

BIOACTIVE COMPOUNDS POTENTIATION AND ANTIBACTERIAL ACTIVITIES OF A PETALONIA FASCIA EXTRACT ON METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS STRAINS

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ABSTRACT

The bioactive algal components that contribute to their antimicrobial properties have garnered significant attention, particularly in light of the rising incidence of antibiotic resistance. The current study aimed to evaluate the antibacterial activity of a *Petalonia fascia* methanolic extract and to explore the potentiation action of extract-antibiotic combinations with the available antibiotics, as well as the effects of their mixture on multidrug-resistant (MDR) bacteria, namely, five methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The algal crude extract displayed antibacterial activity against all assayed isolates, with an inhibition zone ranging from 16 to 21 mm and the best spectrum of bactericidal effect with a ratio of MBC/MIC < 4 obtained on the five tested bacterial strains. Amoxicillin-extract mixtures decreased the bacterial growth rate dramatically on all five *S. aureus* isolates in a time-dependent manner. These results indicate the ability of the extract to modulate antibiotic activity. However, the possibility of its use to solve the issue of antibiotic-resistant bacterial strains and their application as therapeutic agents against infectious diseases warrants further investigation.

Keywords: *Petalonia fascia*, Antibiotic potentiation activity, MDA, *Staphylococcus aureus*

INTRODUCTION

Many bacterial strains overcome the efficacy of antibiotics via their rapid mutation. However, antibiotics can synergistically act with natural bioactive molecules that boost their efficacies. Algal extracts proved to be more promising antibacterial agents even though their antibacterial activity is milder than that of commercially available antibiotics. Many reports have described active compounds derived from macroalgae that exhibited potential antimicrobial activity (Alghazeer *et al.*, 2013; He *et al.*, 2014; Lee *et al.*, 2014; Morán-Santibañez *et al.*, 2018; Nafis *et al.*, 2021).

Much attention has been paid in the past few years to the bioactive components of algal extracts that contribute to their antimicrobial properties, particularly because of the emergence of antibiotic-resistant bacterial strains (Pérez *et al.*, 2016). It has been reported that these components act on their own or enhance the potency of several antibiotics. Furthermore, the complex nature of the extracts hampers microbial adaptation (Daferera *et al.*, 2003); therefore, these extracts are less likely to induce resistance to their effects.

The antimicrobial compounds derived from brown marine algae consist of a diverse group of chemical compounds, including polyphenols, phlorotannins, flavonoids, alkaloids, tannins, terpenoids, saponins, coumarins, sterols, quinones, polysaccharides, and free fatty acids (A. Brantes *et al.*, 2010; Demirel *et al.*, 2009; Alghazeer *et al.*, 2017; Kim *et al.*, 2017; Ummat *et al.*, 2020; Singkoh *et al.*, 2021). These active constituents are reported to be bactericidal against Gram-negative and Gram-positive bacteria (Nor Affah *et al.*, 2010; Alghazeer *et al.*, 2021; Mahendran *et al.*, 2022).

The bacterial resistance to antibiotics is a serious public health issue worldwide, which has been addressed by various researchers (Lucet *et al.*, 2009; Periasamy

et al., 2021). By and large, the use of antibiotics to treat infectious diseases in humans, animals, plants, or food processing may lead to the development of bacterial resistance against these antibiotics. Some strains of *Staphylococcus aureus* are known to resist methicillin (MRSA strains) (Lucet *et al.*, 2009), thus causing severe infections in humans, often in hospital settings and nosocomial infections. *S. aureus* is a highly multidrug-resistant (MDR) bacterium, partly because of its capacity to form biofilms; therefore, infection with this pathogen is difficult to treat (Kluytmans *et al.*, 1997; Periasamy *et al.*, 2021). *S. aureus* infections are often fatal in nature because of their resistance to many β -lactam antibiotics (Pavillard *et al.*, 1982). However, not much is known about the combination of algal antibacterial extracts and antibiotics and their effects on bacterial cells. Thus, this study aimed to ascertain the antibacterial activity of a *Petalonia fascia* methanolic extract and to explore the potentiation action of the extract-antibiotic combinations with available antibiotics, as well as of their mixture on MDR bacteria, namely, five MRSA isolated from food of animal origin (Nass *et al.*, 2019).

MATERIALS AND METHODS

Sampling area, collection, and identification of the algal material

The *Petalonia fascia* samples were collected during June 2020 at Tajura, on the western coast of Libya (SA 01, N 32°53'45.47 E 13°21'3.16; SA 02, N 32°53'51.95 E 13°21'4.25; SA 03, N 32°53'54.19 E 13°20'54.10; SA 04, N 32°53'46.23 E 13°20'50.90) (Figure 1). The samples were identified and classified at the Marine Biology Research Center, Tajura, Tripoli, Libya. Routine procedures, which included the removal of the necrotic parts and epiphytes followed by rinsing with

sterile distilled water, were carried out on the collected samples. The samples were dried in the shade at ambient temperature for 7 days and immediately subjected to extraction.



Figure 1 Localization of the collection site of the algal material (MBRS; Marine Biology Research Center).

Bacterial strains

The staphylococcal isolates used in this study were originally isolated from meat and meat products and molecularly identified as *S. aureus* at the Department of Food Hygiene, Veterinary Medicine, University of Tripoli, Libya. When tested for antibiotic susceptibility, their antibiogram revealed that they were MDR, and, with the exception of *S. aureus* 120 (S2), all isolates (*S. aureus* 119 (S1), *S. aureus* 121 (S3), *S. aureus* 130 (S4), and *S. aureus* 283 (S5)) were all MRSA (Azawi et al., 2016; Nass et al., 2019).

Antibiotics

Amoxicillin (500 mg) was obtained from Bristol Laboratories Ltd/UK, whereas amoxicillin/clavulanic acid (Augmentin, 1 g) was obtained from Dar Al Dawa, Jordan. The antibiotics were dissolved in 0.01% dimethyl sulfoxide (DMSO).

Extraction and phytochemical screening

A powdered sample of *P. fasciata* was extracted using methanol (99%), with the amount of solvent used being twice the mass of the specimens. The crude extract obtained after three rounds of extraction over a 72-h period at room temperature (25°C–30°C) was filtered through a Whatman-1 filter paper. The extract was concentrated on a rotary evaporator (Stuart RE300) at 40°C under reduced pressure, and the resultant residue was kept at –20°C until use.

The qualitative detection of polyphenols, alkaloids, flavonoids, tannins, saponins, coumarins, terpenoids, quinones, steroids, and anthraquinones was performed according to Kumar et al., (2003). The color intensity of the formed precipitate was used as the analytical response in these tests.

Anti-hemolytic activity

The anti-hemolytic activity of the algal extract was tested against human red blood cells (RBCs) collected from non-smoking healthy volunteers (Da Silva et al., 2004). RBCs, which were obtained after centrifugation of whole blood for 10 min at 3000 rpm, were washed and re-suspended in saline. One hundred milliliters of RBCs were mixed with the algal extract at a concentration of 6.25, 12.5, 25, 50, or 100 mg/mL to obtain 200 mL of 4% erythrocytes. The contents were incubated at 37°C for 1 h, centrifuged, and a clear supernatant was obtained. The absorbance of the supernatant, which represents the released hemoglobin, was measured at 414 nm using a UV–visible spectrophotometer (PerkinElmer, LAMBDA 25, USA). Absolute (100%) and 0% hemolysis was achieved using 0.1% Triton X-100 and normal saline, respectively. Aspirin was used as the negative control. The percentage of hemolysis of the algal extract was calculated using the following formula:

$$\% \text{ Hemolysis} = \frac{\text{Absorbance of the sample solution} - \text{Absorbance of saline}}{\text{Absorbance of 0.1\% Triton X} - \text{Absorbance of normal saline}} \times 100$$

Bacterial strains and culture medium

The antibacterial activity of the methanol algal extract was tested against the five strains using the hole-plate diffusion method (Saravanakumar 2009). The extract (at a concentration of 250 mg/ml) was added to the wells in triplicate and incubated at 37°C for 18 h. The diameters of the inhibition zones (DIZs) were measured, and the mean DIZ value from triplicate experiments was calculated. Methanol was used as the negative control, and amoxicillin (AML) (40 µg) and amoxicillin/clavulanic acid (AMC) (Augmentin; 120 µg) were used as the reference.

Measurement of the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the algal extract

The MIC and MBC of the extract were measured using the two-fold microdilution agar method.

Bacterial strains were cultured in Mueller–Hinton broth (MHB) until they reached approximately 10^6 cfu/ml. A serial dilution of the extract was prepared using MHB as the diluent to achieve a range of concentrations of 30 to 200 mg/mL. The diluted extracts were inoculated with an equal volume of bacterial suspension and incubated at 37°C for 18 h. After this incubation, the lowest concentration that completely inhibited the growth of the tested bacteria within 24 h was considered the MIC, whereas the lowest concentration that prevented any visible growth was noted as the MBC. Each test was carried out thrice at separate times.

Antibiotics and extract preparation

The stock solutions of the test materials were prepared by dissolving the methanol extract of *P. fasciata* to a final concentration of 125 mg/ml in 0.01% DMSO. Similarly, the AML and AMC antibiotics were dissolved in 0.01% DMSO to a final concentration of 40 µg/ml and 120 µg/ml, respectively, and used in the assays. The working solutions of the tested extract and the antibiotics were prepared by calculating the MIC of each one of the stock solutions. The potentiating action of the extract-antibiotic combinations used for the treatment of the *S. aureus* isolates was investigated. The following extract-antibiotic combinations were used. C1, extract: antibiotic (1:1); C2, extract: antibiotic (1:2); and C3, extract: antibiotic (2:1).

Potentiating of antibiotic activity

Two methods were used to evaluate the capability of the *P. fasciata* extract to potentiate the effects of the antibiotics against the *S. aureus* strains: the antimicrobial susceptibility test and the bacterial growth inhibition test (viable counts of bacteria).

Antimicrobial susceptibility

After culturing bacteria in MHB, the samples were incubated for 17 h at 37°C. Cell growth was monitored by measuring the optical density at 600 nm.

Time-kill growth rate (%)

For the viability assay, a bacterial culture was initiated in MHB medium. Each sample was inoculated with a bacterial suspension (6×10^8 CFU/mL) to reach the MIC. A bacterial culture in MHB alone served as the negative control. The cultures were incubated at 37°C with agitation for 0, 40, 80, 120, or 160 min. Subsequently, the cultures were diluted 10-fold and spread on Mueller–Hinton agar (MHA). Colonies were counted to determine cell viability. Each assay was repeated thrice.

Mode of action of the algal extract

Measurement of the potassium ion released

The potassium ions released from bacteria were measured according to Hao et al., (2009). Isolated cells (6×10^8 CFU/mL) were cultured either with the extract (2 × MIC) or solvent (as a negative control) together with the antibiotics at 37°C for varying periods. The potassium ion concentration in the culture supernatant was measured using a flame photometer.

Determination of nucleotide leakage

The effect of the algal extract regarding cell membrane damage was assessed by measuring the nucleotide leakage into the culture supernatant, as per the method described by Tang et al., (2008). The extract (1 × MIC) or solvent (negative control) was added to the bacterial suspension during logarithmic growth (6×10^8 CFU/mL) and incubated at 37°C for different periods. The amount of nucleotide leakage into the culture supernatant was determined by measuring the absorbance at 260 nm using a UV spectrophotometer (PerkinElmer LAMBDA 25, USA).

RESULTS

Phytochemical analysis

The results of the phytochemical analysis of the algal extract are reported in Table 1. In the current study, the methanol crude extract of *P. fascia* was positive for polyphenols, flavonoids, alkaloids, saponins, coumarins, and steroids. The presence of these bioactive phytochemicals, as detected in this study, indicates a wide range of bioactivity applications of the algal extract.

Table 1 Results of the phytochemical screening of *P. fascia*

Metabolites	<i>P. fascia</i>
Polyphenols & Tannins	++
Flavonoids	+++
Alkaloids	++
Saponins	++
Tannins	++
Coumarins	++
Terpenoids	-
Quinones	-
Steroids	+++
Anthraquinones	-

Qualitative phytochemical screening

The total phenol, tannin, phlorotannin, flavonoid, alkaloid, steroid, and coumarin content of the crude methanol extract of *P. fascia* is presented in Table 2. We found that phenols were the most abundant components (1367.44 ± 17.51 mg GAE/g dw), followed by phlorotannin (357.60 ± 38.44 mg PhgE/g dw) and flavonoids (113.22 ± 0.69 mg RE/g dw), whereas alkaloids (1.55 ± 0.08 mg AE/g dw) were the least abundant components of the extract.

Table 2 Mean total content of selected phytochemicals in the *P. fascia* extract

Phytochemicals	Unit	Mean ± SD
Polyphenols	mg GAE/g dw	1367.44 ± 17.51
Tannins	mg GAE/g dw	14.42 ± 0.60
Phlorotannins	mg PhgE/g dw	357.60 ± 38.44
Flavonoids	mg RE/g dw	113.22 ± 0.69
Alkaloids	mg AE/g dw	1.55 ± 0.08
Steroids	mg EE/g dw	89.06 ± 7.87
Coumarins	mg CE/g dw	10.87 ± 0.13

mg GAE/g dw, milligrams of gallic acid equivalent per gram of dry weight; Phg, phloroglucinol; R, rutin; A, atropine; E, estrone; C, coumarin. Data are presented as the mean value ± standard deviation (SD) of triplicate readings (n = 3).

Anti-hemolytic activity of the algal extract

The possibly toxic effect of the *P. fascia* extract was tested on human RBCs (Table 3). No toxic effect of the extract on RBCs was detected at concentrations that yielded antimicrobial activity. Although there was an increase in the hemolytic activity as the concentration of the extract increased, and the observation that the hemolysis activity of the extract was higher than that of the positive control, these results led us to consider that the extract was within the safe level.

Table 3 Anti-hemolytic activity of the *P. fascia* methanol extract against human erythrocytes (HRBCs)

Concentration (µg/ml)	Hemolytic activity (%)	
	<i>P. fascia</i>	Aspirin
62.5	1.74 ± 0.05	0.57 ± 0.03
125	2.68 ± 0.08	0.88 ± 0.02
250	4.73 ± 0.25	1.86 ± 0.01
500	5.84 ± 0.01	2.87 ± 0.10

Data are presented as the mean value ± standard deviation (SD) of triplicate readings (n = 3).

Antibacterial activity of the algal extract

The primary antibacterial screening of the crude extract showed the presence of antibacterial activity against all assayed bacteria, with a range of the inhibition zone of 16–21 mm (Table 4). The zone of inhibition was smaller for the extract against the assayed strains compared with the positive control, with the exception of the *S. aureus* 130 (S4) strain, the inhibition zone of which was larger than that of the positive control. The maximum inhibition zone (21 mm) was observed for the extract against *S. aureus* 283 (S5) (Table 4).

Table 4 Zone of inhibition (mm) in the primary screening of the *P. fascia* extract against five isolates of *Staphylococcus aureus* using the disc diffusion method.

Isolates	<i>P. fascia</i>	Amoxicillin/Clavulanic Acid	Amoxicillin
S1	18 ± 1.00	19	-
S2	18.5 ± 1.0	22	-
S3	16.0 ± 0.00	21	-
S4	19 ± 0.00	14	-
S5	21.0 ± 0.00	32	-

DIZ, diameter of the inhibition zone

Diameter of the hole, 6 mm

Data are presented as the mean ± SD, n = 3

S1, *S. aureus* 119; S2, *S. aureus* 120; S3, *S. aureus* 121; and S4, *S. aureus* 130; S5; *S. aureus* 283

Table 5 lists the MIC and MBC values of the *P. fascia* extract tested against five strains of *S. aureus*. The extract yielded the same MIC and MBC (50 and 100 mg/ml, respectively), with the exception of *S. aureus* 120, which showed a higher MIC and MBC (62.5 and 187.5 mg/ml, respectively). The algal extract also exhibited broad-spectrum bactericidal activity, with a MBC/MIC ratio <4 on the five tested bacterial strains (Table 5)

Table 5 The MIC, MBC, and MIC/MBC ratio of the algal extract against the tested bacterial strains

Isolates	MIC*	MBC*	MBC/MIC
S119	50	100	2
S120	62.5	187.5	3
S121	50	100	2
S130	50	100	2
S283	50	001	2

MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration*mg/ml S1: *S. aureus* 119; S2: *S. aureus* 120; S3: *S. aureus* 121; and S4: *S. aureus* 130; and S5: *S. aureus* 283

Antibacterial activity of three different combinations of the *P. fascia* and amoxicillin

The assayed strains were sensitive to amoxicillin/clavulanic acid (Augmentin) and resistant to amoxicillin; therefore, amoxicillin was used to study the ability of the extract to modulate the activity of the antibiotics. For this, the algal extract was mixed with amoxicillin at three different combination ratios of 1:1 (C1), 1:2 (C2), and 2:1 (C3) and was incubated with the assayed strains for 18 h at 37°C. After the incubation period, bacterial growth was completely inhibited as the absorption of each sample decreased dramatically, indicating the reduction of bacterial growth as an effect of the enhancement of the antibacterial activity of amoxicillin afforded by the algal extract (Figure 2).

To confirm the potentiation of the antibiotic activity by the algal extract when the antibiotic was combined with the extract, the C1, C2, and C3 combinations were subjected to a time-kill growth rate (%) analysis (Table 6). The results pertaining to the viable bacterial population (%) of *S. aureus* strains treated with the algal extract alone, amoxicillin alone, and the extract–amoxicillin combination at different ratios recorded within 180 min of the treatment are presented in Table 6. Generally, the algal extract alone and its combinations (C1, C2, and C3) showed a stronger inhibitory activity on all five *S. aureus* isolates in a time-dependent manner. A positive effect on the antibacterial activity of amoxicillin was observed when this antibiotic was combined with the extract at different ratios, which indicated the ability of the extract to modulate the activity of the antibiotic. Compared with amoxicillin, C1 and C3 significantly enhanced the activity of amoxicillin, as the percentage of viable bacterial cells reached 0% after 80 min of incubation against all assayed staphylococcal isolates. Conversely, C2 yielded a slower decrease in the population of viable bacteria, as the rate of the viable population reached 0% at 160 min of incubation.

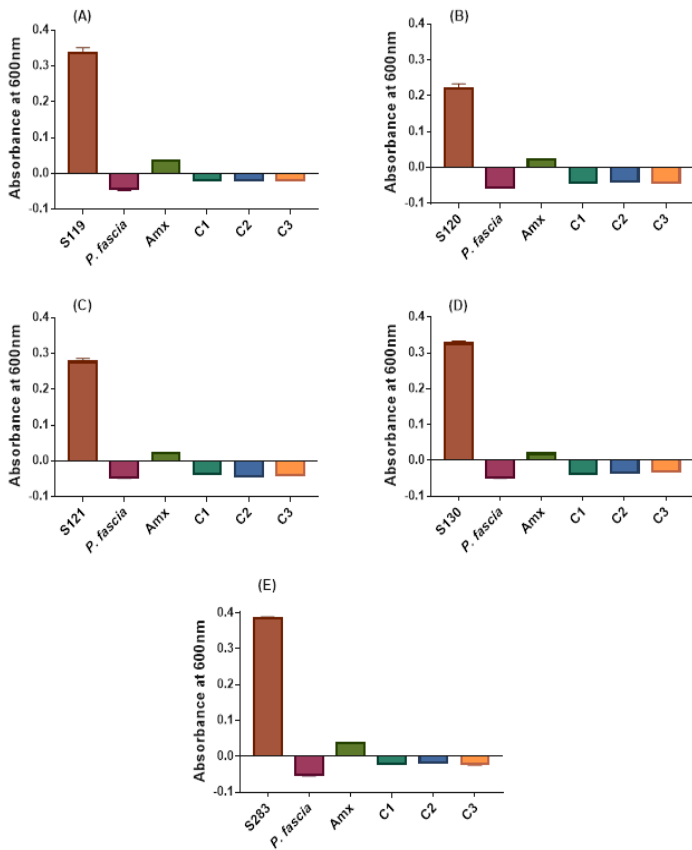


Figure 2 Antibiotic-modifying activity of the *P. fasciata* extract in combination with the amoxicillin beta-lactam antibiotic. S1: *S. aureus* 119; S2: *S. aureus* 120; S3: *S. aureus* 121; S4: *S. aureus* 130; and S5: *S. aureus* 283. Amx, amoxicillin; P, *P. fasciata*; C1, P:Amx = 1:1; C2, P:Amx = 1:2; C3, P:Amx = 2:1. Each data point is

the mean ± SD (n = 3).

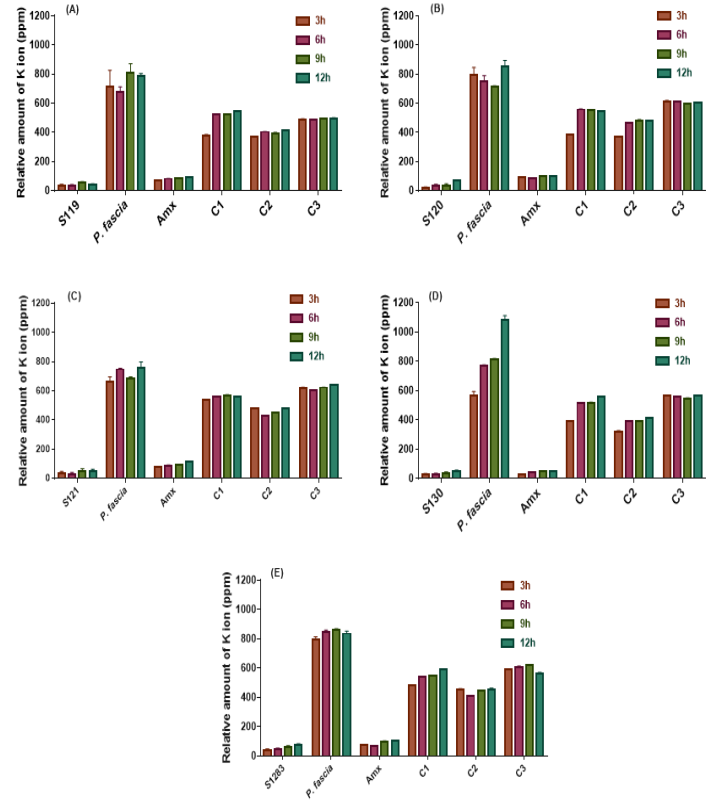


Figure 3 Effect of the MIC of the *P. fasciata* extract and its combination with amoxicillin on potassium ions (K^+) release from *S. aureus* strains. S1: *S. aureus* 119; S2: *S. aureus* 120; S3: *S. aureus* 121; S4: *S. aureus* 130; and S5: *S. aureus* 283. Amx, amoxicillin; P, *P. fasciata*; C1, P:Amx = 1:1; C2, P:Amx = 1:2; C3, P:Amx = 2:1. Each data point is the mean ± SD (n = 3).

Table 6 Growth inhibition rate (%) of the combination of the *P. fasciata* extract with amoxicillin and of amoxicillin alone against MRSA strains.

Samples	Time (min)				
	20	40	80	120	160
S119					
<i>P. fasciata</i> extract	17.24 ± 2.00	000.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amx	88.50 ± 2.00	65.63 ± 3.21	74.00 ± 4.00	74.23 ± 3.78	80.13 ± 4.00
C1	48.00 ± 1.00	17.6 ± 1.00	11.15 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
C2	20.94 ± 2.52	12.14 ± 2.00	14.87 ± 2.52	2.57 ± 0.58	3.1 ± 1.15
C3	27.58 ± 2.00	4.8 ± 1.15	4.59 ± 1.00	5.28 ± 1.53	1.1 ± 1.00
S120					
<i>P. fasciata</i> extract	8.00 ± 2.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amx	66.34 ± 3.00	59.3 ± 2.52	65.38 ± 2.00	65.4 ± 2.00	68.28 ± 2.00
C1	33.65 ± 2.00	.65 ± 2.008	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
C2	41.02 ± 1.53	4.80 ± 1.00	3.84 ± 1.00	5.12 ± 0.58	4.80 ± 1.00
C3	50.00 ± 1.53	13.77 ± 1.53	7.69 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
S121					
<i>P. fasciata</i> extract	15.44 ± 1.53	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amx	64.88 ± 4.00	88.84 ± 2.00	90.33 ± 2.08	88.37 ± 2.52	68.52 ± 2.52
C1	44.79 ± 2.52	14.67 ± 2.08	3.47 ± 2.00	0.00 ± 0.00	0.00 ± 0.00
C2	47.1 ± 3.51	8.1 ± 2.00	7.78 ± 1.53	4.99 ± 2.08	2.31 ± 1.00
C3	30.89 ± 2.52	10.42 ± 2.00	3.85 ± 1.53	0.00 ± 0.00	0.00 ± 0.00
S130					
<i>P. fasciata</i> extract	6.97 ± 2.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amx	67.00 ± 2.00	65.67 ± 3.06	58.67 ± 1.53	70.67 ± 1.53	0.00 ± 0.00
C1	52.6 ± 1.53	9.63 ± 1.53	6.97 ± 1.53	0.00 ± 0.00	0.00 ± 0.00
C2	51.88 ± 2.00	5.18 ± 2.08	7.18 ± 0.58	7.33 ± 1.53	3.33 ± 1.00
C3	50.00 ± 2.00	9.63 ± 2.08	5.18 ± 1.53	0.00 ± 0.00	0.00 ± 0.00
S283					
<i>P. fasciata</i> extract	33.33 ± 2.52	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Amx	63.51 ± 3.00	61.15 ± 1.53	64.91 ± 2.52	62.9 ± 2.00	57.22 ± 3.51
C1	43.56 ± 2.53	24.66 ± 2.08	5.77 ± 1.53	6.29 ± 1.00	2.33 ± 2.00
C2	47.67 ± 1.52	14.07 ± 2.00	8.1 ± 1.53	5.97 ± 1.15	3.3 ± 1.00
C3	38.32 ± 1.53	6.67 ± 2.08	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

S1: *S. aureus* 119; S2: *S. aureus* 120; S3: *S. aureus* 121; S4: *S. aureus* 130; and S5: *S. aureus* 283. Amx, amoxicillin; P, *P. fasciata*; C1, P:Amx = 1:1; C2, P:Amx = 1:2; C3, P:Amx = 2:1. Each data point is the mean ± SD (n = 3).

Mode of action

Figure 3 depicts the potassium ion (K⁺) concentration after *S. aureus* isolates were treated with different concentrations of the *P. fasci*a extract in combination with an antibiotic from 1 to 12 h. The results indicated that, after treating staphylococcal strains with the *P. fasci*a extract combined with amoxicillin (C1, C2, and C3), the release of K⁺ increased significantly in an extract-concentration-dependent manner, whereas no noticeable changes in the level of K⁺ were observed in the control sample. The measurement of the UV absorption value at time intervals can reflect the degree of cell membrane damage.

Figure 4 depicts the increase in the level of the intracellular cell constituents, including DNA and RNA, released after the incubation of the *S. aureus* isolates with the *P. fasci*a extract at three different concentrations together with amoxicillin for 3, 6, 9, and 12 h. *S. aureus* strains incubated with the *P. fasci*a extract combined with amoxicillin showed higher absorption than did the *S. aureus* strains incubated with amoxicillin alone, whereas no obvious changes were observed in the OD260 values of the control.

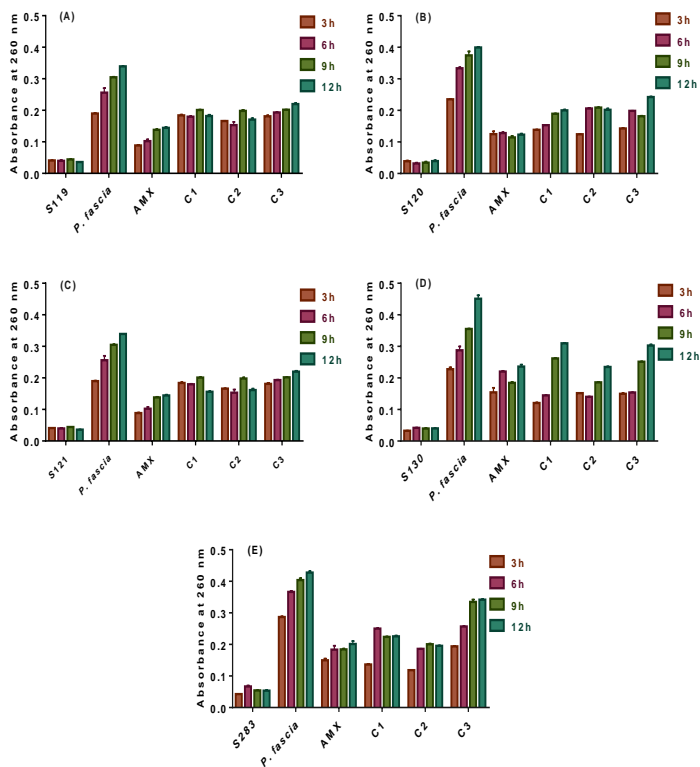


Figure 4 Effect the MIC of the *P. fasci*a extract and its combination with amoxicillin on total nucleotide release from *S. aureus* strains. S1: *S. aureus* 119; S2: *S. aureus* 120; S3: *S. aureus* 121; S4: *S. aureus* 130; and S5: *S. aureus* 283. Amx, amoxicillin; P, *P. fasci*a; C1, P:Amx = 1:1; C2, P:Amx = 1:2; C3, P:Amx = 2:1. Each data point is the mean ± SD (n = 3).

DISCUSSION

The search for alternatives to antibiotics has become of paramount importance considering the increasing resistance of bacteria to antibiotics. Moreover, although the basic and preliminary *in vitro* characteristics of some of these alternatives have been known for some time, these compounds were never exploited for their pharmaceutical properties.

In the current study, qualitative phytochemical screenings of the *P. fasci*a extract revealed the presence of phenols, phlorotannins, tannins, flavonoids, alkaloids, saponins, coumarins, terpenoids, and steroids. In addition, the quantitative screening showed that the extract had a considerable amount of phenolics, flavonoids, phlorotannins, and steroids, which agreed with previous findings (Akgul et al., 2015; Demirel et al., 2009).

Recent studies have reported that brown seaweeds have an *in vitro* antimicrobial action against MRSA (Chandrasekaran et al., 2014; Jegan et al., 2019). Our previous work showed that different extracts of algae were effective as anti-MRSA agents (Alghazeer et al., 2017; Alghazeer et al., 2021). Moreover, previous studies demonstrated that the high antibacterial potential observed for brown algae against MDR isolates was probably attributable to the presence of various bioactive compounds, such as sulfated polysaccharides, peptides, amino acids, lipids, polyphenols, flavonoids, and alkaloids (Alghazeer et al., 2017; Gupta and Abughannam 2011; Vallinayagam et al., 2009; Alghazeer et al., 2021), furthermore, their activity could be directly or indirectly linked to the increase in the activity of antibiotics (Lee et al., 2014). In this study, the evaluation of the antimicrobial

activity of an extract of *P. fasci*a against five isolates of *S. aureus* was attempted. Our results showed that the extract exerted the strongest antibacterial activity on S119, S121, S130, and S283 with a MIC of 50 mg/ml, whereas weaker activity was observed against S120, with a MIC of 62.5 mg/ml. The extract had a bactericidal effect on all tested strains (Table 4). In addition, compared with amoxicillin/clavulanic acid (Augmentin), the extract showed weaker activity against S1, S2, S3, and S5 and stronger activity against S4 than did amoxicillin/clavulanic acid (Augmentin). Conversely, amoxicillin alone had no effect on all tested isolates (Table 6).

Antibiotics are commonly prescribed to treat bacterial infections. However, the rampant use of antibiotics has allowed bacteria to develop antibiotic resistance because of their high degree of genetic variability, thus rendering the antibiotic action ineffective. The emergence of antibiotic-resistant bacteria necessitated the exploration of alternative treatments. To overcome drug resistance, different combinations of antibiotics against various targets have been used (Sibanda and Okoh 2007). In this context, synthetic and natural compounds have displayed synergy with different classes of antibiotics. Several studies concluded that the use of extracts of natural sources in combination with antibacterial agents enhances the activity of the latter by lowering the MIC of antibiotics specific to bacterial strains (Betoni et al., 2006; Nafi et al., 2021; Souza et al., 2011). Various bioactive chemical compounds have been shown to regulate antibiotic activity against many strains of bacteria by negating the antibiotic resistance property of bacteria, thereby eliminating plasmids and attenuating the plasma membrane efflux of antibiotics (Coutinho-Silva et al., 2009). The current study showed that the efficacy of the amoxicillin antibiotic increased against isolates of *S. aureus* when it was combined with the *P. fasci*a extract at different ratios.

It has been shown that many β-lactam antibiotics can penetrate Gram-positive bacteria through protein channels located on the outer membrane, thus allowing the drug to easily reach its receptors on the cell wall and exert its bactericidal effect (Nikaido 1994). The active components of the extract, including flavonoids, alkaloids, tannins, and phlorotannins, may facilitate the penetration of the molecules of the antibiotics to be delivered to their intracellular target by increasing the membrane permeability while contributing to the cessation of bacterial multiplication (Nagayama et al., 2002; Eumkeb and Richards 2005; Hierholtzer et al., 2012; Liu 2020; Alghazeer et al., 2021). In this report, Gram-positive strains were sensitive to the combination of the *P. fasci*a extract with amoxicillin at three different ratios (Figure 2). All combination samples significantly increased the activity of the antibiotic by increasing the membrane permeability, which was demonstrated by the increase in the amount of intracellular constituents released (Figures 3 and 4); in turn, this led to a reduction in the growth rate of the bacteria, which could be explained by the inhibition of the biomolecules that are required for bacterial cell membrane synthesis via alignment with a penicillin-linked protein. This results in the inhibition of the transpeptidase property and consequent inhibition of peptidoglycan synthesis (Tenover 2006).

CONCLUSIONS

The research focusing on marine algae should be expanded by contributions from researchers from different disciplines, to address the possibility of their use to solve the issue of resistant bacterial strains and their application as therapeutic agents against infectious diseases.

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Declaration

Ethical Approval: All authors confirm that this manuscript conforms to the ethical standards and requirements of the journal.

Consent to Participate: This research paper did not utilize any animal testing or human participants.

Consent to Publish: All authors consent to the publication of this work.

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REFERENCE

Abrantes, J. L., Barbosa, J., Cavalcanti, D., Pereira, R. C., Fontes, C. L. F., Teixeira, V. L., ... & Paixão, I. C. (2010). The effects of the diterpenes isolated from the Brazilian brown algae *Dictyota pflaffii* and *Dictyota menstrualis* against the herpes simplex type-1 replicative cycle. *Planta Medica*, 76(04), 339-344. <https://doi.org/10.1055/s-0029-1186144>

Alghazeer, R., Azwai, S., Garbaj, A. M., Amr, A., Elghmasi, S., Sidati, M., ... & Alansari, W. S. (2022). Alkaloids rich extracts from brown algae against multidrug-resistant bacteria by distinctive mode of action. *Arabian Journal for Science and Engineering*, 1-10. <https://doi.org/10.1007/s13369-021-05592-w>

Alghazeer, R., Elmansori, A., Sidati, M., Gammoudi, F., Azwai, S., Naas, H., ... & Eldaghayes, I. (2017). *In vitro* antibacterial activity of flavonoid extracts of two

- selected Libyan algae against multi-drug resistant bacteria isolated from food products. *Journal of Biosciences and Medicines*, 5(01), 26. <https://doi.org/10.4236/jbm.2017.51003>
- Alghazeer, R., Whida, F., Abduelrhman, E., Gammoudi, F., & Azwai, S. (2013). Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. *Natural Science*, 5(1), 7-14. <https://doi.org/10.4236/ns.2013.51002>
- Akgül, R., Kizilkaya, B., Akgül, F., & Erduğan, H. (2015). Total lipid and fatty acid composition of twelve algae from Çanakkale (Turkey).
- Azwai, S. M., Alfallani, E. A., Abolghait, S. K., Garbaj, A. M., Naas, H. T., Moawad, A. A., ... & Eldaghayes, I. M. (2016). Isolation and molecular identification of *Vibrio* spp. by sequencing of 16S rDNA from seafood, meat and meat products in Libya. *Open Veterinary Journal*, 6(1), 36-43. <http://dx.doi.org/10.4314/ovj.v6i1.6>
- Betoni, J. E. C., Mantovani, R. P., Barbosa, L. N., Di Stasi, L. C., & Fernandes Junior, A. (2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Memórias do Instituto Oswaldo Cruz*, 101, 387-390. <https://doi.org/10.1590/s0074-02762006000400007>
- Chandrasekaran, M., Venkatesalu, V., & Raj, G. A. (2014). Anti-MRSA activity of Brown and Red algae from Gulf of Mannar Coast, South India. *International Journal of Life Sciences & Technology*, 7(4).
- Coutinho-Silva, R., Corrêa, G., Sater, A. A., & Ojcius, D. M. (2009). The P2X 7 receptor and intracellular pathogens: a continuing struggle. *Purinergic signalling*, 5, 197-204. <https://doi.org/10.1007/s11302-009-9130-x>
- Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. *Crop protection*, 22(1), 39-44. [https://doi.org/10.1016/s0261-2194\(02\)00095-9](https://doi.org/10.1016/s0261-2194(02)00095-9)
- Da Silva, E., Shahgaldian, P., & Coleman, A. W. (2004). Haemolytic properties of some water-soluble para-sulphonato-calix-[n]-arenes. *International journal of pharmaceuticals*, 273(1-2), 57-62. <https://doi.org/10.1016/j.ijpharm.2003.12.008>
- Demirel, Z., Yilmaz-Koz, F. F., Karabay-Yavasoglu, U. N., Ozdemir, G., & Sukatar, A. (2009). Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. *Journal of the Serbian Chemical Society*, 74(6), 619-628. <https://doi.org/10.2298/jsc0906619d>
- Eumkeb, G., & Richards, R. M. F. (2003, February). Reversing beta-lactam antibiotic resistance with flavonoids in gram-positive bacteria. In *III WOCMAP Congress on Medicinal and Aromatic Plants-Volume 4: Targeted Screening of Medicinal and Aromatic Plants, Economics* 678 (pp. 171-178). <https://doi.org/10.17660/actahortic.2005.678.24>
- Gupta S, Abu-Ghannam N (2011) Bioactive potential and possible health effects of edible brown seaweed. *Trends Food Sci. Technol* 22:315-326. <https://doi.org/10.1016/j.tifs.2011.03.011>
- Hao G, Shi YH, Tang YL, Le GW (2009) The membrane action mechanism of analogs of the antimicrobial peptide Buforin 2. *Peptides* 30: 1421-1427. <https://doi.org/10.1016/j.peptides.2009.05.016>
- He, X., Hwang, H. M., Aker, W. G., Wang, P., Lin, Y., Jiang, X., & He, X. (2014). Synergistic combination of marine oligosaccharides and azithromycin against *Pseudomonas aeruginosa*. *Microbiological research*, 169(9-10), 759-767. <https://doi.org/10.1016/j.micres.2014.01.001>
- Hierholtzer, A., Chatellard, L., Kierans, M., Akunna, J. C., & Collier, P. J. (2013). The impact and mode of action of phenolic compounds extracted from brown seaweed on mixed anaerobic microbial cultures. *Journal of applied microbiology*, 114(4), 964-973. <https://doi.org/10.1111/jam.12114>
- Jegan, S., Raj, G. A., Chandrasekaran, M., & Venkatesalu, V. (2019). Anti-MRSA activity of *Padina tetrastratica*, *Padina gymnospora* from Gulf of Mannar biosphere. *World Scientific News*, 115, 15-26.
- Kim, H. J., Dasagrathi, C., Kim, S. H., Kim, B. G., Eom, S. H., & Kim, Y. M. (2018). In vitro antibacterial activity of phlorotannins from edible brown algae, *Eisenia bicyclis* against streptomycin-resistant *Listeria monocytogenes*. *Indian journal of microbiology*, 58, 105-108. <https://doi.org/10.1007/s12088-017-0693-x>
- Kluytmans, J. A. N., Van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clinical microbiology reviews*, 10(3), 505-520. <https://doi.org/10.1128/cmr.10.3.505>
- Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The scientific world journal*, 2013(1), 162750. <https://doi.org/10.1155/2013/162750>
- Lee, J. H., Eom, S. H., Lee, E. H., Jung, Y. J., Kim, H. J., Jo, M. R., ... & Kim, Y. M. (2014). In vitro antibacterial and synergistic effect of phlorotannins isolated from edible brown seaweed *Eisenia bicyclis* against acne-related bacteria. *Algae*, 29(1), 47-55. <https://doi.org/10.4490/algae.2014.29.1.047>
- Liu, X. (2020). *Extraction and Anti-bacterial Effects of Edible Brown Algae Extracts*. North Carolina State University.
- Lucet, J. C., Paoletti, X., Demontpion, C., Degrave, M., Vanjak, D., Vincent, C., ... & *Staphylococcus aureus* Resistant à la Meticilline en Hospitalisation A Domicile (SARM HAD) Study Group. (2009). Carriage of methicillin-resistant *Staphylococcus aureus* in home care settings: prevalence, duration, and transmission to household members. *Archives of internal medicine*, 169(15), 1372-1378.
- Mahendran, S., Sankaralingam, S., Sethupathi, S. M., Kathiresan, D., Muthamani, M., Kousalya, L., ... & Harinathan, B. (2024). Evaluation of antioxidant and cytotoxicity activities of polyphenol extracted from brown seaweed *Sargassum tenerrimum* biomass. *Biomass Conversion and Biorefinery*, 14(2), 2063-2069. <https://doi.org/10.1007/s13399-022-02301-x>
- Morán-Santibañez, K., Peña-Hernández, M. A., Cruz-Suárez, L. E., Ricque-Marie, D., Skouta, R., Vasquez, A. H., ... & Trejo-Avila, L. M. (2018). Virucidal and synergistic activity of polyphenol-rich extracts of seaweeds against measles virus. *Viruses*, 10(9), 465. <https://doi.org/10.3390/v10090465>
- Nafis, A., El Khalloufi, F., Aknaf, A., Oudra, B., Marraiki, N., Al-Rashed, S., ... & Custódio, L. (2021). In vitro antimicrobial and synergistic effect of essential oil from the macroalgae *Centroceras clavulatum* (C. Agardh) Montagne with conventional antibiotics. *Asian Pacific Journal of Tropical Biomedicine*, 11(9), 414-420. <https://doi.org/10.4103/2221-1691.321129>
- Naas, H. T., Edarhoby, R. A., Garbaj, A. M., Azwai, S. M., Abolghait, S. K., Gammoudi, F. T., ... & Eldaghayes, I. M. (2019). Occurrence, characterization, and antibiogram of *Staphylococcus aureus* in meat, meat products, and some seafood from Libyan retail markets. *Veterinary World*, 12(6), 925. <https://doi.org/10.14202/vetworld.2019.925-931>
- Nagayama, K., Iwamura, Y., Shibata, T., Hirayama, I., & Nakamura, T. (2002). Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *Journal of Antimicrobial Chemotherapy*, 50(6), 889-893. <https://doi.org/10.1093/jac/dkf222>
- Nikaido, H. (1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science*, 264(5157), 382-388. <https://doi.org/10.1126/science.8153625>
- Affiah, S. N., Darah, I., Fariza, S. S., Nordin, M. M. J., & Aili, Z. N. (2010). Antimicrobial activity of various extracts of a tropical chlorophyta macroalgae, *Halimeda discoidea*. <https://doi.org/10.3923/jas.2010.3007.3013>
- Pavillard, R., Harvey, K., Douglas, D., Hewstone, A., Andrew, J., Collopy, B., ... & Tosolini, F. (1982). Epidemic of hospital-acquired infection due to methicillin-resistant *Staphylococcus aureus* in major Victorian hospitals. *Medical Journal of Australia*, 1(11), 451-454. <https://doi.org/10.5694/j.1326-5377.1982.tb132413.x>
- Pérez, M. J., Falqué, E., & Domínguez, H. (2016). Antimicrobial action of compounds from marine seaweed. *Marine drugs*, 14(3), 52. <https://doi.org/10.3390/md14030052>
- Periasamy, S., Joo, H. S., Duong, A. C., Bach, T. H. L., Tan, V. Y., Chatterjee, S. S., ... & Otto, M. (2012). How *Staphylococcus aureus* biofilms develop their characteristic structure. *Proceedings of the National Academy of Sciences*, 109(4), 1281-1286. <https://doi.org/10.1073/pnas.1115006109>
- Saravanakumar, A., Venkateshwaran, K., Vanitha, J., Ganesh, M., Vasudevan, M., & Sivakumar, T. (2009). Evaluation of antibacterial activity, phenol and flavonoid contents of *Thespesia populnea* flower extracts. *Pakistan journal of pharmaceutical sciences*, 22(3).
- Sibanda, T., & Okoh, A. I. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *African Journal of Biotechnology*, 6(25).
- Singkoh, M. F., Katili, D. Y., & Rumondor, M. J. (2021). Phytochemical screening and antibacterial activity of brown algae (*Padina australis*) from Atep Oki Coast, East Lembean of Minahasa Regency. *Aquaculture, Aquarium, Conservation & Legislation*, 14(1), 455-461. <https://doi.org/10.1063/5.0118858>
- Souza, B. W., Cerqueira, M. A., Martins, J. T., Quintas, M. A., Ferreira, A. C., Teixeira, J. A., & Vicente, A. A. (2011). Antioxidant potential of two red seaweeds from the Brazilian coasts. *Journal of agricultural and food chemistry*, 59(10), 5589-5594. <https://doi.org/10.1021/jf200999n>
- Tang, Y. L., Shi, Y. H., Zhao, W., Hao, G., & Le, G. W. (2008). Insertion mode of a novel anionic antimicrobial peptide MDp5 (Val-Glu-Ser-Trp-Val) from Chinese traditional edible larvae of housefly and its effect on surface potential of bacterial membrane. *Journal of pharmaceutical and biomedical analysis*, 48(4), 1187-1194. <https://doi.org/10.1016/j.jpba.2008.09.006>
- Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American journal of medicine*, 119(6), S3-S10. <https://doi.org/10.1016/j.amjmed.2006.03.011>
- Ummat V, Tiwari BK, Jaiswal AK, Condon K, García-Vaquero M, O'Doherty J., O'Donnell C, Rajauria G (2020) Optimisation of Ultrasound Frequency, Extraction Time and Solvent for the Recovery of Polyphenols, Phlorotannins and Associated Antioxidant Activity from Brown Seaweeds. *Mar Drugs* 18: 250. <https://doi.org/10.3390/md18050250>
- Vallinayagam, K., Arumugam, R., Kannan, R. R. R., Thirumaran, G., & Anantharaman, P. (2009). Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. *Global Journal of Pharmacology*, 3(1), 50-52.