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Evaluation of Phytochemical Analysis and Antibacterial Activity of *Chara fibrosa* and *Cladophora crispata*

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ABSTRACT: This study evaluates the antibacterial activities of various solvent extracts from the freshwater algae *Chara fibrosa* and *Cladophora crispata.* The agar well diffusion method was utilized to evaluate the antibacterial activity against three types of bacteria, namely *E. coli*, *S. marcescens* and *S. aureus.* The inhibition zones measured for all crude extracts demonstrated a broad spectrum of antimicrobial activity against the tested pathogens. The fresh water algal biomass's FTIR spectrum investigation revealed the existence of organic chemical groups like C-Br, C-N, C-O and C=C. These findings suggest that the biomass of *Chara fibrosa* and *Cladophora crispata* constitutes a sustainable green resource. The observed antibacterial activity implies that the active constituents within the freshwater algae extracts can be harnessed for the development of innovative pharmaceuticals, potentially benefiting both medical and agricultural applications.

Keywords: freshwater algae, FTIR, antibacterial activity, agar well diffusion method.

INTRODUCTION

Algae are macro-macroscopic, unicellular or multicellular plant-like organisms with a highly variable composition depending on species, habitat, light intensity, temperature, and nutrients nutrient concentrations in the water (Leonel, 2021). They can be classified into three major group: brown algae, red algae, and green algae. Algae are the most vital primary producers in the biosphere. They are fascinating organisms that play a vital role in the environment, but they also offer numerous benefits for human health and industry due to their rich composition of bioactive compounds. These compounds are natural substances that have effects on living organisms and can provide health benefits or serve as raw materials for various applications (Makkae *et al.,* 2022).

Algae have a very attractive source of antibacterial agents and contribute various advantages for antimicrobial investigations due to their fast growth rate and great biodiversity (Karthikeyan *et al.,* 2022). They are rich in a variety of bioactive compounds that hold significant pharmaceutical value. These include proteins, lipids, vitamins, enzymes, sterols, and fatty acids such as oleic acid, linoleic acid, and palmitoleic acid. Additionally, they are sources of essential vitamins like A, C, E, D, and B12, as well as important carotenoids such as β-carotene, phycocyanin, lutein, and zeaxanthin (Menaa *et al.,* 2021; Falaise *et al.,* 2016; Koyande *et al.,* 2019). Among the key bioactive components of algae that demonstrate antimicrobial potential, the most significant include polysaccharides, proteins, polyunsaturated fatty acids (PUFAs) like Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA), as well as antioxidants such as polyphenols, carotenoids, flavonoids, and amino acids (Vahdati *et al.,* 2022; Manirafasha *et al.,* 2016; Shinge and Shivasharana 2019).

The growing scientific and commercial interest in the utilization of genetic resources is also of significant importance to international policymakers. Algae are rich in unusual and fascinating biochemical characteristics, which are crucial to their antibacterial and antifungal actions. Additionally, a wide array of in vitro antibacterial activities of extracts from freshwater algae has been reported (Bhattacharjee, 2016; (Rekha and Sujathamma 2020).

In vitro antibacterial activity against both Gram-positive and Gram-negative bacteria has been demonstrated by cell extracts and active components derived from these freshwater algae. Therefore, algal extracts possess enormous therapeutic potential, serving as effective antibacterial agents with fewer side effects compared to synthetic alternatives (Alsenani *et al.,* 2020).

Algal extracts are rich in various biomolecules FTIR can help characterize these biomolecules, providing insights into their potential health benefits and applications. Combining FTIR analysis with antibacterial studies helps understand the composition of algal extracts and their potential as sources of natural antibacterial agents (Lauritano *et al.,* 2016). This approach aids in identifying the functional groups responsible for antibacterial activity and optimizing extraction methods for maximum efficacy (Safari *et al.,* 2019). This integrated approach not only advances the understanding of the chemical nature and biological

effects of algal extracts but also contributes to the discovery of novel antibacterial substances with potential applications in various industries.

MATERIALS AND METHODS

Study Area. Algal samples were collected from two distinct freshwater sites in the Sabarkantha district, Gujarat, India: Aadesh Pond in Talod Village (Latitude: 23° 20' 16.53" N: Longitude: 72° 56' 51.45" E) and Maucha Pond in Maucha Village (Latitude: 23° 29' 28.23" N: Longitude: 73° 6' 46.74" E). Freshwater algae species *Chara fibrosa* and *Cladophora crispata* were harvested from these locations. The samples were immediately transferred to the laboratory in polyethylene bags containing pond water to minimize degradation. In the laboratory, the algal samples were meticulously washed with tap water to eliminate any extraneous matter, including debris and epiphytes. The cleaned samples were air-dried in the shade to a constant weight to prevent photo degradation of bioactive compounds and subsequently stowed at low temperature for future Study.

Preparation of Algal Extracts. The algal biomass that had been shade-dried was weighed and then ground into a fine powder using a mortar and pestle. Then, ten grams of the powdered algae were extracted using a variety of solvents, including acetone, methanol, ethanol and distilled water. Each solvent extraction materials were soaked in 100 milliliters of the respective solvent for 48 hours at room temperature, with intermittent shaking to ensure maximum extraction of bioactive compounds (Vaou *et al.,* 2021). The mixtures were filtered through Whatman filter paper after extraction to provide clean filtrates. Through the use of a rotating evaporator, these filters were concentrated at lower pressure and stowed at low Temperature in airtight container for study of antibacterial assays.

ANTIBACTERIAL ASSAY

Test of Micro Organisms. Three Bacterial strains were used to assess the algal extracts' antibacterial efficacy and they are *Serratia marcescens* (ATCC 13880), *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). These strains were obtained from the MTCC, ensuring the use of standardized reference strains.

Preparation of Inoculum. To prepare the bacterial inoculum, a loopful of each microorganism from a Nutrient broth (pH 7.4) was used to inoculate a 24-hour-old nutrient agar slant, which was then incubated for 24 hours at 37°C. This development period allowed the bacteria to reach the exponential (log) phase of growth, ensuring a high density of viable cells (Sanders, 2012). The cultures' optical density (OD) was calibrated to the 0.5 McFarland standard in order to standardize the inoculum density for the antibacterial laboratory experiment. Uninoculated nutrient broth served as a negative control throughout the experiments.

Antimicrobial Activity Assessment Using the Disc Diffusion Method. Preparation of Agar Plates. The agar well diffusion method was utilized to assess the antibacterial activity of the fresh water algal extracts. The pH of the produced MHA medium was adjusted to 7.4. After that, medium was sterilized by autoclaved for 15 minutes at 121°C and 15 pressure. After autoclaving, Twenty milliliters of the sterilized MHA were transferred into aseptic Petri dishes and left to harden at room temperature (Aneja, 2003).

Inoculation of Test Microorganisms. The test microorganisms, which included *S. aureus*, *S. marcescens*, and *E. coli*, were cultivated in nutritional broth and incubated for a whole day at 37°C. Once incubated, sterile cotton swabs were used to evenly spread the bacterial suspensions onto the surface of the solidified MHA plates. Each bacterial strain was inoculated on separate MHA dish. To make sure the bacterial inoculum was absorbed into the agar, the infected dish was let to stand for a short while (Petersen and McLaughlin 2016).

Well Preparation and Application of Algal extracts. Sterile cork borers were used to punch wells of 5 mm diameter into the centre of the inoculated MHA plates. Each well was carefully loaded with fifteen microliter of the respective fresh water algal biomass extract. Than extracts were allowed to diffuse into the agar at room temperature for 30 minutes. Control plates, with wells containing no extracts, were also prepared to compare and ensure the validity of the results by excluding any potential contamination.

Incubation and Measurement of Inhibition Zones. The plates loaded with algal extracts were incubated at 37°C for whole day. Post incubation, the appearance of antibacterial activity was showed by clear zones of inhibition surrounding the wells. These zones were calculated in millimeters, together of diameter of the well, using a calibrated ruler. The diameters of the inhibition zones were recorded to evaluate the antibacterial efficacy of the algal extracts against the test microorganisms.

FTIR Spectroscopy Methodology. The FTIR spectroscopy was employed to analyze the functional groups present in the algal extracts of *Cladophora crispata* and *Chara fibrosa*. The rationale behind using FTIR is its ability to provide detailed insights into the chemical composition of the extracts by identifying the specific functional groups and molecular bonds. This information is crucial for understanding the bioactive compounds in these algae, which could be responsible for their antibacterial properties (Patel and Patel 2006).

The algal biomass was shade-dried and then finely ground using an agate mortar and pestle to prevent contamination. Approximately 10 milligrams of the powdered sample was mixed with 100 milligrams of dry KBr to create a homogenous mixture. This mixture was compressed into a thin, transparent pellet using a hydraulic press under a pressure of 10 tons for about 5 minutes. The prepared KBr pellets were analyzed immediately using an FTIR spectrometer, scanning in the spectral range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}. The recorded spectra provided specific absorption bands that correspond to various functional groups, offering insights into the chemical composition and potential bioactive constituents of the algal extracts (Catherine and Rainer 2009).

RESULTS AND DISCUSSION

Results of Qualitative Phytochemical Analysis (FTIR). FTIR spectroscopy was chosen for the qualitative phytochemical analysis of *Chara fibrosa* and *Cladophora crispata* extracts to detect the functional groups present in the extracts. This technique is particularly useful because it allows the identification of chemical bonds within the compounds, offering a direct link between the observed functional groups and the potential biological activities of the algae.

The identification of these functional groups is critical as they contribute to the biological activity of the algal extracts, including their potential as antibacterial agents. The comprehensive functional group analysis presented in Table 1 underscores the diverse range of bioactive compounds in these algal species, highlighting their potential for pharmaceutical applications.

Table 1: Range of major FTIR band assignments reported for *Chara fibrosa* analysis.

Sr. No.	Wave number range	Assignments (stretches/bends)	Functional Group	Comments
1.	437.84	C - Br	Halo compound	Halo compound
2.	713.66	C = C	Alkene	Alkene
3.	875.68	C = C	Alkene	Alkene
4.	958.62	C - H	Alkane	Alkane
5.	1008.77	C - F	Fluoro compound	Fluoro compound
6.	1024.2	C - N	Amine	Amine
7.	1037.7	S = O	Sulfoxide	Sulfoxide
8.	1147.65	C - O	Aliphatic ether	Aliphatic ether
9.	1244.09	C - N	Amine	Amine
10.	1433.11	O - H	Carboxylic acid	Carboxylic acid
11.	1444.68	C - H	Alkane	Alkane
12.	1454.33	C - H	Alkane	Alkane
13.	1795.73	C = O	Conjugatedacidhalide	Conjugatedacidhalide
14.	2513.25	O - H	Carboxylicacid	Carboxylicacid
15.	2848.86	C - H	Alkane	Alkane
16.	3273.2	O - H	Carboxylicacid	Carboxylicacid

The FTIR spectrum analysis of the *Chara fibrosa* extract revealed a variety of functional groups and compound classes, contributing to the chemical profile of the extract. For example, the presence of C-Br bending vibration at 473.84 cm^{-1} and other groups like C=C bending vibrations at 713.66 cm^{-1} and 875 cm^{-1} indicated alkene groups, while the C-F stretching peak at 1008.77 cm^{-1} confirmed fluoro compounds. The presence of amines was indicated by C-N stretching vibrations at 1024.2 cm^{-1} and 1244.09 cm^{-1}. These functional groups, among others, likely contribute to the extract's biological activities.

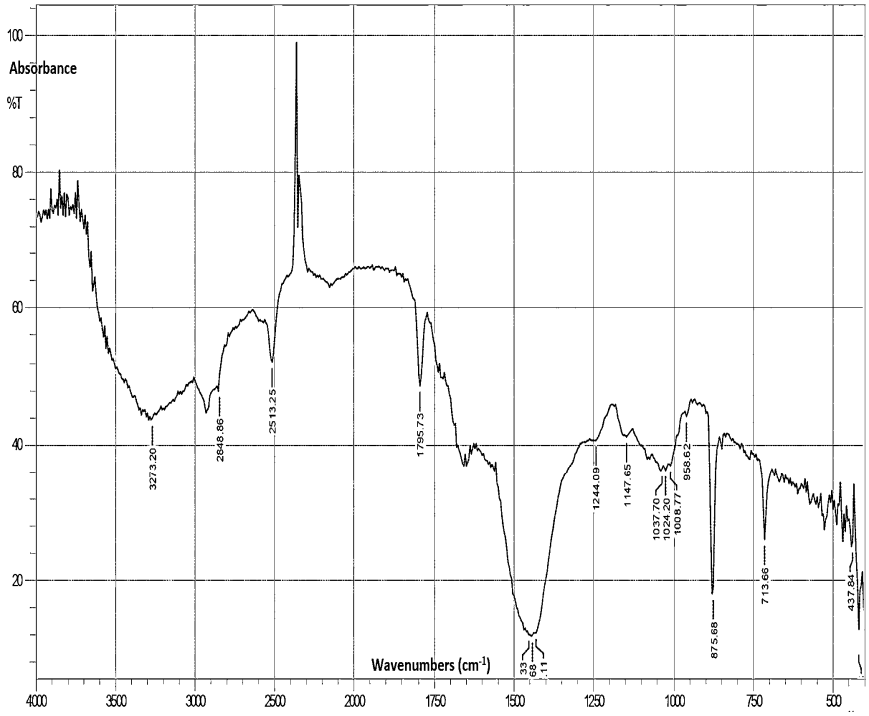

Fig. 1. FTIR Spectra of Algal Extracts from *Chara fibrosa.*

The extract also contained sulfoxides, as evidenced by an S=O stretching peak at 1037.7 cm^{-1}. Aliphatic ethers were identified through a C-O stretching peak at 1147.65 cm^{-1}. Carboxylic acids were confirmed by O-H bowing down to 1433.11 cm^{-1} and other O-H stretching at 2513.25 cm^{-1} and 3273.2 cm^{-1}. Alkane groups were detected with C-H bending vibrations at 958.25 cm cm^{-1}, 1444.68 cm^{-1} and 1454.33 cm^{-1}, and a C-H stretching peak at 2848.86 cm^{-1} (Nandiyanto *et al.,* 2019).

Furthermore, a C=O stretching peak at 1795.73 cm^{-1} indicated the presence of conjugated acid halides. These results highlight the diverse chemical composition of the *Chara fibrosa* extract, reflecting a range of functional groups that may contribute to its biological activity and potential applications in various fields.

Similarly, the FTIR spectrum of the *Cladophora crispata* extract displayed a range of functional groups, including halo compounds indicated by C-Br stretching vibrations and alkenes confirmed by C=C bending vibrations. The presence of various amines, alcohols, esters, and phenols, as detailed in Table 2, suggests a complex chemical composition that could be responsible for the extract's bioactive properties.

The algal extract displayed multiple peaks corresponding to halo compounds, with strong C-Br stretching vibrations observed at 462.20, 526.57, 555.5, 615.29, and 667.37 cm^{-1}. These peaks suggest the presence of brominated halo compounds in the extract. Additionally, the spectrum revealed an alkene group, characterized by a C=C bending vibration at 713.66 cm^{-1} and 875.68 cm^{-1}. Fluoro compounds were identified with a C-F stretching peak at 1008.77 cm^{-1}, although no antibacterial activity was associated with this group. The analysis also identified several amine groups, with C-N stretching vibrations observed at 1022.27, 1035.77, 1076.28, and 1242.16 cm^{-1}. This indicates the presence of various amine derivatives, including aromatic amines, which were identified with a C-N stretching vibration at 1336.67 cm^{-1} (Nandiyanto *et al.,* 2019).

Table 2: Range of major FTIR band assignments reported for *Cladopho racrispata* analysis.

Sr. No.	Wave number range	Assignments (stretches/bends)	Functional Group	Comments
1.	462.20	C - Br	Halo compound	Halo compound
2.	526.57	C - Br	Halo compound	Halo compound
3.	555.50	C - Br	Halo compound	Halo compound
4.	667.37	C - Br	Halo compound	Halo compound
5.	713.66	C - Cl	Alkene	Alkene
6.	875.68	C - Cl	Alkene	Alkene
7.	1008.77	C - F	Fluoro compound	Fluoro compound
8.	1022.27	C - N	Amine	Amine
9.	1035.77	C - N	Amine	Amine
10.	1076.28	C - N	Amine	Amine
11.	1112.93	C - O	Secondary alcohol	Secondary alcohol
12.	1161.15	C - O	Tertiary alcohol	Tertiary alcohol
13.	1207.44	C - O	Ester	Ester
14.	1242.16	C - N	Amine	Amine
15.	1336.67	C - N	Aromatic amine	Aromatic amine
16.	1382.86	O - H	Phenol	Phenol
17.	1433.11	O - H	Carboxylic acid	Carboxylic acid
18.	2852.72	C - H	Aldehyde	Aldehyde
19.	3269.34	O - H	Carboxylic acid	Carboxylic acid

The Functional groups associated with alcohols and esters were also present. Secondary alcohols were indicated by a C-O stretching peak at 1112.93 cm^{-1}, while tertiary alcohols were detected at 1161.15 cm^{-1}. An ester group was identified by a C-O stretching peak at 1207.44 cm^{-1}. An O-H bending peak at 1382.86 cm^{-1} indicated the presence of phenol and carboxylic acids were detected with O-H stretching at 3269.34 cm^{-1} and O-H bending at 1433.11 cm^{-1}. An O-H bending peak at 1382.86 cm^{-1} indicated the presence of phenol and carboxylic acids were detected with O-H stretching at 3269.34 cm^{-1} and O-H bending at 1433.11 cm^{-1}. Furthermore, the extract contained aldehyde groups, indicated by C-H stretching at 2852.72 cm^{-1}. Overall, the FTIR results demonstrate a diverse range of functional groups in *Cladophora crispata* extract, which may contribute to its bioactivity and potential applications.

The identification of these functional groups in both algal extracts supports their potential use in developing novel bioactive compounds for pharmaceutical applications, particularly as antibacterial agents.

Result of antibacterial activity. The agar well diffusion method was used to assess the antibacterial activity of the extracts of *Chara fibrosa* and *Cladophora crispata*. Table 3 provides an overview of the outcomes, which presents the zone of inhibition measured against three bacterial strains: *Escherichia coli*, *Serratia marcescens*, and *Staphylococcus aureus*.

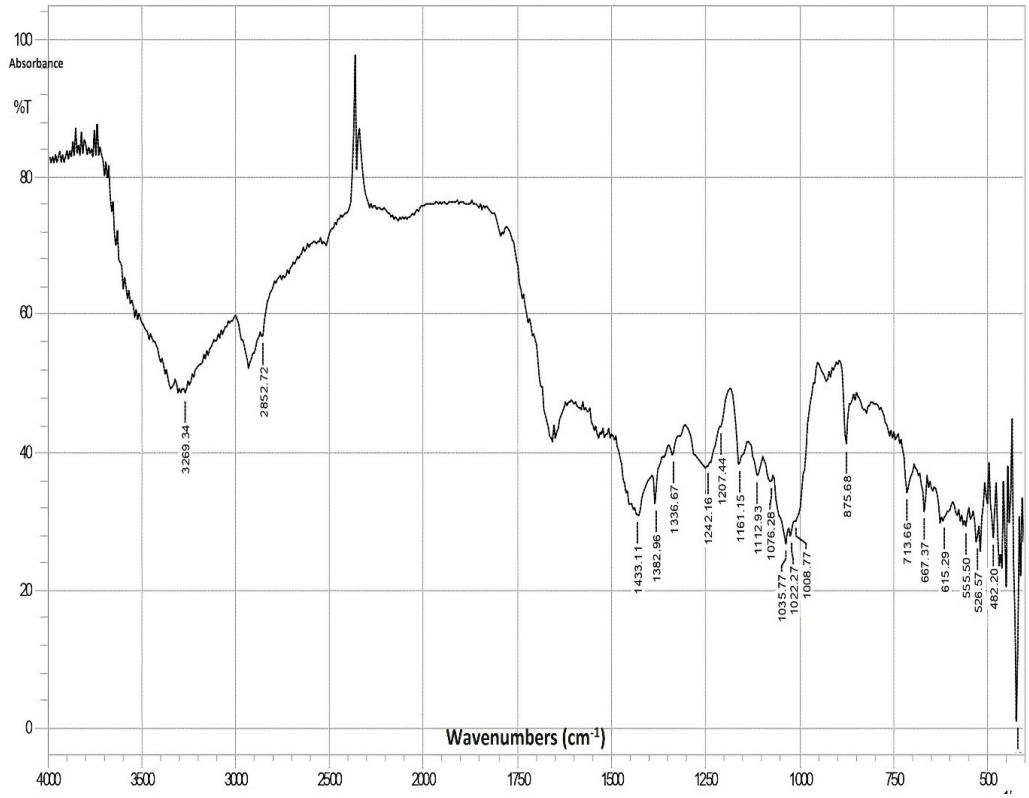

Fig. 2. FTIR Spectrum of *Cladophora crispata* Extract.

Table 3: Data of antibacterial activity of algal extracts

Sr. No.	Name of Sample	Concentration	Test Microorganism	Zone of Inhibition (mm)
1.	*Chara fibrosa*	15 µl	*E. coli*	12.0 ± 0.5
2.	*Cladophora crispata*	15 µl	*E. coli*	15.0 ± 0.7
3.	*Chara fibrosa*	15 µl	*Serratia marcescens*	12.0 ± 0.4
4.	*Cladophora crispata*	15 µl	*Serratia marcescens*	17.0 ± 0.6
5.	*Chara fibrosa*	15 µl	*Staphylococcus aureus*	No zone observed
6.	*Cladophora crispata*	15 µl	*Staphylococcus aureus*	No zone observed

Each value represents the mean of three replicates (n=3) ± standard deviation (SD).
"No zone observed" indicates no antibacterial activity was detected in all replicates

As demonstrated in Table 3, both *Chara fibrosa* and *Cladophora crispata* extracts exhibited *Serratia marcescens, Staphylococcus aureus* and *E. coli* of the antibacterial action. They aqueous extracts showed varying degrees of inhibition. Specifically, *Chara fibrosa* yielded a zone of inhibition of 12 millimeter against *Escherichia coli*, while *Cladophora crispata* produced a slightly larger zone of 15 mm. for *Serratia marcescens*, *Chara fibrosa* exhibited a 12 mm inhibition zone, whereas *Cladophora crispata* demonstrated a more substantial inhibition with a zone measuring 17 mm.

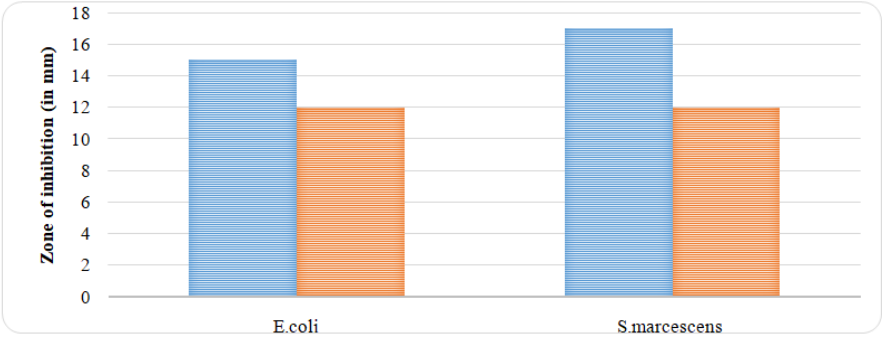

Fig. 3. Comparative antibacterial activity of *Chara fibrosa* and *Cladophora crispate* extracts against *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus.*

Neither *Chara fibrosa* nor *Cladophora crispata* showed any antibacterial activity against *Staphylococcus aureus*, as no inhibition zones were observed for this bacterial strain. In conclusion, the extracts from both algal species effectively inhibited the growth of *E. coli* and *Serratia marcescens*, with *Cladophora crispata* showing superior activity compared to *Chara fibrosa*. The results suggest a higher antibacterial efficacy of both algal extracts against *Serratia marcescens* compared to *E. coli*, indicating that *Serratia marcescens* is more susceptible to the antibacterial effects of these algae.

CONCLUSIONS

This research provides a preliminary investigation into the antibacterial potential of the freshwater microalgae *Chara fibrosa* and *Cladophora crispata*, assessing their efficacy against prominent bacterial pathogens including *Escherichia coli*, *Serrati amarcescens*, and *Staphylococcus aureus*. The antibacterial activity of the algal extracts was assessed using the agar well diffusion method in this investigation.

Which demonstrated notable effectiveness against *E. coli* and *S. marcescens*, but no activity against *S. aureus*. The results highlight the significant antibacterial properties of these microalgae, attributable to the presence of bioactive compounds such as phenolics, amines, aromatic cyclic compounds, and halo-compounds. These compounds were identified through FTIR analysis, revealing diverse functional groups that potentially contribute to the observed antimicrobial activity (Suciyati *et al.,* 2021). Specifically, *Cladophora crispata* exhibited superior antibacterial activity compared to *Chara fibrosa*, particularly against *S. marcescens*, indicating a higher efficacy of the former in inhibiting bacterial growth. This study underscores the potential of utilizing natural phycocompounds from these algal species in the drug discovery process. The promising antibacterial activity observed suggests that *Chara fibrosa* and *Cladophora crispata* could serve as valuable sources of novel antimicrobial agents (Asmaa *et al.,* 2021).

FUTURE SCOPE

Future studies will concentrate on doing additional pharmacological assessments, such as in silico analyses to evaluate the active compounds features related to absorption, metabolism, excretion, and ADMET, as well as molecular docking studies to explore interactions between these non-toxic compounds and target proteins (Chowdhury and Sruthi 2021). Comprehensive assessments like this will open the door for the creation of new, effective antimicrobial drugs derived from these algal resources, potentially contributing to more sustainable and eco-friendly alternatives in drug development.

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Conflicts of Interest. None.

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