

RESEARCH ARTICLE

Antibacterial Potential of Aqueous Extracts and Compounds from Selected Brown Seaweeds

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Abstract: Background: The incident of antibacterial resistance is increasing rapidly. Seaweeds, the marine macroalgae that are rich in bioactive compounds have the potential to be applied as an antimicrobial agent.

Objective: The objective was to assess the potential of brown seaweed aqueous extracts (*Sargassum polycystum* and *Padina australis*) and commercial pure compounds (alginic acid and fucoidan) as antimicrobial agent toward Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* as well as Gram-positive bacteria, *Staphylococcus aureus*.

Methods: Powdered brown seaweeds were macerated with distilled water and followed by filtration. The aqueous extracts were recovered by ethanol precipitation, oven-dried, and stored at 4°C. Aqueous extracts were examined for their qualitative phytochemical content and antibacterial susceptibility test using Kirby–Bauer method.

Results: *S. polycystum* was reported for higher extraction yield (7.00%) than *P. australis* (1.40%). The presence of steroid, phytosterols, and phenols was observed in *S. polycystum* extract while steroid and phytosterols were observed in *P. australis* extract. In antibacterial susceptibility test, there was no inhibition shown by both seaweed extracts against the four bacteria tested. Fucoidan (0.50 mol/L) and alginic acid (0.50 mol/L) were observed to have antibacterial property against *S. aureus* (with inhibition zone of 7.43±0.17 mm and 7.45±0.14 mm, respectively).

Conclusion: The aqueous extracts of *S. polycystum* and *P. australis* have shown low antibacterial activity, as compared to fucoidan and alginic acid. Further studies on the mode of antibacterial activity possessed by fucoidan and alginic acid will be carried out.

Keywords: Bactericidal action; Fucoidan; Alginic acid; *Staphylococcus aureus*; Macroalgae.

1. INTRODUCTION

Marine seaweed has a variety of compounds with pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, and others. Seaweeds are highly active against bacteria, especially Gram-negative bacteria. A variety of seaweed extracts and their bioactive compounds have been explored for their antimicrobial and cytotoxic properties [1].

Padina australis is a brown seaweed (9.6-11.0 cm high) with thallus in leaf-like clusters and fan-shaped blades having

chalky white and light brown alternating bands (**Fig. 1**). In Malaysia, *P. australis* was collected from Port Dickson in Negeri Sembilan, Pulau Besar in Melaka, and Pulau Sibul and Pulau Sibul Tengah in Johor [2]. *P. australis* collected from Malaysia was reported to have an antibacterial effect against Gram-positive bacteria *Bacillus cereus* (Chiao-Wei et al., 2013) and *in vitro* cytotoxic effects to reduce the viability of human lung cancer cell lines with an IC₅₀ value of 2.45 mM [3].

Sargassum polycystum is a bushy seaweed (20.0-100.0 cm) with thallus consists of basal holdfast and spine main stem

with lanceolate blades (Fig. 2). In Malaysia, *S. polycystum* can be found in Port Dickson and Pulau Sibul [2]. The chloroform extract of *S. polycystum* inhibits the activity against *Staphylococcus aureus*, *B. cereus*, *Micrococcus luteus*, *Escherichia coli*, and *Aeromonas hydrophila* effectively with zone of inhibition ranging from 10.00 mm to 11.50 mm [4].

Alginic acids in brown algae are alkali-soluble polysaccharides, which are the main component of cell walls and make up to 50% of the dry weight of seaweed. Fucoidan is a branched polysaccharide sulfate ester with L-fucose 4-sulfate building blocks as the major component [5]. Fucoidan was reported to have antibacterial [6] and antioxidant effects [7].

E. coli, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are Gram-negative rod-shaped bacteria. Some strains of *E. coli* will cause urinary tract infection in the elderly and diarrhea in infants due to the production of enterotoxin. *Klebsiella* has also been incriminated for nosocomial infections. Common sites include the urinary tract, lower respiratory tract, biliary tract, and surgical wound sites [8]. *P. aeruginosa* is a known opportunistic pathogen in infected people with impaired host defense [9]. *S. aureus* is Gram-positive cocci shaped bacteria. *S. aureus* can cause skin infections, heart valve infections, bone infections, and pneumonia.

According to the World Health Organization, antimicrobial resistance threatens the effectiveness of preventative measures and treatment of the infections caused by bacteria, parasite, viruses, and fungi [10]. With the broad usage of beta-lactam drugs and immunosuppressant, it is easier for these pathogenic bacteria to become multidrug resistant. The Centers for Disease Control and Prevention has reported that 40% of pneumococcal

infections are the results of pneumococcal bacteria that were resistant to at least one antibiotic [11].

The objective of this study was to assess the potential of brown seaweed aqueous extracts (*S. polycystum* and *P. australis*) and pure compounds (fucoidan and alginic acid) as antimicrobial agent toward bacteria, namely, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*.

2. MATERIALS AND METHODS

2.1. Seaweed Collection

The brown seaweeds (*S. polycystum* and *P. australis*) were collected from Tanjung Tuan, Port Dickson, Malaysia (2.4089° N, 101.8494° E). The seaweeds were brought back to the laboratory in cooler box.

2.2. Preparation of Seaweed Aqueous Extract and Compounds

The brown seaweeds were cleaned, rinsed with distilled water, dried for 3 days, blended to powder form, and stored at -20°C . Powdered seaweed (5 g) was added to 200 ml of water under stirring (5 h). The solution was filtered. The polysaccharide was recovered by ethanol precipitation, filtered, and then washed down with ethanol and acetone to remove the impurities. Finally, it was dried under hot airflow for 24 h [12]. The mass obtained was used to calculate the yield. The extract was then stored at -20°C for further analysis.

The seaweed compounds, namely, alginic acid and fucoidan were purchased from Sigma-Aldrich (US) with 99% purity.

2.3. Qualitative Phytochemical Screening

Phytochemical test was carried out to identify the presence of flavonoids, alkaloids, saponins, steroids, terpenes, tannins, phytosterols, phenols, and cardiac glycoside qualitatively. Yellow color precipitate was formed after adding few drops of sodium hydroxide and acetic acid into the extract, indicates the presence of flavonoid. Red coloration was visible after adding 2 mL of Dragendorff solution to the extract, indicating the presence of alkaloids. About 2 mL of the seaweed extracts were to be shaken with 2 mL of water. Persistent foam production for 10 min indicates the presence of saponins. Formation of emerald green coloration in the extract that was dissolved in 3 mL of chloroform followed by 2 drops of sulfuric acid indicates the presence of terpenes. Formation of white precipitate in extract (containing 1% of gelation solution in sodium chloride) indicates the presence of tannins. The extracts were dissolved with chloroform and few drops of concentration sulfuric acid, shaken, and allowed to stand. The appearance of golden-yellow coloration indicates the presence of phytosterol. Formation of bluish-black coloration after addition of 3-4 drops of ferric chloride solution indicates the presence of phenols [13]. The appearance of reddish-brown layer after adding 2 mL of chloroform and 2 mL concentrated sulfuric acid into the seaweed extracts indicates the presence of steroid. A brown ring formation between the layers of extract shows the presence of cardiac glycoside after testing with a

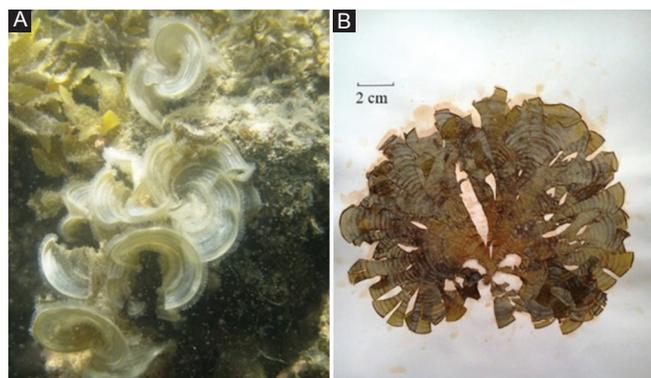


Fig. (1). *Padina australis*: (A) Natural habit and (B) herbarium specimen.

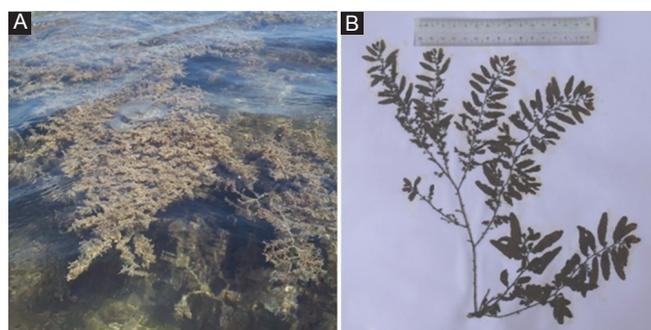


Fig. (2). *Sargassum polycystum*: (A) Natural habit and (B) herbarium specimen.

mixture of 2 mL of glacial acetic acid with 1 drop of ferric chloride and 1 mL of sulfuric acid [14].

2.4. Bacterial Culture

The bacterial culture used in this study was *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. The laboratory strain was purchased from UKM Kesehatan Sdn Bhd and cultured in Microbiology Laboratory, Management and Science University. The purity of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* was confirmed by subculturing in suitable selective agar such as blood agar and MacConkey agar.

2.5. Antibacterial Susceptibility Test

Test solutions, including seaweed aqueous extracts diluted in distilled water in concentrations of 25, 50, and 100 mg/ml, and alginic acids and fucoidan in concentrations of 0.125, 0.25, and 0.50 mol/L, were transferred to sterile Whatman filter paper disc with 10 μ L on each side of the disc. Microbial suspension was prepared by suspending a single colony of cultured bacteria into the sterile saline water. After air drying, the disc was placed on the Mueller-Hilton agar plates that were inoculated with test bacteria. The plate was inoculated with test bacteria prepared in the same medium after the microbial suspension was adjusted to 0.5 based on the McFarland scale. The disc that was soaked with similar volume of distilled water was used as the control. Antibiotic discs (Oxoid, UK), namely, vancomycin was used against *S. aureus*, while gentamicin was used against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. After 24 h of incubation at 37°C, the inhibition zone was measured using Vernier calipers [15]. The test was done in triplicates and repeated for 3 times.

3. RESULTS

3.1. Extraction Yield

S. polycystum (30 g) had higher extraction weight (2.10 g) and yield (7.00%) as compared to *P. australis* (25 g) which has lower extraction weight (0.35 g) and yield (1.40%).

3.2. Phytochemical Screening

Aqueous extract of *S. polycystum* had higher number of phytochemical components compared to *P. australis*. Both seaweeds had steroids and phytosterol. However, phenols are only present in *S. polycystum*. Flavonoids, alkaloids, saponins, terpenes, tannins, and cardiac glycosides were absent in both seaweeds, as shown in **Table 1**.

3.3. Antibacterial Susceptibility Test of Aqueous Extracts of Seaweeds

There was no inhibition zone observed by *S. polycystum* aqueous extract toward *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* at any concentrations of 25, 50, and 100 mg/mL/disc. The positive control showed inhibition zone of 24.90 \pm 0.27 mm toward *E. coli*, 18.98 \pm 0.08 mm toward *K. pneumoniae*, 14.92 \pm 0.65 mm toward *P. aeruginosa*, and 14.56 \pm 0.34 mm toward *S. aureus*.

Table 1. Qualitative phytochemical screening of aqueous extracts of *P. australis* and *S. polycystum*

Phytochemicals	<i>S. polycystum</i>	<i>P. australis</i>
Flavonoids	–	–
Alkaloids	–	–
Saponins	–	–
Steroids	+Reddish-brown layer	+Reddish-brown layer
Terpenes	–	–
Tannins	–	–
Phytosterols	+Golden-yellow color	+Golden-yellow color
Phenols	+Bluish-black color	–
Cardiac glycosides	–	–

+: Presence of phytochemical, –: Absence of phytochemical.

S. polycystum: *Sargassum polycystum*, *P. australis*: *Padina australis*

There was also no inhibition zone observed by *P. australis* aqueous extract toward *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* at any concentrations of 25, 50, and 100 mg/mL/disc. The positive control showed inhibition zone of 21.40 \pm 0.57 mm toward *E. coli*, 36.85 \pm 1.06 mm toward *K. pneumoniae*, 27.32 \pm 1.20 mm toward *P. aeruginosa*, and 17.02 \pm 0.09 mm toward *S. aureus*. The negative control (distilled water) showed no inhibition toward all bacteria tested. The results showed that aqueous extract of *S. polycystum* and *P. australis* was not effective to inhibit *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* (**Table 2**).

3.4. Antibacterial Susceptibility Test of Seaweed Compounds

There was an inhibition zone of 7.43 \pm 0.17 mm shown by fucoidan against *S. aureus* at 0.500 mol/L/disc. However, there was no inhibition zone toward *E. coli*, *K. pneumoniae*, and *P. aeruginosa* at any concentration of 0.125, 0.250, and 0.500 mol/L/disc. The positive control showed inhibition zone of 22.33 \pm 0.40 mm toward *E. coli*, 17.76 \pm 1.66 mm of toward *K. pneumoniae*, 22.62 \pm 0.98 mm toward *P. aeruginosa*, and 17.13 \pm 0.71 mm toward *S. aureus*.

For alginic acid, there was an inhibition zone of 7.45 \pm 0.14 mm against *S. aureus* at 0.500 mol/L/disc. However, there was no inhibition zone toward *E. coli*, *K. pneumoniae*, and *P. aeruginosa* at any concentrations of 0.125, 0.250, and 0.500 mol/L/disc. The positive control showed inhibition zone of 24.22 \pm 1.23 mm toward *E. coli*, 18.51 \pm 0.34 mm toward *K. pneumoniae*, 19.91 \pm 2.68 mm toward *P. aeruginosa*, and 14.45 \pm 0.25 mm toward *S. aureus* (**Fig. 3**).

4. DISCUSSION

In the present study, brown seaweeds *S. polycystum* and *P. australis* were collected from Port Dickson to screen for the

Table 2. Antibacterial activity of *S. polycystum* and *P. australis* against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*

Bacteria	Seaweed	Zone of inhibition (mea±SD) (mm) concentration (mg/mL/disc)				
		25	50	100	Negative control*	Positive control**
<i>E. coli</i>	<i>S. polycystum</i>	0	0	0	0	24.90±0.27
	<i>P. australis</i>	0	0	0	0	21.40±0.57
<i>K. pneumoniae</i>	<i>S. polycystum</i>	0	0	0	0	18.98±0.08
	<i>P. australis</i>	0	0	0	0	36.85±1.06
<i>P. aeruginosa</i>	<i>S. polycystum</i>	0	0	0	0	14.92±0.65
	<i>P. australis</i>	0	0	0	0	27.32±1.20
<i>S. aureus</i>	<i>S. polycystum</i>	0	0	0	0	14.56±0.34
	<i>P. australis</i>	0	0	0	0	17.02±0.09

*Negative control: Distilled water, **Positive control: Vancomycin against *S. aureus*; gentamicin against *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. *S. polycystum*: *Sargassum polycystum*, *P. australis*: *Padina australis*, *E. coli*: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *S. aureus*: *Staphylococcus aureus*

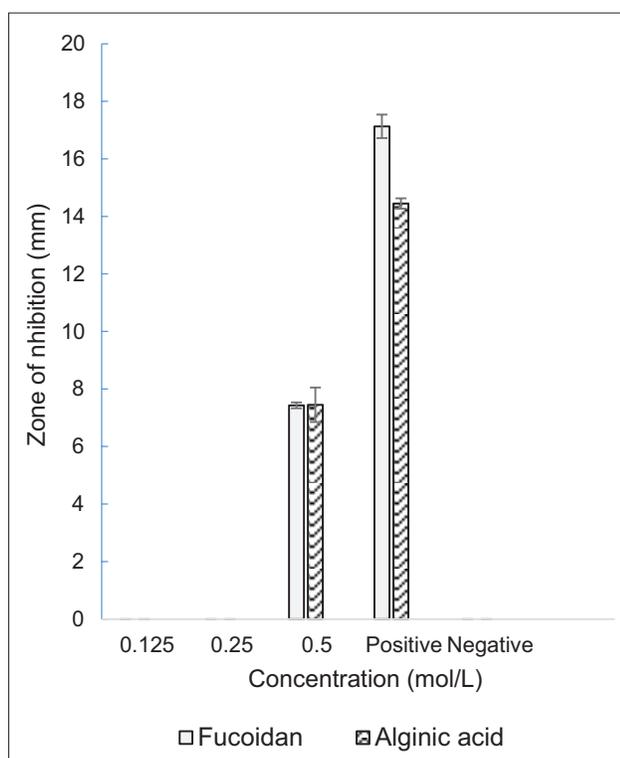


Fig. (3). Antibacterial susceptibility activity of fucoidan and alginic acid toward *Staphylococcus aureus*. Positive control against *S. aureus* was vancomycin.

antimicrobial activity against pathogenic respiratory bacteria. The qualitative phytochemical screening of both seaweeds has been tested and the phytochemical components present were different in each seaweed. The antibacterial activity of the seaweed aqueous extracts and pure compounds (fucoidan and alginic acid) was assessed by the antibacterial susceptibility test (Kirby–Bauer method).

Flavonoids, alkaloids, saponins, steroids, tannins, terpenes, phytosterols, cardiac glycosides, and phenols are the most common phytochemical contents that are present in seaweeds. Elsie and DhanaRajan stated that the phytochemical content of seaweeds has antimicrobial activity, and thus, the seaweeds

could have a significant role in the treatments of microbial infections [16]. Sulfated polysaccharides could be a good source of antibacterial agent [17].

The extraction yield of both seaweeds showed that *S. polycystum* has higher extraction yield (7.00%) as compared to *P. australis* (1.40%). The results indicate that aqueous extract of *S. polycystum* has more compounds extracted compared to aqueous extract of *P. australis*.

In the present study, aqueous extract of *S. polycystum* was observed to have steroid, phytosterols, and phenols. This is in agreement with the study of Kanimozhi et al., which showed the presence of sterol in methanolic extract of *S. polycystum* [18]. However, flavonoids, alkaloids, saponins, terpenes, tannins, and cardiac glycosides are absent in aqueous extract of *S. polycystum*. This is in contrast with the study of Nurhidayah which showed the presence of saponins, steroids, and cardiac glycosides in the ethanol extract of *S. polycystum* [19].

Meanwhile, aqueous extract of *P. australis* was observed to have steroid and phytosterols. This is in agreement with the study of Nurhidayah which showed the presence of steroid in ethanol extract of *P. australis* [19] and the study of Melpha et al. that reported about the presence of steroid in the chloroform extract of *Padina* sp. [20] However, flavonoids, alkaloids, saponins, terpenes, tannins, phenol, and cardiac glycosides are absent in aqueous extract of *P. australis*. This is in contrast with the study of Maharany et al. which showed the presence of flavonoids, tannins, and saponins in methanolic extract of *P. australis* [21].

Based on the results of antibacterial susceptibility test, the aqueous extracts of *S. polycystum* and *P. australis* had no inhibition against the four tested bacteria. However, in contrast to Nurhidayah's study, the study showed that the ethanol extract of *S. polycystum* and *P. australis* has inhibition activity toward *Streptococcus pneumoniae* [19]. This is also in contrast to the study carried out by Chong et al. that showed that the methanol extract of *S. polycystum* has high minimum inhibitory concentration (MIC) activity (0.833±0.29 mg/mL) against *E. coli* while the methanol extract of *P. australis* has low MIC activity (2.083±0.59 mg/m) against *E. coli* [22].

Based on the results of antibacterial susceptibility test on fucoidan and alginic acid in the present study, fucoidan

(0.50 mol/L) and alginic acid (0.50 mol/L) were observed to have antibacterial property against Gram-positive bacteria *S. aureus* (inhibition zone of 7.43±0.17 mm and 7.45±0.14 mm, respectively). This is in agreement with the study of Marudhupandi and Kumar that showed the inhibition of fucoidan against *Vibrio cholerae* (18.6±0.32 mm) and *Salmonella typhi* (8.6±0.26 mm) [23].

5. CONCLUSION

The results have proven the antimicrobial potential of fucoidan and alginic acid against *S. aureus* using antibacterial susceptibility test. The aqueous extracts of *S. polycystum* and *P. australis* have shown low antibacterial activity, as compared to fucoidan and alginic acid. In the future, higher concentration of brown seaweed aqueous extracts will be included for antibacterial susceptibility test. Both seaweed extracts will be tested on other bacteria strains. The assay of time field effect (to determine the duration needed for bactericidal action) and identification of active antibacterial compounds will be carried out.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in any kind.

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AUTHOR CONTRIBUTIONS

K.X.Y. conceived the main ideas and designed the experiments. C.H.N. and S.M.S. verified the analytical methods. S.R.G.R. performed the experiments. S.R.G.R., C.H.N., and K.X.Y. analyzed the data. S.R.G.R. and K.X.Y. wrote the paper. S.M.S. reviewed drafts of the paper. All authors read and approved the final manuscript.

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