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In Vitro Antimicrobial Activity of *Arthrospira platensis*

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Abstract

Arthrospira platensis is a cyanobacteria with the ability to produce a large amount of antimicrobial substances, making it a suitable microorganism for use as a biocontrol agent for pathogenic bacteria. In this research, different amounts of *A. platensis* extracts grown outside (100 µg/µL, 50 µg/µL, and 25 µg/µL) were tested for their ability to kill five types of bacteria: *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella enterica*, and *Staphylococcus aureus*. The tests were done using methanol, aqueous, and chloroform as solvents. The disc diffusion and serial micro-dilution techniques were used to determine the inhibition zone and minimum inhibitory concentration (MIC) of the pathogen bacteria, respectively. The results showed that *A. platensis* extracts significantly inhibited the growth of *E. coli*, with inhibition zones of 24.00 ±1.51 mm, 18.00 ± 0.15 mm and 15.00±0.12 mm at concentrations of 100 µg/µL, 50 µg/µL, and 25 µg/µL, respectively. At the highest concentration, the effect is comparable to that of tetracycline (30 µg). Methanol and aqueous extracts with MIC values of 100-200 µg/µL demonstrate antibacterial properties. However, *A. platensis* extracts did not significantly inhibit the growth of *P. vulgaris*, *S. enterica*, or *S. aureus*. In conclusion, the findings of this study demonstrated that outdoor-cultured *A. platensis* extracts could be a good source for the production of promising antimicrobial agents.

Keyword: Cyanobacteria; *Arthrospira platensis*; antimicrobial; solvent extracts

INTRODUCTION

Infectious diseases caused by pathogenic microorganisms continue to be a significant global health concern, with the emergence of antimicrobial resistance adding complexity to the challenge of treatment. Resistance to bacteria that cause transmitted diseases has made treatment more difficult, necessitating the development of new approaches to combat these infections. Furthermore, the discovery of new and cost-effective sources of antimicrobial agents is critical. *Arthrospira pplatensis,* also known as Spirulina, is a filamentous cyanobacterium that has gained popularity not only as a nutritional supplement but also for its potential therapeutic properties (Gentscheva et al., 2023; Mohd Syahril et al., 2011; Mohd Syahril et al., 2012; Mohd Syahril et al., 2012). It is characterized by its larger cell size, which facilitates simplified cultivation and harvest, along with a readily digestible cell wall. Additionally, it is renowned for its richness in protein, essential amino acids, vitamins, and minerals (Levine & Fleurence, 2018; Roshani Othman & Lokman Shamsudin 2009). Many biologically active hydrophilic and lipophilic substances found in *A. platensis* have therapeutic effects on tissues, blood cells, and organs. Its wide range of biologically active components determine its diverse pharmacological properties, which include antimicrobial effects (Gentscheva et al., 2023). The antimicrobial effects of *A. platensis* make it a promising candidate for low-cost antimicrobial agents. Further research and development in this area can explore the full potential of *A. platensis* in providing affordable solutions for combating microbial infections.

The biochemical composition of *A. platensis*, which includes the production of bioactive compounds and the culture medium, affects biomass production. The genes of microalgae or cyanobacteria affect the culture medium to change the production of bioactive compounds. This creates the best conditions for biotechnological uses and long-term growth (Furmaniak et al., 2017; Mohd Syahril et al., 2014; Roshani et al., 2014). While numerous studies have focused on its cultivation in standard laboratory media, the potential of this microorganism when grown in low-cost cultivation media has been relatively unexplored (Furmaniak et al., 2017). The development of cost-effective cultivation methods not only promises to reduce production costs, but it also increases the accessibility of their bioactive compounds to a wider range of industries and researchers. By utilizing low-cost cultivation media, the barriers to entry for studying and utilizing this microorganism are significantly lowered. Furthermore, the optimization of conditions for biotechnological applications and long-term cultivation can lead to enhanced yield and quality of bioactive compounds, making them more commercially viable. The exploration of low-cost cultivation media for this microalga holds great potential for both economic and scientific advancements and potential antimicrobial applications, particularly in resource-constrained settings (Papadopoulos et al., 2022).

There are bioactive compounds inside and outside of *A. platensis* cells that can kill microbes. These compounds have been successfully extracted (El-Sheekh et al., 2014). Furthermore, extracting bioactive compounds from *A. platensis* can be difficult because the compounds are frequently embedded in the complex cell wall structure. Therefore, meticulous extraction methods are required to obtain the maximum amount of bioactive compounds. In addition, *A. platensis* may have limited antimicrobial specificity, which means that it may also affect beneficial microorganisms in the body. The problem of microbial resistance to antibiotics is still a concern, and natural preservatives can be a valid alternative to address this issue. The choice of culture medium significantly influences the production of antimicrobial agents, as shown in various studies (Maulana et al., 2023; Žalnėravičius & Ramanavicius, 2022). The urgency arises from the alarming surge in infections caused by antibiotic-resistant

microorganisms, prompting the search for cyanobacteria like Spirulina and Arthrospira with antimicrobial potential. Therefore, the aim of this study is to find out what antimicrobial effects *A. platensis* has when it is grown in outdoor culture using a low-cost medium and carefully extracted with different solvents. If successful, this could lead to a new way to treat bacterial infections and fight antibiotic resistance.

METHODOLOGY

Materials

Arthrospira platensis stock culture was obtained from the Marine Biotechnology Laboratory, Faculty of Engineering and Life Sciences, Universiti Selangor (UNISEL), Bestari Jaya, Selangor. The *A. platensis* stock source has been employed for outdoor culture in fiber glass tanks.

Culture Conditions

Arthrospira platensis was initially cultivated in large vessels under controlled indoor conditions with sequential transfers. Subsequently, the cyanobacteria were transferred to an outdoor setting. The culture of *A. platensis* was maintained in a 300 L fiberglass tank filled with a nutrient-rich medium composed of bicarbonate, N:P:K fertilizer, and urea in a 9:3:1 ratio. The culture was continuously aerated and exposed to white fluorescent light with an intensity of 40 W. The *A. platensis* culture was harvested for experimentation when it reached the late-logarithmic growth phase.

Preparation of Cyanobacteria Extracts

Arthrospira platensis biomass was collected using a plankton net, followed by rinsing with distilled water. The cyanobacteria were subsequently dried at 30°C in an oven and ground into a powder. Fifty grams of the powdered samples were separately soaked in three different solvents: methanol (a polar solvent), chloroform (a non-polar solvent), and water (a bipolar solvent), each at a ratio of 1:20 for 72 hours. The resulting solutions were then subjected to evaporation using a rotary evaporator at a temperature range of 40–48 °C. Subsequently, the evaporated crude extracts were transferred into sterile centrifuge tubes and covered with aluminium foil. These crude samples were frozen at -80°C before undergoing the vacuum-drying process. The solidified samples were transferred to a drying machine, a freeze dryer that employed a vacuum to eliminate any residual water or solvent from the crude extract, ultimately converting the extracts into a powdered form. Then, one hundred mg of dried extract was weighed and dissolved in 1 mL of 5% DMSO to make a concentrated stock extract for further analysis, resulting in a concentration of 100 mg/mL. These stock extracts were then utilized in subsequent antibacterial assays.

Bacteria Preparation

Escherichia coli, *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella enterica*, and *Staphylococcus aureus* were obtained from the UNISEL Microbiology Laboratory and sub-cultured onto nutrient agar from the stock culture. Four or five colonies of the same morphological type were aseptically transferred from the nutrient agar plate to separate tubes containing tryptic soy broth (TBS), which had been previously prepared and sterilized by autoclaving at 121°C. This procedure was carried out in separate test tubes, one for each bacterium, to avoid airborne contamination. The broth cultures were then incubated for 24 hours at 37°C.

Disc Diffusion Method

The disc diffusion method was used to qualitatively evaluate the antibacterial activity of *A. platensis* extracts (methanol, chloroform and aqueous) based on the zone of inhibition diameter. Petri plates were first prepared by pouring 20 mL of Mueller-Hinton agar and allowing it to solidify before testing for bacterial susceptibility. After the plates had solidified, a 0.1-mL aliquot of standardized inoculum suspension was evenly spread across the agar surface. Any excess inoculum was carefully drained, and the plates were allowed to dry for five minutes. Following the drying period, circular discs containing the extracts were gently pressed onto the plate's surface with sterile forceps to ensure they made contact with the agar. Tetracycline (30 mg/disc) was used as a positive control, while 5% DMSO was used as a negative control. Following that, the plates were incubated at 37°C for 24 hours. The zones of inhibition were then observed and measured in millimetres. Each assay in these experiments was repeated three times to ensure consistency.

Microdilution assay

In order to measure quantitatively, the minimum inhibitory concentration (MIC) of *A. platensis* extract against the test microorganisms was determined by the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI). Assays were performed in 96-well microtiter plates, and two-fold dilutions of all extracts and standard antibiotics were made in Muller-Hinton Broth (MHB) ranging from 0.4 to 200 µg/mL. Each inoculum was prepared in the same medium with the 0.5 McFarland standard, containing 105 cfu/mL in each well and making the total well volume 200 µL. The plates were incubated at 37°C for 24 hours. Any wells that didn't turn hazy after this incubation were noted as having the greatest dilution of the test ingredient that stopped bacterial growth.

Statistical Analysis

The experimental data were analyzed by using one-way ANOVA in IBM SPSS (Statistical Package for the Social Sciences) version 28.0 and a value of $p < 0.05$ was considered statistically significant. The experiment is accomplished in triplicate, and the result will be expressed as the mean ± SD (standard deviation).

RESULTS AND DISCUSSION

Arthrospira platensis is a cyanobacteria, recognized as a valuable source of natural compounds with potential antibacterial properties. The content and yield of bioactive compounds in cyanobacteria are influenced by various factors such as extraction methods, diversity of bacterial strains, growth conditions and assay protocols (Mohd Syahril et al., 2011; Mohd Syahril et al., 2014; Norazah et al., 2020; Norazah et al., 2021; Roshani et al., 2014; Strieth et al., 2022). In this study, *A. platensis* filaments have shown good antimicrobial activity in aqueous, methanol and chloroform extracts against various microorganisms, revealing varying levels of effectiveness (Table 1). All extracts exhibited activity against *E. coli*. The methanol extract showed broad spectrum activity as the highest effective inhibition zone was recorded against *E. coli* (24.00±1.51 mm) followed by *S. aureus* (20.00±0.57 mm), *B. subtilis* (12.4±1.27 mm) and *Salmonella enterica* (6.8±0.41 mm) for a concentration of 100 µg/µL and no inhibitory effect against *Proteus vulgaris*. Other studies showed that the methanol extract of *Spirulina platensis* against *E. coli* was 12.42±.47 mm and against *S. aureus* was 15.21±.1mm (Kaushik & Chauhan, 2008). Similar results were found for the pure antimicrobial from fraction 11 (which had the highest value compared to the other fractions) that was separated on a silica gel column against *S. aureus*, which was 20±0.03 mm (Elshouny et al., 2017). This shows that the methanol extract of *A. platensis* can inhibit the growth of the majority of gram positive and gram negative bacteria

due to the presence of flavonoids (Biharee et al., 2020). In addition, flavonoids derived from cyanobacteria show potent antibacterial activity with a different mechanism than conventional drugs (Gheda & Ismail, 2020). Due to the fact that the majority of cyanobacterial natural compounds do not initially encode resistance genes, bacterial pathogens cannot easily become resistant, making them crucial for improving antibacterial therapy.

The results of the minimum inhibitory concentration of *A. platensis* extract are shown in Table 2. The results show that the MIC of the methanol extract is 50 µg/mL and 100 µg/mL against *B. subtilis* and *S. enterica* respectively. However, the MIC of this extract was recorded as 200 and >200 µg/mL against *E. coli* and *S. aureus*, respectively. Similar findings were obtained from a previous study performed on Spirulina methanolic extract tested against *S. aureus* and *E. coli* (Kaushik & Chauhan, 2008). Other reports on the antimicrobial activity of methanol extracts of *S. platensis* include effects against *A. flavus* (Souza et al., 2011); against *B. subtilis*, *E. coli*, *P. vulgaris* and *C. albicans* (Medina-Jaritz et al., 2011) and with a higher concentration of 50–100 mg/mL towards *S. aureus*, *Streptococcus pneumoniae*, *Pseudomonas aureginosa*, *P. vulgaris*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *E. coli*, *Bacillus cereus* and *Salmonella typhi* (Al-Ghanayem, 2017). Scientists found that the polar extract of methanol was more effective at killing both gram-positive and gram-negative bacteria than the non-polar extract. This suggests that the polar extract has more bioactive compounds that are strong against these bacteria, such as peptides, polysaccharides, and hydrophilic compounds (Esquivel-Hernández et al., 2017). Furthermore, a study by (Abdel-Moneim et al., (2022) also stated that the antimicrobial and antioxidant activity of *Spirulina* methanol extract was observed to be the most effective.

In this study, the MIC of the aqueous extract was found to be 100 µg/mL and >200 µg/mL against *P. vulgaris* and *E. coli*, respectively. This result indicates the varying sensitivity of these bacteria to the Spirulina extract. Other studies reported that the MIC values of aqueous extracts of *Spirulina* against different strains of *Aeromonas hydrophila*, *Pseudomonas sp*., *Vibrio*, *Escherichia coli* and *Edwardsiella tarda* were 500 µg/mL (Pradhan et al., 2012). In contrast, the antibacterial activity of *S. platensis* is mostly on gram-positive bacteria, with a moderate effect on gram-negative bacteria. There are several things that can change the lack of MIC values found for *B. subtilis* and *S. aureus* using aqueous extracts of *A. platensis* strains. These include compound specificity, complexity in antibacterial action, concentration thresholds, and strain-specific susceptibility. On the other hand, the aqueous extract of Spirulina strains had high antifungal activity against the three tested phytopathogenic fungi (Bencheikh et al., 2022). Meanwhile, the MIC of the chloroform extract was recorded only against *B. subtilis*, 100 µg/mL and no activity was detected for other bacteria in this study. The chloroform extract of *S. platensis* did not kill clinical isolates of *S. typhi* and *S. paratyphi*, which is similar to what (Ahsan et al., 2015) found. According to (Mohy El Din, 2020), *S. platensis* has antimicrobial efficiency, antioxidant capacity, anticancer efficiency, and cholesterol-lowering effect depending on the type of solvent used for cyanobacteria extraction. Acetone is the most effective solvent compared to chloroform for bioactive compound extraction from *S. platensis*. Future studies should be conducted with increasing concentrations of extracts to obtain an increase in the inhibition assay. In order to reduce costs, an aqueous solution can be used for the extraction of chemicals that provide antimicrobial activity from *A. platensis* against *E. coli*. Active ingredients can be isolated and tested to design a new generation of cyanobacterial antibiotics. However, the findings from this study are very promising, given the fact that the methanol extract successfully inhibited the growth of almost all tested bacteria. This suggests that *A. platensis* has great potential as a source for developing effective antibiotics, and further research is warranted to explore its therapeutic applications.

Table 1: Antimicrobial activity of *Arthrospira platensis* extracts at different concentrations

Bacteria	Zone of inhibition diameter in mm										
	A. platensis crude extracts									Positive control (Antibiotic)	Negative control
	Aqueous (µg/µL)			Methanol (µg/µL)			Chloroform (µg/µL)				
Gram positive	100	50	25	100	50	25	100	50	25	Tetracycline	DMSO
Bacillus subtilis	-	-	-	12.40^{b} ± 1.27	8^{b} ± 0.71	4.20^{b} ± 0.71	18.00^{a} ± 1.41	6.00^{a} ± 0.87	3.00^{a} ± 0.60	16.00 ± 2.84	-
Staphylococcus aureus	-	-	-	20.00^{a} ± 0.57	8.00^{a} ± 0.62	4.00^{a} ± 0	-	-	-	5.00 ± 0	-
Gram negative											
Escherichia coli	20.00^{a} ± 1.80	12.50^{a} ± 1.11	6.10^{a} ± 1.19	24.00^{c} ± 1.51	18.00^{c} ± 0.15	15.00^{c} ± 0.12	20.00^{b} ± 1.28	14.20^{b} ± 1.28	8.80^{b} ± 0.14	26.00 ± 0	-
Proteus vulgaris	11.00^{a} ± 1.41	6.10^{a} ± 1.19	2.60^{a} ± 0.35	-	-	-	-	-	-	22.00 ± 3.51	-
Salmonella enterica	-	-	-	6.80^{a} ± 0.41	4.00^{a} ± 0.20	2.02^{a} ± 0.11	-	-	-	14.00 ± 0	-

Note: Value in the same column having the same alphabet are not significantly different at $p<0.05$.
DMSO = Dimethyl Sulfoxide, - = No inhibition zone

Table 2: Minimum inhibitory concentration (MIC) of crude extracts (µg/mL)

	Minimum inhibitory concentration (µg/mL)			
	Organic Extracts			Standard
Microorganisms	Aqueous	Methanol	Chloroform	Vancomycin
E. coli	>200	200±0	ND	<1
B. subtilis	ND	50±0	100±0	<1
P. vulgaris	100±0	ND	ND	1
S. enterica	ND	100±0	ND	1
S. aureus	ND	>200	ND	1

Results are the means of values ± standard deviation, ND-not detected

CONCLUSION

Arthrospira platensis methanolic polar extract might contain a broader spectrum of bioactive compounds with antimicrobial properties, effective against gram-negative bacteria (*E. coli*) and gram-positive (*S. aureus*). While the aqueous extract only inhibited the growth of *E. coli*. The MIC value of *E. coli* is 200 µg/mL using methanol extract and more than 200 µg/mL using aqueous extract, while the methanolic MIC value of *S. aureus* is > 200 µg/mL. Further studies are needed to determine the MIC with mass spectrometry and nuclear magnetic resonance for purification. In vivo experiments and evaluation of cytotoxicity effects are also needed to be involved in future research.

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