Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Determination of Antioxidant, Antimicrobial Properties with Evaluation of Biochemicals and Phytochemicals Present in Oscillatoria limosa of District Jamshoro, Pakistan

Azhar Ali TUNIO[1], S. Habib NAQVI[2], Qamar-u-Nisa TUNİO[3], Tanzeel REHMAN CHARAN[*4], Mihr Ali BHUTTO[5], M. Haneef MUGHARI[6]

[1,2,3,4,5]Institute of Biotechnology & Genetic Engineering, University of Sindh, Jamshoro 76080, Sindh, Pakistan.
[6]Institute of Biochemistry, University of Sindh, Jamshoro 76080, Sindh, Pakistan.

[1]https://orcid.org/0000-0003-3218-1291, [2]https://orcid.org/0000-0002-0797-4436, [3]https://orcid.org/0000-0002-2771-3807, [4]https://orcid.org/0000-0003-4332-5342, [5]https://orcid.org/0000-0001-8617-0710, [6]https://orcid.org/0000-0001-8976-9788

*Corresponding author e-mail: trcharan@scholars.usindh.edu.pk

Article Info

Received: 10.05.2022
Accepted: 18.07.2022
Online published: 15.09.2022
DOI: 10.29133/yyutbd.1112896

Keywords

Antimicrobial,
Antioxidant
Cyanobacteria,
Oscillatoria limosa,
Phytochemicals,

Abstract: *Oscillatoria limosa* is a well-known member of blue-green algae, usually found in open water reservoirs. Ecologically it plays very important roles, like releasing Oxygen and being a supportive alternative source of food for aqua fauna. In research, it is being investigated as a medicinal organism for cancer and infectious diseases. In the current study, we have determined the medicinal and ecological importance of *Oscillatoria limosa* particularly found in the vicinity of district Jamshoro 76080, Sindh, Pakistan. For this purpose, four different solvent extracts of 20% (w/v) dried powder of organism were used to determine the presence of bioactive compounds, phytochemicals, antimicrobial activities, and antioxidant properties through previously well-reported methods. The obtained results of this study prove the presence of phenolic acid, flavonoids, total proteins, total sugar, reducing sugar and one free amino acid, and phytochemicals like alkaloids, phytosterol, tannin, terpenoids, glycosides, and saponin in samples. In this study, remarkable antioxidant properties ranging from 0.248 to 1.080 mg ml^{-1} were observed in all the samples. The antibacterial activities against *S. aureus*, *A. tumefaciens*, *K. aureus*, *E.coli*, and *P.aeruginosa*, and antifungal activities against *A. niger*, *P. notatum,* and *Rhizopus* were observed, which proves it a good antimicrobial organism. It may be concluded from this study that *Oscillatoria limosa* of local vicinity is a potential organism of interest for biotechnological and pharmaceutical industries as an antitumor, antimutagen, free radical scavenger, and possess antimicrobial properties against various kind of bacteria and fungi.

To Cite: Tunio, A A, Naqvi, S H, Tunio, Q N, Charan, T R, Bhutto, M A, Mughari, M H, 2022. Determination of Antioxidant, Antimicrobial Properties with Evaluation of Biochemicals and Phytochemicals Present in Oscillatoria limosa of District Jamshoro, Pakistan. Yuzuncu Yil University Journal of Agricultural Sciences, 32(3): 538-547. DOI: https://doi.org/10.29133/yyutbd.1112896

1. Introduction

Oscillatoria limosa is the filamentous blue-green algae, which is widely distributed in all convincible aquatic habitats on earth and possesses its particular ecological importance of being oxygenic phototrophs and aquatic food of aqua fauna, although some of its species are reported toxic for certain organisms (Abed et al., 2009; Luu et al., 2019). The phycologists can easily identify the freshwater *Oscillatoria limosa* in freshwater reservoirs by its exceptional morphological characteristics like blue-green long, straight, and without mucous membrane filamentous colonies (Luu et al., 2019). Taxonomically *Oscillatoria limosa* belongs to the diversified group of cyanobacteria, which has gained

a lot of attention in recent years, particularly in the field of biotechnology, food sciences, and pharmacological industries.

Currently, many members of cyanobacteria attracted many researchers and scientists worldwide to search it, as these organisms are a rich source of biochemical compounds, phytochemicals, as well as antibiotic and antiviral compounds; they are immunosuppressive agents, anticancer, antiplasmodial, and algicide organisms(Patterson et al., 1994; Papke et al., 1997; Papendorf et al., 1998; Kajiyama et al., 1998; Dahms et al., 2006; Abed et al., 2009). Cyanobacteria, through research, are also being tried to be used as food, biofertilizer, and medicinal organism (Lem & Glick, 1985). Throughout history, many members of algae, fungi, and plants have been widely used in particular to promote and maintain good health, fight against sickness, provide relief from pain, and for treatment of diseases since times immemorial (Hemavani et al., 2012; Charan et al., 2021). These all organisms and their derivatives which are being used as medicines are counted as parts or members of traditional medicines and practices through the centuries, particularly in the countries of the continent of Asia like India, China, Japan, Thailand, Pakistan, etc. (Das, 2016; Piwowar & Harasym, 2020; Wells et al., 2017). Throughout the study of various species of *Oscillatoria,* many researchers have reported various important findings like *Oscillatoria raoi to* possess an antiviral bioactive compound, Acetylated sulfoglyco-lipids (V et al., 1997), *Oscillatory Sp.,* used as food as well as medicinal organism, and further, it has been tested for various purposes in research like for removal of heavy metals, activation of monocytes and B-cell of blood and as antibiotics (Azizi et al., 2012; Swanson-Mungerson et al., 2017; Swanson-Mungerson et al., 2018). However, on the other hand, the scientific study on *Oscillatoria limosa* from district Jamshoro, Pakistan, like other species of cyanobacteria, is still limited, particularly on its bioactive components, antimicrobial agents, and antioxidant properties.

So, the present study aimed to explore the biological activities of *Oscillatoria limosa* found in the vicinity of the district Jamshoro 76080, Pakistan, like antioxidant, antifungal, antibacterial, and cytotoxicity. And to investigate the feature benefits of this organism as an alternative source of food and medicines. The obtained results of the present study confirm the antioxidant, antimicrobial and antifungal activity and the presence of various known and unknown biochemicals and phytochemicals in *Oscillartoria limosa* collected from the said local regions.

2. Material and Methods

2.1. Materials

Formalin, peptone, China grass**,** acetone, dextrose, agar, methanol, ethanol, H_2SO_4, glucose, 80 % phenol, 28 mM sodium phosphate, purchased from Sigma Aldrich Roche, and Yeast extract was obtained from IBGE, ferric chloride, NaCl, sodium hydroxide, acetic acid, alkaline sodium carbonate, copper sulphate-potassium sodium tartrate, alkaline solution, folin-ciocalteu parched from Merk, 10% aluminum chloride, 5% sodium nitrate, 0.1% ferric chloride ($FeCl_3$), 0.2 M phosphate buffer (Ph 6.6), acetic anhydride, all parched from Merck. Dinitrosalicyclic acid (DNS) from Bio Basic INC, 4mM Ammonium Molybdate BDH, sodium acetate, 1% potassium ferricyanide, 10% trichloroacetic acid (TCA), 0.008 M Potassium ferric cyanide and chloroform provided by IBGE. All the reagents and chemicals used were of analytical grade.

2.2. Methodology

2.2.1 Collection or ısolation of algal strains

Oscillatoria limosa algae were collected in plastic bags from natural freshwater reservoirs. Environmental and water temperature were noted along with water pH at the time of sample collection. All samples were collected and stored in black plastic bottles. Then in the laboratory, all the samples were washed twice in tap water and dried at room temperature in the shade. After that, all the collected algal species were stored in 4% formalin (commercial) for further taxonomical or morphological studies.

The collected specimen material was taxonomically verified as *Oscillatoria limosa* with the help of a microscope by a phycological expert from the institute of plant science.

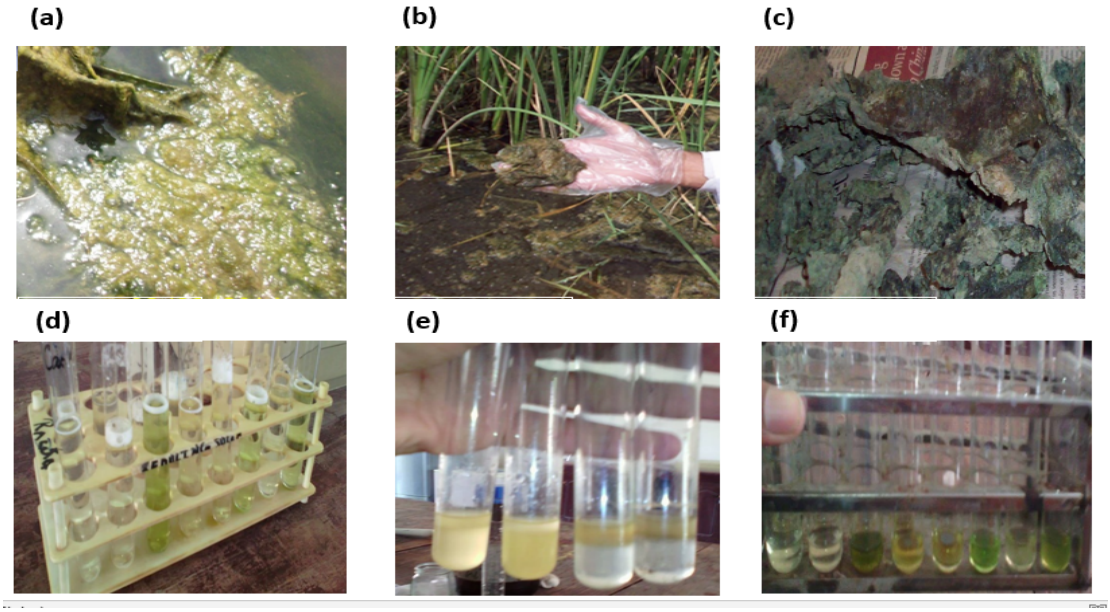

Figure 1. (a), (b) floating specimen at the water surface, (c) dried specimen at the laboratory, (d), (e), (f) various test tubes of 20% *Oscillatoria limosa* extracts at different laboratory testings.

2.2.2. Preparation of 20% extracts

Prepared 20% extracts through the method reported by Habib Naqvi et al., 2011. Briefly, for the preparation of 20% extracts of water, ethanol, methanol, and acetone, the dried material (coarse powder) of *Oscillatoria limosa* was dissolved in all the solvents at the ratio of 5g ml^{-25}, while the purity of all the organic solvents were 70%. For further proper extraction, all the solutions were centrifuged at 6000 rpm for 30 minutes, then stand stored at -40°C before any procedure of experiments or tests.

2.2.3. Determination of total and reducing sugar

Total and reducing sugar contents, analyzed from the extracts of *Oscillatoria limosa,* followed by the reported method of Miller (2002) and Montgomery (1961). Briefly, a single sample of every extract of 0.5 ml was mixed with concentrated H_2SO_4 and 50 µl of 80% phenol solution in separate Eppendorf tubes and rest them at room temperature for complete mixing with each other. Then, the solutions of Eppendorf tubes were taken for determination of total sugar in UV-spectrophotometer at 485 nm, as put up in R, Montgomery's method. Whilst, the test solution of 2.0 ml from every extract was taken in other Eppendorf tubes, mixed with 2.0 ml of dinitrosalicyclic acid for estimating the presence of reducing sugar. Finally, observed the absorbance at 540 nm of samples for verification of the presence of compounds in samples as followed previously reported method of Miller. All the experiments were repeated thrice for further confirmation of the results.

2.2.4. Determination of total protein

Total protein, determined by the method of Lowry et.al, with some variations (Lowry et al., 1951). Briefly, test samples of 0.5 ml of each extract were taken in Eppendorf tubes, then 2.5 ml of alkaline copper reagent was added with folin ciocalteus reagent (1:1 v/v with water) in each tube after that, tubes were left at room temperature for 30 minutes. The results were developed as described in Lowry et al.'s method. The total protein from samples of Eppendorf tubes was read against the blank of bovine serum calibrated a standard curve at 750 nm in UV-spectrophotometer.

2.2.5. Determination of total antioxidant, total phenolic, and total flavonoids

Total antioxidant, total phenolic, and total flavonoid of *Osillatoria limosa* were determined by applying the relevant method of Prieto et al. (1999), Yasoubi et al. (2007) and Djeridane et al. (2008)

with minor changes. Briefly, for total antioxidant activity, samples of 0.2 ml of each extract were mixed separately with 2 ml of reagent solution (28 mM sodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate) in Eppendorf tubes. All Eppendorf tubes were incubated at 95°C for 90 minutes, then kept at room temperature to cool down. The absorbance of each sample was measured at 695 nm against a blank standard curve of α-tocopherol and ascorbic acid, respectively.

Whilst, the 0.2 ml test sample of each extract was mixed with 1 ml of 10-fold diluted Folin-ciocalteu, and 0.8 ml $NaCO_3$ in an Eppendorf tube for quantification of phenolic contents. The result was read against the blank of Gallic acid calibrated a standard curve at 765 nm in UV-spectrophotometer.

The quantity of flavonoid was estimated through flavonoid–aluminum complex solution treated with 0.1 ml of every extract in separate Eppendorf tubes. Here Rutin was used for a standard calibration curve at the absorbance of 430 nm in a UV-spectrophotometer, compared with prepared samples of Eppendorf tubes for total flavonoids.

2.2.6. Antimicrobial activity

Antimicrobial activity of *Oscillatoria limosa* from its extracts in water, ethanol, methanol, and acetone was tested against common pathogenic fungi like *Aspergillus niger*, *Penicillium notatum*, and *Rhizopus spp*. While bacterial species like *Escherichia Coli*, *Agrobacterium tumefaciens*, *Staphylococcus aureus*, *Klebsiella aureus*, and *Pseudomonas aeruginosa*, were chosen for analyzing antibacterial activity. Through the agar-well-diffusion method, previously reported by Mothana and Lindequist (2005). İnitially, all the samples of bacteria and fungi were obtained from the microbiology laboratory of the Institute of Biotechnology and Genetic Engineering, University of Sindh.

2.2.7. Identification of free amino acids through thin layer chromatography (TLC)

Free amino acids were identified by the method of Thin Layer Chromatography (TLC) previously reported by Hudaib et al. (2016). Briefly, silica gel G-60 as stationary phase and butanol: acetic acid: water (5:1:4 v/v and 4:1:5 v/v) as the mobile phase was used. Thin layer gel plates were prepared and activated as described in the earlier method before applying the samples of extracts. The following amino acids, glycine, serine, leucine, cysteine, valine, aspartic acid, tryptophan, tyrosine, threonine, histidine, proline, glutamic acid, cystine, arginine, alanine, glutamine, isoleucine, asparagine, methionine, phenylalanine, hydroxyproline, and lysine were applied as standard amino acids, and their R_F noted through TLC spots, after preparing 2% (w/v) of aqueous amino acid samples.

3. Results and Discussion

The temperatures of water and environment at the spot of the collection of the *Oscillatoria limosa*, were 30 oC and 39 °C, respectively, while the pH of the pond water was 8.5. The pH of dried filamentous extracts was observed in acetone 8.9 pH, water 6.3 pH, methanol 7.1 pH, and ethanol 6.5 pH. The results are presented in Figures 2. (a) and (b).

3.1. Total sugar and reducing sugar

Carbohydrate is the main component of algal organisms which is considered a helpful source of health for humans in the form of nutrients, antioxidants, anticoagulants, and antiviral. Different types of carbohydrates are found abundantly in various algal species (Chennubhotla, 1996). In the present study, the total sugar concentration of *Oscillatoria limosa* was analyzed from extracts of four different solvents (water, acetone, methanol, and ethanol). Our results indicate that acetone extract has a higher concentration of about 9.531 mg ml^{-1} as compared with other extracts such as water, methanol, and ethanol with 5.40, 4.875, and 2.06 mg $ml^{-1,}$ respectively. While the reducing sugar concentration was determined in four extracts, the maximum concentration recorded in acetone was 4.689 mg ml^{-1}, while other samples from methanol, water, and ethanol extracts showed 4.65, 3.67, and 1.93 mg $ml^{-1,}$ respectively.

3.2. Total protein

Total protein concentration was measured from four extracts, the higher concentration of 3.34 mg ml^{-1} was noted in water extract as compared with methanol, acetone, and ethanol having a quantity of 2.86, 2.09, and 1.95 mg $ml^{-1,}$ respectively, by applying bovine serum as standard. All the results of total sugar, reducing sugar, and total protein are presented through graphs in Figure 2 (d).

3.3. Qualitative analysis of phytochemical

Phytochemicals are very important compounds present in plants and algae, generally, humans use them as antioxidants, antitumors, antimutagens, enzyme modulators, free radical scavengers while as well as antimicrobial agents (Rutikanga et al., 2017). Hereby this study, various important phytochemicals of *Oscillatoria Limosa,* like terpenoids, alkaloids, phenolic, flavonoids, phytosterol, glycosides, tannin, saponin, etc., were observed in extracts of solvents (ethanol, methanol, acetone, and water). Briefly, 32 tests were conducted to analyze various phytochemicals; however, we found 25 positive and 7 negative results. The detailed results are prescribed in Table 1.

Table 1. Qualitative analysis of different bioactive compounds from collected algal specie O*scillatoria limosa*

Phytochemicals	Ethanol	Acetone	Methanol	Water
Alkaloids	++	++	+++	+
Phytosterol	++	+	++	++
Phenolic	-	++	+	+
Flavonoids	-	+	+	+
Tannin	++	++	-	-
Terpenoids	+	++	-	-
Glycosides	++	++	-	+
Saponin	+	+++	+++	+

The + and - sign respectively used for the presence and absence of phytochemicals.

3.4.Total flavonoids

Total phenolic acid, flavonoids, and tannins are important biological chemicals present in medicinal plants which develop antioxidant, anti-carcinogenic, anti-atherosclerotic, and anti-inflammatory activities, etc. (Hemalatha et al., 2013). So, the presence of these compounds in any organism authenticates the medicinal significance of that organism. Here in this study, Rutin was used as standard, and the quantification of total flavonoid contents showed the highest result of 8.268 mg ml^{-1} observed in methanolic extract, while samples of ethanol, water, and acetone extracts contained 7.30, 6.99, and 2.35 mg ml^{-1}, respectively.

3.5. Phenolic quantity

For this study, gallic acid was applied as standard, and the maximum concentration of phenolic quantity noted in extracts from acetone of 2.937 mg ml^{-1}, while in water, methanol, and ethanol extracts showed 2.01, 0.33, and 0.26 mg ml^{-1} of phenolic quantity respectively.

3.6. Antioxidant

Total antioxidant activity was checked in all extracts of the organism. The result was noted in mg ml^{-1} by using α-tocopherol standard. The highest antioxidant activity 1.080 mg ml^{-1} was noted in methanolic extracts, followed by ethanol, water, and acetone of 0.559, 0.261, and 0.248 mg ml^{-1}, respectively. The results are presented through graphs in figure 2 (c)

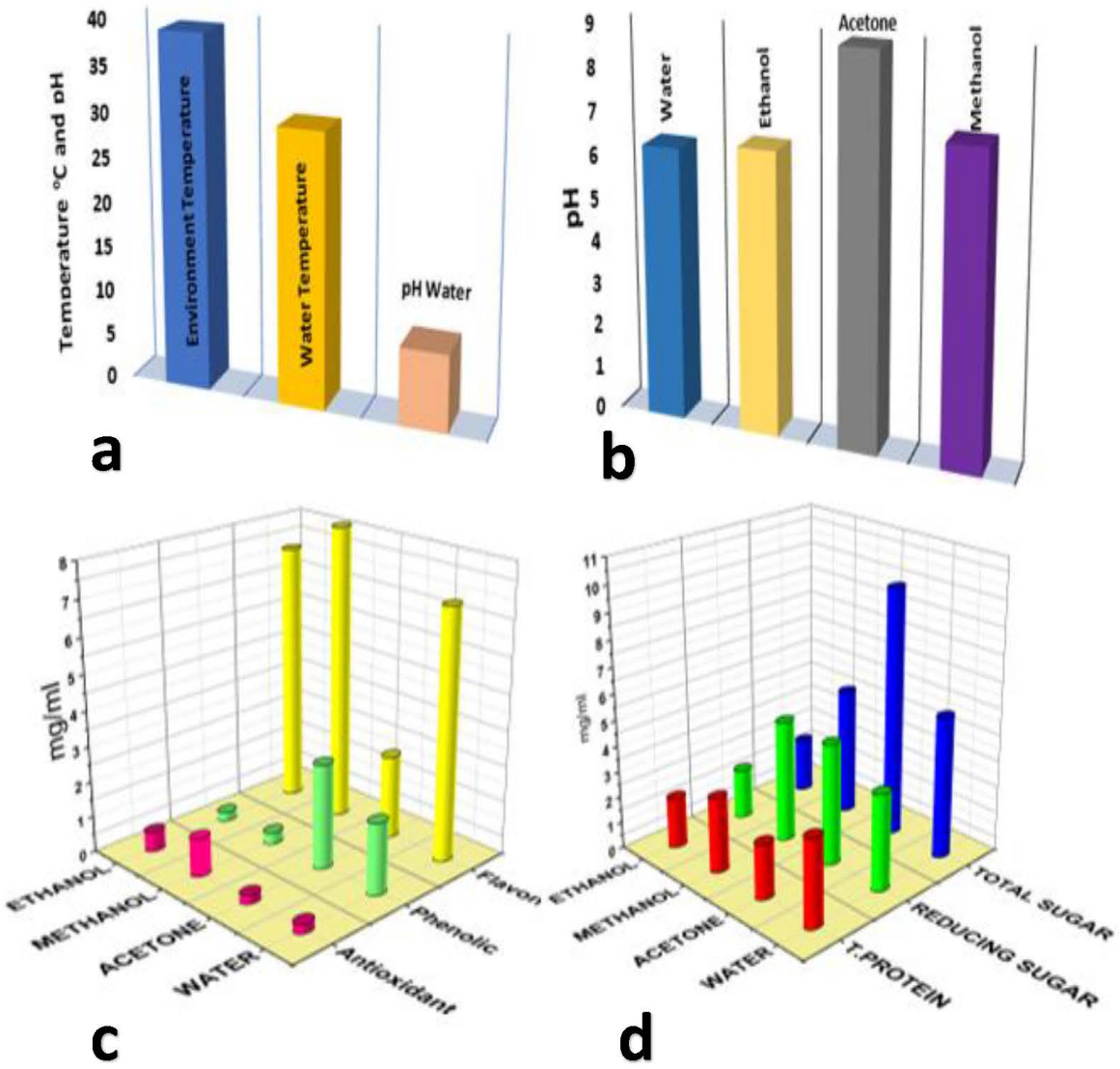

Figure 2. (a) Temperature and pH at the time of sample collection, (b) pH of different extracts of 20% *Oscillatoria limosa* floating specimen at the water surface, (c) antioxidant, phenolic, and flavonoids contents in extracts of different solvents (d) total sugar, reducing sugar and total proteins contents in extracts of different solvents.

3.7. Antimicrobial activity

Antibacterial activity of *Oscillatoria limosa* extracts was examined over five different bacterial species, *E.coli*, *A.tumifaciens*, *S.aerus*, *K.aerus,* and *P.aeruginosa.* The highest zone of inhibitions 31.2 mm was observed from methanol extract. While 10 samples were checked positive from all of the extract's samples, ethanol and *acetone* extracts showed activity against only *S. aureus* and *A.tumifacians,* while methanol extract showed activity against *K. aureus and E.Coli*, while the water extracts showed activity against four bacterial species, *S.areus*, *A.tumifacians, E.Coli,* and *P.aeruginosa.*

Antifungal activity of *Oscillatoria limosa,* over the pathogenic fungi like *A.niger*, *Rhizopus spp,* and *P.notatum* were observed using the agar well diffusion method. Methanol and aqueous extracts strongly inhibited the growth of *Rhizopus* spp and *P.notatum* 24.66 mm. While the minimum inhibition zone was observed from aqueous extracts of 9.0 mm against *P.notatum*. The acetone and methanolic extracts had potential against *A.niger* 11.333mm, 17.66 mm, respectively. In the current study, antifungal activity was checked over 12 samples of *O.limosa* (water, acetone, methanol, and ethanol). However, Six negative results were noted from samples of different extracts, those did not show any inhibition against specific fungus species. The detailed results of antimicrobial activities of *Oscillatoria limosa* are prescribed in Tables 2 and 3.

Table 2. Antibacterial Activity from 20 % algal extract of *Oscillatoria limosa* specie

	Antibacterial activity from 20% *Oscillatoria limosa* algal extracts			
	Ethanol	**Acetone**	**Methanol**	**Water**
Styphylococus aureus	12 ± 3*	14 ± 3*	Negative	26.33 ± 3.21*
A.tumifacians	10.33 ± 1.73*	15 ± 3*	Negative	12 ± 3*
Klebsiella aureus	Negative	Negative	30.4 ± 1.3*	Negative
Escherichia. Coli	Negative	Negative	31.2 ± 0.9*	10 ± 2.73*
Pseudomonas aeruginosa	Negative	Negative	Negative	10.33 ± 3.08*
Control	Negative	Negative	Negative	Negative

Zone of inhibition was measured in mm and ± standard deviation.

Table 3. Antifungal activity from 20% algal extract of *Oscillatoria limosa* specie

	Antifungal activity from 20% *Oscillatoria limosa* algal extracts			
	Ethanol	**Acetone**	**Methanol**	**Water**
Aspergillus Niger	Negative	11.33± 1.52*	17.66± 3.51*	Negative
Rhizopus sp	Negative	Negative	24 ± 2.64*	Negative
Penicillium notatum	Negative	Negative	11.66 ± 1.15*	9 ± 4.35*
Control	Negative	Negative	Negative	Negative

Zone of inhibition was measured in mm and ± standard deviation.

3.8. Free amino acids and free sugar

Free amino acids are generally recognized to serve as the principal currency of protein metabolism in the multicellular organism, and their concentrations are low compared with the quantities present in the protein-bound form. Free amino acid analysis determines the amount of each unbound individual amino acid i.e. not bound in a protein (Christensen, 1964). The free amino acid from each extract (acetone, ethanol, methanol, and water) of the algal species, *Oscillatoria Limosa,* was identified through the Thin-layer chromatography (TLC) method. In the present study, the R_F value of subjected amino acids was noted through TLC as standard. Their R_F values were recorded for comparing and matching with our results (R_F values of standards summarized in Table 4). However, only Leucine was found in the methanolic extract, showed in Table 5.

The standard R_F value of lactose, glucose, fructose, ribose and maltose were compared with R_F of *Oscillatoria Limosa's* extracts for identification of the presence of free Sugars through TLC. Only one R_F value of ethanol extract was matched with lactose standard R_F value, and one unknown R_F value of sugar was noted. These results are presented in Table 6.

Table 4. list of R_F values of various amino acids find out through TLC, used as standards

	Name of Amino acid	***R_F* Value of Standard Amino acids**		**Name of Amino acid**	***R_F* Value of Standard Amino acids**
1	**Aspartic acid**	0.001	12	**Hydroxyproline**	0.312
2	**Threonine**	0.279	13	**Tyrosine**	0.400
3	**Phenylalanine**	0.628	14	**Cysteine**	0.466
4	**Lucien**	0.496	15	**Cystine**	0.439
5	**Glycine**	0.406	16	**Arginine**	0.320
6	**Glutamic acid**	0.076	17	**Valine**	0.409
7	**Alanine**	0.304	18	**Serine**	0.252
8	**Proline**	0.545	19	**Glutamine**	0.426
9	**Lysine**	0.207	20	**Isoleucine**	0.433
10	**Tryptophan**	0.521	21	**Histidine**	0.404
11	**Asparagine's**	0.363	22		

Table 5. Result for free amino acid by TLC, only Lucine was found, with two unknown readings

Extracts	R_F values of Sample	Amino acid
Ethanol	0.91	Unknown
Acetone	No	
Methanol	0.50	Lucine
Water	0.94	Unknown

Table 6. Free sugars from different algal species by thin-layer chromatography (TLC) method

Name of Sugar	R_F Value of Standard Sugar	Extracts	R_F value of samples	Identification of Sugar
Lactose	0.849	Ethanol	0.725	Unknown
Glucose	0.938	Acetone	0.795	Unknown
Fructose	0.938	Methanol	No	
Ribose	0.319	water	No	
Maltose	0.885			

Conclusion

In the current study, we have analyzed bioactive compounds, phytochemicals, antifungal, antibacterial, and antioxidant activities of *Oscillatoria limosa* a species of cyanobacteria found around in the vicinity of district Jamshoro, Pakistan. We concluded that this organism possesses a significant amount of total sugar, reducing sugar, total proteins, and one free amino acid leucine, so it may serve as a better nutritional source for aquatic organisms. We have also observed the remarkable antioxidant activity, and the presence of a variety of phytochemicals, like phenolic acid, flavonoid, tannins, and others, so this organism can be used in pharmaceutical industries as an antitumor, antimutagen, enzyme modulator, and free radical scavenger. We have also observed that this specie can work as a powerful natural antimicrobial agent against a variety of bacteria and fungi.

Acknowledgments

The following research work and write-up of this research article were done in the Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro 76080, Pakistan. We acknowledge and thank Dr. Muhammad Aqeel Bhutto and Dr. Muhammad Rafique for their moral support and help.

References

Abed, R. M. M., Dobretsov, S., & Sudesh, K. (2009). Applications of cyanobacteria in biotechnology. *Journal of Applied Microbiology*, *106*(1), 1–12. https://doi.org/10.1111/J.1365-2672.2008.03918.X

Azizi, S. N., Hosseinzadeh Colagar, A., & Hafeziyan, S. M. (2012). Removal of Cd(II) from aquatic system using Oscillatoria sp. biosorbent. *The Scientific World Journal*, *2012*. https://doi.org/10.1100/2012/347053

Charan, T. R., Bhutto, M. A., Bhutto, M. A., Tunio, A. A., Khuhro, G. M., Khaskheli, S. A., & Mughal, A. A. (2021). "Nanomaterials of curcumin-hyaluronic acid": their various methods of formulations, clinical and therapeutic applications, present gap, and future directions. *Future Journal of Pharmaceutical Sciences*, *7*(1), 126. https://doi.org/10.1186/s43094-021-00281-9

Chennubhotla, V. S. (1996). Seaweeds and their importance. *CMFRI Bulletin-Artificial Reefs and Seafarming Technologies*, *48*, 108–109.

Christensen, H. N. (1964). Free Amino Acids and Peptides in Tissues. *Mammalian Protein Metabolism*, 105–124. https://doi.org/10.1016/B978-1-4832-3209-6.50011-6

Dahms, H. U., Ying, X., & Pfeiffer, C. (2006). Antifouling potential of cyanobacteria: a mini-review. *Biofouling*, *22*(5–6), 317–327. https://doi.org/10.1080/08927010600967261

Das, S. K. (2016). Dietary use of algae among tribal of North-east India: Special reference to the monpa tribe of Arunachal Pradesh. *Indian Journal of Traditional Knowledge*, *15*(3), 509–513.

Djeridane, A., Yousfi, M., Nadjemi, B., Maamri, S., Djireb, F., & Stocker, P. (2006). Phenolic extracts from various Algerian plants as strong inhibitors of porcine liver carboxylesterase. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *21*(6), 719–726. https://doi.org/10.1080/14756360600810399

Habib Naqvi, S., Umar Dahot, M., Rafiq, M., Yakoub Khan, M., Ibrahim, I., Lashari, K. H., Ali, A., & Korai, A. L. (2011). Anti-microbial efficacy and biochemical analysis from different parts of Acacia nilotica L. and Ricinus communis L. extracts. *Journal of Medicinal Plant Research*, *5*(27), 6299–6308. https://doi.org/10.5897/JMPR11.533

Hemalatha, A., Girija, K., Parthiban, C., Saranya, C., & Anantharaman, P. (2013). Antioxidant properties and total phenolic content o f a marine diatom, Navicula clavata and green microalgae, Chlorella marina and Dunaliella salina. *Undefined*.

Hemavani, C., &Thippeswamy, B. (2012). *Evaluation of antimicrobial property of Spirogyra species*. *2*(2), 13–15.

Hudaib, T., Brown, S., Wilson, D., &Eady, P. E. (2016). Identification of Free Amino Acids in Several Crude Extracts of Two Legumes Using Thin-Layer Chromatography. *JPC – Journal of Planar Chromatography – Modern TLC*, *29*(2), 145–147. https://doi.org/10.1556/1006.2016.29.2.9

Kajiyama, S. I., Kanzaki, H., Kawazu, K., & Kobayashi, A. (1998). Nostofungicidine, an antifungal lipopeptide from the field-grown terrestrial blue-green alga Nostoc commune. *Tetrahedron Letters*, *39*(22), 3737–3740. https://doi.org/10.1016/S0040-4039(98)00573-5

Lem, N. W., & Glick, B. R. (1985). Biotechnological uses of cyanobacteria. *Biotechnology Advances*, *3*(2), 195–208. https://doi.org/10.1016/0734-9750(85)90291-5

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Proteın measurement wıth the folın phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Luu, P. T., Duy, N. D., & Long, L. T. (2019). Toxicity of the cyanobacteria Ocillatoria limosa isolated from Tri An reservoir. *Journal of Biology*, *41*(January), 467–474. https://doi.org/10.15625/0866-7160/v41n2se1

Miller, G. L. (2002). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/AC60147A030

Montgomery, R. (1961). Further studies of the phenol-sulfuric acid reagent for carbohydrates. *Biochimica et Biophysica Acta*, *48*(3), 591–593. https://doi.org/10.1016/0006-3002(61)90059-2

Mothana, R. A. A., &Lindequist, U. (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. *Journal of Ethnopharmacology*, *96*(1–2), 177–181. https://doi.org/10.1016/J.JEP.2004.09.006

Papendorf, O., König, G. M., & Wright, A. D. (1998). Hierridin B and 2,4-dimethoxy-6-heptadecyl-phenol, secondary metabolites from the cyanobacterium Phormidium ectocarpi with antiplasmodial activity. *Phytochemistry*, *49*(8), 2383–2386. https://doi.org/10.1016/S0031-9422(98)00440-3

Papke, U., Gross, E. M., & Francke, W. (1997). Isolation, identification and determination of the absolute configuration of Fischerellin B. A new algicide from the freshwater cyanobacterium Fischerella muscicola (Thuret). *Tetrahedron Letters*, *38*(3), 379–382. https://doi.org/10.1016/S0040-4039(96)02284-8

Patterson, G. M. L., Larsen, L. K., & Moore, R. E. (1994). Bioactive natural products from blue-green algae. *Journal of Applied Phycology*, *6*(2), 151–157. https://doi.org/10.1007/BF02186069

Piwowar, A., & Harasym, J. (2020). The importance and prospects of the use of algae in agribusiness. *Sustainability (Switzerland)*, *12*(14), 1–13. https://doi.org/10.3390/su12145669

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, *269*(2), 337–341. https://doi.org/10.1006/ABIO.1999.4019

Reshef, V., Mizrachi, E., Maretzki, T., Silberstein, C., Loya, S., Hizi, A., & Carmeli, S. (1997). New acylated sulfoglycolipids and digalactolipids and related known glycolipids from cyanobacteria with a potential to inhibit the reverse transcriptase of HIV-1. *Journal of Natural Products*, 60(12), 1251-1260.

Rutikanga, A., Gitu, L., Oyaro, N., & Chacha, S. (2017). Mineral composition, antioxidant and antimicrobial activities of freshwater algae (Spirogyra genus). *Journal of Agricultural Science and Technology, JKUAT*. http://localhost/xmlui/handle/123456789/3111

Swanson-Mungerson, M. A., Incrocci, R., Subramaniam, V., Radowska, K., Hall, M., Williams, P., & Mayer, A. (2018). Oscillatoria sp. LPS activates human monocytes in a manner that is distinct from E. coli LPS. *The Journal of Immunology*, *200*(1 Supplement).

Swanson-Mungerson, M., Incrocci, R., Subramaniam, V., Williams, P., Hall, M. L.,& Mayer, A. M. S. (2017). Effects of cyanobacteria Oscillatoria sp. lipopolysaccharide on B cell activation and Toll-like receptor 4 signaling. *Toxicology Letters*, *275*, 101. https://doi.org/10.1016/J.TOXLET.2017.05.013

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., Smith, A. G., Camire, M. E., & Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. In *Journal of Applied Phycology* (Vol. 29, Issue 2, pp. 949–982). Springer Netherlands. https://doi.org/10.1007/s10811-016-0974-5

Yasoubi, P., Barzegar, M., Sahari, M. A., & Azizi, M. H. (2007). Total Phenolic Contents and Antioxidant Activity of Pomegranate (Punica granatum L.) Peel Extracts. *J. Agric. Sci. Technol*, *9*, 35–42.