

ANTIMICROBIAL ACTIVITY OF SOME POTENTIAL GREEN ALGAL STRAINS ISOLATED FROM BUNDELKHAND REGION UTTAR PRADESH

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ABSTRACT

Objective

To study antimicrobial activity of some potential green algal strains viz., *Scenedesmus abundans*, *Nannochloropsis oculata* and *Spirogyra condensata*, against four human bacterial pathogens.

Methods

The characterization of the antimicrobial activity was used disc diffusion method against four human pathogenic bacteria viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*. Streptomycin disc were used as a positive control and only solvent were used as a negative control.

Results

The methanolic extract of *Scenedesmus abundans*, *Nannochloropsis oculata*, and *Spirogyra condensata* showed the antibacterial activity against four pathogenic bacteria viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* with the inhibition zone (15, 16, 22, 17 mm) (15,15,26,17mm) and (25, 16, 26, 20mm) respectively. The ethanolic extract of *Scenedesmus abundans*, *Nannochloropsis oculata*, and *Spirogyra condensata* showed the antibacterial activity against four pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* with the inhibition zone (20, 23, 23,21 mm) (19, 25,33, 24 mm) and (24,23,32,22 mm) respectively. The acetone extract of *Scenedesmus abundans*, *Nannochloropsis oculata*, and *Spirogyra condensata* showed the antibacterial activity against four pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa*, with the inhibition zone (18, 34, 23, 20 mm) (00, 17, 28, 12 mm) and (17,13,15,15) respectively. The di ethyl ether extract of *Scenedesmus abundans*, *Nannochloropsis oculata*, and *Spirogyra condensata* showed the antibacterial activity against four pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* with the inhibition zone (18, 29, 17, 15 mm) (00, 10, 12, 15 mm) and (00,15,8,9 mm) respectively. Streptomycin disc showed the antibacterial activity against four pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* with the inhibition zones of 28, 15, and 22 mm respectively.

Conclusions

The present studies conclude that acetone extract of *Scenedesmus abundans* showed the maximum antibacterial activity against *Staphylococcus aureus* with the inhibition zone 34 mm. The ethanolic extract of *Nannochloropsis oculata* showed the maximum antibacterial activity against *Bacillus cereus* with the inhibition zone 33 mm. The ethanolic extract of *Spirogyra condensata* showed the maximum antibacterial activity against *Bacillus cereus* with the inhibition zone 32 mm with reference to antibiotic disc Streptomycin showed antibacterial activity against *Staphylococcus aureus* with the inhibition zone 15 mm where as antibacterial activity against *Bacillus cereus* with the inhibition zone 28 mm. Further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extract against bacteria.

KEYWORDS: Green Algae *Scenedesmus Abundans*, *Nannochloropsis Oculata*, *Spirogyra Condensata* Antimicrobial Activity, Bundelkhand & Region

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INTRODUCTION

Since ancient times the emergence of modern approaches, the drug discovery and the pace of drug development has slowed down, because of lack of proper lead in biomolecules, which is crucial to designing newer drug [6]. Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug-resistant infectious disease and more and more upcoming disorder causing by bacteria. The terrestrial resources have been greatly explored and the academic and industry researchers are striving to get lead molecules from the inner space of oceans [8]. Microalgae are a diverse group of photosynthetic microorganisms found in everywhere like ocean, lakes, rivers, ponds, puddles, moist surfaces and fresh water etc [11]. Microalgae represent a unique opportunity to discover novel metabolites at lower costs. However microbial metabolites produced by microalgae. Microalgae are highly potential source of bioactive molecules which are able to produce some biological activities such as antibacterial, antiviral, antifungal and anticancer [1]. These organisms are rich source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes and cyclic polysulphides [9, 10]. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interests in the pharmaceutical industry [2, 3, and 4]. The bioactive compound from the algae is investigated for their antimicrobial activities as the pathogenic microbes are becoming resistant to the synthetic drug. According to the world Health Organisation (WHO), approximately 80 % of the world population depends on traditional remedies for their primary health care needs [16]. Microalgae have for long time been used with therapeutic purposes and their systematic screening for biologically active compounds began in 1950s. The different solvent extracts from microalgae were tested against gram positive and gram negative bacteria. The antimicrobial compounds are fatty acids, acrylic acids, halogenated aliphatic compounds, terpenes, sulphur containing hetero cyclic compounds, carbohydrates and phenols [20]. Temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing the production of antimicrobial agents [5]. The antibacterial properties of a Chlorophycean green alga, *Scenedesmus* sp. isolated from a natural pond were tested against three different pathogenic bacterial strains [27]. With this knowledge the present study was aimed to screen the antimicrobial potential of *Scenedesmus abundans*, *Nannochloropsis oculata* and *Spirogyra condensata* against human bacterial pathogens. The first antibacterial compound isolated from a microalgae and chlorella; a mixture of fatty acids, viz., chlorellin which was found to be responsible for the inhibitory activity against Gram +ve and Gram -ve bacteria [21, 7].

MATERIALS AND METHODS

Collection of the Microalgal Strains and Bacterial Strains

Fresh water algal samples were collected with 100 ml capacity plastic bottles during winter session from Bundelkhand region Uttar Pradesh (U.P.) in Central India. It is located between 23° 20' and 26° 20' N latitude and 78° 20' and 81° 40' E longitude. Where *Nannochloropsis oculata* were collected from Chhabi pond of Banda, *Scenedesmus abundans* were collected from Mandakani river of Chitrakoot and *Spirogyra condensata* were collected from atiya tal of

Jhansi. Human bacterial pathogens like *Bacillus cereus*-MCCB0061, *Staphylococcus aureus*-MCCB0045, *Escherichia coli*-MCCB0016 and *Pseudomonas aeruginosa*-MCCB0035 were obtained from department of microbiology & Fermentation Technology SHIATS Allahabad (U.P.), India. Bacterial strains were inoculating on to in nutrient broth and incubated at 37°C for 24 hrs.

Isolation and Growth Condition of Microalgal Strains

Microalgae were isolated from fresh water algal samples. After purification, the culture was grown in BG-11 medium. The cultures were grown autotrophically in the batch culture, in haffkins flasks and were kept in the culture room. Culture condition was maintained such as temperature 27°C \pm 0.5°C; pH rang 7 to 7.5 of culture medium, incubation period 2 months and 3000 lux light intensity.

Identification of Microalgal Strain

Microscopic observation of algal samples was done by Lieca DM. 500 research microscope and microphotography was done with attached camera Ec-3 [17]. Morphological observation presence of chloroplast shape and size of cells were taken into consideration. The identification of taxa was done by referring standard taxonomic manuals of Philipose [18] and Prescott [19].

Growth Analysis and Biomass Productivity

Growth rate of cultures was determined by measuring the optical density (O.D.) at 680 nm using UV-VIS Spectrophotometer (Spectrascan UV 2700, Thermo Scientific). For the measurement of optical density, 2 ml culture was drawn from the culture flask at the regular measure of alternate days. The samples were diluted so that the value of OD₆₈₀ falls between the ranges of 0.2-0.8, actual OD was determined by multiplying the dilution factor with value of OD.

Extraction of Microalgal Biomass

Biomass of microalgae was harvested after 40 and 60 days respectively. The harvested biomass was centrifuged at 10000 rpm for 7 min and pellet was lyophilized. 250 mg of lyophilized algal powder was taken in four different 50ml capacity conical flask and mix with 15ml four different solvents such as methanol, ethanol, acetone and diethyl ether. The mixture was shaken overnight in orbital shaker, centrifuged at 10000 rpm for 10min in twice and supernatant was taken. Supernatant was dried in a rotary evaporator at 40°C. The algal extract dissolved in 1 ml four different solvents used for antimicrobial activity and preserved in 4°C for further use [12].

Disc Diffusion Method

Antibacterial activity of microalgae was used by disc diffusion method [13, 14]. For the disc diffusion assay 400 µL of each bacterial suspension was uniformly spread on a solid nutrient agar medium in a petri dish. Two sterile paper disks (6 mm in diameter whatman filter paper) and one streptomycin disc were placed on the surface of each nutrient agar plate and were impregnated with 20 µL of the diluted algal extract. Plates were incubated for 24 h under appropriate culture conditions [15]. Disc impregnated with algal extract and methanol, ethanol, acetone and diethyl ether served as negative controls and a disk with an antibiotic (Streptomycin) served as a positive control. The antibacterial activity was using (ethanol, methanol, acetone and diethyl ether) algal extract.

RESULTS

Growth Analysis and Biomass Productivity

The biomass contained found in *Scenedesmus abundans* 0.48943 g/L, *Nannochloropsis oculata* 0.53316 g/L and *Spirogyra condensata* 0.37916 g/L.

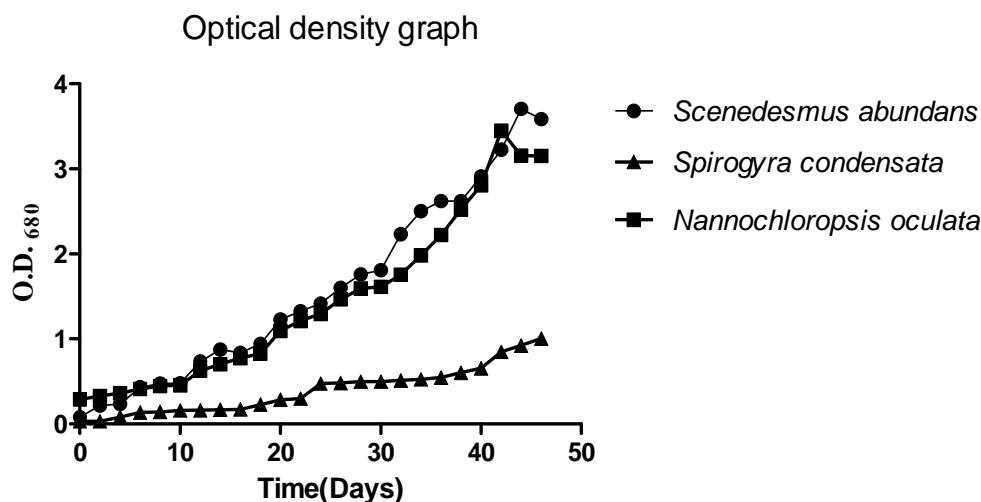


Figure 1

Antibacterial Activity of Different Algal Strain in Different Solvents

In the present study the methanolic, ethanolic, acetone and diethyl ether were tested for antibacterial activity against four human bacterial pathogens. The methanolic extract of *Scenedesmus* sp. showed the antibacterial activity against four bacterial pathogens viz., *B. Cereus*, *P. aeruginosa*, *S. aureus* and *E. Coli*, with the inhibition zone of 22, 17, 16 and 15 mm, respectively. The ethanolic extract of *Scenedesmus abundans* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. Coli* with the inhibition zone of 23, 21, 23 and 20 mm, respectively. The acetone extract of *Scenedesmus abundans* showed the antibacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. Coli* with the zone of inhibition zone of 23, 20, 34 and 18 mm, respectively. The diethyl ether extract of *Scenedesmus abundans* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. Coli* 17, 15, 29 and 18 mm, respectively. The methanolic extract of *Nannochloropsis oculata* showed the antibacterial activity against four bacterial pathogens viz., *B. Cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with the inhibition zone of 26, 17, 15 and 15 mm, respectively. The ethanolic extract of *Nannochloropsis oculata* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with the inhibition zone of 33, 24, 25 and 19 mm, respectively. The acetone extract of *Nannochloropsis oculata* showed the antibacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with the zone of inhibition zone of 28, 12, 17 and 00 mm, respectively. The diethyl ether extract of *Nannochloropsis oculata* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* 12, 15, 10 and 00 mm, respectively. The methanolic extract of *Spirogyra condensata*, showed the antibacterial activity against four bacterial pathogens viz., *B. Cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with inhibition zone of 26, 20, 16, and 25 mm, respectively. The ethanolic extract of *Spirogyra condensata* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. Coli* with the inhibition zone of 32, 22, 23 and 24 mm, respectively. The acetone extract of

Spirogyra condensata, showed the antibacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with the zone of inhibition zone of 15, 15, 13, 17 mm, respectively. The diethyl ether extract of *Spirogyra condensata* showed the antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* with the inhibition zone of 8, 9, 15, 00 mm, respectively. The negative control only for solvents In methanol solvent showed antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* 00, 09, 00 and 07 mm., respectively. In ethanol solvent showed antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* 12, 11, 00 and 07 mm. respectively. In acetone solvent showed antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* 13, 15, 00 and 10 mm. respectively. In diethyl ether solvent showed antibacterial activity against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* 00, 11, 00 and 00 mm. respectively. Source of antibacterial activity keeping Streptomycin disc were used as a positive control against four bacterial pathogens viz., *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli* zone of inhibition 17, 22, 15 and 28 mm. respectively.

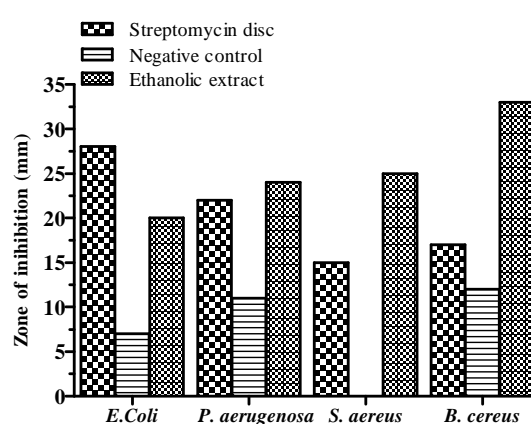
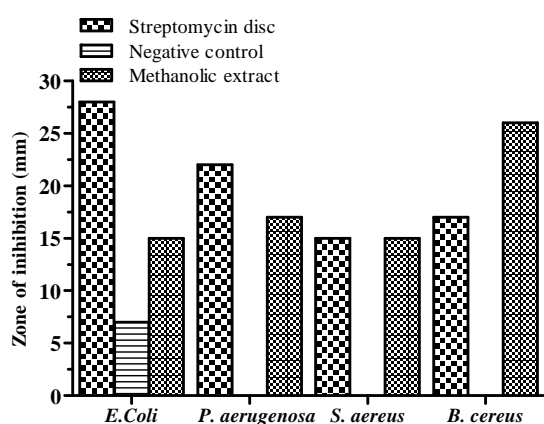
Antibacterial Activity of Various Algal Extract in Different Solvents

Table 1

Algal Species	Extracts	Concentration (mg/mL)	Inhibition Zone Diameter (mm)											
			<i>E. Coli</i>			<i>S. Aureus</i>			<i>P. Aeruginosa</i>			<i>B. Cereus</i>		
			AE	NC	PC	AE	NC	PC	AE	NC	PC	AE	NC	PC
<i>Scenedesmus abundans</i>	Methanol	0.0039	15	7	28	16	-	15	17	9	22	22	-	17
	Ethanol	0.0010	20	7		23	-		21	11		23	12	
	Acetone	0.0010	18	10		34	-		20	15		23	13	
	Diethyl ether	0.0017	18	-		29	-		15	11		17	-	
<i>Nannochloropsis oculata</i>	Methanol	0.0050	15	7	28	15	-	15	17	9	22	26	-	17
	Ethanol	0.0014	19	7		25	-		24	11		33	12	
	Acetone	0.0011	-	10		17	-		12	15		28	13	
	Diethyl ether	0.0003	-	-		10	-		15	11		12	-	
<i>Spirogyra codensata</i>	Methanol	0.0026	25	7	28	16	-	15	20	9	22	26	-	17
	Ethanol	0.0015	24	7		23	-		22	11		32	12	
	Acetone	0.0006	17	10		13	-		15	15		15	13	
	Diethyl ether	0.0014	-	-		15	-		9	11		8	-	

AE: algal extract; NC: Negative control; PC: Positive control

Nannochloropsis Oculata Shows Antibacterial Activity against Four Pathogenic Bacteria in Four Different Solvent



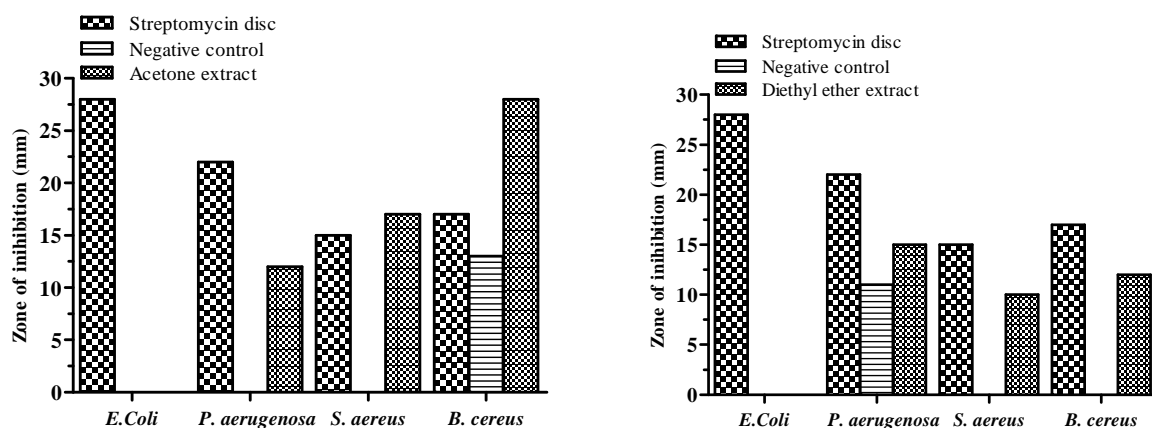
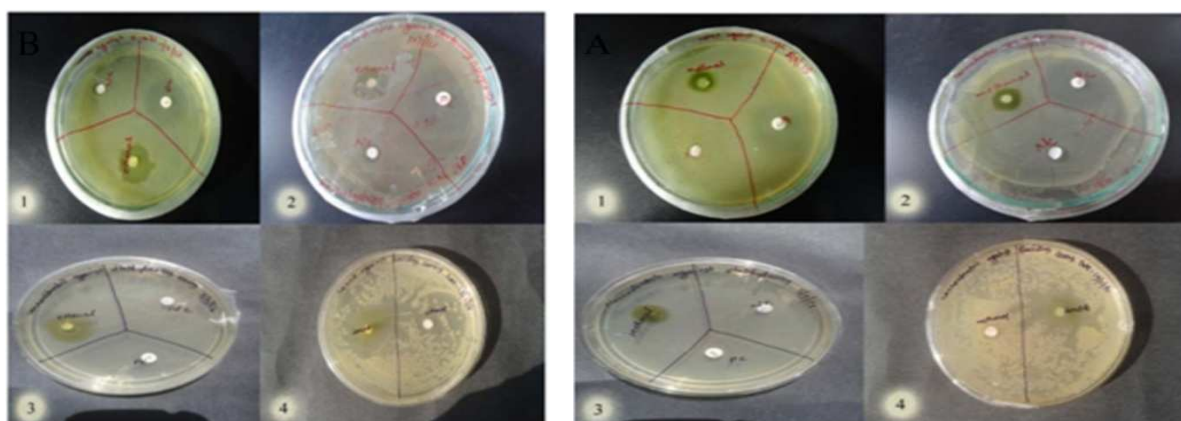


Figure 2

The Plates Showed Antimicrobial Activity of *Nannochloropsis Oculata* against Four Pathogenic Bacteria in Four Different Solvents

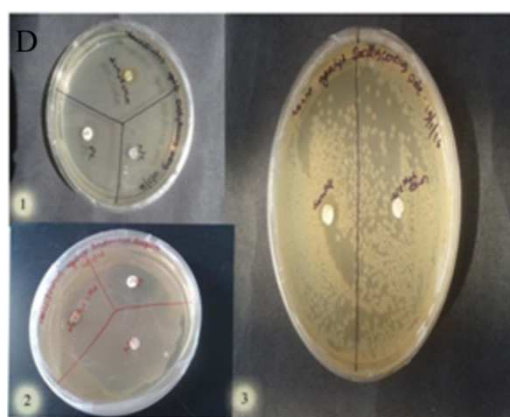


4. *Nannochloropsis Oculata* Shows the Maximum Antibacterial Activity in Ethanolic Extract against *B. Cereus*

4. *Nannochloropsis Oculata* Shows the Maximum Antibacterial Activity in Methanolic Extract against *B. Cereus*



4. *Nannochloropsis Oculata* Shows the Maximum Antibacterial Activity in Acetone Extract against *B. Cereus*



2. *Nannochloropsis Oculata* Shows the Maximum Antibacterial Activity in Di Etylether Extract against *P. Aeruginosa*

Figure 3

A.Methanolic extract of *Nannochloropsis* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S.aereus* (4) *B. cereus*

B. Ethanolic extract of *Nannochloropsis* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S. aereus* (4) *B.cereus*

C.Acetone extract of *Nannochloropsis* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S.aereus* (4) *B. cereus*

D.Diethyl ether extract of *Nannochloropsis* shows antibacterial against four different bacteria (1) *E.coli* (2) *P. aeruginosa* (3) *S.aereus* (4) *B. cereus*

***Scenedesmus Abundans* Shows Antibacterial Activity against Four Pathogenic Bacteria in Four Different Solvent**

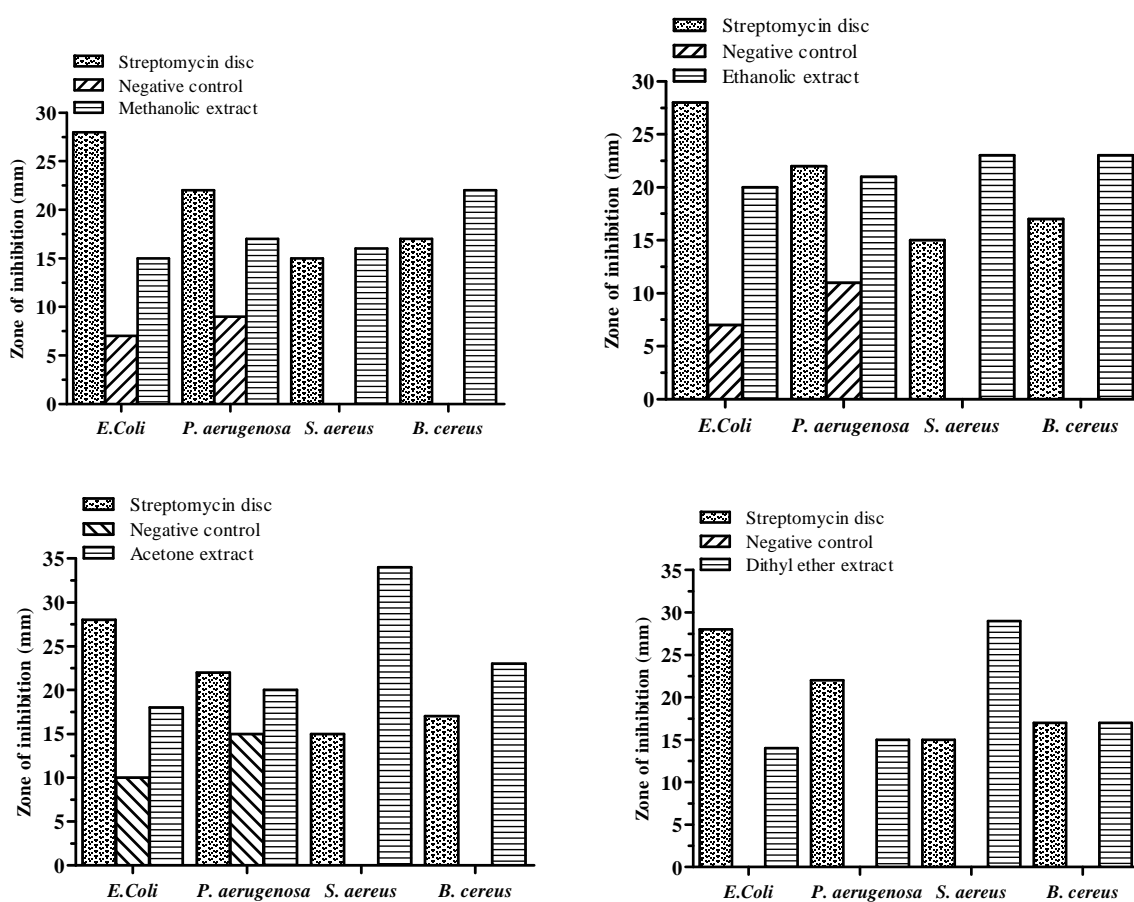
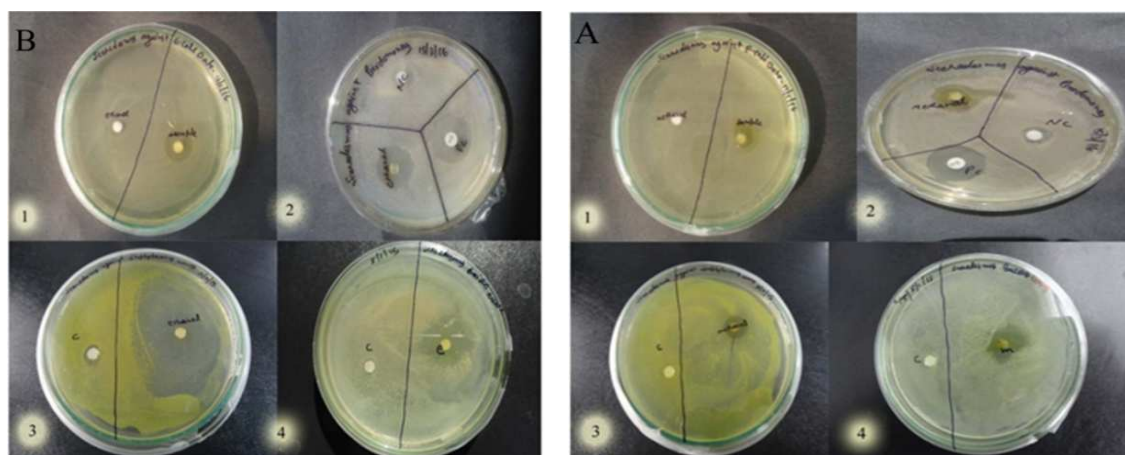


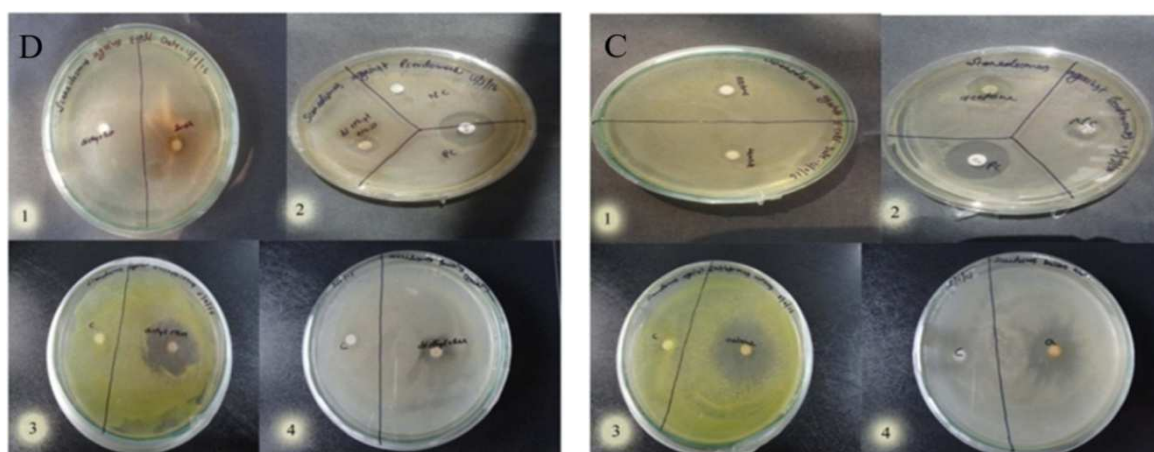
Figure 4

The Plates Showed Antimicrobial Activity of *Scenedesmus Abundans* against Four Pathogenic Bacteria in Four Different Solvents



4. *Scenedesmus Abundans* Shows the Maximum Antibacterial Activity in Methanolic Extract against *B. Cereus*

3&4. *Scenedesmus Abundans* Shows the Maximum Antibacterial Activity in Ethanolic Extract against *S. Aereus* & *B. Cereus* Bacillucereus



4. *Scenedesmus Abundans* Shows the Maximum Antibacterial Activity in Acetone Extract against *S. aureus*

4. *Scenedesmus Abundans* Shows the Maximum Antibacterial Activity in Diethyl Ether Extract against *S. aureus*

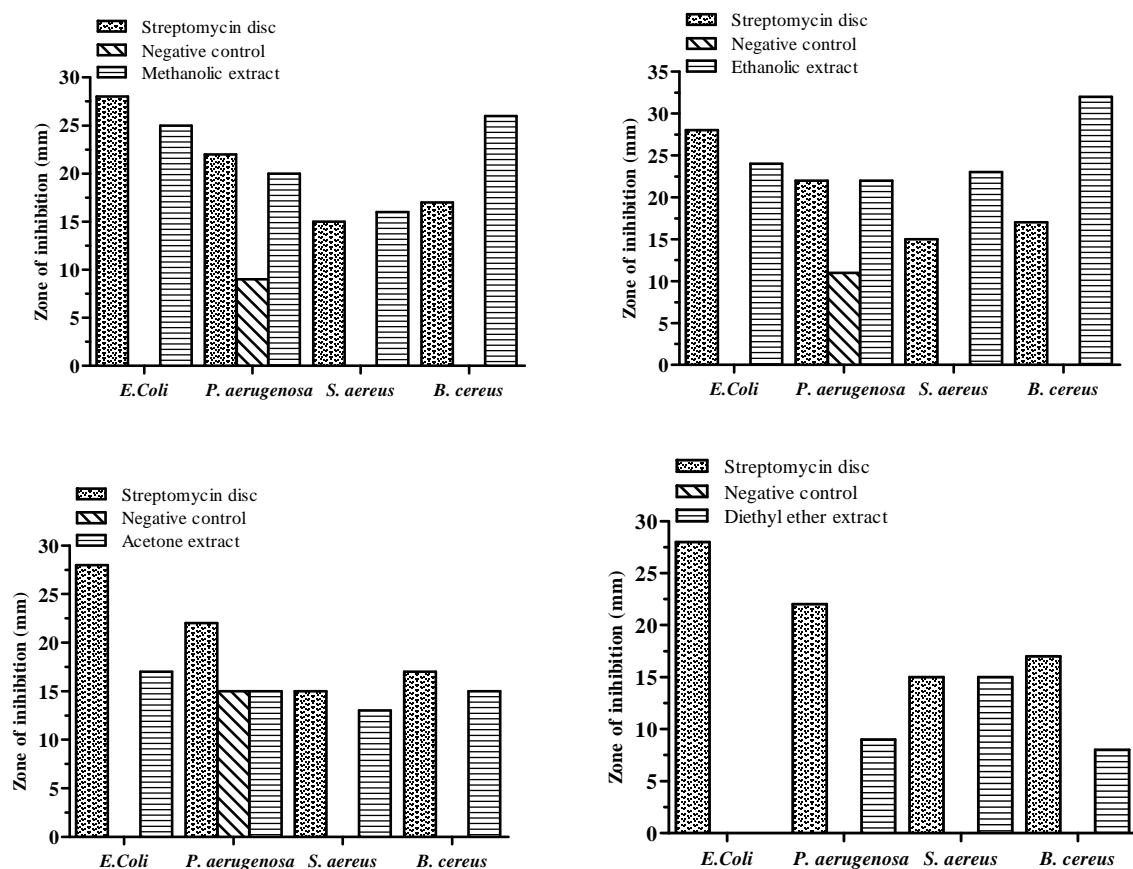
Figure 5

A. Methanolic extract of *Scenedesmus* shows antibacterial against four different bacteria (1) *E. coli* (2) *P. aeruginosa* (3) *S. aureus* (4) *B. cereus*

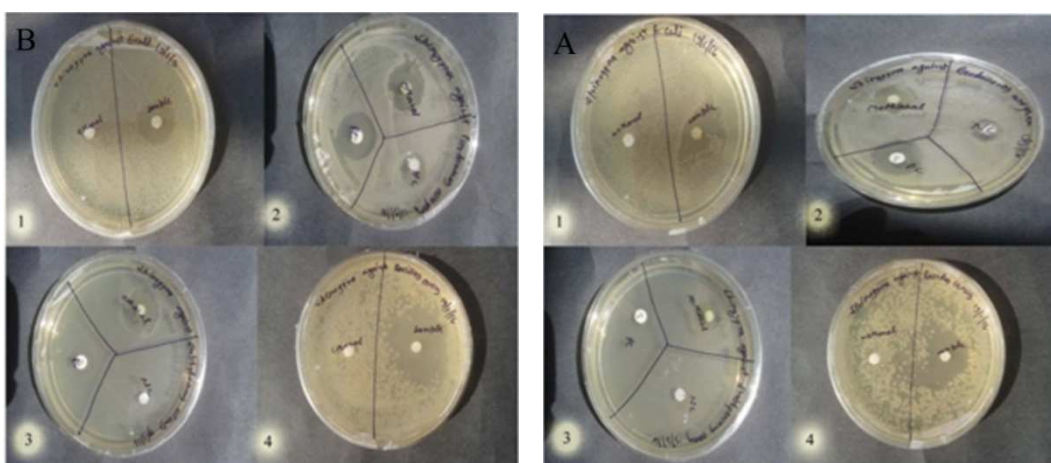
B. Ethanolic extract of *Scenedesmus* shows antibacterial against four different bacteria (1) *E. coli* (2) *P. aeruginosa* (3) *S. aureus* (4) *B. cereus*

C. Acetone extract of *Scenedesmus* shows antibacterial against four different bacteria (1) *E. coli* (2) *P. aeruginosa* (3) *S. aureus* (4) *B. cereus*

D. Diethyl ether extract of *Scenedesmus* shows antibacterial against four different bacteria (1) *E. coli* (2) *P. aeruginosa* (3) *S. aureus* (4) *B. cereus*

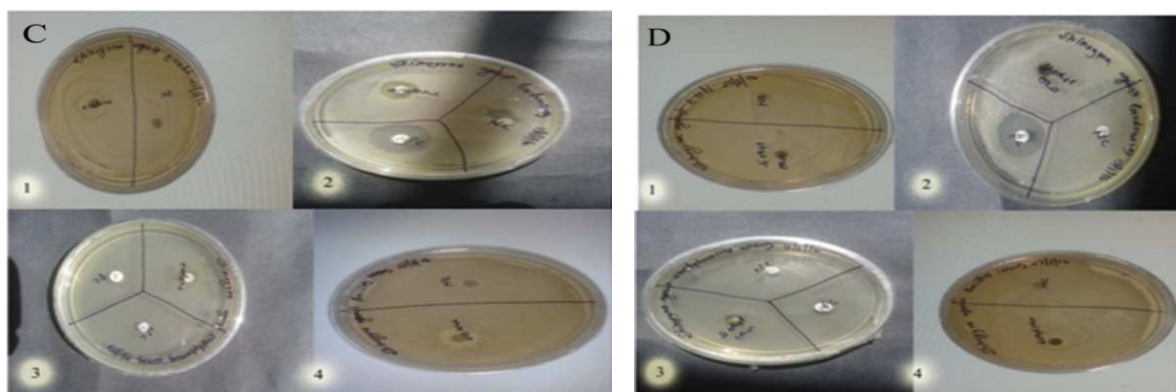
***Spirogyra Condensata* Shows Antibacterial Activity against Four Pathogenic Bacteria in Four Different Solvent****Figure 6**

The plates showed antimicrobial activity of *Spirogyra condensata* against four pathogenic bacteria in four different solvents



4. *Spirogyra Condensata* Shows the Maximum Antibacterial Activity in Methanolic Extract against *B. cereus*

4. *Spirogyra Condensata* Shows the Maximum Antibacterial Activity in Ethanolic Extract against *B. cereus*



1. *Spirogyra Condensata* Shows the Maximum Antibacterial Activity in Acetone Extract against *E. coli*

3. *Spirogyra Condensata* Shows the Maximum Antibacterial Activity in Diethyl Ether Extract against *S. Aureus*

Figure 7

A. Methanolic extract of *Spirogyra* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S.aereus* (4) *B. cereus*

B. Ethanolic extract of *Spirogyra* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S. aureus* (4) *B.cereus*

C. Acetone extract of *Spirogyra* shows antibacterial against four different bacteria (1) *E. coli* (2) *P.aeruginosa* (3) *S.aereus* (4) *B. cereus*

D. Diethyl ether extract of *Spirogyra* shows antibacterial against four different bacteria (1) *E.coli* (2) *P. aeruginosa* (3) *S.aereus* (4) *B. cereus*

DISCUSSIONS

Fresh water microalgae have become part of complementary medicine worldwide, because of their potential sources of bioactive molecules cause many health benefits in human being[25]. The present study results revealed that the ethanolic extract of *Nannochloropsis oculata*, *Scenedesmus abundans* and methanolic extract of *Spirogyra condensata* has shown maximum zone of inhibition against *E. coli*. This bacteria is gram negative, rod shaped caused many types of infection in human being like urinary tract infection, diarrhea, phylogenetic infections and septicemia. The ethanolic extract of *Nannochloropsis oculata*, *Scenedesmus abundans* and methanolic extract of *Spirogyra condensata* has shown maximum zone of inhibition against *P. aeruginosa*. This bacterium is gram negative rod shaped, which caused many types of infection in human being like urinary tract infection, respiratory system infection, soft tissue infections, bone and joint infection. The ethanolic extract of *Nannochloropsis oculata*, *Spirogyra condensata* sp. and acetone extract of *Scenedesmus abundans* has shown maximum zone of inhibition against *S. aureus*. This bacteria is gram positive, rod shaped caused many types of infection in human being like sepsis in wounds and burns, septicemia, pharyngitis, sinusitis and tonsillitis. The ethanolic extract of *Nannochloropsis oculata*, *Scenedesmus abundans* and *Spirogyra condensata* sp. has shown maximum zone of inhibition against *B. cereus*. This bacterium is gram positive, rod shaped produce toxin, these toxins can cause two types of illness: one type characterized by diarrhea and the other, called emetic toxin, by nausea and vomiting[26]. In present study ethanolic crude extract of *Nannochloropsis oculata* was found more potent antibacterial activity against *B. cereus* as compared to antibiotic disc streptomycin. Acetone crude extract of *Scenedesmus abundans*

was found more potent antibacterial activity against *S. aureus* as compared to antibiotic disc streptomycin. Beena et al. 2011 reported, The crude pigment extract of *Scenedesmus abundans* was found to have inhibitory activity against the food borne pathogen *S. aureus*[24] ethanolic crude extract of *Spirogyra condensata* was found more potent antibacterial activity against *B. cereus*, as compared to antibiotic disc streptomycin. It concludes that *B. cereus* and *S. aureus*, Gram – positive bacteria were more inhibited than Gram – negative bacteria *E.coli* and *P. aeruginosa*. Issa (1999) reported the antimicrobial activity of *O. angustissima* and *Calothrix parietal*, against bacteria and fungi. He concluded that *B. cereus* and *S. aureus*, Gram- positive species were more inhibited than gram- negative species *E. coli* and *P. aeruginosa* by the antibiotic applied[23]. Ethanolic crude extract of *Nannochloropsis oculata* and *Spirogyra condensata* were found more potent antibacterial activity against *B.cereus*, *P. aeruginosa*, *S.aereus* and *E.coli* as compared to antibiotic disc streptomycin. Acetone crude extract of *Scenedesmus abundans* was more potent antibacterial activity against *S.aereus* as compared to antibiotic disc streptomycin. The result also proved that ethanol was the best solvent for the extracting the antibacterial and antifungal agents from *Oscillatoria latevirens*, while acetone was the best organic solvents for extracting antibacterial and antifungal agents from *Phormidium corium* and *Lyngbya martensiana*[22].

The present study indicates that the antibacterial property of *Nannochloropsis oculata*, *Scenedesmus abundans* and *Spirogyra condensata* against the selected strains of human pathogenic bacteria varies depending upon the four different solvent medium used for extraction. Further phytochemical studies are needed to elucidate the component responsible for antibacterial activity of these extracts against bacteria.

Conflicts of Interest

We declare that we have no conflict of interest.

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