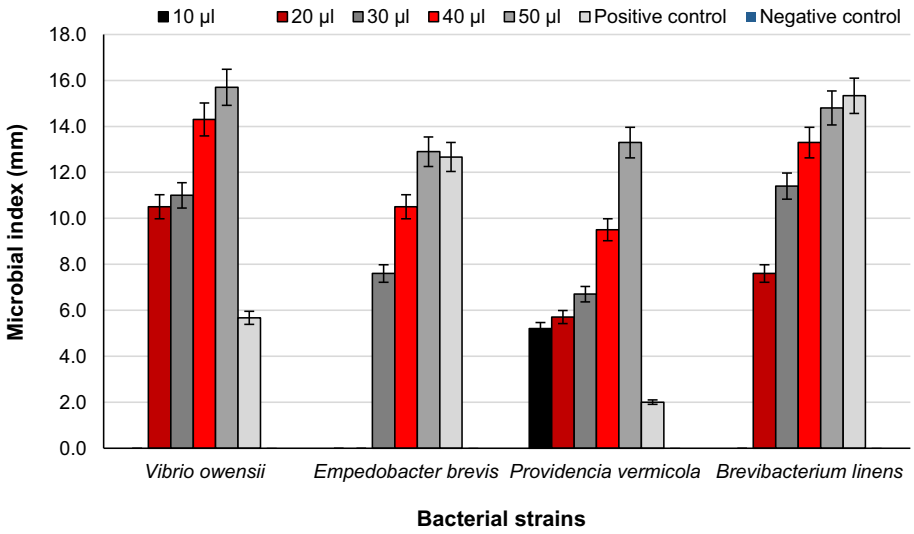


Fig. 4. Microbial indices calculated from LB cultures of white band diseased *Porites lutea* pathogenic bacteria with *S. fusiforme* n-hexane extract. 10 µL, 20 µL, 30 µL, 40 µL, 50 µL of (50 mg/mL) n-hexane extracts from *S. fusiforme* were used while tetracycline (500 µg/mL) was used as a positive control.

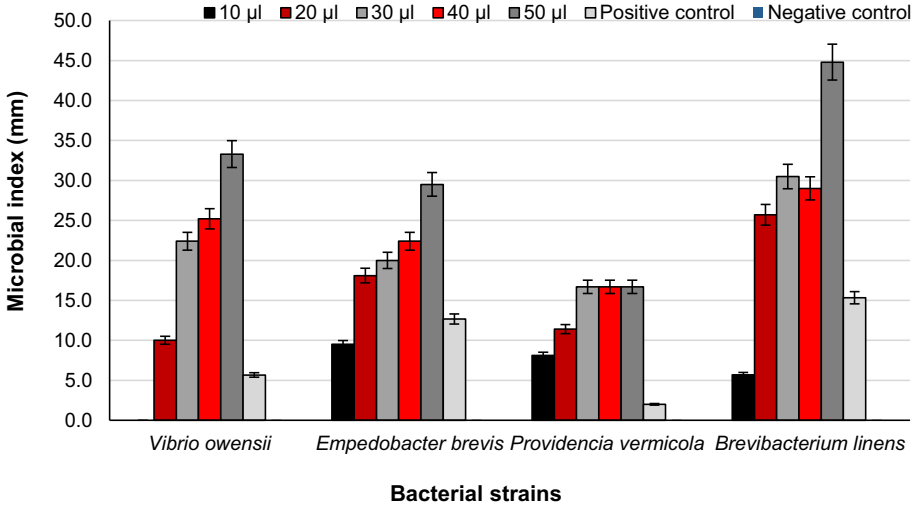


Fig. 5. Microbial indices calculated from LB cultures of white band diseased *Porites lutea* pathogenic bacteria with *S. fusiforme* alkaloid extracts. 10 µL, 20 µL, 30 µL, 40 µL, 50 µL of (50 mg/mL) alkaloid extracts from *S. fusiforme* were used while tetracycline (500 µg/mL) was used as a positive control.

pathogenic bacteria with *S. fusiforme* n-hexane and alkaloid extracts, respectively, as both extracts reported the best results of bacterial inhibition.

Our findings also confirmed that the crude extracts and alkaloids themselves have antimicrobial properties compared to n-hexane, ethyl acetate, and ethanol which were used as negative controls.

3.4. Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of alkaloids, n-hexane, ethyl acetate, and ethanol was reported in (Fig. 6). The sensitivity order of MIC for alkaloids against *Brevibacterium linens*, *Empedobacter brevis,* and *Vibrio owensii* was 0.39063 mg/mL while with *Providencia vermicola* was 0.78125 mg/mL. On the other hand, the sensitivity order of n-hexane against *Vibrio owensii* and *Empedobacter brevis* was 3.125 mg/mL while with *Brevibacterium linen* and *Providencia vermicola* was 1.5625 and 6.25 mg/mL respectively. The minimum inhibitory concentration of ethanol extract against *Empedobacter brevis* was 3.125 mg/mL and ethyl acetate against *Vibrio owensii* was 3.125 mg/mL. The findings of MIC for the potent tested extracts concluded that *S. fusiforme* might be used to prevent and control bacterial coral diseases. Pathogenic bacterial strains included in this study were chosen for their importance in coral diseases which lead to their death so, controlling their growth by natural alternatives might improve the health status of coral species.

3.5. Bacterial growth curve

Data obtained from the optical density values (OD 600 nm), the plate count method (CFU/mL), and the dry weight method (gram) after the isolated bacterial pathogens were cultured in LB culture media showed that the isolated pathogenic bacteria *Providencia vermicola* has a lag phase 0–1 h, log phase 1–4 h, stationary phase 4–6 h, and the decline phase started 6–10 h from the starting of the growth while *Empedobacter brevis* has a lag phase 0–1 h, log phase

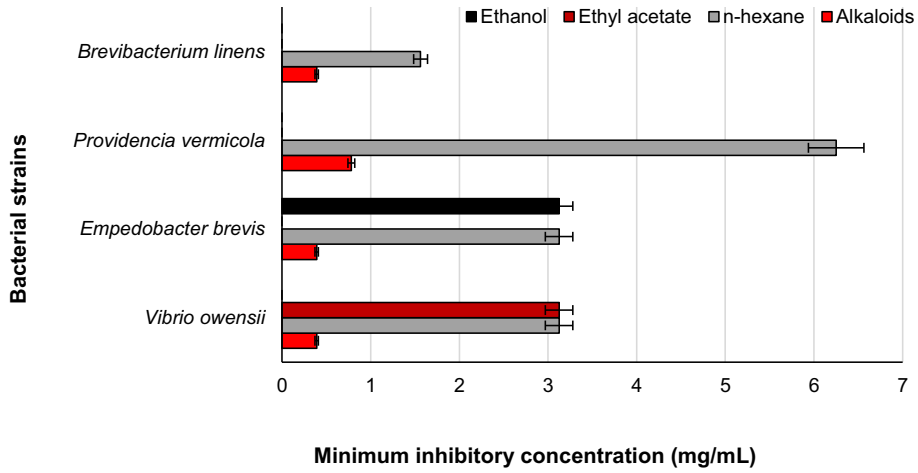


Fig. 6. Minimum inhibitory concentration for white band diseased *Porites lutea* pathogenic bacteria with *S. fusiforme* extracts

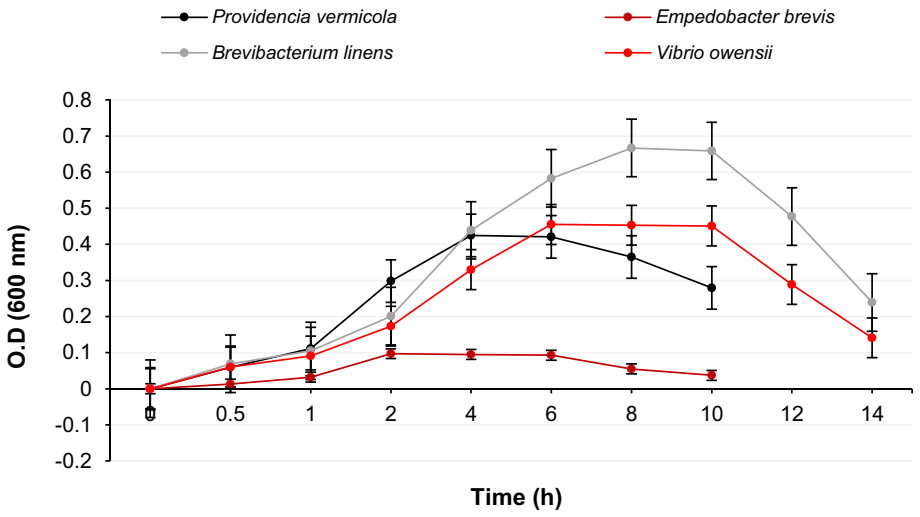


Fig. 7. Growth curve for the isolated bacteria (*Vibrio* owensii, *Empedobacter brevis*, *Providencia vermicola,* and *Brevibacterium linens*) depending on optical density data.

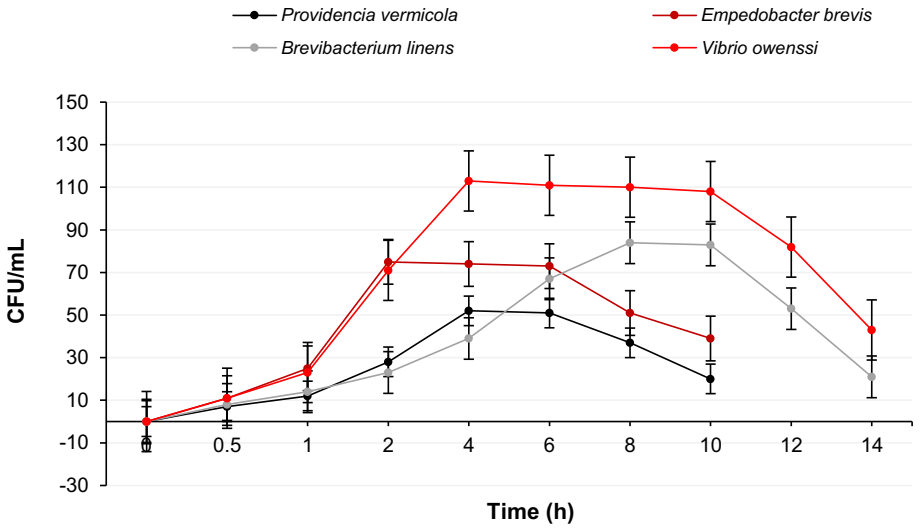


Fig. 8. Growth curve for the isolated bacteria (*Vibrio owensii*, *Empedobacter brevis*, *Providencia vermicola*, and *Brevibacterium linens*) depending on the plate count method in the form of CFU/mL.

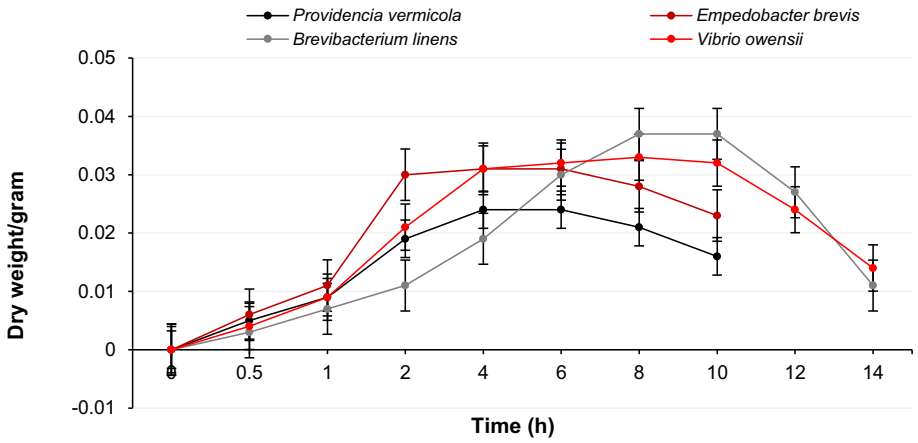


Fig. 9. Growth curve for the isolated bacteria (*Vibrio owensii*, *Empedobacter brevis*, *Providencia vermicola*, and *Brevibacterium linens*) depending on dry weight /gram.

1–2 h, stationary phase 2–6 h, and the decline phase started 6–10 h from the starting of the growth.

As regards *Vibrio owensii,* the results showed that it has a lag phase 0–1 h, log phase 1–6 h, stationary phase 6–10 h, and the decline phase started 10–14 h from the starting of the growth, while *Brevibacterium linens* has a lag phase 0–2 h, log phase 2–8 h, stationary phase 8–10 h, and the decline phase started 10–14 h from the starting of the growth as shown in (Fig. 7, Fig. 8, Fig. 9).

4. Discussion

To our knowledge, there are no previous studies on the antibacterial activity of brown seaweed *S. fusiforme* against coral bacterial pathogens. Therefore, the main objective of this study was to evaluate the impact of the crude extracts, alkaloid and flavonoid extracts from *S. fusiforme* as antimicrobial agents on different isolated coral bacterial pathogens. As mentioned before n-hexane extract exhibited a higher antibacterial activity against tested *Porites lutea* pathogenic bacteria compared to ethanol and ethyl acetate extracts. The result obtained may be more or less similar to the results reported by earlier experiments. For example, n-hexane extracted from *S. polycystum, S. oligocytum, S. cristaefolium*, and *S. crassifolium* showed a moderate inhibition activity against different bacterial strains, while ethanol, ethyl acetate, and dichloromethane extracts showed a higher activity [37]. Another previous study regarding ethanol extract reported that 50 µl ethanol extract of *S. vulgare* exhibited inhibitory activity against *S. aureus* and *K. pneumonia* [31]. Ethanol extract from *S. glaucescensas* in another study produced higher antibacterial activity than n-hexane and methanol extracts [40]. Regarding the efficiency of the solvent used for extraction of the bioactive substances, many authors reported that ethyl acetate extracts from *Chaetomorphalinum, Enteromorpha compressa*, and *Polysiphonia subtilissima* were active against most of the pathogens while ethanol and methanol extracts were active only against *Shigella flexneri* [41]. However, in another report, it was detected that ethyl acetate and methanol were the best solvents for the extraction of antimicrobial compounds from marine algae which are in contrast to our study [42]. Antibacterial activity of brown seaweeds such as *S. tenerrimum* and *S. polycystum* demonstrated significant bioactivity against both gram-negative and gram-positive bacteria [43], which emphasizes the present investigation. Other findings by Bolaños et al. [37] mentioned that the crude extracts isolated from *S. polycystum, S. oligocystum, S. crassifolium*, and *S. cristaefolium* inhibited Gram-negative and Gram-positive bacteria as well as the fungus while the extraction from *S. tenerrimum* inhibited only Gram-negative and Gram-positive bacteria [43]. The difference in results may be due to the existence of diverse antibacterial compounds among different species as proposed by Lustigman and Brown [44], the place and time of sampling collection, and also the ability of the selected protocol to extract the bioactive metabolites [42]. The results obtained by Alghazeer et al. [45] may be in contrast or less similar to our results in which flavonoid extract from brown seaweed *Cystoseira compressa* and *Padina pavonica* exhibited stronger antibacterial activities against *Staphylococcus aureus, Bacillus cereus, Bacillus pumilus, Salmonella enterica, Enterohaemorrhagic*, and *Escherichia coli*. Our results might be different from the previous findings which demonstrated greater activity of flavonoid extracts towards Gram-positive bacteria and Gram-negative bacteria [46]. The probable reason for obtaining different results is the difference in the configuration and permeability of bacterial cell walls and probably due to the severity or improperly applied treatments or the repulsion charge between the cell wall of pathogenic bacteria and secondary metabolites as previously explained by Amorim et al. [47]. It was found that the cell walls of the Gram-positive bacteria are made of teichoic acids and peptidoglycans, while the outer membrane of the Gram-negative cell wall consists of lipopolysaccharides which make the cell wall impermeable to lipophilic solutes [48].

To the best of our knowledge, there is only one report concerning the extraction of flavonoids and evaluation of their antibacterial activity from macro-algae [45]. However, several studies have reported flavonoid extract from higher plants [49,50]. The results obtained in this study regarding alkaloid extract may be more or less similar to the results obtained by earlier experiments. For example, alkaloid extract from *Conocarpus lancifolius* showed antibacterial activity against *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Erwinia amylovora*, and *Agrobacterium tumefaciens* [51]. The results obtained by Alghazeer et al. [52] indicated that alkaloid extracts from brown, green, and red seaweeds (*S. vulgare, D. membranacea*, and *C. barbata),* (*U. lactuca* and *C. tomentosum*) and (*G. latifolium*), respectively, exhibited remarkable antibacterial activity against *Salmonella typhi, S. aureus, S. epidermidis, Bacillus spp., B. subtilis, E. coli, kleb. spp.* and *P. aeruginosa* in which alkaloid extract from brown seaweeds *S. vulgare* and *C. barbata* showed the highest inhibition zone. The antibacterial activity of brown, red, and green macro-algae was well documented by Val et al. [53] as well as their extracted alkaloids [26]. It has been observed that the tested alkaloid extract was highly efficient against *Porites lutea* bacterial pathogens compared to flavonoids and other solvents used.

Recently, there are several studies that reported the antimicrobial properties of marine plants or seaweeds [54,55]. A few report about bioactivities of seaweeds, while this study is the first to report about the antibacterial activity of *S. fusiforme* against different *Porites lutea* bacterial pathogens. Seaweeds are similar to terrestrial plants in the production of bioactive compounds called secondary metabolites, such as alkaloids, carotenoids, terpenes, bromophenols, acetogenins, aromatic, and amino acid compounds [56,57]. Additionally, the polyunsaturated esters might be the bioactive compound responsible for antimicrobial properties in different *Sargassum* species [58]. As mentioned before in many reports, flavonoids [59] and alkaloids [60] found in plants are important antimicrobial agents against a wide range of microorganisms.

Flavonoids are low-molecular-weight secondary metabolites classified as a phenolic group and synthesized by plants because of plant microbial infection which suppresses cytoplasmic membrane function, synthesis of nucleic acid, and energy metabolism of pathogenic bacteria [61]. They are powerful against a broad range of microorganisms [62] due to their ability to constitute complexes with cellular proteins, associated with the bacterial cell membrane and cell wall [63]. The antimicrobial activity of flavonoids has been mentioned in many studies using them in clinical trials as antibiotics alone or combined with traditional antibiotics [64]. In contrast to our study, the results obtained in many studies showed that total flavonoids are a promising approach in future studies to find out a new antibiotic against different pathogens.

On the other hand, alkaloids are a large group of natural compounds that originated from plants, microbes, and animals [65]. More than 18,000 different alkaloids have been detected to date [66]. Alkaloids have been intensely investigated for their bioactivity as antibacterial, antiviral, and anticancer activity in both modern and traditional medicine [67].

As for the infected coral bacteria, it might be worthy to mention that *Vibrio harveyi* [15] and *Vibrio owensii* [68] are coral bacterial pathogens implicated in the appearance of white Syndrome in stony corals. Other studies in aquaria and the wild have noticed a connection between the emergence of the white syndrome and the presence of *Vibrio* species [69,70]. Regarding *Brevibacterium* which is a coral pathogen causing white plague disease and reported to be found in healthy, diseased *Diploria strigosa* and diseased *Siderastrea siderea* [71]. *Bordetella trematum,* which is a species of rare Cocco bacillus Gram-negative bacteria, has been poorly illustrated and comprehended. Related information for this species is rare as a result of a low frequency of isolates [72]. In this study, we have been able to isolate *Bordetella trematum* from the coral reef *Porites lutea* which might be the first to isolate it from the coral aquarium.

The white band and the black band diseases are the most significant coral diseases leading to the devastation of a coral ecosystem [73]. The inhibition of the tested bacterial growth using different crude solvent extracts and alkaloids may be due to the elevated biosynthetic potential of secondary metabolites, which are already known to inhibit bacterial growth like alkaloids and flavonoids that were determined in this study.

Half of the stony coral species on the reef are affected by the stony coral tissue loss disease which results in an infection rate of 60–100% and 100% subsequent mortality [74].

The causative agent for these coral diseases can be polymicrobial infections including various pathogens [75]. This complexity was described by Ushijima et al. [10], where different *Vibrio* species were associated with the severe stony coral tissue loss disease [9,76]. Vibrionaceae, Alteromonadaceae, Rhodobacteraceae, Flavobacteriaceae, Desulfovibrionaceae, and Rhizobiaceae are infectious pathogenic families that become enriched and more dominant in the coral micro-biome during different environmental stresses as water pollution, overfishing, and climate change. These pathogenic families cause a lot of serious diseases to the coral reef ecosystem leading to their decline and death. To overcome this problem, it is important to find out the curative compounds from natural resources to restrict the growth of coral pathogens. As to our knowledge, the present study, is the first to evaluate the effect of crude extracts, alkaloids and flavonoid extracts from seaweed *S. fusiforme* as antimicrobial agents against different coral bacterial pathogens in the *in vitro* assay.

5. Conclusion

Our results investigated the potential bioactivity of different crude extracts (n-hexane, ethyl acetate, and ethanol) alkaloid and flavonoid extracts from *S. fusiforme* as antimicrobial agents against diseased *Porites lutea* bacterial pathogens which are associated with many diseases in stony corals especially white band disease. We suggest that n-hexane extract and total alkaloids provided potent antimicrobial characteristics against tested *Porites lutea* pathogenic bacteria in the *in vitro* assay. To the best of our knowledge, this is the first report about the antibacterial activity of *S. fusiforme* against different *Porites lutea* bacterial pathogens. Overall, this study strongly suggests that *S. fusiforme* extracts might have potential phytopharmaceutical properties which might be used as natural alternatives to improve the health status of commercially important coral species and provide a good point for further in-depth study to overcome the problem of coral diseases.

Financial support

This research was supported by grants from China Asia-Pacific Economic Cooperation (HV180101 and HV01-190101), Xiamen Science and Technology Program (3502Z20161162), and the Specific Fund Project of Xiamen Marine and Fishery Development (19CZP011HJ08).

Conflict of interest

The authors declare no conflict of interest

Acknowledgments

The authors are indebted to Dr. Xinqing Zheng from the laboratory of coral conservation, Third Institute of Oceanography, Ministry of Natural Resources for providing diseased *Porites lutea* coral samples. Special thanks to Dr. Hala F. Mohamed for giving the idea of antibacterial activity against coral pathogens.

References

[1] Brandl SJ, Rasher DB, Côté IM, et al. Coral reef ecosystem functioning: Eight core processes and the role of biodiversity. Front Ecol Environ 2019;17(8):445–54. https://doi.org/10.1002/fee.2088.
[2] Riegl B, Bruckner A, Coles SL, et al. Coral reefs. Threats and conservation in an era of global change. Ann N Y Acad Sci. 2009;1162(1):136-186. 10.1111/j.1749-6632.2009.04493.x PMid: 19432648.
[3] Wilson SK, Adjeroud M, Bellwood DR, et al. Crucial knowledge gaps in current understanding of climate change impacts on coral reef fishes. J Exp Biol 2010;213(6):894–900. https://doi.org/10.1242/jeb.037895 PMid: 20190114.
[4] Hughes TP, Barnes ML, Bellwood DR, et al. Coral reefs in the Anthropocene. Nature 2017;546:82–90. https://doi.org/10.1038/nature22901 PMid: 28569801.
[5] Bruno JF, Côté IM, Toth LT. Climate change, coral loss, and the curious case of the parrotfish paradigm: Why don't marine protected areas improve reef resilience? Ann Rev Mar Sci 2019;11:307–34. https://doi.org/10.1146/annurev-marine-010318-095300 PMid: 30606097.
[6] Mhuantong W, Nuryadi H, Trianto A, et al. Comparative analysis of bacterial communities associated with healthy and diseased corals in the Indonesian sea. PeerJ 2019;7:. https://doi.org/10.7717/peerj.8137 PMid: 31875145e8137.

[7] Loya Y. The coral reefs of Eilat — Past, present and future: Three decades of coral community structure studies. In: Rosenberg E, Loya Y editors, Coral health and disease. Springer, Berlin, Heidelberg. 2004:1-34. 10.1007/978-3-662-06414-6_1.
[8] Rajasekar P, Palanisamy S, Anjali R, et al. Isolation and structural characterization of sulfated polysaccharides from *Spirulina platensis* and its bioactive potential: *In vitro* antioxidant, antibacterial activity and Zebra fish growth and reproductive performance. Int J Biol Macromol 2019;141:809–21. https://doi.org/10.1016/j.ijbiomac.2019.09.024 PMid: 31499110.
[9] Ushijima B, Videau P, Poscablo D, et al. Mutation of the *toxR* or *mshA* genes from *Vibrio coralliilyticus* strain OCN 014 reduces infection of the coral *Acropora cytherea*. Environ Microbiol 2016;18(11):4055–67. https://doi.org/10.1111/1462-2920.13428 PMid: 27348808.
[10] Ushijima B, Meyer JL, Thompson S, et al. Disease diagnostics and potential coinfections by *Vibrio coralliilyticus* during an ongoing coral disease outbreak in Florida. Front Microbiol 2020;11:. https://doi.org/10.3389/fmicb.2020.569354 PMid: 33193161569354.
[11] Ben-Haim Y, Zicherman-Keren M, Rosenberg E. Temperature- regulated bleaching and lysis of the coral *Pocillopora damicornis* by the novel pathogen *Vibrio coralliilyticus*. Appl Environ Microbiol 2003;69(7):4236–42. https://doi.org/10.1128/AEM.69.7.4236-4242.2003 PMid: 12839805.
[12] Patterson KL, Porter JW, Ritchie KB, et al. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, *Acropora palmata*. Proc Natl Acad Sci USA 2002;99(13):8725–30. https://doi.org/10.1073/pnas.092260099 PMid: 12077296.
[13] Denner EB, Smith GW, Busse HJ, et al. *Aurantimonas coralicida* gen. nov. sp. nov., the causative agent of white plague II on Caribbean scleractinian corals. Int J Syst Evol Microbiol. 2003;53(4):1115–1122. 10.1099/ijs.0.02359-0 PMid: 12892136.
[14] Kushmaro A, Rosenberg E, Fine M, et al. Bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. Mar Ecol Prog Ser 1997;147:159–65. https://doi.org/10.3354/meps147159.
[15] Luna GM, Bongiorni L, Gili F, et al. *Vibrio harveyi* as a causative agent of the White Syndrome in tropical stony corals. Environ Microbiol Rep 2010;2(1):120–7. https://doi.org/10.1111/j.1758-2229.2009.00114.x PMid: 23766006.
[16] Zhenyu X, Shaowen K, Chaoqun H, et al. First characterization of bacterial pathogen, *Vibrio alginolyticus*, for *Porites andrewsi* white syndrome in the South China Sea. PLoS ONE 2013;8(9):. https://doi.org/10.1371/journal.pone.0075425 PMid: 24086529e75425.
[17] Iwanowicz DD, Schill WB, Woodley CM, et al. Exploring the stony coral tissue loss disease bacterial pathobiome. bioRxiv 2020.05.27.120469. 10.1101/2020.05.27.120469.
[18] Bhowmick S, Mazumdar A, Moulick A, et al. Algal metabolites: An inevitable substitute for antibiotics. Biotechnol Adv 2019;43:. https://doi.org/10.1016/j.biotechadv.2020.107571 PMid: 32505655107571.
[19] Xia GX, Wu YM, Bi YF, et al. Antimicrobial properties and application of polysaccharides and their derivatives. Chinese J Polym Sci. 2021;39:133–146 10.1007/s10118-021-2506-2.
[20] Eluvakkal T, Sivakuamr SR, Arunkumar K. Fucoidan in some Indian brown seaweeds found along the coast of Gulf of Mannar. J Bot 2010;6(2):176–81. https://doi.org/10.3923/ijb.2010.176.181.
[21] Balboa EM, Conde E, Moure A, et al. *n vitro* antioxidant properties of crude extracts and compounds from brown algae. Food Chem 2013;138(2–3):1764–85. https://doi.org/10.1016/j.foodchem.2012.11.026 PMid: 23411309.
[22] Cortés Y, Hormazábal E, Leal H, et al. 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. Electron J Biotechnol 2014;17(3):126–31. https://doi.org/10.1016/j.ejbt.2014.04.005.
[23] Chakraborthy K, Lipton AP, Raj RP, et al. Antibacterial labdane diterpernoids of *Ulva fasciata* Delile from South-western coast of Indian Peninsula. Food Chem 2010;119(4):1399–408. https://doi.org/10.1016/j.foodchem.2009.09.019.
[24] Patra J, Rath S, Jena K. Evaluation of antioxidant and antimicrobial activity of seaweed (*Sargassum* sp.) extract: A study on inhibition of Glutathione-S transferase activity. Turk J Biol 2008;32(2):119–25.
[25] Kostić DA, Velicković JM, Mitić SS, et al. Phenolic content and antioxidant and antimicrobial activities of *Crataegus oxyacantha* L. (Rosaceae) fruit extract from Southeast Serbia. Trop J Pharm Res 2012;11(1):117–24. https://doi.org/10.4314/tjpr.v11i1.15.
[26] Güven KC, Percot A, Sezik, et al. Alkaloids in marine algae. Mar Drugs 2010;8(2):269–84. https://doi.org/10.3390/md8020269 PMid: 20390105.
[27] Kahkonen MP, Hopia HJ, Vuorela H, et al. Antioxidant activity of plant extract containing phenolic compounds. J Agric Food Chem 1999;47(10):349–62. https://doi.org/10.1021/jf990146l PMid: 10552749.
[28] Zou D, Gao K. Photosynthetic characteristics of the economic brown seaweed *Hizikia fusiforme* (Sargassaceae, Phaeophyta), with special reference to its "leaf" and receptacle. J Appl Phycol 2005;17:255–9. https://doi.org/10.1007/s10811-005-5768-0.
[29] Li Y, Fu X, Duan D, et al. Extraction and identification of Phlorotannins from the brown alga, *Sargassum fusiforme* (Harvey) setchell. Mar Drugs 2017;15(2):49. https://doi.org/10.3390/md15020049 PMid: 28230766.
[30] Zhang Y, Xu M, Hu C, et al. *Sargassum fusiforme* Fucoidan SP2 Extends the lifespan of *Drosophila melanogaster* by up regulating the Nrf 2-mediated antioxidant signaling pathway. Oxid Med Cell Longev 2019;2019:8918914. https://doi.org/10.1155/2019/8918914 PMid: 31485301.
[31] El Shafay SM, Ali SS, El-Sheekh MM. Antimicrobial activity of some seaweeds species from red sea, against multidrug resistant bacteria. Egypt J Aquat Res 2016;42(1):65–74. https://doi.org/10.1016/j.ejar.2015.11.006.
[32] Sugiura Y, Kinoshita Y, Abe M, et al. Suppressive effects of the diethyl ether fraction from a brown alga *Sargassum fusiforme* on allergic and inflammatory reactions. Fish Sci 2016;82:369–77. https://doi.org/10.1007/s12562-016-0969-9.
[33] Luo HY, Wang B, Yu CG, et al. Evaluation of antioxidant activities of five selected brown seaweeds from China. J Med Plant Res 2010;4(23):2557–65. https://doi.org/10.5897/JMPR10.609.
[34] Han YR, Ali MY, Woo MH, et al. Anti-diabetic and anti-inflammatory potential of the edible brown alga *Hizikia fusiformis*. J Food Biochem 2015;39(4):417–28. https://doi.org/10.1111/jfbc.12138.
[35] Paskaleva EE, Lin X, Duus K, et al. *Sargassum fusiforme* fraction is a potent and specific inhibitor of HIV-1 fusion and reverse transcriptase. Virol J 2008;5:8. https://doi.org/10.1186/1743-422X-5-8 PMid: 18197976.
[36] Throndsen J. The dilution-culture method. In: Sournia A, editor. Phytoplankton manual. Paris: Unesco; 1978. p. 218–24.
[37] Bolaños JM, Baleta FN, Cairel JD. Antimicrobial properties of *Sargassum* spp. (Phaeophyceae) against selected aquaculture pathogens. Int. J. Curr. Microbiol. App. Sci. 2017;6(2):1024-1037. 10.20546/ijcmas.2017.602.115.
[38] Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. Plant Pathol J. 1988;48(1):146. Chapman and Hall, London. 10.1046/j.1365-3059.1999.00318.x.
[39] Bohm BA, Koupai-Abyazan R. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinum*. Pacific Sci J 1994;48(4):458–63.
[40] Mahianeh A, Ghaednia B, Mirbakhsh M, et al. The effects of brown alga, *Sargassum glaucescensas* (Agardeh, 1948) against selected bacterial, fungal and yeast pathogens of shrimp. Int. J. Biol. Sci. 2014;5(12):399-405. 10.12692/ijb/5.12.399-405.
[41] Patra JK, Patra AP, Mahapatra NK, et al. Antimicrobial activity of organic solvent extracts of three marine macroalgae from Chilika Lake, Orissa, India. Malays J Microbiol 2009;5(2):128–31. https://doi.org/10.21161/mjm.15409.
[42] Salem WM, Galal H, Nasr EF. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). Afr J Microbiol 2011;5(15):2160–7. https://doi.org/10.5897/AJMR11.390.
[43] Kausalya M, Narasimha Rao GM. Antimicrobial activity of marine algae. JABU 2015;6(1):78–87.
[44] Lustigman B, Brown C. Antibiotic production by marine algae isolated from the New York/New Jersey Coast. Bull Environ Contam Toxicol 1991;46:329–35. https://doi.org/10.1007/BF01688928 PMid: 2031998.
[45] Alghazeer R, Elmansori A, Sidati M, et al. *In vitro* antibacterial activity of flavonoid extracts of two selected Libyan algae against Multi-drug resistant bacteria isolated from food products. J Biosci Med 2017;5(1):26–48. https://doi.org/10.4236/jbm.2017.51003.
[46] Mandalari G, Bennett RN, Bisignano G, et al. Antimicrobial activity of flavonoids extracted from *Bergamot (Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. J Appl Microbiol 2007;103(6):2056–64. https://doi.org/10.1111/j.1365-2672.2007.03456.x PMid: 18045389.
[47] Amorim RNS, Rodrigues JAG, Holanda ML, et al. Antimicrobial effect of a crude sulfated polysaccharide from the red seaweed *Gracilaria ornate*. Braz Arch Biol Technol 2012;55(2):171–81. https://doi.org/10.1590/S1516-89132012000200001.
[48] Rankovic B, Mišić M, Sukdolak S. The antimicrobial activity of substances derived from the lichens *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogymnia physode*. World J Microbiol 2008;24:1239–42. https://doi.org/10.1007/s11274-007-9580-7.
[49] Ayaz FA, Hayirlioglu-Ayaz S, Alpay-Karaoglu S, et al. Phenolic acid contents of Kale (*Brassica oleraceae* L. var. *acephala* DC.) extracts and their antioxidant and antibacterial activities. Food Chem 2008;107(1):19–25. https://doi.org/10.1016/j.foodchem.2007.07.003.
[50] Hendra R, Ahmad S, Sukari A, et al. Flavonoid analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff.) Boerl fruit. Int. J. Mol. Sci. 2011;12(6):3422-3431. 10.3390/ijms12063422 PMid: 21747685.
[51] Ai HM, Salem M, Abdel-Megeed A. *In-vitro* antibacterial activities of alkaloids extract from leaves of *Conocarpus lancifolius* Engl. J Pure Appl Microbiol 2013;7(3):1903–7.
[52] Alghazeer R, Whida F, Abduelrhman E, et al. *In vitro* antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya. Afr J Biotechnol 2013;12(51):7086–91.
[53] Val A, Platas G, Basilio A, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int J Microbiol 2001;4:35–40. https://doi.org/10.1007/s101230100006 PMid: 11770818.
[54] Kulshreshtha G, Critchley A, Rathgeber B, et al. Antimicrobial effects of selected, cultivated red seaweeds and their components in combination with tetracycline, against poultry pathogen *Salmonella enteritidis*. J Mar Sci Eng 2020;8(7):511. https://doi.org/10.3390/jmse8070511.
[55] Imran MAS, Bhuiyan FR, Ahmed SR, et al. Phytochemical constituency profiling and antimicrobial activity screening of seaweeds extracts collected from the Bay of Bengal Sea coasts. JABET 2021;4(1):25–34. https://doi.org/10.5455/jabet.2021.d103.

[56] Rodríguez-Bernaldo de Quirós A, Lage-Yusty MA, López-Hernández J. Determination of phenolic compounds in macroalgae for human consumption. Food Chem 2010;121(2):634–8. https://doi.org/10.1016/j.foodchem.2009.12.078.
[57] Priyadharshini S, Bragadeeswaran S, Prabhu K, et al. Antimicrobial activity and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India. Asian Pac J Trop Biomed 2011;1(1): S38–9. https://doi.org/10.1016/S2221-1691(11)60118-4.
[58] Ambreen K, Hira A, Tariq A, et al. Evaluation of biochemical component and antimicrobial activity of some seaweeeds occurring at Karachi Coast. Pak J Bot 2012;44(5):1799–803.
[59] Górniak I, Bartoszewski R, Króliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev 2018;18:241–72. https://doi.org/10.1007/s11101-018-9591-z.
[60] Casciaro B, Mangiardi L, Cappiello F, et al. Naturally-occurring alkaloids of plant origin as potential antimicrobials against antibiotic-resistant infections. Molecules 2020;25(16):3619. https://doi.org/10.3390/molecules25163619 PMid: 32784887.
[61] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents 2005;26(5):343–56. https://doi.org/10.1016/j.ijantimicag.2005.09.002 PMid: 16323269.
[62] Dixon RA, Dey PM, Lamb CJ. Phytoalexins: enzymology and molecular biology. Adv Enzymol Relat Areas Mol Biol 1983;55:1–136. https://doi.org/10.1002/9780470123010.ch1 PMid: 6353887.
[63] Nogueira LFB, Morais EC, Brito MAD, et al. Evaluation of antibacterial, antifungal and modulatory activity of methanol and ethanol extracts of *Padina sanctae-crucis*. Afr Health Sci 2014;14(2):372–6. https://doi.org/10.4314/ahs.v14i2.12 PMid: 25320586.
[64] Amin MU, Khurram M, Khattak B, et al. Antibiotic additive and synergistic action of ruin, morin and quercetin against methicillin resistant *Staphylococcus aureus*. BMC Complement Altern Med 2015;15(1):59. https://doi.org/10.1186/s12906-015-0580-0 PMid: 25879586.
[65] Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents 2014;44(5):377–86. https://doi.org/10.1016/j.ijantimicag.2014.06.001 PMid: 25130096.
[66] Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. Front Microbiol 2019;10:911. https://doi.org/10.3389/fmicb.2019.00911 PMid: 31156565.
[67] Thawabteh A, Juma S, Bader M, et al. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins 2019;11(11):656. https://doi.org/10.3390/toxins11110656 PMid: 31717922.
[68] Ushijima B, Smith A, Aeby GS, et al. *Vibrio owensii* induces the Tissue Loss Disease *Montipora* White Syndrome in the Hawaiian Reef Coral *Montipora capitata*. PLoS ONE 2012;7(10):. https://doi.org/10.1371/journal.pone.0046717 PMid: 23056419e46717.
[69] Luna GM, Biavasco F, Danovaro R. Bacteria associated with the Rapid Tissue Necrosis (RTN) of stony corals. Environ Microbiol 2007;9(7):1851–7. https://doi.org/10.1111/j.1462-2920.2007.01287.x PMid: 17564618.
[70] Sussman M, Willis BL, Victor S, et al. Coral pathogens identified for White Syndrome (WS) epizootics in the Indo-Pacific. PLoS ONE 2008;3:. https://doi.org/10.1371/journal.pone.0002393e2393.
[71] Cardenas A, Rodriguez LM, Pizarro V, et al. Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. ISME j 2012;6:502–12. https://doi.org/10.1371/journal.pone.0002393 PMid: 18560584.
[72] Castro TR, Martins RCR, Dal Forno NLF, et al. *Bordetella trematum* infection: Case report and review of previous cases. BMC Infect Dis 2019;19:485. https://doi.org/10.1186/s12879-019-4046-8 PMid: 31146691.
[73] Thinesh T, Mathews G, Patterson EJK. Coral disease prevalence in the Palk Bay, Southeastern India-with special emphasis to black band. Indian J Mar Sci 2011;40(6):813–20.
[74] Neely K. Florida Keys Coral Disease Strike Team: FY 19-20 Final Report. Florida DEP. Miami, FY 2020;1-17.
[75] Meyer JL, Paul VJ, Raymundo LJ, et al. Comparative metagenomics of the black band disease of corals. Front Microbiol 2017;8:618. https://doi.org/10.3389/fmicb.2017.00618.
[76] Gignoux-Wolfsohn SA, Precht WF, Peters EC, et al. Ecology, histopathology, and microbial ecology of a white-band disease outbreak in the threatened staghorn coral *Acropora cervicornis*. Dis Aquat 2020;137(3):217–37. https://doi.org/10.3354/dao03441 PMid: 32132275.