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Research Article

Extract from Sargassum fluitans III: A Promisin Valuable resource of Anti-bacterial metabolites

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Abstract

The rise of new bacterial strains resistant to current antibacterials emphasizes the urgency of seeking alternative methods for microbial protection. Plants, including brown algae, have attracted considerable attention due to their intriguing chemical compositions. This study aims to characterize both crude and family extracts of secondary metabolites from an invasive brown algae, *Sargassum fluitans III*. Following the extraction of various compounds, their antibacterial and bactericidal activity against four bacterial strains is assessed. The selected algae exhibit a molecular richness in alkaloids and polyphenols, which, despite their low content, confer high toxicity. This research demonstrates that these extracts possess both bacteriostatic and bactericidal effects against both Gram-positive and Gram-negative bacteria.

Keywords: *Sargassum fluitans III;* Secondary metabolites; Extraction; Phytochemical tests; Antibacterial

Introduction

Present in all environments (industrial, medical, marine...), bacteria develop very quickly and manage to live even in extreme conditions by exploiting the resources available around them but also thanks to their ability to organize in biofilm. This last capability is a huge issue in health, agro-industry and other productive sectors using metal alloys for example, such as oil and gas industries [1] where microbial-induced corrosion is responsible for huge economic losses [2]. In addition to this, about 60% of bacterial infections are due to microbial biofilms that are insensitive to 100% of the chemical antibiotics currently used [3,4]. Indeed, the microbial adhesion to a surface (metal, plastic...) leads to the formation of a very resistant microbial biofilm, hence the need to develop antibacterial strategies to prevent their adhesion or inhibit their growth. Different factors influence bacterial adhesion, namely, the bacterium itself (chemical composition, surface...) [5], the surface of the material (chemical nature, geometry, roughness) [6,7] and the environment (temperature, presence of organic molecules) [8]. Actions could be carried out separately or simultaneously on those three families of factors.

Faced with this significant resistance, researchers are increasingly focusing on antibacterial properties of plant-based compounds. Indeed, plants known to have developed protective mechanisms against bacteria are, composed of antimicrobial molecules (polyphenols, terpenes, alkaloids...) [9–11]. Marine

resources such as macro-algae are also a source of interesting constituents with reported biological activities such as antiviral, antifungal or antibacterial activities [12–16]. Brown algae were reported to contain diverse bioactive components that play a protective role against marine herbivorous organisms [17,18].

Phlorotannins were described as the principal bioactive compound with antibacterial activity in ten Sargassum-belonging brown algae [19,20], nevertheless, other sargassum-containing secondary metabolites such as quinones from *S. paradoxum*, *S. fallax and S. sagamianum* [21–23]. Triterpenes and triterpenoid derivatives from *S. wightii* [24], *S. henslowianum* [25]. Saponins from *S. aquifolium* [26],flavonoids from *S. crassifolium* and *S.oligocystum* [27,28] and phenolic non identified compounds from *S. mangarevense* [29] were described to exhibit antibacterial activity.

Sargassum fluitans III, S. natans I and S. natans VIII are the main holopelagic brown seaweed of which thousands of tons wash up every year on the coasts of the Caribbean islands [30]. These algae strandings caused several damages : ecological (disturbance of the ecosystems), economical (material corrosion, perturbations of touristic activities), but also represents a danger to local populations' health once its decomposition process begins (inhalation of toxic gases) [31]. In order to deal with this issue, the valorization of these algae is strongly encouraged. We recently reported the results of a study examining extraction and electrochemical analysis of natural compounds extracted from *Sargassum fluitans III*. The objective of this research was the development of new organic bio-sourced corrosion inhibitors, ecofriendly and biodegradable. The results obtained showed that the extract of *Sargassum fluitans III* could serve as an effective inhibitor for the C38 steel in acidic media [32]. The current study represents a continuation of this previous research, exploring additional activities that Sargassum extracts can exhibit. Thus, the aim of the present paper is to emphasize for the first time, the antibacterial activity of *Sargassum fluitans III* extract and its secondary metabolites.

Materials and Methods

Sampling

Sargassum collection and preparation

The *Sargassum* is defined as brown macrophytes of the *fucaceae* family, which grow in tropical waters. They are primarily benthic, but some, such as *Sargassum natans* (*I and VIII*) and *Sargassum fluitans III*, involved in sargassum rafts that wash ashore on the coasts of the Caribbean, are called 'holopelagic' because they proliferate on the water surface without attaching to a hard substrate. They gather in the form of rafts or sargassum patches stretching for several kilometers [33,34]. These three species have similar properties but are distinguished by somewhat different morphologies, as shown in Figure 1.

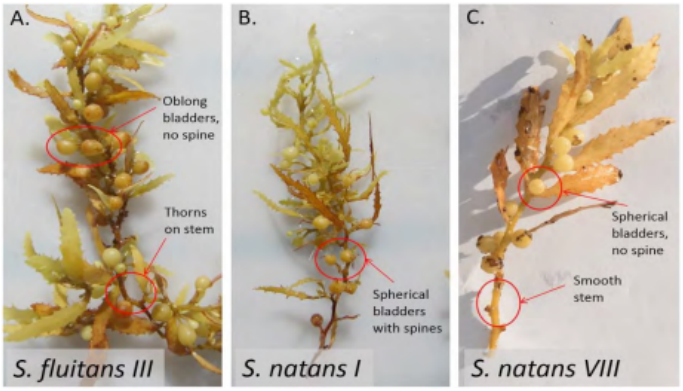

Figure 1: Morphological differences of the three pelagic *Sargassum* species.

The brown macroalgae *S. fluitans III*, *S. natans I* and *S. natans VIII* types were collected by hand picking method in the south-east coastline of Martinique (14° 28 '40.4"N; 61° 01' 45.1"W). They were immediately and thoroughly washed using distilled water to remove salt and superfluous matter and selected to keep only mature, disease free, fresh algae. It was then sorted and identified based on their morphology differences [35] left to dry at room temperature and conserved in vacuum-packed bags until use.

Ethanolic and secondary metabolites extraction

Dried algae were crushed with a Pulverisette 14 rotor crusher. 20g of the grinding product was then used in a reflux system for 3 hours with 400 mL of a green solvent composed of water/ethanol (30/70, 50/50 or 30/70 v/v%). The extracted solution was then filtered (12 µm pore filter paper) under vacuum pump and evaporated using a rotary evaporator (Heidolph®). Similarly, the families of extracted secondary metabolites were obtained by modified extraction protocols initially established by Botosa and Bruneton [11,36]. The alkaloids were extracted using a previously described experimental procedure [37], while flavonoids [38,39], saponins [40], triterpenes [41], anthocyanins [42,43], coumarins [44,45], quinones [46], and tannins [47] were extracted using specific protocols.

The extracted fractions from different families were collected and weighed. Depending on their solubility, the solids obtained were subsequently solubilized, in water or ethanol for microbiological analysis. The yield of the various extractions carried out was calculated as following:

$$R(\%) = \frac{m}{m^{\circ}} \times 100$$

Where m and m° correspond respectively to the mass of dry plants before and after extraction.

Bacterial strains

The bacterial strains *Shewanella fodinae* (Gram negative) [48] was isolated from an electroactive bioanode from a mangrove sediment microbial fuel cell. *Vibrio alginolyticus* (Gram negative) [49], *Staphylococcus warneri* (Gram positive) [50], *Staphylococcus capitis* (Gram positive) [51], *Micrococcus yunnanensis* (Gram positive) [52,53] and *Alteromonas macleodii* (Gram negative) [54,55] were isolated from a biofilm formed on DC01 Carbon steel and 304L stainless steel coupons exposed to marine corrosion in the presence of *Sargassum*. The strains were identified and were frozen at -80°C in Brain and Heart Infusion (BHI) containing 10% glycerol. Before use, bacterial strains were thawed and maintained in a Marine Broth medium (MB) at 27°C for 48h, tested regarding purity before their use.

Antibacterial analysis

Evaluation of antibacterial activity by the disk diffusion method

1 mL containing 10^8 CFU/mL of fresh bacterial suspension of each bacterium was streaked uniformly onto individual plates containing Marine Agar (MA). In parallel to this, a 6mm diameter sterile paper disk was soaked in an increasing volume of ethanolic extract supplemented with 5% DMSO and left to dry for 3h at room temperature under a sterile atmosphere. Then, disks were placed on the inoculated plates and incubated at 27 °C for 48 hours. The bioactivity was determined by the observation of clearance zones and their diameters were measured in millimeters, including the disk. A 5% DMSO disk was used as negative control and Amoxicillin and Benzathine benzylpenicillin as well as $CuSO_4$ (100 µg/mL) containing 5% DMSO were used as positive controls.

Determination of the Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of extracts was determined in triplicate, using 96-wells microdilution method [56]. In brief, 100 µL of a twofold serial dilution of extract ranging from 8mg/mL to 100µg/mL were prepared in 96-wells plate, as following, 100 µL of sterilized MB were pipetted onto all the wells from column 1 to column 12, then, 100 µL of the deserved extract was added to the well 1, mixed well by sucking up and down, next, 100 µL were pipetted from well 1 and transferred onto the well 2. This operation was repeated until column 10 where pipetted 100 µL was discarded. After that, 100 µL of an overnight culture containing 10^6 CFU/mL were added in every well and incubated for 48h at 24 °C, under shaking. A growth control and a sterility control well were also prepared for every bacterial strain. The MIC was determined as the lowest concentration of extract exhibiting no visible growth [57] and confirmed by absorbance at 600nm using a microplate reader. Thus, the MIC of an extract corresponds to an inhibition of bacterial growth greater than 50%. The percentage of inhibition of bacterial growth was calculated as following:

$$Growth\ inhibition = (1 - \frac{A_c}{A_0}) \times 100$$

Where A_c is the optical density (OD) of the sample at the concentration of *S. fluitans III* extract and A_0 is the OD of the negative control (sample without *Sargassum* extract).

In order to assess the sensitivity of bacteria used, Amoxicillin and Benzathine benzylpenicillin at final concentrations ranging from 25 mg/mL to 0.05 mg/mL as well as the $CuSO_4$ at a concentration of 100 µg/mL were used.

Determination of the Minimum Bactericidal Concentration

Minimal bactericidal concentration (MBC) was recorded as the lowest extract concentration killing 99.9% of bacterial inocula after 24h of incubation at 27 °C. MBC was performed on all extracts. 10 µL were taken from the MIC experiment (MIC value) and the two wells around and were spread on MA plates [58]. Once plates were seeded, they were incubated for 48 hours at 27 °C. The number of colonies was counted and the concentration of extracts giving less than 10 colonies was considered as MBC [59].

Results

Extraction and Characterization of *Sargassum* species

Extractions were conducted on three morphotypes of *Sargassum* (*S. fluitans III*, *S. natans I* and *VIII*) through reflux, employing water/ethanol as an economical and environmentally friendly solvent. The extraction yields obtained for different ratios, (30:70), (50:50) and (70:30) (water:ethanol), were 10.20%, 15.80% and 17.08% respectively. The IR analysis of the extracts obtained for the three morphotypes revealed the presence of identical peaks, as illustrated in Figure 2. Most of the observed IR bands are indicative of the presence of various families within the total extract, specifically: 3347–3320 cm^{-1}, intermolecular O-H elongation; 2981–2881 cm^{-1}, symmetrical C-H elongation in an aromatic ring; 1646–1644 cm^{-1}, phenyl-type C=C elongation; 1407–1379 cm^{-1}, phenolic ring stretching from in-plane OH deformation; 1087–1043 cm^{-1}, cyclic ether C-O elongation; 879–638 cm^{-1}, out-of-plane aromatic ring C-H deformation. Additionally, a band around 1650 cm^{-1} is characteristic of quinone derivatives. The IR spectra also exhibit the distinctive absorption bands of an alcoholic hydroxyl function (3450 cm^{-1}), methyl radicals (2960 and 1400 cm^{-1}). The chemical composition remains generally consistent, albeit with slight variations, likely attributed to the extraction solvent ratio. The accumulation of *Sargassum* along the Caribbean Sea coastline was reported to be predominantly comprised of *Sargassum fluitans III* [60,61]. Considering the chemical composition and the high yield using (70:30) water/ethanol together with these findings, our research was centered on the *Sargassum fluitans III* extracted with a 70:30.

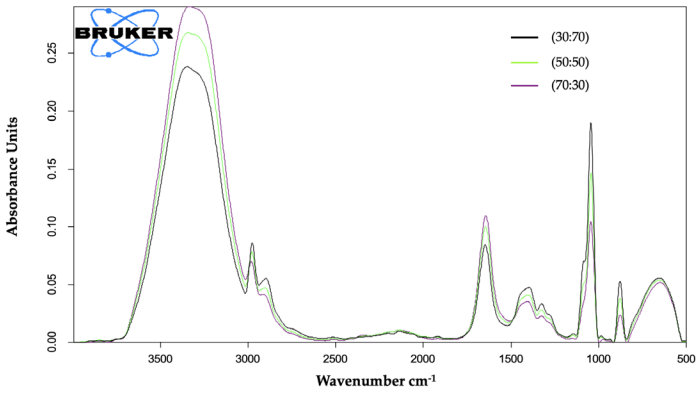

Figure 2: IR spectra of *Sargassum fluitans III* extract.

Secondary metabolites composition of *Sargassum fluitans III* extracts

In order to identify the family of compounds responsible for the antibacterial activity, the different families present in the extract of *S. fluitans III* are isolated, identified and tested. Phytochemical tests were conducted on the *S. fluitans III* extract to identify the several families present in the crude extract, following Grenand's method [62]. Table 1 summarizes the percentage distribution of these families in the total extract.

The phytochemical results revealed the presence of diverse compound families, including coumarins, anthocyanins, quinones, flavonoids, saponins, tannins, and triterpenes. An extraction, as descripted in the material section, was performed for each family to determine their respective ratios. As can be seen from the Table 1, the total relative percentage is 93%, not reaching 100%, which may be attributed to losses during extraction or the presence of unidentified families.

Chemical family	Relative Percentage %
Alkaloids	5.71
Flavonoids	19.8
Triterpenes	18.28
Saponins	22.1
Tannins	7.99
Coumarins	5.85
Quinones	5.88
Anthocyanins	7.39

Table 1: Analysis of phytochemical screening tests of *Sargassum fluitans III.*

Antibacterial analysis of *Sargassum fluitans III*

Screening of antimicrobial activity

To determine the most effective extraction ratio of *S. fluitans III*, we evaluated the antibacterial activity of three extraction ratios [(30:70), (50:50), and (70:30)] on *Shewanella fodinae* using the disk diffusion method. CuSO4 was used as a positive control, showing an average inhibition diameter of 13 mm. The results indicate that higher ethanol content in the solvent corresponds to better antibacterial activity. Specifically, extraction ratios (70:30) and (50:50) demonstrated diameters of less than 10 mm, indicating lower antibacterial activity, regardless of concentration. Conversely, the (30:70) extract exhibited the strongest inhibitory activity, with diameters greater than 10 mm and approaching the value of the positive control's inhibition diameter, even at a concentration of 1 µL of crude extract. Additionally, 5% DMSO did not inhibit bacterial growth, confirming the antibacterial activity specially to the sargassum extract.

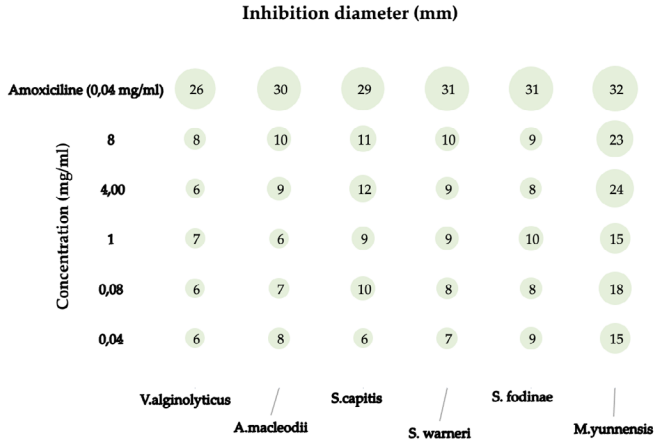

Figure 3: Antibacterial activity of *Sargassum fluitans III* assessed by disk diffusion method. Averages of triplicates obtained for each extraction ratio**.**

To determine the range of minimal inhibitory concentrations, the disk diffusion method was employed with varying amounts of extract. Two concentration ranges were utilized: the first range representing a lower extract amount, below 1 mg, and the second range for a higher extract amount, equal to or above 1 mg. As depicted in Figure 3, with the exception of *V. alginolyticus* and *Alteromonas*, the growth of the other bacteria used in this study was inhibited starting from 1 mg of *S. fluitans III*. However, significant inhibition of *A. macleodii* and *V. alginolyticus* occurred when 4 mg and 8 mg of extract were used, respectively. Therefore, concentrations up to 4 mg/mL were utilized to determine the minimal inhibitory concentration in liquid medium.

Minimal Inhibitory Concentration of ethanolic and secondary metabolites extracts

The antibacterial activity of the ethanolic extract (30:70) of *S. fluitans* III and its secondary metabolites against *S. fodinae, V. alginolyticus,* and *A. macleodii* as Gram-negative bacteria, and *S. warneri, S. capitis*, and *M. yunnanensis* as Gram-positive bacteria, was determined using minimal inhibitory concentrations (MIC) in liquid media, with concentrations ranging from 0 to 4 mg/mL.

Bacteria	Extracts MBC values (in mg/ml) - MIC values (in mg/ml) - MBC/MIC ratio(+ for bactericidal; - for bacteriostatic)									A	BB
	SF	AK	FV	SP	TP	CM	TN	QN	AT		
Shewanella fodinae	0.25	>0.06	>0.06	>1	>0.06	>0.06	>0.06	>0.5	>0.25		
	0.25	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.1	0.78
	1(+)	6(-)	>6(-)	>100(-)	>6(-)	>6(-)	>6(-)	>50(-)	25(-)		
Vibrio alginolyticus	>4	>0.13	>2	>4	>4	>0.5	>0.5	>2	>0.5		
	0.25	0.01	>2	>4	1	0.5	0.06	0.25	0.01	6.25	3.13
	>16(-)	>13(-)	>1(-)	>1(-)	>4(-)	1(-)	>8(-)	8(-)	50(-)		
Alteromonas macleodii	>4	>0.25	>0.25	>0.5	>4	0.5	>0.25	>1	>2		
	1	0.03	>0,25	>0.5	>4	0.5	0.06	0.01	0.01	6.25	25
	>4(-)	>8(-)	>1(-)	>1(-)	>1(-)	1(+)	>4(-)	>100(-)	>200(-)		
Staphylococcus capitis	>2	0.03	0.13	>4	4	>0.06	1	>0.5	0.25		
	1	0.01	0,13	>4	4	0.01	0.13	0.13	0.25	6.25	12.5
	>2	3(+)	1(+)	>1(-)	>1(+)	>6(-)	7(-)	>4(-)	1(+)		
Staphylococcus warneri	0.25	>0.03	>0.25	1	>0.5	>0.02	>0.5	>0.25	>1		
	0.25	0.01	0.25	0.13	0.03	0.01	0.03	0.02	0.01	3.13	6.25
	1(+)	>1(-)	>1(-)	7(-)	>16(-)	>2(-)	>16(-)	>12.5(-)	100(-)		
Micrococcus yunnanensis	>4	>1	>1	4	4	0,5	4	>2	2		
	4	0.5	>1	4	4	0.5	2	2	2	6.25	25
	1(-)	>2(-)	>1(-)	1(+)	1(+)	1(+)	2(+)	>1(-)	1(+)		

SF: crude extract of *Sargassum fluitans III* ; **AK:** Alkaloids ; ***FV:*** Flavonoids ; **SP:** Saponins ; **TP:** Triterpenes ; **CM:** Coumarins ; **TN:** Tannins ; **QN:** Quinones ; **AT:** Anthocyanins ; **A:** Amoxicillin ; **BB:** Benzathine benzylpenicillin.

Table 2: Minimum bactericidal concentrations (MBCs), Minimum inhibitory concentrations (MICs) and MBC/MIC ratio of *S. fluitans III* on the different bacterial strains tested.

The results were compared to the activity of Amoxicillin and Benzathine benzylpenicillin against the same bacteria. As shown in Table 2, for all bacterial strains, the ethanolic extract exhibited an antibacterial effect in a dose-dependent manner. Most strains showed almost complete inhibition of growth in the presence of the crude extract (>90%) for concentrations under 2 mg/mL. Assessment of antibacterial effects revealed a MIC of 0.25 mg/mL for S. warneri and V. alginolyticus, and S. fodinae, indicating strong antibacterial activity [63,64]. Whereas a moderate activity was observed against S. capitis and A. macleodii with MICs of 1 mg/mL, and finally, the weakest activity of *S. fluitans* was recorded against *M. yunnanensis* with the highest MIC of the study at 4 mg/mL.

The comparison of the ethanolic extract of *S. fluitans III* with antibiotics revealed that the extract generally exhibits higher efficacy against the tested bacteria. Except for *S. fodinae*, which showed greater susceptibility to Amoxicillin, the MICs of *S. fluitans III* against all other bacteria were lower than those observed for antibiotics (Table 2).

Furthermore, the antibacterial activity of alkaloids, flavonoids, quinones, coumarins, anthocyanins, saponins, and triterpenes from *S. fluitans III* was tested at concentrations up to 4 mg/mL. Among these secondary metabolites, flavonoids, saponins, and triterpenes showed no antibacterial activity at the concentrations used against *V. alginolyticus, A. macleodii, S. capitis,* and *M. yunnanensis*. None of the tested concentrations allowed for an inhibition greater than 50%. In contrast, alkaloids and coumarins exhibited strong activity, with MICs ranging from (0.01-0.5 mg/mL) and (0.01-2 mg/mL), respectively, surpassing the efficacy of the ethanolic extract against almost all tested bacteria. Tannins and anthocyanins showed activity solely against *S. fodinae* and Staphylococci-belonging bacteria, with higher efficacy against *S. warneri*.

Comparing the efficacy of the secondary metabolites, alkaloids demonstrated stronger activity against Gram-negative bacteria (0.01-0.05 mg/mL) than Gram-positive bacteria (0.1 mg/mL), while coumarins from *S. fluitans III* exhibited broad-spectrum inhibition, with similar efficacy against Gram-positive and Gram-negative bacteria. Quinones were ten-fold more active against Gram-negative bacteria (0.01-0.05 mg/mL) than Gram-positive bacteria (0.1-0.5 mg/mL). These results suggest that alkaloids, coumarins, and quinones extracted from *Sargassum* may have broad-spectrum inhibition, whereas tannins and anthocyanins exhibit more restricted activity.

Determination of the minimal bactericidal concentration of effective extracts

The antibacterial activity was assessed using the minimal bactericidal concentration (MBC) to minimal inhibitory concentration (MIC) ratio of a given extract against a particular bacterium. A bactericidal effect was defined when the MBC/MIC ratio was equal to or less than 4, while a bacteriostatic effect was indicated when the ratio exceeded 4 [65]. Analysis of MBC revealed that *S. fluitans III* inhibited the growth of *V. alginolyticus*, A. *macleodii, S. capitis,* and *M. yunnanensis* in a bacteriostatic manner, while bactericidal activity was observed only against *S. fodinae* and *S. warneri.*

Considering the secondary metabolites, alkaloids, coumarins, and anthocyanins exhibited MBC values within the tested concentration range. Other extracts also demonstrated notable activities, such as tannins and quinones. Coumarins displayed inhibition against all tested bacteria, with bactericidal activity against *A. macleodii* and *M. yunnanensis*, and bacteriostatic activity against the other bacteria. Alkaloids exhibited bactericidal action solely against *S. capitis*. Flavonoids, saponins, and triterpenes showed bactericidal activity against *S. capitis* and *M. yunnanensis*, while quinones demonstrated only bacteriostatic action.

Furthermore, the results for tannins and anthocyanins indicated bactericidal action against *M. yunnanensis* within the tested concentration range. These findings underscore that *S. fluitans III* contains metabolites with potent bacterial inhibition, with coumarins and alkaloids showing promising activity.

Discussion

In addition to their ecological role, brown algae, particularly *Sargassum*, represent a valuable resource due to their secondary metabolites, which have been reported to possess antibacterial activity across various *Sargassum* species. However, the antibacterial potential of *S. fluitans III* has not been explored to date. Our study aims to assess, for the first time, the antibacterial activity of ethanolic and secondary metabolite extracts from *S. fluitans III* against six bacterial species isolated from marine environments.

In our investigation, specific solvent extractions revealed the presence of secondary metabolites, including alkaloids, flavonoids, anthocyanins, coumarins, saponins, tannins, and triterpenes, constituting up to 16% of the dry mass. Previous studies have highlighted the high phenol content in the *Sargassum genus* [66], particularly in tannins, which can reach up to 1% of the dry mass. We observed a higher tannin content of 2.4% in the algae used in our study, indicating variability in biochemical composition influenced by environmental factors such as salinity, temperature, UV exposure, season, geographical location of harvesting, and processing methods [67,68]. Furthermore, solvent polarity and temperature play crucial roles in determining crude composition, with solvent polarity affecting compound solubility and temperature impacting stability [69], consequently affecting bioactive potential.

The IR spectroscopy analysis revealed the presence of various compound families within the total extract, as indicated by most of the observed IR bands. This result is consistent with a previous study that emphasized the presence of the same chemical families among the other species, albeit with a significant difference in their quantity [70].

The screening of antibacterial activity of various crude extracts of *S. fluitans* revealed antibacterial activity across all ratios, with the 30:70 ratio demonstrating higher effectiveness compared to 50:50 and 70:30. This indicates that the total crude extract of *S. fluitans* contains molecules with antibacterial properties. In a prior study, the crude extract of *S. natans* exhibited potent

antibacterial activity [71], with a larger clearance zone (26 mm radius) compared to our study. This variance could be attributed to biochemical differences resulting from the choice of extraction solvent. In contrast to our methodology, the aforementioned study utilized petroleum ether as the extraction solvent. Therefore, while all Sargassum algae in our study were harvested and processed identically, variations in the bioactivity of our crude extracts may stem from differences in biochemical composition due to varying ethanol ratios used for extraction.

The ethanolic crude extract of *S. fluitans III* exhibited strong to moderately effective and broad-spectrum activity against the tested bacteria. A comparison between the antibacterial assessment of *S. fluitans III* ethanolic extracts under agar conditions and broth microdilution revealed different results. While both methods are applicable, MIC values can vary due to factors such as solubility and diffusion. In liquid media, the interaction between compounds and bacterial cells is more likely compared to a solid matrix [72], while growth on agar surfaces may alter oxygen exposure and the microenvironment, affecting the bioavailability of the antibacterial agent [73].

The antibacterial activity of extracts from other *Sargassum* species has been extensively documented [74]. For instance, petroleum ether and ethanolic extracts of *S. crassifolium* were reported to be more efficient against Gram-positive bacteria, while the methanolic extract showed greater efficacy against Gram-negative bacteria [75]. Similarly, ethanolic extracts of *S. aquifolium* and *S. wightii* demonstrated varying degrees of effectiveness against different bacterial strains [76,77]. In our study, the ethanolic extract exhibited inhibition against both Gram-negative and Gram-positive bacteria, with slightly higher efficacy against the former. This outcome may be attributed not only to the chemical composition of the extract but also to the presence of lipopolysaccharides in the outer membrane of Gram-negative bacteria, which provides protection against hydrophobic molecules [78]. Thus, the less hydrophobic molecules would enter more easily inside the bacteria [79].

Ethanolic extraction presents a favorable compromise as it is an environmentally friendly solvent that efficiently extracts secondary metabolites of interest, especially when used in an aqueous phase [80-82]. Additionally, the bioactivity of complex samples like crude extracts is influenced by interactions among multiple compounds, which can lead to synergistic or antagonistic effects [83]. Previous studies have identified phenolic compounds, terpenoids, saponins, flavonoids, tannins, and alkaloids in various *Sargassum* species, attributing biological activity to one or more secondary metabolites based on their biochemical composition [84–89]. However, to our knowledge, anthocyanins and coumarins have not yet been extracted or identified in *Sargassum* algae [90, 91]. Nonetheless, these molecules and their derivatives have been recognized for their antibacterial activity, particularly against Gram-negative bacteria [92,93].

In our study, coumarins, alkaloids had a broad and efficient spectrum against all tested strains, anthocyanins showed greater effectiveness against Gram-negatives whereas triterpenes, saponins and flavonoids were less effective. Previous study had shown that some flavonoid-belonging molecules precipitate when mixed in aqueous solution, reducing the disponibility of the compound. Furthermore, some phenolic molecules could act as aggregators of bacterial cell distorting result of turbidity [94–96]. This result emphasized that the bioactivity did not depend on the amount of bioactive components, since the most active molecules (alkaloids and coumarins) had a weak harvesting (0.11% and 1% respectively), unlike saponins (R = 16.50%) with an effect varying depending on the tested strain. Considering our findings, *S. fluitans III* extracts should be tested against multi-resistance bacteria such as species belonging to *Vibrio*, *Staphylococcus* and *Shewanella* genera [97,98].

S. warneri, *S. capitis*, *S. fodinae*, *V. alginolyticus*, *M. yannensis* and *A. macdeleii* are good biofilm forming bacteria, involved in food contamination [99], human and animal infectious diseases [100,101] or corrosion [102]. In our study, all strains were isolated from environment-exposed conditions. *V. alginolyticus*, one of the known multi-drugs resistant bacterium, is ubiquitous in marine environment and is known to be opportunist in animal and human [103]. It was recently subdivided into two lineages, with the lineage 2 significantly enriched in the antibacterial resistance gene thioredoxin and fos [104]. In our study, *V. alginolyticus* showed a good sensitivity to *S. fluitans* extract and seemed to be sensitive to amoxicillin and benzathine benzylpenicillin. In addition to physicochemical parameters [105], this result could be explained by at least two hypotheses or a combination of the two. First of all, the strain used in this study might belong to lineage 1, less resistant to antibiotics. Secondly, *S. fluitans* as *S. wightii* and *S. crassifolium* might contain a strong antibacterial compound, or several compounds that, by acting in synergy, can potentiate the antibacterial effect of the *Sargassum* extract. In fact, previous works, highlighted the ability of some exogenous sugars and amino acids to revert antibiotics sensibility in *V. alginolyticus* [106–108]. *V. alginolyticus* was recognized as an emerging pathogen which provokes vibriosis in humans and marine animals [109]. It was also described in marine corrosion biofilm, associated to the availability of iron [110]. *S. fodinae* is an iron reducing bacteria capable of enhancing corrosion in specific conditions [111]. According to our results, the strain used in the present study did not show an antibiotic resistance to the tested antibiotics, however, *Shewanella* genus is known to contain several antibiotic resistant species. *A. macdeleii* is a copiotrophic bacteria which had been found in corrosion-inducing biofilm but not directly implicated in corrosion. *M. yunnanensis* had been reclassified by Chien-Hsun

Huang and its colleagues to belong to *M. luteus* species [112]. Interestingly, *M. luteus* had been reported to be for interest in metal bioremediation and might be indirectly associated with corrosion under a specific condition [113,114].

Our findings up new possibilities for the valorization of *Sargassum* seaweed, the stranding of which represents a major threat to the environment, human healthcare in Caribbean. It would therefore be interesting to carry out an in-depth study on the molecules composing the chemical families of this algae giving it its antibacterial properties. In addition, the examination of their biocompatibility with higher organisms and their safety regarding the heavy-metal contamination [115–117], should be taken into consideration to allow their valorization in the food and health industry.

Conclusions

Our study highlighted the antibacterial activity of *S. fluitans III* extract and its efficacy against both Gram-negative and Gram-positive bacteria. The phytochemical analysis of secondary metabolites revealed the presence of compounds previously described in *Sargassum* species. However, to our knowledge, this is the first report of coumarins and anthocyanins being extracted from *Sargassum* algae. These secondary metabolites, along with alkaloids, exhibited significant antibacterial activity against bacteria isolated from corrosion-associated biofilm, which are often implicated in various metal-using industries. Therefore, the ethanol extract of *S. fluitans III* could serve as an eco-friendly antibacterial agent suitable for applications in the food and health industries.

Disclosure

Author Contributions: Conceptualization, M.L; methodology, P.L.,F.R. and M.L; software, P.L. and F.R.; validation, F.R. and M.L.; for-mal analysis, P.L., F.R. and M.L.; investigation, P.L., F.R. and M.L; resources, C.R. and M.L.; data curation, P.L.,P.S. and M.L.; writing—original draft preparation, P.L., F.R. and M.L.; writing—review and editing, P.L., F.R. and M.L.; visualization, P.L., F.R., C.R. and M.L; supervision, F.R. and M.L; project administration, F.R., C.R., P.S. and M.L.; funding acquisition, All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: None.

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