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Assessment of Antibacterial Activity of a Cyanobacterium *Phormidium fragile* (Meneghini) Gomont

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Received Date: October 05, 2024 | Accepted Date: November 01, 2024 | Published Date: November 11, 2024

Citation: Janardhan N. Nehul, (2024), Assessment of Antibacterial Activity of a Cyanobacterium *Phormidium fragile* (Meneghini) Gomont, *Clinical Trials and Case Studies*, 3(6); **DOI:**10.31579/2835-835X/090

Abstract:

A *cyanobacterium* known as *Phormidium fragile* was found in soil samples taken from various places in the Ahmednagar region of Maharashtra state (India). For identification, morphological variation and taxonomy techniques in line with Desikachary (1959) and Prescott (1962) were employed. We used Bolch and Blackburn's (1996) recommended methodology to generate the axenic culture of *Phormidium* fragile. According to [17] investigation, the isolated *Phormidium fragile* was grown autotropically in BG-11 media, with the medium kept at 30±2°C. An air blower and a double-layered muslin cloth filter were used to extract the biomass after 25 days. The antibacterial efficacy against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Micrococcus flavus, Staphylococcus aureus* and *Proteus mirabilis* was evaluated using the biomass of this *Phormidium fragile* species.The disc diffusion method was used to evaluate the antibacterial activity (Anonymous, 1996). *Phormidium fragile* methanol extract proved efficient against every tested bacterial strain. *Phormidium fragile* methanol extract had the largest zone of inhibition (20±1.5mm) when it came to *Bacillus subtilis*. The MICs of each microbe were 64 or 256 µg/ml.

Keywords: *Phormidium fragile; Pseudomonas aeruginosa; Bacillus subtilis; Staphylococcus aureus; Escherichia coli;* Antibacterial Activity*; BG-11.*

Introduction

One of the reasons behind the increased death rate in emerging nations and the global community is infectious illnesses. Heart-related disorders are in first place. When scientists started focusing their attention on developing medications that might get rid of the germs causing the illness in the late 1800s, the hunt for antibiotics officially started. Creating a "magic bullet" or "wonder drug" that would kill microorganisms without endangering the user was the aim of this kind of study. These days, bacteria that can be treated with readily available drugs cause the majority of infections. The target organism's resistance mechanism makes it necessary to continue researching and developing new, powerful antibiotics to combat microbial illnesses. Because of this, attempts have been conducted all around the world to find new compounds, which has led to the development of a structure that may be used directly or through modification to make new medications.

Recently, there has been interest in the cyanobacteria's ability to produce chemicals that are both pharmacologically active and important to industry [20]. As such, pertinent materials can be manufactured in controlled circumstances with expertise [8]. It has been shown that the first partially recognized antibacterial component in algae is found in the unicellular green alga Chlorella. A substance found in the disputed algae was known as "chlorellin," and it had inhibitory effects on bacteria of both the gram-positive and gram-negative varieties. [14] identified these organisms as *Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus pyogenes, and Staphylococcus aureus*. There has been a noticeable decrease in gram-positive bacteria in lakes during cyanobacterial water-bloom occurrences; this could be explained by the synthesis of antibiotic compounds.

It is common knowledge that cyanobacteria may grow in a variety of microbial settings, such as freshwater, saltwater, land, and areas with fierce competition. They can also face a broad range of microbial diseases, such as those caused by bacteria, viruses, and fungi, in addition to predators. They can adapt to a wide range of environmental obstacles, nutrition sources, growth conditions, and habitats thank to their flexible metabolism. Their ability to adapt accounts for the diversity and abundance of chemical substances that have been extracted from them [7,20] The secondary metabolites of cyanobacteria, which include peptides, terpenes, aromatic molecules, and alkaloids, among other structural classes, have been suggested to have therapeutic use. All of these compounds exhibit some degree of biological action [10]. Their production of a diverse range of toxins is widely acknowledged, with lipopeptides being 40% of the total. The cyanobacterial lipopeptides are reported to contain a variety of compounds, including immunosuppressive agents, antimalerials, antimycotics, multi-drug resistance reversers, anti-feedants, and herbicides, as well as antitumor (13%), antibiotics (12%), cytotoxic (41%), antiviral (4%), and other compounds. These reports are based on studies conducted by [4]. Anaephenes A–C (anaephene B), which are polyketides containing alkynes, were found to be effective against MRSA after being synthesized

[11] and isolated [3]. These instances demonstrate the efficacy of cyanobacterial polyketides in treating illnesses brought on by resistant strains.

There are two uses for cyanobacteria bioactive chemical isolation. First, new materials need to be discovered for agriculture, medicine, or biocontrol. Gaining more knowledge about the relationships that different species have with one another in their natural environments is the second goal. For each of these uses, identifying the number and distribution of bioactive strains requires screening recently culturable species. Many research search for antibacterial compounds by examining various families of cyanobacteria found in freshwater, marine settings, and terrestrial environments. The findings of screening *Phormidium fragile* against harmful bacteria are presented in this study.

Materials and Methods

Cyanobacteria: Collection, Isolation and Identification

Soil samples collected from multiple locations were found to contain *Phormidium fragile*. As stated by [17], the isolated *Phormidium fragile* was cultivated on BG-11 medium, and the incubator's temperature was maintained at 30±2°C. Morphological variation and taxonomy methods in accordance with Desikachary (1959) and Prescott (1962) were used to obtain identification. *Phormidium fragile* was grown in BG-11 culture medium in order to produce biomass on a big scale. The biomass was collected using a double-layered muslin cloth filter after 25 days, and an air blower was used to dry it. The biomass of *Phormidium* fragile was employed to evaluate its antibacterial activity.

Extraction procedure

Fifty milliliters of hexane, chloroform, methanol, and water were used to extract five grams of finely powdered *Phormidium fragile* biomass over the course of a 24-hour period using a soxhlet device at 40°C.

At 40°C, the filtered extract was concentrated while under vacuum. Using the appropriate solvents, the extract was eventually transferred into a milliliter.

Standard antibiotic

Hi Media (India) supplied the standard antibiotic disc (10 µg/ml streptomycin) utilized in this investigation. These discs were maintained using nutrient agar media that had a predetermined concentration of bacteria.

Test organisms

The National Chemical Laboratory (NCL), Pune's National Collection of Industrial Microorganisms (NCIM) provided pure cultures of the bacteria utilized in antibacterial tests. *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus flavus, and Proteus mirabilis* are some of these bacteria. When maintaining cultures, the NCIM, NCL, and Pune criteria were adhered to.

Culture Medium Preparation

Hi-media Laboratories in Pune, India provided the ingredients needed to make the nutrition agar medium. The medium's composition is as follows:

Ingredient	g L^{-1}
Peptone	10.00
NaCl	5.00
Agar	20.00
Beef	10.00

Using a standardized pH meter and either 0.1 N HCL or 0.1 N NaOH, the pH was brought to 7.5. The culture medium was autoclaved for 20 minutes at 1.06 kg cm-2 pressure to sanitize it. Additionally, every piece of equipment that was required—such as pipettes, forceps, conical flasks, Petri dishes, and so forth—was autoclave-sanitized for 30 minutes at a pressure of 1.06 kg cm–2.

Preparation of Inoculum

The pH was adjusted to 7.5 using a standardized pH meter and either 0.1 N HCL or 0.1 N NaOH. To sanitize the culture media, it was autoclaved for 20 minutes at 1.06 kg cm-2 pressure. All necessary equipment, including pipettes, forceps, conical flasks, Petri dishes, and so on, was also autoclave-sanitized for 30 minutes at 1.06 kg cm-2 pressure.

Antibacterial assay

The agar diffusion assay was used for the antibacterial inquiry (Anonymous, 1996). Paper discs with a diameter of 6.4 mm (Whatman No. 41) were created and autoclave-sanitized. After cooling to 45°C, 20 µl bacterial cultures, or approximately 1.5 X 10^8 colony forming units (CFU), were introduced to the 10 ml molten nutrient agar medium. After that, the mixture was put into a sterile petri dish. After this had time to set, each plate was labeled with the name of the infected organism. Four discs in all, containing 400µg of extract per milliliter, were produced. The solvent was given time to evaporate. Once they had hardened, the discs were arranged in petri dishes uniformly. For every organism, duplicate plates, standard plates, and control (solvent) plates were created using the same process. Standard plates were treated with 10µg/ml of streptomycin as an antibacterial agent. To allow the samples to disperse, the plates were incubated at 4°C for eight hours. After that, the plates were incubated at 37° C for a full day. After 24 hours, the diameter of the inhibitory zone was measured to the closest millimeter. The test extract's and the standard's activity were compared based on the diameter of the zone of inhibition. Every exam was given three times in a sterile setting.

Minimum Inhibitory Concentration (MIC) determination

Using the micro broth dilution method, the antibacterial activity of crude extracts of *Phormidium fragile* biomass against both Gram positive and Gram-negative bacteria was assessed (Sahm and Washington, 1991). The nutrient broth containing dimethylsulphoxide (DMSO) at concentrations varying from 1 to 400 µg ml-1 was used to dilute the crude extracts. In 96-well plates, the extract solutions were diluted one at a time. Bacteria were injected into each well at a concentration of approximately 1.5 x10^8 colony forming units (CFUs) ml -1. By calculating the lowest concentration turbidity (by measuring absorbance at 600 nm) during the course of a 24-hour incubation period at 37°C, the final MIC was determined. The positive control was streptomycin, and the negative control was DMSO.

Results & Discussion

The disc diffusion method was used to investigate the antibacterial activity of *Phormidium fragile* (Anonymous, 1996). Based on the size of the inhibition zone, Table 1 displays the antibacterial activity of various *Phormidium fragile* extracts against both gram positive and gram negative bacteria at a concentration of 400µg/ml. In this case, streptomycin was the positive control. The methanol extracts' antibacterial activity displayed a variety of inhibitory patterns with standard positive control, contingent upon the sensitivity of the bacterium under investigation. A methanol extract from *Phormidium fragile* showed efficacy against all of the tested strains of bacteria. Using methanol extract, the greatest zone of inhibition (20±1.5.4mm) against Bacillus subtilis was found. Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis were all successfully combatted by *Phormidium fragile's* chloroform extract. Chloroform extract stimulated *Staphylococcus aureus* more potently. At 400µg/ml concentration, hexane extract was shown to be effective against all bacteria except *Escherichia coli.* No indications of bacterial activity were seen in the aqueous extract.

Human health has been significantly improved by antibiotics, the most effective treatment for bacterial infections. However, due to the emergence of drug-resistant bacteria in recent decades and the decreasing efficacy of many commonly used antibiotics against specific illnesses, not to mention the dangerous side effects of many of them, these health benefits are now in peril. Researching more recent medications with lower

resistance is crucial. Six pathogenic bacteria were used to test the *Phormidium fragile* extracts' antibacterial properties. Hexane, water, chloroform, and methanol were the solvents utilized in the extraction process. Three of the six bacterial strains evaluated exhibited inhibitory action against each extract. *Phormidium fragile* exhibited the highest activity of *Bacillus subtilis* at the effective zone of inhibition (20±1.5 mm). A significant level of inhibition against *Bacillus subtilis* was found upon analysis of the methanol extract of *Phormidium fragile*. However, there was a noticeable decrease in activity in the *Phormidium fragile* chloroform extract. It is understandable why methanol extract is more potent than other extracts and has a stronger antibacterial effect on microorganisms. The antibacterial activity of methanolic extraction is stronger than that of hexane and other solvents, according to studies by Rosell et al. (1987) and Moreau et al. (1988); however, some investigations claimed that chloroform is superior than methanol and benzene (Febles et al., 1995). It is clear that organic solvents provide better extraction efficiency for antibacterially active chemicals than water-based methods [1,5].

Upon analyzing organic solvent extracts of several cyanobacteria against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* Cannell et al. (1988) found activity in five of the cyanobacterial cultures. Phormidium fragile had the highest degree of activity (13±1.8 mm) in the effective zone of inhibition when it comes to inhibiting Staphylococcus aureus in chloroform extract. *Tolypothrix fragilis* chloroform extract showed a modest level of efficacy against every tested pathogen. This implies that of the group, the antibacterial chemical can have the lowest concentration. It was discovered that the chloroform extract was less efficient than the methanolic extract. Compared to the methanolic and chloroform extracts, the hexane extract of *Phormidium fragile* biomass shown superior efficacy against the studied microorganisms. Hexane extract showed little activity against E. coli up to a concentration of 400µg/ml.

Falch and colleagues (1995) performed a series of extractions using progressively more polar solvents. Petroleum ether, ethyl acetate, methanol, dichloromethane, etc. The antibacterial efficacy of these extracts against B. subtilis, E. coli, and Micrococcus luteus varied in a bioautographic investigation. We found that methanol was a more effective than chloroform and hexane at killing both gram positive and gram-negative bacteria.

Bacterium	Diameter of effective zone of inhibition (mm)				
	Methanol extract	Chloroform extract	Hexane extract	Aqueous(water) Extract	Streptomycin (10 µg/ml)
Escherichia coli	10±1.1	-	-	-	17±2.4
Bacillus subtilis	20±1.5	9±1.2	18±1.6	-	25±1.3
Staphylococcus aureus	16±1.4	13±1.8	16±1.8	-	23±1.7
Pseudomonas aeruginosa	10±0.4	6±1.1	14±1.5	-	22±1.6
micrococcus flavus	12±1.3	-	15±1.7	-	20±1.2
Proteus mirabilis	11±2.1	-	13±1.7	-	19±1.2

Table-1. Different Phormidium fragile extracts have antibacterial action against both gram positive and gram negative bacteria at a dosage of 400µg/ml.

MIC (Minimum Inhibitory Concentration)

The ingredient's minimum inhibitory concentration, or MIC, is the concentration at which no appreciable macroscopic growth was seen. The MIC values for a particular isolate were either the same or within one dilution. Using methanol, chloroform, hexane, and water, the current study investigated the effects of biomass extracts on a variety of gram positive and gram negative bacteria. It was shown that the solvent had an impact on the MIC for *Phormidium fragile*. The MICs of each microorganism ranged from 64 to more than 400 µg/ml. The minimum inhibitory concentration (MIC) of 64 µg/ml for the *Phormidium fragile* methanol extract was lower than that of *Staphylococcus aureus* and *Bacillus subtilis*. The chloroform extract had its peak activity at MIC 256 µg/ml. Chloroform extracts had a greater minimum inhibitory concentration (MIC) than methanol extracts. The hexane extract's MIC was from 128 to over 400 µg/ml. Up to 400 µg/ml, the *Phormidium fragile* aqueous extract showed no action at any MIC

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Bacterium	Concentration of extracts in µg/ml.			
	Methanol extract	Chloroform extract	Hexane extract	Aqueous(water) Extract
Bacillus subtilis	64	325	128	>400
Escherichia coli	256	>400	>400	>400
Staphylococcus aureus	64	256	256	>400

Micrococcus flavus	128	>400	256	>400
Pseudomonas aeruginosa	256	325	256	>400
Proteus mirabilis	256	>400	256	>400

Table 2: Phormidium fragile extracts' minimum inhibitory concentrations (MIC) against various harmful microorganisms were measured. Extract concentrations are reported in µg/ml.

The type of chemical responsible for *Phormidium fragile's* antibacterial action was revealed by positive screening results. The most effective Phormidium fragile strains have been demonstrated to exhibit a wide range of activity spectra and the ability to inhibit the development of *Pseudomonas aeruginosa, Bacillus subtilis, and Staphylococcus aureus.* These bacteria also create metabolites of antibiotics. The results presented are in line with previous research [23, 25-26] that shows the potential of cyanobacteria as a copious source of physiologically active chemicals.

Conclusion

Using a cyanobacterium as an example tne excellent source of bioactive antibacterial metabolites is *Phrommidium fragile*. Water and chloroform extracts are less effective than those derived from methanol and hexane. *Phormidium fragile* chloroform extract has less effectiveness against all of the bacteria that have been examined. The bioactive metabolites in the aqueous extract are insoluble in water, making it inactive against all tested bacteria up to 400 µg/ml. The extract made of methanol has the lowest MIC value.

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