IJPSR (2012), Vol. 3, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 August, 2012; received in revised form 19 September, 2012; accepted 28 November, 2012

ANTIMICROBIAL AND ANTIOXIDANT PROPERTY OF COMMONLY FOUND MICROALGAE *SPIRULINA PLATENSIS*, *NOSTOC MUSCORUM* AND *CHLORELLA PYRENOIDOSA* AGAINST SOME PATHOGENIC BACTERIA AND FUNGI

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Keywords:
Green algae, Antimicrobial activity, Antioxidant activity, Secondary metabolites

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QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

In the present study, three algal species (*Spirulina platensis, Chlorella pyrenoidosa* and *Nostoc muscorum* (ATCC 2789) were used to investigate their antimicrobial and antioxidant properties against some human pathogenic bacteria and fungi (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus* and fungus *Aspergillus luchuensis*, *Aspergillus niger*, *Fusarium oxysporum)*. Four different solvents namely methanol, acetone, n-hexane and water were used for extraction. The present investigation showed that methanolic extract was more effective against the pathogenic bacteria and fungi. Methanolic extract of *C. pyrenoidosa* and *N. muscorum* was found to be most effective against *P. aeruginosa* while methanolic extract of *S. platensis* showed maximum activity against *S. aureus*. *S. platensis* showed maximum antifungal activity in comparison to other algal extracts. Antioxidant activity was determined by free radical scavenging activity (Nitric oxide radical scavenging activity) and it was found that methanolic extract of *S. platensis* showed maximum antioxidant activity.

INTRODUCTION: Algae are photosynthetic organisms, which constitute a total of twenty-five to thirty thousand species, with a great diversity of forms and sizes ranging from unicellular microscopic organisms (microalgae) to multicellular organisms of great size (macroalgae). They can double every few hours during their exponential growth period [1].

During the peak growth phase, some microalgae can double even every 3.5 hrs [2]. In fact, some algae are organisms that live in complex habitats submitted to extreme conditions as they can easily adapt to the new environmental conditions and are known to produce a great variety of secondary (biologically active) metabolites, which cannot be found in other organisms [3].

Algae can be a very interesting natural source of new compounds with biological activity that could be used as functional ingredients [4]. Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antibacterial and antifungal activities [5, 6].

The search for similar antimicrobial activity in other algal species has gained importance in recent years due to growing worldwide concern about antibiotic-resistant micro-organisms. Efforts are being made to extract substances with antibacterial, antiviral, fungicidal, enzyme inhibitory, immunosuppressive and cytotoxic and algicidal property from the low cost algal biomass [7-10].

Among algal species, *Spirulina* has been reported to prevent oxidative damage by scavenging free radicals and active oxygen, and hence can indirectly reduce cancer formation in human body [11, 12]. In this respect, the increased consumption of foods characterized by free radical scavenging activity, leads up to a doubling of protection against many common types of cancer formulation. The antimicrobial activity and antioxidant status of microalgae as whole is considered a tool for evaluation of pharmaceutical potential of the algal extracts [13, 14].

Bacterial infection causes high rate of mortality in human population and aquaculture organisms. For an example, *Bacillus subtilis* is responsible for causing food borne gastroenteritis [15]. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion, respiratory complications including some life threatening illness [16], while *Salmonella sp.* causes diarrhea and typhoid fever [17, 18]. Some fungi are opportunistic while others are pathogenic, causing disease if the immune system is not healthy [19]. Preventing disease outbreaks or treating the diseases with drugs or chemicals alone may not be sufficient to tackles these problems as the microorganisms develop resistance against the applied chemical drugs [20]. It becomes a greater problem of giving treatment against resistant pathogenic bacteria [21].

Microalgae being a rich source of novel bioactive compounds, have recently found immense application in human and animal medicine. A promising strategy for the replacement of antibacterial and antifungal chemicals is to promote the natural biological control products obtained from microalgae. The present work is an effort to evaluate the pharmaceutical potential of some common fresh water microalgae against some pathogenic bacterial and fungal strains. In addition to this, antioxidant potential of these algal strains has also been evaluated.

MATERIAL AND METHODS:

Collection and culturing of Algae: Fresh water, unicellular, green algae *Spirulina platensis, Chlorella pyrenoidosa* and *Nostoc muscorum* (ATCC 2789) were used for the study. *Spirulina platensis* culture was maintained in Zarrouk's medium [22], while *Chlorella pyrenoidosa* and *Nostoc muscorum* was maintained in BG-11 medium [23]. Algal culturing was carried out in 1000 mL conical flask containing 600 mL respective medium, all the cultures were kept under fluorescent light (20 µmol $m^{-2}s^{-1}$) with 16 h light period and at 25 ±2°C temperature.

Preparation of Algal Crude Extracts: The algal cultures were centrifuged at 2500 rpm for 10 minutes to harvest the algal biomass. After centrifugation, algal pastes were air dried in oven at 60°C. One gram of dried algal samples was extracted with 10 mL of the solvents (Methanol, acetone, n-Hexane, water). The dried biomass was taken in sterile screw-capped bottles of 50 mL volume and was soaked in the solvents for 48 h. The mixture was then centrifuged at 2000 rpm for 10 min at 4°C.The supernatants were filtered through a sterile funnel and sterile Whatman filter paper No. 1. The filtrate was sterilized using 0.2 µm membrane filter. The extract obtained was used for screening of their antimicrobial potential [24].

Test Microorganisms: Gram negative bacterial strains *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae),* Gram positive bacterial strains *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Bacillus cereus* (*B. cereus*), *Micrococcus luteus* (*M. luteus*) and fungus *Aspergillus luchuensis* (*A. luchuensis*), *Aspergillus niger* (*A. niger*), *Fusarium oxysporum* (*F. oxysporum*) were used for the study. The parent cultures were obtained from NCIM, Pune and the subcultures were maintained in the nutrient media (Nutrient agar media (bacteria) and Czapek dox media (fungus)) once in 15 days.

Agar Well Diffusion Method: The antibacterial activity was performed by agar well diffusion method. The respective bacterial and fungal cultures were poured into the Muller Hinton agar plates and Czapax dox agar plates respectively, for uniform distribution of microorganisms. Using the sterile well puncture, 6 mm wide well was made on each agar plates. 0.1 ml of organic crude extracts from the stock were poured into each well using a sterile micropipette [25]. Respective organic solvents were used as a negative control for each extract. Amoxicillin were used as standards. The plates were incubated for 24 hours at 37°C for bacteria and at 28°C for 3 days for fungus. At the end of incubation period, the zone of inhibition was measured.

Growth measurement of Fungus: Measuring the fungal growth was made according to the method described by Kane and Mullins [26]. The flasks of each triplicate were filtered off using Whatman filter paper after the end of the incubation period and the fungal mats were washed carefully and dried up to a constant dry weight at 70 - 80˚C for 24 h.

Qualitative analysis of Biochemicals in the Algal Extracts: Qualitative analysis of biochemicals in the methanolic extract of *Chlorella pyrenoidosa, Spirulina platensis* and *Nostoc muscorun* was done.

1. **Detection of Proteins and Amino Acids:** The dried extract (100 mg) was dissolved in 10 mL of distilled water and filtered through Whatman no.1 filter paper and the filtrate was subjected to tests for proteins and amino acids. To 2 mL of filtrate, few drops of Millon's reagent were added and the observations were made for white precipitate (Millon's test) [27].

2. **Detection of Carbohydrates:** The extract (100 mg) is dissolved in 5 mL of water and filtered. To 2 mL of filtrate two drops of alcoholic solution of ά-naphthol were added, the mixture is shaken well and 1 mL of concentrated sulfuric acid was added slowly along the sides of the test tube and allowed to stand, then observed for the formation of violet ring (Molish's test).

3. **Detection of Phytosterols:** The extract (50 mg) was dissolved in 2 mL of acetic anhydride. To this 1 or 2 drops of concentrated sulfuric acid was added slowly along the sides of the tube and observed for an array of colour [28].

4. **Detection of Phenolic Compounds:** The extract (50 mg) was dissolved in 5 mL of distilled water. To this few drops of neutral ferric chloride solution was added and observed for a dark green coloration [29].

5. **Detection of Alkaloids:** Preparation of filtrate solvent free extract (50 mg) is stirred with 2 mL of dilute hydrochloric acid and filtered. To 1 mL of filtrate, a drop of Mayer's reagent was added by the side of tube and then observed for a white creamy precipitate [30].

6. **Detection of Flavonoids:** To 5 mL of dilute ammonia solution a portion of the aqueous filtrate of each algal extract followed by addition of concentrated sulfuric acid. Then was observed for a yellow coloration. The yellow coloration disappears on standing [31, 32].

7. **Detection for Saponins:** Two grams of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered. 10 mL of filtrate was mixed with 5 mL of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion [33].

8. **Test Glycosides:** In the Keller-Killani test, 5 mL of each extract was treated with 2 mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 mL of concentrated sulfuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout thin layer [34].

Antioxidant Activity:

***In vitro* Nitric Oxide Radical (NO) Scavenging Assay:** Nitric oxide radical scavenging activity was estimated on the basis of Griess Illosvey reaction using method followed by Govindarajan et al., [35]. Reaction mixture (3ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5ml, pH 7.4) and 0.5 ml of AVP (20- 120µg/mg) or standard ascorbic acid solution (0.5ml, 20-120µg/ml) was incubated at 25°C for 150 min.

After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 29% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride (0.1% w/v) was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed in diffused light was measured at 540 nm against the corresponding blank solutions.

RESULTS: In the present study , antimicrobial activity of algal species was tested against the human pathogenic bacteria and plant pathogenic fungus by agar well diffusion method. Single concentration was used for the study which was 0.1 g/ml. The inhibition zones formed by the extracts at different concentrations against the specific test organisms were measured. The extract restricted the growth of pathogen on the media around the well.

Antimicrobial activity of *Chlorella pyrenidosa* extract: To check the antimicrobial activity of *C. pyrenoidosa* four different solvents extracts (methanol, acetone, n-hexane and water) were used against six different human pathogenic bacteria and three different plant pathogenic fungi. It was observed that methanolic extract of *C. pyrenoidosa* was most effective against *P. aeruginosa*, with maximum zone of inhibition of (2.5 cm), while the minimum size of inhibition zone (0.2cm) was found in case of *S. aureus*. *B. subtilis* and, *K. pneumoniae* were insensitive. The fungi *A. niger* and *F. oxysporum* were also found to be insensitive against methanolic extract of *C. pyrenoidosa* (**Table 1**). *A .luchuensis* showed zone of inhibition with a diameter of (1.8 cm).

In case of acetone extract zone of inhibition against *B. subtilis* was (1.5 cm) wide, while it was 1.0 cm in case of *B. cereus* and *M. luteus*. *P. aeruginosa* showed complete resistance against acetone extract of *C. pyrenoidosa*. In case of antifungal activity of acetone extract of *C. pyrenoidosa*, the *A. luchuensis* showed maximum sensitivity with (2.5 cm) wide zone of inhibition. *A. niger* was resistant against methanolic extract of *C. pyrenoidosa*.

Hexane extract was not very effective against selected pathogenic bacteria and fungi. Only *B. subtilis* among the tested bacteria showed 2.5 cm wide zone of inhibition, followed by *K. pneumoniae*. Aqueous extract of C*. pyrenoidosa* was not effective against any selected pathogenic microbes (Table 1).

TABLE 1: ANTIMICROBIAL ACTIVITY AROUND THE WELLS OF DIFFERENT ORGANIC EXTRACTS OF *C. PYRENOIDOSA*. INHIBITION ZONE IN DIAMETER (CM). (DATA PRESENTED ARE THE MEAN OF TRIPLICATES ±STANDARD DEVIATION (SD))

Test organism	Methanol	Acetone	n-Hexane
B. subtilis	no zone	1.5 ± 0.15	2.5 ± 0.25
B. cereus	1.2 ± 0.1	1 ± 0.17	no zone
P. aeruginosa	2.5 ± 0.25	no zone	no zone
M. luteus	1.2 ± 0.1	1±0.12	no zone
S. aureus	0.2 ± 0.05	no zone	no zone
K. pneumoniae	no zone	no zone	1.4 ± 0.1
A. niger	no zone	no zone	no zone
A. luchuensis	1.8 ± 0.15	2.5 ± 0.25	no zone
F. oxysporum	no zone	1.5 ± 0.15	no zone

Antimicrobial activity of *Spirulina platensis* extract: Antimicrobial activity of extract of *S. platensis* against pathogenic bacteria and fungi revealed that *S. aureus* was relatively more sensitive (1.5 cm zone of inhibition) to methanolic extract. While *B. subtilis*, *B. cereus*, and *M. luteus* showed moderate sensitivity (1.0 cm for each) *K. pneumoniae* was found to be resistant against methanolic extract of *S. platensis*.

Among three fungi, only *A. niger* was the most sensitive, which showed inhibition zone of 1.5 cm, while *A. luchuensis* and *F. oxysporum* were resistant against the methanolic extract.

In case of acetone extract of *S. platensis*; *B. subtilis*, *K. pneumoniae* were found to be resistant, while *P. aeruginosa* showed the maximum zone of inhibition of 1.3 cm, followed by *B. cereus* and *M. luteus* exhibiting 1.2 cm wide zone ((**Table 2**).

In case of antifungal activity, only *A. niger* exhibited maximum sensitivity to acetone extract with zone of inhibition of 2 cm diameter, while *A. niger* and *F. oxysporum* were found to be resistant against acetone extract of *S. platensis*. In case of hexane extract, only *B. subtilis* showed sensitivity with the zone of inhibition of 1 cm diameter. The other tested bacteria and fungi were found to be insensitive towards the aqueous extract (data not shown).

TABLE 2: ANTIMICROBIAL ACTIVITY AROUND THE WELLS OF DIFFERENT ORGANIC EXTRACTS OF *S. PLATENSIS*. Inhibition zone is in diameter (cm). (Data presented are the mean of triplicates ±standard deviation (sd))

Test microorganism	Methanol	Acetone	n-Hexane
B. subtilis	1 ± 0.17	no zone	1 ±0.05
B. cereus	1 ± 0.25	1.2 ± 0.25	no zone
P. aeruginosa	1.4 ± 0.05	1.3 ± 0.03	no zone
M. luteus	1 ± 0.1	1.2 ± 0.25	no zone
S. aureus	1.5 ± 0.1	1 ±0.17	no zone
K. pneumoniae	no zone	no zone	no zone
A. niger	1.5 ± 0.1	1 ± 0.1	no zone
A. luchuensis	no zone	2 ± 0.26	no zone
F. oxysporum	no zone	no zone	no zone

Antimicrobial activity of *Nostoc muscorum* (ATCC 2789): Antimicrobial activity of *N. muscorum* showed that among selected bacteria *P. aeruginosa* showed maximum zone of inhibition i.e. (2.0 cm), whereas *B. cereus*, *M. luteus* and *S.aureus* showed 0.8 cm wide zone of inhibition (0.8 cm). On the other hand, *B. subtilis* and *K. pneumonia* were found to be resistant to methanolic extract of *N. muscorum*. In antifungal assay, *A. niger* and *A. luchuensis* were resistant, while *F. oxysporum* showed the zone of inhibition of 1.2 cm in diameter. In case of acetone extract, *P. aeruginosa* showed maximum zone of inhibition with 1.8 cm diameter, followed by *S. aureus* and *K. pneumoniae* (1.6 and 1.0 cm, respectively). The bacterial strains *B. subtilis* and *B. cereus* were found to be resistant against acetone extract of *N. muscorum*. In anti-fungal activity, *A. niger* showed the maximum inhibition zone of 2.0 cm, while other two fungi *A. luchuensis* and *F. oxysporum* showed resistance against the acetone extract. Extract with n-hexane was not very effective in inhibiting the growth of the pathogens. Only *B. subtilis* showed samall zone of inhibition of 1.4 cm diameter. In case of aqueous extract of *N. muscorum*, all the pathogenic microbes showed resistance (**Table 3**).

TABLE 3: ANTIMICROBIAL ACTIVITY AROUND THE WELLS OF DIFFERENT ORGANIC EXTRACTS OF *N. MUSCORUM*. Inhibition zone is in diameter (cm). (Data presented are the mean of triplicates ±standard deviation (SD))

Test organism	Methanol	Acetone	n-hexane
B. subtilis	no zone	no zone	1.4 ± 0.1
B. cereus	0.8 ± 0.076	no zone	no zone
P. aeruginosa	2 ± 0.1	1.8 ± 0.1	no zone
M. luteus	0.8 ± 0.076	no zone	no zone
S.aureus	0.8 ± 0.076	1.6 ± 0.1	no zone
K. pneumoniae	no zone	1 ± 0.2	no zone
A. niger	no zone	2 ± 0.2	no zone
A. luchuensis	no zone	no zone	no zone
F. oxysporum	1.2 ± 0.2	no zone	no zone

Antimicrobial Index of Algal Extracts: The antimicrobial efficacy of different algal extracts was calculated in terms of antimicrobial index by comparing the antimicrobial effect of all extracts with the effect known standard antibiotic amoxicillin;

Antimicrobial Index=

$$\frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of standard}} \times 100$$

We found that methanolic extract of *C. pyrenoidosa* showed (**Fig. 1**) maximum percentage of inhibition (66.6%) against *B. cereus*, followed by *S. platensis* (55.5%) and *N. muscorum* (44.4%). The second most sensitive bacterial strain was *S. aureus* which showed maximum sensitivity to methanolic extract of *S. platensis* (35%), followed by *C. pyrenoidosa* (20%). Whereas bacterial strains *B. subtilis*, *P. aeruginosa* and *K. pneumoniae* showed no sensitivity against methanolic extract of all the three algal strains selected for antimicrobial assay. From the foregoing results, it was deduced that gram-positive bacteria were more sensitive to methanolic extract of algal species than gram-negative bacteria. *B. cereus* showed maximum percentage of inhibition, followed by *S. aureus*, *M. luteus*.

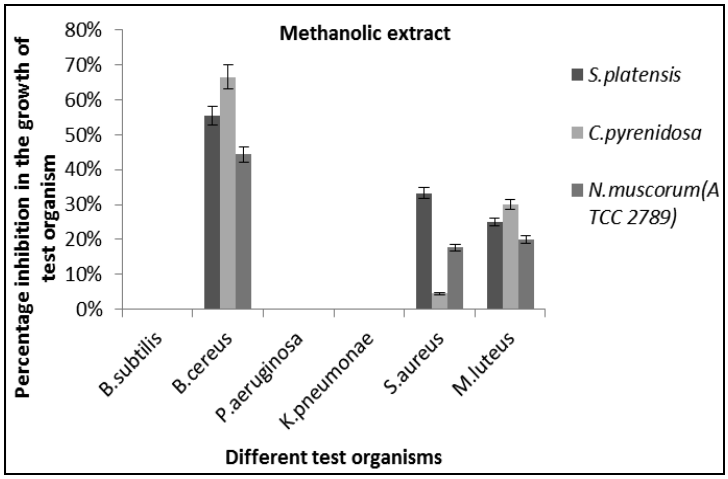

FIG. 1: ANTIMICROBIAL INDEX OF THE METHANOLIC EXTRACTS OF ALGAE AGAINST SELECTED PATHOGENS. (DATA PRESENTED ARE THE MEAN OF TRIPLICATES WITH STANDARD ERROR (5%))

Antimicrobial index of acetone extract (**Fig. 2**) of all selected algae revealed that *B. cereus* was the most sensitive strain. *K. pneumoniae* was more sensitive to extract of *N. muscorum* only (50% inhibition). By observing the antimicrobial index we found that *B. subtilis*, *P. aeruginosa* were relatively more resistant against all the algal extract.

Based on the foregoing results, it may be concluded that the antibacterial efficiency of methanolic and acetone extracts was higher than the n-Hexane extract, particularly for gram-positive bacteria.

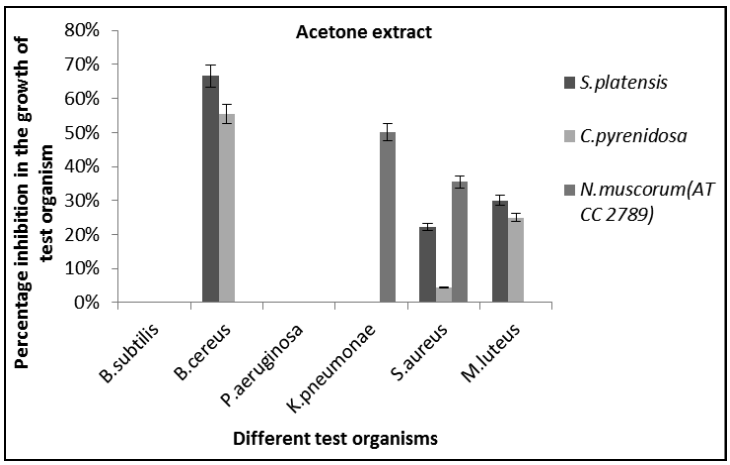

FIG. 2: ANTIMICROBIAL INDEX OF THE ACETONE EXTRACTS OF ALGAE AGAINST SELECTED PATHOGENS. (DATA PRESENTED ARE THE MEAN OF TRIPLICATES WITH STANDARD ERROR (5%))

Effect of Algal Extract on Fungal Growth: To see the antifungal activity of algal extracts, the dry weight of fungal mat and protein content of the broth was determined. The methanolic extract of *C. pyrenoidosa*, *S. platensis* and *N. muscorum* was used to show the antifungal potential of the selected algae.

In case of *Fusarium*, the methanolic extract of *Spirulina platensis* showed the maximum reduction (57.44%) in the dry wt., followed by *Nostoc muscorum* (16.06%) and *Chlorella pyrenoidosa* (1.77%). The order of effectiveness was *S. platensis* (57.4%) > *N. muscorum* (16.06%) > *C. pyrenoidosa* (1.77%). In case of *A. niger*, the maximum growth inhibition was caused by the extract of *Spirulina platensis* (49.7%), followed by *Nostoc muscorum* and *Chlorella pyrenoidos* (**Fig. 3**).

A. luchuensis was most sensitive to *Spirulina platensis* extract (31.49%), followed by *Chlorella pyrenoidosa* (3.53%) and *Nostoc muscorum* (13.12%). On comparison of the antifungal results of different algal extracts, it was found that *Spirulina platensis* extract was more effective than the *Nostoc muscorum* and *Chlorella pyrenoidosa* extract.

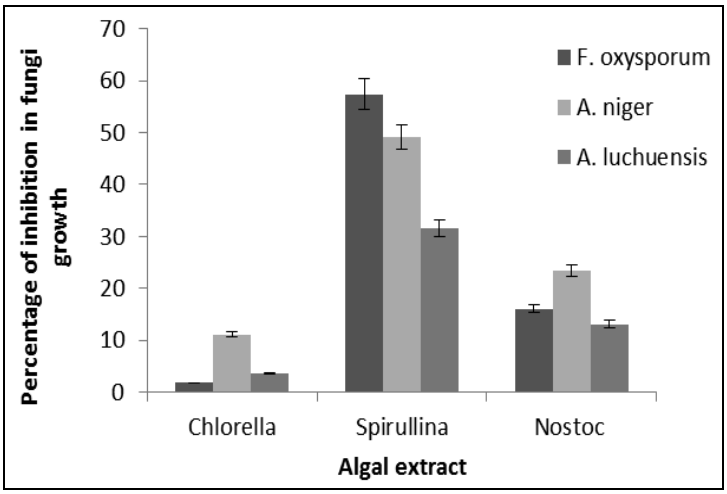

FIG. 3: PERCENTAGE INHIBITION OF FUNGUS GROWTH IN PRESENCE OF METHANOLIC EXTRACT OF DIFFERENT ALGAE (DATA PRESENTED ARE THE MEAN OF TRIPLICATES WITH STANDARD ERROR (5%))

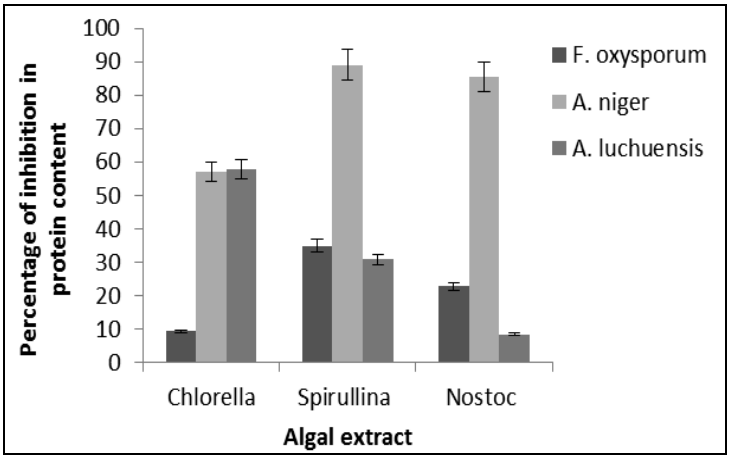

FIG. 4: PERCENTAGE INHIBITION IN PROTEIN CONTENT IN PRESENCE OF METHANOLIC EXTRACT OF DIFFERENT ALGAE (DATA PRESENTED ARE THE MEAN OF TRIPLICATES WITH STANDARD ERROR (5%))

Antioxidant activity: Antioxidant activity was determined, by free radical scavenging (Nitric oxide radical scavenging activity) in the methanolic extract *C. pyrenoidosa, S. platensis and N. muscorum*. It can be said that high percentage of NO scavenging activity was in *S. platensis* (40%) than the *C. pyrenoidsa* (18.85%) and *N. muscorum* (23.58%) (**Figure 5**). Qualitative analysis was done to check the presence of various biochemical constituents present in the algal extract obtained with different organic solvenst (methanol, acetone, hexane,). In case of methanolic and acetone extract, *C. pyrenoidosa*, *S. platensis* and *N. muscorum* showed the presence of glycosides, phenolic compounds, amino acid and protein.

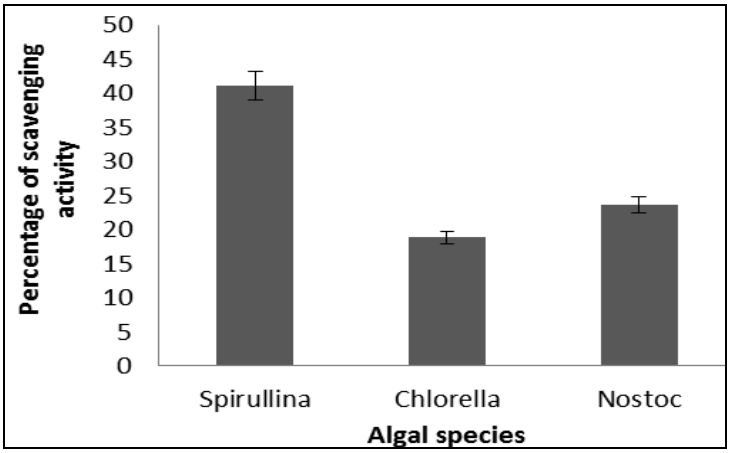

FIG. 5: NITRIC OXIDE SCAVENGING ACTIVITY IN METHANOLIC EXTRACT OF ALGAE (DATA PRESENTED ARE THE MEAN OF TRIPLICATES WITH STANDARD ERROR (5%))

TABLE 4: QUALITATIVE ANALYSIS OF BIOCHEMICALS IN THE VARIOUS EXTRACTS OF *S. PLATENSIS*, *C. PYRENOIDOSA* AND *N. MUSCORUM*

	Glycoside	Saponin	Phenolic compound	Amino acid & protein	Alkaloids	Carbohydrate	Flavonoids
Methanol extract							
C. pyrenidosa	+	–	+	+	–	+	–
S.platensis	+	–	+	+	–	+	–
N. muscorum	+	–	+	+	–	+	–
Acetone extract					–		
C. pyrenidosa	+	–	+		–	+	–
S.platensis	+	–	+	+	–	+	–
N. muscorum	+	–	+	+	–	+	–
n-Hexane extract					–		
C. pyrenidosa	+	–	+	+	–	+	–
S.platensis	+	–	+	+	–	+	–
N. muscorum	–	–	+	+	–	+	–

+ Present, - Absent.

Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibitory effect on microbial growth according to their constitution and concentration.

In case of hexane extract glycosides is absent only in *N. muscorum*, Saponins and flavonoids were absent in all the extracts. Glycosides, protein, amino acids, phenolic compounds were present in all the extract (**Table 4**).

Alkaloids are commonly found to have antimicrobial properties [36] against both Gram-positive and Gram-negative bacteria [37]. Presence of alkaloids in all the extracts might be exerting a remarkable antibacterial activity against Gram-positive (*S. aureus)* and Gram-negative (*K. pneumoniae*) bacteria.

DISCUSSION: Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemically unique compounds of marine origin with various biological activities have been isolated to develop new pharmaceuticals [38]. The cell extracts and active constituents of various algae shown to have both antibacterial and antifungal activities [39, 40]. The extracts of *C. pyrenidosa, S. platensis* and *N. muscorum* possessed noticeable better activity against Gram positive and Gram negative bacteria when compared with amoxicillin.

The antimicrobial index for methanolic extract of *C. pyrenoidosa* showed maximum percentage of inhibition (66.6%) against *B. cereus*, followed by *S. platensis* (55.5%) and *N. muscorum* (44.4%). However, effect of acetone extract of all selected algae revealed that *B. cereus* was the most sensitive strain.

K. pneumoniae was relatively more sensitive to extract of *N. muscorum* only (50% inhibition). On comparison of the results on antifungal activity of different algal extracts showed that *S. platensis* extract was more effective than that of *N. muscorum* and *C. pyrenoidosa* extract. The n-hexane extract and aqueous extracts were least effective against microorganisms. The present results indicated that gram-positive bacteria are more sensitive to methanolic extract of algal species than gram-negative bacteria. Earlier many workers demonstrated that crude extracts of Indian seaweeds are more active against Gram-positive bacteria [41].

Earlier the terpenoids, quinones and phenols have been identified as biologically active compounds against the microorganisms [42]. Earlier Ely *et al*., [39] showed that the methanolic extract of *Cladophora prolifera* was very effective antimicrobial agent with moderate bactericidal activity against *S. aureus* and *Vibrio cholerea*. Freile-Pelegrin and Morales [43] found that the ethanolic extract obtained from different regions of *Caulerpa spp* thallus (apical, basal and stolon) exhibit antibacterial activity. However, extract obtained from stolon region exhibited the highest bactericidal activity. A logical reason for variation in the antibacterial activity of different algal extracts could be due to specific distribution of antimicrobial substances as suggested by Lustigman and Brown [44]. A higher antimicrobial activity in the methanolic extract algal strains than the acetone extract may be due to abundance of some lipophilic, but polar compounds.

Previous investigations also reported that the compounds such as 1-Octadecene, 1-Heptadeceane present in both algae and higher plants are responsible for their anticancer, antioxidant and antimicrobial activities [45, 46]. It has been suggested that the lipids and fatty acids present in the algal strains could also be responsible for antimicrobial property [47, 48]. Fatty acids isolated from microalgae have been known to exhibit antibacterial activity [49].

Nitric oxide radical scavenging activities in the different algal extracts have been used as an indicator of antioxidant property of different algal extracts [50]. The present results revealed high percentage of NO scavenging activity in *S. platensis* (40%), followed by *C. pyrenoidsa* (18.85%).

The antioxidant potential in the extract of *Spirulina* and *Nostoc* strains might be due to the total phycocyanin, triterpenoids and carotenoids present in the algal extracts.

The antioxidant activity of *Spirulina* has been very well documented by Abd El-Baky *et al*., [51]; Khan *et al*., [52]; Athukorela *et al*., [53]. Other macroalgae also synthesize antioxidant molecules such as ascorbate, glutathione (GSH) as well as many more stable molecules including carotenoids, mycosporine-like amino acids, catechins, phlorotannins [54, 55, 56]. Cellular presence of phenolic compounds has also been coupled with both antioxidant and antibacterial activities [55].

In view of foregoing results, it is concluded that algal extracts have potential to work as antimicrobial agents, which may be exploited either by application of individual extracts or in combination with chemical antibiotics. The antioxidative attributes of algal extracts would always contribute positively in pharmaceutical of the algal extracts.

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How to cite this article:
Arun N, Gupta S and Singh DP: Antimicrobial and Antioxidant Property of Commonly found Microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some Pathogenic Bacteria and Fungi. *Int J Pharm Sci Res*. 3(10); 4866-4875.