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THE INVESTIGATION OF ELEMENTS AFFECTING CERTAIN MICROALG'S DEVELOPMENT AND POSSIBLE ANTIBACTERIAL PROPERTIES

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ABSTRACT:

During the previous few decades, the pursuit has experienced an increase for novel marine-derived molecules with potential use in the pharmaceutical, human or animal nutrition, cosmetics, and bioenergy industries. The current study aimed at obtaining the optimum growth rate of some microalgal isolates and finding their antibacterial activity. The microalgae were purified in BG11 medium and the biomass extracted using ethanol, acetone, diethyl ether and methanol was then examined for antimicrobial activity by disc diffusion and minimum inhibitory concentration methods.The optimum growth rate obtained at pH 8, temperature 26-28 ℃, and 2000 lux for all algal isolates. All microalgae extracts showed antimicrobial activity against tested bacteria with different solvents. The higher antibacterial activity obtained with ethanol extracts while water extract showed low antibacterial activity. *Chlorella* sp. and *Spyrogyra* sp. showed higher antibacterial rather than other isolates. Overall, these results imply that microalgae extracts, particularly those from Spirogyra sp. and *Chlorella* sp., may be sources of antibacterial chemicals. To identify and describe the precise bioactive substances in charge of the reported inhibitory effects, additional study is required. Further research into the mechanisms of action, safety, and possible uses of these microalgae extracts in bacterial infection management would be advantageous.

KEYWORD: Microalgae, Antibacterial, Inhibition zone, MIC, optimum growth, BG11.

1. INTRODUCTION

One of the leading advancements in human healthcare has been the synthesis and discovery of antibiotics, which have made it possible for most patients to prevent and treat a variety of infections at a reasonable cost. Biomedical substances are wrapped or loaded with numerous bacterial-destroying chemicals when the antibacterial property is required. Numerous research studies have been conducted to graft different antibacterial moieties, such as enzymes and saccharides, as well as metal ions, onto polymeric substrates (PLA, PE, PS, PP) to give them antibacterial characteristics (Abdo *et al.*, 2013). The success of antibiotics has led to the proliferation of bacterial resistance to many commonly used broad and narrow-range medications (Abdulkareem & Anwer, 2021; Adenan, Yusoff, & Shariff, 2013; Anwer & Abdulkareem, 2014).

In order to counter this threat, there is a growing need to create novel antimicrobial agents, especially by extracting them from medicinal plants and autotrophic organisms. Bacteria, for the most part, have a genetic capacity to both develop and share resistance to medications used as treatments.The term "microalgae" is used to refer to a large category of microscopic, unicellular organisms that are frequently found in water (Anwer, 2023). Green microalgae may live in harsh conditions. These microorganisms must adapt to external changes in order to maintain a constant intracellular environment.

This adaptation can increase energy consumption, which lowers photosynthetic metabolism (Bashir *et al*., 2018; Slavin *et al*., 2017). In addition, it has the potential to result in the accumulation of intermediate compounds within cells, which are later modified by subsequent pathways to generate secondary metabolites. A wide range of compounds are known to display other significant biological features, such as antibacterial activity, and are not required for basic cellular function (Benedetti *et al*., 2018). Now, numerous microalgae species are used in a variety of applications, including CO2 mitigation, human nutrition, biofuel production, wastewater treatment, and antimicrobial compounds. Even though the bioactivities of algae have been recognized, given the heterogeneity of the commercial feed products based on algae and the intra- and interspecific variations among algae, it is still necessary to assess their functional properties, identify the suitable species, and determine whether they can be combined to have a greater impact (Rai, Gautom & Sharma, 2015).The current study aimed to measure the antibacterial efficiency against specific pathogenic bacteria using extracts of microalgae.

2. MATERIAL AND METHOD

2.1 Isolation and Identification of Microalgae:

Sample of microalgae were taken from different points along the Gomaspan River in the Ebil, Iraq region. These water samples were then placed on BG11 plates with 1.5% agar-agar and incubated at 25°C, pH 8, and 2500 lux of light. After two weeks, a single colony was chosen and identified by examining its morphology using a light microscope (CDC- Centers for Disease Control and Prevention Antibiotic Resistance threats in the United States 2019). Morphological characteristics of isolated microalgae were photographically registered at 10–40X magnification using an Olympus BL51 microscope and identified as described by (Anwer, 2023).

2.2 Determination of Dry Weight under Optimum Growth

Algal cultures were nurtured in BG11 medium under different sets of conditions, including variations in pH (ranging from 6 to 10), light intensity (ranging from 1000 to 3000), and temperature (ranging from 20°C to 35°C), in order to study their effects on chlorophyll and biomass. The pH was regulated using

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NaOH and HCl, and the biomass was subsequently dried and preserved for later (Salem, 2011; Cortés *et al*., 2018; Dantas *et al*., 2019).

2.3 Preparation of Inoculum:

Bacterial cultures of *Staphylococcus aureus* and *E. coli* were acquired from medical microbiology lab. College of Health Sciences, sub-cultured to obtain single colony and stained with gram stain to confirm the purity of bacterial samples (Giddings & Newman, 2015). Furthermore, the pure colony from overnight cultured to 3 ml of sterile saline in a clear test tube and the turbidity measured by using the 0.5 McFrland turbidity meter.

2.4 Analytical Experiments:

2.4.1 Estimation of Chlorophyll

Chlorophyll was estimated according to Issa (1999). Every three days for the past two weeks, 5 ml of culture was taken from each flask of the sample, centrifuged at 6000 rpm for five minutes, and the supernatant was discarded. The cell was then suspended in 5 ml of ethanol, and the concentration of chlorophyll a+b of the pigment extract at the specified wavelength (A) was calculated by comparing it to the empty solvent using the equation provided below:

Chlorophyll a+b = (7.12 × A660) + (16.8× A643) ……1

Cell dry-weight and fresh -weight Estimation:
According to Oswald (1988), the specimens drawn at 100 ml of BG11 medium after 2 weeks were taken at 5 ml of the sample and absorbance read at 650 nm. To determine the dry weight of the cells collected after centrifugation for10 minutes, the sample was dried in the oven at 80 ° C and weighed rapidly after drying (to prevent moisture absorption) (Jafari-Sales *et al*., 2020).

2.4.2 Preparation of Microalgae Extracts:

Using a soxhlete extractor, the dry biomass of each algal isolate was progressively extracted for 4 hours with 100, 200, 300, 400, 500 ml of 96% ethanol, acetone, diethyl ether, methanol, and water extracts (Juneja, Ceballos & Murthy, 2013). The solvent was then eliminated by incubating at 60 °C and stored at 4 °C for further use.

2.4.3 Disc diffusion method determined as follow

In the well diffusion method, 100µl of each microalgae extract was introduced to inoculate the bacterial culture on Muller Hinton agar. Subsequently, the agar plates were incubated at 37°C for 18-24 hours. The measurement of the diameter of the inhibition zones was carried out with a ruler. The assessment of inhibitory zones and the comparison of antibacterial activity results with the control were performed in accordance with the standards provided by the National Committee for Clinical Laboratory Standards (NCCLS) (Khan *et al*., 2018).
-Minimum inhibitory concentration: The minimum inhibitory concentrations of microalgae isolates were tested against *E. coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes*. A volume of 100 µl of each extract was added to a 96-well plate containing 100 µl of nutrient broth using dilution methods, and 100 µl of bacterial strains was added into the suspension. The plate was then incubated at 37°C for 18-24 hours. For the negative control, microtiter plates were prepared with the medium but without any inoculation. In contrast, the positive control was established by using the standard drug (Krzemińska *et al*., 2014; Sarwa & Verma, 2017).

3. RESULTS AND DISCUSSION

A total of five genera of microalgae from various locations in the Smaqoli dam were isolated and identified based on their morphological characteristics. The following genera were recognized after observation under a microscope: *Phormidium* sp., *Oscillatoria* sp., *Spirogyra* sp., *Scenedesmus* sp., and *Chlorella* sp. Microalgae were identified based on their morphological characteristics, and microscopic forms are shown in Figures (1,2,3,4,5). In particular, some of the isolated cyanophyta lacked akinetes and heterocysts. The cultures varied in color from blue-green to green.

Maulood and Aziz (1997) recorded a total of 142 taxa of filamentous green algae in Kurdistan. In the province of Erbil, Toma and Bahram (2013) documented a comprehensive compilation of 244 species of blue-green seaweed. Aziz and Muhammed (2016) recorded a total of 151 algal species in various springs located in the Safeen Mountain Area. During the year 2019, Aziz and Yasin (2019) conducted a study that documented a comprehensive total of 116 species encompassing 58 genera, 31 families, 19 orders, 9 classes, and 8 divisions. This research was carried out across eight man-made fish ponds situated in Erbil.

The filaments are unbranched, long cylinder, with straight blue green Trichomes, the cells appeared square and linked end-to-end filaments are rounded. with mucilaginous layers. Conical or capitated apical cells lacking calyptra. No akinet, heterocyst appeared. Identified as *Phormiudium* sp. Figure (1).

Figure 1: Phormiudium sp. observed under the light microscope showing (a) disc-cell and (b)apical cell. Microscopic images at 40X magnification. Scale bar: 50 µm.

Elongated, filamentous, multicellular green algae made up of individual cells joined together to form lengthy filaments. The filaments are unbranched, the cells are rod shape contains spiral shaped chloroplast, pyrenoids appear in chloroplast, the cell walls appeared as 2 layers and is identified as *Spyrogyra* sp. Figure (2)

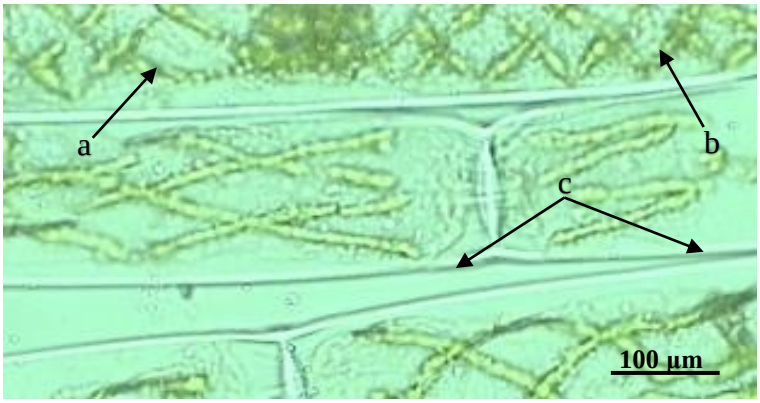

Figure 2:Spirogyra sp .observed under the light microscope showing (a)pyrenoid, (b)chloroplast and(c)cell wall. Microscopic images at 10X magnification.Scale bar: 100 µm.

Scenedesmus sp. appeared under microscope as a tiny, non-motile colonial green coloured alga with cells arranged in a flat plate as coenobium. The colonies usually consist of four elongated cells , more lunate, fusiform, the cells are typically cylindrical there was no hormoginia, akinet and heterocyst's, as seen in Figure (3).

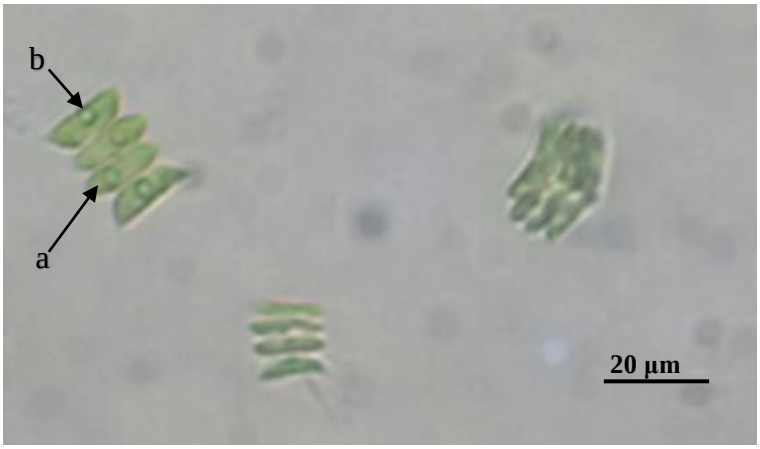

Figure 3: Scenedesmus sp. observed under the light microscope *showing* (a) cell wall and (b) pyrenoid. Microscopic images at 10X magnification.Scale bar: 20 µm.

The cells are appeared as single, spherical shape, green colored. The chloroplast was cup-shaped and they contain chlorophyll a and b as their photosynthetic pigments. There were no flagella, akinet, and hormogonia seen, on the base of the microscopic examination the algae identified as *Chlorella* sp. Figure (4).

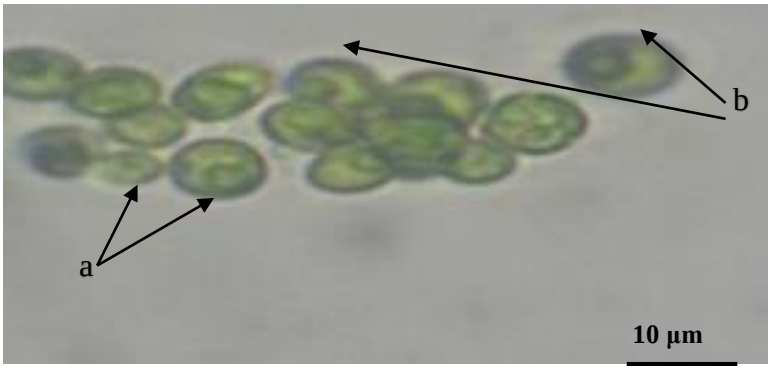

Figure 4: Chlorella sp. observed under the light microscope showing (a)chloroplast and(b)pyrenoid. Microscopic images at 40X magnification. Scale bar:10µm.

Trichomes appeared free and cylindrical without sheath. Short, discoid cells with a coin-like shape. Cell ends were rounded, blue-green in color. Cells lack organelles, but granules appeared on cells and identified as *Oscillatoria* sp. as shown under microscope in Figure 5.

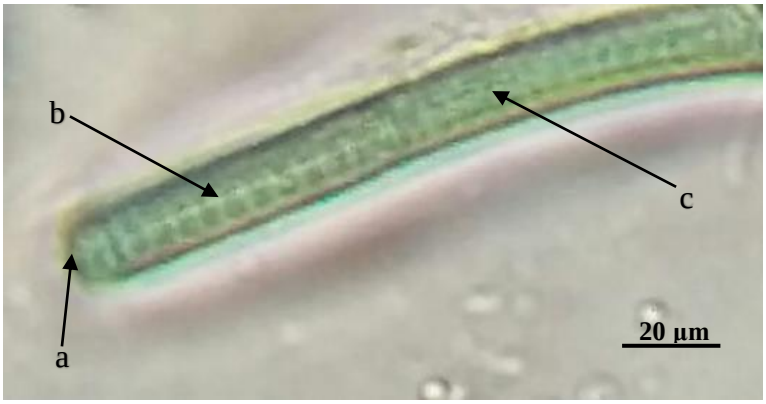

Figure 5: Oscillatoria sp. . observed under the light microscope. showing (a)capitate, (b)cell and (c)granules. Microscopic images at 40X magnification. Scale bar: 20 µm.

Effect of Different condition on the microalgae growth:

By measuring the biomass Chlorophyll content (cell dry weight and fresh weight) in isolated species, the impact of pH

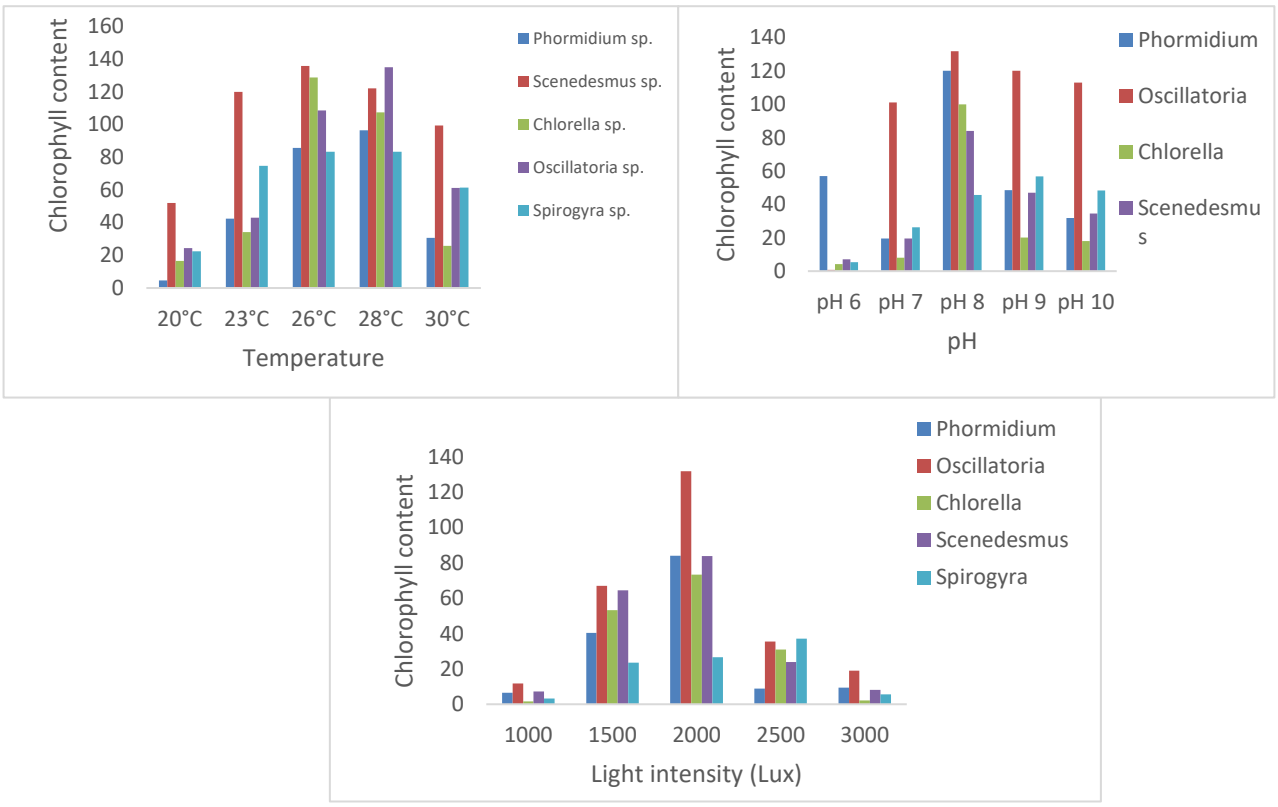

Figure 6: Effect of a-temperature, b- pH, and c- Light intensity to growth of microalgae isolates.

Figure (6) displays the development of isolates at various pH levels and the optimum growth rates within the temperature range of 26 °C and 28 °C, respectively, for microalgae that are unicellular and filamentous. Microalgae grow best in the 25–34 °C temperature range. Their growth proceeds via many distinct phases within this temperature range: a brief exponential phase, a linear phase, and finally a stationary phase, which usually happens around day 14.

As temperature rises above 20 °C, chlorophyll and cell dry weight increase, while below this point, chlorophyll and cell density with cell dry weight decrease. The sluggish growth rate of microalgae might be attributed to the increase in temperature, which leads to a corresponding rise in respiration beyond the species' optimal level. This outcome aligns with the discovery made by Rai and Rajashekhar (2014). In Table (1), the growth of the microalgae strain is demonstrated with respect to pH levels. Microalgae exhibit growth across a wide range of pH values. The best growth determined at pH 8 for all microalgae strains (unicellular & filamentous) and the maximum biomass obtained from *Oscillatoria* sp. similar result obtained by Lauritano *et al*.

(2016) and Luepke *et al*. (2017). It was mentioned that the pH of water plays a major role in determining the relative concentrations of carbonaceous species. Increased pH limits the availability of carbon from CO2, which inhibits the growth of algae in higher pH environments (9–10). In these conditions, carbonates are the main form that algae use to absorb carbon.

An increase in pH causes algae to be less drawn to free CO_2. One of the most important determinants of microorganism growth rate and biomass is light intensity and photoperiod, which have a significant impact on each other (Maderia, 2017). The optimum growth rate of chlorophyll content, turbidity and (cell dry and fresh weight) was recorded at 2000lux for filamentous and unicellular and lowest growth rate recorded at 1000 lux. While the growth rate under escalating light intensity varies according to the strain and culture temperature, algae's growth rate is highest when exposed to saturation intensity. However, it diminishes with both heightened and reduced light intensities. Moreover, light intensity influences the cellular composition of algae. For instance, when the light intensity is elevated up to 3000 lux, the growth rate drops due to reaching the point of saturation.

Antibacterial assay:

The inhibition zone against three bacteria by each of microalgae species with different extracts shown in table 1. Lager inhibition zone obtained by using Ethanol extract of *Chlorella* sp. (26, 26, and 29mm) against *P. aeruginosa, S. pyogenes* and *E. coli* respectively. While lower inhibition zone showed with water extract of algal strains.

Among the microalgae species, water extracts of *Spirogyra* sp. generally demonstrated moderate to strong inhibitory effects were observed against *E. coli* and *P. aeruginosa*, with higher volumes resulting in larger zone sizes. The extract showed a moderate inhibitory effect against *S. pyogenes*. The zone of inhibition against *E. coli* ranged from 13 mm to 17 mm, with increasing values observed at higher volumes. Against *Pseudomonas aeruginosa*, the range of inhibition zones varied between 7 mm and 15 mm, while against *S. pyogenes*, it ranged from 10 mm to 14 mm. *Spirogyra* sp. was subjected to screening against three bacterial strains, namely *Pseudomonas solanacearum, E. coli*, and *Clavibacter michiganense*, as well as three plant pathogenic fungi, including *Fusarium oxysporum*, Curvularia species, and *Aspergillus niger*. The study revealed its potent antimicrobial property against all the tested organisms (Marrez *et al*., 2019). Additionally, the phytochemical components of Spirogyra, including alkaloids, steroids, flavonoids, tannins, and terpenoids, have demonstrated antimicrobial activity against *E. coli* and *Candida albicans* (Montie *et al*., 2016).

Acetone, diethyl ether, methanol, and ethanol extracts of *Spirogyra* sp. exhibited moderate to strong inhibitory effects against all three bacterial strains, with larger zone sizes observed at higher volumes. By using acetone extract, the range of inhibition zones against *E. coli* varied from 12 mm to 21 mm. Against *Pseudomonas aeruginosa*, the zone of inhibition extended from 10 mm to 17 mm, while against *S. pyogenes*, it ranged from 12 mm to 17 mm. In the case of using diethyl ether extract the zone of inhibition against *E. coli* ranged from 14 mm to 25 mm. Against *Pseudomonas aeruginosa*, the zone of inhibition ranged from 11 mm to 24 mm, while against S. pyogenes, ranged from 10 mm to 18 mm. In their study, Munteanu *et al*. (2014) found that the methanol extraction method yielded the most effective inhibition of *E. coli* and *Candida albicans* growth rate when using Spirogyra sp. extract.

This information is pertinent to the current context. Reverse with the *Spirogyra* sp. extract from hot water technique. By the utilizing of methanol extract, the zone of inhibition against *E. coli* vary from 14 mm to 23 mm. Against *Pseudomonas aeruginosa*, the zone of inhibition vary from 7 mm to 16 mm, while against *S. pyogenes*, it extended from 11 mm to 17 mm. While using ethanol extract the zone of inhibition against *E coli* vary from 20 mm to 28 mm. Against *Pseudomonas aeruginosa*, the zone of inhibition ranged from 13 mm to 19 mm, while against *S. pyogenes*, it ranged from 5 mm to 23 mm. An investigation revealed the significant effectiveness of an ethanol extract from the Indian plant *Gracilaria corticata* against *Vibrio cholerae* and *Vibrio parahaemolyticus* bacteria. However, it exhibited reduced efficacy when tested against *Pseudomonas aeruginosa* and *Shigella flexneri* (Patel *et al*., 2019).

Among the microalgae species, water extracts of the *Chlorella* sp. showed a variable inhibitory effect against *E. coli*, ranging from moderate to very strong inhibition. Moderate inhibitory effects were observed against *Pseudomonas aeruginosa* and *Streptococcus pyogenes.* The zone of inhibition against *E. coli* ranged from 15 mm to 21 mm, with higher values observed at higher volumes. Against *Pseudomonas aeruginosa*, the zone of inhibition ranged from 9 mm to 14 mm, while against *Streptococcus pyogenes*, it ranged from 8 mm to 13 mm. A prior investigation demonstrated that extracts derived from Phormidium and Microcoleus species exhibited promising antibacterial activity against *Salmonella enteritidis* and *E. coli* bacteria (Patil & Kaliwal, 2019). Water extract of *Scenedesmu*s sp. inhibitory effect was weak to moderate against *Escherichia coli*, with larger zone sizes observed at higher volumes. The extract showed a weak to moderate inhibitory effect against *Streptococcus pyogenes*. The zone of inhibition against *E. coli* ranged from 6 mm to 10 mm, with increasing values observed at higher volumes. Against *Streptococcus pyogenes*, the zone of inhibition ranged from 6 mm to 16 mm. Acetone, diethyl ether, methanol, and ethanol extracts of *Scenedesmus sp*. exhibited moderate inhibitory effects against both bacterial strains, with little variation in zone sizes across different volume. Tufa *et al.* (2022) concluded that solvent amount and type influence the antimicrobial activity of any extracts.

Water extracts *Phormidium* sp. showed moderate inhibitory effects against *E. coli* and *Pseudomonas aeruginosa*, with larger zone sizes at higher volumes. The extract showed a weak inhibitory effect against *Streptococcus pyogenes.* The study conducted by Jusidin *et al.* (2022) underscored the successful targeting of the highly virulent V. harveyi by hydrophilic compounds present in microalgae extractsThis bacterium is responsible for causing vibriosis, a severe ailment affecting farmed fish and aquaculture practices worldwide.

Unfortunately, no data was provided for the zone of inhibition for *Oscillatoria* sp. water extract. However, the other solvent extracts of *Oscillatoria* sp. displayed weak to moderate inhibitory effects against the tested bacteria (Bhuyar *et al*., 2020).

Table 1: Zone of inhibition against some bacteria strains by different extract of microalgae isolates

Microalgae isolates	Solvents	100ml	200ml	300ml	400ml	500ml	100ml	200ml	300ml	400ml	500ml	100ml	200ml	300ml	400ml	500ml
		E. coli					*Pseudomonas aeruginosa*					*Streptococcus* pyogenes				
Spirogyra sp.	Water	13	13	16	16	17	7	12	13	14	15	10	13	15	15	14
	Acetone	12	16	17	17	21	10	13	15	15	17	12	13	16	17	17
	Diethyl ether	14	17	19	18	25	11	17	18	19	24	10	12	15	17	18
	Methanol	14	20	20	21	23	7	13	13	15	16	11	12	16	16	17
	Ethanol	20	24	27	27	28	13	16	16	18	19	5	13	16	19	23
Chlorella sp.	Water	15	18	19	20	21	9	11	13	14	14	8	9	11	11	13
	Acetone	16	15	17	17	18	10	11	11	13	15	10	11	12	12	14
	Diethyl ether	21	23	24	25	28	10	12	12	14	14	13	15	15	16	18
	Methanol	19	21	25	27	27	10	13	15	15	171	9	19	18	19	20
	Ethanol	23	28	29	29	29	10	16	18	24	26	16	16	18	21	26
Scenedesmus sp.	Water	6	9	6	7	10	-	-	6	10	16	2	6	7	9	10
	Acetone	11	12	13	16	16	-	7	12	12	9	6	9	13	15	15
	Diethyl ether	10	12	14	14	14	5	9	13	14	14	9	9	10	12	14
	Methanol	5	7	9	10	10	10	13	15	15	16	10	12	13	13	13
	Ethanol	12	12	14	16	16	10	11	11	12	12	9	11	12	12	13
Phormidium sp.	Water	12	15	16	17	19	3	5	6	5	9	5	8	10	12	12
	Acetone	14	16	19	21	21	6	6	10	11	14	9	11	14	17	17
	Diethyl ether	14	15	18	18	20	-	-	8	9	12	10	11	11	12	15
	Methanol	15	16	20	20	23	10	11	10	12	12	9	10	14	14	14
	Ethanol	16	20	23	24	25	7	7	9	14	15	10	15	16	16	18
Oscillatoria sp.	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetone	10	10	12	12	13	10	12	14	14	18	8	11	13	15	19
	Diethyl ether	2	5	7	8	8	10	13	15	15	19	7	12	17	18	18
	Methanol	4	7	7	9	14	9	11	11	13	13	8	7	12	13	15
	Ethanol	5	8	12	15	18	11	9	12	13	14	10	11	15	15	18

Table 2: Minimum Inhibitory Concentration (MIC) against some bacteria strains by different extract of microalgae isolates.

Bacterial strains	**Minimum inhibitor concentration (MIC) µg/ml by *Spirogyra* sp.**			
	Water	**Ethanol extract**	**Methanol extract**	**Diethyl ether**
Escherichia coli	-	100	200	200
Pseudomonas aeruginosa	1000	100	200	300
Streptococcus pyogenes	1000	300	450	400
Bacterial strains	**Minimum inhibitor concentration (MIC) µg/ml by *Chlorella* sp**			
	Water	**Ethanol extract**	**Methanol extract**	**Diethyl ether**
Escherichia coli	1000	400	400	200
Pseudomonas aeruginosa	400	400	200	200
Streptococcus pyogenes	-	500	500	300
Bacterial strains	**Minimum inhibitor concentration (MIC) µg/ml by *Scenedesmus* sp.**			
	Water	**Ethanol extract**	**Methanol extract**	**Diethyl ether**
Escherichia coli	500	1000	200	200
Pseudomonas aeruginosa	700	300	600	500
Streptococcus pyogenes	-	500	800	700
Bacterial strains	**Minimum inhibitor concentration (MIC) µg/ml by *Phormidium* sp.**			
	Water	**Ethanol extract**	**Methanol extract**	**Diethyl ether**
Escherichia coli	1000	500	500	300
Pseudomonas aeruginosa	125	500	500	500
Streptococcus pyogenes	-	1000	500	500
Bacterial strains	**Minimum inhibitor concentration (MIC) µg/ml by *Oscillatoria* sp**			
	Water	**Ethanol extract**	**Methanol extract**	**Diethyl ether**
Escherichia coli	-	1000	500	500
Pseudomonas aeruginosa	1000	500	300	700
Streptococcus pyogenes	-	700	500	500

Note: "-" indicates that MIC values were not provided.

To assess the growth-inhibiting impact of solvent extracts on three pathogenic bacteria, *Streptococcus. pyogenes*, *E. coli*, and *P. aeruginosa*, various dilutions were employed. The provided data presents Minimum Inhibitory Concentration (MIC) values in µg/mL for different bacterial strains against various extracts of microalgae isolates, as presented in Table (2).

The water extract of *Spirogyra* sp. did not exhibit any inhibitory effect against *E. coli*. However, the ethanol, methanol, and diethyl ether extracts demonstrated MIC values of 100 µg/mL, 200 µg/mL, and 200 µg/mL, respectively. Similarly, according to Ratikanga, Gitu and Oyaro (2014), the methanolic extract showed strong antibacterial activity against *E. coli* with inhibition zone of 9.3, 11.4, 13.4, 15.2 in the concentrations 3, 5, 10, 20 mg/ml respectively. The ethanolic extract exhibited a moderate level of antibacterial activity against *E. coli*. The water extract against *P. aeruginosa* showed the highest MIC value of 1000 µg/mL. The ethanol and methanol extracts exhibited MIC values of 100 µg/mL and 200 µg/mL, respectively. The diethyl ether extract, on the other hand, demonstrated a minimum inhibitory concentration (MIC) value of 300 µg/mL. Previous research indicated the activity of *Spirogyra* sp. methanol extracts contrary to Gram-positive bacteria (Champa *et al*., 2016). While water extract against *St. pyogenes* did not provide MIC values. The ethanol extract showed an MIC value of 700 µg/mL, the methanol extract exhibited an MIC value of 500 µg/mL, and the diethyl ether extract had a MIC value of 500 µg/mL.

The water extract of *Chlorella* sp. displayed an MIC value of 1000 µg/mL against *E. coli*. The ethanol and methanol extracts exhibited MIC values of 400 µg/mL, while the diethyl ether extract demonstrated an MIC value of 200 µg/mL. Previous research demonstrated the effectiveness of methanol extracts from seaweeds Enteromorpha intestinalis and Gracilaria corticata against Gram-positive bacteria (Rao & Parekh, 1981). MIC value of 400 µg/mL demonstrated with the ethanol and methanol, and water extracts and the diethyl ether extract had an MIC value of 200 µg/mL. The MIC for *Streptococcus pyogenes* with ethanol and methanol extracts exhibited as 500 µg/mL, while the diethyl ether extract showed an MIC value of 300 µg/mL.

The MIC by *Scenedesmus* sp. against *E. coli* with the methanol extract and the diethyl ether extract exhibited an MIC value of 200 µg/mL, respectively. The antimicrobial efficacy of extracts from *Scenedesmus sp*. aligns with the findings of Dantas *et al*. (2019) who observed significant inhibitory effects against *E. coli* using various organic extracts. The freshwater microalgae *Scenedesmus* sp. has demonstrated remarkable antimicrobial capabilities against diverse bacterial pathogens. Notably, Beena and Krishnika (2011) reported that acetone and methanol extracts exhibited mild inhibitory effects on *Pseudomonas* sp.

The MIC against studied bacteria by *Phormidium* sp. ranged between 300-1000 µg/mL for *E. coli* and *P. aeruginosa*: Among the extracts, the water extract exhibited the most favorable MIC value of 125 µg/mL. The ethanol and methanol extracts exhibited MIC values of 500 µg/mL, the diethyl ether extract, on the other hand, displayed an MIC value of 500 µg/Ml *S. pyogenes*. The water extract did not provide MIC values for this bacterial strain. The ethanol extract exhibited an MIC value of 1000 µg/mL, the methanol extract showed an MIC value of 500 µg/mL, and the diethyl ether extract had an MIC value of 500 µg/mL. The results of (Tanase *et al*., 2019) were in agreement with our findings that gradient solvent extracts of cyanobacteria showed effective bioactivity against both gram positive and negative organisms

Oscillatoria sp. showed MIC rate of 500 µg/mL, with methanol and the diethyl ether extract against *E. coli* and *S. progenies.* Against *P. aeruginosa*, the methanol extract exhibited an MIC value of 300 µg/mL. Drawing from the research conducted by Prakash Bhuyar *et al*. (2020), marine cyanobacteria (*Oscillatoria* sp.) have exhibited noteworthy capabilities as antimicrobial agents, demonstrating potential in areas such as anticancer, antioxidant, and antitumor activities. The minimal inhibitory concentration (MIC) values for gram-positive bacteria were observed at 30 µg/ml, while for gram-negative bacteria, the value was 25 µg/ml. Previous studies have indicated that the extracts from the algae examined in their investigation exhibited more effective control over Gram-positive bacteria in contrast to Gram-negative bacteria (Tuney *et al.,* 2006). Solubility of Active Compounds, extraction efficiency different solvents can extract different quantities and types of compounds, Bioavailability, chemical stability and polarity.

CONCLUSION

A comparative analysis of the MIC values among various microalgae extracts further underscores these findings. In the present study, Ethanol extracts generally exhibited better inhibitory effects compared to other solvents, we could observe variations in the inhibitory effects against different bacterial strains. Each microalgae extract may have different bioactive compounds that contribute to its antimicrobial activity. The differences in MIC values can be attributed to variations in the chemical composition and concentrations of bioactive compounds in the extracts, as well as the susceptibility of the bacterial strains tested. More study is needed to pinpoint and characterize the precise bioactive substances causing the reported inhibitory effects. Furthermore, it would be beneficial to investigate the mechanisms of action, evaluate safety, and consider prospective uses of these microalgae extracts for the treatment of bacterial infections. It is also necessary to develop a molecular tool that is sensitive enough to identify extremely minute amounts of algal DNA.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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